Doc Ophthalmol
https://doi.org/10.1007/s10633-018-9625-2

ORIGINAL RESEARCH ARTICLE

Comparison between multifocal ERG and C-Scan SD-OCT ("en face" OCT) in patients with a suspicion of antimalarial retinal toxicity: preliminary results

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Received: 25 April 2017 / Accepted: 25 January 2018

Abstract

Purpose Pericentral visual field changes and disruption of the ellipsoid layer on spectral domain optical coherence tomography (SD-OCT) are the main features of antimalarial retinal toxicity. C-Scan OCT or "en face" enables a topographic frontal view of the changes observed within the different retinal layers in particular the ellipsoid layer. The aim of this prospective study was to compare multifocal ERG (mfERG) responses with the results of C-Scan OCT ("en face" OCT) in patients with abnormal visual field and to analyze relationships between the structural and functional abnormalities.

Methods In 354 consecutive patients screened for antimalarial toxicity between January 1, 2014 and December 31, 2016, central visual field, mfERG recording, C-Scan OCT and short-wavelength fundus autofluorescent imaging were performed.

Results Among the 17/354 patients with abnormal central visual field results, all presented with abnormalities on the mfERG at least in one eye. In 16/33 eyes, there was a good concordance between focal loss of the mfERG response and the disruption of the ellipsoid layer on C-Scan OCT. In one eye with characteristic changes in the ellipsoid layer on the C-Scan OCT, the mfERG was normal, whereas in three eyes the mfERG was abnormal in eyes with a normal C-Scan OCT.

Conclusions The contribution of the C-Scan OCT changes remains difficult to establish as there is no strict concordance with the local ERG responses. Although C-Scan OCT technology provides a new approach in analyzing focal abnormalities in the photoreceptor–retinal pigment epithelium interface, the sensitivity of this method compared with mfERG and other tests (central visual field, B-Scan OCT) needs to be evaluated. This study is still ongoing on a larger cohort.

Keywords Hydroxychloroquine retinopathy · Multifocal electroretinogram · Optical coherence tomography · Ring ratio · En face OCT

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Introduction

Chloroquine (CQ)- and hydroxychloroquine (HCQ)-induced retinal toxicities have been described for the

