Assessment of microvascular involvement in lupus nephritis patients by retinal OCT-angiography and kidney biopsies

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Abstract

Objective

Ocular and renal microvascular damage in lupus nephritis (LN) share similar physiopathological pathways that have investigated using traditional fundus examination and high-resolution colour electroretinography. Optical coherence tomography angiography (OCTA) is a recent, non-invasive technique for imaging the microvasculature of retina and choroid. Aim of the study was to investigate through OCTA analysis the relationship between retinal microvasculature alterations and renal function and histologic features.

Methods

Systemic lupus erythematosus (SLE) patients with LN, SLE without renal involvement and healthy controls were recruited and accomplished an ophthalmological evaluation, including OCTA. SLE-LN patients underwent a rheumatological evaluation, including disease-related clinical and laboratory features collection and kidney biopsy examination.

Results

This cross-sectional study enrolled forty-six eyes of 23 LN patients, thirty-two eyes of 16 SLE patients and forty-two eyes of 21 controls. Thirteen SLE-LN patients (56.5%) displayed lupus retinopathy, 10 at moderate (77%) and 3 at severe stage (23%) by fundus oculi examination. Analysis of OCTA data showed with high/moderate accuracy a reduction of retinal capillary vessel density in both SLE and SLE-LN patients compared to controls in superficial and deep plexi. A reduction in fovea thickness and an increase in foveal avascular zone were also detected. OCTA data of LN patients correlated with LN duration, disease activity, kidney function and the presence of LN-vascular lesions at kidney biopsy.

Conclusion

Our results suggest the role of OCTA in early detection of systemic vascular involvement in SLE-LN patients and related kidney functional-histological impairment.

Key words

eye, lupus nephritis, retina, systemic lupus erythematosus, optical coherence tomographic angiography

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Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease involving different organs and systems and lupus nephritis (LN) is its most common organ-threatening manifestation. LN develops in up to 40% of SLE patients, usually appearing in the first three years from onset (1), and kidney biopsy is still the gold standard for diagnosis (2). Lupus nephritis pathogenesis comprises a broad range of different mechanisms as immune-complex deposition or immune-complex formation in situ, both involved in glomerular and tubulointerstitial damage. (3)

Lupus retinopathy has a prevalence of 7–29% among the SLE population. It is the second most-common SLE ocular manifestation and can be associated with a wide range of clinical patterns, from subclinical involvement to visual loss (4). Lupus retinopathy may correlate with systemic disease activity, since immune-complexes depositions, inflammation and subsequent microvascular damage (vasculopathy) proved to have an important role in its pathophysiology. (4-5). The standard for diagnosis is fundoscopy (4), however in the last few years optical coherence tomography angiography (OCTA), has been investigated for its ability to detect retinal vascular damage at its preclinical stage in systemic diseases, including SLE (6-9). OCTA is a non-invasive technique that, in addition to the measure of the thickness of the different retinal layers, is able to visualise the retinal microvasculature detecting blood flow without intravenous dye injection (10).

The aim of this study was to investigate a potential correlation between ocular and renal microvascular damage in SLE-LN patients through OCTA analysis of retinal microvasculature and evaluation of renal functional and histologic features.

Methods

The study was designed as a pilot study. We recruited SLE patients referred to our rheumatology clinic of the Policlinico of Rome Tor Vergata, Italy between 1 January 2017 and 31 December 2018. SLE was classified according to the 2012 Systemic Lupus International Collaborating Clinics classification criteria (11).

The inclusion criteria were:

1. age between 18 and 65 years old;
2. LN according to the International Society of Nephrology/Renal Pathology Society classification for lupus nephritis (12) at kidney biopsy or an history of persistent proteinuria level ≥500 mg at 24-hour urine collection;
3. best-corrected visual acuity (BCVA) >0.5 LogMAR.

The exclusion criteria were:

1. the coexistence of other rheumatic diseases, such as Sjögren's syndrome, systemic sclerosis, mixed connective tissue disease, rheumatoid arthritis;
2. retinal toxicity due to antimalarials according to the 2016 American Academy of Ophthalmology criteria (13);
3. a diagnosed primary ophthalmic pathology, the presence of lens opacities, drusen-like deposits, focal atrophy, retinal pigment epithelium detachment, an history of ocular trauma or surgery;
4. presence of systemic disorders such as diabetes, cardiovascular disease, uncontrolled dyslipidaemia (total cholesterol ≥240 mg/dL, LDL ≥160 mg/dL, HDL <40 mg/dL), or uncontrolled hypertension (systolic blood pressure >140 mm Hg, or diastolic blood pressure >90 mm Hg).

A population of SLE patients without nephritis referred to the rheumatologic clinic and healthy controls (HC) were also recruited at the Ophthalmology Clinic of the Policlinic of Rome Tor Vergata, Italy, matched for sex and age with the SLE-LN population.

