UNIVERSITÁ DEGLI STUDI DI PADOVA

CORSO DI LAUREA MAGISTRALE IN MEDICINA E CHIRURGIA

DIPARTIMENTO DI MEDICINA

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TESI DI LAUREA

ANTIMALARIALS AND THEIR ASSOCIATION WITH REMISSION, DAMAGE, AND GLUCOCORTICOID USE IN SYSTEMIC LUPUS ERYTHEMATOSUS

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ANNO ACCADEMICO 2022/2023

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ABSTRACT

Introduction: Systemic lupus erythematosus (SLE) is an autoimmune rheumatic disease with a protean clinical phenotype. Antimalarials (AMs) are a cornerstone in SLE treatment, and over the years have been associated with many benefits. Nevertheless, clinicians and patients not infrequently discontinue AMs therapy, due to remission, AM-induced retinopathy, and non-adherence.

Objective: to assess the prevalence and use patterns of AMs and to examine their differences in disease activity, attainment of remission and low disease activity, glucocorticoid (GC) use, and damage accumulation.

Methods: We performed a retrospective analysis of 455 SLE patients referring to Padua Clinic between 1980 and 2020. Disease activity was assessed by SLE Disease Activity Index 2000 (SLEDAI-2K) and damage accumulation by Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI). Remission on GCs was defined as clinical SLEDAI-2K=0, stable background therapy, and prednisone (PDN) ≤ 5 mg/day. Low disease activity status (LLDAS) was defined according to Franklyn et al., modified by excluding PGA. Cumulative SDI, SLEDAI-2K, achievement of remission, and PDN therapy at the end of follow-up were examined. AM therapy status was defined as: AMs never prescribed, prescribed and never stopped, prescribed and stopped. Among the latter, we identified patients who discontinued AMs due to retinopathy or due to other reasons. Retinopathy was defined according to a certified ophthalmologist evaluation contraindicating further AM therapy. Time without AMs (difference between SLE duration and AM therapy duration) and fraction of time off AMs (time without AMs divided by SLE duration) were also calculated. The Cox regression model was used to explore predictors of retinopathy. The association between AM therapy status and SDI was evaluated in a multivariable logistic regression model.

Results: Less than 5% of the enrolled patients never assumed AMs. In this group, neurological manifestations, vasculitis, the use of immunosuppressants and SDI ≥2 were more frequent. Patients who had stopped AMs were less likely to be off GCs (44.9% vs. 68%, p=0.0001) and more likely to have an active SLE (20% vs. 9%, p=0.0003). SDI ≥2 was also highly discordant (47.9% vs. 27.89%, p<0.0001). Moreover, patients who stopped AMs were less likely to be free of chronic kidney disease (CKD) (81.6% vs. 91.1%, p=0.002). The prevalence of discontinuation of

AM therapy due to confirmed retinopathy was 6.4% with a median duration of therapy of 9 years (IQR: 4-19). At Cox regression analysis, older age in years (per unit change: HR 1.04, 95% CI 1.01-1.07 p=0.04) and anti-U1RNP positivity (HR 3.2, 95% CI 1.38-7.46, p=0.006) were predictors of retinopathy. Furthermore, patients with eGFR <30 ml/min or history of renal transplantation showed a HR of 10.1 (95% CI 1.24-82.4) towards retinopathy vs. patients with no eGFR reduction. Patients with damage at the end of follow-up had an incrementally higher mean fraction of SLE duration spent without AM exposure (0.18 vs. 0.24 vs. 0.35 for SDI =0, SDI =1, and SDI ≥2, respectively, p=0.0034). A similar pattern emerged regarding GC dose (p=0.0013) and disease activity status (p=0.0009).

Conclusions: Time spent without AMs during SLE is associated with increased disease activity, damage accumulation, and GC use. The development of retinopathy is infrequent below the threshold of 5 mg/kg/day of HCQ.

1. Systemic lupus erythematosus

1.1 Definition

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with potential multisystem involvement ranging from mild mucocutaneous manifestations to life-threatening renal or neuropsychiatric conditions.

The etiology of SLE is the result of intricate interactions among genetic and epigenetic, ethnic and hormonal, as well as environmental variables. Many important aspects of these multifactorial relationships remain unexplained.

Numerous autoantibodies, the subsequent formation and deposition of immune complexes (ICs), and other immunological processes are related to SLE clinical phenotypes.

Like other autoimmune conditions, SLE is a female-predominant disease, and certain ethnic groups like Afro-Americans, Asians, and Aboriginals have a higher incidence, morbidity, and mortality.

The disease course is unpredictable, and progressive damage accrual often occurs due to disease activity and side effects of the chronic therapies these patients are necessarily subjected to.

1.2 Epidemiology

A well-defined epidemiological characterization of SLE is hampered by several factors. The heterogeneity of the clinical presentation, the lack of a standardized methodology for case identification, and ethnic differences are some examples.

1.2.1 Incidence and prevalence

In European states, epidemiological studies are frequently confined to a single state, and due to the different nature of the health systems in place, the data obtained may not be comparable.

A larger registry study in the UK reported an incidence of 4.9 per 100,000 persons-years. [1] The lowest incidence is recorded by a study from Estonia (1.5-1.8 per 100000 person-years) [2], while the highest is found in a study conducted in Crete, Greece (7.9 per 100000 person-years). [3]

The prevalence estimated by European studies varies between 29 [3] and 210 [4] per 100,000 individuals.

In a population-based study conducted in the Veneto region between 2012 and 2020, the incidence amounted to 2.8 per 100,000 person-years. The point prevalence increased from 63.5 to 70.6 per 100,000 inhabitants during the study period. [5]

In females, incidence and prevalence are five and nine times higher respectively, and up to 93% of SLE patients are women [6]. Moreover, in men, the onset occurs generally later (52 years) than in women (45 years), in whom the peak of incidence is between the ages of 30 and 39. [1],[5]

Several American studies have emphasized the higher frequency of SLE in ethnic minorities.

In 2017, data from the Manhattan Lupus Surveillance Project, a population-based registry, confirmed the previously known gender and ethnic disparities. This study underlines how the highest prevalence of SLE affected non-Hispanic black women (221.4 per 100,000 individuals); in second place are Hispanic women and non-Hispanic Asian women (142.7 and 118.5 per 100,000 individuals, respectively). The prevalence of SLE in non-Hispanic white males is considerably lower (6.3 per 100,000 individuals). [7]

1.2.2 Mortality and its determinants

In 2013, the USA age-standardised annual mortality rate (ASMR) was 0.34 per 100,000 individuals. In 1968 the ASMR was 0.45 per 100,000 individuals. [8] This shows a decrease in mortality compared to the past; indeed, 5-year survival was 50% in the 1950s, exceeding 90% in the 1990s. Several factors are responsible for this improvement, and examples are earlier diagnosis and treatment, more prudent use of glucocorticoids (GCs) and immunosuppressive drugs, and finally better management of disease complications.[9] However, SLE is a significant cause of death among young women [10], and in both sexes the mortality rate is still unacceptably high, varying from two to three times compared to that of the general population. [11]–[13]

Black patients compared to Caucasians have a higher mortality risk, mitigated with adjustment for comorbidities and socioeconomic factors. [14] Despite low socioeconomic status, Hispanic patients paradoxically have a lower mortality risk, possibly for lower all-cause mortality. [15]

In addition to ethnicity, other risk factors associated with higher mortality have been identified. Juvenile onset, lupus nephritis [16], damage accrual [13], [17], and

chronic therapy are the main factors [9]. The main causes of death in Europe are cardiovascular diseases and infections, responsible for 27-52% and 15-43% of deaths respectively [11]. Cancer is also a major cause of death.

Mortality rates for cardiovascular disease and infection are higher than in the general population [12], and to a large extent, we can explain this gap with the side effects of drugs.

Chronic therapy with immunosuppressive drugs exposes patients to increased infectious, oncological, and cardiovascular risks. It has also been demonstrated that higher cumulative doses of glucocorticoids correlate with higher mortality, while the introduction of hydroxychloroquine reduces this risk. [9]

1.3 Etiopathogenesis

The exact etiopathogenesis of SLE is unknown but the trend of autoimmune disorders to cluster within families raises the possibility that shared environmental variables and genetics play a key role.[18]

1.3.1 Genetic factors

Over one hundred genes participate in the loss of immunotolerance against self-antigens, leading to pathogenic autoantibodies that cause tissue damage through multiple mechanisms. The multitude of genes involved partly contributes to the clinical heterogeneity of SLE. In addition, an association between specific genetic variants, clinical phenotype, and age of onset has been recognized.

Some molecular mechanisms influenced by the genetic basis of SLE are Toll-Like Receptor(TLR)/type one interferon (IFN-1) signaling, NF-κB signaling, T-Cell and B-Cell signaling and interaction, self-antigen clearance, immunocomplexes (IC) clearance, and DNA repair.[19]

In SLE patients, the mechanisms of apoptosis by extrinsic pathway and NETosis are deregulated, and as a result, there is an accumulation of autoantigens recognizable by the immune system.

The abnormal expression of Fas/Fas Ligand causes increased production of apoptotic bodies which, added to the clearance deficit of the same, increases the pool of autoantigens.[20]

NETosis is a cell death mechanism that plays a role in protecting against microorganisms and controlling inflammation. In SLE patients, its dysfunction contributes to the release of autoantigens.[21]

Alterations in B and T cell signaling added to the presence of many autoantigens results in the formation of autoreactive clones and autoantibodies which are responsible for tissue damage through many mechanisms, including the formation of immune complexes.

Type 1 IFN genes, of which IFN- α is the major subtype, are of current interest. The IFN-α regulator (IRF) is upregulated in SLE, and this pathway appears to play a role in disease induction. [22], [23]

Genes residing on the X chromosome could also contribute to the pathogenesis of the disease owing to the female prevalence and the 14-fold increased risk in Klinefelter (XXY) patients of developing SLE.[24]

Nevertheless, the importance of genetic-environment interaction is clear considering the relatively low concordance between monozygotic twins (11%-57%) [25] and the low penetrance of genetic variants [26].

1.3.2 Environmental factors

Environmental exposure probably induces biological mechanisms such as oxidative stress, systemic inflammation, and hormonal as well as epigenetic modifications leading to the development of the disease in predisposed individuals.

The main environmental factors related to an increased incidence of SLE are cigarette smoking, viruses, and hormonal therapies.

There is a relationship, albeit less strong, with other factors such as pollution and UV light. [27]

- **Cigarette smoke**

  Cigarette smoke generates immunogenic DNA adducts and enhances the expression of CD95 on the surface of B cells and CD4 T cells, potentially causing autoimmunity.[27]

- **Viruses**

  Viruses activate innate immunity, through the TLRs of intracellular plasmacytoid dendritic cells, which produce IFN-I. Type I IFN has an immunostimulatory and amplifying effect, which, in an already hyper-reactive immune system, has the effect of fuel on the fire, promoting the activation of adaptive immunity. Over time, particular attention has been given to Epstein-Barr virus (EBV) whose seropositivity rates are significantly greater in adults and children with SLE compared to age-matched controls.

Possible pathways include molecular mimicry between EBV and SLE antigens, EBV RNA/protein SSB complexes activating IFN-I via the TLR 3 [28], furthermore, plasmacytoid dendritic cells and CD69+CD4+ T cells in SLE patients produce irregular cytokine in response to EBV.[29]

- **Sex hormones**

  Both sex hormones and genes hosted by the X chromosome are responsible for the higher incidence of SLE in females. Furthermore, the disease tends to begin or worsen during pregnancy, therefore pre-conception counseling is indicated to all affected women with pregnancy desire.[27]

- **UV exposure and vitamin D deficiency**

  The role of UV exposure is controversial. We know that photosensitivity is a feature of SLE patients that often precedes diagnosis, and it is known that sun exposure can act as a trigger for disease flare-ups, but the role of UV light as a cause of the disease remains unclear.

