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Validity and sensitivity to change of Laser Doppler imaging as a novel objective outcome measure for cutaneous lupus erythematosus

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ABSTRACT

Objectives: To assess reliability of a novel objective outcome measure, Laser Doppler imaging (LDI), its validity against skin biopsy histology, and other clinical instruments including Localised Cutaneous Lupus Disease Area and Severity Index (L-CLASI), Visual Analogue Scale (VAS) score of photographs and its responsiveness to clinical change with therapy.

Methods: A prospective observational cohort study was conducted in 30 active CLE patients. At baseline and 3 months, disease activity was assessed using L-CLASI and a high resolution LDI system by two assessors. Skin biopsy was scored as 0=non-active, 1=mild activity and 2=active. Photographs were assessed by two clinicians using 100mm VAS. Inter-rater reliability was analysed using Bland-Altman limits of agreement. Correlation between histology and LDI, L-CLASI and VAS and sensitivity to change of LDI with physician subjective assessment of change (PSAC) at 3 months were analysed using Kendall's tau-a.

Results: Of 30 patients with CLE, 28 (93%) were female, mean (SD) age 48.4 (11.5) years, 25 (83%) were Caucasians, 25 (83%) had concurrent SLE and 16 (53%) were smokers. CLE subtypes were acute=9, subacute=8 and chronic=13. Inter-rater agreement for LDI was fair but for VAS score of photographs was poor. In 20 patients with biopsy, correlation with histology was better for LDI (tau-a=0.56) than L-CLASI [0.26; difference 0.27 (90% CI 0.05, 0.49)] or VAS score of photographs [0.17; 0.36 (0.04, 0.68)]. There was a moderate correlation between PSAC score and change in LDI (tau-a=0.56, 90% CI 0.38, 0.74; $p<0.001$, n=15).

Conclusion: LDI provides a reliable, valid and responsive quantitative measure of inflammation in CLE. It has a better correlation with histology compared to clinical instruments. LDI provides an objective outcome measure for clinical trials.

(274 words)

INTRODUCTION

Cutaneous lupus erythematosus (CLE) comprises a wide of range of dermatologic manifestations seen in patients with or without systemic lupus erythematosus (SLE). In general, the prognosis of isolated CLE is considered more favourable than SLE but it may evolve into SLE in about 20% of the patients [1]. CLE can be divided into LE-specific lesions i.e. Acute Cutaneous LE (ACLE), Subacute Cutaneous LE (SCLE) and Chronic Cutaneous LE (CCLE) and LE-nonspecific skin lesions (e.g. vasculitis) [2]. Deposition of immune complexes containing IgM, IgG and complement C3 at the dermo-epidermal junction is pathognomonic in the former. However, not all CLE is driven by B-cell dysfunction [3]. There is also a markedly greater increase in Type I interferon (IFN-I) activity in the skin compared to blood, which precedes onset of clinical symptoms [4].

Given this immunological heterogeneity, it is not surprising that responses to therapy vary. Cutaneous response rates to the licensed biologic in SLE are mixed in clinical trials with lower numbers of British Isles Lupus Activity Group Disease Activity Index version 2004 (BILAG-2004) and SLE Disease Activity Index version 2000 (SLEDAI-2K) improvements compared to the musculoskeletal domain [5]. We previously showed that poor response or worsening could be observed in the skin of patients who otherwise responded well to rituximab in other organ systems [6].

Current methods for assessing CLE activity include validated clinical indices such as the CLE Disease Area and Severity Index (CLASI) [7] and the revised CLASI [8]. For SLE, specific assessment for mucocutaneous components are included in the BILAG-2004 [9] and SLEDAI-2K [10]. However, these indices depend on the physicians' judgement and if each symptom is due to active CLE, damage or another disease. They also require adequate training and may be complex for inexperienced clinicians to use. SLEDAI-2K cannot capture partial response to

therapy or differentiate mild, moderate or severe disease [11]. The BILAG has a more limited ability to capture partial response, since a wide range of severity and change in the skin fall within the BILAG B rating, and improvement relies on the assessors' ability to recall previous appearances. These instruments were developed to document activity in the whole body rather than localised lesions. Thus, validated, objective, quantitative outcome measures are needed. These would allow us to more sensitively detect improvements with therapy, and to compare the different targeted therapies proposed, with less influence by expertise and recall of the assessors.