Published online: 13 March 2018

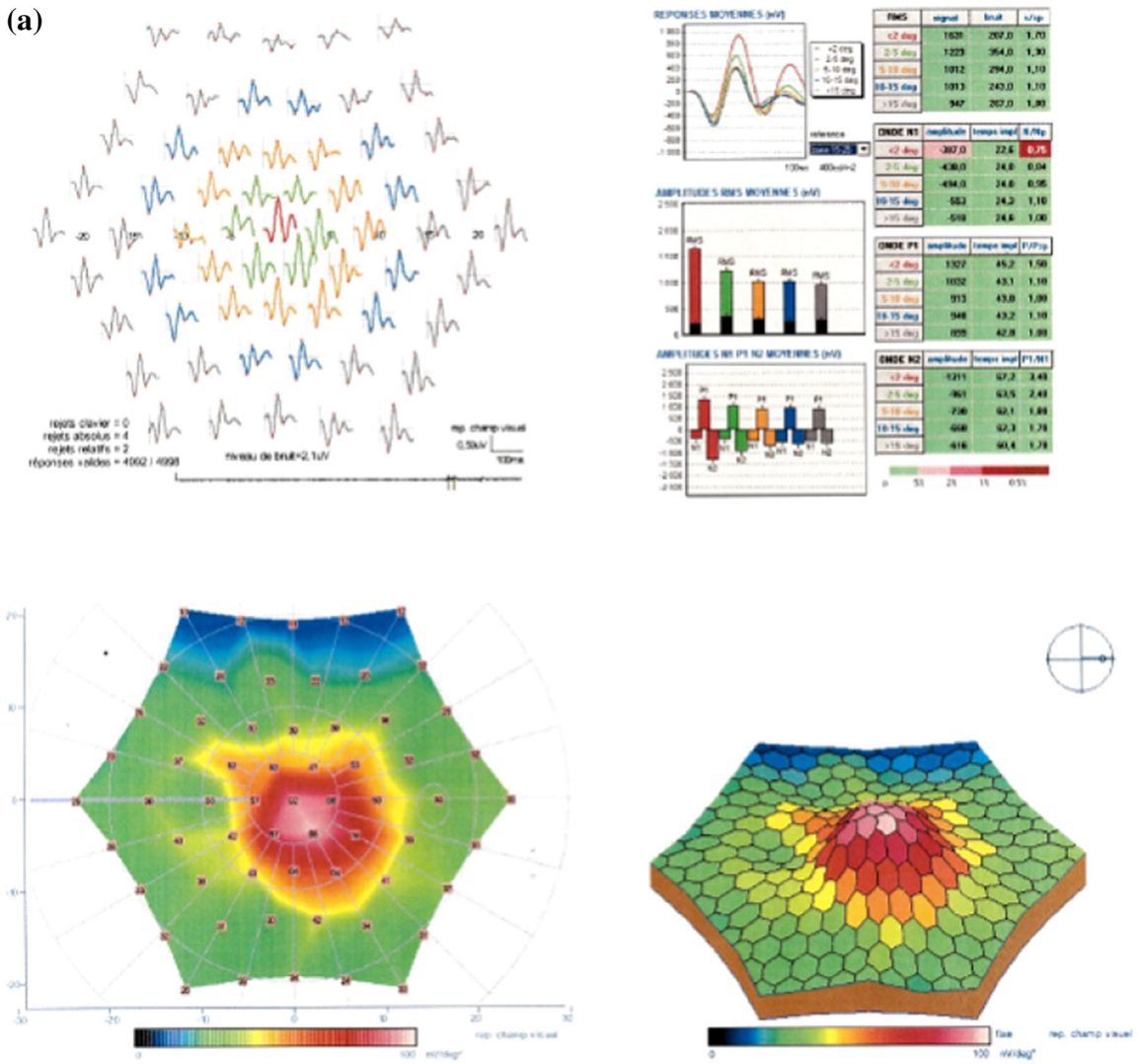

Fig. 1 Eighty-six-year-old female patient, Gougerot-Sjögren syndrome, hydroxychloroquine treatment for 6 years, 400 mg/day, 3.67 mg/kg (estimated cumulative dose: 877 g). **a** Normal multifocal ERG (field view), R2/R5 ratio (1.14) > 1, R3/R5 ratio (1.02) > 1. **b** Both retinal biomicroscopy and the infrared image retinal were normal besides retinal wrinkling in relation with an epiretinal membrane. **c** Normal C-Scan OCT of the ellipsoid layer. **d** Superposition of the C-Scan OCT and the local responses obtained with the multifocal ERG

first time in 1957 [1]. Hydroxychloroquine is widely used in various inflammatory conditions such as systemic lupus and rheumatoid arthritis [2] because of its excellent systemic tolerance.

When the first symptoms occur, the macular changes are irreversible and may continue to deteriorate even when antimalarial (AM) treatment is discontinued for several years [3]. Therefore, detection of maculopathy requires screening for early preclinical changes. Conversely, when hydroxychloroquine treatment is interrupted at an early stage, the limited changes observed on the multifocal electroretinogram (mfERG) may be reversible over a period of 6 months [4].

The main identified risk factors are a high dose and a long duration of treatment [5]. The risk of toxicity depends on the daily dose (5 mg/kg of HCQ or 2.3 mg/kg of CQ) [5]. Duration of treatment exceeding 5 years, kidney failure, concomitant tamoxifen

