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Are there distinct clinical and pathological features distinguishing Idiopathic from Drug-Induced Subacute Cutaneous Lupus Erythematosus? A European retrospective multicenter study

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Abstract:	BBackground: Clinical and pathological criteria to distinguish drug-induced subacute lupus erythematosus (DI-SCLE) from idiopathic (I-SCLE) are controversial. Objective: Aim of the survey was a retrospective analysis of a consistent number of iatrogenous and idiopathic SCLE cases, by means of clinical and histopathological investigation. Methods: Eleven European University Dermatology Units collected all diagnosed cases from January 2000 to December 2016. Board certified dermatopathologists reviewed the histopathologic specimens. Statistical analysis included Student's t-test, exact test of goodness-of-fit, Fisher's test, Cochran-Mantel-Haenszel for repeated measures. Results: Out of 232 patients, 67 (29%) belonged to the DI-SCLE group. Patients with DI-SCLE were significantly older and complained more systemic symptoms than those

	with I-SCLE. No statistical differences were found for presentation pattern or serology, while histopathology showed for I-SCLE a significant association of mucin deposition (p=0, 000083) and direct immunofluorescence positivity for granular IgM, C3 deposits on the basement membrane zone (p=0, 0041), and of leukocytoclastic vasculitis (p=0, 0018) for DI-SCLE. Limitations: This is a retrospective study. Conclusion: An integrated clinical and immunopathological evaluation is useful to differentiate I-SCLE from DI-SCLE. Older age at onset and more frequent systemic symptoms characterize DI-SCLE. Mucin deposition and immunofluorescence findings are found in I-SCLE, while leukocytoclastic vasculitis in DI-SCLE.

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Cover letter

Dear Editor,

I thank so much the reviewer for the interest in our study, and great effort to improve it.

Ref.: Ms. No. JAAD-D-18-00992R3
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MAJOR REVISION: The answers to the reviewer comments and changes to the manuscript are listed in the following tables for clarity. Changes have been highlighted in yellow in the manuscript.

Reviewer #3: The authors' revisions improved the manuscript. However, following are follow-up questions to further clarify the statistical methods used in the manuscript.

Reviewer question	Author answer
Line 153: goodness-of-fit test is typically used to compare the observed values to the expected values from a statistical model. Which model was used to estimate the expected values?	line 153: the implicit model used by the exact test of goodness-of-fit to estimate the expected values is that of equiprobability.
Line 154: It appears that each variable was measured only once per patient. Which variables were measured repeatedly and analyzed using Cochran-Mantel-Haenzsel test?	line 154: all variables were measured once, but we used Cochran-Mantel-Haenszel test because we wanted to test if there were consistent differences in proportion across the repeated locations.
Tables 2 and 5: What are the values in the column labeled Bonferroni? It's not clear. (The Bonferroni adjusted critical values should be the same for all hypotheses included in tests, and should be less than 0.05.)	tables 2 and 5. According to your suggestions we revised the tables and we eliminated the last column reporting Bonferroni's correction, that we maintained only in text. We added symbols referring to p-values, to increase table's readability.
Line 172: "suggested a statistical significance" might be misleading, because the Bonferroni adjustment, the differences are not significant.	The sentence has been changed to: "The Fisher's exact test ~~suggested a statistical significance in favor of~~ showed a more frequent presence in DI-SCLE for annular distribution with bullae (p=0.023), pityriasis-like (p=0.02), and erythema multiform-like pattern (p=0.039); however, the Bonferroni correction for multiple comparisons (eight hypothesis test), gave an adjusted-critical value of 0.0062, and differences were not significant.
Line 160: 32 women and 14 men do not add up to 67 patients.	We are sorry for the embarrassing mistake. The women were 53, not 32, as clearly expressed in table 1. The sentence has been corrected: "The study cohort (Table 1) consisted of 232 patients, 174 women, and 58 men divided into group 1, which included 67 patients with DI-SCLE (53 woman, 14 men;

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	mean age, 53.3 years), and group 2 with the remaining 165 I-SCLE patients (121 women, 44 men; mean age, 40.6 years)."