Microcirculatory abnormalities contribute to the pathophysiology of many autoimmune rheumatic diseases including CLE [12]. Accordingly, various therapies target this and have pharmacologic effect on skin microcirculation [13, 14]. High Resolution Laser Doppler imaging (LDI) is a single-probe, non-invasive imaging modality that monitors the total local microcirculatory blood perfusion including the perfusion in capillaries, arterioles, venules and shunting vessels based on a phenomenon known as the "Doppler effect" [15]. LDI has been used to assess responsiveness after pharmacological stimuli in patients with Raynaud's phenomenon [16]. Alteration in peripheral blood flow (as measured by LDI) has been shown to correlate with inflammation in skin psoriasis [17]. Nevertheless, LDI has not previously been investigated in CLE or cutaneous manifestations of SLE.

The objectives of this study were to assess the reliability of LDI, its criterion and construct validity and responsiveness to clinical change with therapy, with a view to providing a tool that can objectively document response to therapies in clinical trials.

METHODS

Design and Patients

A prospective observational cohort study was undertaken in consecutive patients with active CLE in Leeds, UK between October 2014 and October 2017. Inclusion criteria were (i) adults (≥18 years old); (ii) active CLE as defined by CLASI≥4; and (iii) underwent a change in therapy either increment in dosage or commencement on new immunosuppressive therapies.

Ethical Approval

All patients provided informed written consent and this research was carried out in compliance with the Declaration of Helsinki. All research activities were performed under ethical approval, REC 10/H1306/88, National Research Ethics Committee Yorkshire and Humber–Leeds East, UK. The University of Leeds was contracted with administrative sponsorship. Written consent to publication of photographs from the patients were obtained using Leeds Teaching Hospitals NHS Trust consent form.

Demographic and laboratory assessment

The following demographic and clinical parameters were recorded: age, gender, ethnicity, smoking history, concurrent diagnosis of SLE and concomitant therapies including corticosteroid.

Anti-nuclear antibody (ANA) and a panel of nuclear autoantibodies including anti-dsDNA and extract nuclear antigens (ENAs, including Ro52, Ro60, La, Sm, Chromatin, RNP, Sm/RNP and Ribosomal P) were tested using Bioplex 2200 Immunoassay. Complement levels (C3 and C4) were measured by nephelometry.

Clinical assessment

All patients were assessed in a combined rheumatology–dermatology connective tissue disease clinic at baseline and 3 months post-treatment. The Gilliam classification was used by

consultant dermatologists to grade CLE as ACLE, SCLE, CCLE and non-specific LE (e.g. vasculitis) [2].

Physician outcome measures

CLE disease activity was initially assessed using CLASI to determine eligibility for inclusion into the study. Since only one area with the highest CLASI score was assessed using the LDI, a modified Localised CLASI (L-CLASI), [18] which consists of the sum of erythema and scaling/hypertrophy scores of the selected area, with a maximum total score possible of 5 was used for assessment and analysis.

At 3 months, the Physician Subjective Assessment of Change (PSAC) [19] was used by a dermatologist (independent of L-CLASI scoring) to grade changes in activity as worsening, unchanged or improved since the last visit, the physician's subjective assessment of the patient's skin disease.

Digital Photographs Score

Digital photographs of the CLE lesions were taken using a macro digital camera, Canon EOS 600D during pre- and post-treatment. These were assessed by a dermatologist and a rheumatologist who were blinded to patients' clinical status and sequence. Both clinicians rated the overall CLE disease activity using a visual analogue scale (VAS) of 100mm; where 0=very good and asymptomatic and 100=very poor and severe symptoms. The two raters then provided the agreed scores for each photograph. Significant discrepancies were resolved by consensus.

