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Remissione, low disease activity e sospensione della terapia immunosoppressiva nel lupus eritematoso sistemico. Risultati dalla coorte prospettica di Padova.

Remission, low disease activity and immunosuppressant withdrawal in systemic lupus erythematosus. Results from the Padua prospective Lupus cohort.

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TESI DI DOTTORATO

Dottorato di Ricerca in *Scienze cliniche e sperimentali*, curriculum di *Scienze reumatologiche e laboratoristiche*, 30° ciclo

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The effect of different durations of remission on damage accrual: results from a prospective monocentric cohort of Caucasian patients.

Zen Margherita, Iaccarino Luca, Gatto Mariele, Bettio Silvano, Saccon Francesca, Ghirardello Anna, Punzi Leonardo, Doria Andrea.

Annals of Rheumatic Diseases 2017;76(3):562-565.

Study 2

Lupus low disease activity state is associated with a decrease in damage progression in Caucasian SLE patients, but overlaps with remission.

Zen Margherita, Iaccarino Luca, Gatto Mariele, Saccon Francesca, Larosa Maddalena, Ghirardello Anna, Punzi Leonardo, Doria Andrea.

Annals of Rheumatic Diseases 2018; 77(1):104-110.

Other papers (review articles, letters) on the topic

Remission in SLE: the duration depends on multiple factors, including the definition.

Doria Andrea, Zen Margherita, Iaccarino Luca.

Annals of Rheumatic Diseases 2016;75(12):e77.

The Management of Systemic Lupus Erythematosus Patients in Remission.

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Zen Margherita, Doria Andrea.

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Derivation and validation of the SLE Disease Activity Score (SLE-DAS): a new SLE continuous measure with high sensitivity for changes in disease activity.

Jesus Diogo, Matos Ana, Henriques Carla, Zen Margherita, Larosa Maddalena, Iaccarino Luca, Da Silva José António Pereira, Doria Andrea, Inês Luís Sousa.

Annals of Rheumatic Diseases 2019. pii: annrheumdis-2018-214502. doi: 10.1136/annrheumdis-2018-214502. [Epub ahead of print]

New therapeutic strategies in systemic lupus erythematosus management.

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Nature Review Rheumatology 2019;15:30-48.

SUMMARY

Background. The effect of different durations of remission and LDA on SLE outcomes such as damage accrual has never been evaluated. Unsolved issues concern the treatment of patients achieving remission, being the choice and timing of drug tapering until withdrawal still a matter of debate.

Aims. To assess the prevalence, duration and predictive effect on damage of remission and LDA in a monocentric cohort of patients with SLE. In addition, to evaluate the rate of immunosuppressant (IS) withdrawal and the potential predictors of a subsequent flare and flare-free survival.

Patients and methods. Two cohort were identified: 1) patients diagnosed with SLE between 1990 and 2009 and seen from 2009 to 2015 for remission and LDA evaluation; 2) patients diagnosed with SLE between 1990 and 2018, treated with IS over the disease course and seen at least once in 2017 or 2018 for IS withdrawal evaluation.

Disease activity was assessed by SLE Disease Activity Index (SLEDAI)-2K and physician global assessment (PGA), flare by SELENA-SLEDAI flare index, and damage by SLICC/ACR Damage Index (SDI).

Three levels of remission were defined according to clinical disease activity, serological activity and treatment: complete remission, i.e. no disease activity in corticosteroid- and IS-free patients; clinical remission off-corticosteroids, i.e. serologic active clinical quiescent (SACQ) disease in corticosteroid-free patients; clinical remission on corticosteroids, i.e. clinical quiescent disease with or without serological abnormalities in patients taking prednisone 1-5 mg/day.

LDA was defined according to the “lupus low disease activity state” (LLDAS) definition: SLEDAI–2K≤4 without major organ activity, no new disease activity,

PGA (0–3)≤1, prednisone ≤7.5 mg/day, and well-tolerated IS dosages. Five range of durations of remission and LLDAS were evaluated, i.e. lasting 1, 2, 3, 4, 5 or more consecutive years. The effect of remission and LLDAS on SDI was evaluated by multivariate logistic regression analysis.

IS discontinuation was defined as complete withdrawal of any IS. Reasons for discontinuation were classified as remission or poor compliance/side effects. Predictors of a subsequent flare and flare-free survival were analyzed by multivariate logistic regression and Cox regression analyses, respectively.

Results. 293 three patients were included in the cohort for remission and LLDAS evaluation: 253 (86.3%) were female, mean±SD disease duration 11.1±7.8 years. Among patients achieving 1-year (27, 9.2%), 2-year (47, 16%), 3-year (45, 13.4%), 4-year (26, 8.8%) remission, damage was similar irrespective of the level of remission achieved, whereas among patients achieving ≥5-year remission (113, 38.6%) damage was higher in those in clinical remission on-corticosteroids ($p<0.001$). At multivariate analysis, ≥2 consecutive year remission was protective against damage [Odds ratio (95% CI)]: 0.228 (0.061–0.850).

LLDAS lasting 1-, 2-, 3-, 4-, or ≥5-consecutive years was achieved by 33 (11.3%), 43 (14.7%), 39 (13.3%), 31 (10.6%), and 109 (37.2%) patients, respectively. Patients who spent at least 2 consecutive years in were significantly less likely to have an increase in SDI (Odds ratio 0.160, 95% CI 0.060 to 0.426, $p<0.001$).

Among 456 patients seen at least once in 2017-2018, 319 were ever treated with IS (70%). Of these, 139 patients (43.5%) withdrew IS; among them, 105 (75.5%) discontinued IS due to remission, and 34 (24.5%) due to poor compliance/side effects. Mean±SD follow-up after IS withdrawal was 91±71 months (range 6-372). Among patients who discontinued IS, 26/105 remitted (24.7%) and 23/34 unremitted patients

(67.6%) experienced a flare (p<0.001). Maintenance therapy with antimalarials (OR 0.243, 95% CI 0.070-0.842, p=0.026) was the strongest independent protective factor against disease flares.

Conclusions. Remission and LLDAS were frequently observed and were protective against damage progression over the follow-up. One third of our patients treated with IS discontinued the drug during the follow-up. Antimalarial therapy was the strongest protective factor against flare after IS discontinuation.

INTRODUCTION

Remission in SLE

Systemic lupus erythematosus (SLE) is an autoimmune rheumatic disease characterized by a wide range of clinical manifestations, since any organ can potentially be affected by the disease.[1,2] An increase in survival rate and short term prognosis has been observed over the last decades, nevertheless SLE patients are still at risk of disease-related complications, damage accrual, and premature death.[3,4]

Disease activity is one of the major determinant of morbidity, accrual of new organ damage, and mortality in SLE patients.[5,6] Three patterns of disease activity have previously been reported in SLE using the SLE Disease Activity index (SLEDAI) or SLEDAI-2000 (SLEDAI-2K):[5-10] chronic active (CAD), relapsing-remitting (RRD), and clinical quiescent (CQD) disease.

It has been demonstrated that an active disease, either presenting as CAD or RRD, leads to damage accrual.[5,7,9,11-34]

In recent years, remission has emerged as a key concept in monitoring disease activity and in evaluating treat-to-target therapies in several autoimmune rheumatic diseases. Originally, the term “remission” was used in oncology to describe the absence of detectable tumour; when referring to autoimmune inflammatory diseases, remission can be described as the disease state one would ideally like to achieve when a ‘cure’ cannot realistically be hoped for. Specific definitions of remission have been developed for different rheumatic disease; for example, the definition of remission for patients with rheumatoid arthritis was published by the American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) definition of remission in 2011.[35]

The great effort put in searching for a definition of remission was linked to the need of a shared target/goal for therapeutic interventions and for evaluation of clinical trials.

In SLE, the concept of remission has extensively been discussed, but a generally accepted definition has not been formulated yet. Indeed, which disease and treatment variables should be considered and which activity score(s) should be used to define a patient as in remission have not been established yet.[4,36]

A number of different ad hoc definitions of remission has been used in cohort studies carried out in SLE patients.[5,7,9,37-50] (Table 1)

Table 1. An overview of studies of remission in SLE and different definitions of remission used.

Author(s)	Remission definition	Serological Activity permitted	Treatments permitted	Duration of remission required	Number of total patients	% of patients achieving Remission
Dubois,[37] 1956	According to rheumatologist's impression	Not specified	Not specified	No	520	1.7
Dubois et al,[38] 1964	According to rheumatologist's impression	Not specified	Not specified	No	520	35.0
Gladman et al,[39] 1979	Asymptomatic patient	Yes	None	No	NA	NA
Tozman et al,[40] 1982	Absence of clinical SLE manifestations	No	None	No	160	2.5
Heller and Schur,[9] 1985	Asymptomatic without active organ involvement	No	Antimalarials and low-dose glucocorticoids	No	305	4.0
LeBlanc et al,[41] 1994	Clinical SLEDAI=O	Yes	Any	≥3 consecutive clinic visits	609	13.0
Drenkard et al,[42] 1996	Lack of disease activity permitting SLE treatment withdrawal	Yes	None	≥1 year	667	23.4
Barr et al,[7] 1999	Clinical SLEDAI=0 or PGA <1.0	Yes	Not specified	≥1 year	204	44 (PGA) or 28 (SLEDAI)
Formiga et al,[43] 1999	Lack of disease activity permitted SLE treatment withdrawal	Yes	None	≥1 year	100	24.0
Swaak et al,[44] 1999	Absence of disease-related signs with no need for treatment	Not specified	None	No	187	0
Urowitz et al,[45] 2005	Clinical SLEDAI=O	Yes	None	≥1 years; ≥5 years	703	2.8; 14.5
Urowitz et al,[45] 2005	SLEDAI=O	No	None	≥1 years; ≥5 years	703	6.5; 1.7
Nossent et al,[5] 2010	Physician assessed	Not specified	Not specified	No	200	27.5

(continuing)

(Table 1. continuing)

Author(s)	Remission definition	Serological Activity permitted	Treatments permitted	Duration of remission required	Number of total patients	% of patients achieving Remission
Steiman et al,[46] 2010	Clinical SLEDAI-2K=O	Yes	Antimalarials only	≥2 years	924	6.1
Conti et al,[47] 2012	Clinical SLEDAI-2K=O	Yes	Antimalarials only	≥2 years	45	2.2
Steiman et al,[48] 2014	Clinical SLEDAI-2K=O	Yes	Antimalarials only	≥5 years	1613	2.4
Zen et al,[49] 2015	Clinical SLEDAI-2K=O	Yes	Antimalarials, stable IS, 1- 5 mg prednisone daily	≥5 years	224	38.0
Medina-Quiñones et al,[50] 2015	BILAG scores of C, D or E only	No	Antimalarials only	≥3 years	532	14.5
Medina-Quiñones CV et al,[50] 2015	BILAG scores of C, D or E only	Yes	Antimalarials only	≥3 years	532	23.0
das Chagas Medeiros MM et al,[51] 2016	Absence of any clinical manifestation or laboratory finding indicating active disease	Not specified	Not specified	No (disease remission evaluated upon the last consultation)	338	57.4

SLE, systemic lupus erythematosus; SLEDAI, SLE disease activity index; PGA; physician global assessment; BILAG, The British Isles Lupus Assessment Group index; NA, not available

Notably, there are five controversial areas in the definition of remission: whether or not to use a measure of disease activity (disease activity indices), and which index should be used, serological activity, treatment, and duration. These discrepancies prevent valid interstudy comparisons in most cases.

Measures of disease activity

Several validated index have been used to define remission in SLE, which differ in the ability to capture different aspects of disease activity. Physician global assessment (PGA), SLEDAI, SLEDAI-2K, M-SLEDAI, the European Consensus Lupus Activity Measurement (ECLAM), and the British Isles Lupus Assessment Group (BILAG) are the most common ones. Notably, none of them has been selected as the "best one" in the identification of remission definition.

SLEDAI, or one of its variants, has largely been used in observational studies and it has been included in the SLE responder index for clinical trials. It is a feasible and sensitive instrument, but it has two major limitations, i.e. it lacks the ability to capture the severity of disease activity within a individual organ system, and it does not assess some manifestations which can be observed in SLE patients (myelitis, gastrointestinal lupus, haemolytic anaemia, and lupus lung involvement). Different cut-off for the definition of remission have been proposed (see below).

Although BILAG is the more comprehensive and sensitive index, it is time-consuming and it remains complex and unfeasible for use in the everyday clinical practice. By BILAG, remission has been defined as having categories D and E only, or as having C, D and E categories.[50]

Clinical disease activity.

A definition of remission could require complete absence of clinical manifestations of the disease, in other words the absence of any signs or symptoms of SLE. Alternatively, it could be accepted a minimal amount of symptoms including mild fatigue, mild myalgia, mild alopecia.

Notably, despite the use of the same validated index of disease activity, different cut-off in the definition of remission have been proposed in different studies, preventing clear comparisons.

Serological activity.

Serological activity is commonly defined as the detection of anti-double stranded DNA antibodies (anti-dsDNA Abs) and/or hypocomplementaemia attributable to complement activation. The "serological activity with clinical quiescence" (SACQ) has been defined as a state where serological, but not clinical, activity is present. It has been included in some definitions of remission, but not in others. This is a key point when evaluating the results of observational studies on remission in SLE, since the proportion of patients achieving remission greatly varied if serological activity is allowed or not.[49]

Treatment.