I and my coauthors Federica Guicciardi, MD, Angelo Valerio Marzano, MD, Simona Tavecchio, MD, Giampiero Girolomoni, MD, Chiara Colato MD, Axel Patrice Villani, MD, Jean Kanitakis, MD, Christina Mitteldorf, MD, Rosanna Satta, MD8, Bernard Cribier, MD, Laurence Gusdorf, MD, Maria Teresa Rossi, MD, Piergiacomo Calzavara-Pinton, MD, Isabel Bielsa, MD, Maria Teresa Fernandez-Figueras, MD, Werner Kempf, MD, Giorgio Filosa, MD, Franco Rongioletti, MD, state that the article is original, never submitted elsewhere.

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Kind regards

Laura Atzori

Capsule summary

- Distinguishing drug-induced from idiopathic subacute lupus erythematosus is challenging, as their clinical, histopathological and laboratory presentation can be similar.
- Our results show that older age at onset and leukocytoclastic vasculitis are more commonly seen in drug-induced cases, while mucin deposition and positive immunofluorescence are clues to the idiopathic form.

TITLE PAGE

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Title: Are there distinct clinical and pathological features distinguishing Idiopathic from Drug-Induced Subacute Cutaneous Lupus Erythematosus? A European retrospective multicenter study

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Abstract

Background: Clinical and pathological criteria to distinguish drug-induced subacute lupus erythematosus (DI-SCLE) from idiopathic (I-SCLE) are controversial.

Objective: Aim of the survey was a retrospective analysis of a consistent number of iatrogenous and idiopathic SCLE cases, by means of clinical and histopathological investigation.

Methods: Eleven European University Dermatology Units collected all diagnosed cases from January 2000 to December 2016. Board certified dermatopathologists reviewed the histopathologic specimens. Statistical analysis included Student's t-test, exact test of goodness-of-fit, Fisher's test, Cochran-Mantel-Haenszel for repeated measures.

Results: Out of 232 patients, 67 (29%) belonged to the DI-SCLE group. Patients with DI-SCLE were significantly older and complained more systemic symptoms than those with I-SCLE. No statistical differences were found for presentation pattern or serology, while histopathology showed for I-SCLE a significant association of mucin deposition (p=0, 000083) and direct immunofluorescence positivity for granular IgM, C3 deposits on the basement membrane zone (p=0, 0041), and of leukocytoclastic vasculitis (p=0, 0018) for DI-SCLE.

Limitations: This is a retrospective study.

Conclusion: An integrated clinical and immunopathological evaluation is useful to differentiate I-SCLE from DI-SCLE. Older age at onset and more frequent systemic symptoms characterize DI-SCLE. Mucin deposition and immunofluorescence findings are found in I-SCLE, while leukocytoclastic vasculitis in DI-SCLE.

Introduction

Drug-induced lupus erythematosus (DI-LE) is an autoimmune syndrome occurring in the setting of chronic drug exposure and resolving after discontinuation of the culprit drug (1-5). Persistence despite long-term removal of the drug is sometimes observed, and referred as unmasked LE, which support the view that the drug works as a triggering agent on the individual predisposition to develop the autoimmune disorder (6).

DI-LE can be classified as systemic (SLE), subacute cutaneous (SCLE), chronic cutaneous lupus (7), which is similar to idiopathic LE. The most frequent variant is drug-induced SCLE (DI-SCLE), with 70–80% of cases, firstly recognized in 1985 in association with hydrochlorothiazide (8). The list of drugs has evolved over time to include several commonly used categories, such as antihypertensive, antidepressants, and proton pump inhibitors (7-11), but the association for many active substances remains anecdotal. In fact, the causality assessment following standard pharmacovigilance scores (12), usually concludes for a possible association, because highly probable or certain association require information on re-exposure (rechallenge). The administration of the same drug supposed to have induced an adverse effect is not usually performed for safety and ethical reasons (13). In fact, this approach potentially exposes the patient to the risk of more severe reactions, which is acceptable only for irreplaceable life-saving medications, and with the explicit consent of the patient.

