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Lupus Band Test can be used in combination with anti-chromatin antibodies and complement analysis to predict transition from cutaneous to systemic lupus

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Abstract

The lupus band test (LBT) is frequently performed for patients with lupus erythematosus (LE) but its capacity to discriminate cutaneous (C)LE from systemic (S)LE is debated, as well as its association with serum antinuclear antibodies (ANA) and complement reduction. Among 158 patients, 56 received retrospectively a diagnosis of CLE, 37 have SLE and 65 other skin disorders. Considering 29 clinical, histopathologic, LBT, and serological parameters: 5 parameters were effective in distinguishing LE from other skin disorders (e.g. skin photosensitivity, LBT positivity, basal vacuolar changes, thickening of the basement membrane, and anti-SSA-60kDa); and 8 parameters were able to separate SLE from CLE (e.g. arthritis, lupus nephritis, hematological manifestations, Raynaud/sicca manifestations, anti-chromatin, anti-dsDNA, and low levels of C3/4). A positive LBT was further determined to be associated with systemic manifestations when associated with anti-chromatin response and complement reduction in the profile of patients evolving to a systemic form of lupus.

Key words: lupus erythematosus, lupus band test, anti-chromatin, complement

Highlights:

- Lupus band test (LBT) is effective for classifying patients with lupus erythematosus (LE).
- LBT is associated with lupus nephritis and hematological manifestations in SLE.
- When associated with anti-chromatin antibodies and complement reduction, LBT is predictive for SLE development.

1. Introduction

Among inflammatory skin disorders, lupus erythematosus (LE) is an autoimmune disease classically encountered in women at childbearing age but LE can also occur in both sexes and at more mature ages. Diagnosis and subclassification of LE are based on the combination of clinical, histopathological, and serological (autoantibodies, complement) abnormalities^{1,2}. Clinical presentation in LE represents a continuum from skin lesions unaccompanied by extra-cutaneous signs, referred to as cutaneous (C)LE, to progressive multi-systemic (S)LE. Following LE diagnosis, 10-25% patients will develop SLE within 3 years³. Histopathological LE skin analysis reveals abundant apoptotic bodies along the dermo-epidermal junction, creating an excessive keratinocyte apoptotic process leading to basal vacuolar epidermal changes⁴. This results from immunological alterations based on immunoglobulin (Ig) deposition, perifollicular T and B lymphocytes tagging along the dermo-epidermal junction, and local inflammation leading to a thickening of the basement membrane in relation to apoptosis of keratinocytes. Introduced in 1963⁵, the lupus band test (LBT) explores Ig presence and C3 complement fraction deposition at the dermo-epidermal junction. A positive LBT associated with LE typically includes IgM, IgG +/- IgA isotypes +/- C3 deposition in a granular and abundant pattern⁶⁻¹⁰.

Recent advances in the pathophysiology of LE prompt us to redefine the interpretation criteria of the LBT in association with clinical, histological and serological data¹¹⁻¹³. To this end, a retrospective study was designed by selecting 158 individuals tested for LBT and suspected of LE. Next, 29 criteria (including LBT) to differentiate LE from other skin disorders and to dichotomize CLE from SLE patients were evaluated. Moreover, associations between LBT with systemic clinical and biological parameters were further appreciated according to the LE status and the subsequent development into a systemic form.

2. Material and methods

2.1. Patient selection

A retrospective monocentric study was conducted from January 2010 until December 2020 in the Immunology Laboratory of Toulouse University Hospital. Patients tested for direct immunofluorescence on skin biopsies, at the request of the clinicians, were selected using

two criteria: a suspicion of LE and/or a positive LBT assay with non-linear immunoglobulin deposition. As reported in figure 1 and following exclusion of patients without available clinical data or unknown biopsy site localization, 158 patients were classified according to their medical records into 3 groups: other skin disorders (non-LE patients), CLE patients¹⁴, and SLE patients based on the 1997 American College of Rheumatology criteria¹⁵. Data collected from the medical records include demographic data (sex, age), medications (hydroxychloroquine, steroids, and immunosuppressants), clinical manifestations (based on 2012 EULAR criteria: skin photosensitivity, skin manifestations [defined as malar or discoid rashes, papulo-squamous lesions, annular lesions or undefined rash], arthritis, biopsy proven lupus nephritis, hematological manifestations, neurological manifestations, oral ulcers, pleural/pericardial effusion), and association with Raynaud/sicca manifestations. This study has received the authorization number DC20162804 from the French ethical Southwest and Overseas committee (SOOM 2).

2.2. Lupus band test (LBT)

LBT was performed on 3 to 4 mm punch biopsy specimens, for which skin biopsies were cryopreserved, cut (5 µm) and processed for direct immunofluorescence using a fluorescein-coupled polyvalent antiglobulin against human heavy chains (ref:74511, Bio-Rad Laboratories, Hercules, CA), anti-human IgG (ref:F0202, Dako, Agilent Technologies, Singapore), anti-human IgA (ref:30240, Bio-Rad), anti-human IgM (ref:30243, Bio-Rad) and anti-C3c fraction (ref:F0201, Dako) as previously described¹⁶. Positive LBT was defined by the presence at the dermo-epidermal junction of IgM and IgG +/- IgA deposits. LBT abundance, granular staining and C3 staining were further reported.

2.3. Histological and serological data

Histopathological reports and serological parameters were selected closest in time to the LBT biopsy date (<1 month), when available. The following parameters were analyzed according to the histological definition of cutaneous lupus¹⁷: basal vacuolar change, hyperkeratosis, thickening of the basement membrane, mucin imbibition in the dermis, apoptotic keratinocytes, and pigmentary incontinence in the dermis. Blood samples were tested for presence of anti-nuclear antibodies (ANA) by indirect immunofluorescence (IFI) on human epithelial type 2 (HEp-2) cells with an optimal cut off value $\geq 1:160$ (ref: 26102, Bio-

Rad). Specific autoantibody (Ab) investigations for anti-chromatin, anti-double-stranded (ds)DNA, anti-ribosome, and extractable nuclear Abs (ENA: anti-SSA 60 kDa, -SSA 52 kDa, -SSB, -Sm, -Sm-RNP, -RNP A, -RNP 68) were performed on BioPlex© (Bio-Rad). As recommended by the manufacturer, the positive cut-off was fixed at 0.9 Ab index (value range 0-9) and at 10 IU/ml for anti-dsDNA Abs. Ponderal complement fraction reduction for C3 (<0.72 mg/L) and C4 (< 0.11 mg/L) were assessed on COBAS® 8000 (Roche).

2.4. Statistics

For qualitative data, Fisher's exact test was performed and, if necessary, Odds ratio (OR) and 95% confidence interval (CI) were calculated. Quantitative data, presented as mean +/- standard error of the mean (SEM), were compared using the Student's assay or ANOVA when more than two groups were analyzed. Post-hoc corrections were used for multiple corrections to consider positivity at $p < 0.01$ for Fisher's exact test with a false Discovery Rate (FDR, <https://www.sdmproject.com/>), and a $p < 0.05$ for ANOVA with Tukey's method. We performed a multivariate binary logistic regression model (using SPSS 25.0, SPSS Inc, Chicago, IL, USA) to identify the impact of serological parameters combined to LBT positivity to discriminate between SLE and CLE. Variables of interest were selected based on the previously calculated OR with a p -value < 0.2 . All other tests and figures were built on Prism 9.0 (GraphPad Software, La Jolla, CA).

3. Results

3.1. Patients' characteristics

158 patients were included in the study. Among them, the final diagnosis was as follows: 56 CLE patients (including 16 confirmed after 2 years of follow-up), 37 SLE patients (including 7 initially defined as CLE) and 65 patients with other skin disorders (non-LE patients). The mean age at the date of skin biopsy was 49.4 +/- 1.32 years and among them 117/158 (74%) were women (Table 1). Age, sex and use of steroids and immunosuppressive treatments were equally distributed between CLE, SLE and other skin disorders. An increased use of hydroxychloroquine was reported in the LE group ($p = 0.0001$). Among the 65 other skin disorders subgroup, immune dermatoses were predominant 42/65 (64.6%) including psoriasis ($n = 7$), alopecia ($n = 4$), lichen planus ($n = 4$), and bullous pemphigoid ($n = 2$); followed by systemic diseases 13/65 (20%), such as dermatomyositis ($n = 6$), and Sjögren's syndrome ($n = 2$); and non-immune diseases 10/65 (15.4%).