A critical aspect in the definition of remission is whether to consider lupus treatment, particularly in the definition of complete remission. In fact, remission in patients on treatment is more frequent than in those free of therapy.[46]

Nowadays, since the general feeling is that antimalarials should be considered as long-term maintenance therapy in SLE, treatment with antimalarials does not preclude the patient from being considered to be in remission. The main discussion regards the use of corticosteroids and immunosuppressants, where a consensus has not been achieved.

In particular, although patients treated with moderate-dose or high-dose corticosteroids are usually not considered to be in remission (even if they would fulfil other criteria for remission) there is still a disagreement regarding the maximum acceptable daily dose of corticosteroids to be allowed in the definition of remission. Some studies proposed a prednisone daily dose of 1-5 mg.[49] It should be noted that definitions of remission in other autoimmune diseases do not exclude the use at stable doses of specific antirheumatic medications, immunosuppressives or biologics.

Duration.

Duration of remission represents a particularly hot topic in defining remission in SLE. A number of different durations of remission have been proposed, and no consensus on a definite length of time has been achieved. In particular, it is reasonable that remission maintained for a longer duration is better than a remission maintained for a short period, but the predictive effect of a range of durations of remission on outcomes has not yet been studied and how long the remission should last to yield significant benefits on patients' outcome has not been proven yet. The durability of remission also varied from study to study, ranging from six months to five years.[9,40,42,45,46,48,52] Moreover, some studies assessed remission as a “consecutive period” of no activity, but others considered remission duration as the “total time” spent in remission in an interval of time, allowing periods of disease activity among periods of remission. It stands to reason that two definitions of remission based on these different approaches would not include the same type of patients, preventing effective comparison, and could potentially have a different impact on outcomes.

Recently, the treat-to-target for SLE (T2T/SLE) initiative identified “remission of systemic symptoms and organ manifestations” as one of the major therapeutic targets in SLE. However, it was recognised that an agreed-upon definition of remission does not currently exist, and therefore, the T2T/SLE panel recommended the definition of remission as a research priority for SLE.

As a result, an initiative to achieve consensus on a definition of remission was undertaken by an international task force (Definition Of Remission In SLE, DORIS).[53] For the DORIS task force remission in SLE can be considered as a “desirable disease state for patients with, at the very least, the absence of major symptoms and signs of SLE”. The task force did not provide a definition of remission, but it support three principles to guide the development of remission definitions: 1) remission should be a durable state; 2) for defining remission, a validated index must be used (e.g. clinical SLEDAI, clinical ECLAM, BILAG), completed with PGA with routine laboratory tests included; 3) a distinction should be made between the definition of remission off therapy, which allowed no other treatment for SLE than antimalarials, and remission on therapy, which includes antimalarials, stable prednisone ≤5 mg/day, maintenance immunosuppressives and/or stable biologics.

In this context, the beneficial effect of achieving a disease remission on damage accrual has not been fully elucidated.

Damage

In SLE, damage is assessed using the Systemic Lupus International Collaborating Clinics (SLICC)/ACR Damage Index (SDI), whose items represent chronic irreversible damage that has occurred after the diagnosis of SLE. However, an item

does not have to be attributable to lupus. The mean SDI tends to increase over time [54] and it predicts mortality.[55-57]

Current evidence pinpoints that both higher disease activity and medication-related toxicity are associated with increased damage accrual.[5,11-34] Notably, long lasting corticosteroid therapy, even at very low doses, can be a risk factor of comorbidity and damage.[58-59]

Conversely, strategies aimed at reducing disease activity and GCs intake have been proved to exert a protective effect on damage progression.[60]

On the other hand, damage reduces patients' quality of life in different cohorts [61-63] and it was shown to predict the accrual of more damage and mortality.[12,63-67]

In a previous study [49] we addressed the consequences of having prolonged remission on damage. We evaluated the prevalence and outcomes of prolonged remission, defined as a 5 consecutive year period of no clinical disease activity. We defined three levels of remission, based on SLEDAI-2K: complete remission, having no clinical and serological activity and no treatment other than antimalarials; clinical remission off corticosteroids, where serological activity and stable immunosuppressive therapy were allowed; clinical remission on corticosteroids, where also prednisone 1-5 mg daily was allowed.

We demonstrated that damage accrual was significantly higher among patients who did not achieve a prolonged remission compared with those who achieved one of the three levels of remission.

These findings support the validity of the definitions of remission we proposed, as our definition should identify patients with a better prognosis.

In the light of these data, we thought that it could be of interest to look at the predictive effect of a range of durations of remission on damage. Indeed, it would be useful to know if achieving remission for shorter periods of time is also associated with significantly better outcome.

Low disease activity in SLE

The concept of LDA was recently applied to SLE,[68-73] and preliminary data suggest that patients achieving LDA have better short-term outcomes (Table 2), while no data on long-term outcomes are available yet.

Three definitions of LDA have recently been proposed, as reviewed in Table 2.

Table 2. Different definitions of LDA in systemic lupus erythematosus

	Name	Disease activity			Treatment			Effect on damage, OR (95% CI)
		Clinical	Serological	PGA	Prednisone	AM (allowed)	IS (allowed)	Multivariate analysis
Polackek et al. [72]	Low disease activity	Yes/No[+]	Yes	-	0	Yes	No	-
Franklyn et al.[68]	Lupus low disease activity state	Yes/No"	Yes	≤1	≤7.5 mg/day	Yes	Yes	0.47 (0.28-0.79) if ≥50% follow-up[§] in Franklyn et al[68] 0.52 (0.28, 0.99) if ≥50% follow-up[§] in Tsang-A-Sjoe et al[69]
Ugarte-Gil et al. (GLADEL)[73]	Lupus low disease activity status	Yes/No°°	Yes	-	≤7.5 mg/day	Yes	Yes	0.66 (0.48-0.9)° in Ugarte-Gil et al.[73]

PGA, physician global assessment; AM, antimalarials; IS, immunosuppressants; GLADEL: Gruppo Latino Americano de Estudio de Lupus;

[+] SLEDAI-2K≤2, including only 1 clinical manifestation of rash, alopecia, mucosal ulcers, pleurisy, pericarditis, fever, thrombocytopenia, or leukopenia

" SLEDAI-2K≤4, with no activity in major organ systems and no haemolytic anemia or gastrointestinal activity; PLUS no new features of lupus disease activity compared to the previous assessment PLUS PGA≤1 (scale 0-3)

[°°] SELENA-SLEDAI≤4

[§] mean (SD) duration of follow-up was 3.90 (2.0) in Franklyn cohort, and median follow-up duration was 5.0 in Tsang-A-Tjoe cohort

° the effect of LDA was evaluated as cumulative time spent by all patients (as a whole, and not by each single patient) in this status

As the effect of remission and Low disease activity on damage is concerned, the OR refer to multivariate analyses. For Polackek et al definition, no multivariate analysis was provided. At univariate analysis, the mean damage was significantly lower in a combined group which included patients in LDA and remission.

Although no agreed-upon definition of lupus LDA exists, an increasing number of studies applied the definition by Franklyn et al, which showed a good performance. In fact, the Lupus LDA State (LLDAS) has been frequently attained: 88.5% of 191 patients had at least one episode of LLDAS and 38.2% were in LLDAS ≥50% of the time (mean ±SD follow-up duration was 3.90 ±2.0) in Franklyn cohort;[68] 64.5% of 183 patients were in LLDAS ≥50% of the time in the study by Tsang-A-Sjoe et al (median follow-up was 5 years);[69] 43.9% of 107 patients achieved LLDAS 6 months after diagnosis and treatment initiation in the study on early lupus by Piga et al.[70].

LLDAS has also been proved to be protective against damage progression in the short term: patients with LLDAS in ≥50% of observations had a lower risk of damage accrual in the studies by Franklyn and Tsang-A-Sjoe (RR 0.47, 95% CI 0.28, 0.79 and OR 0.52, 95% CI 0.28, 0.99, respectively); failure to achieve LLDAS at 6 months was an independent predictor of early damage in the study by Piga (OR 5.0, 95% CI 1.5, 16.6). Recently, Petri M et al.[71] found that LLDAS was achieved in 50% of follow-up visit in a cohort of 1356 SLE patients followed between 1987 and 2016. The rate of damage declined as the percentage of time spent in LLDAS increased, and patients with LLDAS in ≥50% of observations had a low rate of damage progression (RR 0.39 to 0.47). Notably, a similar protective effect on damage (RR 0.54, 95% CI 0.44 to 0.67) was observed with a duration of clinical remission (<25% of follow-up time) shorter than that of LLDAS, which means that remission is superior to LLDAS in hampering damage progression.

The definition of LDA recently proposed by Polackek et al.[72] is quite different, since the Authors suggested to score for definition only clinical items of SLEDAI-2K, and not serology. Consequently, they used as the cut-off for LDA a clinical

SLEDAI-2K≤2, including only one clinical manifestation of skin or mucosal involvement, pleurisy, pericarditis, thrombocytopenia, leukopenia, or fever. Antimalarial were the only medication allowed in this definition. As such, LDA was associated with reduced mean SLEDAI-2K score, organ involvement, SDI score, mortality, and therapies after 2 years of follow up in the original cohort. No external validation has been published yet.

These data support the validity of LDA definitions, as they identify patients with a better prognosis. However, what is the shortest duration of LDA associated with improvement in disease outcome has not been investigated.

Immunosuppressant withdrawal in SLE

Treatment of patients with inactive disease, in particular those achieving durable/prolonged remission, is still a matter of debate. The benefits of drug tapering and discontinuation have been definitely proven for GCs,[74] but no sufficient strength of evidence is available regarding antimalarials and immunosuppressants.

As a general consideration, during treatment tapering and withdrawal, a close surveillance should be planned in the first few months, in order to detect early signs or symptoms of disease relapse, and patients should be informed that, although they have inactive disease, they should perform routine laboratory tests and regularly attend their clinical evaluations.

Few data are available on withdrawal of immunosuppressive agents in remitted patients, especially in non-renal SLE. In 1996 Drenkard et al. found that 156 out of 667 patients achieved at least one period lasting ≥1 year of drug-free clinical remission.[75] Remission was achieved mainly in patients with mild disease, but also some patients with renal and neuropsychiatric involvement, severe

thrombocytopenia and hemolytic anemia could achieve and maintain drug-free remission.

Different results were obtained in a small controlled trial of azathioprine withdrawal in 9 patients with stable disease. Disease flares were observed in 7 patients after a mean interval of 10.5 weeks from drug withdrawal.[76] Data from the Toronto Lupus Cohort showed that immunosuppressant-free prolonged remission (> 2 years) was rarely observed.[77]

Nevertheless, the results of these studies should be critically considered, owing to the improvement of our strategies in lupus management, including attempts for an earlier lupus diagnosis, treatments tailored on different manifestations as well as the availability of evidence- and expert-based recommendations; thus, the scenario depicted by the aforementioned studies might be outdated.

Further studies on this topic, aimed at identifying predictive factors of disease quiescence after drug discontinuation (e.g. type and duration of remission, manifestations requiring immunosuppressive therapy, pattern of disease activity) would be timely. Based on available data, we can conclude that no sufficient strength of evidence is available regarding the benefits of immunosuppressants tapering and discontinuation in non-renal SLE.

Some data are available on immunosuppressive discontinuation in lupus nephritis (LN). Pablos et al.[78] withdrawn cyclophosphamide 2 years after complete renal remission in 11 patients with class IV LN and observed a relapse in 36 % of them. In another study, 15 out of 33 patients (45%) with class IV LN who withdrawn immunosuppressive therapy after treatment with intravenous (iv) cyclophosphamide and GCs experienced a renal flare.[79] Roccatello et al. administered four doses of rituximab, two cyclophosphamide pulses and iv GCs followed by oral prednisone, 50

mg for 2 weeks, tapered to 5 mg/day in 2 months. Two more doses of rituximab were administered 1 and 2 months later. At month 3, patients received prednisone 5 mg/day as maintenance therapy. Two out 8 patients (25%) relapsed and were retreated with rituximab. After a follow up of 15–59 months, all patients were in remission.[80]

A progressive discontinuation of therapy was tried in a study involving 52 patients with LN who achieved a durable renal remission. Immunosuppressive drugs were de-escalated and withdrawn firstly, then GC tapering was started: 32 out of 52 patients did not experience any flare during a median follow-up time of 101.8 months after drug discontinuation. Patients who did not flare had a significantly longer treatment duration and a significantly longer duration of remission before withdrawal, and they were concomitantly treated with chloroquine. Ten patients who flared up after first drug withdrawal could later withdraw therapies and were free of immunosuppressive and GC therapy after a median follow-up time of 286 months.[81] Importantly, the Authors suggested a tight follow-up during therapy de-escalation and even closer after complete withdrawal of immunosuppressive agents and GCs (i.e. every 15 days for the first 2 months, every month for 6 months, and then every 2–3 months).

THE THESIS

AIMS OF THE THESIS

The main aims of the thesis were:

1) to assess the effect of different durations of remission on damage accrual, in order to identify the shortest duration of remission associated with a decrease in damage progression in SLE (*Study 1*).

2) to assess the prevalence of LLDAS, to evaluate its protective effect against damage, and to identify the shortest duration of LLDAS associated with a reduction in damage progression in SLE (*Study 2*).

3) to assess the rate of immunosuppressant discontinuation in SLE; in particular, to identify the rate of immunosuppressant discontinuation after remission achievement and to evaluate the effect of immunosuppressant withdrawal on damage accrual. Moreover, to identify the predictors of a subsequent flare and flare-free survival in remitted patients who discontinued immunosuppressive agents (*Study 3*).

PATIENTS AND METHODS

Study 1 and Study 2

Study cohort.