Considering the limitations of the causality assessment, definition of distinctive features for the drug-induced SCLE, not expressed in the idiopathic disease (I-SCLE) might increase the force of the association. Recently, Marzano et al. (14) suggested some clinical and immunological

hallmarks that could be used to identify DI-SCLE. However, the study did not confirm the previously suggested histopathologic criteria for DI-SCLE (15).

The present multicenter observational study aimed to widen the collection of medical and histopathologic records, further investigating whether clinical, immunological, or pathological differences exist between DI-SCLE and I-SCLE.

Materials and Methods

Eleven European Dermatology units retrospectively reviewed all cases of SCLE diagnosed from January 1, 2000 to December 31, 2016. The Coordinating center, responsible for all data collection and analysis was the Dermatology Clinic of Cagliari University, which submitted the study to the local Ethical Independent Committee of the AOU of Cagliari for approval (code Prot. PG/2018/6063). Local IRB approval was not necessary for the limited number of cases, completely anonymous, collected from each participating Institution.

Clinical data

Each center assigned a code to the cases, such that only the recruiting center could identify the source of the data recorded on the shared electronic sheet. Inclusion criteria were: (I) clinical evidence of SCLE, (II) histopathological findings consistent with SCLE, and (III) a dermatologist's diagnosis of SCLE. An additional criterion (IV) was the absence/presence of drug exposure (history of new drug introduction within 6 months). Patients without a skin biopsy were excluded. Cases were divided into DI-SCLE and I-SCLE groups on the base of the IV criterion. The causality drug assessment followed the Jones algorithm (16), a global introspection method chosen for being adaptable to the retrospective nature of the study: enough detailed to be

conclusive, even with few information available. It consists of 4 questions with yes or no answers, progressing from unrelated to related adverse events: 1- plausibility of time relation between drug exposure and manifestations onset; 2- exclusion of alternative explanation for the events; 3- evaluation of the response to the interruption and 4- reintroduction of the suspected drug (dechallenge and rechallenge).

Histopathologic analysis

The pathology slides were assigned a study number, corresponding to the patient code, but blinded for the diagnosis, such that the dermatopathologists were unware of the clinical data. The following changes were evaluated: 1, epidermal atrophy/acanthosis; 2, hyper-orthokeratosis; 3, vacuolar degeneration at the basal-cell epidermal layer; 4, epidermal keratinocyte necrosis/apoptosis; 5, pattern and density of lymphocytic infiltration considering (a) superficial, junctional, and perivascular infiltrate (interface reaction pattern), (b) Periadnexal involvement, and (c) superficial and deep involvement; 6, presence of eosinophils; 7, mucin deposition; 8, leukocytoclastic vasculitis.

Direct immunofluorescence (DIF) was performed on the same site of the diagnostic biopsy, on lesional skin. From the medical chart, the nature of the immune deposits (IgG/IgA/IgM/C3), localization (epidermis or basement membrane zone [BMZ]/sub epidermal blood vessels), and pattern (granular/linear) were retrieved.

Statistical analysis

Categorical variables were expressed as numbers and percentage means. The Student's t-test was used for continuous variables; the exact test of goodness-of-fit for single nominal variables compared to the expected values estimated on the basis of the implicit equiprobability model;

the Fisher's exact test for dual nominal variables, and Cochran–Mantel–Haenszel test to analyze if there were consistent differences in proportion across the repeated locations. Adjustment for multiple comparison was applied by mean of the Bonferroni test, to avoid false positives due to chance. A p-value <0.05 was considered significant.

Results

The study cohort (Table 1) consisted of 232 patients, 174 women, and 58 men divided into group 1, which included 67 patients with DI-SCLE (53 woman, 14 men; mean age, 53.3 years), and group 2 with the remaining 165 I-SCLE patients (121 women, 44 men; mean age, 40.6 years). Cases of DI-SCLE represented 28.98% of the whole cohort, with a mean age at onset one decade over I-SCLE patients, supported by Student's t test (p 0.007).