Written informed consent was obtained from patients and controls according to the Declaration of Helsinki (updated 2008) and the study was approved by the Scientific Ethics Committee of the University of Tor Vergata.

Rheumatological evaluation

All SLE patients underwent a rheumatological evaluation. Disease activity and cumulative organ damage were evaluated using respectively the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) (14) and the Systemic Lupus International Collaborating Clinics Damage

Index (SLICC-DI) (15). Renal disease activity was assessed using the renal domain score of the SLEDAI (16).

Disease duration and time since the first clinical evidence of kidney involvement were assessed. Other patients' clinical information collected were: age, sex, history of anti-rheumatic treatment, including hydroxychloroquine (HCQ, daily and cumulative doses), prednisone (PDN, daily and cumulative doses), immunosuppressive drugs (azathioprine, methotrexate, cyclosporine, mycophenolate, cyclophosphamide, leflunomide) and biologic treatment (rituximab and belimumab).

The following laboratory parameters were also tested: presence of disease characterising autoantibodies, such as anticardiolipin antibodies (IgG ≥40 GPL units, IgM ≥40 MPL units) by commercial enzyme-linked immunosorbent assay (ELISA, Inova Diagnostics, San Diego, CA, USA), anti-β2-glycoprotein I antibodies (IgG ≥40 GPL units; IgM ≥40 MPL units) by commercial ELISA (QUANTA LiteTM; Inova Diagnostics), anti-dsDNA antibodies (IU/mL, over twice the upper limit of normal) by commercial ELISA, anti-RNP antibodies by immunoblotting and anti-nuclear antibodies (ANA ≥ 1:160) by direct immunofluorescence (QUANTA LiteTM; Inova Diagnostics), the presence of lupus anticoagulant by dilute Russel's viper venom time and confirmatory mixing studies if prolonged and plasmatic complement components C3 and C4 (mg/dl; normal values: 83–193 and 15–57, respectively), measured by immunoturbidimetry. Kidney function was evaluated through the following biochemical assays: serum creatinine (mg/dl; normal values: 0.55–1.02) and blood urea nitrogen (BUN; mg/dl; normal values: 15-40) detected by enzymatic colorimetric assay, estimated glomerular filtration rate (eGFR; ml/min), calculated through the Modification of Diet in Renal Disease Study (MDRD) equation, creatinine clearance (ml/min; normal values: 59–151) and 24 hours proteinuria (PTU; mg/24h; normal values: ≤300 mg) measured by enzymatic colorimetric assay and spot urine analysis with the examination of urinary sediment.

Histology of kidney biopsy reports were evaluated by two examiners (PC and SF). Biopsies were analysed by standard optical microscopy, electron microscopy and direct immunofluorescence tests.

Histological lesions characterising each class of LN were evaluated and categorised in active, chronic and vascular lesions according to the 2003 ISN/RPS classification criteria of lupus nephritis (17, 18). Renal histologic lesions were defined according to the presence or absence (1/0) of the specific lesion. All the information collected were included in a database.

Ophthalmologic evaluation

A standard LogMAR chart is used to determine the BCVA in each eye to test central visual acuity according to the Early Treatment of Diabetic Retinopathy Study (ETDRS) protocol. All subjects underwent Goldmann applanation tonometry to assess the intraocular pressure using a Haag-Streit tonometer with slit lamp. Ocular fundus examination by biomicroscopic examination at slit-lamp and high-resolution color retinography (Canon CF-1 digital retinal camera, Canon Inc.) were performed in order to define presence or absence (1/0) and grade of lupus retinopathy (4).

OCTA was performed both in the SLE-LN patients and HC, by the Avanti AngioVue Imaging System (Optovue, Inc., software version 2018.1.0.22, Fremont, CA), using the Angio Retina mode 6x6 mm volumetric scan in macular area with an A-scan rate of 70 kHz, a light source centered on 840 nm and a bandwidth of 45–50 nm as previously shown (6). The segmentation software automatically identified the boundaries of the retinal layers from the structural OCT cross-sectional images. Retinal layer segmentation was checked for artifacts by the same experienced operator. Only images with quality >8 were considered for the study (19).

The vascular density was evaluated and expressed as % vessel density both in superficial and deep retinal plexi, each of these analysing firstly the whole image, then the parafoveal and the foveal region. The fovea avascular zone (FAZ) area (mm^2) and perimeter (mm) of the two groups were also assessed. Moreover, thickness at the whole image, foveal, and parafoveal zone were measured by OCTA (7, 10).

Statistical analysis

To test normality of the data sets, the D'Agostino and Pearson omnibus test were used. Normally distributed variables were summarised using means ± SD. Non-normally distributed variables were summarised using medians with percentile ranges. Categorical variables were presented with absolute frequencies and percentages.

The variability between the two eyes of the individuals was checked by one-way ANOVA and we considered for each parameter of the individuals the mean of the two eyes.