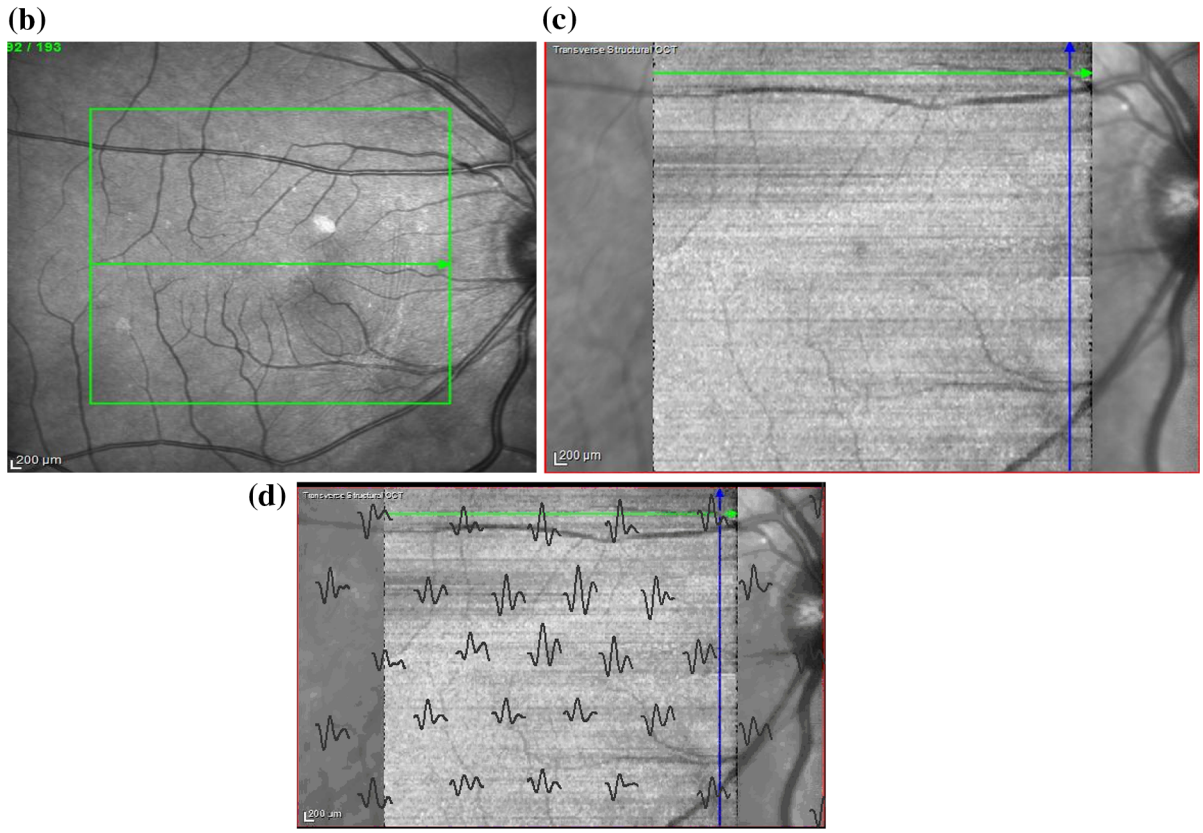

Fig. 1 continued

treatment and a preexisting maculopathy are other commonly accepted risk factors [5].

For HCQ/CQ toxicity screening purposes, various tests have been used [6]. The recently updated recommendations of the American Academy of Ophthalmology emphasize an initial examination with visual acuity, fundus imaging, central visual field and B-Scan OCT in the first year of treatment. [5] If no risk factors are present, then the next evaluation should take place 5 years after the onset of the treatment. In case of uncertain toxicity, objective tests such as mfERG or fundus autofluorescence (FAF) can provide additional data [6].

Among the different tests, mfERG has been considered as the most sensitive test enabling to detect preclinical changes in order to avoid irreversible lesions with dramatic functional consequences [7].

An alternative screening method would be FAF. However, the sensitivity of this test appears to be non-sufficient [8]. Among other recent imaging techniques, C-Scan OCT ("en face" OCT) enables to visualize different layers of the retina in a frontal plane. To the author's best knowledge, the correlation between C-Scan OCT findings and mfERG has not yet been evaluated in patients treated with antimalarial drugs.

The purpose of this study was to analyze the existence of a spatial correlation between mfERG changes and C-Scan OCT abnormalities in case of antimalarial toxicity detected with central visual field testing.

Table 1 Demographic and clinical parameters of the 17 patients with suspected toxicity on the basis of visual field screening

Patients	Age	Gender	Weight (kg)	Height (m)	BMI	Indication of treatment	Risk factors (*n*)	Daily dose (mg)	Daily dose (mg/kg)	Duration of treatment (years)	Cumulative dose (g)
1	43	F	64	1.60	25.15	Lupus	1	400	8.00	0.75	110
2	52	F	56	1.52	24.24	Lupus	2	200	3.57	6.00	438
3	26	F	60	1.65	22.01	Lupus	1	400	6.67	0.75	109
4	44	F	116.7	1.55	48.6	Lupus	1	400	3.43	4.00	584
5	41	F	46.2	1.68	16.4	Sclerodermia	1	200	4.33	7.00	511
6	53	F	58	1.70	20.07	Lupus	1	400	6.90	0.30	44
7	64	F	71.1	1.54	30	Rheumatoid arthritis	2	400	5.63	13.00	1898
8	79	F	58	1.67	20.80	Gougerot-Sjögren syndrome	0	200	3.45	2.00	146
9	47	F	54.5	1.68	19.33	Lupus	2	400	3.67	6.00	877
10	60	F	66.86	1.73	22.34	Lupus	3	400	2.99	10.00	1461
11	54	F	68	1.75	22.35	Lupus	3	400	5.84	15.00	2190
12	49	F	51.7	1.76	16.7	Lupus	3	400	7.74	23.00	3358
13	60	F	58	1.7	20.07	Gougerot-Sjögren syndrome	0	200	3.45	2.00	146
14	58	M	81	1.82	24.47	Lupus		400	4.94	3.00	438
15	59	F	64	1.61	24.71	Lupus	2	400	6.25	14.00	2045
16	53	F	48.3	1.58	19.35	Lupus	1	400	8.28	1.30	190
17	59	F	67.4	1.74	22.27	Lupus	1	400	5.93	1.70	248