Clinical feature analysis

In the overall cohort (Table 2), almost one-third of the patients presented with typical annular-polycyclic or papulosquamous lesions, followed by annular polycyclic and papulosquamous features overlap (14%); other atypical presentations, such as annular with malar rash, annular with bullae, annular with erythema multiforme, pityriasis-like and toxic epidermal necrolysis-like were less frequent.

When the two groups were analyzed separately, the proportion of annular polycyclic or papulosquamous patterns remained similar, while atypical variants were more frequent in DI-SCLE. The Fisher's exact test showed a more frequent presence in DI-SCLE of annular distribution with bullae (p=0.023), pityriasis-like (p=0.02), and erythema multiform-like pattern

(p=0.039); however, the Bonferroni correction for multiple comparisons (eight hypothesis test), gave an adjusted-critical value of 0.0062, and differences were not significant.

As shown in Table 2, lesions were distributed in sun-exposed areas in 101 patients (49.5%), while 65 patients (31.9%) also presented with widespread lesions on covered areas. The DI-SCLE group showed a prevalence of widespread lesions, supported by Fisher's exact test (p=0.017), but not after the Bonferroni correction (seven hypothesis test), that adjusted the critical value to 0.0071.

Systemic symptoms were present in 53 patients (27%) (Table 3), with prevalence in DI-SCLE patients supported by highly significant Fisher's exact test.

Arthralgia/arthritis was the most frequent symptom in both groups (12.1% in I-SCLE, 25.4% in DI-SCLE), followed by Raynaud phenomenon, and non-specific symptoms such as fever and malaise. The DI-SCLE group had a greater number of reported xerostomia (11.9%) and nephropathy (6%) compared to the I-SCLE group. However, a comparison of the single symptoms showed no significance because of the small numbers in both groups.

The search of autoantibodies was the most variable finding among the participating centers, with limited number of patients tested (Table 3). The most performed testing was for antinuclear antibody (ANA) titer with a positivity slightly in favor of DI-SCLE (82.4% instead of 68.6%), and extractable nuclear antigens (ENA) screening, which did not show any difference among the groups. Analysis for anti-Ro/SSA was performed in 158 patients overall, with a slight prevalence in DI-SCLE (69.6% positive versus 42.1% of I-SCLE). Anti-histone was tested in 85 patients, with similar positivity in both groups. Neither the Fisher's exact test nor the Cochran–Mantel–Haenszel test showed significant differences between the two groups.

Culprit drugs included 76 molecules, with contemporary exposure to two/four active substances in some patients (Table 4). Diuretics were the most represented class (11.8%), followed by biologics, cardiologics, and chemotherapies (10.5%). The top single active substance was hydrochlorothiazide, followed by leflunomide, estro-progestinics, and terbinafine. The application of the Jones' algorithm revealed four (5%) active principles (carboplatin, gemcitabine, lamotrigine, desloratadine) with a certain association, while a causal relation was probable for 25 drugs (33%) and possible for the remaining substances (62%).

Histopathologic analysis and direct immunofluorescence findings

No differences between the two groups (Table 5) were found except for epidermal acanthosis (p=0.024), keratinocyte necrosis/apoptosis (p=0.017), cytoid bodies (p=0.018), mucin deposition (p=0.000005), and leukocytoclastic vasculitis (p=0.00013). However, adjustment for eleven hypothesis test (Bonferroni) gave a critical value of 0.0045, and the statistical significance was confirmed only for mucin deposition (odds ratio [OR] 2.28) in favor of I-SCLE, and leukocytoclastic vasculitis (OR: 0.118) in favor of DI-SCLE.

Data on direct immunofluorescence were available in 133 of 232 cases (57%) (Table 5), and the most relevant difference was the combined presence of C3c and IgM at the dermo-epidermal junction in 52.2% of I-SCLE patients *vs* 20.9% of DI-SCLE. The finding was statistically significant, with an OR of 1.093 in favor of I-SCLE.

