UNIVERSITÀ DEGLI STUDI DI PADOVA

SCUOLA DI MEDICINA E CHIRURGIA

CORSO DI LAUREA MAGISTRALE IN MEDICINA E CHIRURGIA

DIPARTIMENTO DI MEDICINA

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TESI DI LAUREA

Predictors of minimal, moderate, and major response to Belimumab evaluated by CLASI, DAS28 and SLE-DAS. A retrospective analysis of a large nationwide multicentric cohort of SLE patients prospectively followed

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Anno accademico 2021/2022

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ABSTRACT

Background Systemic lupus erythematosus (SLE) is an autoimmune disease involving different organs and systems, including skin and joints. Belimumab is an anti-B lymphocyte stimulator protein monoclonal antibody and is the first biological drug approved for SLE after 50 years.

Aims The aim of this study was to assess rates and predictors of organ response to Belimumab in patients with cutaneous and articular manifestations by using graded specific-organ indices, CLASI for skin manifestations, DAS-28 CRP for joint manifestations (*Study A*), and SLE-DAS, an overall activity score (*Study B*).

Methods. *Study A* involves patients with active disease, CLASI>0 and DAS-28>1.32 from the Italian BeRLiSS (Belimumab in Real Life Setting Study) multicentric cohort treated with Belimumab as add-on therapy. CLASI and DAS28 20, 50, 70 defined by a decrease of at least 20%, 50% and 70% in each score were evaluated at 6, 12, 24 months and at 36 and 48 months for patients with joint manifestations. Logistic regression analysis was carried out to find baseline predictors of different outcomes.

Study B involves patients with DAS-28>1.32, available SLE-DAS treated with Belimumab and followed at the Padua Lupus Clinic from 2019. SLEDAS 20, 50, 70 values at 6, 12, 24 months and their relationship with DAS 28 20, 50, 70 were analysed.

Results. *Study A* In the analysis were included 272 patients with joint and 147 patients with skin manifestations.

Joint involvement DAS20 at 6, 12, 24, 36, 48 months was achieved by 57.7%, 71.2%, 84.2%, 79.7% and 82.1%, respectively; DAS50 at 6, 12, 24, 36, 48 months was achieved by 18.0%, 28.4%, 44.7%, 52.5% and 57.1%, respectively; DAS2870 at 6,12, 24, 36, 48 months was achieved by 1.1%, 3.7%, 10.5%, 10.2% and 17.9%, respectively. An association, although not significant, was found between early lupus (disease onset ≤ 2 years) and DAS28 20 (p=0.055) and DAS28 50 responses (p=0.057) at 6 months and DAS 20 response at 12 months (p=0.058). CAD (chronic active disease) pattern was a baseline negative predictor of DAS28 50 response at 6 (p=0.026) months and DAS28 20 response at 24 months (p=<0.001). Daily

prednisone intake ≥ 5 mg was a baseline negative predictor of DAS28 50 response at 6 months (p=0.014) and DAS28 50 response at 12 months (p=0.022). DAS-28 score ≥ 5.1 was a baseline positive predictor of DAS 2850 response at 6 months (p=0.017), DAS28 20 (p=0.033) and DAS28 50 responses (p=<0.001) at 12 months, DAS28 50 (p=0.006) and DAS28 70 responses (p=0.004) at 24 months. SLEDAI at recruitment was independent predictor of DAS28 20 response at 36 months (p=0.037).

Cutaneous involvement CLASI 20 at 6, 12, 24 months was achieved by 70.7%, 83.9%, 91.3%, respectively; CLASI 50 at 6, 12, 24 months was achieved by 52.4%, 72.0%, 84.1%, respectively; CLASI 70 at 6, 12, 24 months was achieved by 41.5%, 61.0%, 75.4%, respectively. There was a trend for an association between CLASI 50 response at 6 months and early lupus (p=0.077). A longer disease duration was a negative predictor of CLASI 70 response at 24 months (p=0.011). Patients with CAD were less likely to achieve CLASI 50 response at 6 months (p=0.052). SLEDAI at baseline was a positive predictor of CLASI 70 responses at 6 months (p=0.025) and at 12 months (p=0.032); conversely, it was a negative predictor of CLASI 50 response at 24 months (p=0.046). CLASI ≥ 10 was a negative predictor of CLASI 70 responses at all the time-points considered: 6 (p=0.047), 12 (p=0.039) and 24 months (p=0.011).

Study B Thirty-three patients were analysed. SLEDAS20 at 6, 12, 24 months was achieved by 48.5%, 76.9% and 84.2%, respectively; SLEDAS50 at 6, 12, 24 months was achieved by 27.3%, 53.8% and 52.6%, respectively; SLEDAS70 at 6, 12, 24 months was achieved by 24.2%, 42.3% and 52.6%, respectively. A positive correlation between the value of DAS28 and SLEDAS values was found at 24 months (ρ=0.022).

Conclusions In patients with joint manifestations the best Belimumab response was found for patients with early and active disease characterized by DAS28 ≥5.1, low daily prednisone intake and with relapsing-remitting pattern. On the other hand, patients with skin manifestations were better responders to Belimumab in case of early disease and relapsing-remitting pattern of disease. No clear correlations between SLE-DAS and DAS28 were found, therefore both should be analysed in clinical practice in order to evaluate response in patients with joint manifestations.

RIASSUNTO

Presupposti dello studio Il lupus eritematoso sistemico (LES) è una malattia autoimmune che coinvolge differenti organi, inclusi la cute e le articolazioni. Il Belimumab, un inibitore dello stimolatore dei linfociti B, è stato il primo anticorpo monoclonale approvato per il trattamento del LES dopo 50 anni.

Scopo dello studio Valutare le risposte al Belimumab e i loro predittori nei pazienti con interessamento cutaneo e articolare utilizzando degli indici graduati di malattia organo-specifici, il CLASI per la le manifestazioni cutanee, il DAS-28 per quelle articolari (*Study A*) e lo SLE-DAS, un indice di malattia globale (*Study B*).

Materiali e metodi Lo *Studio A* ha coinvolto pazienti con malattia attiva, CLASI>0 e DAS-28 CPR>1.32 selezionati dalla coorte dello studio multicentrico BeRLiSS (Belimumab in Real Life Setting Study) e trattati con Belimumab in aggiunta allo standard of care. CLASI e DAS-28 20, 50, 70 definiscono rispettivamente una riduzione di almeno il 20%, 50%, 70% in ciascun indice e sono stati valutati ai mesi 6, 12, 24 e, solo per i pazienti articolari, anche a 36 e 48. I predittori di risposta basali per i differenti outcomes sono stati ricercati con regressioni logistiche.

Lo *Studio B* ha coinvolto pazienti con malattia attiva in trattamento con il farmaco, DAS-28 CPR > 1.32 e con SLE-DAS disponibile seguiti a Padova dal 2019. I valori SLE-DAS 20, 50, 70 e la loro associazione con DAS-28 20, 50, 70 sono stati analizzati a 6, 12, 24 mesi.

Risultati *Studio A* Nell'analisi sono stati inclusi 272 pazienti con interessamento articolare e 147 pazienti con interessamento cutaneo.

Interessamento articolare Il DAS28 20 a 6, 12, 24, 36, 48 mesi è stato raggiunto dal 57.7%, 71.2%, 84.2%, 79.7% e 82.1%, rispettivamente; il DAS28 50 a 6, 12, 24, 36, 48 mesi è stato raggiunto dal 18.0%, 28.4%, 44.7%, 52.5% e 57.1%, rispettivamente; il DAS28 70 a 6, 12, 24, 36, 48 mesi è stato raggiunto dal 1.1%, 3.7%, 10.5%, 10.2% e 17.9%, rispettivamente. Un'associazione, seppur non significativa, è stata identificata tra l'early lupus (esordio $\leq$ 2 anni) e risposta DAS28 20 (p=0.055) e DAS28 50 (p=0.057) a 6 mesi e risposta DAS28 20 a 12 mesi (p=0.058). Il pattern di malattia cronicamente attivo è stato identificato come un predittore negativo di risposta DAS28 50 a 6 mesi (p=0.026) e di risposta DAS28

20 a 24 mesi (p=<0.001). Una dose giornaliera di prednisone ≥ 5 mg al baseline è risultato un predittore negativo di risposta DAS28 50 a 6 mesi (p=0.014) e di risposta DAS28 50 a 12 mesi (p=0.022). Un punteggio ≥ 5.1 di DAS28 al basale è risultato un predittore positivo delle risposte DAS 28 50 a 6 mesi (p=0.017), DAS28 20 (p=0.033) e DAS28 50 (p=<0.001) a 12 mesi, DAS28 50 (p=0.006) e DAS28 70 (p=0.004) a 24 mesi. Il punteggio SLEDAI al reclutamento risulta un predittore indipendente di risposta DAS 20 a 36 mesi (p=0.037).

Interessamento cutaneo CLASI 20 a 6, 12, 24 mesi è stato raggiunto dal 70.7%, 83.9%, 91.3% rispettivamente; CLASI 50 a 6, 12, 24 mesi è stato raggiunto dal 52.4%, 72.0%, 84.1% rispettivamente; CLASI 70 a 6, 12, 24 mesi è stato raggiunto dal 41.5%, 61.0%, 75.4% rispettivamente a 6, 12, 24. È stato evidenziato un trend positivo tra la risposta CLASI 50 a 6 mesi e l'early lupus (p=0.077). Una durata di malattia maggiore era un predittore negativo di risposta CLASI 70 a 24 mesi (p=0.011). Pazienti con un pattern di malattia cronicamente attivo tendevano a non raggiungere la risposta CLASI 50 a 6 mesi (p=0.052). Lo SLEDAI al baseline è un predittore positivo di risposta CLASI 70 a 6 mesi (p=0.025) e a 12 mesi (p=0.032); tuttavia, è anche un predittore negativo di risposta CLASI 50 a 24 mesi (p=0.046). Un predittore negativo al baseline di risposta CLASI 70 a tutti i time-points considerati è un punteggio CLASI ≥ 10 (per i 6 mesi p=0.047, per i 12 p=0.039, per i 24 p=0.011). *Studio B* 33 pazienti sono stati analizzati. Lo SLE-DAS 20 a 6, 12, 24 mesi è stato raggiunto dal 48.5%, 76.9% and 84.2%, rispettivamente; lo SLE-DAS 50 a 6, 12, 24 mesi è stato raggiunto dal 27.3%, 53.8% e 52.6%, rispettivamente; lo SLE-DAS 70 a 6, 12, 24 mesi è stato raggiunto dal 24.2%, 42.3% e 52.6%, rispettivamente. Una correlazione positiva è stata trovata tra i valori di SLE-DAS e DAS28 24 mesi.

Conclusioni I pazienti con interessamento articolare che rispondono meglio presentano esordio recente, malattia attiva con DAS28 ≥ 5.1 e un basso dosaggio di prednisone giornaliero, e un pattern relapsing-remitting. Invece per le manifestazioni cutanee la risposta è migliore in caso di recente diagnosi e malattia relapsing-remitting. Non è stata identificata una correlazione chiara tra SLE-DAS e DAS-28, pertanto entrambi devono essere analizzati nella pratica clinica per valutare la riposta al trattamento nei pazienti con manifestazioni articolari.

1. Systemic lupus erythematosus

1.1 Definition

Systemic lupus erythematosus (SLE) is a multisystemic, chronic autoimmune disease affecting different organ and systems and encompasses either mild or life-threatening manifestations. In predisposed individuals, usually after encountering a trigger agent, the disease leads to the loss of immunological tolerance and the immune system to activate toward self-antigens and the expansion of autoreactive cell-clones (1). The production of a broad, heterogeneous group of autoantibodies, responsible for tissue damage through multiple mechanisms, is a key feature of the disease (1–3). The expansion of autoreactive clones is elicited by abnormal apoptosis stimulating the release of autoantigens (4).

Clinical manifestations in patients with SLE can be attributable not only to the disease activity itself, but also to damage accrual, drug side effects and co-morbidities (5).

Systemic lupus erythematosus is classified as a connective tissue disease (CTD) and it is distinguished by alternating periods of flare, and periods of remission, but some patients have continuous disease activity (6).

1.2 Epidemiology

SLE is more prevalent and severe in non-Caucasian population, including Afroamerican and Asian ethnicity: the prevalence of SLE in US population is higher in Asian-Pacific Islander individuals (90.5 per 100000) than in white individuals (55.2 per 100000) (7). Similarly, black ethnicity is a risk factor for the development of the disease and serious manifestations (8). In Western countries prevalence of SLE ranges from 20 to 150 cases per 100.000, depending on ethical and environmental background (9). In particular, the incidence in Europe and United States is estimated to be between 7 and 150 cases per 100 000(10). Prevalence is nine times higher for women. SLE patients in 65% cases are between the ages 16 and 55, 20% are younger than 20, and 15% are older than 55 (4).

1.3 Etiopathogenesis

The pathogenesis of SLE involves a multitude of cells and molecules that participate in apoptosis, innate and adaptive immune response (4).

Even though the etiology of the disease is not fully understood, it is largely believed to have a multifactorial pathogenesis with genetic, environmental, and hormonal factors contributing to its development (3).

Genetic background in SLE patients confers a predisposition to exaggerated response to different stimuli, i.e. infections. Therefore, especially after encountering an environmental trigger agent such as EBV, irreversible break in immunological tolerance and immune response against endogenous nuclear antigens occurs (1).

In addition, deregulated apoptosis in lymphocytes, mediated by abnormal expression of Fas/Fas Ligand and extrinsic apoptosis pathway, leads to an increase in apoptotic material. Since a defective clearance of cellular debris allows extracellular modifications that increase their immunogenicity and create new epitopes, apoptotic remnants may enrich the autoantigen pool. Not only apoptosis is dysregulated in SLE, but also NETosis, which is a novel death-cell mechanism that involves web-like-structures called NETs. NETs in healthy subjects contribute to keep under control inflammation, damage and attacks from microorganisms. Alterations of this mechanisms lead to a release of even more autoantigens (10).

In the pathogenesis of SLE, B and T cell signalling abnormalities play a pivotal role and contribute to the intrinsic hyperactivity and hyper-responsiveness typical of SLE patients, promoting the productive auto reactive B and T cell clones (1).

First, levels of BAFF in sera of SLE patients are extremely high: BLyS is a member of the TNF ligand superfamily, which supports survival and differentiation of B-cell, including autoreactive cells. Then, B regulatory cell function and T cell modulation appears to be impaired (10).

T cells activations, by means of the secreted cytokines, help B cells to produce antibodies. Recent data support T cell-independent mechanism of B cell stimulation via combined B cell antigen receptor (BCR) and TLR signalling. Finally, this leads to a production of a broad spectrum of autoantibodies responsible for tissue inflammation and damage accrual through different pathogenetic mechanism (4).

Among these, the most common are deposition of immune complexes in target organs, direct cellular lysis and induction of inflammation via complement fixation and activation (10).

1.3.1 Genetic factors

More than 30 genes or loci have been associated with the disease and the genetic susceptibility is a result of different interrelated allelic variations, rather than highly penetrant mutations. These last include deficiencies of complement components (C1q, C2, C4A, C4B), Fcγ receptor and mutations in DNA exonuclease (TREX1), but are responsible for very few cases (1). Among gene mutations considered to be associated with SLE, HLA-genes play an important role. There is a strong connection between class I and II HLA's alleles and the development of LES, mostly due to autoantibodies production (10).

Case-controlled genetic studies have identified genes involved in type I interferon signalling, production and response as SLE-associated loci. The type I interferon pathway has been implicated in the pathogenesis of SLE and has been linked with SLE initiations. IFNα is the predominant circulating type I interferon and its regulatory genes as IRF (IFN regulatory factor) are often upregulated in SLE patients (11). Gain of function variants of IRF5 correlate with increased production of IFNα, triggered by endosomal Toll-like receptor activation in plasmacytoid dendritic cells (p DC). Notably, autoantibodies in SLE targeting RNA binding protein and chromatin form immune complexes with nucleic acids. Their binding to Fc receptor and endocytosis stimulate TLR 7/9 and downstream IRF pathway, boosting the production of cytochines and B-lymphocyte autoantibodies (4,10,11). In addition, the interplay between B cell receptor (BCR), TLR and BAFF contributes to the activation of autoreactive B cells and accumulation of more autoantibodies and immune complexes that adds fuel the ongoing immune response and create a vicious circle (1,10).

In the pathogenesis of SLE epigenetic effects such as DNA methylation, histone modifications and microRNA interference may be involved. Differences in the methylation status of genes may explain, at least in part, the discrepancies observed in some identical twins that are discordant for SLE (4,10).

The risk of SLE is 14 times higher in Klinefelter syndrome (47, XXY). This suggests an association with genes on the X-chromosomes, however the exact genes have not been identified yet (2).

1.3.2 Environmental factors

Genetic factors confer critical susceptibility to SLE, albeit they do not fully clarify its pathogenesis, as demonstrated by the incomplete concordance of the disease in twins. Recognition of environmental factors provides further information on the disease's development and allows preventive measures, where necessary (12).

- Sunlight is the most obvious environmental element that may exacerbate SLE. Ultraviolet rays and sun exposure are well-known triggers for SLE and lead to increased cell apoptosis (2).
- EBV (Epstein-Barr virus) has been identified as a possible factor in the development of lupus, since elevated IFNα levels need to be produced to control chronic viral infection. This is confirmed by the higher prevalence of antibodies against EBV in children and adults with SLE compared to the general population (4).
- Over 100 drugs are well-known culprits of drug-induced lupus (DIL), such as procainamide and hydralazine; sulfa-drugs can cause flares in SLE patients (2,6).
- Female sex hormones are a significant risk factor for SLE: estrogen and prolactine promote autoimmunity and increase B-cell activation. Pregnancy may cause in some cases a lupus flare (1,4). Women experience more flares than men, attesting that female hormones are important drivers of initiation and maintenance of lupus activity (13).
- Other potential risk factors include smoking and vitamin D deficiency (10).

1.4 Clinical manifestations

The disease has a wide heterogeneity of clinical manifestations and several phenotypes. The clinical presentations in patients range from mild mucocutaneous manifestations to multiorgan and severe central nervous involvement that have different impact on patients' quality-life (2,10).

1.4.1 General

Constitutional symptoms are seen in more than 90% of patients with SLE and are often the initial presenting feature. Fatigue, malaise, fever, anorexia and weight loss are common (2).

1.4.2 Mucocutaneous

Skin involvement in SLE occurs in more than 80% of patients: SLE skin lesions may be lupus specific or non-specific. A key characteristic of cutaneous lupus erythematosus is the photosensitive distribution (14).

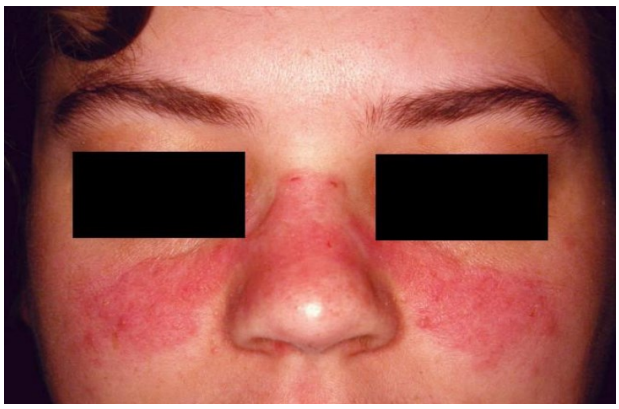
Specific mucocutaneous manifestations	**Aspecific mucocutaneous manifestations**
1. Acute cutaneous lupus erythematosus (ACLE) - Localized rash - Generalized rash	Photosensitivity
	Leucocytoclasic vasculitis - Palpable purpura - Urticarial vasculitis
	Diffuse alopecia (“lupus hair”)
2. Subacute cutaneous lupus erythematosus (SCLE) - Annular - Papulosquamous (psoriasiform)	Thrombophlebitis
	Occlusive vasculopathy
	Raynaud phenomenon
	Periungual telangiectasia
3. Chronic cutaneous lupus erythematosus - Discoid lupus erythematosus (DLE) a. Classical (localized/generalized) b. Hypertrofic or verrucous - Chilblain lupus erythematosus (CHLE)	Livaedo reticularis
	Calcinosis cutis
	Papulonodular mucinosis
	Erythema multiforme
	LE non-specific bullous lesions
	Erythromelalgia
	Leg ulcers
	Lichen planus
	Sclerodactyly

- Lupus erythematosus profundus or panniculitis (LEP) - Lupus tumidus - Mucosal lupus erythematosus	Rheumatoid nodules
	Acanthosis nigricans
	Urticaria

Table I. Classification of lupus erythematosus (SLE) associated skin lesions

Lupus specific lesions include acute, subacute and chronic cutaneous lupus erythematosus.

Acute cutaneous lupus erythematosus (ACLE) can be localized or generalized and is photosensitive. The 'butterfly rash' is the hallmark ACLE lesion and consists in a localized erythematous and edematous pruritic or painful rash spreading symmetrically over the nasal bridge and the cheeks, sparing the nasolabial folds. The differential diagnosis include rosacea, erysipelas, seborrheic dermatitis, and perioral dermatitis (2,4). Considering that the butterfly rash is an active sign of the disease, it is often associated with other inflammatory manifestations and fluctuates with disease activity. Therefore, these lesions are usually transient and heal without scarring when the systemic disease is under control (10).



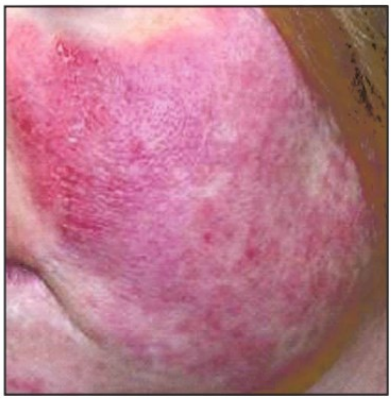


Figure 1, 2. ACLE: acute cutaneous lupus erythematosus(10,15)

Subacute cutaneous lupus erythematosus (SCLE) is a photosensitive rash that commonly affects shoulders, forearms, neck, chest, estensory part of the arms and face. Two types of skin lesions has been described: papulosquamous (psoriasiform skin lesion) or annular-polycyclic (4). SCLE can be an isolated manifestation or can be part of a systemic disease (50% of cases) or Sjogren syndrome. It lasts several months, but usually heal without scarring. It is most frequent among smokers, in

90% of the cases is found in positive anti-Ro (SSA) patients, and some drugs, such as hydroclorothiazide, can trigger its development (2,10).

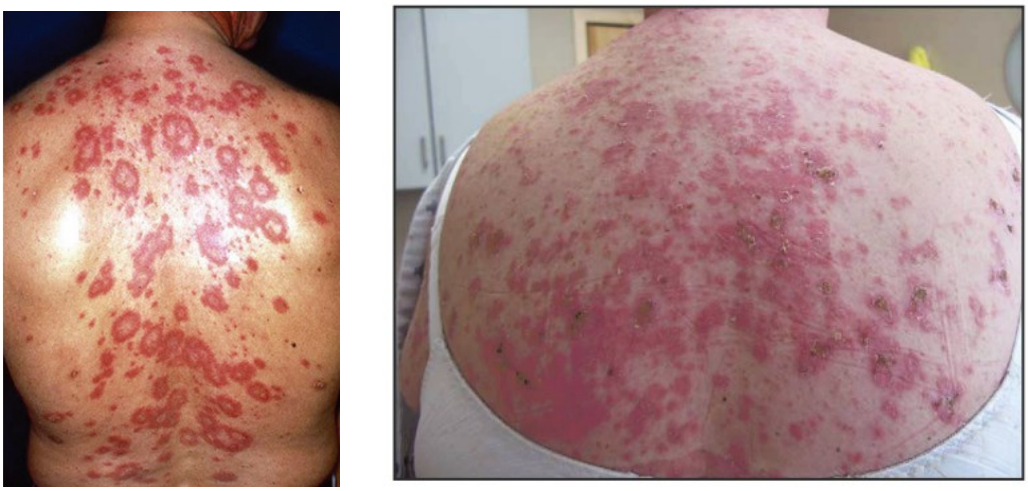


Figure 3, 4. SCLE: on the left annular-polycyclic and on the right papulosquamous form (4,15)

Classic discoid lupus erythematosus (DLE) is the most common form of chronic cutaneous lupus erythematosus (CCLE). Notably, it can occur with (10%) or without systemic disease (90%) and can be either localized (only head and neck) or generalized (above and below the neck) (10). The lesions are sharply bordered, disk-shaped erythematous elevated papules surrounded by adherent scaling that tend to extend into dilated hair follicles and expands centrifugally. After healing, DLE leaves depressed scars, atrophy, dyspigmentation. DLE should be distingued from hypertrophic lichen planus, eczema, actinic keratosis, psoriasis (4).

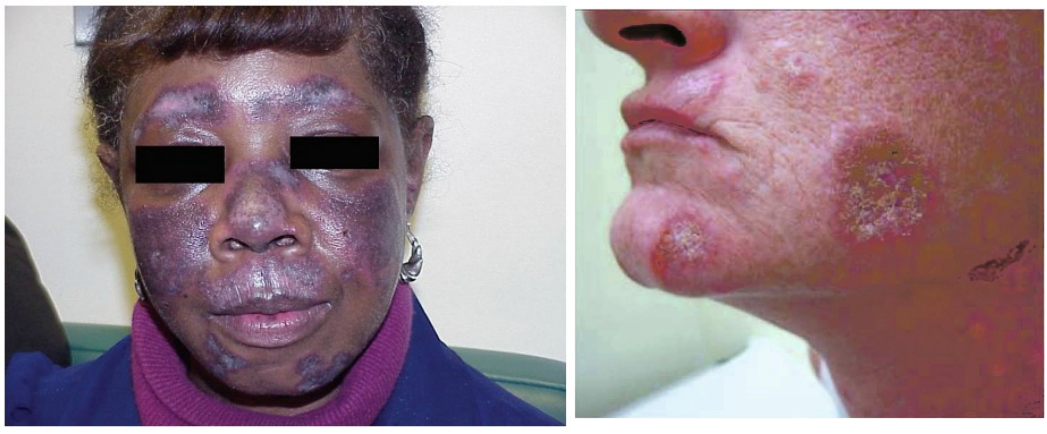


Figure 5, 6. CCLE: chronic cutaneous lupus erythematosus (4,15)

Among other CCLE lesions:

- Thick lesions characterize hyperkeratotic (verrucous) DLE. Body regions most commonly affected are the extensor surface of arms, face and hands; it may mimic keratoacanthoma, hypertrophic lichen planus and squamous cell carcinoma histologically (2,15).
- Chillblain lupus erythematosus (CHLE) is revealed by purple or erythematous tender papules, plaques or nodules mainly in fingers and toes, which are the most exposed anatomical areas to the environmental triggers of this manifestation, i.e. cold, damp (2,15);
- LE panniculitis appears as firm, depressed areas. Nearly half of patients have associated DLE lesions overlying them, resulting in what is known as lupus erythematosus profundus (LEP) (2,15). These lesions are often localized on the scalp, face, arms, trunk, thighs, glutes and cause pain (4).
- LE tumidus is characterised by erythematous photo distributed plaques without superficial involvement (4,15).
- Mucosal lesions are a common finding in patients diagnosed with DLE. Oral DLE affects mainly labial and buccal mucosa in the form of white plaques with a central red area and bordering radiating white striae. In such case, the differential diagnosis with lichen planus might be problematic. Non-specific oral lesions affecting the nasal, conjunctival and the palate with erythema and superficial ulcerations occur in a great percentage of SLE patients (4,15).

  In the oral cavity, the presence of lesions is reported between 7 and 52% of SLE patients; these can be directly related to the disease process or linked to others factors, such as treatment, intraoral infections, or associated Sjogren's syndrome. Mucosal alterations correlated with disease activity are categorized into three different classes, i.e. erythematosus, discoid and ulcerative type. Oral lesions might be asymptomatic and therefore accurate examination of oral cavity is required to detect them (16).

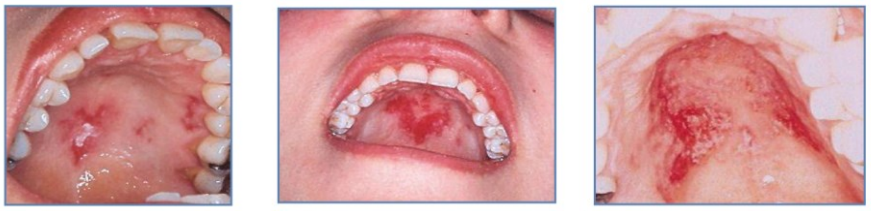


Figure 7. Oral lesions in SLE (10)

LE non-specific cutaneous manifestations include:

- Photosensitivity, which can be described as the outbreak of rashes after exposure to ultraviolet radiation (4). The definition reported in the ACR criteria is: 'skin rash as a result of unusual reaction to sunlight, by patient history or physician observation';
- Alopecia, in particular scarring alopecia is a common result of DLE. Non-scarring alopecia is frequent; generally, alopecia occurs in most SLE patients and it may involve eyebrows, eyelashes, beard and body hair (4);
- Cutaneous vasculitis. The most frequent is a small-vessel cutaneous leukocytoclastic vasculitis, which appears clinically as a palpable purpura from the hip to the toes. Less commonly, in the context of LE, a medium-vessel vasculitis is reported and typical symptoms of this condition are mononeuritis multiplex, ulceration and visceral vasculitis (15);

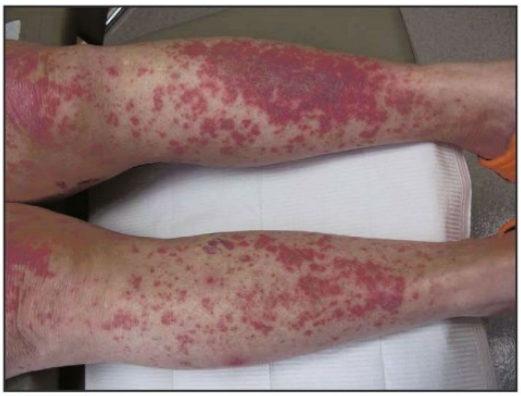


Figure 8. Lupus vasculitis (15)

- Frequent digital manifestations are livedo reticularis, periungual teleangectasia and Raynaud's phenomenon, the last-mentioned in up to 60% of patients. Livaedo affects one third of SLE patients, especially those positive for antiphospolipid antibodies, and reveals the presence of cutaneous and nailfold

vasculitis. The presence of periungual teleangectasia might raise the suspect of others connective tissues diseases such as dermatomyositis and systemic sclerosis (15);

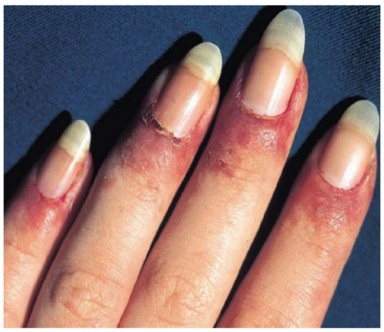


Figure 9. Livedo reticularis and periungual teleangectasia (15)

- Bullous SLE consist of papillary microabscesses filled with neutrophils, manifested as vesciculobullous skin eruptions (15).