Categorical variables were compared using the χ^2test. Continuous variables were compared using the parametric unpaired *t*-test or the non-parametric Mann-Whitney U-test when appropriate. The significance of any correlation was determined by the Pearson correlation test or Spearman's rank correlation coefficient where appropriate. For the evaluation of OCTA densities as a diagnostic test, receiver operating characteristic (ROC) curve analysis was performed using densities of both SLE-LN patients and HC; accuracy was measured by the area under the ROC curve (AUC). Youden's index with likelihood ratio >2 was considered for choosing the optimal threshold value for which sensitivity and specificity are maximised. *p*-values <0.05 were considered significant. All statistical analyses were performed using GraphPad Prism (v. 8; GraphPad software).

Results

Forty-six eyes of 23 SLE-LN patients, thirty-two eyes of 16 SLE without kidney involvement and forty-two eyes of 21 HC were evaluated. Table I shows all the demographic, clinical and ophthalmological parameters evaluated in the study population. Patients and controls were matched for age and sex.

The mean disease duration was 177.6± 126.6 months in SLE-LN and 197±107 months in SLE, while the mean time since LN clinical onset was 108±97

Table I. Demographic, clinical and laboratory parameters of the study population.

	SLE-LN n=23	SLE n=16	HC n=21	*p*-value
Age (years)	44.4 ± 13.8	44.24 ± 9	38.3 ± 10.4	NS
Female (n/%)	21/91.3	14/87.5	17/81	NS
Disease duration (months)	177.6 ± 126.6	197 ± 107	NA	NS
LN duration (months)	108 ± 97	NA	NA	
C3 (mg/dl)	98.8 ± 28.8	97 ± 35	NA	NS
C4 (mg/dl)	22.1 ± 10	19 ± 8	NA	NS
Anti-dsDNA (n/%)	18/78.2	12/75	NA	NS
Anti-PL (n/%)	9/39	5/31.2	NA	NS
Anti-RNP	6/26	4/25	NA	NS
SLEDAI-2K	6.8 ± 5	5.7 ± 3	NA	NS
rSLEDAI	1.18 ± 1.12	NA	NA	
SLICC-DI	2.2 ± 1	1 ± 0.6	NA	NS
Serum creatinine (mg/dl)	0.9 ± 0.3	–	NA	
BUN (mg/dl)	39,6 ± 17,6	–	NA	
Creatinine clearance (ml/min)	99.2 ± 53.7	–	NA	
Proteinuria (mg/24h)	432.8 ± 524.5	–	NA	
HCQ (n/%)	21/91.3	13/81.2	NA	
HCQ cumulative dose (g)	518,9 ± 590	703 ± 155	NA	
PDN >5 mg/die (n/%)	11/47.8	6/37.5	NA	
PDN cumulative dose (g)	14 ± 11,6 g	14.6 ± 22.6	NA	
Immunosuppressive treatment (n/%)	19/82.6	10/62.5	NA	
BCVA (logMAR)	0.01 ± 0.05	0.01 ± 0.02	0.0 ± 0.1 log	NS

SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000; rSLEDAI: renal Systemic Lupus Erythematosus Disease Activity Index; SLICC-DI: Systemic Lupus International Collaborating Clinics damage index; BUN: blood urinary nitrogen; HCQ: hydroxychloroquine; PDN: prednisone; BCVA: best-corrected visual acuity; NA: not applicable; NS: not significant.

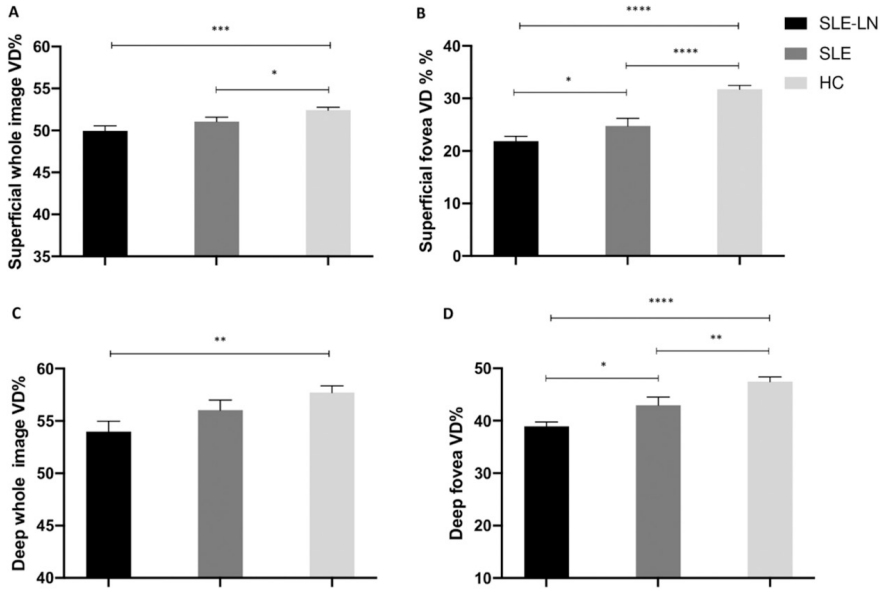

Fig. 1. Analysis of optical coherence tomography angiography (OCTA) data in patients affected by systemic lupus erythematosus (SLE) and healthy controls (HC). Comparative analysis of OCTA vessel density in superficial (**A**-**B**) and deep (**C**-**D**) retinal plexus in SLE patients, SLE with lupus nephritis (LN) and healthy controls (HC). VD: vessel density; * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$.

months. The mean SLEDAI-2K value was 6.8±5 in SLE-LN and 5.7±3 in SLE, consistent with moderate disease activity, while the SLICC-DI value was 2.2±1 in SLE-LN and 1±0.6 in SLE, respectively.