We used our Lupus Database which includes patients recruited in Padua Lupus cohort between 1970 and 2018 and prospectively followed.

We analysed a 7-year period from January 2009 to December 2015. The reasons for having selected this timeframe is that we started to prospectively store patients' data in our electronic database since January 2009, and analyses were performed in June 2016.

Patients attending our outpatient clinic diagnosed with SLE were included in the study if they fulfilled the following inclusion criteria: 1) at least four of the revised American College of Rheumatology (ACR) Classification Criteria for SLE;[82] 2) Caucasian ethnicity; 3) diagnosis of SLE between 1990 and 2009; 4) active disease at study entry or remission lasting no more than 12 months at study entry (in 2009); 5) at least three visits per year between January 2009 and December 2015, no more than 5 months apart. Each patient signed the informed consent for the use of clinical and laboratory data for study purposes.

Methods

Data collection.

Information collected at baseline included demographics (age, gender, year of first symptom, year of diagnosis), disease manifestations at baseline and over the patients' disease course, current and previous therapies, complement (C3 and C4) serum levels, antinuclear antibody, anti-extractable nuclear antigen antibodies, anti-double stranded DNA (anti-dsDNA) and antiphospholipid antibodies. The cumulative prednisone dose (g) taken by the patients before baseline was calculated.

Clinical and laboratory findings (complete blood cell count, urea and electrolytes, liver function tests, complement and anti-dsDNA serum levels, urinary sediment and 24-hours proteinuria) and data regarding therapy were recorded at each visit. according to a standardized protocol and were stored in a dedicated database. Clinical manifestations were defined using ACR definitions.[83]

Laboratory testing.

Antinuclear antibodies (ANA) were determined by indirect immunofluorescence on Hep-2 cell monolayers. A cut-off at 1:160 was considered as clinically significant. Anti-dsDNA antibodies were measured by an enzyme linked immunosorbent assay (ELISA). Standard laboratory tests were used to determine haemoglobin, white cell and platelet counts, blood urea nitrogen, creatinine and creatinine clearance, C Reactive Protein, erythrocyte sedimentation rate, serum proteins, transaminases, C3, C4, and urinalysis. Coombs test and 24 hour-proteinuria were performed and recorded as needed.

Disease activity measurement.

Disease activity was monitored using the SLEDAI-2K index, which was calculated at each visit (Table 3).

Table 3. SLEDAI-2K activity index.

8 ❑	Seizure. Recent onset (last 10 days). Exclude metabolic, infectious or drug cause, or seizure due to past irreversible CNS damage.
8 ❑	Psychosis. Altered ability to function in normal activity due to severe disturbance in the perception of reality. Include hallucinations, incoherence, marked loose associations, impoverished thought content, marked illogical thinking, bizarre, disorganized, or catatonic behavior. Exclude uremia and drug causes.
8 ❑	Organic Brain Syndrome. Altered mental function with impaired orientation, memory or other intellectual function with rapid onset and fluctuating clinical features. Include clouding of consciousness with reduced capacity to focus and inability to sustain attention to environment, plus at least 2 of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, increased or decreased psychomotor activity. Exclude metabolic, infectious, or drug causes.
8 ❑	Visual Disturbance. Retinal and eye changes of SLE. Include cytoid bodies, retinal hemorrhages, serous exudate or hemorrhages in the choroid, optic neuritis, scleritis or episcleritis. Exclude hypertension, infection, or drug causes.
8 ❑	Cranial Nerve Disorder. New onset of sensory or motor neuropathy involving cranial nerves.

	Include vertigo due to lupus.
8 ❑	Lupus Headache. Severe persistent headache: may be migrainous, but must be non-responsive to narcotic analgesia.
8 ❑	CVA. New onset of cerebrovascular accident(s) (CVA). Exclude arteriosclerosis or hypertensive causes.
8 ❑	Vasculitis. Ulceration, gangrene, tender finger nodules, periungual infarction, splinter hemorrhages, or biopsy or angiogram proof of vasculitis.
4 ❑	Arthritis. More than 2 joints with pain & signs of inflammation (i.e., tenderness, swelling or effusion).
4 ❑	Myositis. Proximal muscle aching/weakness associated with elevated creatine phosphokinase/aldolase or electromyogram changes or a biopsy showing myositis.
4 ❑	Urinary Casts. Heme-granular or red blood cell casts.
4 ❑	Hematuria. > 5 red blood cells/high power field. Exclude stone, infection, or other cause.
4 ❑	Proteinuria. New onset or recurrence of proteinuria more than 0.5 gm/24-hours.
4 ❑	Pyuria. > 5 white blood cells/high power field. Exclude infection.
2 ❑	Rash. New or ongoing inflammatory lupus rash.
2 ❑	Alopecia. New or ongoing abnormal, patchy or diffuse loss of hair due to active lupus.
2 ❑	Mucosal Ulcers. New or ongoing oral or nasal ulcerations due to active lupus.
2 ❑	Pleurisy. Classic and severe pleuritic chest pain or pleural rub or effusion or new pleural thickening due to lupus.
2 ❑	Pericarditis. Classic and severe pericardial pain or rub or effusion, or electrocardiogram confirmation.
2 ❑	Low Complement. Decrease in CH50, C3, or C4 below the lower limit of normal for testing laboratory.
2 ❑	Increased DNA. Binding > 25% binding by Farr assay or above normal range for testing laboratory.
1 ❑	Fever > 38° C. Exclude infectious cause.
1 ❑	Thrombocytopenia < 100,000 platelets/mm3
1 ❑	Leukopenia < 3,000 white blood cells/mm3. Exclude drug causes.

Definition of remission: identification of three different levels of remission.

We defined remission as a period of no disease activity based on SLEDAI-2K activity index. We defined three levels of remission according to disease activity (clinical and serological) and treatment (Table 4):[49]

Table 4. Definitions of remission according to clinical, serological and therapeutic status. Disease activity was assessed by SLEDAI-2K.

Remission	**Disease activity**		**Treatment**		
	Clinical	Serological	Prednisone	Antimalarials	Immunosuppressants
Complete remission	No	No	No	Yes	No
Clinical remission off corticosteroids	No	Yes	No	Yes	Yes/No
Clinical remission on corticosteroids	No	Yes/No	1-5 mg/day	Yes	Yes/No

a) *complete remission:* no clinical and serologic disease activity (SLEDAI-2K=0) in corticosteroid- and immunosuppressant-free patients; antimalarials were allowed;

b) *clinical remission off corticosteroids:* serologic active clinical quiescent disease (SACQ) according to SLEDAI-2K (complement component decrease and/or positive anti-dsDNA antibodies, in corticosteroid-free patients; immunosuppressants and antimalarials were allowed;

c) *clinical remission on corticosteroids:* clinical quiescent disease according to SLEDAI-2K, in patients taking a daily dose of prednisone or equivalent ≥1 mg and ≤ 5 mg; immunosuppressants and antimalarials were allowed (Table 4).

Moreover, SLE manifestations not considered in the SLEDAI-2K (haemolytic anemia, myelitis, pulmonary, gastrointestinal and ophthalmic involvement) were recorded in the database.

Notably, in Study 2 we classified patients as being in remission or not, irrespective of the level of remission achieved, i.e., patients fulfilling any of the three levels of remission were categorized as remitted. Accordingly, remission was overall defined as clinical SLEDAI–2K=0, prednisone≤5 mg/day in patients who could be on a stable immunosuppressive and/or antimalarial therapy.

Definition of Lupus low disease activity

Lupus low disease activity was defined according to the recent definition of the *lupus low disease activity state*-LLDAS, proposed by Franklyn et al.:[68]

1) SLEDAI-2K≤4, with no activity in major organ systems (renal, central nervous system, cardiopulmonary, vasculitis, fever) and no haemolytic anaemia or gastrointestinal active involvement; 2) no new lupus disease activity compared with the previous assessment; 3) a physician global assessment (PGA, scale 0–3) ≤1; 4) a

current prednisone (or equivalent) dose ≤7.5 mg/day; and 5) well tolerated standard maintenance doses of immunosuppressive drugs and approved biological agents. Achievement of LLDAS was determined at each visit.

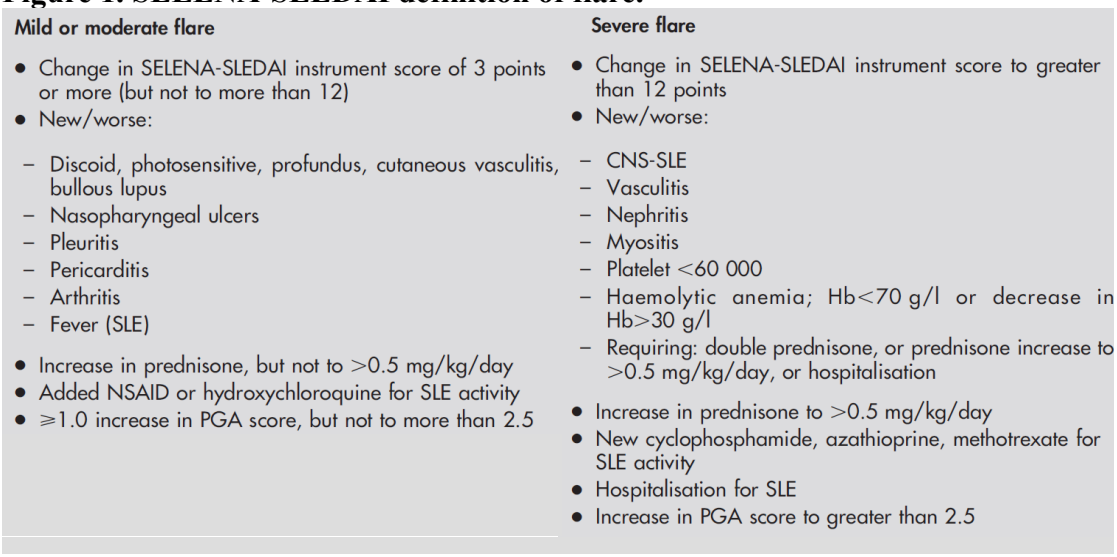
Definition of a range of durations of remission and LLDAS.

Five range of durations of remission and LLDAS were identified, i.e. lasting one, two, three, four, and 5 or more consecutive years. For patients who experienced a relapsing-remitting disease, with periods of activity interspersed with periods of remission/LLDAS, only the longest period of remission or LLDAS achieved during the follow-up was considered in the analysis.

Definition of flare.

Flares were defined according to SELENA-SLEDAI criteria.[84] We registered the organ systems involved at the time of each flare, including renal, musculoskeletal, skin, haematological, serosal, neuropsychiatric, and vasculitic flares (Figure 1).

Figure 1. SELENA-SLEDAI definition of flare.



Definition of damage accrual.

Organ damage was evaluated at baseline and at the end of the follow-up using the Systemic Lupus International Collaborating Clinics/ACR damage index for SLE (SDI) (Table 5). Moreover, damage was categorized into two groups, following definitions by Gladman et al.:[26] related to corticosteroid intake, or independent of corticosteroids. SDI items that were considered corticosteroid-related were ocular, musculoskeletal, cardiovascular, peripheral vascular, neuropsychiatric, and diabetes. SDI items independent of corticosteroids were renal, pulmonary, gastrointestinal, skin, premature gonadal failure, and malignancy.

Moreover, risk factors other than corticosteroids, e.g. personal and family history of some SDI features such as cardiovascular disease and diabetes, were taken into account in the attribution of damage to corticosteroids. When risk factors other than corticosteroids were considered more relevant in triggering the event, damage was recorded as non corticosteroid-related.

Table 5. SLICC/ACR Damage Index

Ocular (either eye, by clinical assessment)	
Any cataract ever	❑ 1
Retinal change or optic atrophy	❑ 1
Neuropsychiatric	
Cognitive impairment (e.g., memory deficit, difficulty with calculation, poor concentration, difficulty in spoken or written language, impaired performance levels)	❑ 1
or major psychosis	❑ 1
Seizures requiring therapy for 6 months	❑ 1 ❑ 2
Cerebrovascular accident ever (score 2 if > 1)	❑ 1
Cranial or peripheral neuropathy (excluding optic)	❑ 1
Transverse myelitis	
Renal	
Estimated or measured glomerular filtration rate < 50%	❑ 1
Proteinuria > 3.5 gm/24 hours	❑ 1
OR	
End-stage renal disease (regardless of dialysis or transplantation)	❑ 3
Pulmonary	
Pulmonary hypertension (right ventricular prominence, or loud P2)	❑ 1
Pulmonary fibrosis (physical and radiograph)	❑ 1
Shrinking lung (radiograph)	❑ 1
Pleural fibrosis (radiograph)	❑ 1
Pulmonary infarction (radiograph)	❑ 1

Cardiovascular	
Angina or coronary artery bypass	❑ 1
Myocardial infarction ever (score 2 if > 1)	❑ 1❑ 2
Cardiomyopathy (ventricular dysfunction)	❑ 1
Valvular disease (diastolic murmur or systolic murmur > 3/6)	❑ 1
Pericarditis for 6 months, or pericardiectomy	❑ 1
Peripheral vascular	
Claudicatio for 6 months	❑ 1
Minor tissue loss (pulp space)	❑ 1
Significant tissue loss ever (e.g. loss of digit or limb) (score 2 if > 1 site)	❑ 1 ❑ 2
Venous thrombosis with swelling, ulceration, or venous stasis	❑ 1
Gastrointestinal	
Infarction or resection of bowel below duodenum, spleen, liver, or gall bladder ever, for cause any (score 2 if > 1 site)	❑ 1 ❑ 2
Mesenteric insufficiency	❑ 1
Chronic peritonitis	❑ 1
Stricture or upper gastrointestinal tract surgery ever	❑ 1
Musculoskeletal	
Muscle atrophy or weakness	❑ 1
Deforming or erosive arthritis (including reducible deformities, excluding avascular necrosis)	❑ 1
Osteoporosis with fracture or vertebral collapse (excluding avascular necrosis)	❑ 1
Avascular necrosis (score 2 if > 1)	❑ 1 ❑ 2
Osteomyelitis	❑ 1
Skin	
Scarring chronic alopecia	❑ 1
Extensive scarring or panniculum other than scalp and pulp space	❑ 1
Skin ulceration (excluding thrombosis) for > 6 months	❑ 1
Premature gonadal failure	❑ 1
Diabetes (regardless of treatment)	❑ 1
Malignancy (exclude dysplasia) (score 2 if > 1 site)	❑ 1 ❑ 2

Statistical analysis.