Other lesions associated with SLE are urticaria, erythromelalgia, sclerodactyly, rheumatoid nodules, calcinosis cutis, erythema multiforme, acanthosis nigricans, lichen planus, leg ulcers, cheilitis and episcleritis (2).

1.4.3 Musculoskeletal

Musculoskeletal disturbance is the one of the most frequent symptom in SLE patients with a prevalence of 70-90% and is commonly associated with disease-flares. Joint involvement comprises joint pain, polyarthritis, Jaccoud's arthropathy and rhupus syndrome; patients can also experience entheseal involvement.

Joint inflammation in SLE firstly involves the hands and clinically presents as arthralgia, characterized by stable joint pain sometimes combined with morning stiffness (3,17).

Lupus arthritis is typically a non-erosive and non-deforming (NDNE) symmetrical inflammatory polyarthritis found in up to 50-60% of patients. It affects predominantly the small joints of the hands, mainly proximal interphalangeal (PIP) and metacarpophalangeal (MP) joints, knees and wrists; shoulders, ankles and feet can be involved as well. The clinical presentation shows the classical signs of

inflammation with tenderness and swelling, but the inflammatory process typically does not lead to erosions and deformity (3,17).

Otherwise, hand and feet deformities can affect nearly 5 to 15% of SLE patients as signs of Jaccoud's arthropathy (JA). Jaccoud arthropaty is the result of the joint capsule and ligament laxity leading to non-erosive hand deformities, i.e. ulnar deviation, swan neck deformity, Z distortions of the thumbs, and subluxation of the metacarpophalangeal joints that may mimic rheumatoid arthritis. This is usually the result of a long-lasting disease with persistent arthritis.

The diagnosis of JA requires the fulfilment of 4 criteria, 1. SLE diagnosis based on the 2019 EULAR/ACR criteria, 2. Classic joint deformities, 3. No erosion or plain radiographs of the joint, 4. Exclusion of RA and other DCTDs based on the newest specific EULAR and ACR criteria; genetic connective tissue diseases, such as Ehlers-Danlos and Marfan's syndrome have to be ruled out (18).

In the 'classical' type, these deformities are reducible, seeing as the result of peri-articular involvement rather than of articular ankylosis. In few cases, patients can developed fixed deformities ('severe') or complex forms, called *mutilans-type* with multiple joint subluxations (3,10).

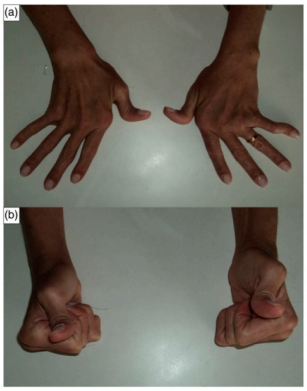


Figure 10. Joint deformities in Jaccoud-type lupus arthropathy: A. ulnar deviation and 'z' of the thumb; B. Reducible pattern of the deformities (18)

The distinguishing feature between articular deformities in RA and SLE is the absence of joint erosions in lupus arthritis.

In a small percentage of patients, radiological assessment identifies erosions in association with anti-cyclic citrullinated peptides antibodies (aCCP) and rheumatoid factor (RF). This condition is known as Rhupus syndrome and describes an overlap between rheumatoid arthritis and SLE, which is rare (5%) (17). The presence of the following features are indicative of rhupus: erosive symmetrical polyarthritis, anti-CCP, clinical signs of SLE in the presence of anti-dsDNA and/or anti-Smith antibodies (19).

To detect joint and tendon inflammation, considered as predictors of MS (musculoskeletal) flare and development of JA, high-resolution US is more sensitive than clinical examination. Therefore, it is often used to identify patients at risk of flares and arthropathy at an early stage in order to establish the appropriate treatment. In addition, given that musculoskeletal flare and Jaccoud's deformities have a major impact on everyday-life, it is a useful tool to prevent poor health related quality of life (HRQoL) and disability (20).

Notably, entheseal involvement it is found in four out of 20 SLE patients with MSUS (musculoskeletal ultrasound); furthermore, the presence of power doppler signal at this level could represent a potential biomarker of SLE disease activity (21,22).

Avascular necrosis can occur in up to 10% of cases, is usually bilateral and involves hip joints, shoulder and knees; many disease-related factors can induce its development such as chronic glucocorticoid use, antiphospholipid syndrome and vasculitis (2,4).

Muscular involvement generally occurs with myalgia; inflammatory myopathy is usually present in less than 10% of SLE cases. Importantly, patients with SLE are at high risk for the development of fibromyalgia with incidences as high as 20% reported (2).

1.4.4 Hematologic

More than 50% of SLE patients suffer from anemia, which correlates with the intensity of the disease activity and it is generally due to chronic illness. Other possible causes of anemia include iron deficiency anemia, ESRD (end-stage renale disease), haemolytic autoimmune anemia detected by positive Coomb's test,

cytotoxic drugs, microangiopathic haemolytic anemia possibly linked to antiphospholipid antibody, aplastic anemia (2,10).

Leukopenia secondary to neutropenia or lymphopenia is also common, especially in active phases of the disease, can be severe and correlates with glucocorticoid use or infection (2,10).

Trombocytopenia can be mild or enough severe to provoke hemorrhagic manifestations, although this is uncommon (5%) and may be associated with antiphospholid antibody syndrome and autoantibodies against platelets, glycoprotein IIb/IIIa receptor or thrombopoietin receptor (2,10).

1.4.5 Lupus nephritis

Kidney involvement occurs in roughly 40% of SLE patients.

It is a severe and detrimental manifestation and, despite the improvement in therapeutic strategies, is responsible for the progression to end stage renal disease in 4.3-10.1% of cases within 5 years from the diagnosis (23). Since glomerulonephritis (GLN) is a major cause of morbidity and mortality in SLE, kidney involvement is highly correlated with prognosis and survival of SLE patients (24,25).

The 2003 ISN/RPS (International Society of Nephrology/Renal Pathology Society) classification of lupus nephritis followed by the 2018 revision outline six histological classes, determined by specific microscopic lesions and distribution of immune complexes (IC):

- Class I LN (Minimal mesangial);
- Class II LN (Mesangial proliferative);
- Class III and IV LN (Focal and Diffuse);
- Class V (Membranous);
- Class VI (Advanced sclerosing).

(25)

Clinical symptoms differ significantly in manifestations and severity from one patient to another: renal involvement might be silent or may manifest as nephrotic,

nephritic syndrome or rapid progression to renal failure. Laboratory urinary tests can be normal or can show abnormalities such as mild proteinuria, haematuria, leukocyturia, cellular casts. The principal factors of renal relapse after appropriate treatment are worth mentioning and include young age at renal disease onset, male gender, African-American ethnicity, delayed treatment or partial response to therapy, high disease activity in other domains and serologically active disease (high anti-dsDNA titer, low complement) (23).

1.4.6 Neuropsychiatric

The ACR Nomenclature for NPSLE (neuropsychiatric SLE) provides case definitions for 19 different neuropsychiatic phenotypes, including CNS and PNS manifestations (4).

If properly investigated even in the slightest form, neurological involvement involves up to 50% of SLE patients and is a negative prognostic factor and a direct cause of death. It can be primary – directly correlated to the development of the disease - or secondary, due to other organs involvement, disease's complications and therapy (10). Data indicates that anti-ribosomal P protein antibodies have high specificity; therefore, they represent one of the most exclusive biomarker of NPSLE (26,27). The most common central nervous system (CNS) manifestations are:

- Headaches, reported in more than 50% cases, but usually not related with SLE. The presence of concomitant 'red flags symptoms or signs' (unusual intensity, fever, confusion, meningeal or focal neurological signs) that could herald severe pathology deserve particular attention and are worth further diagnostic examination (4).
- Cerebrovascular diseases including both stroke and transient ischemic attack, which are frequently associated with antiphospholipid syndrome (10).
- Focal or generalized seizures, which can be associated with disease activity in the setting of active generalized multisystem lupus or as solitary neurological events (2).

Less commonly but worth mentioning are:

- Acute confusional state, which belong to inflammatory neuropsychiatric manifestations (10);
- Cognitive dysfunction reported in up to 20-30% of patient but usually mild (4);
- Psychiatric manifestations range from depression and anxiety to psychosis, which is characterised by the presence of delusions or hallucinations (4).

Finally, rare CNS manifestations (<1%) are aseptic meningitis, demyelinating syndrome encompassing optic neuritis and myelitis, and move disorders such as chorea (2). The PNS manifestations include acute inflammatory demyelinating polyradiculoneuropathy, a syndrome mimicking Guillan-Barrè, autonomic disorders, mononeuropathy, myasthenia gravis, cranial neuropathy, plexopathy and polyneuropathy (4).

The most frequent manifestation of peripheral nervous system among those listed is sensory-motor axonal polyneuropathy, a major cause of morbidity and poorer quality of life (28).

1.4.7 Pulmonary

Pleurisy is the most common pleuropulmonary manifestation affecting nearly half of SLE patients and may constitute the initial sign of the disease. The effusions, if presents, are usually bilateral and evenly allocated between the two hemi thoraces, considering their inflammatory aetiology. Objective sings can be pleuritic chest pain below the diaphragm and pleural frictions at the auscultation (10).

Acute lupus pneumonitis is rare and its typical manifestations are fever, cough, dyspnoea, hypoxemia; it is characterized by a severe prognosis, which justifies an aggressive treatment(10). Infective pneumonitis is more common than in general population due to immunosuppressive treatment (5).

Other pulmonary manifestations include chronic interstitial lung disease (ILD), which is more common than the acute form and in almost all the cases it manifests as interstitial lung pneumonia (NSIP).

The ‘shrinking lung syndrome’ is a rare but typical SLE manifestation characterised by the presence of a restrictive pattern on the patients’ spirometry and no

parenchymal alterations, progressive dyspnoea and small lungs volume on chest radiograph. Diaphragm dysfunction and limited excursion are considered the most likely causes for this syndrome (4,10).

Pulmonary and diffuse alveolar haemorrhage (DAH) as well as pulmonary hypertension (PAH) are uncommon but potentially life-threatening complications. This last can be associated with pulmonary embolism, mostly in patients with antiphospholipid autoantibodies. Clinically, is not distinguishable from pulmonary idiopathic hypertension; the only difference in SLE is the frequent association with Raynaud's phenomenon (2,10).

1.4.8 Cardiovascular

Pericarditis associated with exudative pericardial effusion is the most common SLE cardiovascular manifestation, although tamponade is rare (4).

Vasculitis of the small coronary vessels causes myocarditis and may be detected in patients with anti-Ro (SSA) antibodies in the presence of generalised active lupus. Its treatment should be aggressive to prevent chronic sequelae like congestive heart failure (2,4,29).

SLE patients have a significant increase in morbility and morbidity from acute cardiovascular events, such as myocardial infarction. This is a result of the accelerated and early atherosclerosis typical of the disease, being one of the most important comorbidity and cause of death in young patients. Strict management of traditional cardiovascular factors is therefore paramount (29).

Valvular abnormalities in SLE include Libman-Sacks verrucous endocarditis involving in order of frequency the mitral, aortic, tricuspid and pulmonary valve; association with antiphospholipid antibodies is common (10).

1.4.9 Gastrointestinal

Any part of the gastrointestinal tract can be affected, from the oral cavity to the anus.

Esophagus most common manifestations are dysphagia and dismotility, mainly in the upper one-third, but it can also occur in the inferior third or the whole

esophagus. Esophagitis with ulceration has been observed in 3-5% of SLE patients (10,16).

In the stomach, gastritis and peptide ulcers can be iatrogenic lesions mainly due to anti-inflammatory drugs (NSAID). This explains the necessity for patients undergoing long-term NSAIDs therapy to assume proton pump inhibitors. Lupus enteritis and colitis can be a consequence of small-vessels intestinal vasculitis that can lead to intestinal ischemia, eventually bowel infarction, bleeding, perforation and peritonitis. Early recognition and treatment of gastrointestinal vasculitis is mandatory, considering its role in increasing morbidity, mortality and worsening patients' prognosis (10,16).

Pancreatitis due to lupus may result from vasculitis or thrombosis and occurs in as many as 2-8% of patients; nevertheless an increase in blood amylases can be found in nearly 20% of patients without evident sign of pancreatitis (4,10).

Liver involvement is characterised by abnormal liver chemistries in more than half of patients. SLE associated hepatitis can be caused by the disease itself (lupoid hepatitis) or imputable to treatment. The incidence of hepatomegaly is 12-25% whereas ascites is uncommon and, when detected, is secondary to heart failure, nephrotic syndrome, protein-losing enteropathy, and cirrhosis (4,10).

Abdominal pain accompanied by anorexia, nausea and vomiting is a common finding in up to 50% of patients. It might reveal serious different pathological processes underlying the disease, such as mesenteric vessel thrombosis and intestinal ischemia (often correlated with antiphospholipid syndrome), peritonitis, pancreatitis, inflammatory bowel disease, perforated peptic ulcer, intestinal vasculitis (4,16).

1.5 Diagnosis

The wide heterogeneity of lupus manifestation together with the absence of a unique presentation and the fluctuating symptoms make the diagnosis difficult.

SLE diagnosis is mostly a clinical process in combination with serological analysis, subjective by the clinician's expertise and judgment (26).

Considering the impact of an early SLE detection on the long-term prognosis, understanding which manifestations can be precursors of SLE is fundamental (26). Early SLE diagnosis is crucial for an early therapeutic intervention which can increase the probability of disease remission and improve patient prognosis (30).

The diagnostic process mainly consists in three stages: first a combination of clinical and immunologic features characteristic of SLE, second a distinctive autoantibody profile and in the end, concomitantly ruling out of SLE mimickers.

Several clinical manifestations combined with laboratory tests should raise the suspect of SLE, some examples are elevated erythrocyte sedimentation rate (ESR), γ-globulin and low complements levels (2,10).

The C-reactive protein (CRP) is usually normal in SLE patients. If CRP increases, infections should be investigated (5). Other initial laboratory tests to perform include complete blood count (CBC), urinalysis, liver and renal function test including serum creatinine and a comprehensive metabolic panel (31).

The diagnostic process is then followed by autoantibodies identification and exclusion of possible differential diagnosis.

1.5.1 Serologic tests

1.5.1.1 Antinuclear antibodies (ANA)

The introduction of ANA testing around 1980 has significantly shortened the lag time between the disease onset and diagnosis, helping to improve survival and quality of life. New diagnostic procedures to detect SLE as early as possible to improve the long-term prognosis are still an open challenge (30). The ANAs essay is an ideal screening test thanks to its high sensitivity, ranging from 90% to 95% in SLE patients, and easiness in clinical practice, thus permitting to rule out the diagnosis, if negative. Major drawbacks are inadequate specificity and low positive predictive value, which is only 11-13%, confirmed by their presence in 5-20% of healthy population (especially in elderly people as their formation is age-dependent), as well as in other autoimmune disorders. ANA antibodies are frequently found in patients with scleroderma, polymiositis, dermatomyositis,

rheumatoid arthritis, autoimmune thyroiditis and hepatitis and many other conditions as infections, neoplasms and drugs (4,26).

There are several possible nuclear and cytoplasmic auto-antigens targeted by ANAs and further tests are necessary to detect the precise protein or nucleic acid addressed (2). The main sub specificities of ANA antibodies are anti-dsDNA and anti-ENA (extractable nuclear antigens). Anti-DNA are specific SLE antibodies that target both double stranded (ds) and single stranded (ss) DNA; their circulating levels have recently emerged as a biomarker of the disease *[See forward, Chapter 1.5.2 Biomarkers].*

1.5.1.2 Extractable nuclear antigen antibodies (ENA)

Antibodies to ENA associated with systemic lupus erythematosus include:

- Anti-SM (Smith), SLE-specific antibodies. Their presence is pathognomonic but their sensitivity is low, detected in 20-30% of SLE patients often together with anti-U1-RNP antibodies. Differently from anti-dsDNA they cannot be used as predictors of disease activity (4,26) but they have shown associations with constitutional symptoms, lupus nephritis and central nervous system disease (32);
- Anti-U1-RNP antibodies, which can be seen in overlap connective tissue disease and, unlike anti-SM, are not disease specific (2);
- Anti-Ro (SSA) and anti-La (SSB) antibodies, commonly found in Sjogren syndrome. In SLE they are less common but may be associated with secondary Sjogren syndrome, congenital heart block and neonatal lupus (2,32).

1.5.1.3 Further antibodies

Others autoantibodies associated with the disease are:

- Anti-ribosomial P antibodies (anti-P), whose prevalence is less than 5%. They are considered one of the most exclusive biomarker of neuropsychiatric manifestations (NPSLE) (2,30);

- Antiphospholipid antibodies (aPL), well-known risk factors for thrombosis, obstetric complications and SLE-APS (antiphospholipid syndrome) (4);
- Anti-C1q antibodies, reported mainly in association to a deficiency in apoptotic cell clearance. Their identification is not exclusive of SLE as they are present in many different connective tissue disease and systemic vasculitis. Their levels are directly related to active LN, for which they have a relatively fair sensitivity and specificity; anti C1q levels increase before renal flares and may be better predictors than anti-double-stranded DNA antibodies (25,30).

1.5.2 Biomarkers

Biomarkers currently used in clinical practice are:

- Anti-ds DNA antibodies, associated with general disease activity and renal involvement: serum anti-dsDNA titres are consistent with LN and progression to end-stage renal disease. Despite their high sensitivity, since they exist in about 60-70% of SLE patients, a negative test does not exclude the disease (2,23,32);
- B lymphocyte stimulator (BLyS) or B activating factor (BAFF) is a TNF family member and a key cytokine for B cells differentiation, maturation, proliferation, and survival. BLys serum level are increased in SLE patients and in other autoimmune disease (30). There is a significant correlation between circulating BLys levels, disease activity and anti-dsDNA antibody, therefore it could become a biomarker of forthcoming disease activity and could predict flares. Belimumab is a fully human monoclonal antibody selectively targeting and inhibiting soluble Blys (8) *[See forward, Chapter 3 Belimumab].*
- Decrease in complement fractions C3 and C4 indicates complement consumption and therefore high disease activity. In a recent retrospective case-control study it was demonstrated that low levels of both C3 and C4 and positive ANA assay are highly specific for SLE diagnosis showing better diagnostic performance than isolated low C3 and C4 (26,30,33).

- Specific urinary biomarkers: considering the impact of renal involvement on mortality and morbidity, urinary biomarkers may be instruments of great relevance.Several urinary chemokines and cytokines are interesting candidates, such as monocyte chemoattractant protein-1 (MCP-1), CXCL-10, CXCL-4, CXCL-16, IL-17. Other LN biomarkers include vascular cell adhesion molecule 1 (VCAM-1), whose expression is upregulated, BAFF, tumor necrosis factor-like weak inducer of apoptosis (TWEAK), urinary Neutrophil Gelatinase-Associated Lipocalin (NGAL) (26,34).

In clinical practice serum C3/C4 and anti-dsDNA are the only useful predictors of disease activity and flares and thus are advisable for monitoring SLE patients with SLE (35). The identification of new biomarkers still remains an open challenge (36).

1.5.3 Differential diagnosis

The conditions that most frequently mimic SLE are other autoimmune diseases, rheumatologic-immunologic conditions, infections, neoplasm and medication or vaccine-related diseases.

In the differential diagnosis, other autoimmune diseases to consider are:

- Rheumatoid arthritis (RA). Polyarticular involvement with a predilection for the wrists and small joints of the hands affecting mostly women is a common feature between these two diseases. Typical of RA is the symmetrical involvement of joints, appearing tender and swollen, and the prolonged morning stiffness. (6) (31) Besides the articular involvement, patients with RA can have several extra-articular clinical manifestations and therefore the differential diagnosis with SLE, particularly at an initial stage, can be difficult. Specific autoantibodies can lead the diagnosis: anti-CCP antibodies are usually found in patients with RA and anti-dsDNA in patients with SLE (2,4).
- Undifferentiated, mixed connective tissue diseases and connective tissue diseases (early phase) (31). Anti-U1RNP antibodies are evocative for mixed connective tissue disease(6);

- Skin thickening, Raynaud's phenomenon and micro vascular changes at the nailfold capillaroscopy characterize systemic sclerosis. Performing antibody testing is necessary to differentiate the two connective tissue diseases: LES and SSc share anti-nuclear antibodies but the scleroderma specific ones include anticentromere, Scl-70 and RNA polymerase (31,37).
- Idiopathic thrombocytopenic purpura, autoimmune thyroid disease, antiphospholipid antibody syndrome, autoimmune hepatitis, autoimmune hemolitic anemia, thrombotic thrombocytopenic purpura (4,26).
- Further rheumatologic-immunologic conditions that have to be exclude in the diagnostic process are sarcoidosis, fybromialgia, vasculitis, Behçet disease, adult-onset Still disease and undifferentiated polyarthritis and spondyloarthopaties. The presence of cough, dyspnea, fever, fatigue, night sweats, rash, and uveitis is highly evocative of sarcoidosis, especially if the chest radiography reveals bilateral lymphoadenopathy with biopsy detecting non-caseating granuloma. Among laboratory tests, suggestive for sarcoidosis is the elevated angiotensin-converting enzyme level. Aphthous genital and oral ulcers, uveitis and arthralgia lacking the systemical and serological features of SLE should raise the suspect of Behçet disease (31). Adult-onset Still disease is characterized by a triad of symptoms that include arthralgias/arthritis, high-spiking fever and an evanescent rash; additional features are lympoadenopathy, splenomegaly, hepatomegaly and serositis (38).
- Drug-induced lupus (DIL) can be triggered by a broad range of drugs, encompassing antiarrhythmic (procainamide), antihypertensive (hydralazine), antipsychotic (chlorpromazine), anticonvulsant (carbamazepine), antibiotics (isoniazid, minocycline), anti-inflammatories (sulfasalazine), diuretics (chlorthalidone, hydrochlorothiazide), antihyperlipidaemics (simvastatin), biological agents (TNFα blockers, inferferon α) (4). Positive ANA and anti-histone antibodies associated with systemic symptoms such as fever, arthritis, myalgia, serositis make DIL diagnosis likely. The presence of anti-histone antibodies raise a problem in the differential diagnosis, as they are common feature of both DIL and

idiopathic SLE. Anti-TNF therapies can trigger production of anti-dsDNA antibodies (6). The lack of more serious manifestations such as renal involvement, neurological lupus and haematological abnormalities and the resolution of symptoms after drug discontinuation are hallmarks of DIL (2). Less commonly, various drugs can be a cause of DI-SCLE (Drug Induced Subacute Cutaneous Lupus Erythematosus) characterized by skin lesions in sun-exposed areas in association with anti-SSA antibodies. Distinctive underlying mechanisms seem to determine SCLE and DIL; this justify why photoactive drugs such as leflunomide only in few cases trigger SLE (39).

The second group of diseases mimicking SLE are infections, especially viral: parvovirus B19, hepatitis B and C, EBV, CMV, HIV are among the most common. It is important to include in the differential diagnosis also rare infections such as bacteria (Treponema p. and Borrelia p.), fungi (Tricophynton) and parasites (Leishmania, Toxoplasma).

Fever, inflammatory arthralgias, cytopenias, rash, lymphadenopathy and non-specific autoantibodies (ANA) are associated with both conditions; the final diagnosis is obtained with accurate anamnesis and positive viral serologies (6). Infectious endocarditis can be confused with cardiac SLE manifestation given that its typical symptoms are arthralgia, fever, myalgia, arterial emboli, murmur; positive echocardiography findings and blood cultures can help making the right diagnosis (31).

The third group of diseases mimicking SLE are malignancies, especially non-Hodgkins lymphomas, which share similar clinical and serological findings such as lymphadenopathy, splenomegaly and expansion of monoclonal B cell population. Additional signs typical of cancer are weight loss, fatigue and fever that can be confused with constitutional lupus symptoms and lead to misdiagnosis (4). Elderly patients with a new lupus like syndrome deserve further investigation and the appropriate malignancy screening tests for their age (31).

1.5.4 Further investigations

After the diagnosis, further investigations should be leaded by clinical manifestations and depends on the patients' specific organ involvement (2,5).

To assess renal involvement, urine protein quantification (24-hour proteinuria or spot urine protein/creatinine ratio) is mandatory. If suspecting lupus nephritis, a renal biopsy should be performed (5). Articular involvement can be investigated with synovial fluid aspiration and imaging. Imaging techniques include joint radiographs and musculoskeletal ultrasound as first-line techniques and Magnetic Resonance Imaging as a second-line approach (3). Brain MRI and cerebrospinal fluid analysis are the recommended way to assess patients for NPLSE; ECG, echocardiography, myocardial perfusion scans or angiography and chest imaging with CT-scan are part of the cardiac and pulmonary workup (2,5). In case of atypical or refractory skin lesions, a biopsy should be considered (40).

1.6 Classification criteria

Classification criteria are standardized definitions designed for epidemiological and research purposes aimed to create homogenous cohorts that can entry into clinical trials (41).

1.6.1 ACR 1997 criteria

The American College of Rheumology (ACR) first elaborated 11 classification criteria in 1971, with a revision in 1982, and then again in 1997. According to the 1997 ACR criteria, the classification of lupus requires 4 out of 11 criteria. By fulfilling these criteria, the classification is done with 95% specificity and 85% sensitivity (31). Their sensitivity is high in detecting patients with a longstanding established disease while they are less effective for identification of early lupus (4). The criteria include: malar rash, discoid rash, photosensitivity, alopecia, Raynaud phenomenon, oral/nasal ulcers, arthritis, serositis, renal disease, hematologic disease, neurologic disease, immunologic criteria and antinuclear antibody positivity in the absence of drugs that might be causing DIL. Criteria are cumulative, and therefore they can occur during the course of the disease and not necessarily have to be present concurrently (5).

1.6.2 SLICC 2012 criteria

In 2012 the Systemic Lupus International Collaborating Clinics (SLICC) group remarkably changed the 1997 ACR criteria elaborating some evidence-base criteria with higher clinical relevance and sensitivity in comparison to the previous. These criteria added new items, such as alopecia and low complement, and the classification requires 4 out of the 17 items considered, with at least 1 clinical and 1 immunologic criterion. Another aspect worth mentioning is the special role given to biopsy-proven lupus nephritis, which in combination with ANA or anti-dsDNA is sufficient for classification (42).

1.6.3 EULAR/ACR 2019 criteria

The 2018 EULAR criteria require an ANA of 1:80 or higher as an obligatory entry criterion, followed by weighted criteria scored from 2 to 10 considering different lupus manifestations. A patients needs to score at least 10 point from these criteria for classification. SLE clinical features are assembled in 10 domains: 7 clinical (constitutional, hematologic, neuropsychiatric, mucocutaneous, serosal, musculoskeletal, renal) and 3 immunological (antiphospholipid antibodies, complement proteins, SLE-specific antibodies) (2,14,40).

ACR 1982	ACR 1997	SLICC 2012	EULAR/ACR 2019	
			Mucocutaneous	
1. Malar rash		1. Acute cutaneous LE*	Acute cutaneous LE	6
		or SCLE	SCLE	4
2. Discoid rash		2. Chronic cutaneous LE*	Discoid LE	4
3. Photosensitivity				
4. Oral ulcers		3. Oral ulcers	Oral ulcers	2
		or nasal ulcers		
		4. Non-scarring alopecia	Non-scarring alopecia	2
5. Arthritis		5. Synovitis	Joint involvement	6
6. Serositis		6. Serositis	Serosal	
a) Pleuritis		Pleuritis	Effusion	5
b) Pericarditis		or pericarditis	Acute pericarditis	6
7. Renal disorder		7. Renal	Renal	
a) Persistent proteinuria		Proteinuria	Proteinuria	4
b) Cellular casts		or red cell casts		
		Histology compatible with lupus nephritis	ISN/RPS II/V	8
			ISN/RPS III/IV	10
8.Neurologic disorder		8. Neurologic	Neuropsychiatric	
a) Seizures		Seizures	Seizure	5
b) Psychosis		Psychosis	Psychosis	3
		Mononeuritis multiplex		
		Myelitis		
		Peripheral or cranial neuropathy		
		Acute confusional state	Delirium	2
9. Hematologic disorder			Hematologic	
a) Hemolytic anemia		9. Hemolytic anemia	Coombs+ hemolytic anemia	4
b) Leukopenia		10. Leukopenia	Leukopenia	3
c) Lymphopenia		or lymphopenia		
d) Thrombocytopenia		11. Thrombocytopenia	Thrombocytopenia	4
10. Immunologic disorder				
a) LE cell preparation				
			SLE-specific antibodies	
b) Anti-DNA	a) Anti-DNA	12. Anti-dsDNA	Anti-dsDNA	6
c) Anti-Sm	b) Anti-Sm	13. Anti-Sm	Anti-Sm	6
d) False-positive syphilis serology	c) Anti-phospholipid	14. Anti-phospholipid	Anti-phospholipid	2
		15. Low complements	Low complement	
			C3 or C4 low	3
			C3 and C4 low	4
		16. Coombs test without hemolytic anemia		
11. ANA	11. ANA	17. ANA	Entry criterion ANA	

Table II. Evolution of classification criteria for systemic lupus erythematosus (43)

1.7 Disease progression and prognosis

The survival of SLE patients at 10 years has increased over the last five decades, owing to the recent therapeutic advances and earlier diagnosis. In the 1950s, the survival rate at 10 years was 50% and it is higher than 90% in the 2000s (44). Unfortunately, despite these successes patients with lupus still have 2-5 fold higher mortality when compared with general population (8,44).