Table 2 Results of C-Scan OCT and mfERG in 17 patients; only in two patients an abnormal mfERG in at least one eye was associated with a normal C-Scan OCT in both eyes

17 patients	Normal mfERG in both eyes	Abnormal mfERG in at least one eye
Normal C-Scan OCT in both eyes	0	2
Abnormal C-Scan OCT in at least one eye	0	15

Table 3 Results of C-Scan OCT and mfERG in 33 eyes

33 eyes	Normal mfERG	Abnormal mfERG
Normal C-Scan OCT	2	3
Abnormal C-Scan OCT	1	27

Patients and methods

Currently a multicentric French Protocol (Grenoble, Paris, Reims) is ongoing to compare multifocal ERG and C-Scan SD-OCT ("en face" OCT) in patients with a suspicion of antimalarial retinal toxicity. The protocol is registered at clinicaltrials.gov (identification NCT 02805686). The cohort of consecutive

Table 4 Comparing C-Scan OCT and B-Scan OCT in 33 eyes, a large number of patients had an abnormal C-Scan OCT with a normal B-Scan OCT

33 eyes	Normal B-Scan OCT	Abnormal B-Scan OCT
Normal C-Scan OCT	5	0
Abnormal C-Scan OCT	18	10

In these cases, the retinal biomicroscopy and the infrared image were always normal. No case of a normal C-Scan OCT with an abnormal B-Scan OCT was encountered

patients screened at Reims University Hospital for retinal toxicity in relation with AM treatment was analyzed. A detailed history of the AM treatment (real weight dose, duration) and the other risk factors (kidney failure, preexisting maculopathy, concomitant Tamoxifen use) was taken.

A complete ophthalmologic examination with central visual field (FAST 12 procedure, Vision Monitor, Pérenchies, France), standard B-Scan OCT was performed.

If paracentral visual field defects were detected on two consecutive examinations, retinal toxicity was suspected and the patient was included in the study. If at least two adjacent points presented with a significant loss, then the visual field was considered as being abnormal.

In this case, in addition to the standard procedures, a multifocal ERG (mfERG), a C-Scan OCT and a fundus autofluorescence (FAF) were performed.

The mfERG was performed (Vision Monitor, Metrovision, Pérenchies, France) in accordance with the ISCEV recommendations. Briefly, the response to a 61-hexagon stimulation was recorded and the mean amplitudes of the N1, P1, N2 in 5 concentric areas were analyzed. The R2/R5 and the R3/R5 ratios of the P1 wave were evaluated. If either ratio in either eye was below 1, then the mfERG was considered abnormal [9]. Normal values provided by the manufacturer Metrovision are quite near our normal values. The mean R2/R5 P1 amplitude ratio is 2.07 ± 0.4. If the normal lower limit is fixed at -2 SD, then it would be 1.27, at $-$ 3SD it would be 0.87. If the mean R3/R5 P1 amplitude ratio is 1.60 ± 0.21, then $-$ 2 SD would be 1.18 and $-$ 3SD would be 0.97.

Considering the overlapping of mfERG responses between normals and abnormals and the difficulty in separating between toxicity and impregnation, it was considered that if the amplitude of the response was lower in the paracentral area between 4° and 10° compared with the peripheral area beyond 15°, it was necessarily abnormal, in accordance with the decreasing density of photoreceptors as first described by Østerberg [10].