  Complicating the picture is vitamin D, whose immunomodulatory effects are well known. Indeed, we know that the main source of Vitamin D is sun exposure and that we frequently find low levels in SLE patients, but it is uncertain whether this is a cause or a consequence of the chronic disease[30].

- **Drug-induced lupus**

  In addition, some drugs can also cause lupus-like clinical features, so careful drug anamnesis is necessary at the time of diagnosis. Drug-induced lupus (DIL) is an autoimmune condition in which exposure to a medication causes the development of symptoms like those of SLE. Since hydralazine was the first substance connected to lupus-like symptoms, more than 100 medications have been linked to DIL. When the agent is discontinued, the symptoms of DIL generally vanish, making it less severe than SLE.[31] Table 1 shows some examples of drugs associated with DIL.

drug class	
antiarrhythmics	**procainamide**, quinidine, disopyramide, propafenone, amiodarone
antihypertensives	**hydralazine**, methyldopa, captopril, acebutolol, enalapril, lisinopril, clonidine, atenolol, labetalol, pindolol, minoxidil, prazosin
antipsychotics	chlorpromazine, lithium
antibiotics	Isoniazid, minocycline, sulfamethoxazole, quinine
anticonvulsants	carbamazepine, phenytoin, ethosuximide, valproic acid
antithyroid	propylthiouracil
diuretics	hydrochlorothiazide
biologic	**TNF-alpha inhibitors**, IFN-alpha
miscellaneous	statins, levodopa

Table 1: *Lupus-inducing drugs; in bold the drugs most frequently associated with DIL*

To summarize, in genetically predisposed patients certain environmental factors like viral infections activate innate immunity which amplifies inflammation by recruiting adaptive immunity. Progressively there will be a loss of self-tolerance leading to the development of autoantibodies targeting nucleic acids associated or not with proteins. ICs can be internalized by plasmacytoid dendritic cells through interaction with the receptor for the Fc fragment of Ig. At this point, after entering endosomes, they too can activate TLRs, leading to the production of additional IFN-I. Thus, a positive feedback mechanism occurs, which allows the maintenance of the autoimmune response and antinuclear antibodies (ANA) production.[23]

Here begins the asymptomatic autoimmunity phase in which autoantibodies can be detected in the blood of clinically silent patients until a second hit occurs. Another infection, UV exposure, and cigarette smoking can trigger the first clinical manifestations.

If not diagnosed early, the disease will tend to self-amplify through the recruitment of other immunological pathways, with progressive resistance to therapy and irreversible tissue damage.

1.4 Clinical manifestations and Serum-immunological alterations

The clinical spectrum of SLE ranges from mild organ involvement to life-threatening conditions. More than 50% of patients at onset have a mild disease characterised by skin, joint and hematological involvement. However, as symptoms are common to other clinical conditions, there is a risk of not suspecting SLE, missing the chance of early diagnosis.[32] During the disease's course, there is often an evolution to severe forms, so it is important to make an early diagnosis and promptly set up the right therapy.[33]

1.4.1 General symptoms

Constitutional symptoms such as fever, anorexia, weight loss, and lymphadenopathy are present in more than 90 % of patients and often characterize the early stages of the disease.

1.4.2 Musculoskeletal manifestations

Musculoskeletal involvement is typical of SLE, involving up to 95% throughout the patient's entire disease course. Migratory arthralgia and non-erosive arthritis are the most common manifestations, but bursitis and tenosynovitis are also frequent.[34] Up to 5-10% have Jaccoud's arthropathy involving periarticular tissues leading to a reducible misalignment of the joint heads that over time results in deformity.[35] In some cases, erosive arthritis is observed until overlapping with rheumatoid arthritis (Ruphus syndrome).[36]

1.4.3 Hematologic manifestations

Leukopenia and lymphopenia are the most frequent hematological alterations. Other alterations are non-hemolytic anemia and non-severe thrombocytopenia. One-third of cases will have a positive Coombs test but only 5% have autoimmune hemolytic anemia, indicating that there are many antibodies in the SLE but not all of them are pathogenic.[37]

1.4.4 Skin manifestations

Cutaneous SLE is the second most frequent manifestation and characterises 25% of patients at disease onset. Skin manifestations are divided into specific and non-specific based on histopathological evidence. Non-specific manifestations include photosensitivity, rheumatoid nodules, vasculitis, urticaria, livedo reticularis, and nonscarring alopecia. The specific manifestations that most aid in diagnosis are acute cutaneous lupus (ACLE), subacute cutaneous lupus (SCLE), and chronic cutaneous lupus (CCLE). Typical of ACLE is the butterfly rash which spares the

nasolabial folds and is always an expression of systemic disease. SCLE is a photosensitive manifestation that occurs in photo-exposed areas and correlates with cigarette smoking and the positivity of anti-Ro/SSA. It has two clinical phenotypes, polycyclic annular and papulosquamous, and in 50% is an expression of systemic disease. CCLE in 90% is an isolated disease and presents with telangiectasias, and extensive hyperkeratosis or discoid lupus. Chronic forms are most frequently associated with permanent outcomes.[38]

1.4.5 Renal manifestations

About 50% of SLE patients have renal involvement although more recent studies report a lower prevalence (20% according to Nikolopoulos et al.[39]), nevertheless, it remains one of the most significant causes of morbidity and mortality. Lupus can involve all structures of the kidney but typically affects the glomerulus. [40]

Lupus nephritis (LN) is classified into six histologic classes based on the microscopic lesions and localization of ICs:

-Class I minimal mesangial

-Class II mesangial proliferative

-Class III focal proliferative

-Class IV diffuse proliferative

-Class V membranous

-Class VI advanced sclerosing. [41]

Renal involvement can clinically manifest itself with several phenotypes. Lupus nephritis may manifest as nephritic or nephrotic syndrome, moreover, rapidly progressive forms or chronic renal failure may be encountered. Asymptomatic urinary abnormalities such as hematuria, proteinuria, leukocyturia, or cell casts are also frequent.

Prognosis is closely related to histologic class, but the latter does not always correlate with a precise clinical phenotype. Thus, a biopsy is necessary when persistent urinary changes or rapid deterioration of renal function is observed.

Histologic diagnosis is essential for adequate treatment and differential diagnosis with rarer renal involvement in the course of SLE .[42] Some examples of renal manifestations are IgA nephropathy, anti-phospholipid antibody-associated nephropathy, thrombotic microangiopathy, drug-induced tubulo-interstitial nephritis, diabetic nephropathy, hypertensive nephroangiosclerosis, and podocytopathy.[43]

1.4.6 Neuropsychiatric systemic lupus erythematosus

Neuropsychiatric systemic lupus erythematosus (NPSLE) involves 50% of patients with manifestations ranging from headache to life-threatening conditions. In 1999, the ACR described 19 possible syndromes and recommendations for laboratory testing and imaging. [44] Epilepsy and cerebrovascular disease are the most frequent manifestations (cumulative incidence >5%). Acute confusional state, severe cognitive dysfunction, major depression, and peripheral neuropathy are relatively uncommon (1-5%), and finally, we rarely encounter psychosis, cranial nerve neuropathy, myelitis, or aseptic meningitis.[45] Focal manifestations such as cerebrovascular accidents and epilepsy are often associated with vasculopathy and antiphospholipid antibodies.

However, other autoantibodies are also responsible for diffuse manifestations bypassing the blood-brain barrier and inducing general neuronal damage.[46]

1.4.7 Cardiovascular manifestations

Pericarditis characterises up to 30% of SLE patients. Acceleration of atherosclerosis is one of the most significant co-morbidities of SLE, and cardiovascular events are more frequent and earlier than in the general population. Coronary artery thrombosis is also possible in patients with antiphospholipid antibodies, whereas coronary artery vasculitis is anecdotal.

Myocarditis rarely occurs, but prompt treatment is necessary to avoid chronic consequences on pump function. [47]

In approximately 4% of cases, we may encounter Libman-Sacks endocarditis, a non-bacterial endocarditis that is associated with antiphospholipid antibody positivity, which might be complicated by thromboembolic events.[48], [49]

1.4.8 Pneumological manifestations

In the respiratory system, serositis is the most common manifestation. The lung may be affected by acute interstitial pneumonia and less frequently by chronic interstitial pneumonia, diffuse alveolar hemorrhage, or organising pneumonia. Other presentations include bronchiolitis obliterans, pulmonary hypertension, and shrinking lung syndrome. [47]

1.4.9 Gastrointestinal manifestations

The gastrointestinal tract can also be involved in all its districts. Etiologically, we identify mesenteric vasculitis, thrombosis, and iatrogenic causes. Proton pump inhibitor therapy is recommended in SLE patients who use nonsteroidal anti-inflammatory drugs (NSAID) and glucocorticoids for long term.[50]

Many of the previous clinical features are also common in the general population so there is a risk of over-attributing manifestations to SLE.

Regarding NPSLE, the absence of sense biomarkers complicates the attribution of neuropsychiatric manifestation (NP) to SLE. Thus, the expert clinician's judgment remains paramount; it is also important to approach the diagnosis based on the most epidemiologically frequent causes as in patients without SLE with the help of allocation criteria such as the Italian algorithm for attributing NP events to SLE.[45], [51]

According to the 2019 EULAR recommendations, the attribution of NP manifestations to SLE can be facilitated by consideration of type and timing of the manifestation in relation to lupus onset and other risk factors such as age, non-NP lupus activity, and presence of aPL. Neuroimaging, cerebrospinal fluid analysis, and exclusion of confounding factors are also helpful.[52]

1.4.10 Serum-immunological alterations

Serum-immunological alterations help in differential diagnosis.

ANA positivity and consumption of complement factors (C3, C4, and C1q) are common in SLE patients.

Specific autoantibodies are anti-native-DNA and anti-Sm.

Anti-Sm antibodies have a low sensitivity (20-30%) but their presence is pathognomonic of SLE.[53]

Anti-Ro/SSA autoantibodies are common to many autoimmune rheumatologic diseases and responsible for neonatal lupus erythematosus (NLE). [54]

Anti-U1RNP autoantibodies characterise mixed connective tissue disease (MCTD) [55], and anti-P-ribosomal protein are associated with neurological manifestations. [46]

Quite important are antiphospholipid antibodies, which are present in up to 30% of patients, and in 10-15% of cases these autoantibodies are responsible for life-threatening thrombotic and obstetric complications.[56]

1.5 Diagnosis and classification criteria

In rheumatologic diseases, and especially in SLE, establishing diagnostic criteria is a challenge due to the extreme clinical heterogeneity that hinders reaching all affected patients.

Classifying criteria differ from diagnostic criteria in purpose. Classifying mainly serves to identify those patients with the main features of the disease with the intent of obtaining homogeneous cohorts for clinical research. Otherwise, the scope of diagnosis is to reach every single patient.[57]

Accordingly, classification criteria can only assist, and the diagnostic process may be challenging and requires a thorough knowledge of the clinical manifestations, which may not all be present at the same time. Thus, an adequate remote pathological history is necessary to reconstruct the whole picture and the serum-immunologic changes mentioned above ground the diagnostic suspicion. [58]

SLE-mimicking like other rheumatologic diseases, infections, and hematologic conditions need to be excluded by other tests.[32]

Different classification criteria have followed over time intending to maximize sensitivity and specificity but never succeeded in zeroing out false positives and false negatives. Classification criteria should be applied critically and with the awareness that they are more useful in identifying a florid condition rather than an onset of disease, although newer criteria allow for earlier classification. [59]

1.5.1 The 1982 revised criteria for the classification of SLE

The 1982 revised criteria for the classification of SLE are used for recruiting the patients of this study. Compared with the previous criteria dating back to 1971, changes were made to increase sensitivity and specificity both to 96%.

The ANA immunofluorescence assay (FANA) has a high sensitivity but low specificity (51%) being useful as an entry criterion to the application of the other classificatory criteria. The single clinical manifestations have been merged into an organ system approach with the aim of simplification without loss of accuracy, except for mucocutaneous involvement where the different manifestations remain separate. The immunologic disorders criterion includes positivity to anti-DNA and

anti-Smith autoantibodies, lupus erythematosus (LE) cell assay, and false positivity to a serologic test for syphilis. Skin or kidney biopsies were removed from the criteria because they are rarely performed.