Mortality is due to long-term disease complications and related drug side effects, mostly atherosclerosis, cancer, infections, which represent the main causes of death. This is particularly true for patients with severe disease.

Therefore, disease severity is among the most important prognostic factors influencing survival. Generally, skin manifestations (acute cutaneous lupus, subacute cutaneous lupus and chronic cutaneous lupus), arthritis, haematologic involvement apart from haemolytic and aplastic anemia (i.e. leukopenia and thrombocytopenia) and serositis belong to 'mild disease manifestations'. Instead, major central nervous system manifestations, glomerulonephritis, heart and lung parenchymal manifestations as acute or chronic interstitial pneumonia, myocarditis and pulmonary hypertension, haemolytic or aplastic anemia, WBC <1000/mm^3, platelets <15.000/mm^3 and visceral vasculitis are subclassified as severe SLE (24).

1.7.1 Damage and SLE: prognostic significance

The term 'damage' in SLE refers to the permanent organ impairment secondary to disease activity, drug-related adverse events and comorbidities that endures for a minimum of six months (44). Damage is the common ground of the considerable morbidity, increased mortality, poor quality of life, depression, disability and productivity loss associated with SLE (35,44).

It can occur early and late during the course of the disease and, although a clear discrimination is not possible, in the former case it appears closely related to disease activity, whereas in the latter is more likely to be the result of chronic glucocorticoids exposure (44). The wide variety of chronic tissue damage found in SLE patients reflects the broad spectrum of manifestations, but the most frequent include cardiovascular disease with atherosclerosis and thromboembolic events, end stage renal disease (ESRD) and musculoskeletal affections such as osteoporosis and osteonecrosis (44,45). Damage is scored with SLICC Damage Index (SDI) *[See Chapter 2.4.1]*.

It is estimated that, within 5 years from disease onset, 32 to 50% of patients accumulate organ damage. The main factors associated with damage accrual are older age, high SLE disease activity at diagnosis and during the course of the disease and ethnicity (46). In a monocentric cohort of Caucasian patients followed by Padua Lupus Clinic, it was observed that the main risk factors for damage accrual are age, length of the disease, disease flares, high cumulative dose of

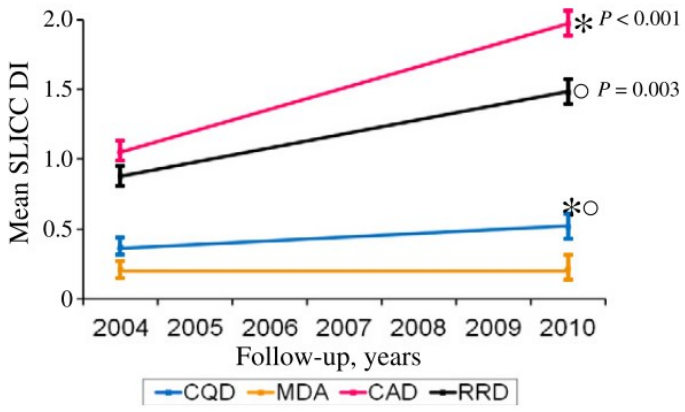
steroids and APS (antiphospholipid syndrome) (47). Early damage accrual, defined by an increase in SDI of two or more points in the first three years of the disease, was an independent predictor of a higher mortality in a monocentric cohort of 388 German patients (44).

These observations suggests that damage is an early event of the disease and its development predicts further damage accrual. Besides, studies around the world clearly established its main contribution to poor long-term prognosis and mortality (48).

1.7.2 Disease activity pattern

As previously mentioned, disease activity is one of the determining factors of damage development.

The Hopkins Lupus Cohort has outlined three main pattern of disease activity in SLE: relapse-remitting (RR), chronic active (CA) and long quiescent (LQ) (49,50). The Padua group has identified a forth pattern named mild disease activity (MDA) to identify patients with mild manifestation mostly haematological (51). The observation of increased organ damage in patients affected with CAD and RRD than those in remission underlines the importance of disease activity in the accumulation of tissue damage (44).



CAD = chronic active disease; CQD = clinically quiescent disease; MDA = minimal disease activity; RRD = relapsing–remitting disease; SLICC DI = Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index.

Figure 11. Association of disease activity pattern and damage accrual (44)

In the study performed in a cohort of Italian SLE patients followed-up for 7 years, definitions of annual disease activity patterns were established according to SLEDAI-2K *[See Chapter 2 for Activity indices]*, as follows (51):

- Serological active clinical quiescent disease (SACQD): a SLEDAI-2K 0 excluding serology in three annual visit;
- Minimal disease activity (MDA): a SLEDAI-2K 1 excluding serology in one or more annual visits (c-SLEDAI=1);
- Chronic active disease (CAD): a SLEDAI-2K ≥ 2 excluding serology in at least two out of the three annual visits (c-SLEDAI ≥ 2);
- Relapse-remitting disease (RRD): a SLEDAI-2K ≥ 2 excluding serology in one out of three annual visits (c-SLEDAI ≥ 2). Flare was defined according to SFI as an increase in SLEDAI-2K ≥ 4 from the previous visit.

Regarding the definition of 'flare', most experts define it as an escalation in disease activity measurable by disease activity indices that require a change in treatment, although a universally definition is lacking (52). [*See Chapter 2 for the definitions of flares according to different disease activity indices]*

The course of the disease remains however unpredictable, since switch from one pattern to another are common and flares can happen any time in the course of the disease, even in previously inactive disease (49,51).

1.7.3 Complications

The leading lupus-related complications are cardiovascular and thromboembolic events, end-stage renal disease, and infections. As previously mentioned, they have a significant impact on patient mortality (24). Notably, the estimated risk of myocardial infarction in SLE population is five times higher than the general population, reaching up to 52-fold increased risk in juvenile women (<45 years) (44). Lupus patients are prone to develop thromboembolic events since the onset of the disease due to the presence of antiphospholipid autoantibodies, accelerated atherosclerosis and nephritis (45). In addition, renal disease is one of the most serious manifestation, which accounts for a decline in survival and worsening of the disease (23). Infections are worth mentioning among comorbidities in SLE

patients, as they are associated with the disease itself and treatment-related factors (52). Symptoms related to SLE activity and infections are quite similar, therefore in a debilitated patient it is crucial to determine whether the deterioration is due to lupus activity or to an infection. Iatrogenic immunosuppression is responsible for most infections, which may involve lungs urinary tract, skin. Opportunistic infections such as herpes zoster, atypical tuberculosis, candidiasis, pneumocystis carinii, cytomegalovirus and fungal infection are among the most frequent. Other factors that may contribute to infections are renal failure, complement deficiencies, splenectomy. Considering the high risk of infection, flu, pneumococcal and herpes zoster vaccinations are advisable and, if the suspect of infections raises, promptly investigation is recommendable (5). Last, since pregnancy is a well-known high risk-situation in women in childbearing age with lupus for the mother's and the foetus' health, obstetric complication must be mentioned. At the current state, most pregnancies are successful but a multidisciplinary team is of paramount importance in providing optimal care. Disease activity at the time of conception is the strongest predictor of poor outcome (53).

1.8 Management and therapy

1.8.1 Goals of treatment and treat-to-target approach

The main goals of SLE treatment are long-term survival, prevention of organ damage, drug-related side effects, disease flares and improved quality of life. Regular clinical and laboratory assessment of disease is fundamental for optimal management, which should aim at remission or at least the lowest possible disease activity (9,52). Medication should be adjusted according to the level of disease activity, usually differentiated in mild, moderate and severe (54).

To maximize the positive therapeutic effects treatment should start in the early stage of the disease, irrespectively of the type of manifestations and drugs used (44).

After its validation in other rheumatic diseases such as rheumatoid arthritis and psoriatic arthritis, treat-to-target approach was proposed in patients with SLE (T2T/SLE) and gained international consensus. It consists of a medical strategy that aims at clinically achievable goals in order to improve the patient's prognosis.

The different steps in the planning process are the identification of the appropriate target, the evaluation of the results once started the therapy and finally, if necessary, adjustment of therapeutic management (44,55).

1.8.1.1 Targets of therapy

The optimal goal of SLE treatment should be both complete remission, both on a serological and clinical level; nevertheless, very few patients achieve this aim, which might be unreal to pursue in clinical practice. (44)

Therefore, the treatment target of SLE should be clinical remission or, if not feasible, low disease activity, which represents the second-best goal. (56) Both conditions are associated with significant less damage accrual; however, remission is the ultimate target because it endows the most protective effect. (57) *[See Chapter 2.5.1 and 2.5.2 for Remission and Low Disease Activity]*

Once achieved the aforementioned targets, the following steps consists in tapering glucocorticoids to the minimum dose necessary and, in case of remission, their discontinuation. In the event of maintained response to therapy over time, immunosuppressant reduction or cessation are plausible objectives (57).

In addition to the above-mentioned therapeutic goals, an international task force displayed further useful recommendations on treat-to-target therapy for lupus, i.e. the early recognition and management of lupus nephritis, the limitation to the least possible of organ injury and coping strategies for symptoms undermining health related quality of life (56).

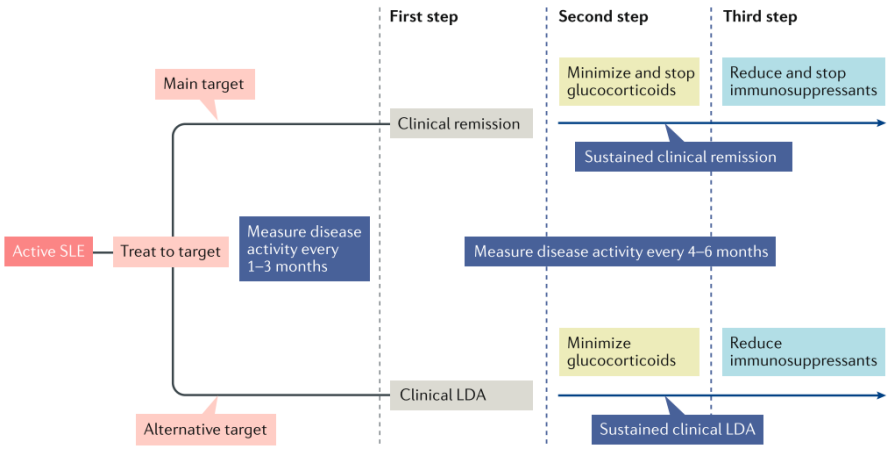


Figure 12: Treat-to-target approach(57)

1.8.2. Preventive measures

Preventive strategies are focused on removal of modifiable risk factors and potential triggers of disease manifestations. Among these, adequate UV protection for sun exposure is fundamental, as well as smoking cessation and elimination of photosensitizing drugs. Due to its immunomodulatory effects, supplement of vitamin D is appropriate. Multiple antiphospholipid antibodies positivity is a well-known risk factor for thromboembolic events, thus needing a prevention therapy with a low-dose aspirin. In patients with a single positivity, special attention must be paid to precipitating conditions such as pregnancy, surgery and prolonged immobilization, where a provisional prevention strategy is recommended (57).

Furthermore, considering that SLE patients are often at risk of infection due to immunosuppressive drugs, EULAR recommendation suggest vaccination for influenza, pneumococcal infection and herpes zoster (52).

As previously mentioned, SLE patients have higher cardiovascular risk than general population, thus stringent monitoring of traditional risk factors seems inevitable:

- High blood pressure strongly affect the prognosis and therefore tight control is mandatory and pressure should be maintained below 130/85 mmHg;
- Body weight, lipids and glucose levels should be at target (52).

Prevention of osteoporosis and thrombosis are also part of the clinical management (5).

1.8.3 Pharmacological treatment

Traditional standard therapies for SLE include hydroxychloroquine, glucocorticoids, immunosuppressive drugs and biological agents.

Antimalarial agents as hydroxychloroquine (HCQ) are advisable for all patient. Their daily dose should not exceed 5 mg/kg real body weight to minimize its retinal toxicity. Ophthalmological examination before starting treatment and once or twice a year screening for toxic retinopathy is mandatory in all patients undergoing antimalarials treatment.

Glucocorticoids are indicated for fast control of disease symptoms. Given the long-term effects of glucocorticoids, tapering down of GC and discontinuation are desirable. When appropriate tapering is not possible, the recommendation is to maintain the lowest dose, as cumulative doses of GC correlate with damage accrual (44). In case of acute organ-threatening manifestations, use of high dosage (0.5 – 1 g) intravenous methylprednisolone (MP) pulses is recommended.

Generally, immumodulatory agents accelerate and facilitate corticosteroids tapering. Immunosuppressive drugs include methotrexate (MTX) and azathioprine (AZA), whose indications are refractory mild manifestations (arthritis or rash $\leq 9\%$) and moderate disease activity as first line therapy. Considering the teratogenic potential of MTX, its withdrawal and switch to AZA should occur at least 6 months before conception in women planning pregnancy. Mycophenolate mofetil (MMF) has shown efficacy in refractory moderate disease and in severe renal and non-renal lupus. Cyclophosphamide is actually used in patients with severe major organ involvement or life threatening conditions.

Biological therapies targeting B-cells currently used in clinical practice are Belimumab and Rituximab. Since its approval in 2019 by FDA and EMA, Belimumab has been widely prescribed in clinical practice, with substantial beneficial effects as an add-on therapy in serologically active patients with refractory manifestation to standard treatment, especially articular and mucocutaneous [*[See forward, Chapter 3 Belimumab*]. Rituximab, an anti-CD20 monoclonal antibody, following the failure of RCTs designed to demonstrate its efficacy, is used off-label. Particularly, it is used in refractory renal disease with inadequate control to the first-line therapy or in extra renal disease, especially in case of haematological, renal and neuropsychiatric involvement, usually after the failure of more than one line strategy.

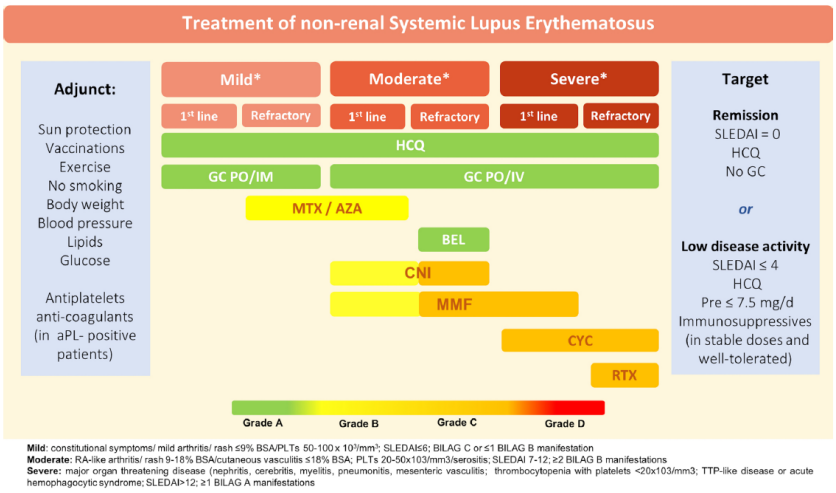


Figure 13. 2019 EULAR recommendations on treatment of non-renal SLE (52)

1.8.3.1 Specific manifestations: skin disease

Other than the already mentioned prevention measures, drug therapy for skin disease comprises topical treatment and systemic drugs. The first-line treatment include topical corticosteroids and calcineurin inhibitors, such as pimecrolimus and tacrolimus; R-Salbutamol cream can also be applied, as well as physical treatment like laser therapy and cryotherapy (52,58).

In case of severe and broad skin involvement, treatment relies on systemic pharmaceutical options. Antimalarials, whose response rate varies from 50 up to 90 percent of patients, are the first-line treatment. HCQ at a maximum daily dose of 5 mg/kg (hydroxychloroquine) is preferred over chloroquine 4mg/kg for its numerous positive effects and lower risk of retinal toxicity. Chloroquine use is therefore restricted to patients not responding to HCQ. In case of refractory manifestations or contraindications, quinacrine as an add-on therapy or an alternative drug is an option. As previously mentioned, patients undergoing antimalarials should be investigated for ocular toxicity (52,58). Indications for systemic GC include highly acute and severe skin lesion, even in addition to antimalarials, considering steroids' slow-acting effect. Standard oral dose for prednisone is 0.5 mg/kg while during exacerbations 3-day intravenous methylprednisolone pulses are available.

First-line therapies fail in around 40% of patients; in such cases, patients can benefit from a wide variety of second-line systemic treatment, especially in case of

refractory-subacute and chronic cutaneous lupus erythematosus. Belimumab, methotrexate (MTX) (59), retinoids, dapsone, and mycophenolate mofetil are part of the armamentarium for refractory skin disease (52,58). Thalidomide, Rituximab, intravenous immunoglobulin and small molecules (baricitinib and tofacitinib) are used as 'rescue' therapies in patients with refractory cases who have failed the previous agents (52,58).

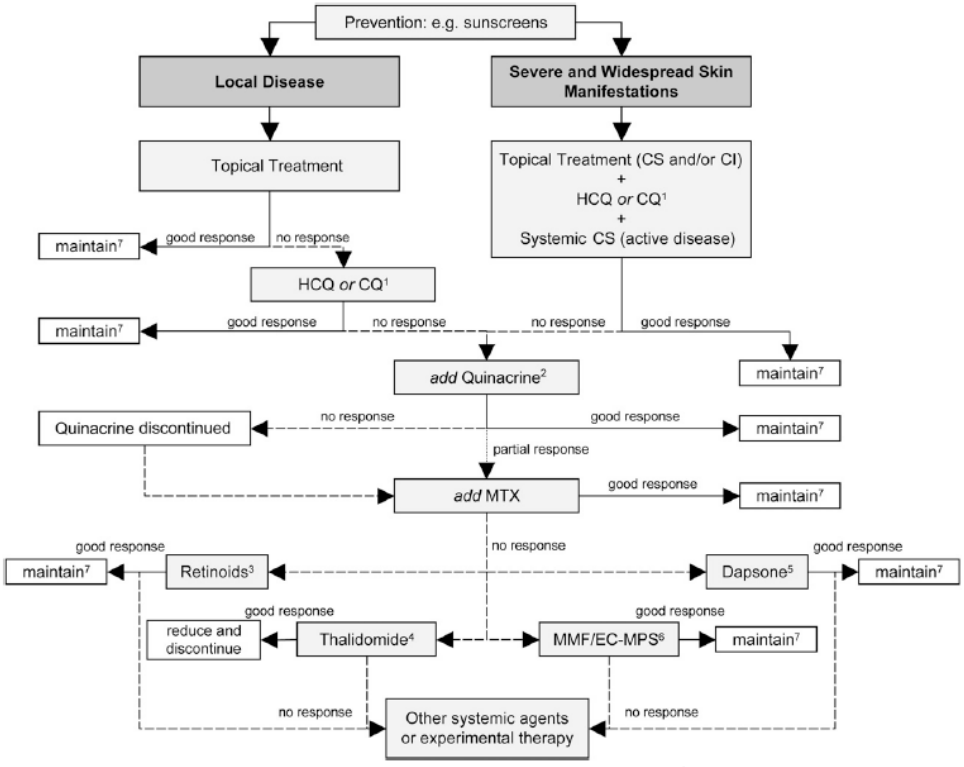


Figure 14. Treatment of cutaneous lupus erythematosus (58)

Anifrolumab, an anti-Interferon-α receptor monoclonal antibody, achieved the primary end-point, consisting in general disease activity improvement measured by SRI4 at week 24 and corticosteroid tapering, and secondary end-points in a phase 2 RCT. Furthermore, the percentage of patients with a baseline Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) ≥ 10 who had a ≥ 50% improvement was greater with Anifrolumab compared with placebo (60). This lead to its approval by FDA in August 2021 for adults with to moderate to severe systemic lupus erythematous.

1.8.3.2 Specific manifestations: musculoskeletal manifestations

Depending on the severity of the disease, the first line treatment is based on non-steroidal anti-inflammatory drugs or oral corticosteroid (prednisone dosage at 0,1-0,5 mg/Kg per day) and antimalarials. The clinical entity more likely to respond is the NDNE, usually considered a benign entity.

In case of persistent joint involvement, multiple therapeutic options are currently available. A systematic review suggests the use of methotrexate for non-organ-threatening SLE, specifically for patients with active arthritis or cutaneous manifestations in case of adverse reactions, failure or suboptimal response to first line therapy (antimalarials) or for those who are unable to appropriately taper GC (59).

The use of Leflunomide in SLE in refractory articular manifestations has demonstrated clinical improvement and decrease of disease flares in numerous open labelled studied. One clinical randomized controlled trial performed in 2014 by Tam et al. has proved reduction in SLEDAI score from baseline (61).

Belimumab provides great improvement in lupus polyarthritis (NDNE lupus arthritis and Jaccoud arthropathy) but not in those with rheumatoid-like joint involvement (Rhupus) (62).

Rituximab, abatacept and small molecules can be prescribed. The Italian Registry documented the efficacy of Rituximab use off-label in inducing both complete and partial articular response in refractory SLE manifestation, in accordance with others European Registry (63). Furthermore, Rituximab as well with Abatacept established a decrease in two disease activity indices (DAS-28 and SLEDAI measuring respectively arthritis and general disease activity) in a group of 6 rhupus patients who hadn't responded to previous line treatments (64).

Besides the positive effects discussed previously, Anifrolumab showed an improvement in swollen and tender joints for patients with ≥ 8 swollen and ≥ 8 tender joints at baseline (60).

Small molecules validated for RA and psoriatic arthritis, such as Tofacitinib and Baricitinib, displayed encouraging preliminary data from RCT in controlling articular manifestations but necessitate further studies.

In conclusion, the pursuit for new treatments for achieving remission, reducing flare frequency, abrogating disease activity, flares and damage accrual is a current issue in SLE scene (62).

2. Activity indices - Clinimetric Evalution

Considering advances in therapies for SLE, there has been a shift from measurement of morbidity to quantification of disease activity and organ damage (49).

Assessing the burden of disease activity over time can be challenging due to both the heterogeneous presentation of the disease and for the disease activity pattern, characterized by flares alternating with remission, but regular assessment of disease activity is essential for an optimal management, prevention of long-term issues disease-related and evaluation of patient response (49,65,66). Measurement of disease activity has a pivotal role in differentiating disease activity from chronic damage, drug side effects, infection and comorbidities. Given that each of these conditions require different and specific measures, clinimetric evaluation is fundamental for the management of the disease and helpful to guide day-to-day drug scheme as well as to evaluate outcomes and effectiveness of new treatments (5). Indeed, recording lupus disease progression in patients undergoing new-targeted therapies is essential in estimating the percentage of patients who can possibly benefit from such therapy (51). Furthermore, disease activity indices are useful to assess disease activity of individual patients and differences among SLE patients groups. Last, they provide some degree of uniformity for longitudinal and clinical studies (67). Standardized and validated indices are widely used in other rheumatologic conditions such as rheumatoid arthritis and psoariatic arthiris while a standard disease assessment tool in SLE is lacking (65,66). Therefore, although there is no universal agreement on which index is the best to assess disease activity from the many present, the most frequently used are SLEDAI, SLEDAI 2000 (SLEDAI-2K), modified SLEDAI (M-SLEDAI) and BILAG (5). Measures of SLE disease activity include global score system such as SLEDAI, which reflects an overall measure of activity, and organ-specific score such as BILAG, where disease activity is assessed in single organs and expressed as separate score for each system involvement. Others organ specific index includes CLASI for cutaneous disease activity and DAS288 for articular involvement (5). It is noteworthy that only items ascribable to active lupus disease, not to damage or comorbid conditions, can be

scored. The length of time considered for recording symptoms varies in different indices (BILAG index examines manifestations during the preceding month, while SLEDAI in the previous 10 days), as well as the haematology, biochemistry and immunology items taken into account (5).

2.2 Global activity indices

2.2.1 PGA

The Physician Global Assessment (PGA) consists of a visual analog scale ranging from 0 to 3 to assess the overall disease activity based on the clinician's judgment, described as absent, mild, moderate and severe. The terms are allocated considering 'severe' as the maximum disease severity universally considered, not in relation to the highest deterioration experienced in the specific patient. Since the absence of a gold standard disease assessment tool for SLE, physician's global assessment was widely used for testing the validity of other disease activity indices (49).

Although EULAR 2019 recommendation advise its use in routine clinical practice, the main limitation consists of the lack of reliability due to its clinician-based nature and the incapability of differentiating a stable disease pattern from a deterioration in one system simultaneous to an improvement in another (49,52). PGA should be assessed before SELENA-SLEDAI and BILAG to increase its validity and reliability (68). The definition of flare based on PGA identified by the Hopkins Lupus Center considers a flare as a modification of at least 1 point in the score within the last 3 months; in addition, different cut-offs reflect different degree of flares. Mild flare will score 1.0 point, moderate flare 2 to 2.5 points while a gain of 3 points stands for a severe reactivation of the disease (50). A clinically significant worsening is defined as an increase of at least 0.3 points in PGA, which corresponds to a 10% increase on the visual analog scale (68).

2.2.2 SLEDAI, SLEDAI 2-K, SLEDAI-M, SLEPDAI

Systemic Lupus Erythematosus Disease Activity Index measures disease activity within the last 10 days and consists of 24 weighted clinical and laboratory items covering 9 organs/system with a global score ranging from 0 to 105.

It does not record subjective symptoms such as fatigue and arthralgia and, unlike BILAG index, includes immunology results (anti-dsDNA, C3 or C4) (5). The original version of SLEDAI was developed in 1985 and was further subjected to modifications, leading to the development of SLEDAI-2000 (2K) and SELENA-SLEDAI. These indices all consider the same descriptors with the equivalent weight but SLEDAI-2K and SELENA-SLEDAI take into account ongoing manifestations such as proteinuria, rash, alopecia and mucosal ulcers, differently from the original SLEDAI, which score such items only if new or recurrent (69). These revisions improved the accuracy in assessing persistent active disease (70).

Weight	Descriptor
8	Seizure
8	Psychosis
8	Organic brain syndrome
8	Visual disturbance
8	Cranial nerve disorder
8	Lupus headache
8	Cerebrovascular accidents
8	Vasculitis
4	Arthritis (>2 joints)
4	Myositis
4	Urinary casts
4	Haematuria (>5 RBC/HPF)
4	Proteinuria
4	Pyuria (>5 WBC/HPF)
2	New rash
2	Alopecia
2	Mucosal ulcers
2	Pleurisy
2	Pericarditis
2	Low complement
2	Increased DNA binding
1	Fever (< 38.5°C)
1	Thrombocytopenia (<100.000/uI)
1	Leukopenia (<3000/uI)

Table III. Original SLEDAI score, descriptors and weight(71)

Descriptor	SLEDAI	SELENA-SLEDAI	SLEDAI-2K
Seizure		Added the exclusion of seizure due to irreversible CNS change	
Visual disturbance		Included scleritis or episcleritis	
Cranial nerve disorder		Included vertigo due to lupus	
Cerebrovascular accident		Added the exclusion of hypertensive causes	
Arthritis	>2 joints	>2 joints	≥2 joints
Proteinuria	>0.5 g/day. New onset or recent increase of >0.5 g/day.	New onset or recent increase of >0.5 g/day	>0.5 g/day
Rash, alopecia, mucosal ulcers	Only new onset or recurrence	Included ongoing symptoms	Included ongoing symptoms
Pleurisy Pericarditis	Subjective AND objective findings	Subjective OR objective findings	Subjective AND objective findings

Table IV. Differences between the three SLEDAI(69)

According to SLEDAI, 5 levels of disease activity have been identified(5):

- SLEDAI 0: inactive disease
- SLEDAI 1-5: mild activity
- SLEDAI 6-10: moderate activity
- SLEDAI 11-19: high activity
- SLEDAI >= 20: very high activity

The SFI (SELENA-SLEDAI Flare Index) defines 'flare' as a minimum of 3 points increase in SLEDAI and discerns mild/moderate flare (3 or more points) from severe (greater than 12) (50).

A simplified version of the SLEDAI omitting serology is the SLEDAI-M in order to place emphasis on clinical activity, the main driver of therapeutic decision (51).

SLE-P-DAI (SLE-Pregnancy Disease Activity Index), a modification of the index for pregnancy use, is suitable to distinguish manifestation due to pregnancy from symptoms related to the disease itself (5).

Overall, SLEDAI index is a simple and reliable tool that allows the assessment of global disease activity retrospectively and the study of different cohorts of patients. In addition, it can provide cut-offs criteria for entry in clinical trials (5). Nevertheless, although being the most widely activity index used, is not devoid of limitations. Namely, the inability to grade the intensity of change, since all items are scored as present or absent, and to capture partial improvement. Then, severe lupus manifestations, such as haemolytic anaemia, pneumonitis or gastrointestinal manifestations are excluded (5). Furthermore, its accuracy in defining low disease activity is limited (54). Last, SLEDAI does not include severity within an organ system and therefore the final score might be the result of little activity in many organs or very high activity in one single organ (70).

2.2.3 SLE-DAS

SLE Disease Activity Score is a new global activity index conceived to overcome the difficulties of SLEDAI and to improve its accuracy. It was developed to provide an appropriate disease assessment tool for clinical setting and an outcome measure for clinical trials (72).

The number of items considered was brought from 24 in the SLEDAI to 17 in the SLEDAS, among which four are scored as continuous variables (arthritis, proteinuria, leukopenia, and thrombocytopenia) and the others dichotomously (72).