Dermatology Life Quality Index

The patients completed the Dermatology Life Quality Index (DLQI) at baseline. This instrument consists of 10 questions concerning the impact of CLE on different aspects of their health related quality of life over the last week. Each question was scored on a 4-point Likert scale as follows: Not at all/Not relevant=0, A little=1, A lot=2 and Very much=3. Scores of

individual items (0-3) are added to yield a total score (0-30); higher scores represent greater impairment of patient's quality of life [20].

Laser Doppler Imaging

An area with the highest L-CLASI score and non-lesional area were evaluated using a high resolution LDI system (moorLDI2-IR, Moor Instruments UK) by either a medical physicist or a rheumatologist; both trained in the operation of LDI and blinded to patients' L-CLASI scores. For some patients, the scans were performed by both operators (within 15 minutes apart) to assess inter-rater reliability. All scans were performed in a designated assessment room after the patients were acclimatised in room temperature (23º Celcius) for 15 minutes. Images were acquired at a distance between 40-70 cm depending on the size of the selected region, using a bandwidth between 250Hz-15KHz and the scan speed of < 4ms/pixel. The region of interests (ROIs) were selected and analysed using Moor LDI2-IR v6.0 software. Typical scan resolutions were set to 170 x 100 pixels but were adjusted to ensure that imaging times were below 2 minutes or less in order to minimise movement artefacts. Gain was set to zero to avoid signal saturation. Appropriate health and safety precautions were taken when using the LDI scanner.

The absolute difference in the mean perfusion between active and non-active CLE lesions was calculated and expressed in perfusion unit (PU). PU is an arbitrary unit, which is proportional to the product of the average speed of the RBCs and their concentrations.

Skin Biopsy Score

Skin biopsy samples obtained from the consenting patients were rated in real-time by a histopathologist, with over 10 years' experience in reporting CLE cases and blinded to patients' clinic status. Since there was no standardised scoring system, the biopsy was scored based on the six classic features of CLE [21, 22] including (i) interface dermatitis; (ii) inflammatory cell

infiltrate in a perivascular, periappendageal or subepidermal location; (iii) vacuolar alteration of the basal layer; (iv) thickening of the basement membrane; (v) follicular plugging and (vi) the presence of immunofluorescence. The first two parameters were rated using a graded scale of 0-2; 0=absent, 1=mild and 2=strong while the remaining four parameters using a binary scale; 0=absent, 1=present, with a maximum total score possible of 8. Since these parameters were not weighted for clinical importance, an overall histology grade was assigned using a graded scale of 0-2; 0=non active, 1=mild and 2=active.

Statistical Analysis

Descriptive statistics were summarised using either mean with SD or median with IQR for continuous variables and proportion for categorical variables. Agreement of the VAS score of photographs between the dermatologist and rheumatologist and inter-reader reliability of the LDI were analysed using Bland-Altman limits of agreement (LOA). The gold standard reference was the overall histology grade. For criterion and construct validity, correlation between the LDI parameter and overall histology grade as well as other outcome measures i.e. L-CLASI and VAS score of photographs were analysed using Kendall's tau-a. This test was also used to assess sensitivity to change of the LDI and expressed in terms of mean difference versus average and 90% confidence interval (CI). 90% CI was chosen to obtain a narrower interval due to sample size and the exploratory nature of this research. Correlation between the two continuous variables, LDI and DLQI was assessed using Pearson correlation coefficient.

All statistical analysis was performed using Stata v.13.1 (StataCorp College Station, Texas, USA) for Windows and Graph Pad Prism v.8.0 (GraphPad, La Jolla, CA, USA).