3.2. LE patients versus non-LE patients, discriminating parameters

The characterization of LE and LE subtypes, in addition to clinical characteristics, relies on histological parameters, LBT results, and serological data as reported in Table 2. Indeed, the 9 clinical parameters, LBT, 6 histological parameters, and the 13 serological parameters were classified into three groups whether: (i) parameters allowed to distinguish LE (CLE+SLE) from other skin disorders, but not CLE from SLE (group 1); (ii) parameters were effective to dichotomize SLE from CLE among LE patients (group 2); and (iii) parameters were equally distributed between groups (group 3).

As presented in Figure 2A/B, 5/31 (16.1%) parameters were effective for distinguishing LE from other skin disorders, but not SLE from CLE. This group's parameters (group 1) included in addition to LBT positivity that will be explored next: skin photosensitivity (OR=11.6; 95% CI: 3.0-51.6, p=0.0001); two histological criteria: presence of vacuolar change involving epidermis basal layer (OR=21.5; 95% CI: 3.1-236, p=0.0003) and a marked thickening of the basement membrane (OR=infinity; 95% CI:[∞], p=0.009); plus anti-SSA 60 kDa Ab detection as serological criteria (OR=18.6; 95% CI:3.0-196, p=0.0001).

3.3. CLE patients versus SLE patients, discriminating parameters

Parameters that were effective to distinguish SLE from CLE patients (Group 2, Figure 2A/C) included the presence of systemic features such as arthritis (OR=11.0; 95% CI:3.5-33.2, p=3x10⁻⁵), biopsy proven lupus nephritis (OR=infinity; 95% CI:[∞], p=0.003), hematological manifestations (OR=9.9; 95% CI: 2.5-34.8, p=0.0005), and the presence of Raynaud/sicca manifestations (OR=5.7; 95% CI: 1.6-20.4, p=0.01). Less common SLE-associated manifestations such as neurological manifestations, oral ulcers, and pleural/pericardial effusion were not reported in our cohort (group 3). No associations were observed with histological parameters and LBT in group 2 parameters. Group 2 immune biological markers included anti-chromatin positivity (OR=16.9; 95% IC:3.5-77.9, p=4x10⁻⁵), anti-dsDNA detection (OR=5.7; 95% CI:1.6-17.5, p=0.008), and a low level of C4/C3 complement fractions (C3: OR = 7.6; 95% CI: 1.9-27.2, p = 0.005; C4: OR=11.3; 95% CI:2.5-53.9, p=0.001).

3.4. Lupus band test

As presented in Figure 3A/B (and data not shown), a positive LBT, defined at least by the deposition of IgM and IgG at the dermo-epidermal junction, was observed in 46/93 (49.4%) LE patients compared to 13/65 (20%) patients with other skin disorders (OR=3.9; 95% CI: 1.9-8.1, p=0.0002). In order to improve the LBT capacity to discriminate LE from other skin disorders, LBT characteristics were compared to LBT positivity alone. Indeed, OR was only slightly increased when considering LBT with an abundant Ig staining (OR=8.3; 95% CI: 1.6-9.4, p=0.0001) and similar OR were retrieved when considering a positive LBT with C3 staining (OR=3.7; 95% CI: 1.6-10.5, p=0.003) and a granular staining (OR=3.1; 95% CI: 1.4-6.7, p=0.004). The LBT capacity to distinguish LE from other skin disorders varies between lesional skin biopsies (122/158, 77.2%: OR=3.5; 95% CI: 1.5-7.9, p=0.002) as compared to non-lesional skin biopsies (36/158, 22.8%: OR=4.66; 95% CI: 0.9-24.3, p=0.12), which suggests giving preference to lesional skin biopsies for LBT. None of these LBT characteristics including biopsy skin location were effective to dichotomize SLE from CLE among LE patients.

3.5. Lupus band test and its combination with clinical, histological, and serological parameters

To further test whether LBT positivity may help to characterize a subset of patients, LBT combinations with clinical, histological, and serological parameters were evaluated. As presented in Figure 4A/B within the whole population (LE and other skin disorders), LBT positivity was associated among the 9 clinical parameters tested with biopsy proven lupus nephritis (OR=8.5; 95% IC: 1.9-40.7, p=0.004) and hematological manifestations (OR=5.5; 95% IC: 1.7-14.7, p=0.002), which are two main SLE-associated systemic manifestations. In addition, LBT positivity associations were retrieved with anti-chromatin Abs (OR=7.42; 95% CI:2.4-21.8, p=0.0009), anti-dsDNA Abs (OR=8.5; 95% CI:2.4-29.3, p=0.0006), low levels of C4/C3 (C4: OR=9.3; 95% IC=1.9-43.8, p=0.002; C3: OR=7; 95% IC:1.8-24.6, p=0.003), anti-Sm Abs (OR=11.3; 95% IC:1.8-129.8, p=0,008), anti-Sm RNP Abs (OR=13,7; 95% CI:1.8-153.9, p=0.003), anti-RNP A Abs (OR=8.8; 95 IC:2.2-41.6, p=0.003), and anti-ribosome Abs (OR=+infinity, 95% CI:2.2-+infinity, p=0.01). No associations were retrieved with ANA, anti-RNP 68 Abs, anti-SSA 60 kDa Abs and anti-SSA 52 kDa Abs, and histological parameters. Interestingly, when using a univariate analysis approach with OR determination (Figure 4C), a positive LBT assay slightly improves serological parameters including anti-chromatin Ab positivity (OR=23.4; 95% CI: 3.5-256, p=0.0001), anti-dsDNA Ab positivity (OR=10.4; 95% CI: 2.4-49.2, p=0.001), C4 reduction (OR=20; 95% CI: 2.7-222.5, p=0.0006) and C3 reduction

(OR=10; 95% CI: 2.1-47.8, $p=0.002$). Other Abs combined with a positive LBT were tested but were not relevant (data not shown).

3.6. Coupling the Lupus Band Test with serology to predict development to SLE

Next, we further explored whether coupling LBT determination with serology may help to predict in a subset of LE patients at diagnosis their development from CLE to SLE. Serological data from anti-chromatin Abs, anti-dsDNA Abs, C3 and C4 fractions were collected close to the time of the skin biopsy (+/- 1 month) and compared between CLE ($n= 26$ LBT+, $n= 21$ LBT-) and SLE ($n= 20$ LBT+, $n= 17$ LBT-) subsets (Figure 5A and data not shown for ENA). Anti-chromatin Abs ($p<10^{-4}$), C3 ($p=0.001$) and C4 ($p=0.007$) reductions characterized the SLE patients with a positive LBT, which was not the case with regards to anti-dsDNA Abs and ENA in this limited cohort. This observation needs however to be weighted since the comparison of regression equations in a multivariate logistic model confirm serological parameter capacity to discriminate SLE from CLE when LBT positivity is added ($R^2=4.41$, 5 ddl $F=25.38$, $p<0.001$) or not ($R^2 = 3.96$, 4 ddl, $F=24.18$, $p<0.001$), in this model LBT capacity to discriminate SLE and CLE was $p=0.107$ (data not shown).

To complete these observations and to evaluate the combination of LBT with serological markers in the prediction of an evolution from CLE to SLE, 6 CLE patients who developed SLE (1 patient was excluded because of missing data) were compared to 16 CLE patients who have not developed SLE in their clinical follow-up (mean 5.06 years ranging from 2 to 11 years). As presented in Figure 5B and within the subset of patients positive in the LBT assay, anti-chromatin Abs (66.7%), anti-dsDNA Abs (33.3%) and a reduction of C3/C4 complement fractions (66.7%) were retrieved in those who developed SLE, which was not the case in those who do not develop SLE.

Altogether, these results support, when a LBT is encountered positive, to further explore anti-chromatin Ab positivity and/or complement fraction reduction to potentially predict a systemic evolution for a subset of LE patients. However, these results need further confirmation.