Raynaud's phenomenon and alopecia were also removed. 4 positive criteria are required to classify a patient with SLE.[60]

1.5.2 2019 EULAR/ACR classification criteria for SLE

The latest revision of the criteria was stipulated in 2019 jointly by the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR), and the general principle to be declined is that in every criterion there should be no better explanation. ANA positivity at least once remains as an entry criterion. The subsequent criteria are grouped into seven clinical domains (constitutional, haematological, neuropsychiatric, mucocutaneous, serous, musculoskeletal, renal) and three immunological (antiphospholipid antibodies, complement proteins, SLE-specific antibodies). Each criterion is weighted from 2 to 10, and patients accumulating more than 10 points are classified. Compared with the 1997 ACR criteria, specificity remains the same, but sensitivity has increased. The importance of not using classificatory criteria as diagnostic criteria is reiterated to avoid the risk of excluding early or latent disease from treatment.[53]

1.6 Disease Course and Clinimetry

In the natural history of SLE, there is usually an alternation from periods of active disease to periods of remission, lasting variable times.

It is important to have a measure of acute disease activity at each follow-up, distinguishing chronic damage and excluding confounding factors such as drug side effects, infections, and other comorbidities. Indeed, this optimizes management, guiding therapy by evaluating its effectiveness.

Several scores are available to measure both global and organ-specific disease activity, some of which are schematized in Table 2.

Global activity indices	Organ specific activity indices
Physician Global Assessment (PGA)	Cutaneous Lupus Erythematous Disease Area and Severity Index (CLASI)
Systemic Lupus Erythematosus Disease Activity Index-2000 (SLEDAI-2K)	British Isles Lupus Assessment Group score (BILAG)
Systemic Lupus Erythematosus Disease Activity Score (SLE-DAS)	Disease Activity Score using 28 joints counts (DAS28)

Table 2: *indices of SLE disease activity.*

Although several SLE activity indices have been validated, there is no consensus on which is more appropriate. The most widely used in clinical practice is SLEDAI-2K.[61]

1.6.1 SLEDAI-2K

The SLE disease activity index 2000 (SLEDAI-2K) considers the different acute clinical and serologic manifestations of SLE by assigning a weight to each and thus providing a disease activity score. (Table 3)[62]

Items	Weight
Seizure	8
Psychosis	8
Organic brain syndrome	8
Visual disturbance	8
Cranial nerve disorder	8
Lupus headache	8
Cerebrovascular accidents	8
Vasculitis	8
Arthritis (>2 joints)	4
Myositis	4
Urinary Casts	4
Hematuria (>5 RBC/HPF)	4
Proteinuria (0,5 G/24H)	4
Pyuria (>5 WBC/HPF)	4
Rash	2
Alopecia	2
Mucosal ulcers	2
Pleurisy	2
Pericarditis	2
Low complement	2
High DNA binding	2
Fever	1
Thrombocytopenia	1
Leukopenia	1

Table 3 *Items and weight of SLEDAI-2K*

Clinical remission can be defined by a clinical SLEDAI-2K (c-SLEDAI-2K) of 0, excluding anti-DNA positivity and complement reduction from the score count.

This score has, however, several limitations to consider during its clinical application. For example, the binomial rating of each variable (present or absent) does not allow for consideration of improvement or worsening of that variable.

In addition, the SLEDAI-2K omits some severe disease manifestations such as hemolytic anemia, gastrointestinal involvement, and pulmonary manifestations other than pneumonia.

1.6.2 Other indices of activity

The limitations of the SLEDAI-2K are partly overcome by the other scores.

In the SLE disease activity score (SLE-DAS), some variables are considered continuous (arthritis, proteinuria, leukopenia, and thrombocytopenia) and two levels of severity are included for vasculitis (systemic or mucocutaneous) and skin involvement (generalized or localized), making it more sensitive to change than the SLEDAI-2K. In addition, some items are added such as hemolytic anemia, cardiac and pulmonary involvement other than serositis, and gastrointestinal manifestations are implied in systemic vasculitis.[63]

The British Isles Lupus Assessment Group (BILAG) score also overcomes the binomial problem. This score that assigns a letter from A to E to each of the 9 systems (general, mucocutaneous, neurologic, musculoskeletal, cardio-respiratory, ocular, renal, haematology, gastrointestinal). The letter A represents the highest degree of severity of a manifestation. The BILAG also correlates better than the SLEDAI with clinical response to a new counter therapy however, the application of this score is much more complex than the others.[64] The easy-BILAG of Carter LM et al. balances the simplicity of the SLEDAI-2K in detecting the most frequent acute clinical manifestations with the sensitivity to change of the BILAG-2004. In parallel, it is time-saving and shows greater inter-rater agreement in each level of disease activity (A-C). [65]

The Physician Global Assessment (PGA) assigns a number from 0 to 3 to the overall severity of the disease according to the clinician's judgment, therein lies its weakness that reduces its inter-rater reliability.[64]

Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) is a skin-specific index of both activity (CLASIa) and damage (CLASId). CLASIa considers erythema, scaling/hypertrophy, mucosal involvement, and hair loss.

CLASId considers chronic lesions such as dyspigmentation, scarring/atrophy, and panniculitis.[66]

The Disease activity score-28 (DAS28) originated for Rheumatoid Arthritis and then was also validated for the evaluation of joint involvement in SLE.[67]

1.6.3 Disease course patterns of SLE

According to Tselios et al., four possible disease patterns are described.

Prolonged remission (PR) is defined in those patients in whom the c-SLEDAI remains zero for 10 consecutive years.

The relapsing-remitting (RR) pattern describes those patients who experience at least 2 periods of remission, each maintained for not less than two consecutive follow-ups.

Persistently active (PA) patients have never experienced a period of remission.

To conclude, Hybrids are those patients who have had a single period of remission in 10 years.

Approximately 70% of SLE patients follow an RR-type disease pattern while the remaining 30% are equally distributed among the remaining three patterns.[68]

1.6.4 SLICC-Damage Index

The different patterns of disease course differ in damage accrual,
which can be measured by the Systemic Lupus International Collaborative Clinics/American College of Rheumatology (SLICC/ACR) Damage Index (SDI).

Damage is defined as a non-reversible change established after the disease's onset. The damage must be ascertained by clinical evaluation, must not be explainable by an acute manifestation of disease, and must last for more than 6 months. Repeated episodes can be counted only if separated by 6 months so as not to risk overestimating the accumulated damage.

SDI consists of 12 categories listed in Table 4, and the maximum achievable score is 46 points.

The SDI correlates with morbidity and mortality. Specifically, renal damage score (DS) at 1 year after diagnosis correlates with an increased risk of developing end-stage renal failure, whereas pulmonary DS at 1 year after diagnosis correlates with an increased mortality within 10 years after diagnosis. Afro-Caribbeans and Asians tend to have higher DS than Caucasians. In particular, Afro-Caribbeans have higher renal DS and Asians have higher neuropsychiatric DS.[69]

Categories	Items
Ocular	Cataract retinal change OR optic atrophy
Neuropsychiatric	Cognitive impairment OR major psychosis seizures requiring therapy ≥6 months Cerebral vascular accident OR resection not for malignancy Cranial or peripheral neuropathy Transverse myelitis
Renal	Estimated or measured GFR <50% Proteinuria >3.5g/24h ESRF (regardless of dialysis or transplantation)
Pulmonary	Pulmonary hypertension, Pulmonary fibrosis, Shrinking lung, Pleural fibrosis, Pulmonary infarction OR resection not for malignancy
Cardiovascular	Angina OR Coronary artery bypass Myocardial infarction, Cardiomyopathy (ventricular dysfunction) Valvular disease Pericarditis OR Pericardiectomy
Peripheral vascular	Claudication, Minor tissue loss (pulp space) Significant tissue loss ever Venous thrombosis with swelling, ulceration OR venous stasis
Gastrointestinal	Infarction or resection of bowel below duodenum, spleen, liver or gall bladder ever Mesenteric insufficiency, Chronic peritonitis Stricture OR Upper gastrointestinal tract surgery Pancreatic insufficiency requiring enzyme replacement OR with pseudocyst
Musculoskeletal	Muscle atrophy OR weakness Deforming or erosive arthritis (including reducible deformities) Osteoporosis with fracture or vertebral collapse Avascular necrosis, Osteomyelitis
Skin	Scarring chronic alopecia Extensive scarring of panniculum other than scalp and pulp space Skin ulceration
Gonadal	Premature gonadal failure
Endocrine	Diabetes requiring therapy, regardless of treatment
Malignancy	Exclude dysplasia

Table 4*: Items of Systemic Lupus International Collaborative Clinics/American College of Rheumatology (SLICC/ACR) Damage Index (SDI)*

Differences in accumulated damage and comorbidity were observed among different disease course patterns. After 2 years of disease the differences are not significant, whereas at 10 years the PA pattern differs by higher cumulative glucocorticoid dose and higher SDI, the latter related to disease activity, especially renal, cardiac, and cutaneous.

After 10 years, osteoporosis, osteonecrosis, and atherosclerotic cardiovascular events are more frequent in PA and RR patterns than in PR type.

Some predictors of PA patterns, and thus worse outcomes, are black ethnicity, greater disease activity at onset, poor adherence to therapy, and skin and musculoskeletal involvement.[68]

According to Zen et al. other factors linked to chronic active disease are: anti-dsDNA positivity, complement consumption, and a long period between onset and diagnosis without therapy, which emphasizes the importance of early diagnosis.[70]

1.6.5 Remission and low disease activity

Several studies have revealed a significant improvement in outcomes given by the treat-to-target approaches. Treating the patient with well-characterized goals of remission or low disease activity correlates with a lower risk of relapse and damage accumulation.[71]–[73]. The international community has widely embraced two recently specified goals: Definitions Of Remission In SLE (DORIS) and Lupus Low Disease Activity State (LLDAS).

According to Franklyn et al., the definition of LLDAS involves clinical activity on the one hand and therapy required to maintain low disease activity on the other.

Low clinical activity requires SLEDAI-2K less or equal to 4 excluding major organ involvement and new clinical manifestations.

Gastrointestinal involvement and hemolytic anemia are also not compatible with LLDAS.

To conclude the clinical aspect, it is necessary to have a SELENA-SLEDAI Physician Global Assessment (PGA) less or equal to 1.

The effects of high doses of glucocorticoids on morbidity are known, so only a prednisone dose (or equivalent) $\leq$7.5 mg per day is accepted in the definition of LLDAS. In addition, the intake of any immunosuppressive agent or biologic must be in a stable dose and without significant intolerance. Indeed, a clinical goal in

which drug intolerance phenomena coexist is undesirable.[72] Table 5 summarises all the criteria needed to define LLDAS.

Two years was the shortest duration of LLDAS associated with a better outcome in terms of damage progression in a Caucasian cohort followed for seven years.[73]

LLDAS definition
1. SLEDAI-2K ≤4, with no activity in major organ systems (renal, CNS, cardiopulmonary, vasculitis, fever) and no haemolytic anaemia or gastrointestinal activity
2. No new features of lupus disease activity compared with the previous assessment
3. SELENA-SLEDAI physician global assessment (PGA, scale 0–3) ≤1
4. Current prednisolone (or equivalent) dose ≤7.5 mg daily
5. Well tolerated standard maintenance doses of immunosuppressive drugs and approved biological agents, excluding investigational drugs

Table 5: *criteria needed to define Lupus Low Disease Activity State (LLDAS)*

Numerous definitions of remission are available, making clinical studies not comparable. The DORIS 2021, developed by international specialists, is recommended in all areas, from clinical practice to research. As in the definition of LLDAS, DORIS also considers clinical and therapeutic aspects. Regarding disease activity the clinical-SLEDAI must be equal to 0 and the PGA <0.5. Concerning therapy, the dose of prednisone (or equivalent) must be less than or equal to 5 mg/day, while the antimalarial, immunosuppressive drug, or biologic must be administered in a stable dose. These criteria are outlined in Table 6 [74], [75]

DORIS
1. clinical- SLEDAI-2K =0
2. SELENA-SLEDAI physician global assessment (PGA, scale 0–3) ≤0,5
3. Current prednisolone (or equivalent) dose ≤5 mg daily
4. Well tolerated standard maintenance doses of immunosuppressive drugs and approved biological agents, excluding investigational drugs

Table 6 *criteria needed to define Definitions Of Remission In SLE (DORIS)*

1.7 Treatment and management

Careful clinical and serum-immunological follow-up is essential in the management of the SLE patient to recognize flares early and treat them appropriately.