The following OCT tests were performed in both eyes (Spectralis, Heidelberg Engineering, Heidelberg, Germany): 2 B-Scans (Line ART100, 30°, HR 180° and 90°), a macular volume for the C-Scan ("en face") of the posterior pole ($15° \times 15°$ mapping, 30 µm between each 2 B-Scans, 145 sections, ART16, HR), a macular mapping (ART9, HS) and a retinal nerve fiber layer (RNFL).

In particular, the appearance of the ellipsoid layer was evaluated on the C-Scan OCT, and pericentral changes were noted. The C-Scan OCT results were compared with the standard retinal examination (clinical biomicroscopy, photographic imaging, B-Scan OCT). Hyporeflectivity changes were considered as an early sign of toxicity. The ellipsoid layer was considered as normal if it appeared as a uniform light gray layer with the projection of the dark shadows due to the overlying retinal vessels. In case of poor segmentation, the fovea could be visible as a central dark spot. There were subjective and objective criteria for determining C-Scan abnormalities at the level of the ellipsoid layer. Subjective criteria were based on the clinical detection and evaluation of diffuse or localized (as spots) areas of hyporeflectivity at this level. Objective criteria were the correspondence of these

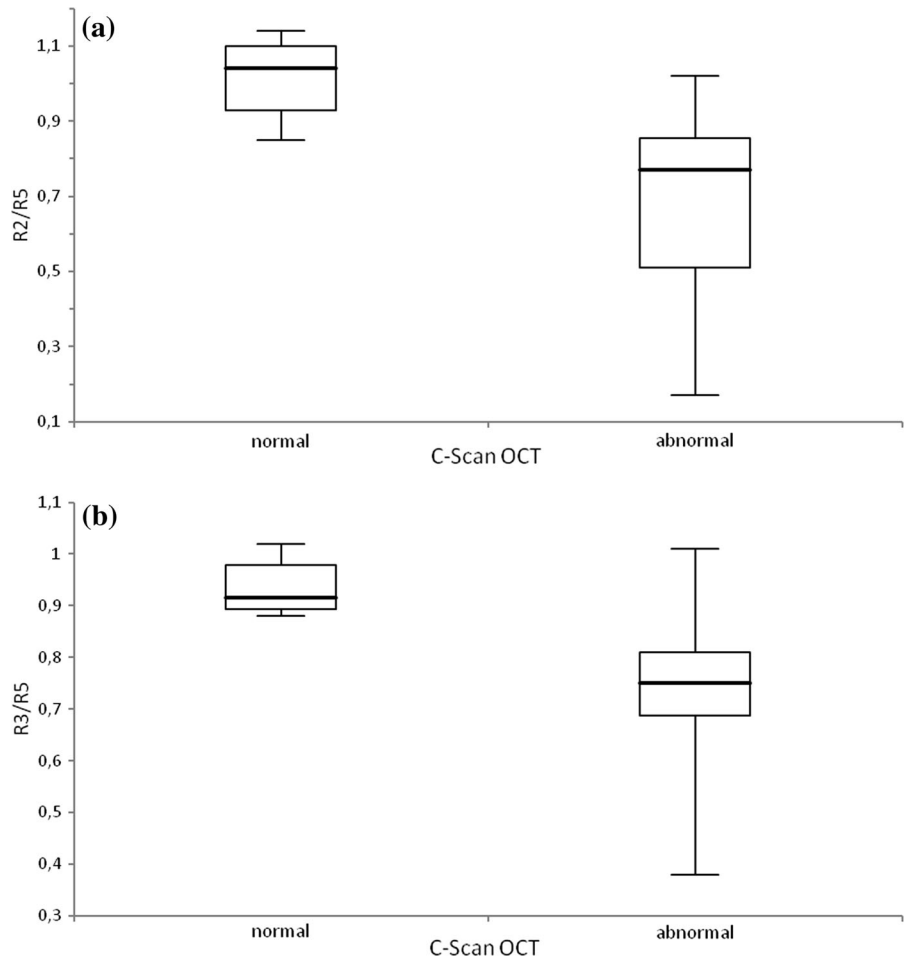

Fig. 2 **a** Box plot comparing R2/R5 P1 amplitude ratios between eyes with normal and abnormal C-Scan OCTs. **b** Box plot comparing R3/R5 P1 amplitude ratios between eyes with normal and abnormal C-Scan OCTs

areas of hyporeflectivity with discrete parafoveal thinning or changes of the ellipsoid or the interdigitation zone on the SD-OCT B-Scan. SD-OCT B-Scan could also easily distinguish areas of hyporeflectivity on C-Scan OCT due to another cause than AM toxicity such as segmentation artifact, shadow artifact (vitreous, retinal vessels, exudates), presence of material of pattern dystrophy, drusen or pseudo drusen. Moreover, SD-OCT changes were considered as a toxicity criteria only when considered clearly abnormal in a typical region (area corresponding to the central 15° around the foveola) and beyond image variability, by both operators. No quantification by software of the hyporeflectivity of the ellipsoid layer was realized.