A retrospective analysis of the prospectively collected data was performed. Comparison of continuous data with a parametric distribution was performed using t-test or one-way analysis of variance (ANOVA) with Bonferroni's post hoc analysis; continuous data with a non-parametric distribution were analysed using the Wilcoxon's test and the Kruskal-Wallis test. Comparison of categorical data was performed using chi-squared test (Fischer's exact test) or the McNemar test for dependent samples. Linear and logistic regression were used to assess the relationship between organ damage accrual during the follow-up and durations of remission or LLDAS, as well as between damage and different levels of remission.

In the analysis of the possible predictors of organ damage accrual the following variables were considered in the univariate analysis: age, gender, disease duration, SDI at baseline, type of organ involvement and type of flare during the follow-up, number of flare during the follow-up, antiphospholipid antibody (aPL Abs) profile, aPL Abs syndrome, therapy including immunosuppressants, corticosteroids, antimalarials, cumulative dose of corticosteroids, and durations and types of remission and LLDAS. Factors with a $p<0.2$ at univariate analysis were entered into the multivariate model. Backward stepwise multivariate logistic regression was performed with damage accrual considered as a dichotomous dependent variable (i.e. final SDI increased or not increased during the follow-up), with significance set at 5%. Spearman' correlation was used to assess the relationship between the duration of remission or LLDAS and damage accrual. Analyses were performed by the SPSS software for Windows (version 22.0 for Study 1 and 23.0 for Study 2, SPSS, Chicago, IL).

Study 3

Study cohort.

We used our Lupus Database which includes patients recruited in Padua Lupus cohort between 1970 and 2018 and prospectively followed.

Patients attending our outpatient clinic diagnosed with SLE were included in the study if they fulfilled the following inclusion criteria: 1) at least four of the revised American College of Rheumatology (ACR) Classification Criteria for SLE;[82] 2) diagnosis of SLE between 1990 and 2018; 3) treatment with at least one immunosuppressant (IS) over the disease course; 4) at least one visit in 2017 or 2018.

Methods.

Data collection.

Data collected included demographics (age, gender, year of diagnosis), age at SLE onset, disease duration, disease manifestations over the patients' disease course, type and reason of ISs use (i.e. manifestation requiring ISs), current and previous therapies (including glucocorticoids, cumulative prednisone dose, antimalarials, ISs, and biologics), date of initiation of first IS and date of discontinuation of last IS, time to achieve remission, level of remission (clinical or complete), reason of IS discontinuation, duration of remission at IS discontinuation, maintenance therapy after IS discontinuation (antimalarials and/or low dose GCs), type of IS discontinued, flare occurrence after IS withdrawal, type of flare (including renal, musculoskeletal, skin, haematological, serosal, neuropsychiatric, and vasculitic flares), flare-free survival (defined as lag-time between IS discontinuation and a subsequent flare, i.e. remission duration without IS), damage accrual at the end of follow-up.

Data regarding serological activity were recorded at each visit, including C3 and C4 serum levels, antinuclear antibodies, and anti-double stranded (ds) DNA antibodies.

Definitions.

Clinical manifestations were defined using ACR definitions.[83] Disease activity was assessed using the SLEDAI-2K index and flares according to SELENA-SLEDAI flare index.[84] Damage was measured by the Systemic Lupus Collaborating Clinics (SLICC) Damage Index (SDI).

IS discontinuation was defined as complete withdrawal of any IS. Reasons of IS discontinuation were classified as 1) remission or 2) poor compliance/side effects.

IS discontinuation because of inefficacy and starting of a new IS (switching) were not considered as IS discontinuation at all.

Clinical remission was defined as *clinical* SLEDAI–2K=0 in patients on a stable immunosuppressive and/or antimalarial therapy and/or on prednisone ≤5 mg/day; complete remission was defined as SLEDAI–2K=0 (no clinical and serological activity) in patients on a stable immunosuppressive and/or antimalarial therapy who were prednisone-free [49].

Statistical analysis.

A retrospective analysis of the prospectively collected data was performed. Comparison of continuous data with a parametric distribution was performed using t-test; continuous data with a non-parametric distribution were analysed using the Wilcoxon's test. Comparison of categorical data was performed using chi-squared test (Fischer's exact test).

Logistic regression was used to identify possible predictors of flare occurrence after IS withdrawal. Factors with a $p<0.2$ at univariate analysis were entered into the

multivariate model. Backward stepwise multivariate logistic regression was performed, with significance set at 5%.

In the analysis of flare-free survival after IS discontinuation, we performed Cox-regression analysis for the identification of possible predictors of better outcome in terms of flare-free survival.

Analyses were performed by the SPSS software for Windows (version 24.0, SPSS, Chicago, IL).

SUMMARY OF RESULTS

Study 1

Among 462 consecutive patients who were evaluated, 293 patients (63.1%) fulfilled inclusion criteria.

Reasons for exclusion were: non-Caucasian ethnicity (15, 3.2%); SLE diagnosis before 1990 (72, 15.6%); remission lasting more than 12 months at study entry (37, 8.0%); less than 3 visits per year during the follow-up (18, 3.8%); incomplete data records (12, 2.6%); lost-to-follow-up (15, 3.2%).

During the 7-year follow-up, 27 patients (9.2%) achieved 1-consecutive year remission, 47 (16.0%) 2-consecutive year remission, 45 (15.4%) 3-consecutive year remission, 26 (8.9%) 4-consecutive year remission, and 113 (38.6%) ≥5-consecutive year remission. Conversely, 35 patients (11.9%) had never been in remission.

Clinical features and treatment according to the durations of remission are reported in Table 6 and Table 7, respectively.

Table 6. Disease manifestations in 293 patients included in the study cohort. Cumulative disease manifestations since the diagnosis and disease manifestations during the follow-up according to the duration, but irrespective to the level of remission achieved are reported.

No. patients	Total study cohort 293	Unremitted disease 35	1-year remission 27	2-year remission 47	3-year remission 45	4-year remission 26	≥5-year remission 113	p
Lagtime onset-diagnosis*, months, mean ±SD	16.6±21.04	18.5±22.2	13.2±10.8	16.4±24.5	18.6±29.3	10.7±5.2	15.7±15.2	n.s.
ANA positivity, No. (%)	293 (100)	35 (100)	27 (100)	47 (100)	45 (100)	26 (100)	113 (100)	n.s.
Anti-dsDNA Ab, No. (%)	246 (83.9)	32 (91.4)	26 (96.3)	40 (85.1)	39 (86.7)	24 (92.3)	85 (75.2)	0.028
Anti-U1RNP Ab, No. (%)	77 (26.2)	16 (45.7)	9 (33.3)	12 (25.5)	10 (22.2)	5 (19.2)	25 (22.5)	0.05
Low C3/C4 levels, No. (%)	257 (87.7)	29 (82.9)	26 (96.3)	41 (87.2)	41 (91.1)	22 (84.6)	98 (86.5)	n.s.
aPL Abs, No. (%)	96 (32.8)	11 (31.4)	11 (40.7)	13 (27.7)	19 (42.2)	7 (26.9)	35 (31.0)	n.s.
Disease manifestations overall								
Constitutional symptoms§, No. (%)	241 (82.2)	28 (80.0)	23 (85.2)	41 (87.2)	38 (86.4)	23 (88.5)	88 (79.3)	n.s.
Skin rashes, No. (%)	182 (62.1)	29 (82.9)	15 (63.6)	32 (68.1)	29 (64.4)	12 (46.2)	65 (57.1)	0.001
Arthritis/Inflammatory arthralgias,No. (%)	218 (74.4)	27 (77.1)	22 (81.5)	35 (74.5)	36 (80)	19 (73.1)	79 (70.5)	n.s.
Serositis, No. (%)	76 (25.9)	14 (40)	11 (40.7)	15 (31.9)	11 (24.49	5 (19.2)	20 (17.9)	0.035
Glomerulonephritis, No. (%)	168 (57.3)	25 (71.4)	21 (77.8)	28 (59.6)	28 (62.2)	14 (53.8)	52 (46.4)	0.019

NP manifestations, No. (%)	39 (13.3)	5 (14.3)	4 (14.8)	9 (19.1)	8 (17.8)	3 (11.5)	10 (8.9)	n.s.
Vasculitis, No. (%)	34 (11.6)	9 (25.7)	4 (14.8)	7 (14.9)	6 (13.3)	3 (11.5)	5 (4.5)	0.021
Haematological involvement, No.(%)	111 (37.8)	14 (40.0)	17 (63.0)	20 (42.6)	18 (40.0)	12 (46.2)	30 (26.5)	0.028
aPL syndrome, No. (%)	40 (13.7)	6 (17.1)	5 (18.5)	4 (8.5)	6 (13.3)	4 (15.4)	15 (13.3)	n.s.
Manifestations during follow-up								
Skin rashes, No. (%)	74 (25.2)	25 (71.4)	12 (44.4)	14 (29.8)	9 (20.0)	6 (23.1)	8 (7.1)	<0.001
Arthritis, No. (%)	82 (28.0)	12 (34.3)	16 (59.3)	21 (44.7)	18 (40.0)	7 (26.9)	8 (7.1)	<0.001
Serositis, No. (%)	17 (5.8)	3 (8.6)	2 (7.4)	5 (10.6)	5(11.1)	1 (3.8)	1 (0.9)	n.s.
Nephritis, No. (%)	103 (35.2)	20 (57.1)	18 (66.7)	23 (48.9)	20 (44.4)	9 (34.6)	13 (11.5)	<0.001
NP manifestations, No. (%)	22 (7.5)	4 (11.4)	2 (7.4)	8 (17.0)	6 (13.3)	1 (3.8)	1 (0.9)	0.005
Vasculitis, No. (%)	17 (5.8)	6 (17.1)	2 (7.4)	6 (12.8)	2 (4.4)	0 (0)	1 (0.9)	0.002
Haematological involvement, No.(%)	49 (16.7)	8 (22.9)	9 (33.3)	13 (27.7)	9 (20.0)	8 (30.8)	2 (1.8)	<0.001

ANA, anti-nuclear antibodies; Anti-dsDNA Ab, anti double-stranded DNA antibodies; C3/C4, complement fractions; aPL, antiphospholipid antibodies, NP, neuropsychiatric; SD, standard deviation.
*Lagtime onset-diagnosis: defined as the time between the onset of the first American Rheumatism Association (ARA) criterion and the medical diagnosis
§constitutional symptoms: fever, anorexia, lymphadenopathy or unintentional weight loss due to SLE
p values refer to ANOVA test for continuous variables and chi square test (5 degrees of freedom) for dichotomous variables freedom (comparison among unremitted, one-, two-, three-, four- and five or more year remitted patients).

Table 7. Treatments over the patients' disease course in the study cohort and according to the duration, but irrespective of the level of remission achieved during the follow-up. Number (%) of patients are reported

Treatments	Total study cohort 293	Unremitted disease 35	1-year remission 27	2-year remission 47	3-year remission 45	4-year remission 26	≥5-year remission 113	p
Hydroxycloroquine	266 (90.8)	30 (85.7)	25 (92.6)	42 (89.4)	39 (86.7)	25 (96.2)	105 (92.9)	n.s.
Methylprednisolone iv	169 (57.7)	25 (71.4)	20 (74.1)	32 (68.1)	21 (46.7)	19 (73.1)	52 (46.0)	0.002
Cumulative average PDN dose ≥180 mg/mo	78 (26.6)	21 (60)	16 (59)	16 (34)	10 (22.2)	4 (15.4)	11 (9.7)	0.02
Immunosuppressives	205 (69.9)	33 (94.3)	23 (85.1)	43 (91.5)	36 (80.0)	17 (65.4)	53 (46.9)	<0.001
Azathioprine	97 (33.1)	19 (54.3)	12 (44.4)	23 (48.9)	10 (22.2)	6 (23.1)	27 (23.0)	0.001
Mycophenolate	127 (43.3)	23 (65.7)	18 (66.7)	25 (53.2)	25 (55.6)	10 (38.5)	26 (23.0)	<0.001
Cyclosporin A	54 (18.4)	10 (28.6)	10 (37.0)	12 (25.5)	7 (15.6)	5 (19.2)	10 (8.8)	0.004
Cyclophosphamide	81 (27.6)	16 (45.7)	9 (33.3)	15 (31.9)	14 (31.1)	5 (19.2)	22 (19.5)	0.042
Methotrexate	47 (18.4)	10 (28.6)	10 (37.0)	10 (21.3)	7 (15.6)	2 (7.7)	8 (7.1)	0.001
Rituximab	22 (7.5)	8 (22.9)	5 (18.5)	7 (14.9)	1 (2.2)	0 (0)	1 (0.9)	<0.001
Belimumab	30 (10.2)	10 (28.6))	8 (29.6)	6 (12.8)	4 (8.9)	1 (3.8)	1 (0.9)	<0.001
Iv Ig	12 (4.1)	4 (11.4)	3 (11.1)	2 (4.3)	0 (0)	2 (7.7)	1 (0.9)	0.016

Iv: intravenous; PDN, prednisone; mo, month; Ig, immunoglobulins.
p values refer to chi-square test with 5 degrees of freedom (comparison among unremitted, one-, two-, three-, four- and five or more year remitted patients).