RESULTS

Patient and treatment characteristics

30 patients with active CLE were recruited into the study. Of these, 28 (93%) were female, mean (SD) age 48.4 (11.5) years, 25 (83%) were Caucasians, 25 (83%) had concurrent SLE and 16 (53%) were smokers. The proportions of CLE subtypes were ACLE=30%, SCLE=27% and CCLE=43%. Baseline characteristics are described in **Table 1.**

Inter-rater agreement for VAS score of photographs

There was a poor agreement in VAS score of digital photographs between the two assessors; mean difference -9.5 mm (90% CI LOA -36.2 to 17.3) versus average **(Figure 1A).**

Inter-rater agreement for LDI

In the 7 patients where the LDI measurements were independently assessed by two assessors, inter-reader reliability of LDI was fair; mean difference 11.5 PU (90% CI LOA -61.6 to 84.5) versus average **(Figure 1B).**

Validity of LDI against histology, L-CLASI and VAS score of photographs

Skin biopsies were performed in 20/30 patients. Of these, correlation with histology was better for LDI (tau-a=0.53, 90% CI 0.35-0.72; p<0.001) than L-CLASI [tau-a= 0.26 (0.06-0.46); p=0.036, difference LDI vs L-CLASI 0.27 (90% CI 0.05-0.49)] or VAS score of photographs [tau-a=0.17 (-0.07 – 0.40); p=0.223, difference LDI vs VAS 0.36 (0.04-0.68)] **(Figure 2A).** Three patients who were mimickers of active CLE indeed had negative histology and low PU using LDI. Examples of images of two patients with active CLE symptoms; each with and without positive histology are illustrated in **Figures 3 and 4** respectively.

Relationship between LDI and DLQI

22/30 patients completed DLQI at baseline visit. Mean (SD) DLQI at baseline was 14.7 (8.1). There was a moderate correlation between LDI and DLQI, r = 0.515; p=0.014.

Clinical response to therapy

15/30 patients had a follow-up at 3 months following a change in therapy. Of these, the proportions of patients who improved, unchanged and worsening based on PSAC score were 7 (47%), 3 (20%) and 5 (33%) respectively. There was a trend to improvement at 3 months using the various therapies employed to treat CLE based on L-CLASI score, mean difference pre- and post-treatment 0.73 (95% CI -0.06 to 1.53); p=0.068. Details of therapies and responses are described in **Table 2.**

Responsiveness to clinical change with therapy between PSAC score and LDI

The percentage change from baseline between pre- and post-treatment perfusion difference in LDI was calculated. There was a moderate correlation in sensitivity to change between PSAC score and the LDI (tau-a=0.56, 90% CI 0.38-0.74; p<0.001). This relationship is depicted in **Figure 2B.**

DISCUSSION

This report presents data on the use of a novel objective outcome measure, LDI in assessing disease activity in various CLE morphologies. We show that LDI appears to be reliable, correlates better with the gold standard reference, the overall histology grade, compared to other clinical indices and is sensitive to change with therapy.

CLE is heterogeneous in term of its pathophysiology. Recent advances have demonstrated the role of keratinocytes and endothelial cells in perpetuating inflammation by expressing adhesion molecules in response to ultraviolet light or other stimuli [23]. Since measurement of endothelial activities are not routinely available, a logical approach is to quantify microvascular blood flow to the inflamed skin, which can be achieved using LDI. Other advantages of LDI include its convenience and non-invasiveness i.e. non-contact compared to a skin biopsy. Indeed, in other

autoimmune skin diseases like psoriasis, changes in LDI outperformed the expression level of CD31, a marker of endothelial activity in active psoriasis plaques [24].

Current CLE disease activity indices such as the CLASI [7] and RCLASI [8] were developed to document activity in the whole body rather than localised lesions. The overall CLASI or RCLASI scores may not be a true representative of the intensity of the activity in localised lesions. Modified limited versions of these indices could be used to assess these lesions but they have not been validated. LDI overcomes this by providing quantitative measures of individual CLE lesions. Moreover, the distribution of CLE lesions is often more localised than generalised as well as less systemic [25]. Despite this, these individual lesions can be difficult to treat and most importantly, affect patients' self-esteem and quality of life [26]. This is especially relevant in cutaneous or systemic lupus since lesions commonly occur on visible areas of the skin such as the face, neck and scalp. Thus, by quantifying the degree of inflammation objectively using LDI, this will guide the clinicians to tailor the intensity of therapy accordingly. Furthermore, in order to minimise toxicity from systemic therapies, various topical [18, 27] and intra-dermal route of drug delivery [28] are currently used and in development to target these individual lesions. Thus, LDI is an ideal tool to document responsiveness to these therapies objectively and can delineate the problem with subjectivity in the assessments of change between pre- and post-treatment.