In the SLE-LN group, twenty-one patients (91.3%) underwent HCQ treatment, with a mean cumulative dose of 518.9±590 g. Eleven patients (47.8%) were taking a PDN dose >5 mg/die, while the mean PDN cumulative dose was 14±11.6 g. Nineteen patients (82.6%) were on immunosuppressive treatment and two patients (8.7%) were on biologic treatment. In the SLE group, thirteen patients (81.2%) were on HCQ treatment, with a mean cumulative dose of 703±155 g, five patients (31.2%) were taking PDN >5 mg/die, with a cumulative dose of 14.6±22.6 g. Ten SLE patients (62.5%) were on immunosuppressive treatment, while no one was on biologic treatment.

Dyslipidaemia was detected in three patients in the SLE-LN group (13%) and two patients in the SLE group (12.5%), all controlled with statins. A total of 11 patients in the SLE-LN group (47.8%) and 7 patients in the SLE group (43.7%) were affected by hypertension, on treatment with ACE-inhibitors. The characteristics of the SLE-LN and SLE patients were matched for age, sex, disease duration and activity, and antibody profile (Table I).

According to SLE-LN biopsy data examination, seventeen patients (74%) met a defined histological diagnosis of LN, fourteen of whom had a diagnosis of class III-IV LN (82%) and three of class III-V LN (17.6%). According to the histological evaluation, eleven patients presented active lesions (61.1%), eleven chronic lesions (61.1%) and six (33.3%) vascular lesions (12, 13) Among patients with biopsy evidence of vascular lesions, intimal hyalinosis was detected in five cases (83.3%).

Ophthalmological evaluation revealed that thirteen SLE-LN patients (56.5%) had evidence of lupus retinopathy, 10 moderate (77%) and 3 at severe stage (23%) at fundus oculi examination and high definition colour retinography.

The analysis of the OCTA data in the superficial and deep retinal plexi showed a significative reduction of vessel density in both SLE groups compared to HC, regarding the following parameters: superficial whole en face density ($p=0.0009$ in SLE-LN, $p=0.02$ in SLE), and superficial fovea density ($p<0.0001$ in SLE-LN, $p<0.0001$ in SLE) (Fig. 1A-B).

A significant reduction of deep whole en face ($p=0.002$ in SLE-LN) and deep fovea density ($p<0.0001$ in SLE-LN,