As mentioned above, the goals of SLE patient management are encapsulated in the treat-to-target approach which namely consists of three different steps. The first step has remission as the main goal and when this is not achievable LLDAS will be targeted. The second step involves steroid reduction, which in the case of sustained clinical remission may be discontinued. The third and final step is similar to the second but involves the reduction and discontinuation of the immunosuppressive agents.

1.7.1 EULAR recommendations of SLE management

The EULAR 2019 recommendations for the treatment of non-renal disease manifestations [52] categorise patients based on clinical severity.

Three classes are defined: mild, moderate, and severe, which characteristics are shown in Table 7.

MILD	Constitutional symptoms, mild arthritis, rash<9%, PLTs 50-100x 10^3/mm^3 SLEDAI≤6, BILAG C or ≤1 BILAG B manifestations
MODERATE	RA-like arthritis, rash 9-18%, cutaneous vasculitis≤18%, PLTs 20-50x 10^3/mm^3 Serositis SLEDAI 7-12, BILAG B manifestations
SEVERE	Major organ-threatening disease, PLTs < 20 x 10^3/mm^3, TTP-like disease, acute hemophagocytic syndrome SLEDAI >12, ≥1 BILAG A manifestations

***Table** 7 The three classes of severity driving the therapy*

1.7.1.1 Pharmacological therapy

Pharmacological therapy of SLE consists of several levels according to the severity of clinical manifestations. Standard therapy involves hydroxychloroquine and glucocorticoids associated or not with immunosuppressants.

Hydroxychloroquine (HCQ) should be administered from onset in all SLE patients unless contraindicated. The 2016 American Academy of Ophthalmology (AAO) guidelines recommend a dose ≤ 5 mg/kg to minimize the risk of retinopathy. They also recommend baseline ophthalmologic screening and then an annual screening after the fifth year of therapy. If risk factors are present, periodic screening should be early and more frequent. In patients with skin manifestations and HCQ retinal toxicity, quinacrine may be opted as an alternative antimalarial.

Glucocorticoids (GCs) therapy should be calibrated based on the severity of organ involvement. For life- or organ-threatening conditions, intravenous pulses of methylprednisolone with a dosage of 250-1000 mg/day and for 1-3 days are recommended. Pulses not only allow for a rapid therapeutic effect but also enable starting with a lower oral dose and faster tapering of GCs. GCs therapy in chronic maintenance should be ≤ 7.5 mg/day of prednisone or equivalent and when possible, implement discontinuation. Reduction and withdrawal of GCs are also facilitated by early treatment with immunosuppressive agents.[9]

Immunosuppressive/immunomodulatory (IS) agents are indicated in those patients not responsive to HCQ in monotherapy or associated with GCs. Even in high steroid threshold patients where GCs cannot be reduced below 7.5 mg/day, the addition of

IS is indicated. Organ-threatening conditions can be managed in first-line therapy with the addition of IS.

Type of manifestation, age, and pregnancy are some elements to consider when choosing IS.

In mild manifestations unresponsive to the combination of HCS and GCs, and as the first line in moderate manifestations, methotrexate (MTX) and azathioprine (AZA) are recommended. Mycophenolate mofetil (MMF) is a potent IS with proven superiority to AZA in inducing remission and reducing flares. However, considering its teratogenicity and higher costs compared with AZA and MTX, there are limitations to universal recommendations in women of childbearing age with non-renal manifestations.

Cyclophosphamide is also a potent IS but is associated with infertility, as well as increased infectious and oncologic risk. Therefore, it can only be used as rescue therapy in refractory non-major organ manifestations, but mostly it is used for life or organ-threatening conditions (especially renal, cardiopulmonary, and neuropsychiatric).

Most of the biological drugs currently in use for SLE target B cells. [76]

Belimumab is a human monoclonal antibody that targets BLyS, a critical molecule for peripheral B cell growth and survival. BLyS levels in the blood correlate with higher title of anti-dsDNA and disease activity.

The recommendations indicate the use of belimumab as add-on therapy in extra-renal as well as in renal manifestations, when, despite the combination of HCQ and GCs with or without IS, the disease flares up or when GCs cannot be reduced below the desired threshold.[52]

A real-life multicenter cohort study has demonstrated increased benefits from belimumab use in the presence of low accumulated damage. [77] In addition, major improvements are observed in musculoskeletal, mucocutaneous, and serological manifestations.[78]

Rituximab is a biological drug used in severe manifestations refractory to immunosuppressants and belimumab or when these are contraindicated. Namely, RTX can be used after the failure of more than one IS in the control of renal and nonrenal manifestations (especially serologic and neuropsychiatric). In severe

autoimmune thrombocytopenia and hemolytic anemia, RTX can be used as first-line therapy. Use is off-label given the failure of randomized, controlled trials.[52]

The EULAR 2019 treatment recommendations for specific clinical manifestations are summarised in Table 8

System	Recommendations
Skin disease	First line: - topical agents (GC, calcineurin inhibitors) - antimalarials (HCQ, quinacrine) - and/or systemic GC In non-responders or those requiring high doses of GC add: - MTX, retinoids, dapsone or MMF
Neuropsychiatric disease	Inflammatory manifestations: - GCs and ISs Atherothrombotic/aPL-related manifestations: - antiplatelets/anticoagulants
Thrombocytopenia	Acute: - high-dose GC (including pulses) and/or intravenous immunoglobulins G Refractory cases: - Rituximab or cyclophosphamide Maintenance therapy: - MMF, AZA, cyclosporine (GC-sparing)
Renal disease	Induction: - MMF or low/high dose of Cyclophosphamide based on the risk of renal failure (GFR and histological features) Maintenance therapy: - MMF or AZA

Table 8: EULAR 2019 guidelines for the treatment of specific clinical conditions

1.7.1.2 Prevention of relapse and comorbidities

Equally important is the prevention of disease recurrence. The effects of smoking and sun exposure on disease activity are well known. Therefore, it will be necessary to educate the patient about adequate UV protection and the absolute necessity of not smoking. [52]

Cardiovascular events are more frequent and early in patients with SLE. An interaction between traditional and disease-related cardiovascular risk factors is implicated in the pathogenesis of cardiovascular disease. Traditional ones are hypertension, hyperlipidemia, smoking, diabetes mellitus, obesity, and a family history of early coronary disease.

Hypertension is frequently observed in SLE patients with renal involvement, and generally in GCs therapies. The latter is also associated with the onset of diabetes.[79]

Therefore, cardiovascular risk reduction based on control of blood pressure, weight, blood glucose, and lipid profile is essential.[16], [47]

The presence and title of antiphospholipid antibodies (aPL) are associated with thrombotic and obstetric complications, as well as an increased risk of damage accumulation.
Treatment follows the indications for primary antiphospholipid antibody syndrome (APS) as there are no specific studies on secondary APS.
Low dosages of aspirin seem to be protective in primary prevention but considering the bleeding risk, it remains unclear whether patients with higher risk such as triple positivity should be selected.
Additional anticoagulant treatment might be useful during periods of increased thrombotic risk (pregnancy or postoperative).
New oral anticoagulants should be avoided considering the link to excess thromboembolic events compared with warfarin observed in an open-label randomized trial.[52]

Infectious diseases also occupy the podium of causes of death in SLE patients. Among the clinical manifestations, we mentioned lymphopenia and hypocomplementemia, factors that together with immunosuppressive therapy work in concert significantly increasing the infectious risk. [12], [37]
EULAR guidelines strongly encourage vaccination for influenza virus, pneumococcus, and herpes zoster. Infectious diseases can sometimes mimic a relapse of SLE so a careful differential diagnosis must be conducted to avoid potentially dangerous therapeutic errors.[80]

Pregnancy in SLE patients can cause relapse resulting in major obstetric morbidity. Appropriate counseling is needed to determine the risk of maternal and fetal complications. Pregnancy planning is also necessary to prioritise periods of disease control and allow therapeutic switching from potentially teratogenic drugs (MTX, MMF, CYC) to pregnancy-compatible drugs (AZA).[40], [81], [82]

SLE is also associated with lower bone mineral density (BMD) and an enhanced risk of fractures, especially asymptomatic vertebral fractures.

Disease-specific predisposing factors include chronic arthritis, reduced physical activity, induction of cytokines that promote bone resorption, renal failure, and endocrine factors.

In addition, low Vitamin D levels due to photoprotection, and medications used (GCs, immunosuppressants, and chronic anticoagulants) participate in the etiopathogenesis. Glucocorticoid-induced osteoporosis is the prevalent finding in SLE patients. [83]

Therefore, it is critical to minimize the dose and duration of exposure to GCs, it is necessary to combine adequate calcium intake, and to maintain blood vitamin D in the range. In cases of osteopenia associated with spontaneous vertebral fracture, it is indicated to undertake treatment for osteoporosis.[84]

2 Hydroxychloroquine

2.1 Mechanism of action

Hydroxychloroquine (HCQ) is a basic, lipophilic drug and as such crosses the plasma membrane, accumulates in lysosomes, and raises their pH. Alkalinization interferes with lysosomal function by damaging essential cellular mechanisms. Indeed, examples of lysosomal functions are receptor recycling, plasma membrane repair, cell signaling, and energy metabolism.

To fully understand the mechanisms through which HCQ acts is usefull to briefly recap the pathogenesis of SLE.

Some features of the complex etiopathogenesis of SLE include accelerated cell death also induced by increased interferon production, which is not balanced by adequate clearance of cellular debris. High exposure to autoantigens activates Toll-Like Receptors (particularly TLR7 and TLR9) leading to the maturation of dendritic cells (DCs). Then, T and B lymphocytes are recruited from which autoantibody production will occur.

HCQ-mediated alkalinization is responsible for the inhibition of some steps listed above. TLR7 and TLR9 are located in the endosomal membrane, and acidification of the endosomal lumen is required for ligand uptake. HCQ also can bind nucleic acids by masking them to TLRs.

Autoantigen must be digested to bind the major histocompatibility complex (MHC) and then be presented to T lymphocytes. Alkalinization disfavors the processing of the autoantigen and thus its presentation to adaptive immunity.

A crucial step in the activation of innate and acquired immunity is protein processing via autophagy mechanisms, which HCQ can hinder by constraining the acidification of the lysosomal compartment. Rand et al. demonstrated that HCQ can inhibit the binding between the antiphospholipid- β2-glycoprotein I antibody complex and phospholipid bilayer, reducing the risk of thrombosis in APS.[85]

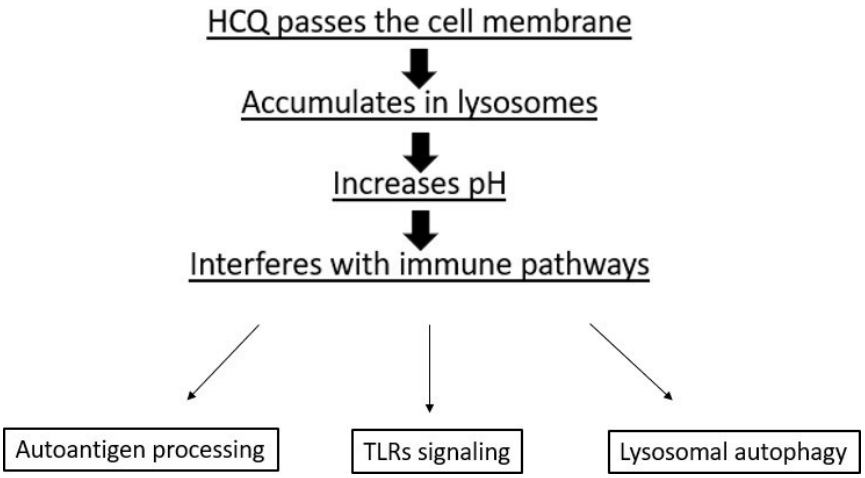


***Figura 1**: HCQ mechanism of action in SLE*

2.2 Efficacy in SLE

It has been more than 50 years since the first publication on the use of HCQ in SLE [86] and nowadays the antimalarials (AMs) may be considered anchor drugs in SLE. In this regard, we have already mentioned how the EULAR recommendations for SLE management broadly support the use of HCQ for the treatment of both renal and extra-renal manifestations, unless contraindicated.[52], [87]

2.2.1 HCQ and SLE outcomes

AMs have been associated with several aspects of a better outcome such as improved survival[88], [89], protection against disease flares[90], [91], and hindrance of damage accrual.