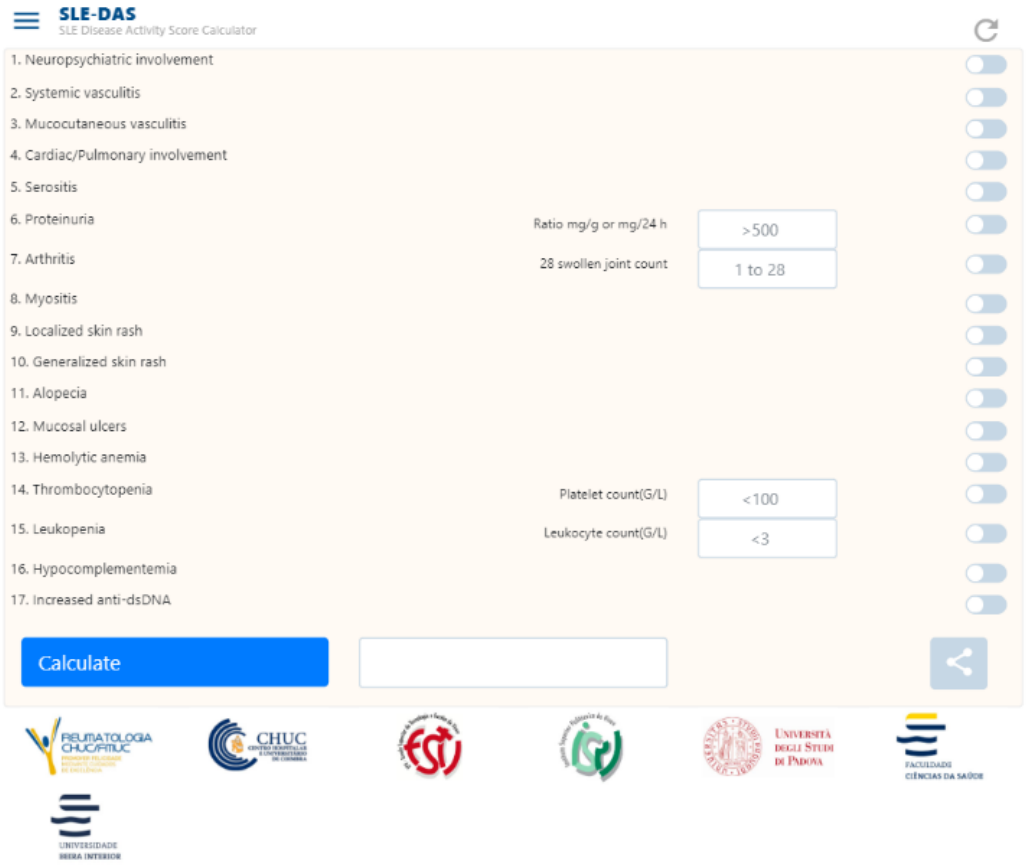


Figure 15: SLE-DAS online calculator

Its continuous nature accounts for the higher sensitivity of SLE-DAS in detecting clinically meaningful changes over time, both at individual and group levels, as compared to SLEDAI-2K, with similar specificity (72).

SLE-DAS shows higher performance than SLEDAI in predicting damage accrual. The improved accuracy in assessing disease activity has two major causes. First,

the burden of vasculitis and rash was re-defined: the presence of systemic vasculitis has different importance in term of scoring points compared with mucocutaneous vasculitis and generalized rash is scored higher than localized rash. Second, relevant parameters excluded from SLEDAI were also included in the computation, i.e. haemolytic anemia, cardiac/pulmonary involvement and gastrointestinal symptoms (comprised in systemic vasculitis) (54). In conclusion, the solid internal-external validation and the demonstrated correlation with PGA and SLEDAI-2K legitimize its use as a validated activity index (72).

2.1 Organ specific activity indices

2.1.1 CLASI

Mucocutaneous involvement in lupus erythematous is diversified into different categories, thus making the global activity scores, even if they include dermatological criteria, unsuitable for grading dermatological activity. The necessity of a separate score for dermatological activity and damage lead to Cutaneous Lupus Erythematous Disease Area and Severity Index (CLASI) development, a valid scoring system used by rheumatologists (67). Overall, CLASI is an appropriate instrument to measure skin disease in terms of activity and damage caused by CLE, although unable to reflect the different disease subtypes (73). The CLASI consists in calculating two separate scores for each patient, one for disease activity (CLASIa) and the second for disease damage (CLASId) (67,73).

Disease activity (CLASIa) is measured by:

- Erythema, on a scale from 0 to 3, and scale/hypertrophy, ranging from 0 to 2, for the skin involvement, divided in 13 anatomical areas listed in rows [scalp, ears, nose (included malar area), v-area of neck (frontal), posterior neck/shoulders, chest, abdomen, back and buttocks, arms, hands, legs, feet] (67).

  Each area is evaluated based upon the intensity of the involvement without recording the lesion extension. In this, it differs from PASI (Psoriasis Area and Severity Index) where a score on a scale of 0 to 6 is given to the area of involvement of each region, depending on the percentage affected (73,74);

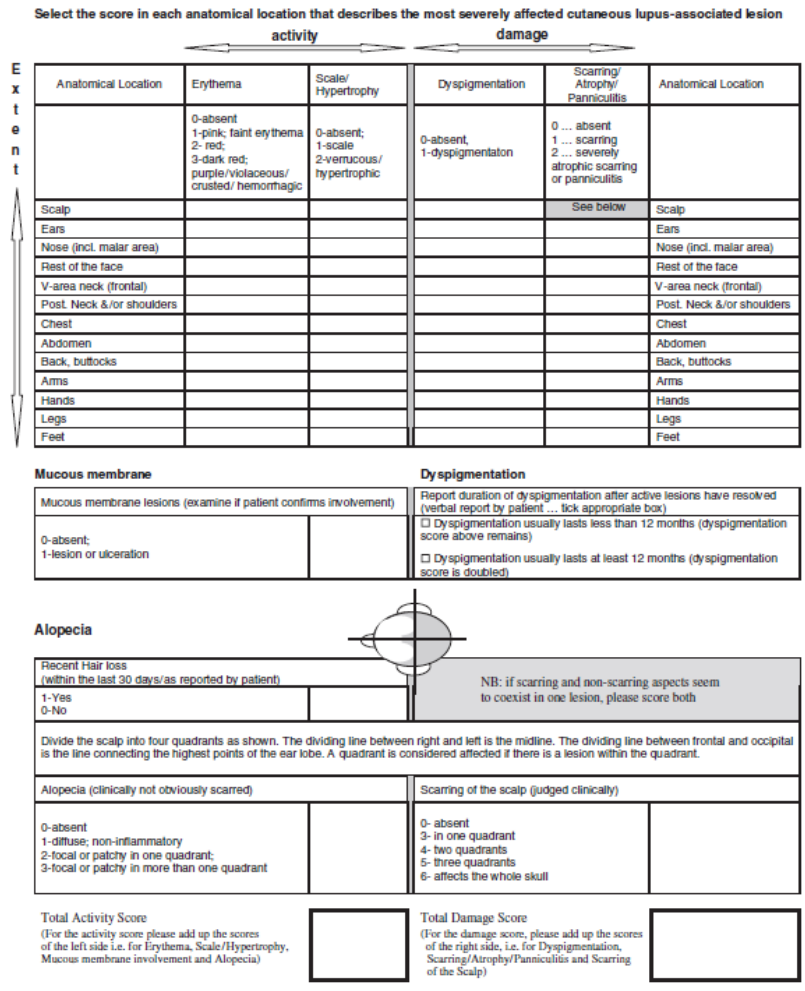
- Mucosal involvement, to whom, if present, is given 1 point;
- Scalp, considering recent hair loss within the last 30 days, yes (1) or no (0) and non-scarring alopecia, on a scale from 0 to 3.

Instead, the total damage score (CLASId) is a result of (67):

- Dyspigmentation [absent (0) or dyspigmentation (1)] and scarring on scale from 0 to 2, where 1 point is given if present and 2 points for severely atrophic scarring or panniculitis, for the 13 skin areas listed above;

- Scarring of the scalp, judged clinically from 3 to 6 when present, according to the numbers of quadrants involved;

- The persistence of dyspigmentation more than 12 months after active lesions have resolved make the dyspigmentation score doubled.



Select the score in each anatomical location that describes the most severely affected cutaneous lupus-associated lesion

activity ⟷ damage

Extent

Anatomical Location	Erythema	Scale/ Hypertrophy	Dyspigmentation	Scarring/ Atrophy/ Panniculitis	Anatomical Location
	0-absent 1-pink; faint erythema 2- red; 3-dark red; purple/violaceous/ crusted/ hemorrhagic	0-absent; 1-scale 2-verrucous/ hypertrophic	0-absent, 1-dyspigmentaton	0 ... absent 1 ... scarring 2 ... severely atrophic scarring or panniculitis	
Scalp				See below	Scalp
Ears					Ears
Nose (incl. malar area)					Nose (incl. malar area)
Rest of the face					Rest of the face
V-area neck (frontal)					V-area neck (frontal)
Post. Neck &/or shoulders					Post. Neck &/or shoulders
Chest					Chest
Abdomen					Abdomen
Back, buttocks					Back, buttocks
Arms					Arms
Hands					Hands
Legs					Legs
Feet					Feet

Mucous membrane

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

Dyspigmentation

Report duration of dyspigmentation after active lesions have resolved (verbal report by patient ... tick appropriate box)

☐ Dyspigmentation usually lasts less than 12 months (dyspigmentation score above remains)

☐ Dyspigmentation usually lasts at least 12 months (dyspigmentation score is doubled)

Alopecia

Recent Hair loss (within the last 30 days/as reported by patient) 1-Yes 0-No	

NB: if scarring and non-scarring aspects seem to coexist in one lesion, please score both

Divide the scalp into four quadrants as shown. 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.

Alopecia (clinically not obviously scarred)		Scarring of the scalp (judged clinically)	
0-absent 1-diffuse; non-inflammatory 2-focal or patchy in one quadrant; 3-focal or patchy in more than one quadrant		0- absent 3- in one quadrant 4- two quadrants 5- three quadrants 6- affects the whole skull	

Total Activity Score (For the activity score please add up the scores of the left side i.e. for Erythema, Scale/Hypertrophy, Mucous membrane involvement and Alopecia)		Total Damage Score (For the damage score, please add up the scores of the right side, i.e. for Dyspigmentation, Scarring/Atrophy/Panniculitis and Scarring of the Scalp)	

Figure 16. CLASI (Cutaneous LE Disease Area and Severity Index)(73)

2.1.2 BILAG

The BILAG index (British Isles Lupus Assessment Group score) was proposed firstly in 1988, based on the physician's principle 'intention to treat' (75). In the last update (BILAG 2004) it explores changes in disease specific manifestations in 9 system: general, mucocutaneous, neurologic, muscoskeletal, cardio-respiratory, ocular, renal, haematology, gastrointestinal. The disease activity for each organ-base system is classified on 5 different levels A-E and to be scored all features must be attributable to active lupus and must have been present within last 4 weeks (70). The alphabetic score reflects disease severity and provides indication for the patient management as indicated below (5):

- BILAG A stands for 'Action' (12) and reflects a severe and very active disease, likely to necessitate a change in therapy such as immunosuppressive drugs or higher prednisolone;
- BILAG B accounts for 'Beware' (8) and indicates a moderately active disease that requires an increase in therapeutic strategy, i.e. low-dose prednisolone or symptomatic treatment with NSAIDs and/or antimalarials;
- BILAG C indicates 'Containment' (1) and represents a mild stable disease dealt with symptomatic therapy;
- BILAG D stands for 'Discount' and reveals a previously active disease but no current disease activity;
- BILAG E indicates 'no Evidence' of current or previous disease activity.

The original score contains 101 items while the updated version in 2009 covers 101 items, including essential laboratory data from the renal (blood pressure, urinalysis by dipstick, serum creatinine) and haematological systems (haemoglobin, white cell count, platelets, neutrophil count, lymphocyte count) without incorporating immunology tests (5,76).

An escalation to an A of any previous score constitutes a severe flare, whereas a raise from C, D or E to a B score in any system identifies a moderate flare (50). Loss of A and B scores in all system and no new A or B score define response to treatment; instead partial response is characterized by the loss of A score with the persistence or development of one or more B scores (5).

BILAG index is more sensitive to change and comprehensive than SLEDAI, being able to assess deterioration and advancement of individual organ system. Considering this, its use is more appropriate in judging the effect of new drugs in clinical trials because the extent of disease activity in each organ domain is graded, unlike the SLEDAI score, where each parameter is a scored dichotomically (present/absent). Differently, SLEDAI is not able to record partial improvement or detect aggravation of an already existing feature (5,72).

Other pros of this index are its validated use as an entry criterion for clinical trials is and the necessity for only a few basic laboratory data.

Major drawbacks are the lack of serology, inadequate sensitivity in overall measure of activity and the length of time and the training necessary for calculation with the British Lupus Integrated Prospective System (BLIPS) computer program.

2.1.3 DAS 28

The Disease Activity Score using 28 joints counts (DAS28) is one of the most employed RA (rheumathoid arthritis) end-point used in randomized clinical trials, alongside ACR20, 50, 70. The DAS28 measures the number of swollen or tender joints in addition to erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) as markers of inflammation and the patient's general assessment of health, estimated on a visual analog scale (VAS) between 0 and 100mm (77).

Swollen joints (0-29)
Tender joints (0-28)
Erythrocyte sedimentation (ESR) or C-reactive protein (CRP)
Visual analog scale (VAS) disease activity (0-100mm)
DAS28 (CRP) = 0.56*√(tender joints) +0.28*√(swollen joints)+0.014*VAS+0.36*ln(CRP+1)+0.96 DAS28 (ESR) = 0.56*√(tender joints) +0.28*√(swollen joints)+0.014*VAS+0.70*ln(ESR)

Table V. Calculation of DAS-28 score

This is a validated and standardized activity index used both in clinical trials and in routine clinical practice in RA patients.

The study carried out in 2014 at the Lupus Clinic of Rome proposed the use of DAS-28 for assessing articular involvement in Systemic Lupus Erythematosus, and the results suggested a higher accuracy in evaluating the wide variety of articular manifestations in SLE patients and sensitivity in detecting articular changes (improvement or deterioration) than SLEDAI-2K. Furthermore, different DAS28 threshold can be useful in categorizing patients in different classes of disease activity, as a great percentage of patients showed values between 3 and 5 and therefore moderate/high disease activity (78).

2.3 Responder indices

2.3.1 SRI

SLE Responder Index (SRI) addresses the need of a reliable and sensitive instrument to measure clinical response to new therapeutic agents in RCTs and to assess their therapeutic efficacy (68). It is a composite scoring system incorporating an overall activity index (SELENA-SLEDAI), a specific organ activity index (BILAG) and Physician Global Assessment (PGA). A responder according to SRI fulfils the following requirements: an improvement of 4 or more points in SELENA-SLEDAI (for this reason the index is also known as the SRI-4) and no concomitant BILAG and PGA worsening (68).

Assessment	Criteria
SELENA-SLEDAI	≥ 4-Point improvement
BILAG	No new A domain score AND no more than 1 new B domain scores
PGA	No worsening (<0.4-point increase)

Table VI. SRI criteria for response

2.3.2 BICLA

BILAG-based Combined Lupus Assessment is a novel composite outcome measure used in clinical trials.

BICLA has a good construct validity, sensitivity and combines different disease activity indices including BILAG-2004, which has a key position in evaluating the efficacy, SLEDAI-2K and PGA. For a patient to be classified as a BICLA responder, meeting all of the following criteria is necessary(79):

1. BILAG 2004 improvement in all systems affected at the starting point;

2. No worsening in disease activity, assessed by BILAG;

3. No worsening of total SLEDAI-2K score from baseline;

4. No significant deterioration in PGA;

5. No treatment failure, described as initiation of non-protocol treatment.

2.4 Disease damage index

2.4.1 SLICC/ACR-DI

As previously stated, persistent disease activity together with related-drug toxicity and comorbidities are the key drivers of damage accrual, which is a well-known prognostic factor in terms of mortality, poor quality of life, work disability, depression and more damage accrual over time (62,80). [*The prognostic significance of damage and SLE has been discussed previously in Chapter 1.7.1]*

The Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI) is a valid and reproducible instrument specifically designed to assess damage in SLE (32). This index includes 41 items and records non-reversible manifestations accumulated across 12 organs system after SLE diagnosis due to disease activity, drugs and comorbidities.

The score ranges from 0 to 46 points and include only irreversible manifestations happening after disease onset and lasting more than 6 months (70). The variables considered are 1. Renal, 2. Neuropsychiatric, 3. Ocular, 4. Musculoskeletal, 5. Peripheral vascular, 6. Skin, 7. Pulmonary, 8. Cardiovascular, 9. Gastrointestinal, 10. Diabetes, 11. Malignancy, 12. Premature gonadal failure.

Score damage are calculated regardless the origin and the total score is a result of 31 parameters scoring a maximum of 1 point, 6 items scoring up to 2 points if repeat episodes occur at least 6 months apart and end-stage renal disease contributing with 3 points. The same lesions can't be scored twice, except in the following cases: more than one cerebrovascular accident, myocardial infarction, significant tissue loss ever, site involved in infarction or resection of bowel, avascular necrosis and malignancy (5).

2.5 Remission and Low Disease Activity

Since disease activity is closely linked to mortality and morbidity in SLE patients, the optimal goal for SLE treatment is remission and if not possible, low disease activity is desirable (52). Complete remission, which implies both clinical and serological healing in absence of treatment, is still infrequent in SLE. However, longitudinal observations demonstrated that clinical remission with the lowest corticosteroid dose and low disease activity ensure an improvement in the patient outcome as well (44).

2.5.1 Remission in SLE

The importance of remission has recently emerged in evaluating the outcome of recent treat-to-target therapies and in assessing disease activity, but a universally accepted definition of remission is still lacking (47).

DORIS, Doria-Zen and SLE-DAS are currently the proposed definitions for remission in SLE.

According to the first two definitions, the mimimum requirements are a clinical SLEDAI = 0 and prednisone ≤ 5 mg/d. It should be noted that treatment with biologics, immunosuppressants, antimalarials and serology active disease does not preclude the patients from being considered in remission.
The DORIS Task Force has reached consensus in 2021 on a definition based upon SLE Disease Activity Index (Clinical SLEDAI=0), physician's global assessment <0.5 (0-3), prednisone 5 mg/day or less, and stable antimalarials, immunosuppressive drugs and biologics, irrespective of serology (81).

Doria-Zen established a validated definition of remission in 2015 that differs from the previous one because it does not consider PGA (physician global assessment). Furthermore, it delineates three categories of remission based on disease activity and treatment (80):

- Complete remission is reached when SLEDAI 2K = 0 in therapy-free patients (glucocorticoids and immunosuppressant). Antimalarials are allowed;

- Clinical remission off glucocorticoids: clinical SLEDAI = 0 and serologically active disease activity (SACQ). Allowed therapies are immunosuppressants and antimalarials.
- Clinical remission on glucocorticoids: the same definition as the above in glucocorticoids-free patients, prednisone up to 5 mg/day is allowed.

Since use of glucocorticoids, even at low doses, is an independent risk factor for damage accrual in the long term, the distinction between 'remission off glucocorticoids' and 'on glucocorticoids' is necessary. In addition, prolonged remission, defined as lasting for five or more years, demonstrated to yield a clinically significant effect on damage accrual in comparison to unremitted disease (80). A different study, aiming to define the shortest period of remission providing clinical positive outcome in terms of damage accrual, has set at least two consecutive years of remission to be protective against damage. If considered the sum of separate periods in remission interspersed by active disease, the length of time raise to three years (47).

The dichotomous nature of SLEDAI-2K limits its usefulness in the definition of remission and different levels of disease activity. Two definitions of remission based upon a new validated index, SLE-DAS, showed excellent performance not only in determining clinical remission but also in grading disease activity. In particular different SLE-DAS cut-offs reflects remission ($\leq$2.08), mild disease activity (2.08<SLE-DAS $\leq$7.4) and moderate/severe disease activity (SLE-DAS>7.64).The continuous nature of this score and the inclusion of important SLE manifestations not included in SLEDAI allows a better accuracy in the identification of clinically meaningful changes, sensitivity and prediction of damage accrual (54).

In a real-life clinical settings these definitions have shown concordance in defining remission achievement (82).

2.5.2 LLDAS: Lupus Low Disease Activity State

A definition of LDA in Lupus was proposed by the Asia-Pacific Lupus Collaboration in 2015 and requires the following criteria(83): 1.SLEDAI-2K $\leq$ 4, excluding activity in major organ systems (renal, central nervous system,

cardiopulmonary, vasculitis, fever), haemolytic anemia or gastrointestinal activity; 2. PGA ≤ 1; 3. Exclusion of new disease activity; 4. Prednisone daily intake ≤ 7,5 5. Antimalarials, immunosuppressants and biologics allowed, if well tolerated and at standard maintenance doses.

A monocentric Caucasian cohort of SLE patients prospectively followed in a study performed by the University of Padua demonstrated the beneficial outcome of LLDAS, defined by Franklin *et al*, in hindering damage accrual over time. The protective effect occurred when LLDAS lasted at least two consecutive years and increased with a longer duration (3,4,5 or more consecutive years). Instead, in keeping with clinical practice, the main negative predictors of low disease activity attainment were higher cumulative and baseline prednisone dose, joint and skin involvement, PGA > 1 and higher SLEDAI-2K. Since in the cohort analysed there was a great overlap between LLDAS and remission, the study suggests that remission is a major contributor to the positive effect of low disease activity on damage accrual. The Authors also raised the issue of defining a more rigorous definition of LLDAS, clearly separated form remission, for future studies. This has been done in other rheumatic conditions such as RA, where Disease Activity Score 28 allows precise discrimination between remitted patients (if the score is lower than 2.6) and LDA (defined by 2.6<DAS-28<3.2) (84).

3. Belimumab

3.1 Mechanism of action of Belimumab

Belimumab is a human IgGλ monoclonal antibody targeting BLyS.
BLyS (also known as BAFF, THANK, TALL-1, TNFSF13B and zTNF4(8)) is a B-lymphocyte stimulator protein crucial for growth and survival of peripheral B cells. The binding and neutralization of the receptor prevents the survival and differentiation of B-cells, thus hindering the growth of autoreactive B cells (85).
BLyS is a biomarker and a predictor of disease activity and flares, therefore elevated BLyS levels in sera herald future activity or reflect current disease. Cross-sectional and longitudinal studies of SLE patients revealed a correlation between circulating BLyS levels, increased titers of anti-double stranded DNA (anti-dsDNA) and risk of flares (8). Instead, low levels are associated with clinical improvement (86).

In 2003, a clone against human BLyS was isolated from screening a phage-display library: the new human monoclonal antibody was firstly named 'LymphoStat B' and later Belimumab. LymphoStatB was tested for its potential to antagonize BAFF (B-cell activating factor) in vitro and in vivo in murine models and cynomolgus monkeys and revealed strong inhibition and prevention of ligand binding to the receptor (8).

Belimumab belongs to a class of drugs known as B-lymphocyte stimulator-specific inhibitors and was approved by FDA (Food and Drug Administration) and EMA (European Medicines Agency) for treatment of active and refractory SLE in 2011. Until 2011 traditional treatment for lupus relied on antimalarials, corticosteroids and immunosuppressants. Since it was the first biological agent and the first new therapy authorized in over 50 years, the introduction of intravenous Belimumab was a considerable step forward (46). In Italy Belimumab has been used in clinical practice since March 2013 for patients with active lupus manifestation refractory to standard therapy, positive and-dsDNA and low C3 or C4 levels (62). It is no longer the only biological agent licensed for SLE treatment as the FDA approved Anifrolumab in August 2021.

3.2 Pivotal studies

A randomized, double-blind, placebo-controlled Phase I escalation study was performed to assess pharmacokinetics and pharmacodynamics of Belimumab. It showed a reduction in anti-dsDNA antibody levels and CD20+ B cells in patients receiving Belimumab, compared with placebo, and evidenced the linear pharmacokinetics of the antibody (87).

This study laid the foundation for a randomised, double-blind, placebo-controlled Phase II study designed to evaluate efficacy of IV Belimumab (1, 4 or 10 mg/kg) plus standard of care (SOC) versus placebo plus SOC. The patients (N=499) were followed for 52 weeks but the primary end-points were unmet, i.e. a 4-point decrease in SELENA-SLEDAI score at week 24 and time to first mild/moderate or severe SLE flare, stated by SFI. Nevertheless, a post hoc analysis suggested that a subgroup of serologically active patients reported greater response to Belimumab than those receiving SOC alone. This paved the way for the development of a new outcome measure, the SRI, used as primary outcome in the Phase III clinical studies (88).

BLISS-52 and BLISS-76 are two multicentre, randomized, placebo-controlled Phase III clinical trials designed to authorize Belimumab use in clinical practice.

Patients were randomised to either Belimumab ev 1 mg/kg, 10 mg/kg plus SOC or placebo plus SOC on days 0, 14, 28 and then every 28 days: BLISS-52 was carried out in Latin America, Asia-Pacific, Eastern Europe whereas BLISS-76 recruited patients from Wester Europe, Northern/Central America. The follow-up time was 48 and 76 weeks for BLISS-52 and BLISS-76, respectively. Both studies achieved the primary end-point (46,89).

In a pivotal, double-blinded, placebo-controlled Phase III study (BLISS-SC) a subcutaneous self-injectable formulation of Belimumab 200 mg together with SOC showed greater SRI-4 response, time to and risk of severe flare and steroid-sparing effect compared to placebo (90).

BLISS-NEA was designed to evaluate the efficacy and safety of IV Belimumab 10 mg/kg in patients from North East Asia and a significant higher percentage of

patients achieved the primary end-point (SRI-4 at week 52) and experienced a decline in flares, compared to placebo-treated patients (46).

BLISS-LN was a randomised, double-blind, placebo-controlled study enrolling 488 adult patients with class III, IV and/or V lupus nephritis, followed for 104 weeks that demonstrated superiority of Belimumab plus SOC in achieving the primary end-point (Primary Efficacy Renal Response) compared to placebo plus ST (91).

The Efficacy of BeliMumab in Subjects of Black RACE (EMBRACE) trial was conducted to study drug efficacy in Black African ancestry with SLE, since this category was underrepresented in BLISS-52 and BLISS-76. This 52 week, randomised, double-blind, placebo-controlled, Phase III/IV study failed its primary end-point, although improvement, especially in patients with high disease activity, was reported (92).

The PLUTO study concluded that the use of Belimumab is safe and effective in 5 years-children and older (46).

3.3 Effectiveness and Safety of Belimumab

Strong evidence from four large phase III pivotal studies and real-life experience studies have established the key role of Belimumab in reducing disease activity, preventing disease flares and tapering steroid use. These beneficial effects might be accountable for its ability in hindering or delaying long-term damage accrual, mainly caused by flares and drug toxicity. Owing to this, Belimumab has been depicted as a disease modifier in the treatment armamentarium for SLE (46,62).

Real-life setting and long-term extension of the BLISS studies proved that Belimumab's efficacy in the long term is maintained. A 7 year extension study has explored the enduring positive effects of Belimumab, showing that disease control and prevention of flares are long-lasting effects throughout the overall observation time (93). Furthermore, a reduction in work disability and meaningful improvements in fatigue score and heath related quality of life have been reported when the disease is controlled. Thereby, Belimumab enables cost reduction, in terms of job absenteeism and medical resources, since it is associated with a lower

number of unplanned medical visits, access to the emergency care and hospitalization (85,94).

Wallace et al. have demonstrated the good benefit-risk profile of Belimumab in long-term extension studies. A double-blind, randomised, placebo-controlled phase IV trial (BASE study) recently confirmed the good safety profile of the antibody. Furthermore, real life experience supported the excellent tolerability of Belimumab use (8). Data from BASE trial and a pooled analysis from the Phase II and III clinical studies show similar adverse events and their incidence was comparable to the placebo arm. In addition, severe infusion and hypersensitivity reactions were very rare (46).

3.4 Baseline predictors of response

Multivariate analysis were carried out in the pooled population of the BLISS-52 and 76 trials in order to identify predictors of greater drug response. Data showed that Belimumab displays higher efficacy in patients with elevated degree of disease activity at baseline, described by the following characteristics: SELENA–SLEDAI scores of 10 or higher, low complement levels, anti-dsDNA positivity and the requirement for corticosteroid treatment. This subgroup of patients benefit more from Belimumab, experiencing decline in disease activity, reduced risk of flares and an effect on steroid reduction (35).

Clinical and serological variables of a large multicentre Italian cohort of 188 SLE patients during treatment were analysed by multivariate logistic regression analysis to outline the profile of the 'best responder' to Belimumab. High disease activity (SLEDAI-2K $\geq$ 10) and high corticosteroid intake, in conformity with the pooled analysis, were independent predictors of SRI-4 response, whereas immunosuppressant showed a negative correlation. Clinical features independently associated with drug response were polyarthritis and relapse-remitting disease (94). Numerous post hoc analysis of patients from the BLISS-52 and BLISS-76 trials provided evidenced that musculoskeletal and skin involvement were the clinical manifestations most likely to gain remarkable improvement in belimumab-treated patients (85,95). The BeRLiSS *[See forward Chapter 3.7.1]* and several other studies confirmed these data (96,97).

This is supported by the significant decline in DAS28 and CLASI shown in 2 Italian prospective cohort of patients affected by active lupus. In this study, a striking improvement occurred in classical lupus polyarthritis measured by DAS-28 as well as acute and subacute cutaneous manifestations, evaluated by CLASI score. Instead, the same improvement did not happened for rhupus syndrome and chronic cutaneous manifestation. Furthermore, arthritis and skin flare rates markedly reduced after starting Belimumab (62).