4. Discussion

In this retrospective monocentric study, a re-evaluation of the utility of the LBT in the diagnosis of LE was investigated. We found that, in our cohort, a positive LBT (i) is effective in discriminating LE patients from other skin disorders but not effective in discriminating CLE

from SLE patients; (ii) is associated with systemic clinical manifestations such as lupus nephritis, hematological manifestations, and SLE-associated immunological parameters as anti-chromatin/dsDNA Abs and low levels of C3/C4 complement fractions; (iii) represents a complementary tool to diagnose SLE patients when combined with serological examination; and (iv) may be predictive for systemic development when combined with anti-chromatin Abs and/or reduced levels of C3 and C4 complement fractions. LBT/anti-chromatin/complement combination needs to be explored further and understood at a physiological level in the context of a possible association with a systemic evolution of LE.

Diagnosis of LE relies on the association of clinical, serological, histopathological features, and the detection of tissue bound immune complexes through direct immunofluorescence in the LBT assay. In agreement with the literature¹³, our study retrieved key elements (group 1 parameters) associated with LE including skin photosensitivity that results from an unusual reaction to sunlight exposure, leading to enhanced epithelial cell apoptosis (corresponding to vacuolar degeneration in basal cells) with apoptotic blebs containing the autoantigens SSA 60 kDa (Ro) and SSB (La) antigens. Therefore, anti-SSA 60 kDa Abs (with or without anti-SSB Abs) represent the hallmark of LE from 60-80% in CLE to 40% in SLE¹⁸ and can be detected up to 9.4 years from first detection to SLE diagnosis¹. A protective role for Ro/SSA 60 kDa is further suspected based on the observation that mice lacking *Trove2* gene (encoding for SSA 60 kDa) develop a systemic lupus-like syndrome with glomerulonephritis coupled with the detection of anti-chromatin Abs¹⁹. Within LE group 1 parameters, we have further reported that LBT positivity was independent from skin photosensitivity, histological parameters revealing epithelial apoptosis and anti-SSA 60 kDa Ab detection in agreement with some previous studies^{20,21}.

How can it be explained that LBT is associated with systemic LE manifestations including kidney nephritis and hematological manifestations? A common process of pathogenesis between skin and kidney was recently proposed through the comparative analysis of skin and kidney biopsies at a single-cell RNA sequencing level¹¹. In addition, it has been further described that part of the Ig spectrum implicated in LBT and lupus nephritis is related to anti-chromatin Abs^{22,23} and a relation also exists between anti-chromatin Abs and leukopenia²⁴. Indeed, anti-chromatin Abs have been demonstrated to bind skin and kidney

basement membrane structures with high affinity. In skin, UV radiation activates matrix metalloproteases that disrupt epidermal membranes and, in turn, make epidermal membranes and apoptotic keratinocyte bodies accessible to chromatin immune complexes^{25,26}. Accordingly, a question that remains to be solved is related to the origin of the chromatin in the skin from local production, from circulating immune complex deposition, or from a defective skin chromatin degradation process due to the loss of DNase I activity. Another question is related to the association reported between LBT and disease activity, in previous studies^{7,9}. Answering these questions may help to understand our observations showing that LBT is associated with anti-chromatin Abs in a subset of patients with systemic manifestations. In addition, the question of immunoglobulin specificities in forming immune complexes along the dermo-epidermal junction in CLE remains to be explored further.

In our study, the LBT assay demonstrated potential to assist with the diagnosis of LE, while being ineffective in determining CLE from SLE, as previously described by other authors^{27,20}. Important discrepancies are however reported between studies regarding LBT capacity to differentiate LE subsets, which may be explained in part by the characteristics of the skin biopsies: lesional/non-lesional areas; sun exposure or not; and body location. These parameters were not well controlled in our retrospective study since lesional skin biopsies represented 77.2% of the biopsies and data related to location and sun exposure were not always available. In order to solve this point, a consensus is emerging to favor lesional and non-exposed skin biopsies^{7,10}. Another debate is related to the nature of the deposited components (IgM, IgG, IgA, C3), morphology (granular, linear), and brightness of the fluorescent band^{7-9,20,28-30}. Indeed, LBT characteristics complete the specificity of the LBT when present, but their capacity to discriminate LE subtypes seems to be moderate as reported in our study and need to be combined with other parameters.

Our study had limitations (retrospective study, small number of patients, no control on the biopsy origin, and no follow-up for some patients), which are counterbalanced by an extensive clinical examination, histological analysis, and LBT realization coupled with laboratory tests including ANA determination and related ANA specific Abs in order to better characterize the LE spectrum.

In conclusion, because the LE clinical spectrum is wide, the use of biomarkers to facilitate early diagnosis, disease management but also identify LE patients with poor prognosis (systemic evolution) is warranted. Accordingly, and in addition to the tryptic LBT positivity, anti-chromatin Abs and complement reduction as described in this manuscript, new biomarkers are currently under development in SLE as well as CLE in order to answer these questions. They include skin transcriptomic profiles^{31,32}, proteome analysis³³, and microbiome studies³⁴.

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Figures

Figure 1. Flow chart of the retrospective study conducted between January 2010 and December 2020 in order to re-evaluate the place of the lupus band test (LBT) for lupus erythematosus diagnosis and potential development to cutaneous (C)LE or systemic (S)LE.

Figure 2. Discriminating parameters for lupus erythematosus (LE) and LE subset discrimination between cutaneous (C)LE and systemic (S)LE. A. Clinical parameters, lupus band test (LBT), histological, and serological associations compared between lupus erythematosus (LE) and other skin disorders (in blue) and between systemic (S)LE and cutaneous (C)LE (in red). Data are represented as $-\log(p \text{ value})$ with a significant threshold fixed at 2 corresponding to $p \leq 0.01$ as established using a post-hoc FDR (False Discovery Rate) test. **B.** Odds ratio and confidence interval (95% CI) are indicated for significant ($p \leq 0.01$) LE associated parameters (group 1) that allow discrimination of LE from other skin disorders. **C.** Group 2 SLE associated parameters ($p \leq 0.01$) that allow discrimination of CLE from SLE patients.

Figure 3. Lupus band test (LBT) capacity to discriminate LE and LE subsets. A. Positive Lupus Band Test (LBT) and LBT parameters according to C3 complement deposition, abundance, granular deposition and skin characteristics (lesional, non-lesional) compared between Lupus Erythematosus (LE) and other skin disorders (in blue) and between systemic (S)LE and cutaneous (C)LE (in red). Data are represented as $-\log(p \text{ value})$ with a significant threshold fixed at 2 corresponding to $p \leq 0.01$ as established using a post-hoc FDR (False Discovery Rate) test. **B.** Odds ratio and confidence interval (95% CI) are indicated for significant LBT parameters ($p \leq 0.01$) that allow to discriminate LE from other skin disorders.

Figure 4. Lupus band test (LBT) associations. A. LBT associations with clinical, histological and serological parameters among the whole patient group (lupus erythematosus [LE] and other skin disorders, purple) and within the LE subgroup (cutaneous and systemic LE, green). Data are represented as $-\log(p \text{ value})$ with a significant threshold fixed at 2 corresponding to $p \leq 0.01$ as established using a post-hoc FDR (False Discovery Rate) test. **B.** Odds ratio and confidence interval (95% CI) are indicated for significant clinical and serological parameters

($p \leq 0.01$) associated with a positive LBT in the whole patient group. **C.** Odds ratio and 95% CI are indicated for serologic parameters coupled with a positive LBT to discriminate SLE from CLE.

Figure 5. Lupus band test (LBT) association with anti-chromatin antibodies and/or complement reduction highlights lupus erythematosus (LE) patients with systemic evolutions. A. Quantitative values for anti-chromatin antibodies, anti-double strand (ds)DNA antibodies, C3 and C4 complement fractions among SLE patients (LBT positive $n=20$, LBT negative $n=17$) and CLE patients (LBT positive [+] $n=26$, LBT negative [-] $n=21$), test performed with ordinary one-way ANOVA using the post-hoc Tukey's correction ($\alpha=0.05$) **B.** Anti-chromatin Abs, anti-dsDNA Abs, C3 and C4 complement fraction expressed as a percentage of positivity (values in each box) according to the LBT and clinical status. Patients mentioned as "CLE→CLE" are those who have not evolved to a systemic form of LE, "CLE→SLE" those who clinically evolved to SLE. LE patients were compared to non-LE with serological data available.

Table 1. Clinical characteristics of lupus erythematosus (LE) patients compared to non-LE patients. LE subgroup includes patients with cutaneous (C)LE and systemic (S)LE.