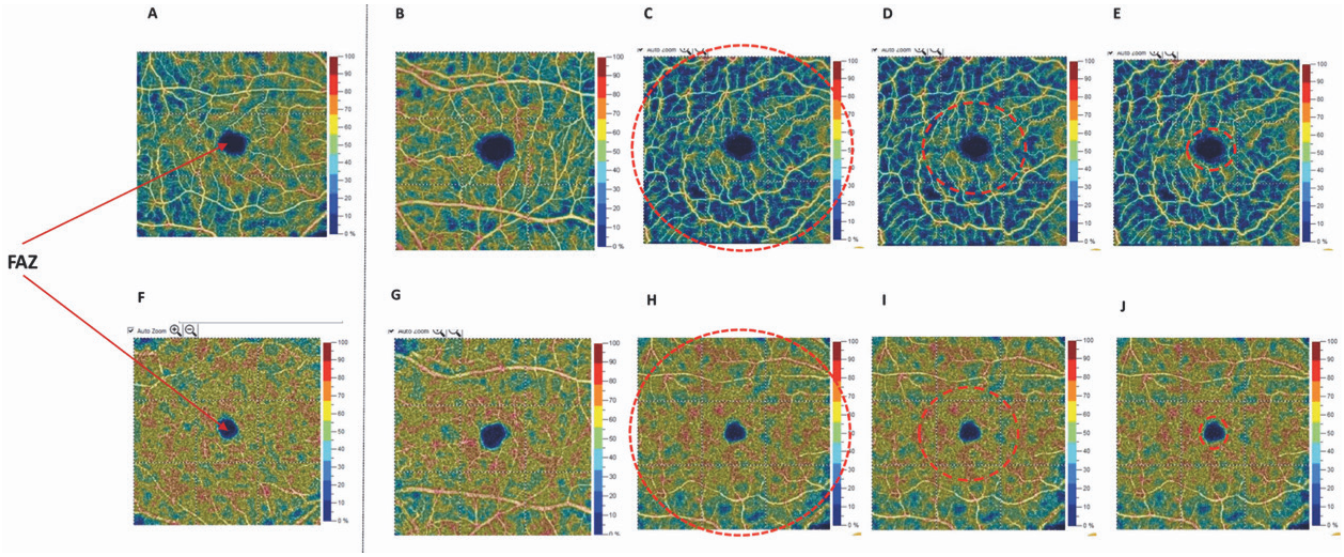

Fig. 2. Representative OCTA colour-coded images. On the left side of the line, **A** and **F** show the whole vascular pattern of 6x6 OCTA scansion of both superficial and deep retinal plexi in a healthy subject and the corresponding fovea avascular zone (FAZ), signed by arrods.
On the right side of the line, the whole image vessel density of both superficial and deep retinal plexi in a systemic lupus erythematosus (SLE) patient is displayed (**B**, **G**). Regarding the following panels, red sketched lines indicate the whole image (**C**, **G**), parafoveal (panels **D**, **H**) and foveal (panels **E**, **J**) vessel density in SLE-LN patient superficial and deep retinal plexi.

p=0.004 in SLE) was detected compared to HC, while no significant difference in deep whole en face density between SLE patients without kidney involvement and HC was observed (Fig. 1C-D).
Moreover, OCTA evaluation showed a significant reduction in superficial fovea (p=0.03) and deep fovea (*p*=0.01) vessel density in SLE patients with LN compared to SLE without kidney involvement. (Fig. 1B, D)
Analysis of parafovea density data showed a significant reduction in superficial plexus in SLE group compared to HC (*p*=0.01 in SLE-LN, *p*=0.03 in SLE), while no significant differences were found in the deep parafovea plexus (Supplementary Fig. S1 A-B).
Figure 2 shows OCTA colour-coded images of HC, SLE and SLE-LN superficial (panels A-E) and deep (panels F-J) retinal plexi. SLE-LN images are divided in whole image capillary, parafoveal and foveal areas.
Fovea thickness was also evaluated through OCTA and a significant thinning was observed in both SLE groups (*p*<0.0001 in SLE-LN, *p*=0.003 in SLE) compared to HC (Fig. 3). Likewise, parafovea thickness in SLE patients resulted to be significantly reduced compared to HC (*p*=0.0005 in SLE-LN, *p*=0.03 in SLE; Suppl. Fig. S2).

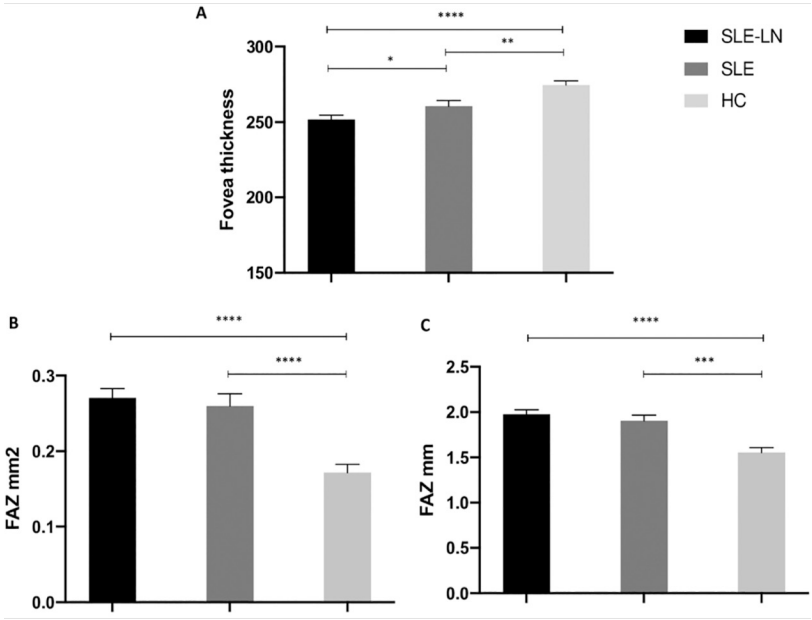

Fig. 3. Analysis of parafovea and fovea thickness and fovea avascular zone (FAZ) data from optical coherence tomography angiography (OCTA) in patients affected by systemic lupus erythematosus (SLE) and healthy controls (HC).
Comparative analysis of fovea (**A**) thickness detected through OCTA in SLE with and without kidney involvement and HC. FAZ area (**B**) and perimeter (**C**) in both SLE groups and HC were also examined. FAZ: fovea avascular zone. * *p*<0.05; ** *p*<0.01; *** *p*<0.001; **** *p*<0.0001.