Discussion

The association between drug intake and the occurrence of SCLE has been increasingly reported, and poses the problem of the risk's evaluation for the general population, exposed to

certain active substances or categories of drugs. A recent Denmark survey estimated that DI-SCLE accounts for 20% of all SCLE cases (17), and other authors suggested that the condition might occur more frequently than that reported (9). The present multicenter study largely confirms these findings, as 29% of our patients fulfilled the criteria for DI-SCLE, suggesting that for every four patients with SCLE, one possibly has a drug-induced disease. The literature concerning the criteria to identify DI-SCLE as a separate entity from I-SCLE is still unclear. A systematic review concluded that DI-SCLE does not differ clinically, histopathologically, or immunologically from I-SCLE (15). However, Marzano et al (14) observed that the age at disease onset was higher in patients with DI-SCLE compared with those with I-SCLE, and our data concurred, with a decade between patients with I-SCLE and DI-SCLE, and a significant p-value (Table 1). This finding has been hypothesized to be consistent with the increasing frequency and number of co-medications with age (15). Other suggested criteria include a more heterogeneous widespread clinical presentation, involving areas usually spared by I-SCLE (14), with bullous and erythema multiform-like patterns, as well as the presence of SLE-like malar rash, purpura, and necrotic-ulcerative lesions (14, 18-22). In contrast, the prevalence of systemic involvement was considered characteristic of I-SCLE (23-25). We could not confirm these individual criteria, as we found no significant differences in clinical presentation, pattern, and distribution of lesions, while systemic symptoms as a whole were almost four times more frequent in the DI-SCLE group than in the I-SCLE (Table 3). However, by performing the analysis for single symptom, there were no statistical differences between the two groups. A possible explanation for this apparently contrasting evidence is that a wider spectrum of symptoms, not just cutaneous are reported in DI-SCLE, probably related to older age or comorbidities.

Although the low number of patients tested could make conclusions not accurate, the serological profile in most of our patients was in line with literature findings for SCLE (11, 14-15, 29), including ANA positivity associated with anti-Ro/SSA antibodies, without significant differences between DI-SCLE and I-SCLE.

Few studies compared the different pathologic features of drug-induced and idiopathic SCLE. Marzano et al (14) provided a description of DI-SCLE histopathologic findings, with no attempt to describe the differences from I-SCLE. Other studies suggested an increased positive dust-like granular IgG deposition along the basement membrane zone in DI-SCLE (28,29). The first author to propose distinctive microscopic clues, such as tissue eosinophilia, was Callen (10). In our study, no significant differences were found in the mean eosinophil content, basal cell vacuolar liquefaction, keratinocyte necrosis, depth and pattern of inflammatory infiltration. The only significant associations were with mucin deposition in the dermis and positive direct immunofluorescence for both IgM and C3c along the basement membrane zone in I-SCLE, and the presence of leukocytoclastic vasculitis in DI-SCLE.

The pathogenesis of DI-SCLE remains uncovered, but active principles or their metabolites probably unchain the autoreactive process, superimposable to the idiopathic disease, in predisposed individual, carrying the HLA-DR3 antigen. Many drugs, primarily hydrochlorothiazide, are potential photosensitizers, while others interfere with the immune balance or induce an enzymatic and endocrine dysregulation, favoring the loss of self-tolerance against cell nuclei antigens (8, 30-32).

Our study included patients with many of the associated drugs as reported elsewhere (1-7, 17, 31-39): hydrochlorothiazide, terbinafine and biologics, especially TNFα antagonists, anti-

epileptics, and proton pump inhibitors. Additional drugs frequently associated with DI-SCLE include non-steroidal anti-inflammatory drugs and antihypertensive drugs, such as calcium channel blockers and angiotensin-converting enzyme inhibitors (39-43). The second most frequent active substance in our study was leflunomide, an immune-modulating agent that suppresses the production of pro-inflammatory cytokines, especially TNFα, with a mechanism similar to modern anti-TNFα biologic drugs. Only 3 cases of leflunomide DI-SCLE were retrieved in prior Medline database (20, 44, 45), and we report 4 more cases. At least two other culprit agents deserve attention, because of a sort of paradoxical reaction: certolizumab-pegol and intravenous immunoglobulins (IVIg). Literature retrieval found no previous reports of SCLE certolizumab-pegol induction, and surprisingly, the switch to this fusion-humanized protein was indicated in patients with inflammatory bowel diseases who developed lupus-like symptoms from anti-TNFα (46). As for IVIg, considered among therapeutic options for patient with severe resistant LE cases (47), there is a six cases series of disseminated cutaneous LE induced by IVIg (48).