	LE versus other skin disorders (non-LE patients)		LE subsets	
	All LE (CLE + SLE) n = 93	Other skin disorders N=65	CLE n = 56	SLE n = 37
Mean age (+/- SEM) at the date of skin biopsy	48.8 +/- 1.7	50.2 +/- 2.1	51.4 +/- 2	45 +/- 2.9
Sex ratio (women/men)	3.04	2.61	2.11	6.4
Acute/subacute LE	-	-	20/40 (50%)	-
Chronic/discoid LE	-	-	19/40 (47.5%)	-
Lupus tumidus	-	-	1/40 (2.5%)	-
Skin photosensitivity, n (%)	18/67 (26.8)*	2/65 (3%)	11/37 (29.7%)	7/30 (23.3%)
Skin manifestations, n (%)	62/67 (92.5%)	54/65 (83.1%)	34/37 (91.8%)	28/30 (93.3%)
Arthritis, n (%)	24/67(35.8%)*	2/65 (3%)	5/37 (13.5%)	19/30 (63.3%)#
Lupus nephritis, n (%)	10/67 (14.9%)*	0/65	0/37	9/30 (30%)#
Hematological manifestations ¹ , n (%)	17/67 (25.3%)*	0/65	3/37 (8.1%)	14/30 (46.6%)#
Neurological manifestations, n (%)	4/67 (5.9%)	0/65	0/37	4/30 (13.3%)
Oral ulcers, n (%)	6/67 (8.9%)	0/65	1/37 (2.7%)	5/30 (16.6%)
Pleural/pericardial effusion, n (%)	3/67 (4.5%)	0/65	0/37	3/30 (10%)
Raynaud/sicca syndrome, n (%)	13/67 (19.4%)*	1/65 (1.5%)	3/37 (8%)	10/30 (33%)#
Hydroxychloroquine treatment, n (%)	38/69 (55.1%)*	8/65 (12.3%)	22/39 (56.4%)	16/30 (53.4%)
Steroids treatment, n (%)	10/69 (14.5%)	3/65(4.6%)	3/39 (7.7%)	7/30 (23.3%)
Immunosuppressive treatment, n (%)	8/69 (11.6%)	6/65 (9.2%)	1/39 (2.5%)	7/30 (23.3%)

¹Hematological manifestations characterized by one or multiple of the following criteria: anemia, thrombopenia, lymphopenia. Statistics are indicated when $p < 0.01$ between LE and non-LE patients (*) and between CLE and SLE patients (#).

	LE versus other skin disorders (non-LE patients)			LE subsets		
	All LE (CLE + SLE) n = 93	Other skin disorders N=65	P value	CLE n = 56	SLE n = 37	P value
Lupus band test (LBT)						
Positive LBT	46/93 (49.4%)	13/65 (20%)	0.0002	26/56 (46%)	20/37 (54%)	0.52
Positive LBT with C3 staining	29/93 (31.2%)	7/65 (10/8%)	0.003	16/56 (28.6%)	13/37 (35.1%)	0.65
Positive LBT with abundant staining	38/93 (40.9%)	5/65 (7.7%)	0.0001	21/56 (37.5%)	17/37 (45.9%)	0.52
Positive LBT with granular staining	36/93 (38.7%)	11/65 (16.9%)	0.004	19/56 (33.9%)	17/37 (45.9%)	0.28
Histological parameters						
Basal vacuolar change	17/32 (53.1%)*	1/20 (5%)	0.0003	11/19 (57.8%)	6/13 (46.1%)	0.72
Hyperkeratosis	8/32 (25%)	9/20 (45%)	0.12	6/19 (31.6%)	2/13 (15.3%)	0.42
Thickening of the basement membrane	9/32 (28.1%)*	0/20	0.008	6/19 (31.6%)	3/13 (23%)	0.70
Mucin imbibition in the dermis	15/32 (46.8%)	5/20 (25%)	0.15	11/19 (57.8%)	4/13 (30.7%)	0.16
Apoptotic keratinocytes	15/32 (46.8%)	3/20 (15%)	0.03	10/19 (52.6%)	5/13 (38.5%)	0.49
Pigmentary incontinence in the dermis	9/32 (28.1%)	2/20 (10%)	0.17	6/19 (31.6%)	3/13 (23%)	0.70
Serological parameters						
ANA (titer \geq 1/160)	65/79 (82.3%)	28/38 (73,8%)	0.33	34/45 (75,5%)	31/34 (91.2%)	0.08
Anti-chromatin Ab	17/69 (24.6%)	1/27 (3.7%)	0.02	2/38 (5.3%)	15/31 (48.3%)#	0.0001
Anti-dsDNA Ab	16/68 (23.5%)	0/28	0.005	4/38 (10.5%)*	12/30 (40%)#	0.008
C4 < 0.11 g/L	13/62 (20.9%)	0/24	0.012	2/35 (5.7%)	11/27 (40.7%)#	0.001
C3 < 0.72 g/L	14/63 (22.2%)	1/24 (4.2%)	0.006	3/36 (8.3%)	11/27 (40.7%)#	0.005
Anti-Sm Ab	7/77 (9.0%)	1/36 (2.7%)	0.43	2/42 (4.8%)	5/35 (14.2%)	0.23
Anti-Sm RNP Ab	8/77 (10.4%)	1/35 (2.8%)	0.27	2/42 (4.8%)	6/35 (17.1%)	0.13
Anti-RNP A Ab	10/77 (12.9%)	2/35 (5.7%)	0.33	2/42 (4.7%)	8/35 (22.8%)	0.04

Anti-RNP 68kDa Ab	1/75 (1.3%)	1/35 (2.8%)	0.54	0/42	1/33 (3%)	0.44
Anti-SSA 60 kDa Ab	26/75 (34.6%)*	1/36 (2.7%)	0.0001	14/40 (35%)	12/35 (34%)	1
Anti-SSA 52 kDa Ab	15/77 (19.4%)	1/36 (2.7%)	0.02	6/42 (14.2%)	9/35 (25.7%)	0.25
Anti-SSB Ab	11/32 (34.4%)	2/36 (5.5%)	0.22	6/42 (14.3%)	5/35 (8.6%)	1
Anti-ribosome Ab	5/77 (6.5%)	0/36	0.17	0/42	5/35 (14.3%)	0.02

Table 2. Lupus band test (LBT), histological and biological characteristics among patients with lupus erythematosus (LE) as compared to other non-LE patients. LE subgroup includes patients with cutaneous (C)LE and systemic (S)LE.