Demographic characteristics and damage are summarized in Table 8.

Table 8. Demographic characteristics and damage in total study cohort and in subgroups of patients according to the durations of remission (irrespective of the level of remission) achieved during the follow-up (upper part); damage accrual according to the levels of remission for each duration of remission (lower part).

	Total study cohort 293	Unremitted disease 35	1-year remission 27	2-year remission 47	3-year remission 45	4-year remission 26	≥5-year remission 113	p
Age in 2009, years, mean ±SD	39.1 ±12.5	38.6±9.4	31.8±11.1	41.4±13.2	42.2±12.8	42.4±14.8	40.7±12.1	0.048
Female, No. (%)	253 (86.3)	28 (80%)	21 (77.8)	41 (87.2)	37 (82.2)	24 (92.3)	102 (90.3)	n.s.
SLE duration at 2015, years, mean ±SD	11.1±7.8	19.4±7.6	15.5±8.5	19.8±7.8	19.2±8.7	17.5±7.8	20.0±7.3	n.s.
SDI at study entry	0 (0-8)	0 (0-3)	0 (0-3)	1 (0-4)	0 (0-4)	0 (0-4)	0 (0-8)	n.s.
SDI at the end of study	1 (0-9)	2 (0-7)	2 (0-6)	2 (0-5)	1 (0-7)	1 (0-5)	0 (0-9)	<0.001
Median (range) SDI increase	1 (0-6)	1 (0-6)	1 (0-3)	1 (0-3)	1 (0-3)	0 (0-3)	0 (0-3)	<0.001
Increase in SDI, No. patients (%)	151 (51.5)	31 (88.6)	22 (81.5)	31 (66.0)	23 (51.1)	13 (50.0)	31 (27.4)	<0.001
Increase in SDI ≥2, No. patients (%)	51 (17.4)	14 (40.0)	12 (44.4)	11 (23.4)	6 (13.3)	4 (15.3)	4 (3.6)	<0.001
Median (range) SDI increase according to the level of remission:								
-Clinical remission on-CS	-	-	1 (0-3)	1 (0-3)	1 (0-2)	1 (0-3)	1 (0-3)	-
-Clinical remission off-CS			1 (0-2)	1 (0-2)	0 (0-3)	0 (0-3)	0 (0-1)	
-Complete remission			2 (1-3)	0 (0-0)	0 (0-2)	0 (0-0)	0 (0-2)	
			p=n.s.	p=n.s.	p=n.s.	p=n.s.	p<0.001	

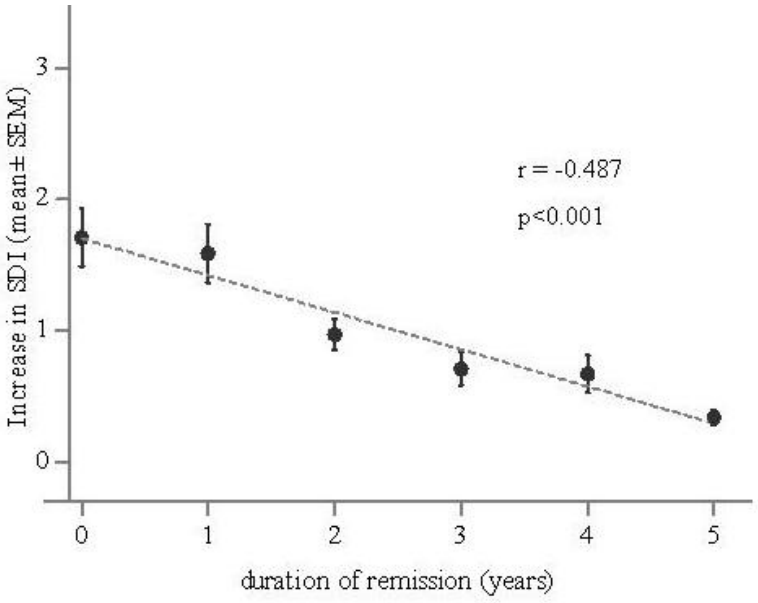
SD, standard deviation; SDI, SLICC/ACR damage index; CS, corticosteroids.

Upper part: variables with a parametric (age, SLE duration) and non-parametric distribution (SDI) were analysed by ANOVA test and Wilcoxon-test for multiple comparisons with 5 degrees of freedom, respectively.

Lower part: the comparison of SDI among patients with different levels of remission (complete remission, clinical remission on- and off-corticosteroids) for each duration of remission was performed by Wilcoxon-test for multiple comparisons with 2 degrees of freedom.

SDI significantly increased during the 7-year follow-up in the study cohort ($p<0.001$): the median SDI increase was higher in unremitted patients compared with patients in remission for 2 ($p=0.005$), 3 ($p<0.001$), 4 ($p=0.001$), and ≥5 consecutive years ($p<0.001$). Conversely, median change in SDI was similar in 1-year remitted and unremitted patients. An inverse correlation between the duration of remission and damage accrual was observed (Figure 2).

Figure 2. Correlation between the duration of remission (irrespective of the level of remission) and damage accrual in the study cohort. Damage accrual (y axis) is expressed as mean ± standard error of mean (SEM). Points stand for mean SDI increase in patients with different durations of remission.



Among patients achieving 1-, 2-, 3-, and 4-consecutive year remission, damage accrual was similar in patients with complete remission, clinical remission off-corticosteroids or on-corticosteroids (Table 8). Among patients achieving ≥5-year remission, patients in clinical remission off-corticosteroids or in complete remission accrued less damage ($p<0.001$) than patients in clinical remission on-corticosteroids. However, when analyzed altogether, no difference among the three levels of remission in terms of damage progression was found.

At multivariate analysis, a remission lasting ≥2 consecutive years was protective against damage (Table 9); conversely, a cumulative prednisone dose ≥180 mg/month, APS, vasculitis ever, number of flare/patient/year, disease duration, and age were independent predictors of new damage (Table 9).

Table 9. Multivariate analysis: independent risk factors and protective factors for damage accrual over the follow-up.

	B	p value	OR	95% CI	
≥ 5 consecutive year remission	-3.128	**<0.001**	**0.044**	**0.012**	**0.159**
4 consecutive year remission	-2.135	**0.005**	**0.118**	**0.027**	**0.519**
3 consecutive year remission	-2.158	**0.001**	**0.116**	**0.031**	**0.436**
2 consecutive year remission	-1.479	**0.028**	**0.228**	**0.061**	**0.850**
1 year remission	-0.054	0.946	0.947	0.199	4.520
Disease duration	0.056	**0.008**	**1.057**	**1.014**	**1.102**
Antiphospholipid antibody syndrome	1.708	**0.001**	**5.517**	**2.092**	**14.546**
Cumulative average PDN dose ≥180 mg/month	1.143	**0.013**	**3.136**	**1.276**	**7.707**
Number of flare/patient/year	2.171	**0.019**	**8.769**	**1.692**	**45.449**
Vasculitis ever	1.134	**0.044**	**3.107**	**1.030**	**9.379**
Cyclophosphamide	0.619	0.067	1.857	0.957	3.607
SDI at baseline	0.065	0.665	1.067	0.795	1.433
Age	0.040	**0.002**	**1.041**	**1.015**	**1.068**
Constant	-2.054	0.024	0.128		

Significant variables are given in bold; 95% CI, 95% confidence interval; PDN, prednisone; SDI, SLICC/ACR damage index.

Study 2

Two-hundred ninety-three patients were considered in the study. During the 7-year follow-up, 33 patients (11.3%) achieved 1-consecutive year LLDAS, 43 (14.7%) 2-consecutive year LLDAS, 39 (13.3%) 3-consecutive year LLDAS, 31 (10.6%) 4-consecutive year LLDAS, and 109 (37.2%) ≥5-consecutive year LLDAS. Conversely, 38 patients (13.3%) had never been in LLDAS.

Clinical features and treatment in the cohort, according to the duration of LLDAS achieved, are reported in Table 10 and Table 11, and demographic characteristics and damage in Table 12.

Table 10. Disease manifestations in 293 patients included in the study cohort. Cumulative disease manifestations since the diagnosis (ever) according to the duration of LLDAS achieved are reported.

	Never in LLDAS 38 (13.3)	1-year LLDAS 35 (11.9)	2-year LLDAS 47 (16.0)	3-year LLDAS 39 (13.3)	4-year LLDAS 25 (9.5)	≥5-year LLDAS 109 (37.2)	p
Number (%) of patients	38 (13.3)	33 (11.3)	43 (14.7)	39 (13.3)	31 (10.6)	109 (37.2)	
ANA positivity, No. (%)	38 (100)	35 (100)	47 (100)	39 (100)	25 (100)	109 (100)	n.s.
Anti-dsDNA Ab, No. (%)	33 (86.8)	30 (90.9)	36 (83.7)	36 (92.3)	26 (83.9)	85 (77.3)	n.s.
Low C3/C4 levels, No. (%)	33 (86.8)	29 (87.9)	37 (86.0)	36 (92.3)	28 (90.3)	94 (86.2)	n.s.
aPL Abs, No. (%)	11 (28.9)	14 (42.4)	11 (25.6)	18 (46.2)	9 (29.0)	33 (30.3)	n.s.
Disease manifestations ever							
Skin rashes, No. (%)	29 (76.3)	24 (68.6)	32 (68.1)	23 (59.0)	15 (60.0)	59 (54.1)	n.s.
Arthritis/Inflammatory arthralgias,No. (%)	31 (81.6)	28 (84.8)	33 (76.7)	28 (71.8)	23 (74.2)	75 (68.8)	n.s.
Serositis, No. (%)	15 (39.5)	10 (30.3)	15 (34.9)	12 (30.8)	9 (29.0)	15 (13.8)	0.008
Glomerulonephritis, No. (%)	22 (58.0)	23 (69.7)	29 (67.4)	23 (59.0)	16 (51.6)	55 (50.5)	n.s.
NP manifestations, No. (%)	5 (13.2)	7 (21.2)	10 (23.3)	6 (15.4)	3 (9.7)	8 (7.3)	n.s.
Vasculitis, No. (%)	11 (30.0)	1 (3.0)	7 (16.3)	6 (15.3)	4 (12.9)	5 (4.6)	0.001
Haematological involvement,No.(%)	15 (39.5)	17 (51.5)	20 (46.5)	14 (36.0)	11 (35.5)	34 (31.2)	n.s.
aPL syndrome, No. (%)	5 (12.8)	6 (18.2)	5 (11.6)	5 (12.8)	6 (19.4)	13 (11.8)	n.s.

ANA, anti-nuclear antibodies; Anti-dsDNA Ab, anti double-stranded DNA antibodies; C3/C4, complement fractions; aPL, antiphospholipid; NP, neuropsychiatric.
p values refer to ANOVA test for continuous variables and chi square test (5 degrees of freedom) for dichotomous variables

Table 11. Treatments during the seven-year follow-up in the study cohort, according to the duration of LLDAS achieved.

	Never in LLDAS 38	1-year LLDAS 35	2-year LLDAS 47	3-year LLDAS 39	4-year LLDAS 25	≥5-year LLDAS 109	p
Hydroxycloroquine, No. (%)	30 (78.9)	32 (87.5)	41 (87.2)	34 (87.2)	25 (100)	95 (87.2)	n.s.
Methylprednisone iv, No. (%)	15 (39.4)	7 (17.2)	12 (25.5)	6 (15.3)	5 (20.0)	7 (5.5)	<0.05*
Cumulative average PDN dose ≥180 mg/month, No. (%)	19 (50)	14 (40)	14 (29.8)	10 (25.6)	5 (20)	11 (10.1)	<0.001
Immunosppressives							
Azatioprine, No. (%)	5 (21.1)	13 (37.4)	14 (29.8)	13 (33.3)	8 (32.0)	17 (15.6)	n.s.
Mycophenolate, No. (%)	21 (55.2)	23 (65.7)	28 (59.5)	20 (51.3)	7 (28.0)	8 (7.3)	0.003
Cyclosporin A, No. (%)	17 (44.7)	4 (11.4)	5 (10.6)	4 (10.2)	0 (0)	0 (0)	0.005
Cyclophosphamide, No. (%)	2 (5.3)	1 (2.8)	3 (6.4)	1 (2.6)	3 (12)	0 (0)	n.s.
Methotrexate, No. (%)	17 (44.7)	9 (25.7)	0 (0)	0 (0)	0 (0)	0 (0)	<0.001
Rituximab, No. (%)	6 (15.7)	2 (5.7)	3 (6.4)	4 (10.2)	1 (4.0)	2 (1.8)	n.s.
Belimumab, No. (%)	1 (2.6)	4 (11.4)	4 (8.5)	5 (12.8)	3 (12.0)	13 (11.9)	n.s.
Iv immunoglobulins, No. (%)	2 (5.2)	1 (2.8)	0 (0)	1 (2.6)	0 (0)	1 (1.1)	n.s.
Anti-aggregant/anti-coagulant therapy, No. (%)	14 (36.8)	17 (48.6)	21 (44.6)	13 (33.3)	14 (48.0)	53 (48.6)	n.s.