In particular, many positive effects have been described regarding renal involvement. When HCQ introduction precedes renal manifestation, the risk of renal failure and death is lower.[92] In addition, HCQ increases the probability of

renal remission [93]and reduces the risk of renal flare when plasma levels are 0.6 mg/L[94], compared with immunosuppressant use as a single treatment.

2.2.2 HCQ against SLE comorbidities

The effects of HCQ are not confined to controlling disease activity but the data also show efficacy on comorbidities, which weigh heavily on morbidity and mortality. The increased cardiovascular risk that contradistinguishes SLE patients has already been widely discussed. In several studies HCQ has demonstrated its antiplatelet and antithrombotic role, in part by reducing the title of antiphospholipid antibodies, and without repercussion on bleeding time.[95]

HCQ counteracts accelerated atherosclerosis in SLE patients, through mechanisms partly unknown and partly as a probable consequence of anti-inflammatory and anti-thrombotic effects. Other antiatherogenic effects described are favorable modulation of lipid and blood glucose profiles, as well as endothelial stabilization. [96]

A prospective study by Clowse et al. evaluated obstetric morbidity and disease activity during pregnancy in 257 women. Disease activity was significantly higher in women who had discontinued HCQ emphasizing the need for continued HCQ therapy during pregnancy. The same study showed a lower average daily dose of prednisone in the group of women who continued to take HCQ during pregnancy compared with those who had discontinued. [97] This finding is in line with what has been observed in other studies.[98]

HCQ passes the placental barrier reaching concentrations in fetal blood equal to maternal concentrations. Nevertheless, several studies have demonstrated the drug's safety in pregnancy, and a reduced risk of cardiac manifestations of neonatal lupus associated with anti-SSA/Ro antibodies, has also been observed.[99]

The American College of Rheumatology (ACR) 2020 guideline for reproductive health management in rheumatic and musculoskeletal diseases recommends the HCQ use during pregnancy and lactation, especially in cases of anti-Ro/SS-A and anti-La/SS-B antibody positivity, as well as in SLE women with refractory obstetric APS. [100]

Despite the benefits listed, physicians and patients often consider reducing or discontinuing HCQ, and reasons leading to these choices may include:

(a) retinal toxicity from cumulative exposure

(b) clinical remission of the disease

(d) intolerance, changes in skin pigmentation, or other adverse effects

(e) other reasons (e.g., health care access problems, drug insurance problems, nonadherence).

2.3 Safety

HCQ use is considered safe. Adverse events are mostly dose-dependent, and other factors that may act synergistically are concomitant therapies and associated comorbidities.[101], [102]

Ruiz et al. conducted a systematic review of the English literature between 1982 and 2007 using MEDLINE and EMBASE to delineate the benefits and risks of using AMs. All studies (two retrospective, one prospective, and one RCT) agreed on the low prevalence of AMs toxicity, significantly more frequent in the use of chloroquine (CQ) than HCQ (total side effects 28.4% vs 14.7%, $p<0.001$[92]). The main toxicities involved the skin and the gastrointestinal tract, and in both cases were mild.

Digestive intolerance can occur from the first day of intake and is the most frequently observed early effect. An example of long-term damage that is not uncommon is HCQ skin hyperpigmentation, promoted by bruising, antiplatelet drugs, and anticoagulants. [101]

The most clinically relevant toxicity is retinopathy, which will be discussed in a dedicated chapter (2.3.1). Neuropsychiatric involvement is more typical in the elderly, and it is not easy to attribute the clinical manifestation to HCQ toxicity because several confounding factors coexist (i.e. other drugs, alcohol intake, disease activity).[102]

Although rare, the main cardiac side effects encountered were conduction disturbances (85%), followed by heart failure (26.8%). Only 44% recovered normal cardiac function after discontinuation of the antimalarial drug.[103]

To conclude, the following manifestations have been described only in case-reports:

- severe liver failure[104]
- erythema multiforme[105]

- erythroderma [106]
- dark skin rash [107]
- grey skin [108]
- podocytopathy mimicking Fabry disease [109]

All side effects are summarized in Table 9 and Table 10.

System	Adverse effect Short term	 Long term	References
Digestive intolerance	Nausea, vomiting, diarrhea, bloating		Ruiz-Irastorza et al.[89]
Dermatologic	pruritus, urticaria, rashes, exanthematous pustulosis, toxic epidermal necrolysis, Stevens-Johnson syndrome	hyperpigmentation	Ruiz-Irastorza et al.[89]
Hematological	Bone marrow toxicity, cytopenia	Bone marrow toxicity, cytopenia	Fiehn et al. [90]
Metabolic	hypoglycemia		Ruiz-Irastorza et al.[89]
Cardiovascular	Prolonged QT Overdose: cardiovascular shock, collapse	Conduction troubles, cardiomyopathy, vacuolar myopathy, valvular disorders	Ruiz-Irastorza et al.[89]

***Table 9**: Rare adverse effects of antimalarials*

System	Adverse effect Short term	Long term	References
Neuropsychiatric	confusion, disorientation, hallucination Overdose: psychosis, seizure	agitation, bradyphrenia, delirium, disorientation, drowsiness, confusion, pseudo-parkinsonism	Fiehn et al. [90]
Neuromuscular	increase of creatine kinase	myositis, muscle weakness	Fiehn et al. [90]
Ophthalmologic	eye accommodation troubles	retinopathy (maculopathy)	Marmor et al. [99]
Otorhinolaryngology	ototoxicity, tinnitus		Fiehn et al. [90]
Only case reports	Fulminant hepatic failure; erythema multiforme; erythroderma; dark rash, gray skin; podocytopathy mimicking Fabry disease;		Makin et al. [91]; Koumaki et al. [92]; Pai et al. [93]; Pelechas et al. [94]; Ivo et al. [95]; Serre et al. [99]

***Table 10**: Rare adverse effects of antimalarials*

2.3.1 HCQ retinal toxicity

2.3.1.1 Definition and epidemiology

HCQ retinopathy is the main concern of prolonged drug exposure. It consists of irreversible damage to the pigmented epithelial cells of the retina that results in loss of vision. Thus, it is necessary to detect and manage the risk early before the damage manifests clinically.

The most recent studies, using highly sensitive techniques for early detection of retinal damage, have shown an increased prevalence of retinal toxicity from AMs. A recent prospective study by Petri et al, used sensitive screening tests, such as optical coherence tomography (OCT), multifocal electroretinogram (mf ERG), and fundus autofluorescence, found an overall prevalence of confirmed HCQ ocular toxicity of 4.3%.

In the same study, the risk of retinopathy within 5 years was estimated to be 1%, and after 21 years of use this risk rises to 8%.[110]

2.3.1.2 Etiopathology and risk factors

According to the hypothesis, hydroxychloroquine harms the retina by preventing autophagy in retinal pigment epithelium (RPE) cells.

HCQ increases lysosomal pH in RPE cells, consequently, a fusion between autophagosomes and lysosomes and thus autophagy is prevented. Interference with the autophagy process seems to result in the accumulation of lipofuscin in RPE cells which leads to the degradation of photoreceptors.

The activity of organic anion transport polypeptide 1A2 (OATP1A2) is also hindered by HCQ. Thus, an important step in the visual cycle, namely, all-trans-retinol recycling in RPE cells, is disrupted.[111] Different patterns of retinopathy can be identified, but mainly toxic damage is at the parafoveal area. In Asians, the extramacular site is equally typical so if the area of the retina analyzed in the screening is not ample, this type of damage can be missed.[112]

Several factors are associated with AMs retinal toxicity. Some of them are pre-existing retinal or macular disease, chronic kidney disease, tamoxifen exposure, age, and Body Mass Index (BMI). [110]

Combined use of HCQ and tamoxifen for more than 6 months is not recommended as both inhibit lysosomal enzymes increasing HCQ-mediated retinal toxicity. [113]

Ethnicity may also play a role. Indeed, a higher risk is observed in sub-Saharan African and East Indian patients. However, the most determining and major factor is summarized in dose (mg/kg) and duration of treatment. [112]

High blood levels of HCQ appear to predict future retinal damage, suggesting the usefulness of monitoring to adjust the dose of HCQ and increase the frequency of ophthalmic follow-up in those patients with higher blood levels of HCQ.[110]

2.3.1.3 American Academy of Ophthalmology (AAO) guidelines

Guidelines have been updated as a consequence of newly introduced screening techniques and thus the finding of a higher prevalence of HCQ toxicity. American Academy of Ophthalmology (AAO) 2016 guidelines [112] recommend a maximum daily HCQ use of ≤5.0 mg/kg real weight. The guidelines also suggest baseline screening and yearly evaluations after the fifth year of therapy for patients without risk factors, but practice varies by country and by center.

2.4 HCQ reduction or withdrawal

Several studies have evaluated the effect of HCQ reduction or discontinuation in SLE patients and the possible effects on disease activity.

As early as 1991, a randomized, double-blind controlled trial showed that discontinuation of AMs in clinically stable SLE patients led to more frequent

disease flare, defined as a new manifestation of disease or worsening of a known condition. A 2.5-fold increase in clinical flare risk was observed in the placebo group compared with patients who continued taking HCQ. [114] The limitation of this study was the heterogeneity in disease activity of the patients recruited.

An extension of this study was performed by Tsakonas et al. where the risk of major flare after HCQ discontinuation was evaluated. Long-term HCQ therapy improved all endpoints considered (flare, flare subtype, and hospitalization). However, as a probable consequence of the small sample size, it did not reach statistical significance.[115]

A prospective study of 1460 patients from the Systemic Lupus International Collaborating Clinics (SLICC) cohort compared the time-to-flare of those who reduced or discontinued HCQ with those who maintained HCQ. Flare was defined as any need for increased therapy, an increase of ≥ 4 in SLEDAI-2K, or hospitalization for SLE. The adjusted Hazard Ratios (aHRs) with 95% CIs for the first flare of SLE was 1.20 (95% CI 1.04 to 1.38) for the HCQ reduction groups and 1.56 (95% CI 1.31 to 1.86) for the HCQ discontinuation groups, versus HCQ maintenance. In multivariable hazard regressions, some factors associated with flare emerged. It was seen that a low level of education and the use of prednisone at time zero increased the risk of flare.[116]

From the data presented, the importance of carefully assessing the possibility of reducing or discontinuing HCQ emerges. Risk assessment is important, and in addition to the factors already considered (i.e., prednisone use and schooling) Fasano et al. suggest periodic monitoring of HCQ levels for safe dose adjustment. [117]

More studies are needed to assess in which patients HCQ reduction or discontinuation can be considered safe.

For example, a retrospective study of a population of stable elderly SLE patients on long-term HCQ therapy showed that discontinuation does not significantly correlate with disease reactivation.[118]

AIM OF THE THESIS

We sought to evaluate AM treatment prevalence and characterize AM treatment patterns and discontinuation events in a large SLE inception cohort. We also explored whether AM therapy was associated with differences at the end of follow-up in terms of disease activity, rates of remission and Lupus low disease activity state (LLDAS) achievement, glucocorticoid (GC) intake, and organ damage.