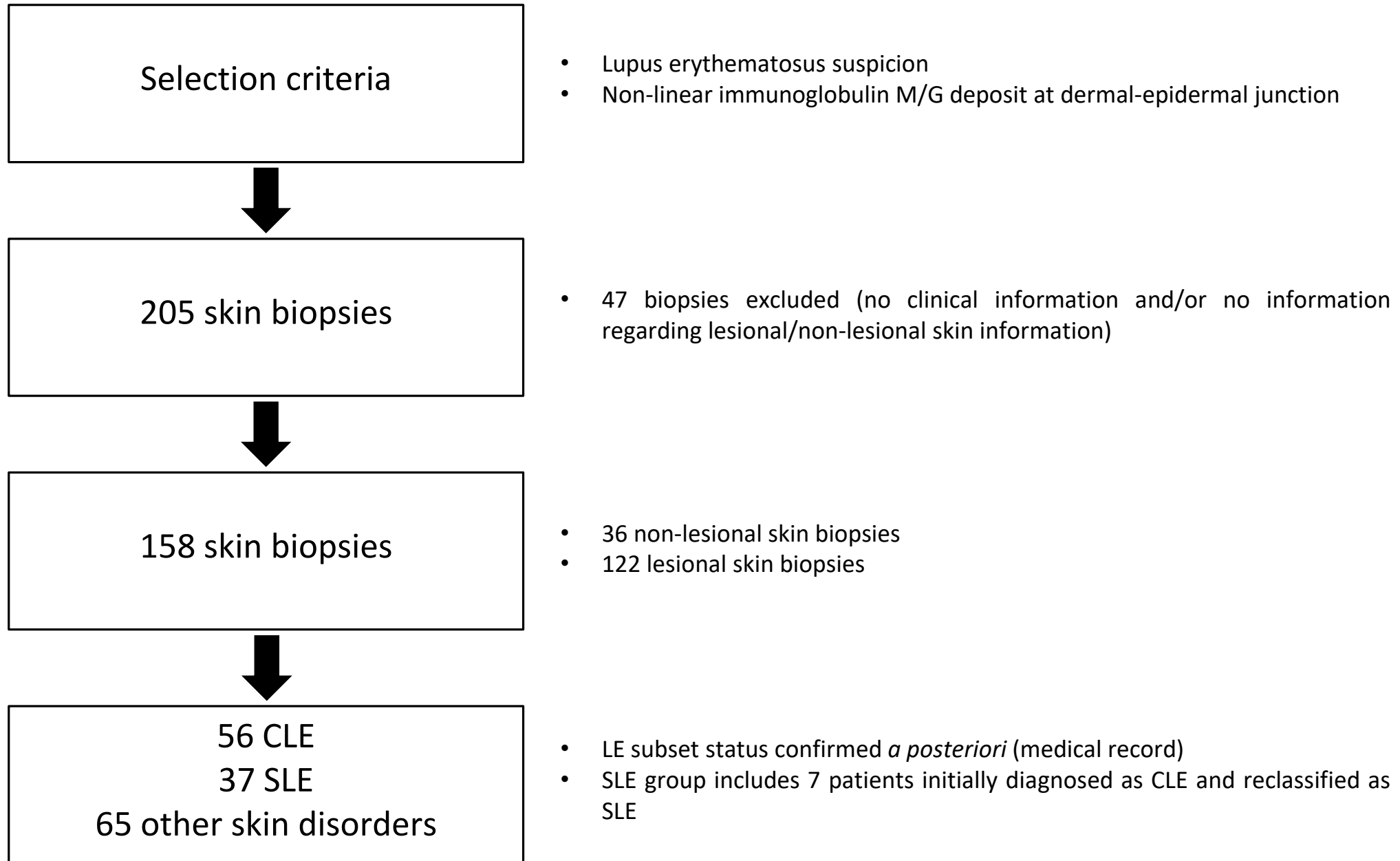
Statistics are indicated when $p < 0.01$ between LE and non-LE patients (*) and between CLE and SLE patients (#). Abbreviations: LE : Lupus Erythematosus (including CLE+SLE patients) ; SLE : Systemic Lupus Erythematosus ; CLE : Cutaneous Lupus Erythematosus; Ab: autoantibody, ANA: antinuclear Ab.

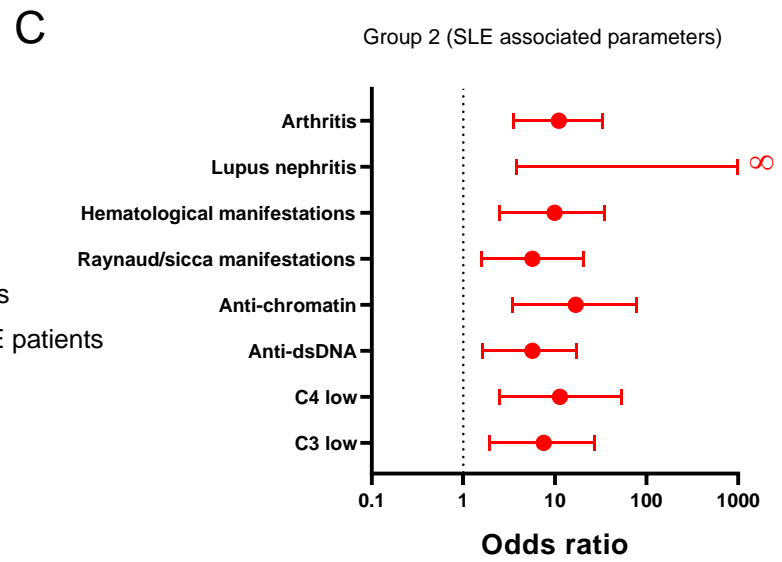
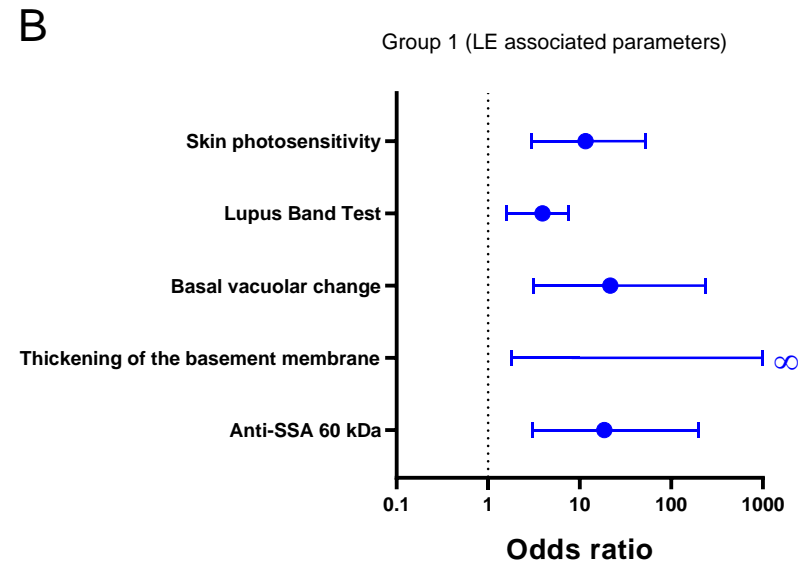
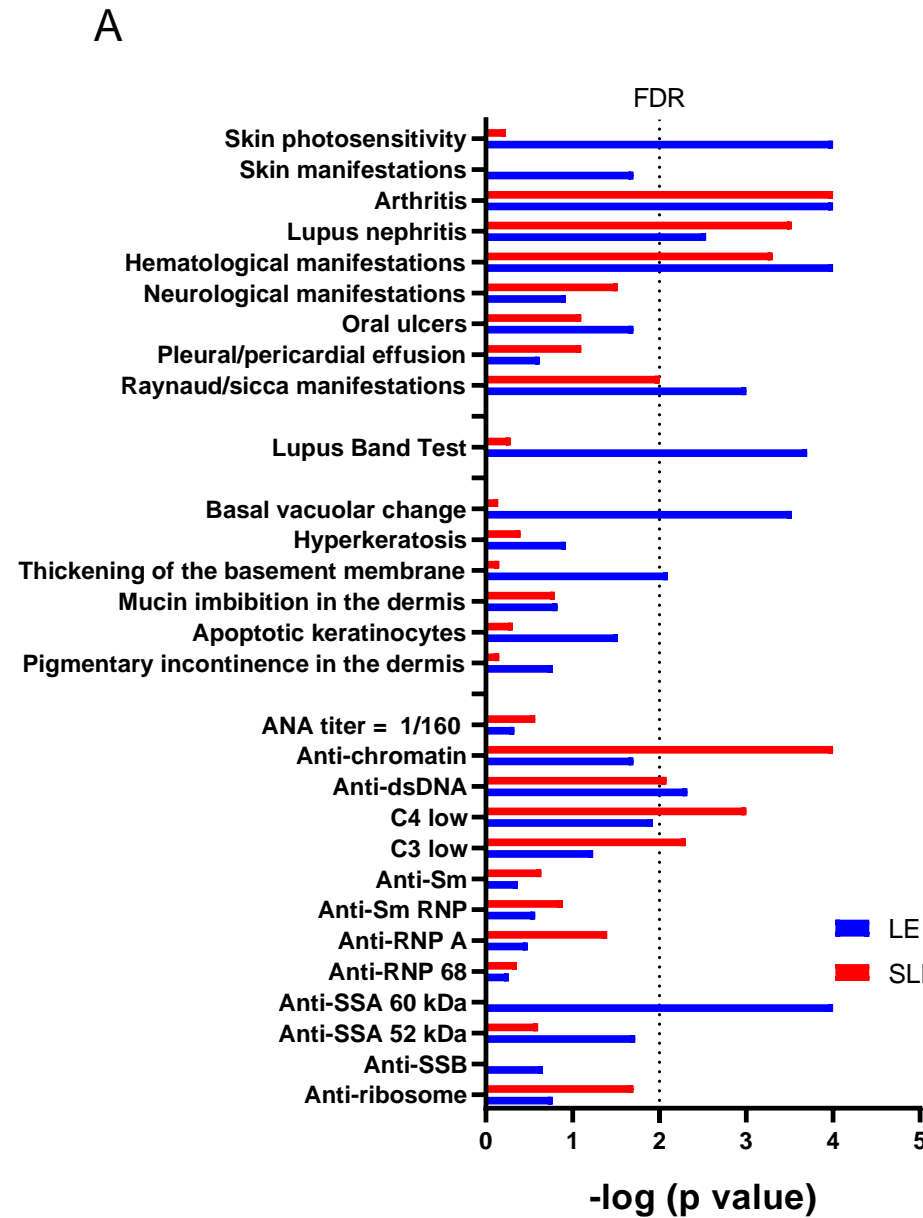
Bibliography