PDN, prednisone; iv: intravenous.
p values refer to chi-square test with 5 degrees of freedom (comparison among patients never in LLDAS, in LLDAS for one-, two-, three-, four- and five or more year).
* 4-year LLDAS vs ≥5-year LLDAS n.s.

Damage more frequently occurred in ocular (17%), neuropsychiatric (16.4%), musculoskeletal (14%), and renal (11.3%) domains, followed by cutaneous (9.5%) and cardiovascular (6.9%) domains; damage in other organs and malignancies was more rarely observed.

Table 12. Demographic features and damage increase according to the durations of LLDAS achieved during the follow-up.

	Never in LLDAS	1-year LLDAS	2-year LLDAS	3-year LLDAS	4-year LLDAS	≥5-year LLDAS	p
Number of patients (%)	38 (13.3)	33 (11.3)	43 (14.7)	39 (13.3)	31 (10.6)	109 (37.2)	
Age at recruitment, mean ±SD years	39.9±13	36.6±16.4	40.0±13.0	42.4±11.7	41.9±11.2	39.8±12.5	n.s.
Female, No. (%)	31 (83.7)	27 (81.8)	39 (90.7)	31 (79.5)	25 (80.6)	100 (91.7)	n.s.
SLE duration at baseline, mean ±SD years	10.8±7.2	10.2±7.1	11.8±6.7	11.4±6.1	10.2±6.3	12.0±5.4	n.s.
SDI at recruitment, mean±SD	0.87±1.53	0.68±0.90	0.97±1.14	0.75±0.94	0.48±0.85	0.42±0.88	n.s.

SDI increase, mean±SD	1.67±1.35	1.20±0.90	0.91±0.89	0.90±0.87	0.45±0.69	0.27±0.49	<0.05*
Increase in SDI≥1, No. patients (%)	32 (84.2)	26 (78.8)	27 (62.8)	23 (58.9)	13 (41.9)	30 (27.5)	<0.05§
Increase in SDI≥2, No. patients (%)	17 (44.7)	9 (27.3)	11 (25.6)	10 (25.6)	2 (8.0)	2 (1.8)	<0.05°

LLDAS: lupus low disease activity state; SLE, systemic lupus erythematosus; SD, standard deviation; SDI, SLICC/ACR damage index. P values refer to ANOVA test with 5 degrees of freedom.

* never in LLDAS vs1-year LLDAS, p=n.s.; never in LLDAS vs 2-year LLDAS, p=0.001; never in LLDAS vs 3-year LLDAS, p=0.001; never in LLDAS vs 4-year LLDAS, p<0.001; never in LLDAS vs ≥5-year LLDAS, p<0.001.

§ never in LLDAS vs1-year LLDAS, p=n.s.; never in LLDAS vs 2-year LLDAS, p=0.02; never in LLDAS vs 3-year LLDAS, p=0.01; never in LLDAS vs 4-year LLDAS, p<0.001; never in LLDAS vs ≥5-year LLDAS, p<0.001.

° never in LLDAS vs1-year LLDAS, p=n.s.; never in LLDAS vs 2-year LLDAS, p=n.s.; never in LLDAS vs 3-year LLDAS, p=n.s.; never in LLDAS vs 4-year LLDAS, p<0.001; never in LLDAS vs ≥5-year LLDAS, p<0.001.

The mean SDI increase was lower in patients achieving LLDAS for at least 2 consecutive years compared with patients never in LLDAS (p<0.001), whereas it was similar in patients with 1-year LLDAS and in those who had never been in LLDAS.

The proportion of patients with damage accrual progressively decreased as the duration of LLDAS increased, both in terms of SDI increase ≥1 or ≥2 (high damage accrual) (Table 12).

In a multivariate logistic regression model including LLDAS and baseline characteristics, a LLDAS lasting 2, 3, 4, 5 or more consecutive years was protective against damage, whereas age, the use of mycophenolate, a higher cumulative prednisone dose and antiphospholipid antibody syndrome were independent predictors of damage accrual (Table 13).

Table 13. Multivariate analysis: protective factors and risk factors for damage accrual over the follow-up.
Baseline predictors and LLDAS.

	OR	95% CI	p value
≥ 5 consecutive year LLDAS	**0.071**	**0.023- 0.217**	**<0.001**
4 consecutive year LLDAS	**0.122**	**0.034- 0.443**	**0.001**
3 consecutive year LLDAS	**0.252**	**0.075- 0.842**	**0.025**
2 consecutive year LLDAS	**0.279**	**0.085- 0.920**	**0.036**

1 year LLDAS	0.899	0.232- 3.480	0.877
Age	**1.038**	**1.015- 1.062**	**0.001**
Mycophenolate	**2.173**	**1.062- 4.446**	**0.034**
Antiphospholipid antibody syndrome	**4.008**	**1.648- 9.749**	**0.002**
Cumulative PDN dose, grams	**1.016**	**1.003- 1.033**	**0.049**

Variables entered in the multivariate analysis were duration of LLDAS (categorical variable with 6 levels), and the following baseline characteristics: age, disease duration, SDI, mean SLEDAI-2K, skin involvement, vasculitis, use of mycophenolate and antimalarials, mean prednisone dose, antiphospholipid antibody syndrome, cumulative prednisone dose.

Significant variables are given in bold; OR, odds ratio; 95% CI, 95% confidence interval; LLDAS: lupus low disease activity state; PDN, prednisone.

As predictors of LLDAS attainment are concern, patients with a higher SLEDAI-2K ($p<0.001$), PGA>1 ($p<0.001$), joint and skin involvement ($p=0.01$) and those treated with methotrexate ($p=0.013$), cyclosporine ($p=0.016$) and a higher prednisone dose ($p<0.001$) were less likely to achieve a LLDAS lasting 2 or more consecutive years.

The multivariate logistic regression model including baseline characteristics showed that a higher cumulative prednisone dose, skin involvement and PGA higher than one were the three most significant negative predictors of LLDAS attainment (OR 0.302, 95% CI 0.151-0.605, p=0.001; OR 0.333, 95% CI 0.148 -0.748, p=0.008; OR 0.093, 95% CI 0.151 -0.605, p=<0.001, respectively)".

We also evaluated the proportion of patients attaining the LLDAS who concomitantly fulfilled the criteria for remission.

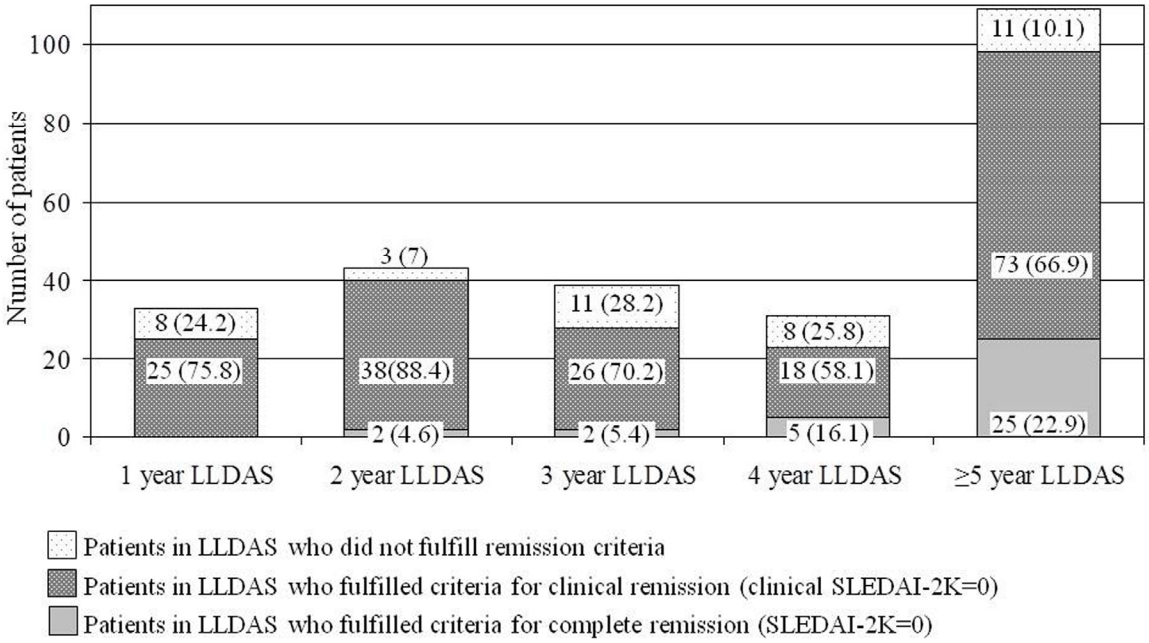
Among the 255 patients achieving the definition of LLDAS for at least 1 year during the follow-up, 246 patients (96.5%) satisfied the definition of remission for the same length of time.

Overall, 214 patients (83.9%) experienced a remission being as long as their LLDAS, suggesting a high overlap exists between the two conditions (Figure 3).

Interestingly, remitted patients accrued significantly less damage than did other LLDAS patients (0.59± 0.78 vs. 0.90± 0.89, p=0.021).

Only one death was observed during follow-up, thus the evaluation of the relationship between LLDAS achievement and mortality was not possible.

Figure 3. Proportion of patients with different durations of LLDAS who fulfilled or not the definition of a remission lasting at least the same number of consecutive year(s). Number of patients (%) are reported



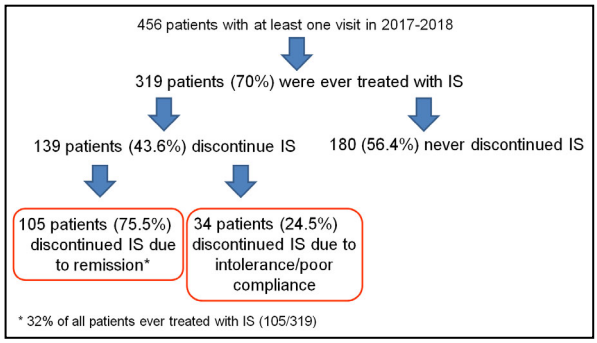
Study 3

In June 2018, Padua Lupus cohort includes 521 patients seen at least twice between 2008 and 2018. Among them, 456 patients (402 female, 88.2%, mean±SD age 45±13 years, mean±SD disease duration 174±9 years) attended at least one visit between 1st June, 2017 – 30th June, 2018 and were considered for inclusion in the study.

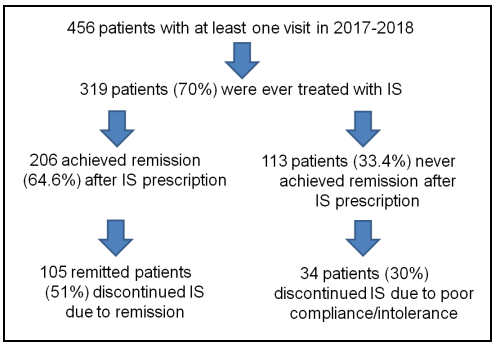
319 out of 456 (70%) were ever treated with immunosuppressants (IS) and were included in the study (Figure 4a and 4b).

Figure 4. Flow-chart for patient inclusion in the study, according to discontinuation of immunosuppressants (a) and according to achievement of remission (b)

a



b



IS, immunosuppressants;

The reasons of the first use of IS were nephritis in 150 patients (47.0%), arthritis in 50 (15.7%), haematological abnormalities in 17 (5.3%), skin rash in 13 (4.1%), skin rash and arthritis in 7 (2.2%), neuroSLE in 6 (1.9%), vasculitis in 4 (1.3%), and serositis in 2 (0.6%); 70 patients (21.9%) had a multisystemic involvement, i.e. an involvement in more than two organs.

Notably, the majority of patients took more than one IS along their disease course, while more than one third of patients (135/319, 42.3%) took only one type of IS over the disease history. Overall, 209 patients were treated with mycophenolate mofetil (MMF), 136 with azathioprine (AZA), 95 with cyclophosphamide (CYC), 84 with methotrexate (MTX), 65 with cyclosporine A (CsA), and 10 with leflunomide.

Immunosuppressant discontinuation

Among the 319 patients ever treated with IS, 139 patients (43.6%) discontinued IS: MMF in 61 (43.8%), AZA in 35 (25.2%), MTX in 21 (15.1%), CsA in 13 (9.3%), CYC in 6 (4.3%) and leflunomide in 3 (2.1%). Mean±SD follow-up duration after IS withdrawal was 91.4±71.9 months (range 6-372). Mean±SD age at IS withdrawal was 35.4±11.4 years.

Mean±SD duration of treatment with the last IS in years was 5.5±3.7 for MMF, 6.5±5.0 for AZA, 5.7±5 for MTX, 5.3±2.3 for CsA, 1.3±0.55 for CYC.

Notably, 105/139 patients (75.5%) discontinued ISs due to remission and 34/139 (24.5%) due to poor compliance or side effects, all of them unremitted at the time of IS discontinuation.

Mean follow-up duration after IS discontinuation was 95.52±69.72 (range 12-324) and 65.54±34.8 (range 12-108) months in remitted and unremitted patients, respectively.

Side effects led to IS discontinuation in 22 patients, as summarized in Table 14A.

Table 14B reports side effects which caused IS discontinuation according to the type of IS.

Table 14. Reason for IS withdrawal in patients who discontinued IS due to side effects.
17A. Types of side effects which led to Is discontinuation
17B. Side effects which led to Is discontinuation according to the type of IS discontinued.