PATIENTS AND METHODS

We used the Lupus Database of the rheumatology clinic in Padua, which includes SLE patients recruited between 1980 and 2020 and classified according to the presence of ≥4 1982 revised ACR criteria. Patients enrolled in the Padua Cohort were followed prospectively by receiving a complete examination at a frequency dictated by their clinical status (on average every 3-6 months). At each visit, clinical, laboratory, and treatment data were recorded according to a standardised protocol. We performed a retrospective analysis of prospectively collected data.

Disease activity was assessed with the SLE Disease Activity Index-2000 (SLEDAI-2K). Organ damage was defined according to the Systemic Lupus Collaborating Clinics (SLICC) Damage Index (SDI). The accumulation of clinically significant damage was defined as SDI greater than or equal to 2.

Remission was defined as clinical SLEDAI-2K=0, stable background therapy, and prednisone (PDN) ≤ 5 mg/day. Remission off GC was defined as clinical SLEDAI2K=0 without PDN and stable background therapy. LLDAS was defined according to the definition of Franklyn et al. [72], modified by excluding PGA. Active SLE was defined as clinical SLEDAI-2K >0 with SLEDAI-2K≥4 and/or PDN > 7.5 mg/day, regardless of background antimalarial or immunosuppressive therapy.

Cumulative SDI, SLEDAI-2K, achievement of remission, and whether patients were on PDN therapy at the end of follow-up were examined.

Screening for retinopathy was recommended at the start of AM therapy (T0) and then yearly thereafter. Visual field testing was suggested at T0 and 5 years later (T5). Retinopathy was defined according to a certified ophthalmologist evaluation contraindicating further AM therapy; OCT, visual field, ERG, and funduscopic examination were employed as indicated by the ophthalmology specialist.

Antimalarial therapy status was defined as: AMs never prescribed, prescribed and never stopped, prescribed and stopped. Among the latter, we identified patients who discontinued AMs due to retinopathy or due to other adverse events (AEs) or intolerance. Delay in prescribing AMs, defined as the lag time between diagnosis of SLE and AM prescription, was recorded. Time without AMs (defined as the difference between SLE duration and antimalarial therapy duration) and the fraction of time off AMs (defined as time without AMs divided by SLE duration) were also calculated.

STATISTICAL ANALYSIS

A retrospective analysis of the prospectively collected data was performed for continuous variables by t-test if normally distributed, and Mann-Whitney test if not. Categorical variables were analysed with the Chi-square test; when less than 5 events were recorded in a 2x2 table cell, an exact Fisher's test was used. Unless otherwise indicated, data were censored at 2020.

Clinically relevant known risk factors for retinopathy and factors with a $p<0.2$ at univariate analysis were entered into a Cox regression model exploring predictors of retinopathy.

The association between AM prescribing groups and damage accrual was evaluated in a multivariable logistic regression model with age, disease duration, state of activity, PDN regimen at the last follow-up, and immunosuppressive therapy as covariates.

The collinearity of variables was tested before running the multivariate model using Spearman and Pearson correlations for categorical and continuous variables, respectively, with a rho value higher than 0.6 set as significant.

Analyses were performed by the SPSS software (v. 26.0, SPSS, Chicago, IL).

All patients provided informed consent before their inclusion in the study. The study complies with the declaration of Helsinki 2000.

RESULTS

Four hundred and fifty-five (455) consecutive SLE patients were included in the study. Clinical characteristics according to AM therapy status are shown in **Table 1**. Overall, more than 95% of patients were prescribed AMs at some point during

the course of SLE (n=435). Use of HCQ was largely predominant, with only 5 patients (1,1%) employing chloroquine (CQ).

Table 1. *Clinical characteristics of 455 patients according to their AM therapy status. P-value refers to the comparison between the three patterns of AM use through Chi-square test or Kruskal-Wallis test (not assuming normality) as appropriate.*

	AM kept n=337	**AM stopped n=98**	**AM never n=20**	**p-value**
Female, n (%)	298 (88.43%)	81 (82.65%)	14 (70%)	0.0315
Age at SLE onset, years (median, IQR)	27 (20-36)	26 (19-37)	35 (23-44)	0.0576
Age at last follow-up (median, IQR)	47 (38-55)	50 (42-58)	57 (44-62)	0.0113
SLE duration at last follow-up (median, IQR)	18 (10-25)	21 (15-29)	15 (5-29)	0.0179
AM duration, years (median, IQR)	13 (7-22)	6 (1-15)	-	< 0.0001
AM delay, years, median (mean, IQR)	0 (mean 3.4 IQR 0-4)	0 (mean 4.04 IQR 0-5.5)	-	0.727
Fraction of SLE duration spent off AMs, median (mean, IQR)	0 (mean 0.17 IQR 0-0.31)	0.6 (mean 0.54 IQR 0.12-0.93)	1	< 0.0001
Overweight + Obesityt, n (%)	72+12 (22.3+3.7)	22+7 (22.9+7.3)	7+1 (35+5)	0.401
Anti-dsDNA+, n (%)	243 (73%)	65 (67%)	11 (55%)	0.146
Antiphospholipid Syndrome, n (%)	40 (11.9%)	10 (10.2%)	2 (10%)	0.951
CKD, n (%)	30 (8.9%)	18 (18.37%)	5 (25%)	0.006
Renal involvement, n (%)	166 (49.3%)	51 (52%)	10 (50%)	0.926
Skin involvement, n (%)	185 (55.5%)	52 (53.6%)	8 (40%)	0.392

Joint involvement, n (%)	244 (73.3%)	70 (72.2%)	16 (80%)	0.392
Serositis, n (%)	68 (20.4%)	17 (17.53%)	4 (20%)	0.819
Neurological, n (%)	49 (14.7%)	21 (21.6%)	8 (40%)	0.0067
Vasculitis n (%)	30 (9%)	6 (6.2%)	5 (25%)	0.0287
Immunosuppressive therapy n, (%)	220 (65.5%)	79 (80.6%)	19 (95%)	0.0007
Immunosuppressive therapy not discontinued, n (%)	115 (34.12%)	46 (46.94%)	18 (90%)	0.0001
Mycophenolate mofetil, n (%)	140 (41.7)	52 (53%)	15 (75%)	0.0017
Azathioprine, n (%)	98 (29.2%)	29 (29.6%)	9 (45%)	0.0021
Cyclosporine, n (%)	33 (9.8%)	27 (27.5%)	5 (25%)	<0.0001
Cyclophosphamide, n (%)	63 (18.8%)	25 (25.5%)	7 (35%)	0.0021
Methotrexate, n (%)	60 (17.9%)	24 (24.5%)	0 (0)	<0.0001
Leflunomide, n (%)	4 (1.2%)	6 (6.1%)	0 (0)	0.0002
Activity at end of follow-up				
GC-free remission, n (%)	213 (80.68%)	41 (41.84%)	10 (50%)	0.0027
Remission on GCs, n (%)	56 (16.82%)	28 (28.57%)	4 (20%)	0.0027
LLDAS, n (%)	88 (26.43%)	37 (37.76%)	6 (30%)	0.0012
Active, n (%)	32 (9.61%)	20 (20.41%)	4 (20%)	0.0027
Damage at end of follow-up				
SDI = 0, n (%)	164 (48.6%)	25 (25.5%)	2 (10%)	<0.0001
SDI ≥2, n (%)	94 (27.9%)	47 (48%)	15 (75%)	<0.0001

AM: antimalarials. IQR: Interquartile Range. GCs: Glucocorticoids. LLDAS: Lupus Low Disease Activity State. CKD: Chronic Kidney Disease (eGFR <50%).

1. Patients with no antimalarial exposure

Only 20 patients never received AMs (**Table 1**) and the underlying reasons for this choice are listed in **Supplementary Table 1**. The perception of contraindications by the treating physician due to multiple concomitant factors was the main reason for not receiving AMs. In this group, the diagnosis tended to be prior to 2010 but this finding did not reach statistical significance. Disease duration was also not significantly different (median 14.5 vs. 19 years, p=0.2). Compared to the rest of the cohort, these patients showed clinical, immunological, and therapeutic differences. Namely, they had more frequent neurological involvement and vasculitis (42 vs 16% p=0.004 and 23 vs 8% p=0.01, respectively), had a higher representation of males (p= 0.016), were less likely to be anti-SSA positive (p=0.025) and were more likely to receive immunosuppressants (p=0.01). Furthermore, the majority of patients (15/20, 75%) had an SDI of at least 2 at the end of follow-up, compared to 32% of patients who were prescribed antimalarials overall (p=0.0002).

These individuals were removed from further investigation due to their unusual characteristics, which suggested an overall more severe condition with a quick and progressive accumulation of damage.

Supplementary Table 1.

Reasons for not prescribing AMs in the 20 patients not exposed to AMs.

Reason for not prescribing AMs	**N. of patients (%)**
Low eGFR	3 (15%)
Neurologic	2 (10%)
Organ transplant	2 (10%)
Pre-existing retinal damage	1 (5%)
Concern over muscle toxicity	2 (10%)
Concern over myelosuppression	1 (5%)
Favism	1 (5%)
Combination of more than one factor	8 (40%)
Total	20

eGFR: estimated Glomerular Filtration Rate.

2. Patients exposed to antimalarials

Of the 435 patients that were prescribed AMs, 98 (22.5%) discontinued this class of drugs, of which 32 (32.6%, 7.3% of all AM-prescribed patients) due to concerns over retinopathy. Other causes of discontinuation were gastrointestinal intolerance (15/98, 15%) and cutaneous hypersensitivity (16/98, 16%), of which 2 were severe (Stevens-Johnson's). In a considerable proportion of patients, non-adherence was the cause for discontinuation of AM therapy (9/98, 9.1%).

The AM delay was 0 for more than half of the cohort, but we observed an increasing trend in patients diagnosed before 2010 compared to more recent diagnoses. (median 0 years in both groups, with a mean of 4.4 years vs. 0,6 years, p <0.0001).

At univariate analyses, factors associated with AM discontinuation were older age (median 50 vs. 47 years, p=0.03), longer disease duration (median 21 vs. 18 years, p=0.0065), leukopenia expressed as ACR item (51% vs. 38.7%, p=0.0243), immunosuppressant prescription (80% vs. 65%, p=0.0043).

Namely, patients on immunosuppressants who discontinued AMs were more likely to subsequently be maintained on these drugs (46.9% vs 34.1%, p=0.025). Furthermore, although Lupus Nephritis (LN) was equally distributed (52% vs. 49%, p=0.6), patients who discontinued AMs were less likely to be free of chronic kidney disease (CKD) at last follow-up (81.6% vs. 91.1%, p=0.002).

At end of follow-up, after a median overall disease duration of 19 years, patients who stopped AMs were less likely to be off GCs (44.9% vs. 68%, p=0.0001), less likely to be in remission off-GCs (41.8% vs. 63.9%, p=0.0003) and more likely to have active SLE (20% vs. 9%, p=0.0003). Finally, remission on GCs was conversely more frequent (28.6% vs. 16.8%) in patients who stopped AMs.