1. Arbuckle, M. R. *et al.* Development of Autoantibodies before the Clinical Onset of Systemic Lupus Erythematosus. *N Engl J Med* **349**, 1526–1533 (2003).
2. Dema, B. & Charles, N. Autoantibodies in SLE: Specificities, Isotypes and Receptors. *Antibodies (Basel)* **5**, E2 (2016).
3. Fabr e, V. C., Lear, S., Reichlin, M., Hodge, S. J. & Callen, J. P. Twenty percent of biopsy specimens from sun-exposed skin of normal young adults demonstrate positive immunofluorescence. *Arch Dermatol* **127**, 1006–1011 (1991).
4. Pablos, J. L. *et al.* Keratinocyte apoptosis and p53 expression in cutaneous lupus and dermatomyositis. *J Pathol* **188**, 63–68 (1999).
5. Burnham, T. K., Neblett, T. R. & Fine, G. The Application of the Fluorescent Antibody Technic to the Investigation of Lupus Erythematosus and Various Dermatoses. *Journal of Investigative Dermatology* **41**, 451–456 (1963).
6. Cormane, R. H. " BOUND " GLOBULIN IN THE SKIN OF PATIENTS WITH CHRONIC DISCOID LUPUS ERYTHEMATOSUS AND SYSTEMIC LUPUS ERYTHEMATOSUS. *The Lancet* **283**, 534–535 (1964).
7. Reich, A., Marcinow, K. & Bialynicki-Birula, R. The lupus band test in systemic lupus erythematosus patients. *TCRM* **27** (2011) doi:10.2147/TCRM.S10145.
8. Cardinali, C., Caproni, M. & Fabbri, P. The composition of the lupus band test (LBT) on the sun-protected non-lesional (SPNL) skin in patients with cutaneous lupus erythematosus (CLE). *Lupus* **8**, 755–760 (1999).
9. Zec evic, R. D., Pavlovic, M. D. & Stefanovic, D. Lupus band test and disease activity in systemic lupus erythematosus: does it still matter? *Clinical and Experimental Dermatology* **3** (2006).
10. Mehta, V., Sarda, A. & Balachandran, C. Lupus band test. *Indian J Dermatol Venereol Leprol* **76**, 298 (2010).
11. Der, E. *et al.* Tubular cell and keratinocyte single-cell transcriptomics applied to lupus nephritis reveal type I IFN and fibrosis relevant pathways. *Nat Immunol* **20**, 915–927 (2019).
12. Dolff, S., Abdulahad, W. H., Bijl, M. & Kallenberg, C. G. M. Regulators of B-cell activity in SLE: a better target for treatment than B-cell depletion? *Lupus* **18**, 575–580 (2009).

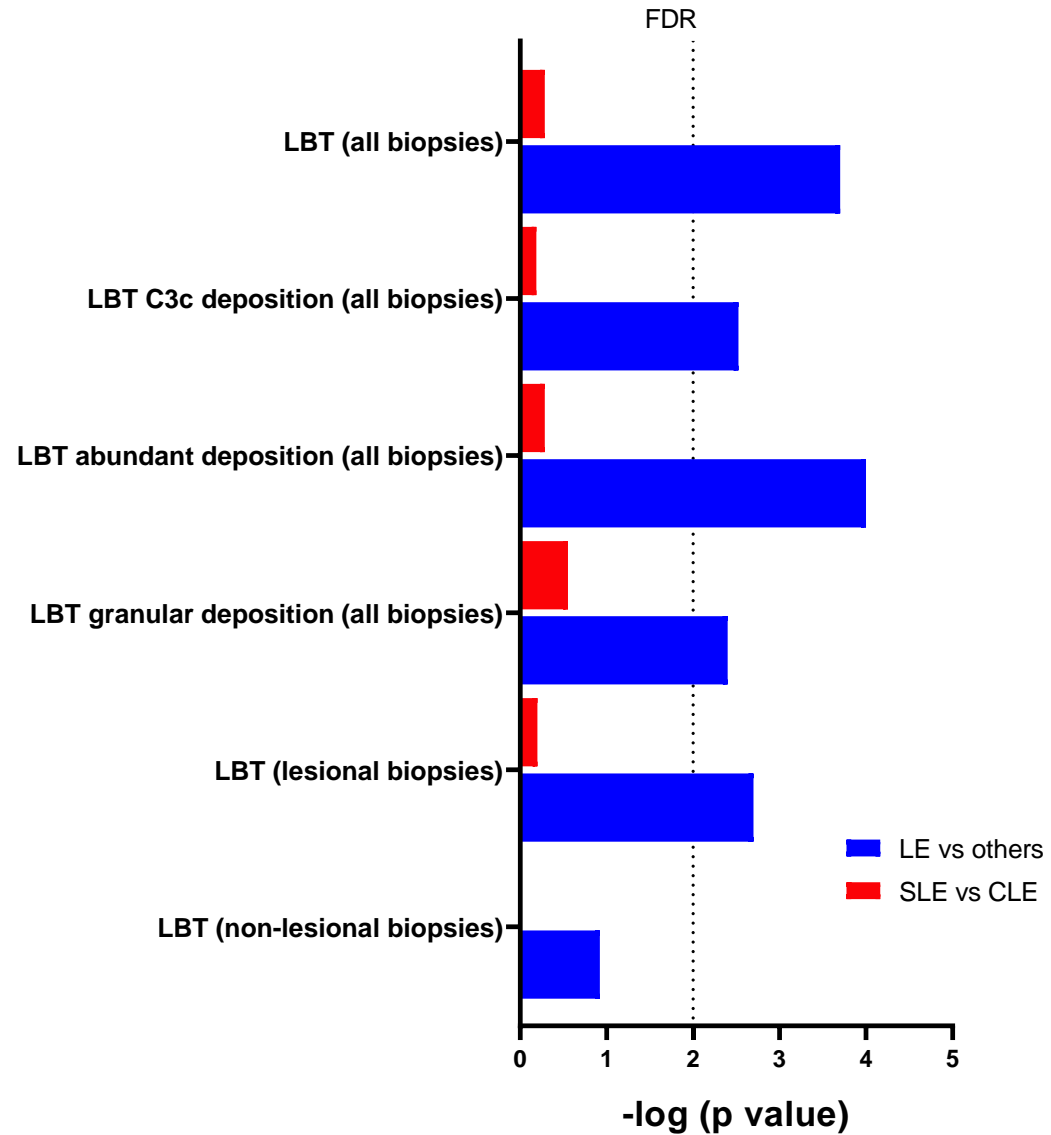
13. Wenzel, J. Cutaneous lupus erythematosus: new insights into pathogenesis and therapeutic strategies. *Nat Rev Rheumatol* **15**, 519–532 (2019).
14. Kuhn, A. & Landmann, A. The classification and diagnosis of cutaneous lupus erythematosus. *Journal of Autoimmunity* **48–49**, 14–19 (2014).
15. Hochberg, M. C. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* **40**, 1725 (1997).
16. Velthuis, P. J., Kater, L., van der Tweel, I., de la Faille, H. B. & van Vloten, W. A. Immunofluorescence microscopy of healthy skin from patients with systemic lupus erythematosus: more than just the lupus band. *Annals of the Rheumatic Diseases* **51**, 720–725 (1992).
17. Baltaci, M. & Fritsch, P. Histologic features of cutaneous lupus erythematosus. *Autoimmunity Reviews* **8**, 467–473 (2009).
18. McCauliffe, D. Cutaneous diseases in adults associated with Anti-Ro/SS-A autoantibody production. *Lupus* **6**, 158–166 (1997).
19. Xue, D. *et al.* A lupus-like syndrome develops in mice lacking the Ro 60-kDa protein, a major lupus autoantigen. *Proc Natl Acad Sci U S A* **100**, 7503–7508 (2003).
20. Provost, T. T., Andres, G., Maddison, P. J. & Reichlin, M. Lupus Band Test in Untreated SLE Patients: Correlation of Immunoglobulin Deposition in the Skin of the Extensor Forearm with Clinical Renal Disease and Serological Abnormalities. *Journal of Investigative Dermatology* **74**, 407–412 (1980).
21. Gangaram, H. B. Lupus Band Test in Systemic Lupus Erythematosus. **59**, 11 (2004).
22. Fismen, S. *et al.* Circulating chromatin-anti-chromatin antibody complexes bind with high affinity to dermo-epidermal structures in murine and human lupus nephritis. *Lupus* **18**, 597–607 (2009).
23. Hanrotel-Saliou, C., Segalen, I., Le Meur, Y., Youinou, P. & Renaudineau, Y. Glomerular antibodies in lupus nephritis. *Clin Rev Allergy Immunol* **40**, 151–158 (2011).
24. Correa-Rodríguez, M. *et al.* Clinical and serological associations of autoantibodies in patients with systemic lupus erythematosus. *J Investig Med* jim-2021-001887 (2021) doi:10.1136/jim-2021-001887.
25. Hedberg, A., Fismen, S., Fenton, K. A., Mortensen, E. S. & Rekvig, O. P. Deposition of chromatin-IgG complexes in skin of nephritic MRL-lpr/lpr mice is associated with increased local matrix metalloprotease activities. *Exp Dermatol* **19**, e265-274 (2010).