The causality assessment of adverse drug reactions is a multistep process, based on four cardinal principles: temporal relationship, biological plausibility, amelioration after withdrawal (dechallenge), and worsening after rechallenge. Several causality assessment tools (CATs) support the clinician in the correlation judgement (13), and the adoption of the Jones algorithm (16) in our study identified four drugs (5%) with a certain association, three of which with previous reports (gemcitabine, carboplatin, and lamotrigine), and another (desloratadine) not currently listed, which warrants further evaluation. A final judgment of a probable association characterized 25 active substances (32%), including hydrochlorothiazide, several cardiologics,

anti-inflammatory drugs, hydroxychloroquine, and terbinafine. For all other drugs (62%), the association remained only possible. If confirmed by other prospective studies, the histopathology assessment might be a useful criterion for implementing DI-SCLE diagnostic accuracy and causality judgment.

Discontinuation of the culprit drug remains the major therapeutic intervention in any adverse drug reaction, including DI-SCLE, which, unlike idiopathic SCLE, usually result in recovery within 8 to 12 weeks (14, 17, 39), although Ro/SSa antibodies might remain positive for months or even years (15). Persistence of clinical manifestations despite long-term removal of the drug, namely drug unmasked LE, and other refractory cases might require pharmacological treatment (6). Systemic corticosteroids are supplied at doses commonly used for I-SCLE, followed by antimalarials, and other immunosuppressants, such as azathioprine, thalidomide, or mycophenolate-mofetil. Topical steroids have also been used with variable success (49). Present survey was not expressively designed to give information about long-term monitoring, but all cases improved at dechallenge, and none of the centers reported persistence of manifestations after definite withdrawal.

Conclusions

Over the last decade, the awareness that a distinct subset of subacute lupus erythematosus might be associated with drugs challenged the definition of clinical and laboratory features that are useful to differentiate DI-SCLE from its idiopathic counterpart, with contradictory findings. The present multicenter study found minimal, but significant differences in clinical features, such as age at onset and non-specific systemic complaints, and histopathological findings.

Mucin deposition and IgM and C3 positivity at the basement membrane zone were microscopic clues of I-SCLE, while leukocytoclastic vasculitis of DI-SCLE. The multistep drug causality assessment might benefit of the integrated evaluation of additional clinical, histopathological and immunofluorescence findings, which support DI-SCLE diagnosis.

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Table legends

Table 1. Demographic data of SCLE patients

	Total cohort **N=232**	**I-SCLE** **N=165**	**DI-SCLE** **N= 67**	**Student's t-test** **p-value**
Female	**174 (75%)**	**121 (73%)**	**53 (79%)**	**0.232**
Male	**58 (25%)**	**44 (27%)**	**14 (21%)**	**0.09**
Age (mean)	**51.5**	**40.3**	**53.3**	**0.007**
DI-SCLE/SCLE	**67/232 (28.9%)**			

Table 2. Clinical features of the two patients' groups

	Tot. cohort	**N° of cases (%)**		
Clinical presentation	N (%)	I-SCLE (n=126)	DI-SCLE (n=63)	p-value
Annular polycyclic	66 (34.9)	49 (38.9)	17 (26.9)	0.283
Papulosquamous	64 (33.9)	44 (34.9)	20 (31.7)	0.528
Overlap	27 (14.3)	21 (16.7)	6 (9.5)	0.073
Annular with malar rash	9 (4.8)	6 (4.8)	3 (4.8)	0.346
Annular with bullae	8 (4.2)	2 (1.6)	6 (9.5)	0.023*
Annular with erythema multiforme	8 (4.2)	3 (2.4)	5 (7.9)	0.068
Pityriasis-like	4 (2.1)	1 (0.8)	3 (4.8)	0.02*
Toxic Epidermal Necrolysis-like	3 (1.6)	0 (0)	3 (48)	0.039*
Involved areas	N (%)	I-SCLE (n=142)	DI-SCLE (n=64)	p-value
Sun-exposed	101 (49.5)	78 (54.9)	23 (35.9)	1
Widespread	65 (31.9)	34 (23.9)	31 (48.4)	0.017*
Head-neck	14 (6.9)	8 (5.7)	6 (9.4)	0.382
Upper limbs	13 (6.4)	12 (8.5)	1 (1.6)	0.115
Chest	9 (4.4)	6 (4.2)	3 (4.7)	1
Back	3 (1.5)	3 (2.1)	0 (0)	0.554
Lower limbs	1 (0.5)	1 (0.7)	0 (0)	1