3.6 Belimumab in the SLE treatment paradigm

The most recent EULAR recommendations propose the use of Belimumab as an add-on therapy for extra-renal manifestations with inadequate control to first line therapies (typically a steroid combined with antimalarial agent, with or without immunosuppressant), assessed by ongoing disease activity or frequent flares, and inability to taper GC daily dose to a maximum of 7.5 mg/day (52). Belimumab is effective in patients with active and refractory lupus manifestation, especially if they experience classical lupus polyarthritis and skin involvement in the context of a relapse-remitting disease (62).

3.7 Real-life studies

Following the BLISS trials several observational studies were carried out to provide real-world insights on the characteristics of patients receiving Belimumab, as well as its effectiveness and safety and overall patterns of SLE disease activity in routine cirumstances (66).

The OBSErve (evaluation Of use of Belimumab in clinical practice Setting) is a large multicentre study program involving 6 countries (Argentina, Canada, Germany, Spain, Switzerland, and USA) which support the results of RCT and provide a more realistic picture in clinical practice. These retrospective medical chart reviews confirmed a considerable improvement in disease activity, estimated according to physicians' global assessment and SLEDAI-2K, a steroid-sparing effect after 6 months of Belimumab, and a reduction in frequency of flares and organ damage accumulation (65).

3.7.1 BeRLiSS

The Belimumab in Real Life Setting Study (BeRLiSS) is a national multicentre cohort study to investigate efficacy and predictors of response in a cohort of 466 SLE patients treated with Belimumab in Real Life Setting from 24 Italian references centres for treatment of the disease. Participants were prospectively followed-up for a mean of 18 months *[See forward PATIENTS AND METHODS for Inclusion criteria and Data collection and management].*

The patients enrolled in the study displayed active refractory disease manifestations; in line with previous observation, the most frequent involvements requiring Belimumab treatment were articular (N = 200 patients, 42.9%) and cutaneous (N = 110, 23.6%). The rest of the patients exhibit renal, haematological, constitutional and serosal symptoms.

The objectives of the study were to identify predictors of response, remission, low disease activity (LDA), damage and drug discontinuation.

The results of the study confirmed the effectiveness of Belimumab, which reduces disease activity, decreases the incidences of flares and hampers damage accrual. This study showed that a considerable proportion of patients experienced clinical improvement and attained LDA and remission [Figure 17 and 18].

Response to Belimumab was evaluated by SRI-4 at months 6, 12, 24, 36 and 48 and was achieved by 49.2%, 61.3%, 69.7%, 69.9%, and 66.7% patients, respectively. Responders were more likely to have higher disease activity at the time of the first infusion (SLEDAI $\geq$ 10) while non-responders tended to have longer disease duration and chronic manifestations. A great proportion of patients, i.e. 66.1% and 44.3% of subjects spent more than half time of follow-up in low disease activity or remission, correspondingly. SLEDAI-2K score < 10 and an SDI score of 0 at baseline were found impactful predictors on both outcomes. Furthermore, the achievement of remission for more than 25% of follow-up or low disease activity for at least half of the time of the study lead to a significant less organ damage accumulation; conversely, a high SDI score at baseline predicted damage accrual.

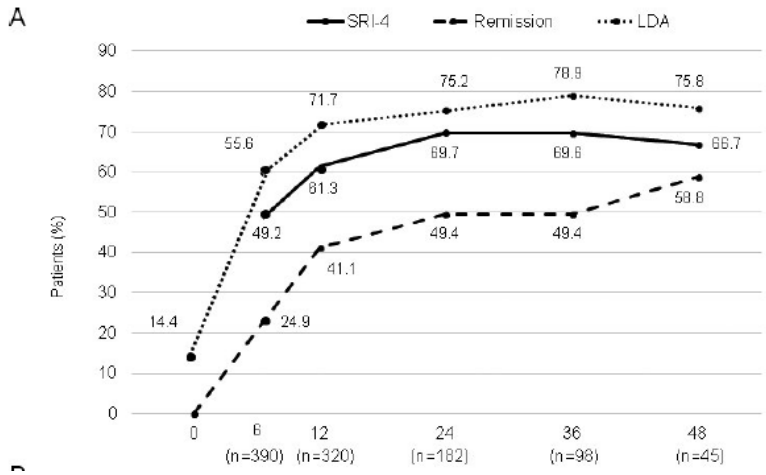


Figure 17. Responses rates of patients enrolled in the study.

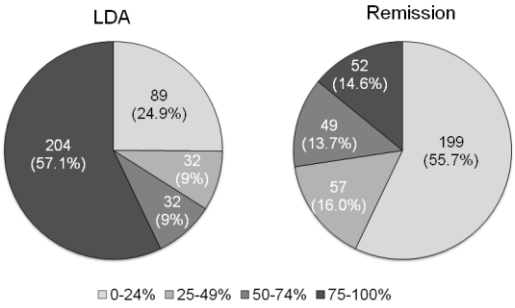


Figure 18. The four groups for each pie chart refers to the proportion of follow-up time (0-24%, 25-49%, 50-74%, and 75-100%) spent by patients in LDA or remission.

In summary, BeRLiSS study provides evidence that low baseline damage is a predictor of remission and LDA under Belimumab; oppositely, a higher baseline damage score is associated with a reduced likelihood of their attainment. Therefore, patients with active manifestations in the early phase of the disease who undergo treatment before damage formation stand the higher chance of a gaining a prompt clinical benefit from this drug.

The greatest efficacy of intervention was observed in patients with musculoskeletal and mucocutaneous involvement, demonstrated by marked reduction in DAS-28 in CLASI, respectively [Table VII]. Instead, patients affected by Rhupus syndrome had higher rate of discontinuation due to inefficacy.

	N pts*	Baseline	12 months	24 months	36 months	48 months	*p*
DAS-28 score	293	4.13±1.07	2.45±1.15	2.01±1.13	1.72±1.14	1.65±1.35	*<0.001*
CLASI activity score	177	4 (2-7.5)	0 (0-3)	0 (0-1)	0 (0-1.5)	0 (0-0.5)	*<0.001*

Table VII. Proportion of patients achieving each time point is reported. Data are expressed as mean ±SD or median (25°-75°). DAS 28 score and CLASI activity score were analysed for patients with musculoskeletal and cutaneous involvement.

3.7.7.1 Effects of Belimumab on specific organ involvements

Recently, the Rheumatology Unit of Padua performed a retrospective post hoc subgroup analysis on patients displaying renal involvement enrolled in the BeRLiSS cohort by prospectively collecting their data since Belimumab initiation. The aim of the study was to assess Belimumab renal response, already witnessed by the BLISS-LN and the BeRLiSS study, in a real life setting and to assess safety and predictors of response. BeRLiSS-LN (lupus glomerulonephritis) suggested the drug beneficial effect as an add-on therapy in clinical practice. Patients with serum creatinine, proteinuria, hypertension and smoke habit were unlikely to achieve PERR (Primary efficacy renal response), which was the primary outcome analysed and consist of proteinuria ≤ 0.7 g/24h, e GFR ≥ 60 ml/min/1.73 m² and no rescue therapy. PERR was achieved by 66.1% of patients after 24-month follow-up (98).

In conclusion, the BeRLiSS study portraits the use Belimumab in a tertiary referral centre where patients with different degrees of disease activity were referred to. The aim of the study was to assess the proportion of remission and low disease activity attainment, achieved by the vast majority of participants.

The most commonly affected organ domains at the study entry reflect the overall scenario of SLE patients, who mostly display articular and cutaneous involvements. Nevertheless, the study do not examine single organ domains improvement thus making necessary the design of a distinct subanalysis, which is currently lacking, in order to evaluate the trend of articular and cutaneous specific activity indices in real-life.

AIM OF THE THESIS

The aims of the thesis were:

1) To assess rates and predictors of response to Belimumab in patients with cutaneous and articular involvement included in the BeRLiSS study employing two organ specific activity indices: CLASI and DAS-28. As remission and LDA were previously evaluated, we used graded indices to test drug-effectiveness, i.e. CLASI 20,50,70 and DAS28 CRP 20,50, 70, which correspond to a 20, 50, 70% improvement in dermatological and articular response (*Study A*).

2) To investigate the performance and attainability of these different thresholds of response, in order to inform whether they can be suitable outcomes to be included in clinical trials (*Study A*).

3) To evaluate the performance of SLE-DAS versus DAS-28 in predicting Belimumab response and to assess correlations between these two disease activity indices at different time-points (*Study B*).

PATIENTS AND METHODS

1. BeRLiSS

1.1 Participants

The BeRLiSS study included a total of 466 SLE patients. Inclusion criteria were as follows: 1) Fulfillment of the American College of Rheumatology (ACR) 1982 revised criteria for SLE (Table II) or the Systemic Lupus International Collaborating Clinics (SLICC)/ACR classification criteria for SLE of 2012 (Table II); 2) Active disease, defined by a clinical SLE Disease Activity Index (SLEDAI) score of >0, that is refractory to a standard of care regimen; 3) IV belimumab (10 mg/kg on days 1, 14, and 28, and then every 28 days) as adjunct therapy; 4) Monthly follow-up due to infusion schedule.

Standard of care was defined according to the 2019 EULAR recommendations for the management of SLE as glucocorticoids and antimalarials (if not absolutely contraindicated), with or without immunosuppressive agents.

1.2 Data collection and management

Patients were followed up in a prospective manner from 1st of January 2013 to the 31st of March 2019 according to EULAR 2019 recommendations for monitoring of SLE patients in clinical practice and observational studies. Anonymized patient data were collected in an ad hoc database since Belimumab initiation and were regularly updated. Clinical and laboratory variables collected at baseline and every 6 months were as follows: SLEDAI 2000 (SLEDAI-2K) score, fatigue (0-10 on a visual analog scale), daily prednisone intake, complete blood cell count, 24-hour proteinuria, levels of anti-double-stranded DNA (determined by ELISA, CLIA or Farr assay), levels of C3 and C4, number of tender and swollen joints, DAS-CRP 28, CLASI, PGA, and concomitant medications. All compiled data were systematically and regularly evaluated. Patient data that did not fulfill inclusion and qualitative control criteria were excluded.

The study was approved by the University of Padua Ethics Committee and was carried out in accordance with the Declaration of Helsinki. Informed consent was obtained from each patient regarding personal data treatment.

2. Study A

2.1 Participants

In this post-hoc analysis patients from the BeRLiSS cohort with musculoskeletal and cutaneous involvement were included. We considered patient with at least 6 months of follow-up, with data regarding joint (DAS-28 CRP) or skin involvement (CLASI) at baseline and at month six, twelve, twenty-four, thirty-six, and forty-eight thereafter. Patients were included in the analysis until the last available infusion. Since some centres provided DAS-28 at baseline and at month 12 (no data at month 6), patients with DAS-28 measured at least at months 6 *or* month 12 were included.

2.2 Methods

We included patients with sign of skin or joint involvement, i.e. having a DAS-28 CRP>1.32 or a CLASI>0.

Articular response was evaluated by DAS-28 CRP, and patients were grouped in different classes of response in line with the decrease in the activity index. Patients reaching DAS-28 CRP 20, 50, 70 responses had a reduction in DAS-28 of at least 20%, 50% and 70%. The three classes were not mutually exclusive.

Similarly, we evaluated skin involvement by CLASI. Cutaneous response was quantified through a change in CLASI score, and patients were categorized in different levels of response according to the improvement from baseline values. CLASI 20, 50, 70 define a decrease of at least 20%, 50%, and 70% in the CLASI score, respectively. The three levels were not mutually exclusive, thus patients achieving CLASI 70 were also included in CLASI 20 and CLASI 50 categories.

3. Study B

3.1 Participants

An adjunctive analysis including patients treated with Belimumab and followed at the Padua Lupus Clinic (Division of Rheumatology, University of Padua, Italy) was carried out to evaluate the performance of SLE-DAS in comparison to DAS-28 CRP in defining response to treatment. Inclusion criteria were as follows: 1) Fulfillment of the American College of Rheumatology (ACR) 1982 revised criteria for SLE (Table II) or the Systemic Lupus International Collaborating Clinics (SLICC)/ACR classification criteria for SLE of 2012 (Table II); 2) Active disease, defined by a clinical SLE Disease Activity Index (SLEDAI) score of >0, that is refractory to a standard of care regimen; 3) Belimumab treatment plus standard of care; 4) Articular involvement defined by DAS-28>1.32; 5) At least 6 months of follow-up. Since SLE-DAS as a measure of disease activity was validated in 2019, only patients whose SLE-DAS was prospectively collected after that year were enrolled for this analysis. Therefore, we did not consider the BeRLiSS cohort in order to avoid retrospective calculation bias.

3.2 Methods

SLE-DAS is a recent overall activity measure to evaluate disease activity and response to treatment. SLE-DAS was available in Padua Lupus cohort at baseline and at months 6, 12, 24. Cut-offs for SLE-DAS 20, 50, 70 response (i.e., a reduction in SLE-DAS of 20%, 50%, and 70%) were calculated. The relationship between SLE-DAS values and responses and DAS-28 20, 50, 70 values and responses at different time-points were investigated. In the Padua Lupus Cohort SLE-DAS20, 50, 70 and DAS2820, 50, 70 were recorded at 6, 12, 24 months.

Statistical analysis

Parametric and non-parametric tests were used according to the types of variables. Comparisons of continuous data with a parametric distribution were performed using t-test, *t*-test for paired data and one-way analysis of variance (ANOVA) with

Bonferroni's post hoc analysis. Continuous data with a non-parametric distribution were analysed using the Wilcoxon's rank sum test and Wilcoxon's test for paired data. Comparisons of categorical data was performed using χ^2 test (Pearson test). Correlations were evaluated by Spearman's test. We investigated baseline predictors of response at 6, 12, 24, 36 months, according to DAS28 20, 50, 70. Similarly, we investigated baseline predictors of response at 6, 12 and 24 months according to CLASI 20, 50 and 70.

The following variables were included in the univariate analysis: gender, early lupus (disease onset ≤ 2 years), age at SLE onset, disease duration, age at the first infusion of Belimumab, chronic active disease activity pattern, number of medications and involvements before the study, concomitant immunosuppressant (yes/no), antimalarial use (yes/no), prednisone use (yes/no), prednisone dose ≥ 5 mg/day, prednisone dose ≥ 7.5 mg/day, methotrexate use (yes/no), SLEDAI-2K score, anti-ds DNA antibodies, anti-U1RNP antibodies, complement consumption, C3 and C4 consumption. For the analysis of DAS-28 only further variables considered were: fatigue, DAS28 ≥ 5.1, presence of anti-ds-DNA and complement consumption at 6, 12 and 24 months, complement consumption, C3 and C4 consumption at 6, 12 and 24 months, fibromyalgia, Rhupus syndrome.

For the analysis of skin involvement only were additionally included: smoking, azathioprine use (yes/no), mycophenolate mofetil (yes/no), cyclosporine use, SLICC > 0, SLICC > 1, SLICC > 2, CLASI a score, CLASI d score at baseline and 6, 12 and 24 months, CLASI ≥ 10, anti-Sm antibodies, anti-SSA antibodies, anti-SSB antibodies, anti-ribosomal P antibodies, anti-phospholipid antibodies, antiphospholipid syndrome.

Variables with a p value <0.2 at univariate analysis were included in multivariate models.

Backward stepwise logistic regression was employed to identify predictors of response with DAS28 20 50 70 and CLASI 20 50 70 as a dichotomous dependent variable, with significance set at 5%.

We also analysed the relationship between DAS 28 responses (20, 50 and 70) at different time-points (6, 12, 24 months) using logistic regression.

Cohen's kappa coefficient (κ) was used to evaluate the concordance of DAS-28 50/70 and DAS-28 remission, which was considered as the reference standard. Crosstabs were used to describe the relationship between two categorical variables and scatter-plots to describe correlations.

Variations in the SLEDAS score were analysed using Friedman's tests for repeated measures. Logistic regression was used to assess the relationship between DAS28 and SLEDAS at baseline and DAS 28 and SLEDAS responders (20, 50, 70) at different time-points (6, 12, 24 months).

Statics were performed using the SPSS (version 28.0) software. P values less than 0.05 were considered statistically significant.

RESULTS

4. Study A

The present study included a total of 272 patients with joint involvement and 147 with skin involvement from the BeRLiSS cohort.

1.1 Joint involvement

From the BeRLiSS study, two hundred seventy two patients displaying articular involvement at baseline (DAS28>1.32) with DAS-28 scores at the different time-points were included in the statistical analysis. Demographic, clinical and serological features and concomitant treatment at baseline are reported in Table VIII. Two hundred seventy two patients completed 6-month (98.6%), 215 12 month (77.9%), 114 24-month (41.3%), 59 36-month (21.6%), 28 48-month (10.1%) and 2 60-month (0.7%) of follow-up. The mean follow-up period was 23.69 ± 14.25 months.

	Baseline
Patients, N	272
Female, N (%)	253(91.7)
Male, N (%)	23(8.3)
Age at SLE diagnosis, years, mean ± SD	30.43± 11.56
Age at the first infusion, years, mean ± SD	42.39 ± 10.98
Disease duration at recruitment, mean ± SD	12 ± 9
Disease duration ≤ 2, N (%)	49(17.8)
Follow-up duration, mean ± SD, months	23.69 ± 14.25
Discontinuation, N (%)	92(33.3)
Number of previous medication, mean, ± SD	2.35 ± 1.67
Number of previous involvement, mean, ± SD	3 ± 1
Concomitant treatment	
- Oral corticosteroid, N (%)	268(97.1)
- Antimalarials, N (%)	182(65.9)

- Immunosuppressants, N (%)	179(64.9)
• Mycophenolate mofetil, N (%)	73(26.4)
• Methotrexate, N (%)	48(17.4)
• Azathioprine, N (%)	38(13.8)
• Cyclosporine, N (%)	22(8.0)
Disease features at baseline	
SLEDAI-2K score ≥ 10	129(46.7)
Daily PDN intake ≥ 7.5mg	175(63.4)
Daily PDN intake ≥ 5 mg	257(93.1)
Daily PDN intake, mean ± SD, mg	10.20 ± 7.01
DAS28 score ≥ 5.1, N patients (%)	41(14.9)
DAS28 score, mean, ± SD	3.84 ± 1.24
Fatigue, mean ± SD	5.37 ± 2.69
Chronic active disease, N (%)	103(37.3)
SLICC-DI score, mean ± SD	1 ± 2
SLEDAI score, mean ± SD	10 ± 3
Low complement levels, N (%)	234(85.7)
Low C3 levels, N (%)	169(62.1)
Low C4 levels, N (%)	217(79.8)
Autoantibodies at baseline	
- Anti-dsDNA, N (%)	221(80.7)
- Anti-Sm, N (%)	71(25.8)
- Anti-SSA, N (%)	124(45.1)
- Anti-SSB, N (%)	50(18.2)
- Anti-U1RNP, N (%)	87(31.6)
- Antiphospholipid Abs, N (%)	96(35.0)
Antiphospholipid syndrome, N (%)	43(15.9)
Rhupus syndrome, N (%)	11(4.0)
Fibromyalgia, N (%)	13(4.7)

Table VIII. Demographic, clinical and serological features of 272 articular patients treated with Belimumab.

Median and percentiles of DAS scores and daily prednisone intake at different end-point are reported in table IX.

	Mean ± SD	Median	25th Percentile	75th Percentile
DAS28-CPR				
Baseline	3.84±1,24	3.77	3.00	4.50
6 months	2.83±1.21	2.60	2.01	3.42
12 months	2.48±1.00	2.30	1.74	3.00
24 months	2.15±0.97	1.90	1.42	2.7
36 months	1.99±0.84	1.74	1.36	2.50
48 months	1.97±1.03	1.70	1.36	2.47
60 months	1.58±0.31	1.58	1.36	1.80
Prednisone daily dose, mg				
Baseline	10.20±7.01	10.00	5.00	12.50
6 months	6.48±4.37	5.00	5.00	7.50
12 months	5.31±4.95	5.00	2.50	6.25
24 months	3.60±3.84	3.50	0	5.00
36 months	3.45±5.11	2.20	0	5.00
48 months	3.60±5.36	1.13	0	5.00

Table IX. Disease activity variables in 272 articular patients treated with Belimumab.

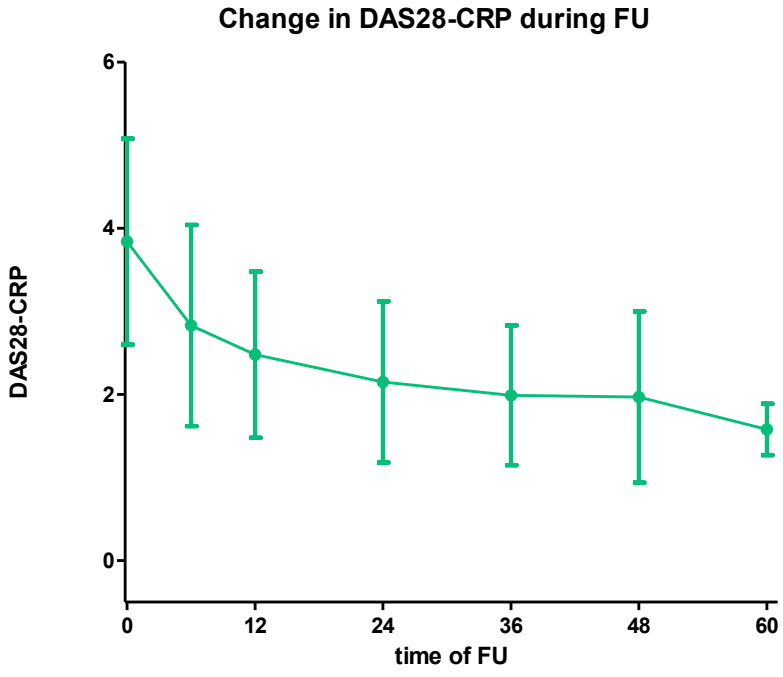


Figure 19. Change in DAS-28 CRP during follow-up. Values are reported in table IX.

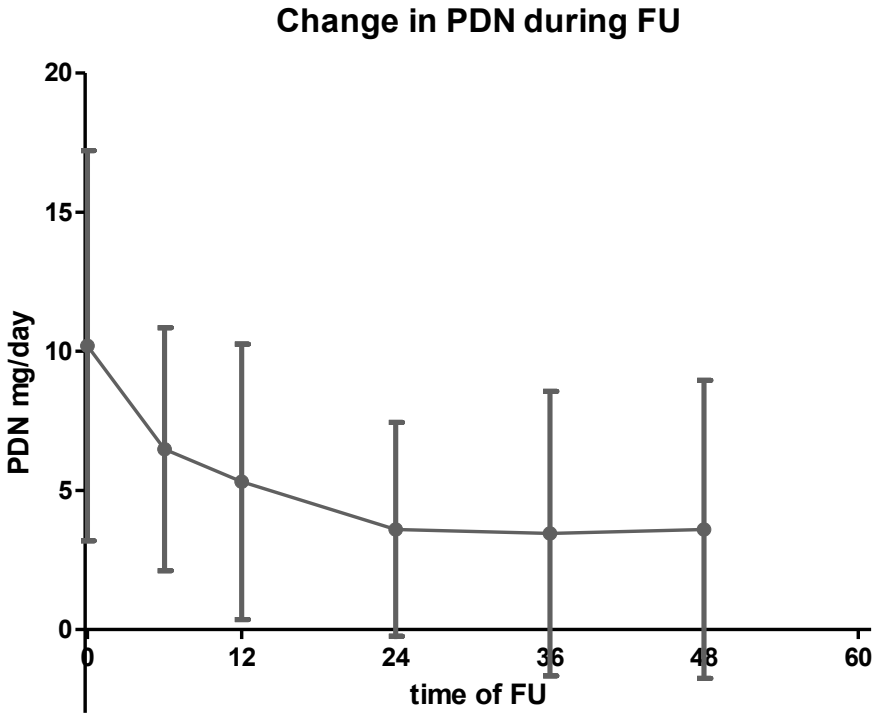


Figure 20. Change in PDN during follow-up. Values are reported in table IX.

Proportion of patients who achieve DAS28 20, 50, 70 and remission defined as DAS 28 < 2.6 at different time-points are reported in Table X.

	6 months	12 months	24 months	36 months	48 months
DAS28_20	157(57.7)	153(71.2)	96(84.2)	47(79.7)	23(82.1)
DAS28_50	49(18.0)	61(28.4)	51(44.7)	31(52.5)	16(57.1)
DAS28_70	3(1.1)	8(3.7)	12(10.5)	6(10.2)	5(17.9)
Remission	137(50.4)	130(60.5)	84 (73.7)	47 (79.7)	23 (82.1)

Table X. Number of patients (%) achieving DAS28 20, 50 and 70 at different time-points.

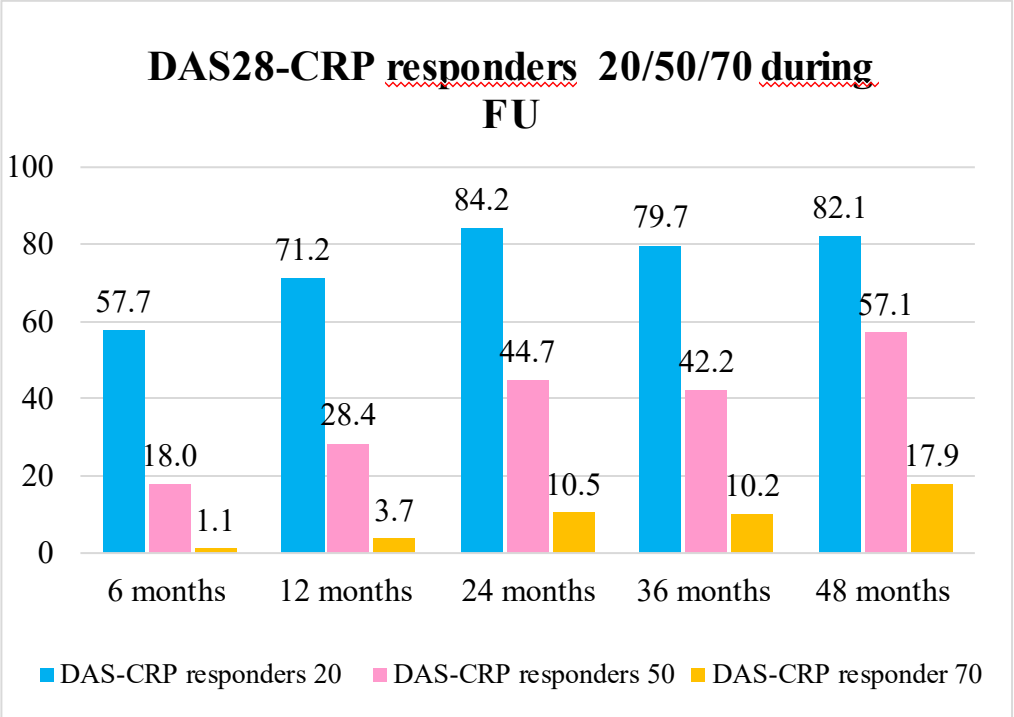


Figure 21. Proportion of patients achieving DAS28-20, DAS28-50 and DAS28-70 during follow-up.

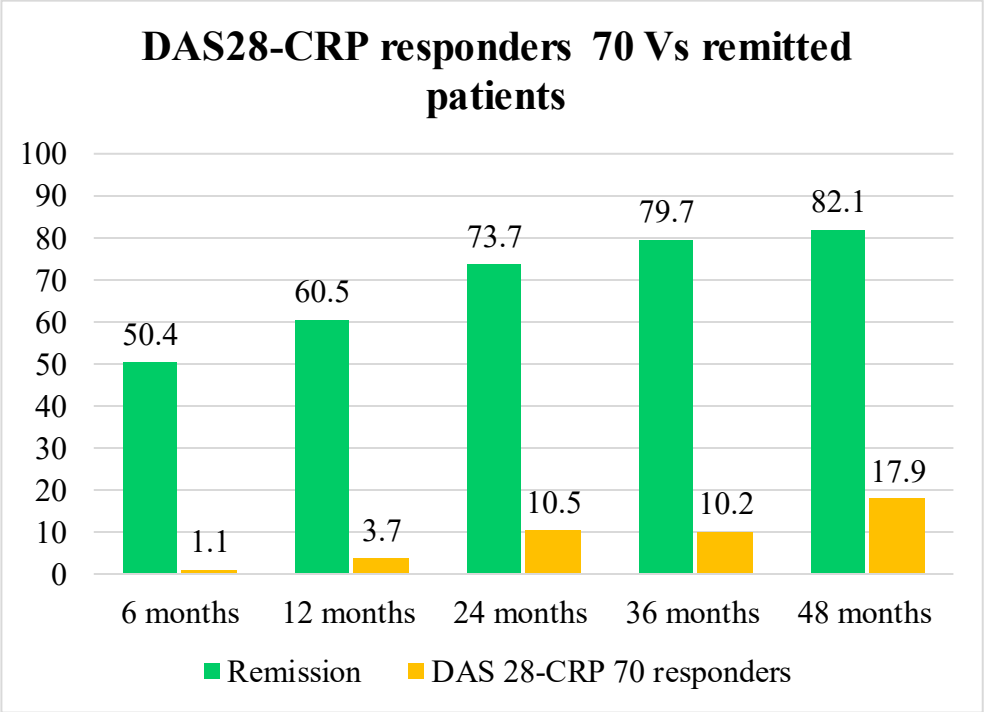


Figure 22. Comparison between patients achieving DAS28-70 and remission during follow-up.

Univariate analysis

- **6 months**

At the univariate analysis, early lupus (disease duration < 2 years), DAS score 5.1, earlier age at the start of Belimumab and lower fatigue score at baseline were more frequently observed in patients who reached DAS28 20 and 50 responses at 6 months compared with non-responders patients. Responder patients displayed more frequently a relapse-remitting disease activity pattern rather than chronic active. Associations were found between serological variables and non-responder patients (anti-ds DNA and low complement levels at baseline) at this time point.

No significant differences were observed among other variables in terms of achieving response 20, 50, and 70 at this time-point.