26. Lee, Y. *et al.* Ultraviolet irradiation-induced inhibition of histone deacetylase 4 increases the expression of matrix metalloproteinase-1 but decreases that of type I procollagen via activating JNK in human dermal fibroblasts. *J Dermatol Sci* **101**, 107–114 (2021).
27. Cardinali, C., Caproni, M. & Fabbri, P. The utility of the lupus band test on sun-protected non-lesional skin for the diagnosis of systemic lupus erythematosus. 6.
28. Gilliam, J. N. The Significance of Cutaneous Immunoglobulin Deposits In Lupus Erythematosus And Nzb/Nzw F1 Hybrid Mice. *Journal of Investigative Dermatology* **65**, 154–161 (1975).
29. Harrist, T. J. & Mihm, M. C. The specificity and clinical usefulness of the lupus band test. *Arthritis & Rheumatism* **23**, 479–490 (1980).
30. George, R., Mathai, R. & Kurian, S. CUTANEOUS LUPUS ERYTHEMATOSUS IN INDIA: IMMUNOFLUORESCENCE PROFILE. *Int J Dermatol* **31**, 265–269 (1992).
31. Merola, J. F. *et al.* RNA tape sampling in cutaneous lupus erythematosus discriminates affected from unaffected and healthy volunteer skin. *Lupus Sci Med* **8**, e000428 (2021).
32. Xiang, M. *et al.* Bioinformatic analysis of key biomarkers and immune filtration of skin biopsy in discoid lupus erythematosus. *Lupus* **30**, 807–817 (2021).
33. Niewold, T. B. *et al.* Proteome study of cutaneous lupus erythematosus (CLE) and dermatomyositis skin lesions reveals IL-16 is differentially upregulated in CLE. *Arthritis Res Ther* **23**, 132 (2021).
34. Huang, C. *et al.* Disordered cutaneous microbiota in systemic lupus erythematosus. *J Autoimmun* **108**, 102391 (2020).