Accordingly, LLDAS was less prevalent in the group who had stopped AMs (79.6% vs. 90.4%, p<0,0001). Among LLDAS patients, we also analysed the percentage of patients who met the definition of LLDAS but not that of remission. (status defined as LLDAS/no remission): patients who continued taking AM were more frequently in LLDAS and remission while those who discontinued AM were more often in LLDAS/no remission status (37,76% vs.26,43%, p=0,0012)

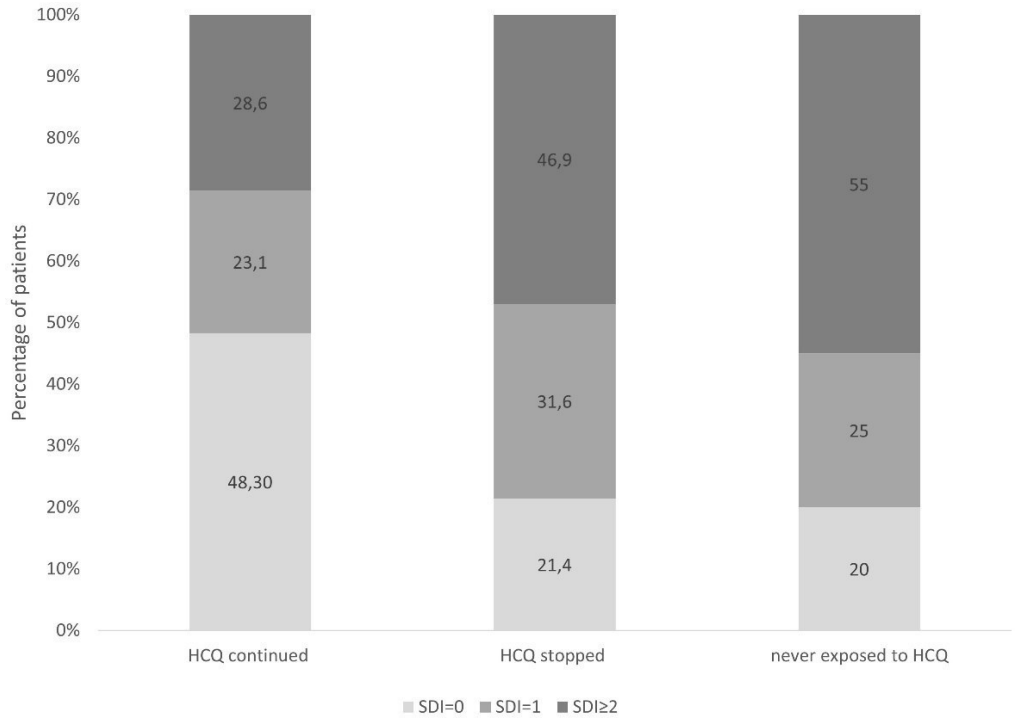
Total damage accrual at last follow-up was also revealed to be highly discordant, with 47/98 (47.9%) patients who discontinued AMs exhibiting an SDI ≥2 compared to 94/337 (27.89%) who did not discontinue these agents (p<0.0001) (**Figure 1**).

As a post-hoc sensitivity analysis, to determine whether differences in organ damage scores were caused by retinopathy (which would be scored in the SDI under the "ocular change" item), we subtracted 1 point to the SDI score of patients with retinopathy: after this adjustment the difference in damage remained consistent, with an SDI ≥2 for 28/98 (38.78%) patients who had stopped AMs vs. 94/337 (27.89%) patients who did not withdraw the drug (p= 0.017).

At multivariate logistic regression, stopping AMs remained independently predictive of the development of significant damage (OR 1.68, 95% CI 1.1-2.83, p=0.04) together with increasing age (per unit change in years: OR 1.06, 95% CI 1.04-1.08) and use of immunosuppressants through the disease course (OR 3.14, 95% CI 1.68-5.87), while having achieved GC-free remission was protective (OR 0.39, 95% CI 0.18-0.85, p=0.018).

Figure 1

Antimalarial prescribing groups distribution according to accrued organ damage at last follow-up. Percentages of the different groups in each SDI category are reported. SDI: SLICC-Damage Index. P-value: Chi-square test.



3 Retinopathy

Characteristics of the 32 patients discontinuing AMs due to concerns of retinopathy are reported in **Table 2**. On further review, 28 showed retinopathy. Among the 4 remaining patients, two were suspicions based on fundus findings unconfirmed at later OCT, and two had retinal changes unrelated to AMs. Overall observed prevalence of AM therapy discontinuation due to retinopathy in patients exposed to AMs was 32/435 (7.3%) with a median duration of therapy of 8 years (IQR: 3-14); when considering 28/435 patients with confirmed retinopathy, prevalence was 6.4% with a median duration of 9 years (IQR: 4-19). At univariate analyses, patients who discontinued AMs due to retinopathy were older, had longer disease duration, had more frequently leukopenia and positive anti-U1RNP, and less frequently had serositis. No patient stopping AM therapy due to retinopathy had the antiphospholipid syndrome (**Table 2**).

On Cox regression analysis, older age in years (per unit change: HR 1.04, 95% CI 1.01-1.07 p=0.04) and anti-U1RNP positivity (HR 3.2, 95% CI 1.38-7.46, p=0.006) were predictors of retinopathy. Although chronic kidney disease (CKD) did not show an association with retinopathy (**Table 3**), events were very few and, within the CKD category, patients with eGFR <30 ml/min or history of renal transplantation showed a HR of 10.1 (95% CI 1.24-82.4) towards retinopathy vs patients with no eGFR reduction.

Table 2. *Clinical characteristics of 32 patients who discontinued AM due to suspected retinopathy.*

	AM stopped due to retinopathy n=32, of which retinopathy n=28	**Exposed to AM n= 403**	**p-value**
Female, n (%)	30 (92.2%)	349 (86.6%)	0.245
Age at SLE onset, years, median (IQR)	28 (19-40)	27 (20-36)	0.650
Age at last follow-up (median, IQR)	51 (42.5-64.75)	47.5 (39-55)	0.0379
SLE duration at last follow-up (median, IQR)	23 (15-30)	18 (11-25)	0.030

AM duration, years (median, IQR)	8 (3-14)	All AM exposed: 12 (7-21) AM stopped due to reasons other than retinopathy: 5 (1-15)	0.0126
Overweight + Obesity, n (%)	6 + 3 (30%)	88 + 16 (26.7%)	0.348
Anti-dsDNA+, n (%)	21 (65.6%)	287 (72.1%)	0.434
Anti-U1RNP+, n (%)	16 (50%)	101 (25.3%)	0.003
Antiphospholipid Syndrome, n (%)	0	50 (12.4%)	0.056
Renal involvement, n (%)	15 (46.9%)	202 (50.1%)	0.858
Skin involvement, n (%)	20 (62.5%)	217 (54.5%)	0.383
Joint involvement, n (%)	24 (75%)	290 (72.9%)	0.933
Serositis, n (%)	2 (6.3%)	83 (20.9%)	0.0616
Neurological, n (%)	4 (12.5%)	66 (16.6%)	0.547
Vasculitis, n (%)	1 (3.1%)	35 (8.8%)	0.265
Tamoxifen exposure, n (%)	0	4 (1%)	0.571
CKD + ESRD or Renal transplant	2 + 1 (6.25%+3.13%)	36 + 9 (8.9%+2.2%)	0.910
Immunosuppressive therapy n, (%)	23 (71.88%)	276 (68.66%)	0.705
Mycophenolate mofetil, n (%)	13 (40.6%)	179 (44.5%)	0.535
Azathioprine, n (%)	10 (31.3%)	117 (29.1%)	0.873
Cyclosporine, n (%)	8 (25%)	52 (12.9%)	0.164
Cyclophosphamide, n (%)	5 (15.6%)	83 (20.7%)	0.588
Methotrexate, n (%)	9 (28.1%)	75 (18.7%)	0.425
Leflunomide, n (%)	2 (6.3%)	8 (2%)	0.278
Damage at end of follow-up			
SDI ≥2, n (%)	14 (43.8%)	127 (31.5%)	0.350

Table 3 *Multivariable models for significant organ damage (logistic regression) and retinopathy (Cox regression). For Cox: Time-to-event: AM therapy duration. Censoring according to retinopathy. Range ORs and HRs are reported. The item for use of GCs at last follow-up, showing high collinearity (rho > 0,8) with the item for Activity status, was excluded.*

	Range OR (95% CI) for SDI≥2	**p-value**
Age at last follow-up, years	57.79 (13.39-249.26)	0.0001
Disease duration, years	1.31 (0.36-4.78)	0.677
Immunosuppressant, yes/no	3.14 (1.68-5.87)	0.0003
Fraction of SLE spent off-AMs	2.59 (1.23-5.44)	0.012
Activity status at last follow-up (GC-free remission vs Active)	0.39 (0.18-0.85)	0.018
	Range HR (95% CI) for Retinopathy	**p-value**
Age at last follow-up, years	12 (1.1-117)	0.035
Disease duration, years	0.28 (0.03-2.52)	0.26
Anti-U1RNP yes/no	3.2 (1.37-7.46)	0.008
eGFR<50% yes/no	0.82 (0.19-3.53)	0.78

4 Fraction of SLE duration spent without antimalarials

Time spent with a diagnosis of SLE and without exposure to AMs was indexed to disease duration, obtaining a fraction. As stated, patients who never received AMs (i.e. with a fraction equal to 1, or 100%) were conservatively excluded from these analyses. Fraction of disease without antimalarials was slightly lower in patients stopping AMs due to retinopathy than due to other reasons (mean 0.52 vs. 0.55). Patients who had developed significant damage by end of follow-up had an incrementally higher mean fraction of SLE duration spent without AM exposure (0.18 vs. 0.24 vs. 0.35 for SDI =0, SDI =1, and SDI ≥2, respectively, p=0.0034) (**Figure 2a**).

A similar pattern emerged regarding disease activity status (p=0.0009) (**Figure 2b**) and steroid dose (p=0.0013) at last follow-up (**Figure 2c**), displaying better outcomes for patients on AMs for a longer proportion of disease duration.

At multivariate logistic regression analysis (**Table 3**), the fraction of SLE duration spent without AMs remained independently associated with a SDI ≥2 (OR 2.59, 95% CI 1.23-5.44, p=0.012); moreover, patients who spent > 50% of SLE duration off AMs had a higher probability of developing organ damage than patients who had spent less than 50% of SLE off AMs, with an OR of 3.89 (95% CI 1.89-7.97, p=0.009).

Figure 2a

Fraction of SLE duration spent off antimalarials (as a result of AM delay or AM discontinuation) according to accrued organ damage (SDI) at last follow-up. Mean and 95% CI for the mean are reported. P-values: Kruskal-Wallis test for multiple group comparisons.

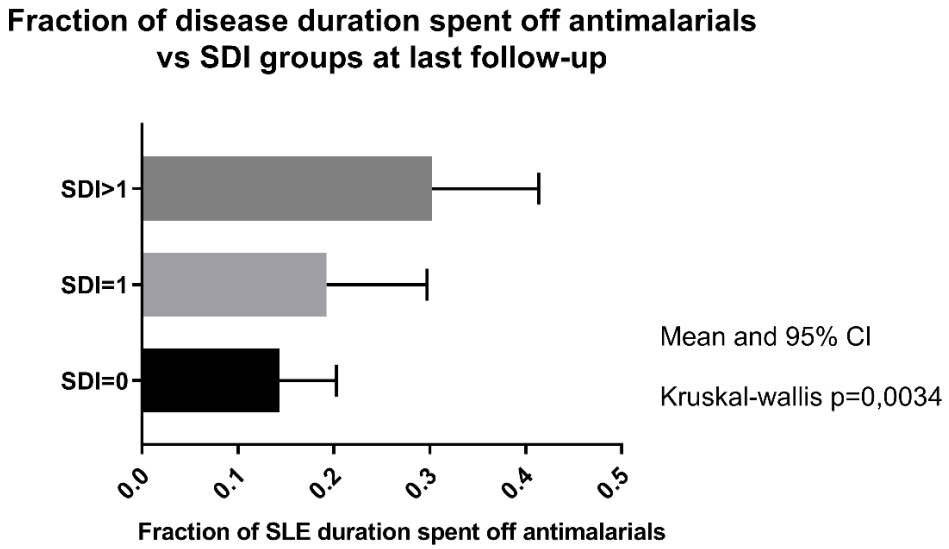


Figure 2b

Fraction of SLE duration spent off antimalarials according to disease activity at last follow-up.