*** p < 0.05; Bonferroni adjusted-critical value 0.0062 for t (8); 0.0071 for t (7) hypothesis.**

Table 3. Systemic symptoms and autoantibodies panel in the two patients' groups

	Total cohort	I-SCLE	DI-SCLE	
	N (%)	N (%)	N (%)	p-value
Total of patients with symptoms	53 (22.8)	21 (12.7)	32 (47.8)	0.00000005***
Arthralgia/Arthritis	37 (15.9)	20 (12.1)	17 (25.4)	0.017*
Raynaud phenomenon	14 (6)	9 (5.4)	5 (7.5)	0.553
Xerostomia	14 (6)	6 (3.6)	8 (11.9)	0.029*
Non-specific symptoms (fever, malaise)	13 (5.6)	8 (4.8)	5 (7.5)	0.529
Xerophthalmia	9 (3.9)	4 (2.4)	5 (7.5)	0.125
Nephropathy	7 (3)	3 (1.8)	4 (6)	0.109
Serositis	0 (0)	0 (0)	0 (0)	1
Autoantibodies panel	N° tot tests	I-SCLE N°pos /tot (%)	DI-SCLE N°pos/tot (%)	p-value
ANA	178	83/121 (68.6)	47/57 (82.4)	0.07
ENA	176	80/119 (67.2)	42/57 (73.7)	0.485
Ro/SSA	158	68/102 (42.1)	39/56 (69.6)	0.726
La/SSB	146	23/91 (25.3)	14/55 (25.4)	1.00
dsDNA	137	12 /93(12.9)	4/44 (9.1)	0.584
anti-SM	129	6/77 (7.8)	4/52(7.7)	1.00

LAC	94	7/54 (13)	2/40 (5)	0.293
anti-histone	85	6/45 (13.3)	9/40 (22.5)	0.393

*** p-value < 0.05; *** p-value < 0.01; Bonferroni adjusted-critical value 0.0071 for t (7) hypothesis.**

Table 4. List of drugs and causality assessment according to the Jones' algorithm.

Drug Categories	**Cases (%)**	**Active principle**	**N**	**Algorithm of Jones**		
				Certain	**Probable**	**Possible**
Diuretics	9/76 (11.8%)	Hydrochlorothiazide	8	0	2	6
		Furosemide	1	0	0	1
Biologics	8/76 (10.5%)	Etanercept	2	0	0	2
		Adalimumab	1	0	0	1
		Infliximab	1	0	0	1
		Rituximab	1	0	0	1
		Nivolumab	1	0	0	1
		Bevacizumab	1	0	1	0
		Certolizumab	1	0	0	1
Cardiologics	8/76 (10.5%)	Amlodipine	2	0	0	2
		Nitrendipine	1	0	1	0
		Ramipril	1	0	1	0
		Enalapril	1	0	1	0