- **12, 24, 36 months**

The rate of response to Belimumab was higher in patients with DAS ≥ 5.1, early disease (disease onset < 2 years) and earlier age at the start of Belimumab, antimalarial use at 12 months and 24 months. Chronic disease activity was associated with lower response rate. No associations were found with serological variables. At 36 months a positive association was found between higher SLEDAI score at baseline and response rate.

Characteristics of non-responder and responder patients at 6 and 12 months are reported in tables XI-XVII. Tables for responders 36 were not reported as their trend is comparable to 24-month variables.

Characteristics of non-responders and responders DAS28 20 patients at 6 months Values are expressed as number (%) if not otherwise stated			
Baseline variable	**NON-RESPONDERS DAS28 20 6 MONTHS**	**RESPONDERS DAS28 20 6 MONTHS**	P
Patients	115	157	
Female	109(94.8)	141(89.8)	0.137
Disease duration ≤ 2 years	15(13.0)	32(20.4)	0.114
Immunosuppressant use	70(60.9)	105(66.9)	0.31
Antimalarial use	82(71.3)	98(62.4)	0.126
MTX use	19(16.5)	26(16.6)	0.99
Chronic active disease	42(36.5)	58(36.9)	0.94
PDN use	113(98.3)	151(96.2)	0.32
Daily PDN intake ≥ 7.5 mg	70(60.9)	103(65.6)	0.42
Daily PDN intake ≥ 5 mg	110(95.7)	145(92.4)	0.27
DAS 28 Score ≥ 5.1	15(13)	26(16.6)	0.42
Rhupus	5(4.3)	6(3.8)	0.83
Anti-U1RNP	31(27.2)	54(34.4)	0.21
Fibromyalgia	2(1.7)	11(7.0)	**0.044**
Anti-dsDNA	95(83.3)	122(78.2)	0.295
Low complement levels	106(92.2)	146(93.0)	0.80
Low C3 levels	95(82.6)	128(81.5)	0.82
Low C4 levels	101(87.8)	140(89.2)	0.30
Age at the first infusion, years, mean ± SD	43.3±10.6	41.7±11.4	0.73
Age at SLE diagnosis, years, ± SD	29.8±11.1	30.8±12.0	0.52
Disease duration at recruitment, ± SD	13.5±9.6	11±9	**0.029**
Fatigue, mean ± SD	5.8±2.8	5.1±2.6	0.109
Number of previous treatment, mean ± SD	2.2±1.7	2.4±1.6	0.37
SLEDAI at recruitment, mean ± SD	9.8±3.0	9.7±3.0	0.80
Daily PDN intake, mean ± SD, mg	9.65±6.08	10.59±7.55	0.40

Table XI. MTX, methotrexate; PDN, prednisone

Characteristics of non-responders and responders DAS28 50 patients at 6 months Values are expressed as number (%) if not otherwise stated			
Baseline variable	**NON-RESPONDERS DAS28 50 6 MONTHS**	**RESPONDERS DAS2850 6 MONTHS**	P
Patients	223	49	
Female	204(91.5)	46(93.9)	0.58
Disease duration ≤ 2 years	36(16.1)	11(22.4)	0.29
Immunosuppressant use	145(65.0)	30(61.2)	0.62
Antimalarial use	146(65.5)	34(69.4)	0.60
MTX use	39(17.5)	6(12.2)	0.37
Chronic active disease	89(39.9)	11(22.4)	**0.022**
PDN use	217(97.3)	47(95.9)	0.60
Daily PDN intake ≥ 7.5 mg	141(63.2)	32(65.3)	0.78
Daily PDN intake ≥ 5 mg	212(95.1)	43(87.8)	0.056
DAS 28 Score ≥ 5.1	28(12.6)	13(26.5)	**0.013**
Rhupus	9(4.0)	2(4.1)	0.99
Anti-U1RNP baseline	69(31.1)	16(32.7)	0.83
Fibromyalgia	10(4.5)	3(6.1)	0.63
Anti-dsDNA baseline	176(79.6)	41(83.7)	0.52
Low complement levels	208(93.3)	44(89.8)	0.40
Low C3 levels	186(83.4)	37(75.5)	0.20
Low C4 levels	199(89.2)	42(85.7)	0.48
Age at the first infusion, years, mean ± SD	42.7±10.7	41.0±12.4	0.37
Age at SLE diagnosis, years, ± SD	30.7±11.1	29.0±13.6	0.36
Disease duration at recruitment, ± SD	12.0±9.5	12.0±8.8	0.98
Fatigue, mean ± SD	5.6±2.7	4.5±2.4	**0.034**
Number of previous treatment, mean ± SD	2.3±1.6	2.3±1.7	0.87
SLEDAI at recruitment, mean ± SD	10.0±3.1	9.4±2.9	0.46
Daily PDN intake, mean ± SD, mg	10.09±6.78	10.66±7.85	0.96

Table XII. MTX, methotrexate; PDN, prednisone

Characteristics of non-responders and responders DAS28 20 patients at 12 months Values are expressed as number (%) if not otherwise stated			
Baseline variable	**NON-RESPONDERS DAS28 20 12 MONTHS**	**RESPONDERS DAS2820 12 MONTHS**	P
Patients	62	153	
Female	55(88.7)	141(92.2)	0.42
Disease duration ≤2 years	5(8.1)	27(17.6)	0.074
Immunosuppressant use	39(62.9)	98(64.1)	0.87
Antimalarial use	40(64.5)	103(67.3)	0.69
MTX use	11(17.7)	27(17.6)	0.99
Chronic active disease	23(37.1)	54(35.3)	0.80
Daily PDN intake ≥ 7.5 mg	40(64.5)	94(61.4)	0.67
Daily PDN intake ≥ 5 mg	57(91.9)	141(92.2)	0.96
PDN use	60(96.8)	147(96.1)	0.81
DAS 28 Score ≥ 5.1	5(8.1)	30(19.6)	**0.038**
Rhupus	3(4.8)	5(3.3)	0.58
Anti-U1RNP baseline	17(27.4)	54(35.5)	0.25
Fibromyalgia	1(1.6)	6(3.9)	0.39
Anti-dsDNA baseline	45(75.0)	127(83.0)	0.182
Low complement levels	55(90.2)	128(84.8)	0.30
Low C3 levels	42(68.9)	89(58.9)	0.179
Low C4 levels	50(82.0)	120(79.5)	0.68
Age at the first infusion, years, mean ± SD	42.4±11.1	43.4±11.1	0.56
Age at SLE diagnosis, years, ± SD	28.5±10.8	31.8±11	**0.047**
Disease duration at recruitment, ± SD	14±9	12±9	0.092
Fatigue, mean ± SD	5±2.8	5.9±2.6	0.090
Number of previous treatment, mean ± SD	2.2±1.8	2.4±1.7	0.29
SLEDAI at recruitment, mean ± SD	10±3	10±3	0.36
Daily PDN intake, mean ± SD, mg	9.8±6.3	9.9±7	0.97

Table XIII. MTX, methotrexate; PDN, prednisone

Characteristics of non-responders and responders DAS28 50 patients at 12 months Values are expressed as number (%) if not otherwise stated			
Baseline variable	**NON-RESPONDERS DAS2850 12 MONTHS**	**RESPONDERS DAS2850 12 MONTHS**	P
Patients	154	61	
Female	138(89.6)	58(95.1)	0.20
Disease duration ≤ 2 years	21(13.6)	11(18.0)	0.41
Immunosuppressant use	98(63.6)	39(63.9)	0.97
Antimalarial use	98(63.6)	45(73.8)	0.156
MTX use	24(15.6)	14(23.0)	0.20
Chronic active disease	54(35.1)	23(37.7)	0.72
Daily PDN intake ≥ 7.5 mg	99(64.3)	35(57.4)	0.35
Daily PDN intake ≥ 5 mg	146(94.8)	52(85.2)	**0.019**
PDN use	150(97.4)	57(93.4)	0.167
DAS 28 Score ≥ 5.1	16(10.4)	19(31.1)	**<0.001**
Rhupus	8(5.2)	0(0.0)	0.070
Anti-U1RNP baseline	54(35.1)	17(28.3)	0.39
Fibromyalgia	5(3.2)	2(3.3)	0.99
Anti-dsDNA baseline	122(80.3)	50(82.0)	0.78
Low complement levels consumption	132(87.4)	51(83.6)	0.47
Low C3 levels	93(61.6)	38(63.3)	0.92
Low C4 levels	122(80.8)	48(78.7)	0.73
Age at the first infusion, years, mean ± SD	43.0±11.2	43.3±10.6	0.85
Age at SLE diagnosis, years, ± SD	29.9±10.8	33.0±11.5	0.070
Disease duration at recruitment, ± SD	13.1±1.0	10.3±7.1	**0.046**
Fatigue, mean ± SD	5.6±2.7	4.9±2.5	**0.049**
Number of previous treatment, mean ± SD	2.3±1.7	2.5±1.6	0.38
SLEDAI at recruitment, mean ± SD	9.9±3.1	9.5±2.5	0.33
Daily PDN intake, mean ± SD, mg	10.04±6.23	9.60±7.98	0.27

Table XIV. MTX, methotrexate; PDN, prednisone

Characteristics of non-responders and responders DAS28 20 patients at 24 months Values are expressed as number (%) if not otherwise stated			
Baseline variable	**NON-RESPONDERS DAS2820 24 MONTHS**	**RESPONDERS DAS2820 24 MONTHS**	P
Patients	18	96	
Female	18(100)	90(93.8)	0.28
Disease duration ≤ 2 years	4(22.2)	14(14.6)	0.90
Immunosuppressant use	14(77.8)	58(60.4)	0.61
Antimalarial use	11(61.1)	67(69.8)	0.47
MTX use	3(16.7)	12(12.5)	0.63
Chronic active disease	11(61.1)	30(31.3)	**0.015**
Daily PDN intake ≥ 7.5 mg	14(77.8)	57(59.4)	0.14
Daily PDN intake ≥ 5 mg	18(100)	89(92.7)	0.24
PDN use	18(100)	94(97.9)	0.54
DAS 28 Score ≥ 5.1	16(10.4)	19(19.8)	0.15
Rhupus	0(0)	1(1.0)	0.66
Anti-U1RNP baseline	6(33.3)	33(34.7)	0.91
Fibromyalgia	1(5.6)	3(3.1)	0.61
Anti-dsDNA baseline	15(83.3)	83(86.5)	0.73
Low complement levels consumption	17(94.4)	81(85.3)	0.29
Low C3 levels	11(61.1)	60(63.2)	0.87
Low C4 levels	17(94.4)	73(76.8)	0.73
Age at the first infusion, years, mean ± SD	39.9±9.8	42.6±11.3	0.30
Age at SLE diagnosis, years, ± SD	29.3±12.4	30.8±11.2	0.64
Disease duration at recruitment, ± SD	10.6±9.0	11.8±8.6	0.60
Fatigue, mean ± SD	5.7±2.3	6.3±2.6	0.46
Number of previous treatment, mean ± SD	2.3±1.7	2.3±1.6	0.94
SLEDAI at recruitment, mean ± SD	10.4±3.6	10.0±2.5	0.45

Table XV. MTX, methotrexate; PDN, prednisone

Characteristics of non-responders and responders DAS28 50 patients at 24 months Values are expressed as number (%) if not otherwise stated			
Baseline variable	**NON-RESPONDERS DAS2850 24 MONTHS**	**RESPONDERS DAS2850 24 MONTHS**	P
Patients	63	51	
Female	60(95.2)	48(94.1)	0.79
Disease duration ≤ 2 years	9(14.3)	9(17.6)	0.56
Immunosuppressant use	35(55.6)	37(72.5)	0.06
Antimalarial use	41(65.1)	37(72.5)	0.39
MTX use	6(9.5)	9(17.6)	0.20
Chronic active disease	22(34.9)	19(37.3)	0.79
Daily PDN intake ≥ 7.5 mg	40(63.5)	31(60.8)	0.76
Daily PDN intake ≥ 5 mg	60(95.2)	47(92.2)	0.50
PDN use	62(98.4)	50(98.0)	0.88
DAS 28 Score ≥ 5.1	5(7.9)	15(29.4)	**0.003**
Rhupus	1(1.6)	0(0.0)	0.37
Anti-U1RNP baseline	20(31.7)	19(38.0)	0.49
Fibromyalgia	2(3.2)	2(3.9)	0.83
Anti-dsDNA baseline	55(87.3)	43(84.3)	0.65
Low complement levels consumption	56(88.9)	42(84.0)	0.45
Low C3 levels	40(63.5)	31(62.0)	0.87
Low C4 levels	51(81.0)	39(78.0)	0.70
Age at the first infusion, years, mean ± SD	42.2±11.1	42.2±11.1	0.97
Age at SLE diagnosis, years, ± SD	30.8±10.5	30.3±12.4	0.83
Disease duration at recruitment, ± SD	11.4±8.1	11.9±9.2	0.76
Fatigue, mean ± SD	6.3±2.6	6.2±2.6	0.93
Number of previous treatment, mean ± SD	2.2±1.6	2.4±1.6	0.61
SLEDAI at recruitment, mean ± SD	9.8±2.8	10.1±2.5	0.51

Table XVI. MTX, methotrexate; PDN, prednisone

Characteristics of non-responders and responders DAS28 70 patients at 24 months Values are expressed as number (%) if not otherwise stated			
Baseline variable	**NON-RESPONDERS DAS2870 24 MONTHS**	**RESPONDERS DAS2870 24 MONTHS**	P
Patients	63	51	
Female	60(95.2)	48(94.1)	0.62
Disease duration ≤ 2 years	9(14.3)	9(17.6)	0.06
Immunosuppressant use	35(55.6)	37(72.5)	0.71
Antimalarial use	41(65.1)	37(72.5)	0.89
MTX use	6(9.5)	9(17.6)	0.60
Chronic active disease	22(34.9)	19(37.3)	0.66
Daily PDN intake ≥ 7.5 mg	40(63.5)	31(60.8)	0.34
Daily PDN intake ≥ 5 mg	60(95.2)	47(92.2)	0.24
PDN use	62(98.4)	50(98.0)	0.62
DAS 28 Score ≥ 5.1	5(7.9)	15(29.4)	**0.002**
Rhupus	1(1.6)	0(0.0)	0.37
Anti-U1RNP baseline	20(31.7)	19(38.0)	0.46
Fibromyalgia	2(3.2)	2(3.9)	0.34
Anti-dsDNA baseline	55(87.3)	43(84.3)	0.55
Low complement levels consumption	56(88.9)	42(84.0)	0.67
Low C3 levels	40(63.5)	31(62.0)	0.48
Low C4 levels	51(81.0)	39(78.0)	0.33
Age at the first infusion, years, mean ± SD	42.2±11.1	42.2±11.1	0.97
Age at SLE diagnosis, years, ± SD	30.8±10.5	30.3±12.4	0.83
Disease duration at recruitment, ± SD	11.4±8.1	11.9±9.2	0.76
Fatigue, mean ± SD	6.3±2.6	6.2±2.6	0.93
Number of previous treatment, mean ± SD	2.2±1.6	2.4±1.6	0.61
SLEDAI at recruitment, mean ± SD	9.8±2.8	10.1±2.5	0.51

Table XVII. MTX, methotrexate; PDN, prednisone

Multivariate analysis – Predictors of response

By multivariate logistic regression analysis, patients with early lupus, defined as disease duration ≤ 2 years, were more likely to achieve DAS 20 response (OR 2.41, 95% CI 0.98 – 5.91, p = 0.055) and DAS28 50 response (0R 3.01, 95% CI 0.97 – 9.35, p = 0.057) to Belimumab at 6 months, although not significantly. This trend was also confirmed for DAS28 20 response (OR 2.65, 95% CI 0.97 – 7.30, p = 0.058) at 12 months and for DAS28 70 response (OR 3.74, 95% CI 0.89-15.75, p = 0.072) at 24 months.

Chronic disease activity pattern was protective against DAS 50 response at 6 months (OR 0.29, 95% CI 0.09 – 0.86, p = 0.026) and DAS20 response at 24 months (OR 0.29, 95% CI 0.10 – 0.8, p <0.001). Similarly, daily prednisone intake ≥ 5 mg was a negative predictor of DAS 50 attainment at 6 (OR 0.20, 95% CI 0.05 – 0.72, p = 0.014) and DAS 50 at 12 months (OR 0.30, 95% CI 0.11 – 0.84, p = 0.022).

In this model, DAS score ≥ 5.1 was a positive predictor of DAS 50 response at 6 months (OR 3.56, 95% CI 1.26 – 10.05, p = 0.017), DAS 20 response (OR 2.98, 95% CI 1.10 – 8.13, p = 0.033) and DAS 50 response (or 4.01, 95% CI 1.87 – 8.57, p < 0.001) at 12 months. These data were proved valid also for DAS 50 (OR 4.75, 95% CI 1.57 – 14.38, p = 0.006) and DAS 70 (OR 6.97, 95% CI 1.87 – 26.99, p = 0.004) responses at 24 months.

In a multivariate logistic regression model including DAS20 and DAS50 response at 36 months and baseline characteristics, SLEDAI at recruitment was significantly associated with DAS 20 response (OR 1.41, 95% CI 1.02 – 1.94, p = **0.037**). Variables entered in the multivariate analysis for 36 months were: early lupus, immunosuppressant and antimalarial use at baseline, chronic active disease pattern and SLEDAI at recruitment. No other variables were found to be statistically significant in the multivariate model.

Results are reported in table XVIII.

	6 months		12 months		24 months		
DAS28 CPR	DAS 20	DAS 50	DAS 20	DAS 50	DAS 20	DAS 50	DAS 70
Variable at baseline	OR (95% CI) p		OR (95% CI) p		OR (95% CI) p		
Early lupus	2.41 (0.98-5.91) 0.055	3.01 (0.97-9.35) 0.057	2.65 (0.97-7.30) 0.058	ns	ns	ns	3.74 (0.89-15.75) 0.072
CA disease pattern	ns	0.29 (0.09-0.86) **0.026**	ns	ns	0.29 (0.10-0.80) **<0.001**	ns	ns
Daily PDN intake ≥ 5 mg	ns	0.20 (0.05-0.72) **0.014**	ns	0.30 (0.11-0.84) **0.022**	-	-	-
DAS ≥ 5.1	ns	3.56 (1.26-10.05) **0.017**	2.98 (1.10-8.13) **0.033**	4.01 (1.87-8.57) **<0.001**	ns	4.75 (1.57-14.38) **0.006**	6.97 (1.87-26.00) **0.004**
Fatigue	ns	0.84 (0.71-0.99) **0.030**	-	-	-	-	-

Table XVIII. Variables entered in the multivariate analysis only for 6 months: anti-ds-DNA, low complement levels at baseline. Age at the first infusion, immunosuppressant and antimalarial use were included in the analysis of 6, 12 and 24 months. IS, immunosuppressant; CA: chronic active, PDN: prednisone

DAS20 response at 6 months predicted DAS20 response at 12 months (OR 6.78, 95% CI 3.47 – 13.24, p=<0.001). Among 122 DAS20 responders at 6 months, 16 were non-responders at 12 months; therefore 87% of patients had a durable response. Instead, 44 (49.4%) of 89 patients who were non-responders DAS20 at 6 months became responders at 12 months. DAS50 response at 6 months predicted DAS50 response at 12 months (OR 9.78, 95% CI 4.38 – 21.85, p=<0.001). We found that among DAS50 responders at 6 months, 11 (30.5%) lost their response and 142 non-responder patients (81%) at 12 months maintained the absence of their response. Finally, also DAS70 response at 6 months predicted DAS70 response at 12 months (OR 16.83, 95% CI 1.33 – 212.18). At 6 months, DAS70 responders had 67% of probability of experiencing a loss of their response at 12 months while among non-responders, 6 patients (2.9%) gained response in the following 6-month period. Conversely, non-response DAS20 at 12 months was a negative predictor of DAS20 response at 24 months (OR 0.73, 95% CI 0.022 – 0.24, p=<0.001) and non-responders DAS50 at 12 months were at risk of not achieving DAS50 response at 24 months (OR 0.86, 95% CI 0.03 – 0.22, p=<0.001). DAS70 non-response at 12 months was negatively associated with DAS70 response at 24 months (OR 0.05, 95% CI 0.004 – 0.61, p=0.019). After 6 months of follow-up 137 patients achieved remission; 98% of them were non-responders DAS70. Only three patients reached DAS70 after 6-month of follow-up and all of them were in remission. At 12 months, 100% of patients who attained DAS70 response (8 patients) were also in remission; non-responders DAS70 patients were 207 and 60% of them were in remission (122 patients). The percentage of responders DAS70 remitted patients at 24 months was 85%; only 12 patients achieved DAS70 response and remission at 24 months (15%). Remitted patients at 36 months were 47: of those, only 6 patients were responders DAS70 and the rest (87%) did not reach DAS70 response. No patient among non-responders DAS70 was in remission. Finally, at 48 months follow-up 23 patients were in remission, which in 5 patients overlapped DAS70 response (21.7%). The strength of the agreement between DAS 70 response and remission at 6 months assessed by Cohen's kappa (0.022) was slight; the data was confirmed for 12 months (0.049), 24 months (0.081) 36 months (0.056) and 48 months (0.090). Contingency table are reported in Supplementary material.

1.2 Cutaneous involvement

From the BeRLiSS study, one hundred forty seven patients displaying cutaneous involvement at baseline (CLASI>0) with CLASI scores at the different time-points were included in the statistical analysis. Demographic, clinical and serological features and concomitant treatment at baseline are reported in Table XIX.

One hundred forty seven patients completed 6-month, 118 12-month (80.3%), 69 24-month (46.9%) of follow-up. The mean follow-up period was 25.86 ± 15.66 months.

	Baseline
Patients, N	147
Female, N (%)	137 (93.2)
Male, N (%)	10(6.8)
Age at SLE diagnosis, years, mean ± SD	29.33±10.89
Age at the first infusion, years, mean ± SD	40.46±10.09
Disease duration at recruitment, mean ± SD	11±9
Disease duration ≤ 2, N (%)	28(19)
Follow-up duration, mean ± SD, months	25.86±15.66
Discontinuation, N (%)	48(32.7)
Number of previous medication, mean, ± SD	2.41±1.73
Number of previous involvement, mean, ± SD	3±1
Concomitant treatment	
- Oral corticosteroid, N (%)	144(98)
- Antimalarials, N (%)	107(72.8)
- Immunosuppressants, N (%)	95(64.4)
• Mycophenolate mofetil, N (%)	41(27.9)
• Methotrexate, N (%)	20(13.6)
• Azathioprine, N (%)	23(15.6)
• Cyclosporine, N (%)	10(6.8)
Disease features at baseline	
SLEDAI-2K score ≥ 10	92(62.6)

Daily PDN intake ≥ 7.5mg	100(68)
Daily PDN intake ≥ 5 mg	140(95.2)
Daily PDN intake, mean ± SD, mg	11.14±8.24
CLASI score ≥ 10, N patients (%)	20(13.6)
CLASI a score, mean, ± SD	5.19±4.24
CLASI d score, mean ± SD	0.87±1.78
Fatigue, mean ± SD	5.12±2.67
Chronic active disease, N (%)	57(38.8)
SLICC-DI score, mean ± SD	1±1
SLEDAI score, mean ± SD	10±4
Low complement levels, N (%)	126(86.3)
Low C3 levels, N (%)	95(65.1)
Low C4 levels, N (%)	118(80.8)
Autoantibodies at baseline	
- Anti-dsDNA, N (%)	122(83.0)
- Anti-Sm, N (%)	45(30.8)
- Anti-SSA, N (%)	82(56.2)
- Anti-SSB, N (%)	36(24.7)
- Anti-U1RNP, N (%)	52(35.6)
- Anti-ribosomal P, N (%)	11(7.5)
- Antiphospholipid, N (%)	40(27.4)
Antiphospholipid syndrome, N (%)	15(10.5)

Table XIX. Baseline features in 147 patients with skin involvement

CLASI 20, 50, 70 responses at different time-points are reported in Table XX.

	6 months	12 months	24 months
CLASI_20	104(70.7)	99(83.9)	63(91.3)
CLASI_50	77(52.4)	85(72.0)	58(84.1)
CLASI_70	61(41.5)	72(61.0)	52(75.4)

Table XX. Number of patients (%) achieving CLASI 20, 50 and 70 at different time-points.

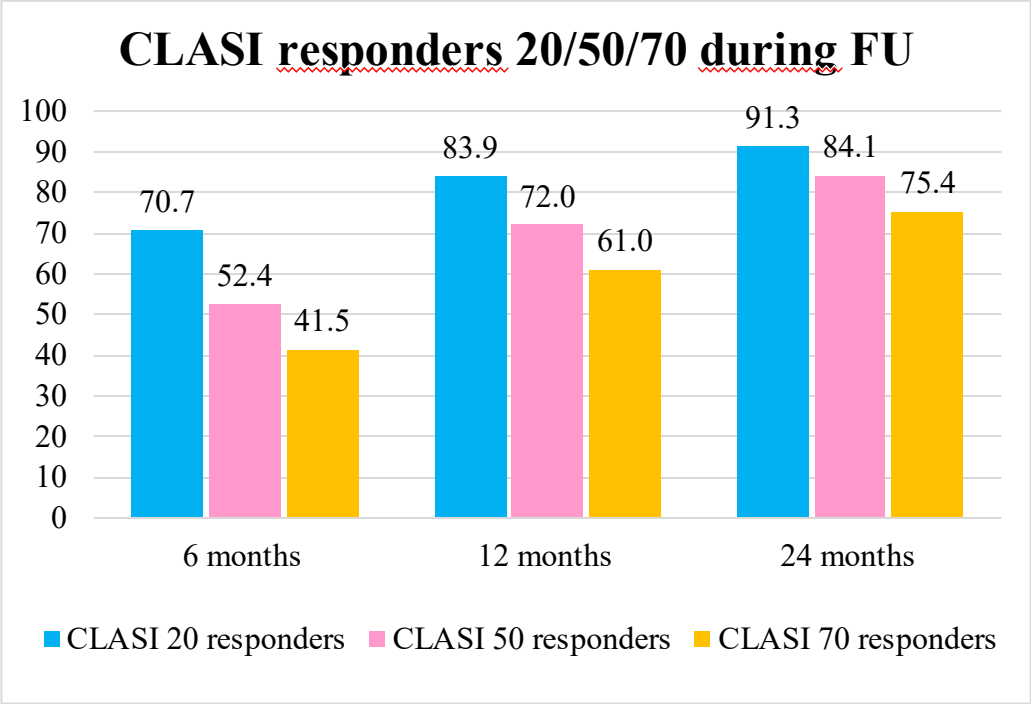


Figure 23. Proportion of patients achieving CLASI-20, CLASI-50 and CLASI-70 during follow-up.

Univariate analysis

- **6 months**

At the univariate analysis, early lupus (disease duration < 2 years) was more frequently observed in responder patients. Concomitant treatment with methotrexate, prednisone use > 5 mg/daily, CLASI ≥ 10 and chronic disease activity pattern were associated with lower response rate. Considering serological variables, the rate of response was higher in anti-Sm positive patients. Higher SLEDAI score and number of previous treatment at baseline showed greater prevalence in responder patients.

No other significant associations are worth pointing out.

- **12 and 24 months**

Responders displayed recent lupus onset and number of previous treatment, compared to non-responders. Conversely, CLASI ≥ 10 was associated with lower response rate. Serological abnormalities (low complement levels) were also observed among non-responders patients.

No significant differences were observed among other variables in terms of achieving response 20, 50, and 70.

Characteristics of non-responders and responders CLASI 20, 50 and 70 at different time-points are reported in table XXI-XXIX.