FAF was also performed with Spectralis (Heidelberg Engineering, Heidelberg, Germany).

For each patient, both the mfERG and the C-Scan OCT were evaluated separately by two independent operators. (CA and SB for mfERG and VV and MMF for SD-OCT). If mfERG or SD-OCT interpretations were divergent, a new interpretation was realized until accordance was obtained between the two operators.

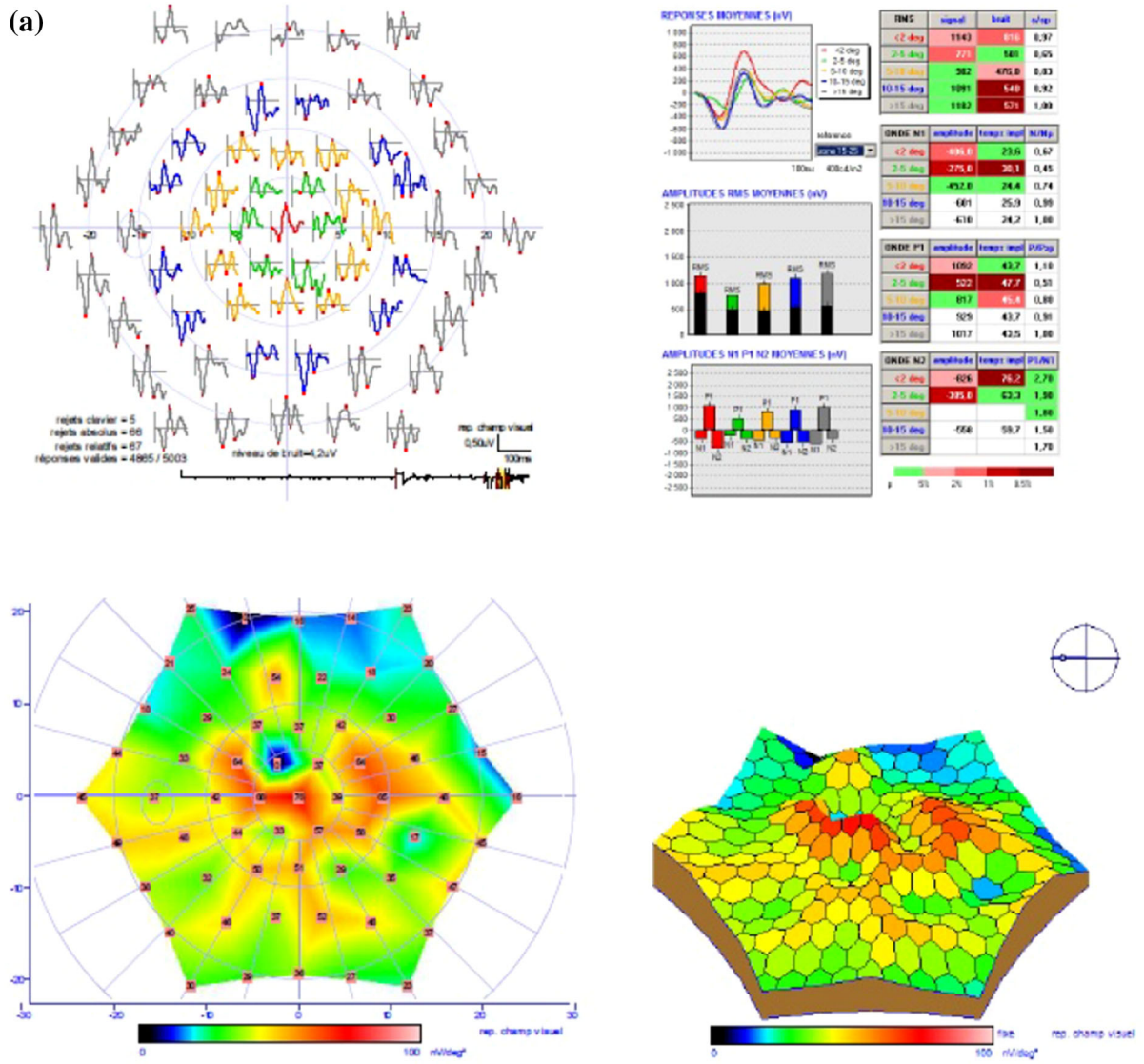

Fig. 3 Sixty-six-year-old female patient, rheumatoid arthritis, hydroxychloroquine treatment for 14 years, 400mg/day, 5.88mg/kg (estimated cumulative dose: 2045 g). **a** Abnormal multifocal ERG (field view), the R2/R5 ratio (0.51) and the R3/R5 ratio (0.80) are both below 1. **b** Pericentral retinal atrophy