Although the CLASI has been validated and is widely used, another limitation of this index is the element of training-dependent subjectivity in differentiating active disease, damage and non-inflammatory pathologies. There is also potential for bias by incorporating patient-reported symptoms in the score (i.e. recent hair loss within the last 30 days, and duration of dyspigmentation after active lesions have resolved [7]) with total scores being adjusted based on these verbal reports. We found the inter-rater agreement in VAS score of photographs was

poor, which could be contributed to inability to detect salient morphology like hypertrophy of CLE through photo images. On the other hand, although one may argue that LDI may be operator-dependent, the inter-rater reliability between a medical physicist and a rheumatologist was fair in this study. The apparent wide confidence interval shown has to be interpreted in the context of a wide range of possible PU values, ranging from 0 to 1500.

This study has some limitations. First, the number of patients available to assess responsiveness to change using LDI was too small (n=15) to calculate the optimal threshold of changes in LDI against the magnitude of improvement by PSAC. However, we had shown that the percentage change in LDI parameter and PSAC score was moderately correlated. Next, although LDI was correlated with DLQI at baseline, its minimal clinically important difference post-treatment could not be estimated due to the sample size. Lastly, LDI was performed in selected areas with the highest CLASI score for patients although many had generalised CLE. This may limit the validity of measurement of response in widespread disease where responses may differ between body areas. However, for topical therapies or early phase assessment of novel systemic therapies, assessment of a selected target lesion has been used successfully. Further studies would be needed to explore its use in patients with generalised CLE, the feasibility of capturing multiple images throughout the body with respect to resources and tolerability as well as flare prediction in areas with high PU value but clinically asymptomatic.

In conclusion, LDI provides a reliable, valid quantitative measure of inflammation in CLE and appears to be responsive to change with therapy. LDI has a better correlation with histology compared to currently available clinical instruments. Further validation and longitudinal analysis will provide evidence on its usefulness in clinical trials and quantitative assessment of treatment response.

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FIGURE LEGENDS:

Figure 1: Inter-rater agreement of VAS score of photographs and LDI. Both graphs display a scatter diagram of the differences plotted against the averages of the two measurements. Horizontal lines are drawn at the mean difference (blue), and at the limits of agreement (green). A) There was a poor agreement of VAS score of photographs using the 100mm scale between the two raters. B) The agreement for LDI between the two assessors was fair. LDI: Laser Doppler imaging, PU: perfusion unit, VAS: visual analogue scale

Figure 2: Criterion and construct validity of LDI and its responsiveness to change with therapy. A) Of 20 patients with skin biopsy, correlation with histology was better for LDI than L-CLASI and VAS score of photographs. The bars represent box and whiskers plots while the error bars denote tukey. B) Of 15 patients with a follow-up at 3 months, the change in perfusion unit of LDI between baseline and 3 months corresponded to PSAC score. L-CLASI: modified localised cutaneous lupus disease area and severity index, LDI: Laser Doppler imaging, PSAC: physician subjective assessment of change, PU: perfusion unit, VAS: visual analogue scale

Figure 3: LDI images of a patient with clinically active CLE, positive histology and high PU. Her L-CLASI score was 3 (Erythema=2, Scale/Hypertrophy=1). ROIs 1-3 denote active lesions while 4-5 represent non-lesional areas. Absolute difference was calculated by subtracting non-lesional with the smallest ROI from active lesion with the largest ROI. PU: perfusion unit, ROI: region of interest

Figure 4: LDI images of a patient perceived clinically active CLE but with negative histology and low PU. Her L-CLASI score was 2 (Erythema=1, Scale/Hypertrophy=1). ROIs 1, 4 and 5 denote clinically active lesions while 2-3 represent non-lesional areas. PU: perfusion unit, ROI: region of interest