Characteristics of non-responders and responders CLASI 20 patients at 6 months Values are expressed as number (%) if not otherwise stated			
Baseline variable	**NON-RESPONDERS CLASI 20 6 MONTHS**	**RESPONDERS CLASI 20 6 MONTHS**	P
Patients	43	104	
Female, N (%)	38(88.4)	99(95.2)	0.135
Smoking	12(28.6)	19(18.6)	0.187
Disease duration ≤ 2 years	5(11.6)	23(22.1)	0.141
Immunosuppressant use	29(67.4)	66(63.5)	0.65
Antimalarial use	28(65.1)	79(76.0)	0.179
MTX use	8(18.6)	12(11.5)	0.256
Azathioprine use	6(14)	17(16.3)	0.72
MMF use	12(27.9)	29(27.9)	0.10
Cyclosporine use	2(4.7)	8(7.7)	0.51
Chronic active disease	14(32.6)	43(41.3)	0.32
PDN use	43(100)	101(97.1)	0.26
Daily PDN intake ≥ 7.5 mg	26(60.5)	74(71.2)	0.21
Daily PDN intake ≥ 5 mg	43(100)	97(93.3)	0.081
CLASI ≥ 10	8(18.6)	12(11.5)	0.26
CLASI a at 6 months, mean ± SD	5.84±4.25	1.55±2.52	**<0.001**
CLASI d at 6 months, mean ± SD	1.21±2.08	0.71±1.63	0.067
Anti-Sm	9(21.4)	36(34.6)	0.12
Anti-U1RNP	12(28.6)	40(38.5)	0.26
Anti-dsDNA	35(81.4)	87(83.7)	0.74
Anti-ribosomal P	2(4.7)	9(8.7)	0.39
Anti-SSA	21(50)	61(58.7)	0.34
Anti-SSB	11(26.2)	25(24.0)	0.79
Antiphospholipid Abs	13(31)	27(26.0)	0.54
Antiphospholipid syndrome	4(9.8)	11(10.8)	0.86
Low complement levels	36(83.7)	90(87.4)	0.56
Low C3 levels	27(62.8)	68(66.0)	0.71
Low C4 levels	34(79.1)	84(81.6)	0.73
Age at the first infusion, years, mean ± SD	40.44±8.31	40.46±10.78	0.83
Age at SLE diagnosis, years, ± SD	27.30±9.25	30.16±11.43	0.29

Disease duration at recruitment, mean ± SD	13±9	10±8	0.087
Number of previous treatment, mean ± SD	2.26±1.54	2.47±1.81	0.66
SLEDAI at recruitment, mean ± SD	10±3	10±4	0.60
CLASI a at recruitment	5.40±4.11	5.11±4.30	0.76
CLASI d at recruitment	1.02±1.91	0.81±1.74	0.59
SLEDAI ≥ 10	26(60)	66(63)	0.73
SLICC>0	24(58.5)	57(60.6)	0.82
SLICC>1	9(22)	22(23.4)	0.85
SLICC>2	6(14.6)	13(13.8)	0.90

Table XXI. MTX, methotrexate; PDN, prednisone; MMF, mycophenolate mofetil

Characteristics of non-responders and responders CLASI 50 patients at 6 months Values are expressed as number (%) if not otherwise stated			
Baseline variable	**NON-RESPONDERS CLASI 50 6 MONTHS**	**RESPONDERS CLASI 50 6 MONTHS**	P
Patients	70	77	
Female	63(90.0)	74(96.1)	0.14
Smoking	13(18)	18(24)	0.45
Disease duration ≤ 2 years	10(14.3)	18(23.4)	0.161
Immunosuppressant use	44(62.9)	51(66.2)	0.67
Antimalarial use	51(72.9)	56(72.7)	0.99
MTX use	11(15.7)	9(11.7)	0.48
Azathioprine use	11(15.7)	12(15.6)	0.98
MMF use	18(25.7)	23(29.9)	0.58
Cyclosporine use	3(4.3)	7(9.1)	0.25
Chronic active disease	31(44.3)	26(33.8)	0.19
PDN use	69(98.6)	75(97.4)	0.62
Daily PDN intake ≥ 7.5 mg	46(65.7)	54(70.1)	0.57
Daily PDN intake ≥ 5 mg	69(98.6)	71(92.2)	0.07
CLASI ≥ 10	13(18.6)	7(9.1)	0.09
CLASI a 6 months, mean ± SD	0.95±1.50	6.00±4.10	**<0.001**
CLASI d 6 months, mean ± SD	0.56±1.43	1.36±2.18	**0.027**
Anti-Sm	19(27.5)	26(33.8)	0.106
Anti-U1RNP	19(27.5)	33(42.9)	0.080
Anti-dsDNA	59(84.3)	63(81.8)	0.69
Anti-SSA	33(47.8)	49(63.6)	0.34
Anti-SSB	19(27.5)	17(22.1)	0.71
Low complement levels	58(84.0)	68(88)	0.46
Low C3 levels	42(60.9)	53(68.8)	0.31
Low C4 levels	53(76.8)	65(84.4)	0.24
Age at the first infusion, years, mean ± SD	39.97±11.13	41.30±8.03	0.31
Age at SLE diagnosis, years, ± SD	28.16±10.07	30.39±11.54	0.285
Disease duration at recruitment, ± SD	13±9.0	9±7	**0.011**
Number of previous treatment, mean ± SD	1.96±1.56	2.82±1.78	0.002
SLEDAI at recruitment, mean ± SD	10.0±3.0	11.0±4	0.11
CLASI a at recruitment	4.51±3.79	6.37±4.72	**0.038**

CLASI d at recruitment	0.66±1.54	1.23±2.11	0.40
SLEDAI ≥10	40(57.1)	52(67.5)	0.19
SLICC>0	41(61)	40(58)	0.78
SLICC>1	16(23)	15(22)	0.80
SLICC>2	8(12)	11(16)	0.48

Table XXII. MTX, methotrexate; PDN, prednisone; MMF, mycophenolate mofetil

Characteristics of non-responders and responders CLASI 70 patients at 6 months Values are expressed as number (%) if not otherwise stated			
Baseline variable	**NON-RESPONDERS CLASI 70 6 MONTHS**	**RESPONDERS CLASI 70 6 MONTHS**	P
Patients	86	61	
Female	79(91.9)	58(95.1)	0.45
Smoking, n (%)	17(20.0)	14(23.7)	0.59
Early lupus (disease duration ≤ 2 years)	14(16.3)	14(23.0)	0.31
Immunosuppressant use	57(66.3)	38(62.3)	0.62
Antimalarial use	65(75.5)	42(68.9)	0.37
MTX use	15(17.4)	5(8.2)	0.107
Azathioprine use	14(16.3)	9(14.8)	0.80
MMF	20(23.3)	21(34.4)	0.137
Cyclosporine	6(7.0)	4(6.6)	0.92
Chronic active disease	35(40.7)	22(36.1)	0.57
PDN at baseline, N (%)	85(98.8)	59(96.7)	0.37
Daily PDN intake ≥ 7.5 mg, N (%)	56(65.1)	44(72.1)	0.37
Daily PDN intake ≥ 5 mg, N (%)	85(98.8)	55(90.2)	**0.015**
CLASI ≥ 10	16(18.6)	4(6.6)	**0.036**
CLASI a at recruitment, mean ± SD	6.27 ± 4.48	3.67±3.35	**<0.001**
CLASI d at recruitment, mean ± SD	1.01±1.89	0.67±1.62	0.23
Anti-Sm	24(28.2)	21(34.4)	0.42
Anti-U1RNP	28(32.9)	24(39.3)	0.43
Anti-dsDNA	72(83.7)	50(82.0)	0.78
Anti-ribosomal P	5(5.9)	6(9.8)	0.37
Anti-SSA	45(52.9)	37(60.7)	0.35
Anti-SSB	26(30.6)	10(16.4)	0.05
Antiphospholipid antibodies	23(27.1)	17(27.9)	0.91
Antiphospholipid syndrome	8(9.5)	7(11.9)	0.65

Low complement levels	73(85.9)	53(86.9)	0.86
Low C3 levels	54(63.5)	41(67.2)	0.65
Low C4 levels	68(80.0)	50(82.0)	0.77
Age at the first infusion, years, mean ± SD	41.37±8.77	39.16±11.66	0.26
Age at SLE diagnosis, years, ± SD	28.85±10.33	30.00±11.68	0.70
Disease duration at recruitment, ± SD	13±9	9±7	**0.038**
Number of previous treatment, mean ± SD	2.16±1.62	2.75±1.83	0.057
SLEDAI at recruitment, mean ± SD	10±3	11±4	0.08
CLASI a at 6 months, mean ± SD	4.70±3.78	0.13±0.43	**<0.001**
CLASI d at 6 months, mean ± SD	1.11±1.98	0.51±1.41	**0.018**
SLEDAI≥10	50(58)	42(69)	0.19
SLICC>0	51(63.7)	30(54.5)	0.28
SLICC>1	19(23.8)	12(21.8)	0.79
SLICC>2	10(12.5)	9(16.4)	0.53

Table XXIII. MTX, methotrexate; PDN, prednisone; MMF, mycophenolate mofetil

Characteristics of non-responders and responders CLASI 20 patients at 12 months Values are expressed as number (%) if not otherwise stated			
Baseline variable	**NON-RESPONDERS CLASI 20 12 MONTHS**	**RESPONDERS CLASI 20 12 MONTHS**	P
Patients	19	99	
Female	18(94.7)	91(91.9)	0.67
Smoking	4(21.1)	19(19.6)	0.88
Disease duration ≤ 2 years	2(10.5)	18(18.2)	0.42
Immunosuppressant use	15(78.9)	62(62.2)	0.17
Antimalarial use	13(68.4)	70(70.7)	0.84
MTX use	2(10.5)	12(12.1)	0.84
Azathioprine use	4(21.1)	13(13.1)	0.37
MMF use	8(42.1)	28(28.3)	0.23
Cyclosporine use	1(5.3)	8(8.1)	0.67
Chronic active disease	9(47.4)	37(37.4)	0.41
PDN use	19(100)	97(98.0)	0.53
Daily PDN intake ≥ 7.5 mg	13(68.4)	67(67.7)	0.95
Daily PDN intake ≥ 5 mg	19(100)	94(94.9)	0.32
CLASI ≥ 10	0(0)	17(17.2)	**0.05**
CLASI a 12 months, mean ± SD	3.95±2.84	1.16±2.24	**<0.001**
CLASI d 12 months, mean ± SD	1.16±1.98	0.70±1.61	0.272
Anti-Sm	8(42.1)	26(26.5)	0.171
Anti-U1RNP	7(36.8)	31(31.6)	0.66
Anti-dsDNA	13(68.4)	84(84.8)	0.86
Anti-ribosomal P	0(0)	31(31.6)	0.23
Anti-SSA	12(63.2)	56(57.1)	0.63
Anti-SSB	6(31.6)	23(23.5)	0.45
Antiphospholipid antibodies	3(15.8)	29(29.6)	0.22
Antiphospholipid syndrome	0(0)	14(14.7)	0.07
Low complement levels	17(89.5)	81(82.7)	0.46
Low C3 levels	12(63.2)	59(60.2)	0.81
Low C4 levels	17(89.5)	74(75.5)	0.18

Age at the first infusion, years, mean ± SD	42.58±9.92	41.38±10.56	0.81
Age at SLE diagnosis, years, ± SD	31.53±9.70	29.79±11.39	0.55
Disease duration at recruitment, ± SD	11±8	12±9	0.87
Number of previous treatment, mean ± SD	2.47±1.71	2.42±1.80	0.76
SLEDAI at recruitment, mean ± SD	10±3	10±4	0.29
CLASI a at recruitment	3.32±2.65	5.29±4.31	**0.046**
CLASI d at recruitment	0.89±1.88	0.85±1.78	0.81
SLEDAI≥10	10(52)	65(66)	0.28
SLICC>0	13(72.2)	58(63.0)	0.46
SLICC>1	4(22.2)	24(26.1)	0.73
SLICC>2	4(22.2)	14(15.2)	0.46

Table XXIV. MTX, methotrexate; PDN, prednisone; MMF, mycophenolate mofetil

Characteristics of non-responders and responders CLASI 50 patients at 12 months Values are expressed as number (%) if not otherwise stated			
Baseline variable	**NON-RESPONDERS CLASI 50 12 MONTHS**	**RESPONDERS CLASI 50 12 MONTHS**	P
Patients	33	85	
Female	31(93.9)	78(91.8)	0.64
Smoking	8(24)	15(18)	0.45
Disease duration ≤ 2 years	4(12.1)	16(18.8)	0.39
Immunosuppressant use	23(69.7)	54(63.5)	0.53
Antimalarial use	23(69.7)	60(70.6)	0.92
MTX use	4(12.1)	10(11.8)	0.96
Azathioprine use	5(15.2)	12(14.1)	0.89
MMF use	12(36.4)	24(28.2)	0.39
Cyclosporine use	2(6.1)	7(8.2)	0.69
Chronic active disease	13(39.4)	33(38.8)	0.96
PDN use	33(100)	83(97.6)	0.38
Daily PDN intake ≥ 7.5 mg	21(63.6)	59(69.4)	0.55
Daily PDN intake ≥ 5 mg	33(100)	80(94.1)	0.16
CLASI ≥ 10	7(21.2)	10(11.8)	0.19
CLASI a 12 months, mean ± SD	0.70±1.53	4.67±2.92	**<0.001**
CLASI d 12 months, mean ± SD	0.60±1.49	1.37±2.10	0.073
Anti-Sm	11(34.4)	23(27.1)	0.44
Anti-U1RNP	9(28.1)	29(34.1)	0.54
Anti-dsDNA	25(75.8)	72(84.7)	0.25
Anti-SSA	19(59.4)	49(57.6)	0.87
Anti-SSB	9(28.1)	20(23.5)	0.60
Low complement levels	24(72)	74(88)	**0.043**
Low C3 levels	13(39.4)	58(69.0)	**0.003**
Low C4 levels	24(72.7)	67(79.8)	0.41
Age at the first infusion, years, mean ± SD	40.90±10.72	43.85±9.18	0.27
Age at SLE diagnosis, years, ± SD	29.76±9.84	30.19±11.63	0.47

Disease duration at recruitment, ± SD	14±10	11±8	0.68
Number of previous treatment, mean ± SD	2.24±1.68	2.51±1.82	0.56
SLEDAI at recruitment, mean ± SD	10±3	10±4	0.20
CLASI a at recruitment, mean ± SD	4.87±4.05	5.33±4.49	0.37
CLASI d at recruitment, mean ± SD	0.82±1.79	0.96±1.83	0.97
SLEDAI≥10	19(57)	56(65.9)	0.40
SLICC>0	21(66)	50(64)	0.88
SLICC>1	9(28)	19(24)	0.68
SLICC>2	6(19)	12(15)	0.66

Table XXV. MTX, methotrexate; PDN, prednisone; MMF, mycophenolate mofetil

Characteristics of non-responders and responders CLASI 70 patients at 12 months Values are expressed as number (%) if not otherwise stated			
Baseline variable	**NON-RESPONDERS CLASI 70 12 MONTHS**	**RESPONDERS CLASI 70 12 MONTHS**	P
Patients	46	72	
Sex (female)	43(93.5)	66(91.7)	0.72
Smoking	10(22.2)	13(18.3)	0.61
Disease duration ≤ 2 years	6(13.0)	14(19.4)	0.37
Immunosuppressant	33(71.7)	44(61.1)	0.237
Antimalarial	33(71.7)	50(69.4)	0.79
MTX	7(15.2)	7(9.7)	0.37
Azathioprine	7(15.2)	10(13.9)	0.84
MMF	15(32.6)	21(29.2)	0.69
Cyclosporine	2(4.3)	7(9.7)	0.28
Chronic active disease	19(41.3)	27(37.5)	0.68
PDN at baseline	46(100)	70(97.2)	0.25
Daily PDN intake ≥7.5 mg	31(67.4)	49(68.1)	0.94
Daily PDN intake ≥ 5 mg	46(100)	67(93.1)	0.068
CLASI ≥ 10	11(23.9)	6(8.3)	0.019
CLASI a 12 months, mean ± SD	3.98±2.70	0.10±0.34	<0.001
CLASI d 12 months, mean ± SD	1.40±2.18	0.38±1.10	0.004
Anti-Sm	15(33.3)	19(26.4)	0.42
Anti-U1RNP	14(31.1)	24(33.3)	0.80
Anti-dsDNA	34(73.9)	63(87.5)	
Anti-ribosomal P	1(2.2)	6(8.3)	0.175
Anti-SSA	26(57.8)	42(58.3)	0.95
Anti-SSB	13(28.9)	16(22.2)	0.42
Antiphospholipid antibodies	9(20.0)	23(31.9)	0.16
Antiphospholipid syndrome	3(6.8)	11(15.7)	0.16
Low complement levels	35(77.8)	63(87.5)	0.16
Low C3 levels	23(51.1)	48(66.7)	0.09
Low C4 levels	32(71.1)	59(81.9)	0.17

Age at the first infusion, years, mean ± SD	43.33±8.46	40.46±11.43	0.17
Age at SLE diagnosis, years, ± SD	30.72±10.02	29.65±11.82	0.52
Disease duration at recruitment, ± SD	13.0±9.0	11.0±8.0	0.35
Number of previous treatment, mean ± SD	2.09±1.62	2.65±1.85	0.11
SLEDAI at recruitment, mean ± SD	10±3	11±4	0.15
CLASI a at recruitment	6.39±4.82	4.07±3.37	0.006
CLASI d at recruitment	1.18±2.05	0.65±1.59	0.167
SLEDAI≥10	27(59)	48(66)	0.38
SLICC>0	30(68.2)	41(62.1)	0.52
SLICC>1	14(31.8)	14(21.2)	0.21
SLICC>2	9(20.5)	9(13.6)	0.34

Table XXVI. MTX, methotrexate; PDN, prednisone; MMF, mycophenolate mofetil

Characteristics of non-responders and responders CLASI 20 patients at 24 months Values are expressed as number (%) if not otherwise stated			
Baseline variable	**NON-RESPONDERS CLASI 20 24 MONTHS**	**RESPONDERS CLASI 20 24 MONTHS**	P
Patients	6	63	
Female, N (%)	6(100.0)	58(92.1)	0.47
Smoking	0(0.0)	11(17.7)	0.30
Disease duration ≤ 2 years	0(0.0)	10(15.9)	0.29
Immunosuppressant use	3(50.0)	46(73.0)	0.24
Antimalarial use	4(66.7)	43(68.3)	0.94
MTX use	1(16.7)	7(11.1)	0.69
Azathioprine use	0(0.0)	14(22.2)	0.20
MMF use	2(33.3)	19(30.2)	0.87
Cyclosporine use	0(0.0)	6(9.5)	0.43
Chronic active disease	3(50.0)	26(41.3)	0.68
PDN use	6(100.0)	62(98.4)	0.76
Daily PDN intake ≥ 7.5 mg	4(66.7)	45(71.4)	0.81
Daily PDN intake ≥ 5 mg	6(100.0)	61(96.8)	0.66
CLASI ≥ 10	0(0.0)	8(12.7)	0.34
CLASI a at 24 months, mean ± SD	5.33±4.5	0.67±1.49	
Anti-Sm	2(33.3)	15(24.2)	0.62
Anti-U1RNP	2(33.3)	20(32.3)	0.96
Anti-dsDNA	4(66.7)	53(84.1)	0.28
Anti-ribosomal P	2(33.3)	4(6.5)	**0.027**
Anti-SSA	4(66.7)	32(51.6)	0.48
Anti-SSB	1(16.7)	16(25.8)	0.62
Antiphospholipid Abs	0(0.0)	22(35.5)	0.076
Antiphospholipid syndrome	0(0.0)	9(15.3)	0.30
Low complement levels	3(50.0)	53(84.1)	**0.029**
Low C3 levels	2(33.3)	44(71.0)	0.06
Low C4 levels	3(50.0)	46(74.2)	0.21
SLEDAI ≥ 10	3(50.0)	42(66.7)	0.41
SLICC>0	2(33.3)	38(67.9)	0.93
SLICC>1	0(0.0)	15(26.8)	0.14
SLICC>2	0(0.0)	8(14.3)	0.32

Table XXVII. MTX, methotrexate; PDN, prednisone; MMF, mycophenolate mofetil

Characteristics of non-responders and responders CLASI 50 patients at 24 months Values are expressed as number (%) if not otherwise stated			
Baseline variable	**NON-RESPONDERS CLASI 50 24 MONTHS**	**RESPONDERS CLASI 50 24 MONTHS**	P
Patients	11	58	
Female	11(100.0)	78(91.8)	0.31
Disease duration ≤ 2 years	0(0.0)	16(18.8)	0.14
Immunosuppressant use	7(63.6)	54(63.5)	0.56
Antimalarial use	6(54.4)	60(70.6)	0.29
MTX use	1(9.1)	10(11.8)	0.78
Azathioprine use	2(18.2)	12(14.1)	0.85
MMF use	4(36.4)	24(28.2)	0.64
Cyclosporine use	0(0.0)	7(8.2)	0.26
Chronic active disease	6(54.4)	33(38.8)	0.36
PDN use	11(100.0)	57(98.3)	0.66
Daily PDN intake ≥ 7.5 mg	7(63.6)	59(69.4)	0.56
Daily PDN intake ≥ 5 mg	11(100)	80(94.1)	0.53
CLASI ≥ 10	1(9.1)	7(12.1)	0.77
Anti-Sm	2(20.0)	15(25.9)	0.69
Anti-U1RNP	2(20.0)	20(34.5)	0.37
Anti-dsDNA	9(81.8)	48(82.8)	0.94
Anti-SSA	7(70.0)	29(50.0)	0.24
Anti-SSB	3(30.0)	14(24.1)	0.69
Low C3 levels	4(36.4)	42(73.7)	**0.015**
Low C4 levels	7(63.3)	42(73.7)	0.50
Age at SLE diagnosis, years, ± SD	29.6±10.29	30.40±10.9	0.94
Disease duration at recruitment, ± SD	13±9	10±7	0.37
Number of previous treatment, mean ± SD	1.55±1.51	2.8±1.93	**0.033**
SLEDAI at recruitment, mean ± SD	11±5	10±3	0.71
SLEDAI≥10	7(63.6)	38(65.5)	0.90

Table XXVIII. MTX, methotrexate; PDN, prednisone; MMF, mycophenolate mofetil

Characteristics of non-responders and responders CLASI 70 patients at 24 months Values are expressed as number (%) if not otherwise stated			
Baseline variable	**NON-RESPONDERS CLASI 70 24 MONTHS**	**RESPONDERS CLASI 70 24 MONTHS**	P
Patients	17	52	
Female	16(94.1)	48(92.3)	0.80
Smoking	3(18.8)	8(15.7)	0.77
Disease duration ≤ 2 years	1(5.9)	9(17.3)	0.25
Immunosuppressant	13(76.5)	36(69.2)	0.57
MTX	2(11.8)	6(11.5)	0.98
Azathioprine	2(11.8)	12(23.1)	0.31
MMF	7(41.2)	14(26.9)	0.27
Antimalarial	11(64.7)	36(69.2)	0.72
Cyclosporine	1(5.9)	5(9.6)	0.64
Chronic active disease	8(47.1)	21(40.4)	0.63
PDN at baseline	17(100.0)	51(98.1)	0.57
Daily PDN intake ≥ 7.5 mg	11(64.7)	38(73.1)	0.51
Daily PDN intake ≥ 5 mg	17(100.0)	50(96.2)	0.41
CLASI ≥ 10	4(23.5)	4(7.7)	0.07
Anti-Sm	3(18.8)	14(26.9)	0.51
Anti-U1RNP	4(25.0)	18(34.6)	0.47
Anti-dsDNA	13(76.5)	44(84.6)	0.44
Anti-ribosomal P	2(11.8)	4(7.8)	0.62
Anti-SSA	10(62.5)	26(50.0)	0.38
Anti-SSB	5(31.3)	12(23.1)	0.51
Antiphospholipid antibodies	1(6.3)	21(40.4)	**0.011**
Antiphospholipid syndrome	1(6.7)	8(16.0)	0.36
Low complement levels	11(64.7)	45(88.2)	**0.028**
Low C3 levels	7(41.2)	39(76.5)	**0.007**
Low C4 levels	9(52.9)	40(78.4)	**0.043**
Age at the first infusion, years, mean ± SD	43.5±8.4	40.3±10.7	0.28
Age at SLE diagnosis, years, ± SD	30.1±9.1	30.3±11.3	0.86

Disease duration at recruitment, ± SD	13.0±8.0	10.0±7.0	0.16
Number of previous treatment, mean ± SD	2.1±1.6	2.7±2.00	0.24
SLEDAI at recruitment, mean ± SD	10±4	10±3	0.44
SLICC>0	8(50.0)	41(62.1)	0.16
SLICC>1	2(12.5)	14(21.2)	0.21
SLICC>2	0(0.0)	9(13.6)	0.07

Table XXIX. MTX, methotrexate; PDN, prednisone; MMF, mycophenolate mofetil

Multivariate analysis - Predictors of response

As demonstrated in the previous multivariate analysis, patients with early lupus, defined as a disease duration ≤ 2 years, were more likely to achieve DAS50 response at 6 months (OR 2.29, 95% CI 0.91 – 5.74, p=0.077), although not significantly. Furthermore, a longer duration of disease at Belimumab initiation defined a trend toward statistical significance and tended to be negatively associated with CLASI 50 response (OR 0.90, 95% CI 0.79 – 1.01, p=0.076) at 24 months. This negative association was significant for CLASI 70 response (OR 0.87, 95% CI 0.79 – 0.97, p = 0.011) at 24 months.

Patients with chronic active disease showed a negative trend in CLASI50 response at 6 months (OR 0.48, 95% CI 0.23 – 1.01, p = 0.052), almost significant.

A CLASI score ≥ 10 was an indepedent protective factor against CLASI 70 response to Belimumab at 6 (OR 0.30, 95% CI 0.09 – 0.99, p = 0.047), 12 months (OR 0.31, 95% CI 0.10 – 0.94, p=0.039) and 24 months (OR 0.05, 95% 0.004 – 0.49, p=0.011).

In this model, a higher SLEDAI at baseline was positively associated with CLASI 70 response at 6 (OR 1.12, 95% CI 1.02 – 1.24, p = 0.025) and 12 months (OR 1.16, 95% 1.01 – 1.32, p = 0.032). Conversely, a lower SLEDAI at baseline was shown to be negatively associated with CLASI 50 response at 24 months (OR 0.80, 95% CI 0.64 – 1.00, p=0.046).

In addition, the number of treatments before Belimumab was a positive predictor of CLASI 50 (OR 1.40, 95% CI 1.12 – 1.74, p=0.003) and CLASI 50 at 24 months (OR 1.19, 95% CI 1.02 – 3.60, p=0.043). A positive trend for patients with different line therapies before Belimumab was shown for CLASI 70 response (OR 1.12, 95% CI 0.99-1.49, p=0.063), nearly significant.

Results are reported in table XXX.

	6 months			12 months			24 months		
CLASI	20	50	70	20	50	70	20	50	70
Variable at baseline	OR (95% CI) p			OR (95% CI) p			OR (95% CI) p		
Early lupus	ns	2.29 (0.91-5.74) 0.077	ns	ns	ns	ns	-	-	-
Disease duration at recruitment	-	-	-	-	-	-	ns	0.90 (0.79-1.01) 0.076	0.87 (0.79-0.97) **0.011**
Chronic acive	-	0.48 (0.23-1.01) 0.052	-	-	-	-	-	-	-
CLASI≥10	ns	ns	0.30 (0.09-0.99) **0.047**	-*	ns	0.31 (0.10-0.94) **0.039**	ns	ns	0.05 (0.004-0.49) **0.011**
SLEDAI at baseline	ns	1.092 (0.99-1.21) **0.079**	1.12 (1.02-1.24) **0.025**	ns	ns	1.16 (1.01-1.32) **0.032**	ns	0.80 (0.64- 1.00) **0.046**	ns
Number of previous treatment	ns	1.40 (1.12-1.74) **0.003**	1.21 (0.99-1.49) 0.063	ns	ns	ns	ns	1.92(1.02-3.60) **0.043**	ns

Table XXX. Variables entered in the multivariate analysis for DAS20 and DAS 70: low complement levels, methotrexate use at baseline, anti-Sm, disease, SLEDAI>10 (only for 12 and 24 month). IS, immunosuppressant; PDN: prednisone. *For DAS20 response CLASI≥10 was not considered at 12 months because of the limitated numerosity of nonresponders

5. Study B

Thirty-three patients with lupus followed at Padua Lupus Clinic with DAS28>1.32 and SLEDAS (prospectively calculated since 2019) scores at baseline and at different time-points were included in the study. Twenty-six patients completed 6-month, 19 patients completed 12-month follow-up and 12 completed 24-month follow-up. Mean, median and percentiles values for SLE-DAS and DAS-28 responses at baseline and different time-points are reported in Table XXXI.

	Mean	Median	25th Percentile	75th Percentile
SLEDAS				
Baseline	9.31	9.05	6.48	11.07
6 months	6.34	5.66	2.08	9.12
12 months	4.18	4.34	1.32	6.27
24 months	15.37	2.08	1.12	6.27
DAS28				
Baseline	3.89	3.89	3.03	4.50
6 months	2.94	2.29	2.08	3.91
12 months	2.49	2.29	1.93	3.28
24 months	2	2	2	3

Table XXXI. Mean, median and percentiles of SLE-DAS and DAS 28.

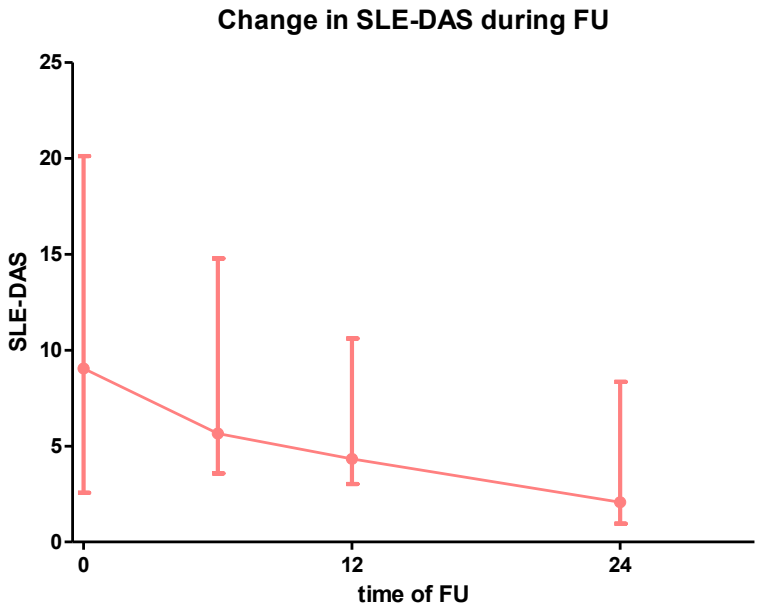


Figure 24. Change In SLE-DAS during follow-up.

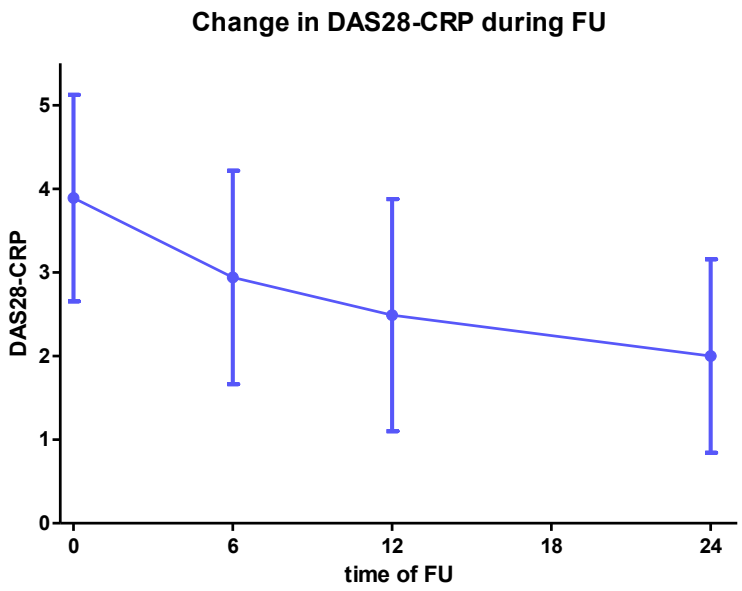


Figure 25. Change in DAS-28 CRP during follow-up.