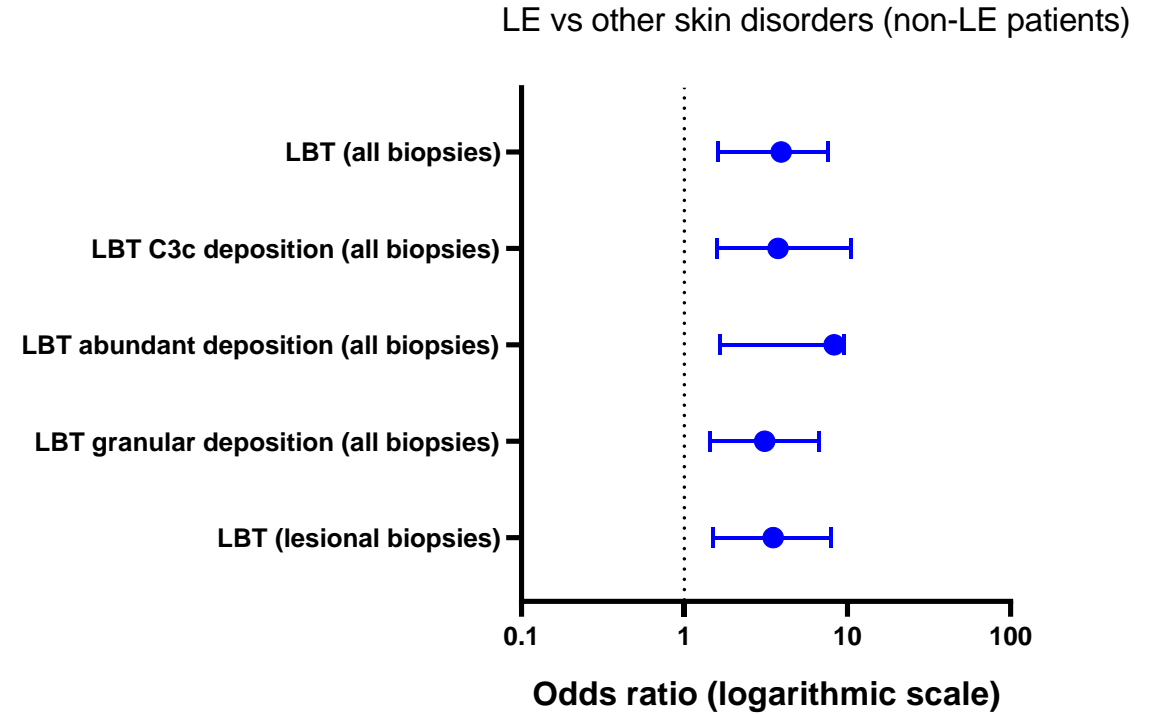


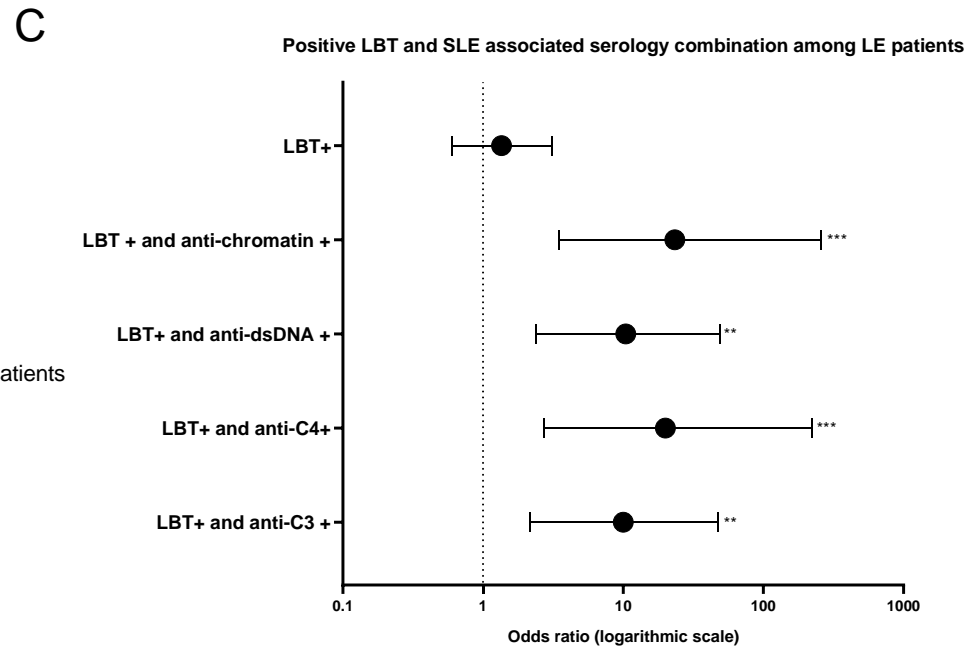
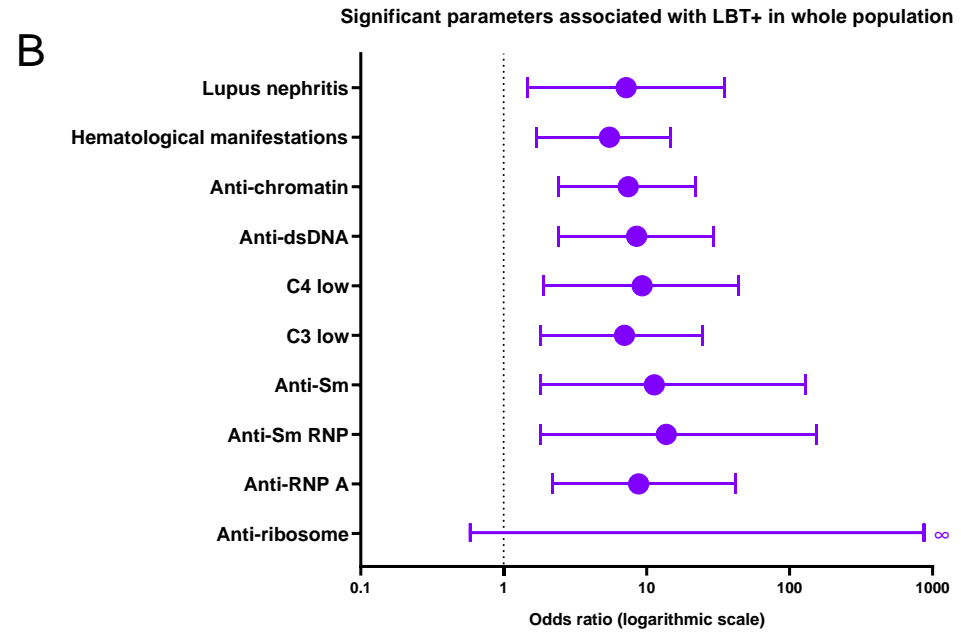
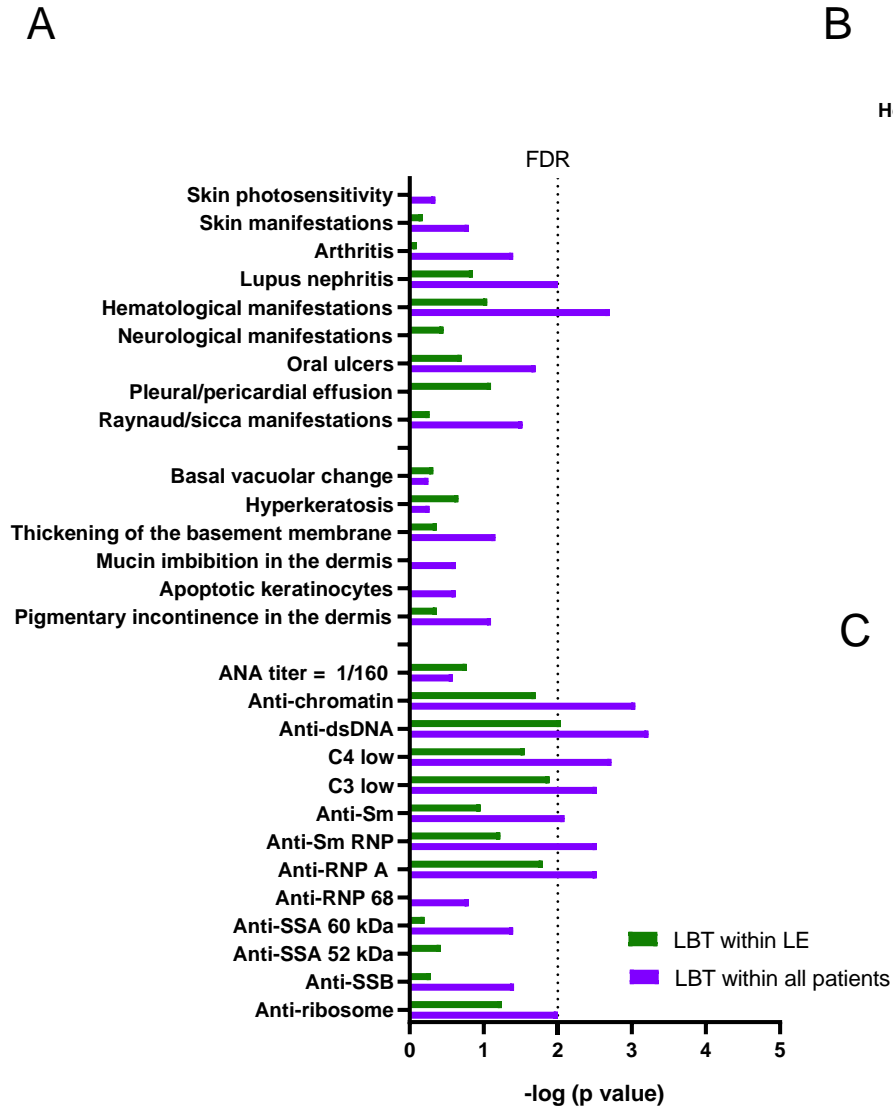


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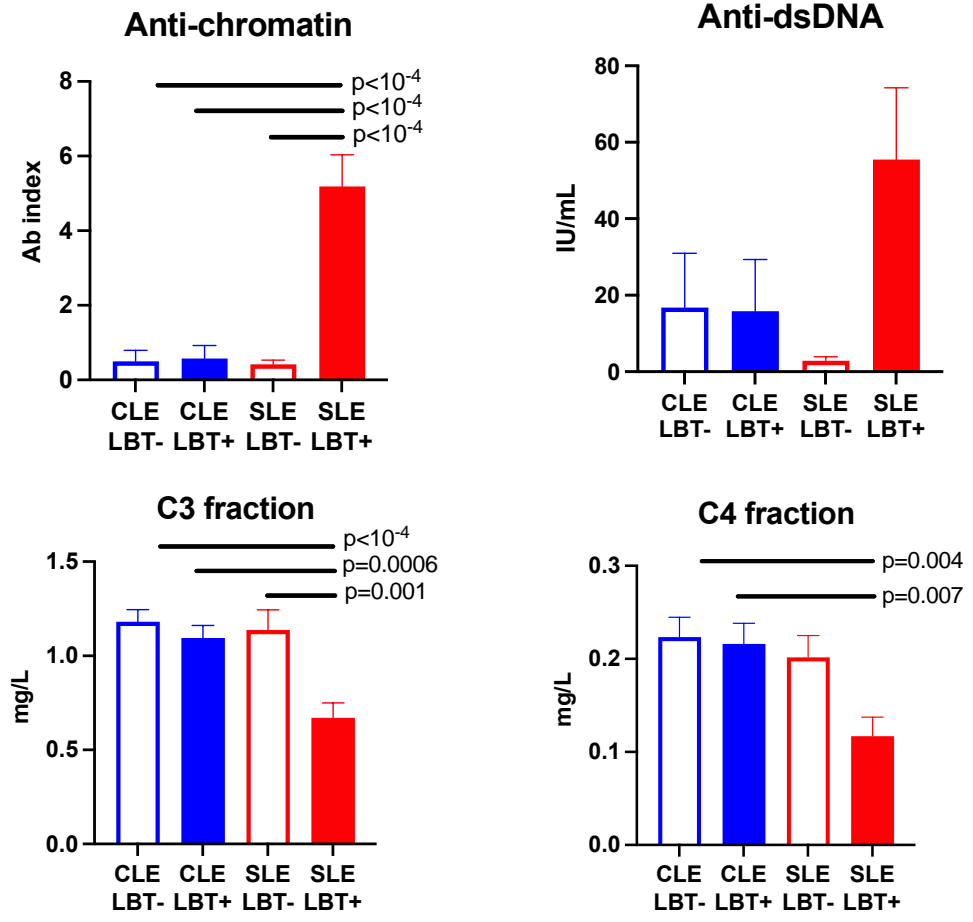


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