Table 1: Baseline characteristics of the 30 patients with active CLE

Characteristics	Values
Age, years; mean (SD)	48.4 (11.5)
Female; N (%)	28 (93)
Ethnicity; N (%)	
Caucasian	25 (83)
Indian/South Asian	5 (17)
Diagnosis; N (%)	
Systemic lupus erythematosus	25 (83)
Cutaneous lupus erythematosus only	5 (17)
Type of cutaneous lupus erythematosus; N (%)	
Acute cutaneous lupus erythematosus	9 (30)
Sub-acute cutaneous lupus erythematosus	8 (27)
Chronic cutaneous lupus erythematosus	13 (43)
Positive ANA at Baseline assessment; N (%)	25 (83)
Positive ANA specificities; N (%)	
anti-dsDNA	3 (10)
anti-Ro	16 (53)
anti-La	6 (20)
anti-Smith	4 (13)
anti-Chromatin	9 (30)
anti-RNP	4 (13)
anti-Ribosomal P	1 (3)
Low complement levels (C3 or C4); N (%)	2 (7)
Ever smoked; N (%)	16 (53)
Concomitant immunosuppressant; N (%)	
Anti-malarials	20 (67)
Methotrexate	4 (13)
Mycophenolate Mofetil	11 (37)
Abatacept	1 (3)
Belimumab	1 (3)

Characteristics	Values
Rituximab	7 (23)
Other investigational drugs	4 (13)
Location of Index CLE lesion for assessment; N (%)	
Scalp	8 (27)
Face	9 (30)
Neck	3 (10)
Arms	4 (13)
Back	6 (20)

ANA: Anti-nuclear antibody, CLE: cutaneous lupus erythematosus, dsDNA: Double stranded deoxyribonucleic acid, LDI: Laser Doppler imaging, RNP: Ribonucleic protein.

Table 2: Details of treatment prescribed for CLE and response

ID	CLE subtype	Conventional or Biological Disease Modifying Anti-Rheumatic Drugs	Pre-Treatment		Post-Treatment			PSAC score
			L-CLASI	Absolute Difference in LDI (PU)	L-CLASI	Absolute Difference in LDI (PU)	Percentage change from baseline in LDI (%)	
1	CCLE	Belimumab	3	704.3	5	1072.8	-52.3	Worsening
2	CCLE	Investigational Drug	4	580.5	4	735.6	-26.7	Worsening
3	ACLE	HCQ, MMF	4	221.6	2	508.0	-129.2	Worsening
4	CCLE	HCQ	4	120.0	4	246.3	-105.3	Worsening
5	SCLE	HCQ, MMF, RTX	2	321.2	5	608.8	-89.5	Worsening
6	CCLE	HCQ,MMF	3	533.9	4	600.5	-12.5	No change
7	CCLE	MTX	4	874.8	4	397.6	54.6	No change
8	SCLE	HCQ, MTX	3	10.9	1	14.1	-29.4	No change
9	CCLE	Investigational Drug	5	732.0	3	228.6	68.8	Improved
10	CCLE	Investigational Drug	4	640.8	2	415.5	35.2	Improved
11	SCLE	HCQ, MMF	2	242.4	1	213.6	11.9	Improved
12	CCLE	RTX	3	259.3	1	144.9	44.1	Improved
13	CCLE	HCQ	3	380.0	2	85.3	77.6	Improved
14	CCLE	HCQ	3	351.3	2	170.0	51.6	Improved
15	ACLE	HCQ, MMF, RTX	2	301.9	0	176.8	41.4	Improved

ACLE: acute cutaneous lupus erythematosus, CCLE: chronic cutaneous lupus erythematosus, HCQ: hydroxychloroquine, L-CLASI: modified localised cutaneous lupus disease area and severity index, LDI: Laser Doppler imaging, MMF: mycophenolate mofetil, MTX: methotrexate, RTX: rituximab, PSAC: physician subjective assessment of change, PU: perfusion unit, SCLE: sub-acute cutaneous lupus erythematosus