A

Side effects	Number of patients(%)
Anemia	6 (27.3)
Leucopenia	3 (13.6)
Infections	4 (18.2)
Neoplasia	2 (9.1)
GI intolerance	7 (31.8)

B

	Types of side effects, N.
MMF	Anemia, 2; Infections, 1; Neoplasia, 1; Leucopenia, 1; GI intolerance, 3.
AZA	Anemia, 3; Infections, 1; Leucopenia, 2; GI intolerance, 1.
CsA	Anemia, 1; Infections, 1; GI intolerance, 1.
MTX	Infections, 1: Neoplasia, 1; GI intolerance, 2.

GI, gastro-intestinal.

Patients who discontinued IS due to poor compliance/side effects required the maintenance or the increase in GCs daily dose at the time of IS discontinuation.

Among the 105 patients who discontinued IS due to remission, 26 patients (24.8%) experienced a flare after a median (range) of 57 (6 to 264) months after IS discontinuation. Conversely among patients with poor compliance or side effects, 23 (67.7%) relapsed (OR, 95% CI 6.9, 2.94 - 16.59, p<0.001, Figure 5) after a median (range) follow-up of 8 (1-72) months (p=0.009, Figure 6).

Figure 5. Proportion of patients who experienced a flare after IS discontinuation according to the reason for withdrawal.

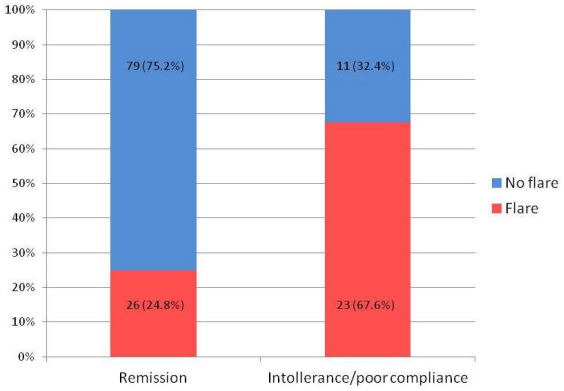
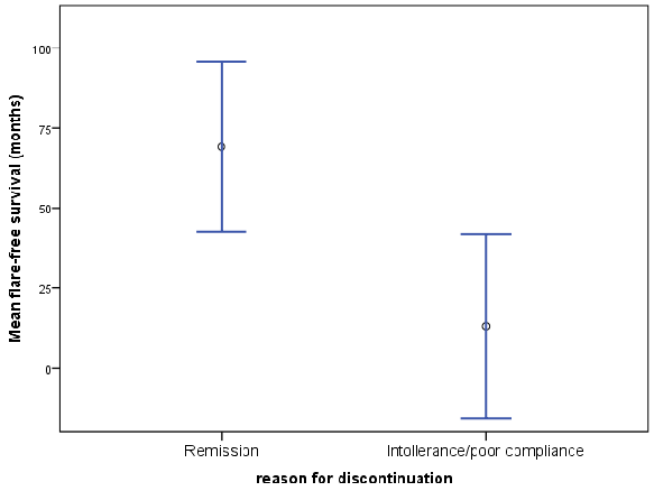
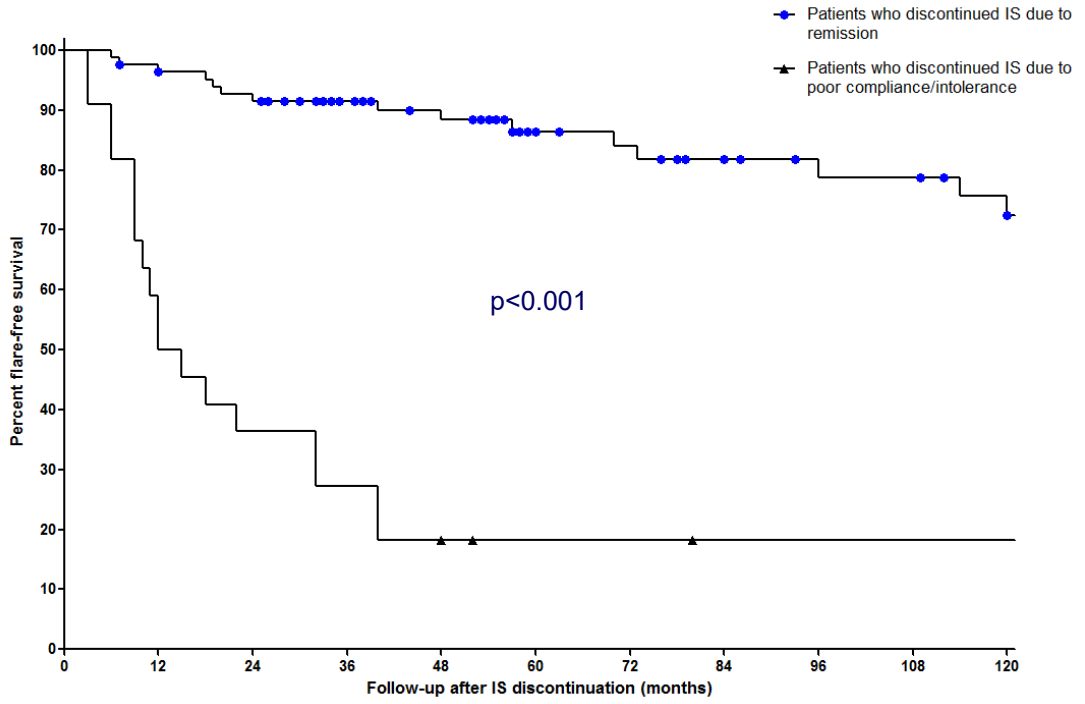


Figure 6. Mean flare-free survival according to the reason for IS discontinuation. Bars refer to 95% CI of means.



By Cox-regression analysis we found that flare-free survival rate was higher when immunosuppressive therapy was discontinued due to remission than when it was due to poor compliance/side effects (p<0.001, Figure 7).

Figure 7. Flare-free survival in patient who discontinued IS due to remission or due to poor compliance/side effects along the follow-up after IS discontinuation.



Using Wilcoxon's rank sum test no difference between patients who flared and did not flare in terms of damage progression was found; indeed SDI was numerically lower (but not statistically significant) in patients who did not flare (median SDI, range 1, 0-6) compared with those who flared (1, 0-8).

IS discontinuation in remitted patients

Among remitted patient, the reasons to start IS therapy were nephritis (68 patients, 64.8%), arthritis (12 patients, 11.4%), skin rash (6 patients, 5.7%), haematological involvement (5 patients, 4.7%), neuroSLE (3 patients, 2.9%), vasculitis (3 patients, 2.9%), multisystemic involvement (8 patients, 7.6%).

In Table 15 the characteristics of remitted patients who discontinued ISs, overall and according to the occurrence of a flare after IS discontinuation are reported.

The proportion of patients who flare up during the follow-up and the time to flare did not differ among patients with different lupus manifestations.

Table 15. Characteristics of remitted patients who discontinued IS, overall and according to the occurrence of a subsequent SLE flare. Data are expressed as mean±SD or number (%).

	Patients			P values
	Remitted (105)	With flare (26)	Without flare (79)	
Female, N (%)	93 (88.6)	22 (84.6)	71 (89.9)	n.s.
Age at SLE onset, years	25±9	22±8	26±9	0.028
Age at 2018, years	44±11	40±11	45±11	0.035
SLE duration at 2018, years	19.5±9.2	18.3±8.7	19.1±9.4	n.s.
SDI at 2018	1.1±1.48	0.96±1.50	1.15±1.50	n.s.
SLE duration at IS discontinuation, years	10.3±8.2	8.8±6.4	11.2±8.6	n.s.
SLE duration at remission, years	5.2±6.1	4.3±4.2	6.4±7.4	n.s.
Remission duration at IS discontinuation, months	42±29	28.4±16.6	46.1±31.2	<0.001
Remission lasting at IS discontinuation > 2 consecutive years, N (%)	66 (63)	12 (46.1)	54 (68.4)	0.027
Time to achieve remission, months	32±43	26±35	34±45	n.s.
Complete remission, N (%)	17 (16.2)	4 (15.4)	13 (16.5)	n.s.
HCQ therapy after IS discontinuation, N (%)	84 (80)	15 (57.7)	68 (87.3)	0.015
Reason for IS therapy				
Lupus Nephritis, N (%)	68 (64.8)	14 (53.8)	54 (68.3)	n.s.
Skin involvement, N (%)	6 (5.7)	2 (7.7)	4 (5.1)	
Arthritis, N (%)	12 (11.4)	5 (19.2)	7 (8.9)	
Haematological involvement, N (%)	5 (4.7)	2 (7.7)	3 (3.9)	
Neuropsychiatric involvement, N (%)	3 (2.9)	1 (3.9)	2 (2.5)	
Vasculitis, N (%)	3 (2.9)	0	3 (3.7)	
Multisystemic involvement, N (%)	8 (7.6)	2 (7.7)	6 (7.7)	
Type of IS discontinued				
Mycophenolate, N (%)	48 (45.7)	10 (38.4)	38 (48.1)	n.s.
Azathioprine, N (%)	30 (28.6)	4 (15.4)	26 (32.9)	<0.05*
Methotrexate, N (%)	14 (13.3)	8 (30.7)	6 (7.6)	<0.05°
Cyclosporine, N (%)	7 (6.7)	3 (11.5)	4 (5.0)	n.s.
Cyclophosphamide, N (%)	6 (5.7)	1 (3.9)	5 (6.3)	n.s.

SLE, systemic lupus erythematosus; SDI, SLICC/ACR damage index; HCQ, hydroxychloroquine; IS, immunosuppressant; Multisystemic: involvement of more than 2 organs requiring IS therapy.

*p value refers to the comparison MTX vs. all other IS; ° p value refers to the comparison AZA vs. MTX (p=0.026) and AZA vs. Cyclosporine (p=0.036)

MMF was discontinued in 48 patients (45.7%), AZA in 30 (28.6%), MTX in 14 (12.4%), CsA in 7 (6.7%), CYC in 6 (5.7%); 1 patient (0.9%) discontinued Leflunomide. Mean±SD treatment duration was 6.7±4.8 years overall, and it did not differ between patients with (5.1±4.5 years) and without flares (7.0±4.4 years).

Mean±SD duration of treatment with last IS was 5.2±2.7 years for MMF, 6.6±5.0 years for AZA, 5.6±4.6 years for MTX, 5.0±2.5 for CsA, and 1.2±0.5 years for CYC.

Patients who discontinued MTX were more likely to flare up during the follow-up compared with patients treated with AZA (p=0.026), and they had a shorter flare-free survival compared with patients treated with AZA (p=0.001) and Cyclosporine (p=0.002). Patients who discontinued MTX were more likely to flare up during the follow-up compared with patients treated with other IS (p=0.026).

Patients who discontinued AZA had a similar flare rate compared to patients treated with MM or CYC and had a lower flare rate compared with those who discontinued Cyclosporine (p=0.030) and MTX (p=0.022).

Notably, the mean time needed to achieve remission was similar in patients who did or did not flare, while the duration of remission at the time of IS discontinuation was longer in patients who did not develop a flare over the follow-up (Table 15, p<0.001).

Disease flare was due to nephritis in 7 cases (26.9%), arthritis in 4 (15.4%), haematological involvement in 4 (15.4%), skin involvement in 2 (7.7%), serosal involvement in 2 (7.7%), vasculitis in 2 (7.7%), and multisystemic involvement in 1 case (3.9%). In 4 cases (15.4%) the type of flare was not known.

Interestingly, 17/105 patients (16.2%) were in complete remission at the time of IS discontinuation; the proportion of patients in complete remission did not differ

between patients who developed a flare (4/26, 15.4%) and those who did not flare (13/79, 16.5%). Patients in complete and clinical remission had a similar flare-free survival (54.0±17.8 and 72.6±70.9, respectively).

There was no difference in flare rate among different manifestations accounting for IS prescription. (Table 16)

Table 16 proportion of patients who flared according to disease manifestation accounting for IS prescription.

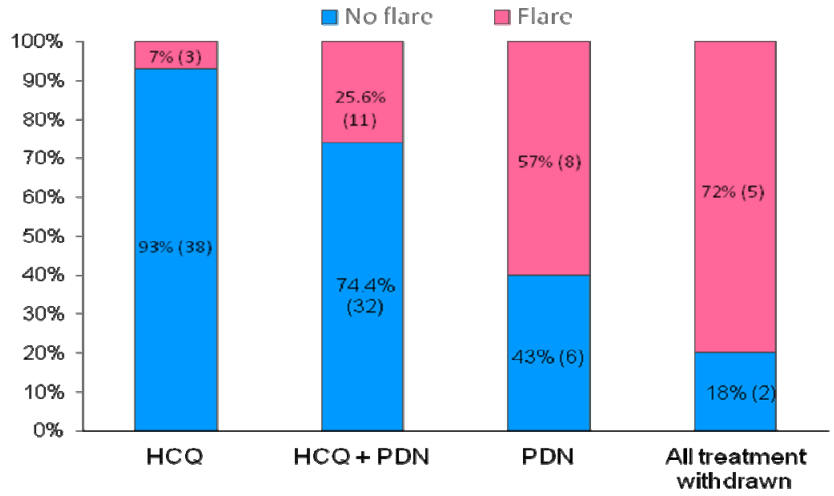
	Number of patients with flare (%)
Lupus nephritis	14/68 (20.6)
Skin involvement	2/6 (22.2)
Arthritis	5/12 (41.6)
Neuropsychiatric SLE	1/3 (33.3)
Heamatological involvement	2/5 (40)
Multisystemic disease	2/8 (25)
Vasculitis	0/3

After IS discontinuation, the majority of patients were treated with hydroxychloroquine (HCQ) alone (41 patients, 39.0%) or in combination with low-dose GCs (43 patients, 40.0%); 13 patients (12.4%) received only low-dose GCs and 8 patients (7.6%) discontinued all treatment after achieving remission.