The comparative analysis of FAZ data showed a significative increase in the fovea avascular zone area of SLE-LN and SLE patients compared to HC (*p*<0.00001 in both groups). The same analysis was performed for FAZ perimeter and it resulted significantly higher in SLE-LN and SLE patients compared to the control group (*p*<0.0001 and *p*=0.0001, respectively; Fig. 3B-C).
The ROC analysis and the corresponding AUC of OCTA densities in SLE-LN patients and HC showed high-moderate accuracy of the diagnostic test for superficial whole en face density (AUC 0.67, 95% CI 0.54–0.79, *p*=0.01, cut-

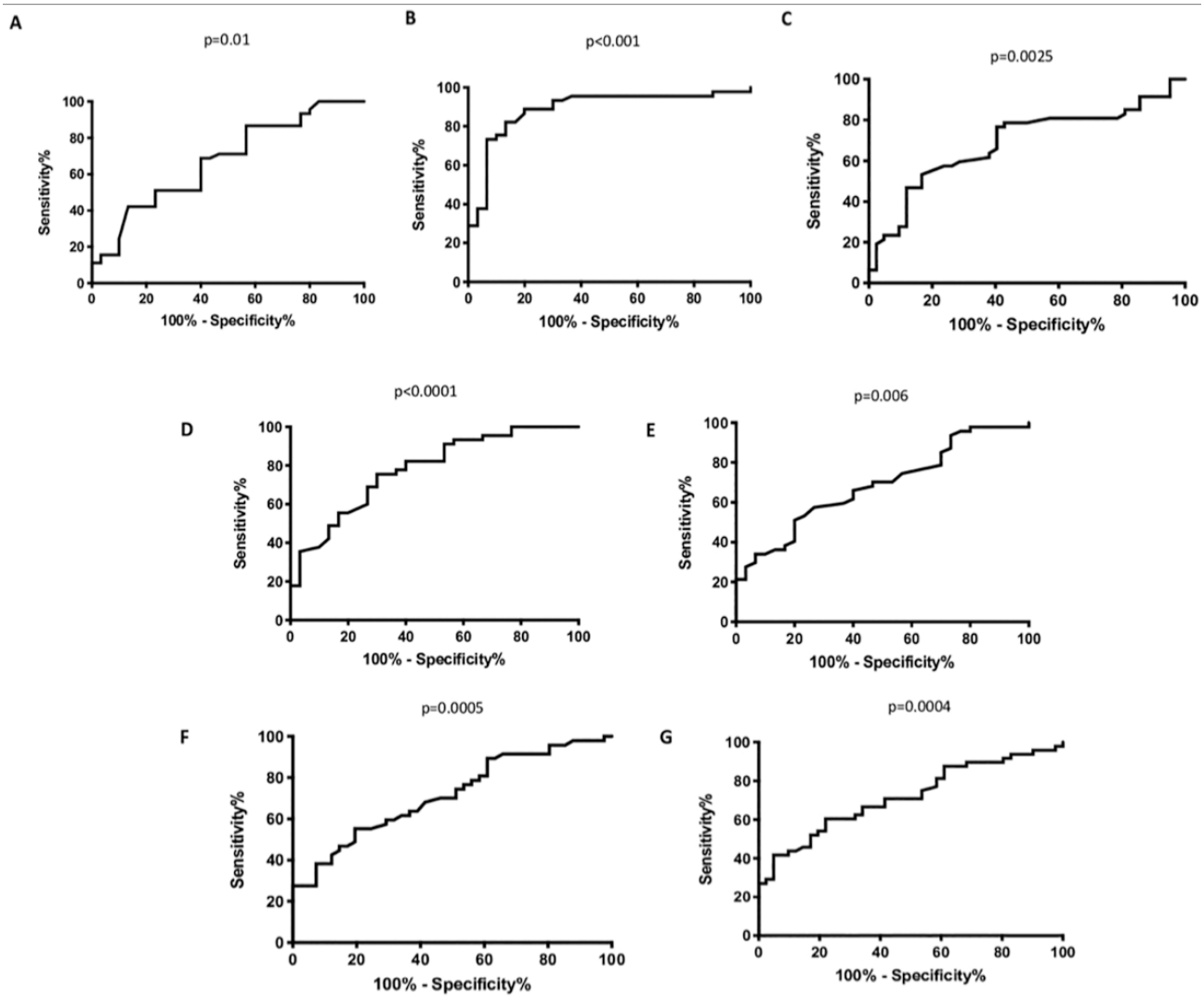

Fig. 4. Receiver operating characteristic (ROC) curve analysis of optical coherence tomography angiography (OCTA) data in patients affected by systemic lupus erythematosus (SLE) with a diagnosed lupus nephritis (LN) and healthy controls (HC).
ROC curve analysis was performed using SLE-LN and HC vessel densities of superficial (**A**-**B**) and deep (**C**-**D**) retinal plexi, fovea thickness (**E**), FAZ area (**F**) and perimeter (**G**).
Accuracy was measured by the area under the ROC curve (AUC). Youden's index with likelihood ratio >2 was considered for choosing the optimal threshold value for which sensitivity and specificity are maximised. *p-v*alues <0.05 were considered significant. FAZ: fovea avascular zone.