Rates of SLEDAS20, 50, 70 and DAS2820, 50, 70 responses are summarized in table XXXII.

	6 months	12 months	24 months
SLE-DAS_20	16(48.5%)	20(76.9%)	16(84.2%)
SLE-DAS_50	9(27.3%)	14(53.8%)	10(52.6%)
SLE-DAS_70	8(24.2%)	11(42.3%)	10(52.6%)
DAS28_20	13 (39.4%)	18(69.2%)	15(78.9%)
DAS28_50	7(21.2%)	10(38.5%)	9(47.4%)
DAS28_70	2(6.1%)	3(11.5%)	3(15.8%)

Table XXXII. Number of patients (%) achieving SLE-DAS and DAS 28 cut-offs during follow-up time

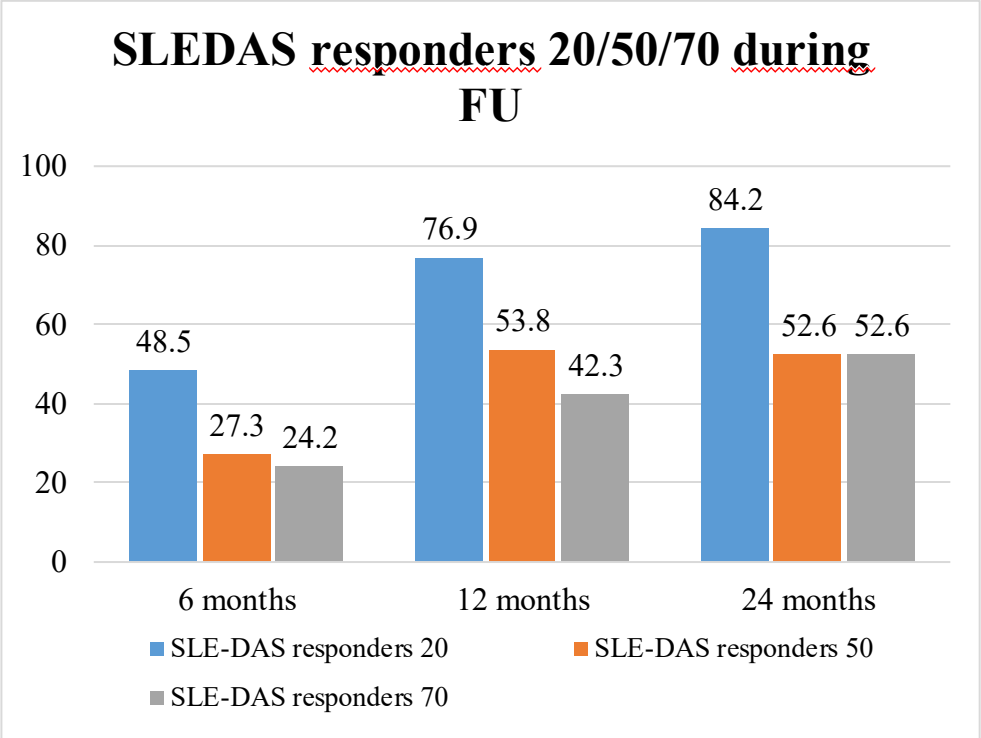


Figure 26. Proportion of patients achieving SLE-DAS 20, SLE-DAS 50, SLE-DAS 70 during follow-up.

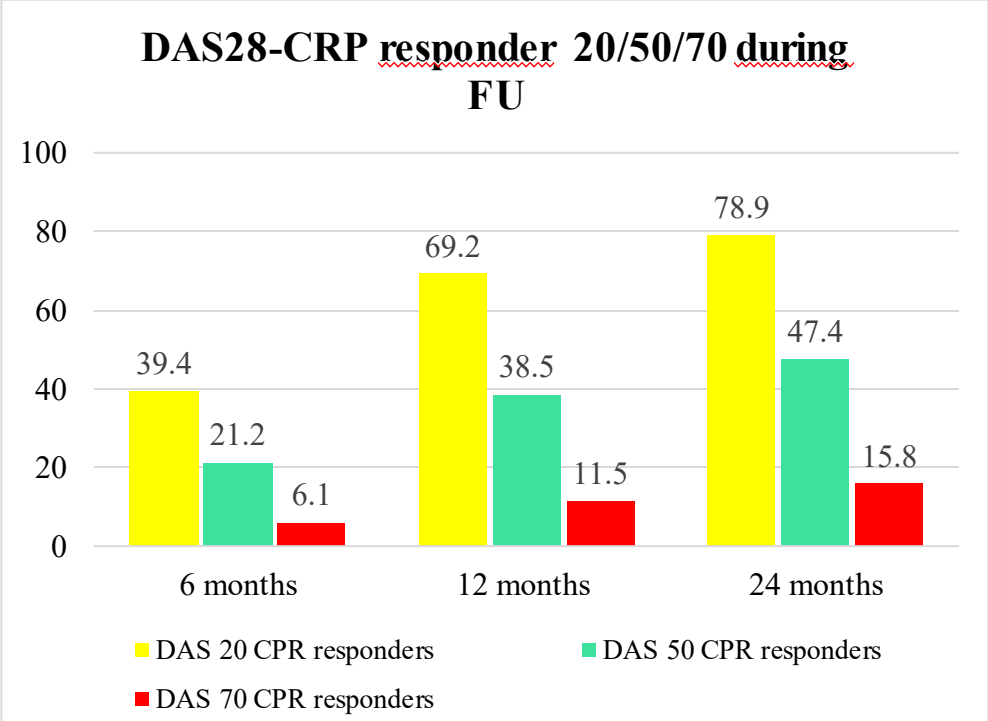


Figure 27. Proportion of patients achieving DAS28-CRP 20, DAS28-CRP 50, DAS28-CRP 70 during follow-up.

SLEDAS 50 response. After 6 months follow-up, 9 patients achieved response and 6 out of 17 of non-responders patients (35%) gained response at 12 months. The probability of losing response after this lag of time was 11%. Non-responders patients at 12 months had 25% probability of achieving response while responders at 12 months experienced a 27.3% probability of losing response at 24-month.

SLEDAS 70 response. After 24 months of follow-up 10 patients achieved response. At 12 months 4 non-responders patients reached the outcome at 24 (40%) and 3 responders experienced loss of response (33%).

DAS 28 50 response. After 6 months follow-up, 7 patients achieved response and non-responders patients at 6 months had 84% probability of not reaching response in the following 6 months.

DAS 28 70 response. Only 2 patients achieve DAS2870 at 6 months and 94% remained non responders at 24 months. Non-responders patients at 6 months had 23% of probability of achieving DAS2870 at 24 months and 77% of maintaining non-response.

All 9 patients who reached DAS28 50 response (47%) after 24 months of follow-up were responders at 12 months as well (100% maintenance of response between month 12 and 24). Similarly, 100% who did not respond at 12 months were non-responders at 24 months as well. DAS28 70 response rate showed the same trend: 3 responders and 16 non-responder at 12 months, which were the same percentages at 24 months. Contingency table are shown in supplementary material.

DAS score at the starting of the study was a predictor of DAS28 50 response at 12 (OR 3.43, p=0.026) and at 24 months (OR 2.73, p=0.067). Contrarily, SLEDAS at baseline was not a predictor of responses of DAS 28 20, 50, 70 at the following time-points: 6, 12, 24 months.

By using Spearman's rho, no correlations were found between DAS 28 and SLEDAS at baseline and at 12 months but a positive correlation between DAS 28 and SLEDAS values was identified at 24 months (ρ=0.022). The test showed a correlation between SLEDAS20, 50, 70 responder and DAS28 20, 50, 70 responders at 6 months (ρ=0.007, 0.002, 0.009 respectively). This relation was confirmed for SLEDAS and DAS28 responders 20 (ρ=0.030) at 12 months.

Scatterplots between SLEDAS 6 and DAS28 at baseline, 6, 12 and 24 are reported in Figure 28-31.

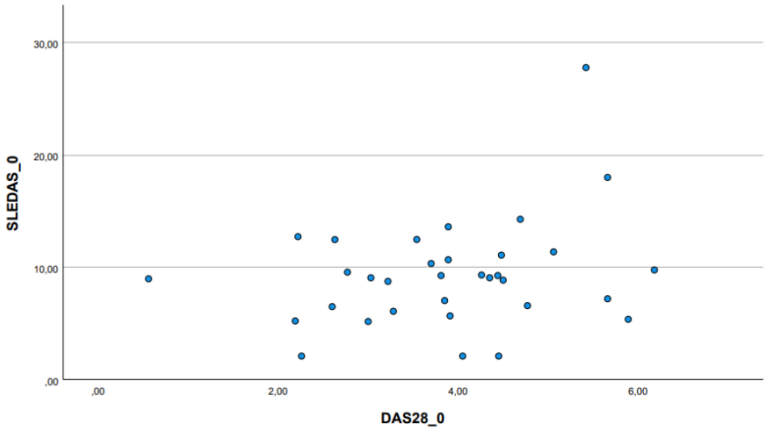


Figure 28. Scatterplot between DAS 28 and SLEDAS at baseline, ρ=0.25

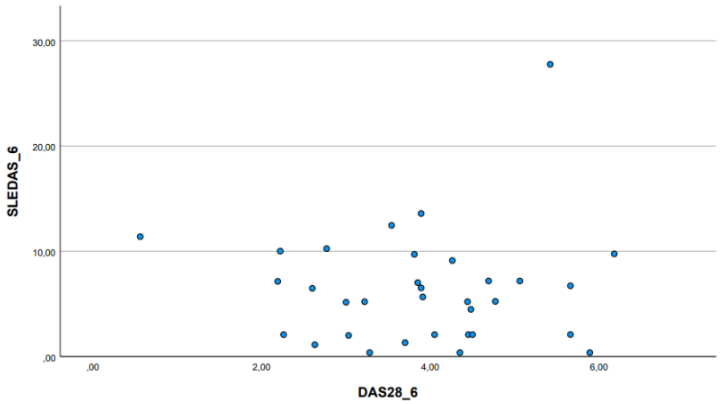


Figure 29. Scatterplot between DAS 28 and SLEDAS after 6-month, ρ=0.764

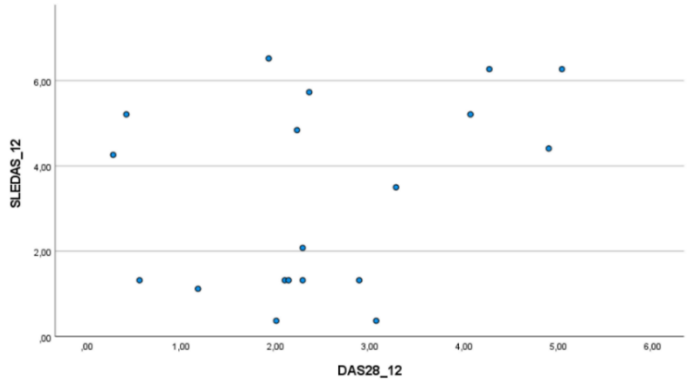


Figure 30. Scatterplot between DAS 28 and SLEDAS after 12-month, ρ=0.399

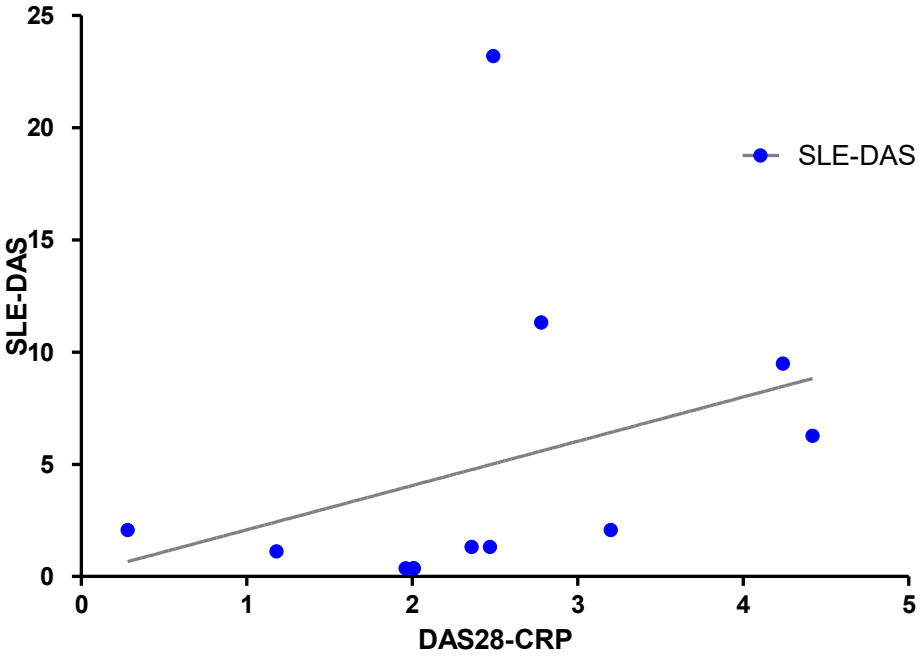


Figure 31. Scatterplot between DAS 28 and SLEDAS after 24-month of follow-up, **ρ=0.22**

DISCUSSION

In the scientific SLE community, predominant emphasis is given to the achievement of remission and LDA as predictors of positive outcome in the disease, whereas little attention has been paid to different disease activity categories and the evaluation of graded response to treatment.

The opposite applies to rheumathoid arthritis and psoriasis, for which different thresholds have been validated to assess clinical efficacy of treatment. Specifically, ACR 20,50 and 70 outcomes are standardized measures widely used in randomized controlled clinical trials to mark improvement or decrease in disease activity and response to therapy. They reflect a 20%, 50% or 70% improvement in tender and swollen joints and concurrently in three of the five following parameters: patient and physician global assessments of overall disease activity, pain scale, a score of physical disability evaluated by a questionnaire, blood acute-phase reactans, such as sedimentation rate (77,99).

The most frequently used outcome in RA trials is the ACR20, whose achievement defines the lowest clinically significant advancement (100). Similarly, in psoriasis the Psoriasis Area and Severity Index (PASI) is the most commonly used quantitative psoriasis measure used in randomized controlled trials as primary and secondary outcomes. A standard cut-off for assessing treatment efficacy in moderate-to-severe psoriatic patients is PASI75, i.e. at least a 75% improvement in PASI score from baseline. In addition to PASI75, PASI90 and PASI 50 are applied in clinical practice. The first was developed to quantify clinical improvement following the most effective emerging therapies and the latter to identify a significant response in clinical trials though less than PASI75 and PASI90 (74,101).

A second aspect of paramount importance in SLE overview is the need for a valid outcome measure closely connected to clinical beneficial effects and clinician's impression of response to test new therapeutic strategies in RCTs. The heterogeneity of SLE manifestations, its unsteady trend characterized by flares and remission and the complications and comorbidities associated result in a complex disease whose activity assessment remains challenging. The lack of a tool as

described above might be the underlying cause of the several RCTs failures, aiming to assess the efficacy of new biologics drugs, such as Rituximab and Tabalumab. As suggested by Reddy et al., one of the leading causes of such disappointing results might be the excessively stringent and non-organ specific clinical response criteria (102). In fact, although remission should be considered the main therapeutic target in lupus, the setting of too high standards in clinical trials might have been the cause for their failures and might prevent appropriate interpretation of the results. Conversely, standardized indices in rheumathoid arthritis such as Disease Activity Score, 28-joints have allowed the approval of several biologic drugs currently available for patients who fail conventional therapies (103).

Altogether, two major aspects inspired our analysis: on one hand the success of ACR20,50,70 and PASI50,75,90 for assessing outcomes and on the other the necessity of a redefinition of SLE clinical trials design and appropriate end-points.

In study A, we assess the response to Belimumab among cutaneous and articular patients included in the BeRLiSS study by evaluating different cut-offs of CLASI and DAS-28 CRP for improvement and their applicability in clinical practice.

Results showed a steady increase in the number of responders from the beginning of the study to the end of the follow-up for both activity indices and cut-offs analysed, except for DAS 20 response which slightly decreased from 84.2% at 24 months to 79.7 at 36 months. In the cohort of patients, DAS70 achievement was rare, occurring in only 3 patients (1.1%) at 6 months, 8 (3.7%) at 12 months, 12 at 24 months (10.5%), 6 at 36 months (10.2%) and 5 (17.9%) at 48 months. Our data would therefore suggest that DAS2870 might be unreasonably stringent in evaluating articular response. We demonstrated the validity of this observation by comparing DAS2870 to a less strict cut-off, that is remission (DAS-28 <2.6). Responders to both outcomes (i.e. DAS70 and remission) were few and among remitted patients only 2%, 6% and 12% were also DAS28 70 responders at 6, 12, 36 months, respectively. These findings confirmed the inconsistency between these two activity assessment tools and the uselessness of DAS28 70 in clinical practice.

Nearly 58% and 71% of patients achieved DAS28 20 response at 6 and 12 months of follow-up, respectively. In addition, among 215 patients with at least 12 months

of treatment, 87% of patients who achieved DAS2820 response at 6 months maintained the response at 12 and nearly half of non-responders patients at 6 months gained response in the following 6 months. This suggested that response at 6 months predicts response at 12 months but nonresponders still stand a 50% of probability of response at 12 months, thus making this last time-point the most appropriate to evaluate Belimumab effectiveness. This observation is in keeping with the daily clinical practice, since many studies confirmed that Belimumab response is likely to persist over time (46,93,96). Furthermore, it supports the rationale for continuing Belimumab, even if the patient has not experienced an improvement yet at 6 month.

With regard to DAS50 response, nearly one third of responder patients at 6 months lost their response at 12 months. This can be attributed to the limited range of DAS-28 values in the cohort analysed in the present study and comparable to other studies (78) that spans from a mean of 3.84 ± 1.24 SD at baseline to 2.15 ± 0.97 to 24 months of follow-up. This makes the maintenance of DAS28 50 response difficult, even in the face of a substantial clinical benefit or, even more, in case of remission. For example, a patient with a DAS28 score of 3.3 at baseline who achieve DAS28 50 at 6 months (1.6 DAS-28 score), may be no longer a responder in case of a minimum raise in DAS28score, i.e. 0.2, though being still in clinical remission.

In accordance with previous studies, early lupus was associated with a positive trend for DAS 28 20, 50 and 70 at different time-points while chronic active disease activity pattern was identified as an independent risk factor associated with lower response (94,96). Since patients with rhupus are less likely to respond to Belimumab, the several patients with overlap disease displaying a persistent active disease might be accountable for the reduced probability of achieving articular response in this subgroup. Another possible characteristic of the chronic refractory lupus patient profile is a high cumulative corticosteroid dose (daily PDN intake ≥ 5 mg), which indeed emerged as a risk factor for non-response. Notably, DAS28 $\geq$ 5.1 was a predictive factor of nearly all the outcomes and this is easily understandable considering that the higher the score at baseline, the higher the probability of experiencing a 20%, 50% and 70% reduction in DAS28 value. A similar implication might explain the positive association between SLEDAI at

baseline and higher response rates at 36 months. Besides that, Belimumab is most effective in lupus classic polyarthritis that comes with a higher DAS-28 score, rather than the rheumatoid-like.

In our cohort, we found that CLASI responders were the majority of patients and showed a gradual growth between consecutive time-points over the 2 years of follow-up. The fewer response rate was observed for CLASI 70 at 6 months, but still nearly one out of two patients achieved this outcome. Stricter cut-offs than the ones analyzed in order to evaluate skin disease improvement in lupus, such as the ones validated for psoriasis (50, 75, 90), might be the objective of future studies, considering the noticeable proportion of positive outcomes in the present study. The percentage of patients achieving CLASI20 was remarkably high, that is 104 patients (70.7%) at 6 months, 99 (83.9%) at 12 months and 63 at 24 months (91.3%). Thus, based on our result, CLASI20 seems too liberal and may be helpful to detect skin improvement only up to one year after Belimumab initiation, because at 2 years nearly all the patients enrolled became responders. On the other hand, it is worth further studying whether or not CLASI20 provides clinical significant improvement to assess its appropriatness in clinical practice.

The multivariate analysis showed that CLASI≥10 was inversely associated with CLASI70 response and prevented its achievement at all the time-points analysed. One explanation could be the substantial reduction required to reach a 70% decrease: for instance, in case of a CLASI score 12, it would mean its fall to 3 points, which is difficult to realize. Furthermore, it is noteworthy that CLASI score has some limits due its numerical nature and therefore is not able to capture the different variety of lesions in SLE (67). A qualitative organ specific index like BILAG has a better performance in assessing the extreme variability of the disease, but it is time consuming and it needs a specific training to be used in clinical practice. In addition, CLASI score does not report the extension of the lesion in each area but only records the number of areas interested while PASI and BILAG rely heavily on the percentage of skin affected and body region involved. Consequently, a certain score might be caused by a small lesion characterized by a high degree of severity or a large but mild lesion. Accordingly, a ten point CLASI score implies either more areas involved or few areas very active. For this reason,

even if there is a strong cutaneous improvement but the areas affected mildly are several, the score remains the same despite consistent response to treatment. A third explanation, although less probable, could be the less effectiveness of Belimumab in certain cutaneous types as the subacute cutaneous lupus.

We observed that patients with higher SLEDAI score at the start of Belimumab treatment were more likely to achieve CLASI 50 response at 6 months and CLASI70 response at 6 and 12 months. This can be attributable to the rapid decline expected to occur in those patients, thereby leading to a faster CLASI70 response. Similar trend was reported in term of SRI-4 responders patients with SLEDAI-2K score ≥ 2 in another study (94). Interestingly, this was not confirmed for CLASI 50 response at 24 months, maybe due to the lower numerosity of responder patients at this time point.

The fact that patients with an increased number of failed therapies before Belimumab seemed to be more likely to achieve response could imply its effectiveness also in patients with a refractory disease, both in the short- and long-term. As evidenced in the articular multivariate analysis, patients with early lupus, a lower disease duration and relapse-remitting pattern were best responders compared to long disease and chronic active disease.

In study B, we considered the application of SLEDAS in clinical practice to evaluate response to Belimumab and its association with DAS-28.

The number of responders patients showed a considerable raise of approximately 25 percentages points from 6 to 12 months (from 48.5% to 76.9%, from 27.3% to 53.8%, from 24.2% to 42.4% for SLEDAS 20, 50 and 70, respectively) and remained reasonably steady between 12 and 24 months (from 76.9% to 84.2%, from 53.8% to 52.6%, from 42.3% to 52.6% for SLEDAS 20, 50 and 70, respectively). For non responders a higher probability of gaining SLEDAS50 response was demonstrated from 6 to 12 months (35%) than from 12 to 24 months (25%) of follow-up. The response was maintained in 75% of the cases for both time-points. Another fact worth noting was the complete juxtaposition between DAS28 50 and 70 responders and non responders at 12 and 24 months. Our data suggest that there is a significant improvement from 6 to 12 months in terms of response to

Belimumab. From then on responders rates undergo a stabilization. Therefore, based on our analysis and in line with previous studies (96,104), Belimumab should be continued for more than 6 months and its efficacy in articular and cutaneous manifestations should be evaluated within the first 12 months of treatment initiation, considering this time-point the most suitable concluding whether there has been an adequate response or not. The previously mentioned stringency of DAS28 70 was confirmed in this Study as well: responders went from 6.1% at 6 months to 11.5% at 12 months and reached a peak at 24 months (15.8%).

Despite the fact that no correlation was identified between SLE-DAS and DAS-28 scores except at 24 months, we found positive correlations among SLE-DAS and DAS 28 responders. Although showing similar trends in responses, SLEDAS was not a predictor of DAS-28 response and therefore it can't be used to estimate Belimumab efficacy as an alternative of DAS-28.

Our study has both strengths and limitations. Limitations include the lack of a control group population, the exclusion of patients for whom data were not available from the analysis of response at that particular time points, and no Patient-Reported Outcomes (PROs) collection. These limitations are mainly connected to the retrospective nature of the study, which poses some objective restrictions to the amount of data that can be inferred. The greatest strengths of the study are the real-life setting, the large cohort of patients analysed and the long follow-up period.

CONCLUSIONS

The two distinct studies in patients with articular and cutaneous manifestations enables us to identify the best responder profile to Belimumab: a patient with high disease activity, especially with polyarticular involvement and high DAS-28 score (≥5.1), not presenting rhupus nor chronic active lupus pattern, treated within two years after the diagnosis. The early intervention during the so-called 'window of opportunity' before patients start to accumulate damage allows to maximize Belimumab articular and cutaneous response. These results are in keeping with the findings of BeRLiSS. In the cutaneous analysis, it seemed that a higher cutaneous activity hampers Belimumab response; this apparent contradictory result is primarily attributable to the nature of CLASI.

The results suggest that DAS-28 20, 50 and CLASI 50, 70 might be valuable tools in daily clinical practice. Conversely, the use of DAS-28 CPR 70 is limited because all responder patients fall into the definition of remission. On the other hand, the applicability of CLASI20 might have some limitations because it seems to be too liberal, and therefore may find its usefulness only within one year from treatment initiation.

No clear correlation between SLE-DAS and DAS-28 was found, therefore both should be used in clinical analysed in clinical practice in order to evaluate clinical response in patients with joint manifestations.

Supplementary material

Study A

Contingency table responder_70_DAS_6 months* remission_DAS28_6 months

		remission_DAS28_6 months		
		,00	1,00	Total
responder_70_DAS_6 months	,00	135	134	269
	1,00	0	3	3
Total		135	137	272

Table I ,00: non-responder; 1,00: responder

Contingency table responder_70_DAS_12 months* remission_DAS28_12 months

		remission_DAS28_12 months		
		,00	1,00	Total
responder_70_DAS_12 months	,00	85	122	207
	1,00	0	8	8
Total		85	130	215

Table II ,00: non-responder; 1,00: responder

Contingency table responder_70_DAS_24 months* remission_DAS28_24 months

		remission_DAS28_24 months		
		,00	1,00	Total
responder_70_DAS_24 months	,00	30	72	102
	1,00	0	12	12
Total		30	84	114

Table III ,00: non-responder; 1,00: responder

Contingency table responder_70_DAS_36 months* remission_DAS28_36 months

		remission_DAS28_36 months		
		,00	1,00	Total
responder_70_DAS_36 months	,00	12	41	53
	1,00	0	6	6
Total		12	47	59

Table IV ,00: non-responder; 1,00: responder

Contingency table responder_70_DAS_48 months* remission_DAS28_48 months

		remission_DAS28_48 months		
		,00	1,00	Total
responder_70_DAS_48 months	,00	5	18	23
	1,00	0	5	5
Total		5	23	28

Table V ,00: non-responder; 1,00: responder

Contingency table responder_20_DAS_12 months* responder_20_DAS28_6 months

		responder 20_DAS28_6 months		
		,00	1,00	Total
responder_20_DAS_12 months	,00	45	16	61
	1,00	44	106	150
Total		89	122	211

Table VI ,00: non-responder; 1,00: responder

Contingency table responder_50_DAS_12 months* responder_20_DAS28_6 months

		responder 50_DAS28_6 months		
		,00	1,00	Total
responder_50_DAS_12 months	,00	142	11	153
	1,00	33	25	58
Total		175	36	211

Table VII ,00: responder; 1,00:responder

Contingency table responder_70_DAS_12 months* responder_70_DAS28_6 months

		responder 70_DAS28_6 months		
		,00	1,00	Total
responder_70_DAS_12 months	,00	202	2	204
	1,00	6	1	7
Total		208	3	211

Table VIII ,00: non- responder; 1,00: responder

Study B

Contingency table responder_50_SLEDAS_6 months* responder_50_SLEDAS_12 months

		responder 50_SLEDAS_12 months		
		,00	1,00	Total
responder_50_SLEDAS_6 months	,00	11	6	17
	1,00	1	8	9
Total		12	14	26

Table IX ,00: non- responder; 1,00: responder

Contingency table responder_50_SLEDAS_12 months* responder_50_SLEDAS_24 months

		responder 50_SLEDAS_24 months		
		,00	1,00	Total
responder_50_SLEDAS_12 months	,00	6	2	8
	1,00	3	8	11
Total		9	10	19

Table X ,00: non- responder; 1,00: responder

Contingency table responder_70_DAS28_6 months* responder_70_DAS28_12 months

		responder 70_DAS28_12 months		
		,00	1,00	Total
responder_70_DAS28_6 months	,00	23	1	24
	1,00	0	2	2
Total		23	3	26

Table XI ,00: non- responder; 1,00: responder

Contingency table responder_50_DAS28_6 months* responder_50_DAS28_24 months

		responder 50 DAS28 24 months		
		,00	1,00	Total
responder_50_DAS28_6 months	,00	10	3	13
	1,00	0	6	6
Total		10	9	19

Table XII ,00: non- responder; 1,00: responder

Contingency table responder_70_DAS28_6 months* responder_70_DAS28_24 months

		responder 70_DAS28_24 months		
		,00	1,00	Total
responder_70_DAS28_6 months	,00	16	1	17
	1,00	0	2	2
Total		16	3	19

Table XIII ,00: non- responder; 1,00: responder

Contingency table responder_50_DAS28_12 months* responder_50_DAS28_24 months

		responder 50_DAS28_24 months		
		,00	1,00	Total
responder_50_DAS28_12 months	,00	10	0	10
	1,00	0	9	9
Total		10	9	19

Table XIV ,00: non- responder; 1,00: responder

Contingency table responder_70_DAS28_12 months* responder_70_DAS28_24 months

		responder 70_DAS28_24 months		
		,00	1,00	Total
responder_70_DAS28_12 months	,00	16	0	16
	1,00	0	3	3
Total		16	3	19

Table XV ,00: non- responder; 1,00: responder

Contingency table responder_50_DAS28_6 months* responder_50_DAS28_12 months

		responder 50_DAS28_12 months		
		,00	1,00	Total
responder_50_DAS28_6 months	,00	16	3	19
	1,00	0	7	7
Total		16	10	26

Table XVI ,00: non- responder; 1,00: responder

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