Interestingly, the frequency of flare was significantly lower in patients treated with HCQ (16/84, 19%) than in patients HCQ-free (10/21, 47.6%, OR 0.22, 95% CI 0.07 - 0.73, p=0.015). Patients on HCQ alone experienced a similar flare rate compared to patients on HCQ plus low dose prednisone (4/41, 9.8% vs. 11/43, 25.5%).

Patients treated with GCs alone experienced a similar flare-rate compared to patients who discontinued all treatment at the time of IS withdrawal (8/14, 57.1% vs. 5/7, 71.4%,) and they were at higher risk of flare compared with other remitted patients (OR 5.8, 95% CI 1.48 - 22.88, p=0.013) (Figure 8).

Figure 8. Proportion of patients who flared according to maintenance therapy after IS discontinuation



HCQ, hydroxychloroquine; PDN; prednisone

In a model including potential predictors of flare occurrence, HCQ maintenance therapy after IS discontinuation was the strongest independent protective factor against flare at multivariate logistic regression analysis (Table 17).

Table 17. Multivariate logistic regression analysis: protective factors of flare occurrence after IS withdrawal.

	OR	95% CI	p value
HCQ therapy after IS discontinuation	0.243	0.070- 0.842	0.026
Duration of remission at IS discontinuation	0.870	0.824- 0.996	0.040
Number of previous ISs	1.553	0.921- 2.619	0.099

IS; immunosuppressant; HCQ, hydroxychloroquine.
Other variables in the model: disease duration at IS discontinuation, reason of IS therapy.

With the aim of evaluating the adjunctive effect of remission duration over HCQ maintenance therapy, we analysed the effect of different durations of remission on flare rate (Table 18). We found that the protective effect of HCQ against flare progressively increased as the duration of remission lengthened (Table 18B). In particular, >1, >2, >3 years remitted patients on antimalarials had a decrease in the risk of flare by 69%, 81% and 86%, respectively, compared with patients

antimalarial-free or with a shorter duration of remission. Thus, the longer the duration of remission on HCQ, the lower the risk of flare.

Table 18. Effect of antimalarial maintenance therapy and different durations of remission before IS discontinuation on flare occurrence.

18A. Risk of flare occurrence in remitted patients according to the duration of remission before IS discontinuation; 18B. Risk of flare occurrence in remitted patients on antimalarials according to the duration of remission before IS discontinuation.

18A

Duration of remission	OR	95% CI	p value
> 1 year remission	0.82	0.255 - 2.628	n.s.
> 2 year remission	0.35	0.132 – 0.953	0.027
> 3 year remission	0.24	0.079 – 0.722	0.005
> 4 year remission	0.14	0.030 – 0.0645	0.004

18B

Duration of remission in patients on HCQ	OR	95% CI	p value
> 1 year remission	0.31	0.115 – 0.859	0.032
> 2 year remission	0.19	0.068 – 0.569	0.003
> 3 year remission	0.14	0.039 – 0.534	0.002
> 4 year remission	0.14	0.032 – 0.686	0.008

HCQ, hydroxychloroquine.

None of the patients in ≥5-consecutive year remission at the time of IS discontinuation (26 patients) developed a flare (p=0.003).

No predictive factors of a longer flare-free survival were identified by multivariate Cox-regression analysis.

We separately analyzed data of non-renal remitted patients (37 patients, 35.2%). Even in this group, HCQ intake was associated with a lower flare rate (OR, 95% CI 0.048, 0.005 to 0.503, p=0.002).

Damage accrual

Median damage accrual was similar in patients who discontinued (139 patients, SDI 1, range 0-8) or not IS (180 patients, SDI 1, range 0-9) after adjusting for age, disease duration and cumulative prednisone dose.

Among the 139 patient who discontinued IS during the follow-up, SDI was numerically lower (but not statistically significant) in patients who did not flare (median SDI, range 1, 0-6) compared with those who flared (1, 0-8).

Among the 105 remitted patients who discontinued IS, no difference in damage progression between remitted patients who flared and did not flare after IS withdrawal was found at the end of follow-up; indeed, the median (range) SDI was 1 (0-5) and 1 (0-6), respectively.

DISCUSSION

One of the major challenges in managing SLE patients is to control disease activity and prevent irreversible organ damage, which impact on patient quality of life and mortality.[70]

Many studies [5, 7, 9, 11-34, 49] assessed the association between active disease and the accrual of organ damage, underlining that risk factors of organ damage include disease activity, flares, and disease severity.[11,12,15,16,20-23,33]

In Study 1, we observed an increase in damage over time, confirming that cumulative corticosteroid dose, antiphospholipid antibody syndrome, and number of flares are major predictors of damage accrual.

Nevertheless, few data are available regarding the predictive role of remission on damage accrual. In Study 1 we analysed the prevalence of different duration of remission, i.e. 1, 2, 3, 4, and 5 or more years, addressing the impact of different length of remission on damage.

We demonstrated that a remission shorter in time than 5 years (in particular, 2 or more consecutive years) had a positive impact on damage. Notably, we observed that the longer the duration of remission, the lower the damage accrual during the follow-up. One-year remitted patients had less damage accrual compared with unremitted patients, but this was not statistically significant.

Thus, based on our result, the shortest duration of remission one should seek to achieve in order to hinder the organ damage accrual should be two consecutive years.

When we considered the predictive factors for damage accrual in multivariate analysis, we observed that remission was an independent protective factor for damage accrual, even after adjustment for clinical manifestations. Thus, it seems that

remission exerts a protective effect on damage accrual irrespective of SLE manifestations which have previously characterized the disease course. This finding has a great prognostic implication, meaning that remission can be an ideal target of SLE management.

In our study, a 2, 3, or 4 year complete remission or clinical remission off corticosteroids did not show to have lower damage accrual compared to clinical remission on corticosteroids. This might be due to the slight difference in corticosteroid cumulative dose taken by the patients in remission on corticosteroids compared with those in remission off corticosteroids during the same period (4 years at most). Conversely, patients in ≥5-year clinical remission off-corticosteroids or complete remission accumulated less total damage and less corticosteroid-dependent damage than patients in clinical remission on-corticosteroids. Thus, a longlasting corticosteroid therapy (at least 5 years), even at very low doses, can represent a risk factor for damage.

Nevertheless, in multivariate linear regression analysis, the amount of damage accrual was inversely associated with the duration of remission irrespective of the level of remission achieved. This means that the duration of the remission has an impact on damage higher than the level of remission achieved.

An implication of this observation could be that a short-lasting low-dose corticosteroid therapy, aimed at achieving or maintaining remission, can be a safe therapeutic strategy in term of risk of damage accrual.

The study in Study 1 has some limitations. We did not include non-Caucasian patients, and our findings can not be generalized to other ethnic groups. Non-SLE related risk factors for organ damage were not considered in the analysis. In particular, we did not include hypertension, which has been proven to be associated

with higher damage accrual and we also lack data on psychosocial factors which may influence damage progression. [12,13,34].

In a treat-to-target approach, the concept of LDA has been attractive in the last few years and an increasing number of papers evaluated its effect on damage accrual.

The effect of LLDAS on damage was firstly evaluated by Franklyn et al.,[68] who found that among patients followed for an average time of 3.9 years, those who spent more than 50% of their follow-up in LLDAS accrued significantly less organ damage than other patients. This finding was confirmed in the study by Tsang-A-Sjoe et al.[69]

However, the protective effect on damage of different durations of LLDAS, the shortest duration of LLDAS resulting in decreased damage progression as well as the overlap between LLDAS and remission have not been evaluated yet.

In Study 2 we demonstrated that 2 consecutive years was the shortest duration of LLDAS associated with a decrease in damage progression. Interestingly, since the mean follow-up of patients in Franklyn's study was 3.9 years,[68] the 50% of their follow-up time corresponds approximately to 2 years.

In our study, we considered different periods of time (1, 2, 3, 4, 5 or more consecutive years) instead of the proportion of follow-up spent in LLDAS because such an analysis has more practical implications and can be used in clinical practice to identify patients at higher/lower risk of developing new organ damage.

In addition, we assessed the proportion of patients in LLDAS fulfilling the criteria of remission. In fact, the definition of LLDAS used in the study by Franklyn et al.,[68] included patients who were in low disease activity but also patients in true remission. This is different from what was defined for RA and other rheumatic diseases, where the definitions of low disease activity and remission do not overlap (e.g., disease

activity score (DAS)-28 in RA identifies remitted patients when DAS-28 is lower than 2.6, while patients in low disease activity have a DAS-28 between 2.6 and 3.2).

In our cohort we found that the great majority of patients in LLDAS were, actually, in remission. This result is relevant since it is likely that the protective effect of remission on new organ damage significantly contributed to the lower damage accrual observed in the LLDAS group. Unfortunately, the number of patients included in our cohort does not allow an explorative analysis on the effect of LLDAS after exclusion of patients in remission.

Recently Petri M et al.[71] were able to demonstrated that patients achieving LLDAS *but not remission* for at least 50% of their follow-up were protected against damage, although in a lower extent when compared with remitted patients.

Based on these very recent findings, we can conclude that nowadays remission and LDA are not rarely observed in SLE, are associated with better prognosis and they thus can be considered suitable outcomes in the management of SLE patients.

In this context, the question of how to manage SLE patients in remission is drawing more and more attention and has become an unmet need in SLE.

In clinical practice, it is common that remitted patients continue the treatment which yielded the remission for an indefinite period of time with the aim of preventing flares. The recommended length of IS use after achieving clinical remission has not exactly been defined yet; indeed, available guidelines and recommendations for the management of SLE underline the importance of GCs progressive tapering until withdrawal [87-89], but the recommended duration of maintenance therapy with IS after remission achievement has not been agreed-upon, ranging from 3 to 6.5 years [88-92]. Thus, the length of IS use after achieving remission largely depends on the physician experience and believe [93].

Our results showed that when IS are withdrawn due to remission achievement, the risk of relapses is reasonably low; in fact, less than one quarter of our remitted patients flared during the follow-up, confirming previous findings in renal SLE.[81]

We demonstrated that maintenance therapy with antimalarials was the strongest independent protective factor against disease flare. This finding is in keeping with recent recommendations, as antimalarials have been regarded as standard of care in all SLE patients unless controindicated, including patients with LN, where antimalarials are proposed as an additional therapy.[92]

Few data on the role of antimalarials in maintaining remission are available [94-97]. The Canadian Hydroxychloroquine Study Group conducted the unique randomized control trial on HCQ withdrawal in 1991, demonstrating that the discontinuation of HCQ was associated with a significant increase in the risk of flare.

More recently, the analysis of whole-blood levels of HCQ showed that therapeutic HCQ levels (>500 ng/ml) tended to be associated with no occurrence of disease flares.[98-100]

The protective role of HCQ therapeutic levels has recently been proven in patients with ISN/RPS class III, IV or V LN. Among patients who achieved remission, those who experienced a renal flare during the follow-up had significantly lower average HCQ levels (0.59 vs 0.81mg/L, p=0.005) compared with those remaining in remission.[99]

We also found that the duration of remission exerted an additional protective effect on the risk of flare occurrence over antimalarial intake, since the longer the remission duration on HCQ, the lower the risk of flare. Notably, being on HCQ and in remission for at least 2 consecutive years was able to reduce by 81% the risk of

flare. The decrease in the risk of flare was even higher in >3 year remitted patients (86%).

In our cohort there was no difference in terms of flare occurrence and flare-free survival between patients in clinical or complete remission at the time of IS withdrawal, meaning that the achievement of negative anti-dsDNA and normal C3 and C4 did not exert an additional protective effect over clinical remission on flare occurrence.

An interesting result of our study is the great difference in the flare-free survival rate between patients who discontinued IS due to remission and those who discontinued due poor compliance/side effects. These findings highlight the importance of adherence to therapy. Notably, misinterpretation of disease flares that are instead related to poor compliance may lead to unnecessary therapeutic changes.[100]

Study 3 on IS discontinuation has some relevant strengths: we studied a large cohort of patients, prospectively and regularly followed-up by the same team; to our knowledge this is the first study aimed at identifying predictors of disease relapse after IS discontinuation in SLE since the validation of the definition of remission. In addition, our analyses of flare predictors included some clinical variables never accounted for in other studies (i.e. remission and related variables). However, our study has also some limitations: we retrospectively analyzed data prospectively collected in a single centre; the number of patients who discontinued IS was relatively low.

In conclusion, a remission and a low disease activity state of two consecutive years could be considered clinically meaningful treat-to target goals in the management of SLE.

Notably, the absence of SLE activity is more relevant than low-dose corticosteroid intake in hindering damage progression in the short-term, while in the long-term even low-dose corticosteroids can contribute to organ damage. Thus, the withdrawal of glucocorticoids should be considered in all SLE remitted patients.[101]

The withdrawal of IS seems not to be applicable to all remitted patients and requires a personalized approach, taking into consideration patient's characteristics, including remission duration and maintenance therapy.

In this regard, long-term background therapy with antimalarials should be advised in all SLE patients.

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