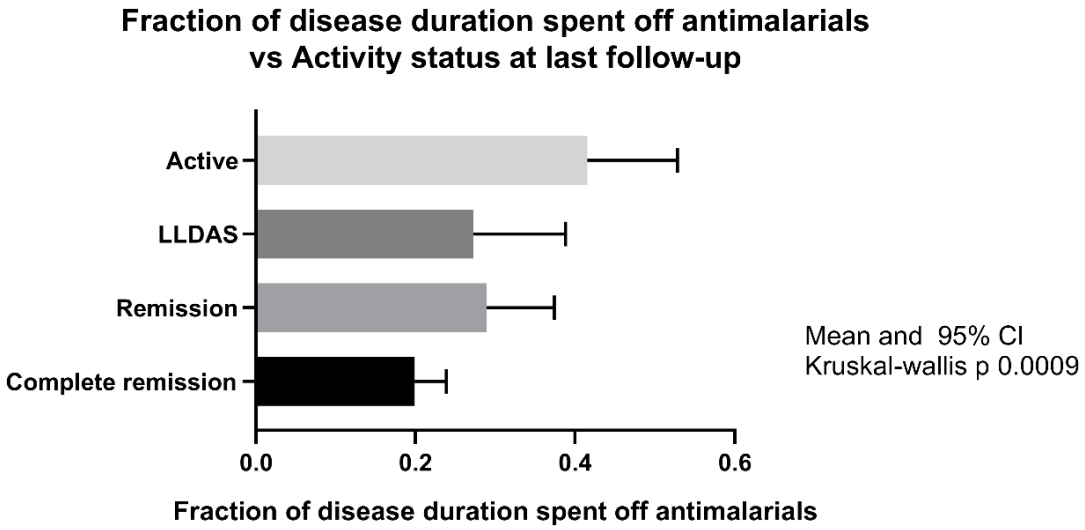
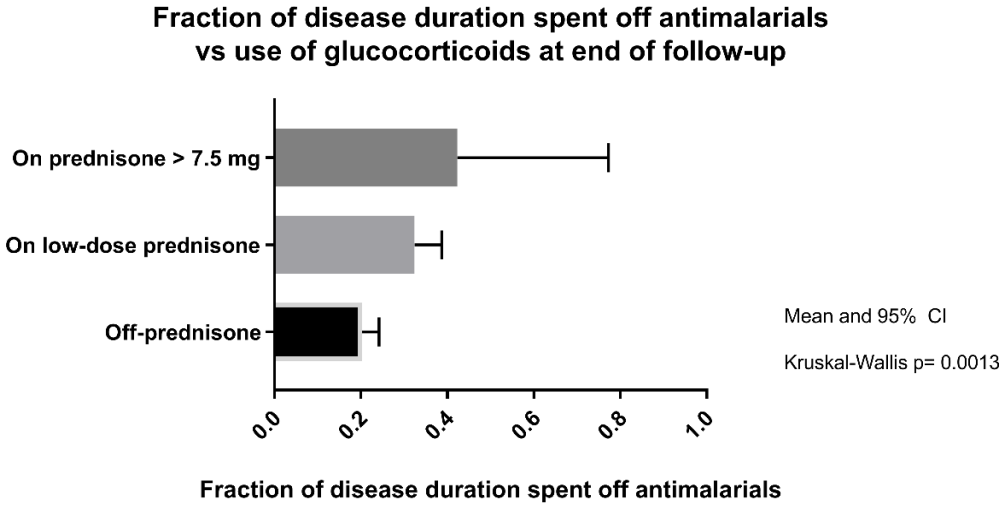


Figure 2c

Fraction of SLE duration spent off antimalarials according to glucocorticoids use at last follow-up.



DISCUSSION

Over the last few decades, the development of novel therapies and the better management of patients with SLE significantly improved life expectancy. Indeed, 5-year survival increased from 50% in 1950 to 90% in 1990. [8] Chronically active disease has become less frequent, and the main disease course pattern is now relapsing-remitting. [68] Achieving remission more frequently is the key to improve the quality and quantity of life in these patients and is therefore the main goal of the SLE treat-to-target approach. [71]

The pleiotropic effects of antimalarials, especially hydroxychloroquine, discussed in Chapter 2.2 of this thesis, account for the major role played by this drug in the EULAR 2019 guidelines.

According to EULAR recommendations all SLE patients should take hydroxychloroquine unless contraindicated and regardless of disease severity and pregnancy status. [52]

Nevertheless, not seldom clinicians and patients try to reduce or discontinue hydroxychloroquine therapy. As early as 1991, the first and only randomised controlled trial of hydroxychloroquine discontinuation demonstrated the association with disease flare. [114] Similar results were found in subsequent studies (see Chapter 2.4).

In this retrospective analysis of a large cohort with a long follow-up, the observed benefits of prolonged antimalarial therapy confirm what has been found in previous studies and are aligned with the EULAR 2019 recommendations on the management and therapy of SLE. [52] In our court less than 5% of patients had never received antimalarial treatment, and the delay in administration was more pronounced in subjects who started the follow-up before 2010. This illustrates how antimalarial drugs has received greater emphasis and early consideration in the treatment of SLE in the last decade.[119]

In the few patients never exposed to AMs, due to contraindications or fear of drug interactions, a more severe disease course was observed with the involvement of critical organs.

The greater severity reported could be the consequence of an aggressive onset that complicates management and therapeutic approach, as well as the lack of opportunity to benefit from the effects of AMs. This could also explain the greater damage observed in this group. This data concurs with the notion that every effort

should be made to reduce the number of SLE patients who do not receive AMs. [116] In addition, the necessity to monitor these patients more intensively emerges, considering their higher overall risk of severe outcomes.

In the 98 patients who stopped antimalarials, we found active disease more frequently than in those who continued therapy. Interestingly, LLDAS, defined by Franklyn et al. [72] was more frequent in patients who continued AM. Moreover, when criteria for remission were excluded from the definition of LLDAS, a higher frequency of LLDAS/no remission emerged in patients who had discontinued AM. This overlap between the definitions of LLDAS and remission had already been described by Zen et al. as a possible source of confounding. [73]

Regarding the remission in the group that stopped AM, we rarely found remission with GCs and even less remission without GCs. Conversely, in the group that continued AM, we achieved GCs-free remission more frequently.

In the treat-to-target approach, the second step after achivemement of remission/LLDAS concerns the need to reduce and possibly even discontinue glucocorticoid therapy, [71] which was also emphasised in the EULAR 2019 guidelines. [52] Indeed, there are many studies in the literature dealing with the effects of prolonged glucocorticoid therapy on the accumulation of damage and thus the consequences on the prognosis of SLE patients. Notably, a dose of > 7.5 mg/day of glucocorticoids has been shown to be associated with damage [120], and according to Gladman et al., the resulting damage tends to occur after 15 years of disease mainly with musculoskeletal, atherosclerotic, and ocular manifestations. [121] Furthermore, we found that prolonged therapy with AMs resulted in more frequent discontinuation of immunosuppressants (IS), while patients on immunosuppressive therapy who discontinued AMs were more likely to be subsequently maintained on these drugs. . These data agree with the findings of two studies by Zen et al. regarding the discontinuation of immunosuppressants in remitted patients. Indeed, it was observed that maintenance therapy with antimalarials was the strongest independent protective factor against disease flares in patients discontinuing IS therapy.[90] This was also confirmed in remitted patients with lupus nephritis in whom the frequency of flare was significantly lower in the HCQ-maintained group than in the HCQ-free group (16.2% vs. 53.3%, p=0.002).[91]

Prolonged maintenance of antimalarial therapy is associated with lower prevalence of significant SDI in our study, irrespective of the weight that AM retinal toxicity has on SDI. Indeed, discontinuation of antimalarials, regardless of the reason, remained associated with damage accumulation in multivariable analyses, and post-hoc correction for retinopathy did not reverse this effect. This reinforces the data available in the literature, where AMs protective effect on organ damage has been demonstrated.[122] This could be largely explained by the effects we observed in this study on disease activity and as a corticosteroid and immunosuppressant sparing agent. In addition, some studies have described the effects of hydroxychloroquine on the lipid profile, glycaemic profile, and the title of antiphospholipid autoantibodies leading to a reduction in atherogenic, and thrombotic risk, thus accounting for AM protective effect on CV events, which are part of the SDI. [95], [96] In our study, we did not performe a separate analysis for CV events, due to the low number of events in our cohort.

HCQ toxicity data in our study confirm the known rarity of adverse events. In fact, only 30 patients had to discontinue therapy for a severe side effect (28 for retinopathy and 2 for Stevens-Johnson syndrome). Thus, the safety of AMs described in previous studies is confirmed.[101], [102]

In this real-world study, retinopathy was defined as a contraindication to further AM exposure received by an ophthalmologist, i.e. an operational definition for the practicing rheumatologist. Funduscopic examination was performed in all cases, and after an in-depth evaluation of the suspected cases, 4 of them were deemed likely to be false positives resulting in discontinuation of AM for a prolonged period and an increased fraction of disease spent without receiving AM. This suggests the importance of ophthalmological follow-up of SLE patients treated with AM in high-volume centres to minimise variability and reporting errors that would lead to harmful therapeutic inertia for the patient. Therefore, the decision to discontinue AMs in SLE patients must be carefully considered in the light of an accurate medical history and a rigorous review of contraindications.

In this cohort, anti-U1RNP positivity showed an association with retinopathy. To our knowledge, this is the first study reporting of this association, and it will require confirmation in larger datasets. No clear pathogenetic mechanisms explaining this finding could be identified through literature review.

In this study, the concept of time spent without AM during SLE was introduced, demonstrating its relationship to disease activity, damage accumulation and GC use after years of follow-up. This underlines how discontinuation or delay in AMs therapy may lead to a worsening prognosis of SLE patients. This measure and its easy-to-understand concept can be an important element of patient education on AM therapy and an aid in doctor-patient communication to maximise adherence. Indeed, concerns about compliance, although reported by the physician and therefore probably underestimated, were relevant in this cohort (9.1%). In Chapter 2.4 of this thesis, several studies in which discontinuation due to non-adherence and other reasons correlated with an increased risk of flare were described [114]–[116], especially when low schooling and at higher GCs dosages coexisted.[116] It is therefore important to investigate and limit non-adherence to AMs in SLE patients using appropriate counseling in which the benefits of therapy and the consequences of discontinuation are explained.

This study has several limitations. Firstly, the retrospective and monocentric nature may be a source of bias. Although this practical real-world approach increases the generalisability of the results, ophthalmological follow-up was not performed for all patients by a single operator, and assessments by low-volume centers were also considered. In addition, HCQ blood levels were not monitored in this cohort, despite being indicative of therapeutic compliance and balancing the risk of relapse and retinopathy. [110], [117] Moreover, HCQ blood monitoring may not be widely available outside research centers. The role of HCQ blood monitoring may be particularly valuable in the context of CKD, where HCQ concentrations may fluctuate unpredictably despite receiving the same drug dosage, and where dosing recommendations are largely based on convention.[87], [123] Finally, the low number of retinopathy cases in our study limited the power and exploration of covariates in predicting risk factors for retinopathy, in addition to age. However, HRs from Cox regression analyses revealed that patients with a very low eGFR presented a HR of 10 for retinopathy compared to patients without eGFR reduction.

CONCLUSIONS

In conclusion, in the large cohort of SLE patients studied, coverage with AM was almost universal, and over the years an increasingly early administration of AM has been observed.

The data presented confirm the central role of HCQ as a safe and effective drug in patients with SLE. From mild manifestations (i.e. constitutional symptoms, arthritis, and rash) to life-threatening conditions, HCQ improves outcomes, being associated with a reduction in disease activity and accumulated damage, even allowing steroid sparing and easier discontinuation of the immunosuppressant.

The development of retinopathy is rare and ophthalmological follow-up should be planned, if possible, with the fewest number of expert operators in the highest volume centers available to minimise avoidable interruptions in AM therapy. HCQ blood monitoring may be useful to reduce the risk of retinopathy in patients with low eGFR, considering their increased vulnerability. Overall, this data reinforces the notion that every effort should be deployed to limit the number of SLE patients not receiving AMs and highlights the importance of contrasting non-adherence improving patient awareness. Finally, the decision to withhold AMs in SLE should be made following an accurate verification of contraindications.

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