off value <49.2%, sensitivity 42.22%, specificity 86.67%, Fig. 4A), deep whole en face density (AUC 0.68, 95% CI 0.57–0.79, *p*=0.0025, cut-off value <55.15%, sensitivity 48.94%, specificity 83.33%, Fig. 4C). According to the fovea data analysis, both superficial fovea density (AUC 0.89, 95% CI 0.8–1.0, *p*<0.001, cut-off value <24.35%, sensitivity 73.3%, specificity 93.3%, Fig. 4B), deep fovea density (AUC 0.78, 95% CI 0.7–0.9, *p*<0.0001, cut-off value <39.15%, sensitivity 55.56%, specificity 83.30%, Fig. 4D) and fovea thickness (AUC 0.71, 95% CI 0.60–0.82; *p*=0.0005, cut-off value <254 mm, sensitivity 51%, specificity 80%, Fig. 4E), showed a similar level of diagnostic accuracy. Likewise, high accuracy by ROC analysis was demonstrated for FAZ area (AUC 0.71, *p*=0.0005, 95% CI 0.6–0.8, cut-off value >0.28 mm^2, sensitivity 48.89 %, specificity 86.67%, Fig. 4F) and FAZ perimeter (AUC 0.71, 95% CI 0.61–0.82, *p*=0.0004, cut-off value >2.010 mm, sensitivity 52.08%, specificity 82.93%, Fig. 4G).

The same analysis was performed for superficial parafovea density (AUC 0.65, 95% CI 0.5–0.8, *p*=0.03, cut-off value <54.05% sensitivity 48.89%, specificity 80%) and parafovea thickness (AUC 0.67, 95% CI 0.54–0.78; *p*=0.01, cut-off value <319.5 mm, sensitivity 46.8%, specificity 76.6%), showing a high-moderately diagnostic accuracy of the test (Suppl. Fig. 3 A-B).

The OCTA data of SLE-LN population were correlated with demographic, clinical, laboratory and histologic features. Results showed a negative correlation between SLE duration and both superficial whole en face density (p=0.03; r=-0.3) and deep whole en face density (*p*=0.004; r=-0.4). Likewise, time elapsed from LN onset negatively correlated with superficial whole en face density (*p*=0.05; r=-0.4), superficial parafovea density (*p*=0.007;

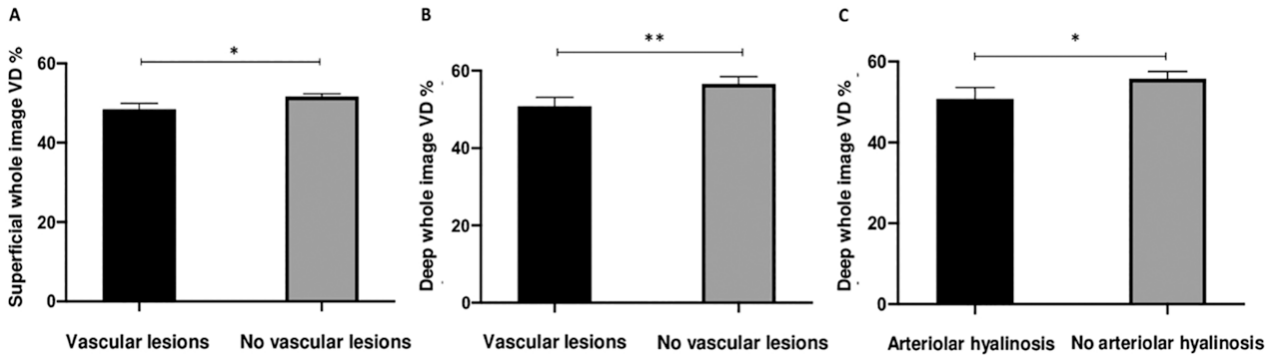

Fig. 5. Analysis of and optical coherence tomography angiography (OCTA) lupus nephritis (LN) biopsy data.
Comparative analysis of OCTA data of superficial and deep retinal plexi and LN biopsies, according to presence or absence of vascular lesions (**A**-**B**), particularly evaluating presence or absence of arteriolar hyalinosis (**C**). VD: vessel density; **p*<0.05; ***p*<0.01.

r=-0.4), deep whole en face density (*p*=0.004; r=-0.4), deep fovea density (*p*=0.01; r=-0.4.) and parafovea thickness (*p*=0.004; r=-0.3), while a positive correlation was found with FAZ area (*p*=0.01; r=0.4). SLEDAI-2K value correlated negatively with superficial and deep fovea density (*p*<0.0001; r=-0.6 and *p*=0.01; r=-0.4, respectively). Kidney function evaluated by BUN showed a negative correlation with superficial whole en face density (*p*=0.03; r=-0.5), superficial parafovea density (*p*=0.004; r=-0.4) and deep fovea density (*p*=0.03; r=-0.3); serum creatinine and deep whole en face (*p*=0.004; r=-0.4). A positive correlation resulted between creatinine clearance and both deep whole en face (*p*=0.05; r=0.3) and deep fovea density (*p*=0.0007; r=0.5). OCTA data were also correlated with other clinical and laboratory features as complement C3 and C4 levels, urinary sediment evaluation, 24 hours proteinuria, renal SLEDAI and SLICC-DI, with no statistically significant results. No significant difference was detected in OCTA data when patients were stratified according to the presence/absence of aPL, anti-dsDNA or anti-RNP antibodies. Moreover, no significant correlations were found between OCTA data and use of medications such as HCQ, PDN (cumulative dose), ACE-inhibitors or statins (data not shown).