	Bisoprolol	1	0	0	1
	Irbesartan	1	0	0	1
	Flecainide	1	0	0	1
Chemotherapies 8/76 (10.5%)	Gemcitabine	2	1	0	1
	Capecitabine	2	0	0	2
	Carboplatin	2	1	0	1
	Cisplatin	1	0	0	1
	Docetaxel	1	0	0	1
Non-steroid anti-inflammatory 7/76 (9.2%)	Ibuprofen	1	0	1	0
	Nimesulide	1	0	1	0
	Diclofenac	1	0	1	0
	Paracetamol	1	0	1	0
	Acetylsalicylic acid	1	0	1	0
	Naproxen	1	0	1	1
	Piroxicam	1	0	0	1
Immunomodulatory 6/76 (7.9%)	Leflunomide	4	0	1	3
	IV-Immunoglobulins	1	0	0	1
	Interferon-α	1	0	0	1
Antibiotics/antifungals 5/76 (6.6%)	Terbinafine	3	0	1	2
	Doxycycline	1	0	1	0
	Amoxicillin clavulinate	1	0	1	0

Antiplatelets/anticoagulants 4/76 (5.3%)	Cardioaspirin	1	0	0	1
	Rivaroxaban	1	0	0	1
	Dabigatran	1	0	0	1
	Prasugrel	1	0	0	1
Proton pump inhibitors (PPI) 4/76 (5.3%)	Omeprazole	2	0	0	2
	Lansoprazole	1	0	0	1
	Pantoprazole	1	0	0	1
Hormones 4/76 (5.3%)	Estro-progestinics	4	0	2	2
Anti-epileptics 3/76 (3.9%)	Lamotrigine	1	1	0	0
	Carbamazepine	1	0	1	0
	Oxcarbazepine	1	0	1	0
Psychotropics 3/76 (3.9%)	Bromazepam	1	0	0	1
	Paroxetine	1	0	0	1
	Fluvoxamine	1	0	0	1
Antimalarials 2/76 (2.6%)	Hydroxychloroquine	2	0	2	0
Uricosurics 2/76 (2.6%)	Allopurinol	2	0	1	1
Hypo-lipidemic 2/76 (2.6%)	Rosuvastatin	1	0	0	1
	Ezetimibe	1	0	1	0
Antihistamines 1/76 (1.3%)	Desloratadine	1	1	0	0
Final Causality Assessment			**4 (5%)**	**25 (33%)**	**47(62%)**

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Table 5. Histological features and direct immunofluorescence panel in the two patients' groups

Histological features	**Tot. cohort N (%)**	**I-SCLE (n=164)**	**DI-SCLE (n=66)**	**Observed p-value**
Epidermal atrophy	149 (64.8)	105 (64)	44 (66.7)	0.761
Epidermal hyperplasia	35 (15.2)	19 (11.6)	16 (24.2)	0.024*
Keratinocyte necrosis/apoptosis	138 (59.5)	90 (54.9)	48 (72,8)	0.017*
Hyper/orthokeratosis	76 (33)	51 (31.1)	25 (37.9)	0.354
Vacuolar degeneration	206 (89.6)	149 (90,8)	57 (86.4)	0.343
Perivascular lymphocytic infiltrate	225 (97.8)	161 (98.2)	64 (97)	0.627
Periadnexal lymphocytic infiltrate	120 (52.2)	91 (55.5)	29 (43.4)	0.144
Cytoid bodies in the dermis	58 (25.2)	34 (20.7)	24 (36.4)	0.018*
Eosinophils	14 (6)	9 (5.5)	5 (7.6)	0.551
Mucin deposition	138 (60)	114 (69.5)	24 (36.4)	0.000005***
Leukocytoclastic vasculitis	7 (3)	0 (0)	7 (10.6)	0.00013***
Direct immunofluorescence	**Tot. cohort N (%)**	**I-SCLE (n=90) (%)**	**DI-SCLE (n=43)(%)**	**p-value**
IgG alone	4 (3)	2 (2.2)	2 (4.7)	0.594

IgM alone	7 (5.3)	6 (6.7)	1 (2.3)	0.427
C3c alone	5 (3.7)	3 (3.3)	2 (4.7)	0.658
IgG + C3c	7 (5.3)	3 (3.3)	4 (9.3)	0.212
IgM + C3c	56 (42.1)	47 (52.2)	9 (20.9)	0.00069***
IgG + IgM + C3c	13 (9.8)	9 (10)	4 (9.3)	1.00
*** p-value < 0.05; *** p-value < 0.01; Bonferroni adjusted-critical value 0.0045 for t (11) hypothesis.**				

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