OCTA data were compared stratifying patients according to the presence or absence of LN-vascular lesions at kidney biopsy; a significative reduction in superficial whole en face density (*p*=0.02) and deep whole en face density (*p*=0.009) was found in patients with vascular lesions compared to the group without vascular involvement (Fig. 5A-B). According to the evaluation of the different histological lesions, SLE biopsies with arteriolar hyalinosis had a reduction in deep whole image vessel density (*p*=0.04) compared to patients without arteriolar hyalinosis (Fig. 5C). The same analysis was performed for the other arterial/arteriolar histologic lesions, including fibrotic intimal thickening, media hyperplasia, rupture of the internal elastic lamina, fibrinoid necrosis, intravascular thrombi, leucocyte infiltration in intima and media and immunofluorescence detection of vascular immune complex deposits and fibrin-deposits and no significant differences in OCTA data were found (data not shown).

Discussion

LN is associated with significant morbidity and mortality in SLE, with these patients having worse survival compared to those without nephritis. LN aetiopathogenesis involves different mechanisms, such as immune-complex deposition inducing activation of endothelial cells, inflammatory response and subsequent pathological vascular changes (18).

Lupus retinopathy may be caused by a vasculitic process involving retinal microvasculature, most commonly immune-complex related (3, 4, 20). Presence of retinopathy is a marker of poor prognosis and appears to be associated with systemic organ involvement, such as kidney and central nervous system (5, 21, 22).

Recent studies have suggested a relationship between lupus retinopathy and kidney involvement, although correlation between ocular findings and functional-histological renal damage have not been evaluated (4, 20). Recently, we reported a high prevalence of functional retinal abnormalities detected through standard automated perimetry in SLE-LN patients (19).

OCTA has been under investigation in the last few years for its ability to detect preclinical ocular vascular damage in systemic conditions, such as arterial hypertension (23), diabetes (24) and chronic kidney disease (25). These conditions can be found more often in SLE patients than in healthy subjects (26), so their presence has to be considered since it could represent a potential confounding factor in evaluation of retinal microvascularity.

Previously, we evaluated the possibility to detect preclinical microvascular damage through OCTA in SLE patients (27). The results obtained from the present study suggest a large variety of observations. Both SLE and SLE-LN patients showed significant reduction in retinal vessel density compared to HC, both in superficial and deep retinal microvascular circulation, as previously assessed (6-8).

Likewise, reduced retinal vessel density was associated for the first time with LN duration and kidney function suggesting a correspondence between retinal microvascular damage and cumulative kidney impairment over the years. Retinal microvascular impairment seemed to be related with systemic disease activity and long disease duration, consistently with previous study results

(6, 22, 28). Indeed, OCTA correlated with BUN, serum creatinine levels and creatinine clearance, as underlined in chronic kidney disease (25). A small percentage of SLE-LN patients had a concomitant dyslipidaemia and half of them were affected by arterial hypertension. These comorbidities, together with other traditional cardiovascular risk factors, could influence the correlation between OCTA data and kidney involvement, therefore a multivariate analysis in a larger cohort should be performed in future studies.

LN vascular lesions are related to different pathogenetic pathways, summarised in vascular immune-complex deposits, arteriosclerosis, thrombotic microangiopathy, non-inflammatory necrotising vasculopathy and true renal vasculitis (18).

Recent studies have re-evaluated the role of vascular damage in SLE, recognising its importance in LN progression and in improving outcome predictions (17).

SLE-LN patients with vascular damage, particularly arteriolar hyalinosis, showed a reduction of retinal vascularisation, suggesting the importance of arteriosclerotic process in renal microvascular damage (18), although the small biopsy sample does not allow an accurate representation of all histologic lesions. Moreover, it would be useful to analyse microvascular alterations by OCTA according to the main parameters by ISN/RPS class of nephritis (*i.e.* III, IV, V, potentially II), since these may differ with regard to the vascular component (most important in class III-IV) (17).

This study has several limitations, including the small size of the population that is relatively heterogeneous, the cross-sectional study design and the absence of longitudinal follow-up data. Nevertheless, these preliminary results support the role of OCTA as a useful non-invasive diagnostic tool in early detection of retinal microvascular alterations and associated renal involvement in SLE-LN patients.

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