was visible on retinal biomicroscopy as on the infrared image. **c** Abnormal C-Scan OCT with pericentral changes in the ellipsoid layer. **d** Superposition of the OCT with the multifocal ERG responses. The local ERG responses correlate with the changes in the ellipsoid layer

The local retinal responses were then superimposed on the C-Scan OCT image of the ellipsoid layer (Fig. 1) using a software provided by Metrovision.

Besides the spatial relationship between mfERG and C-Scan OCT, a kappa test was performed to evaluate the overall concordance between abnormalities in both tests.

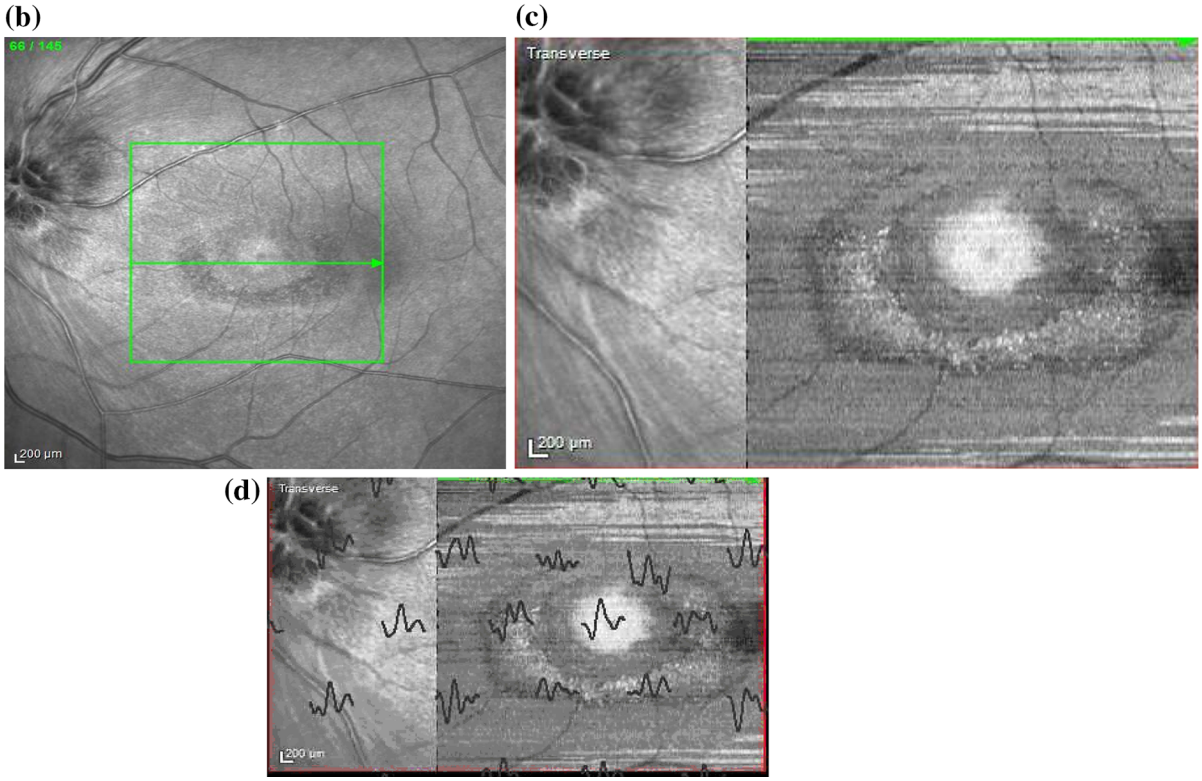

Fig. 3 continued

Results

In a cohort of 354 patients screened for AM toxicity between January 1, 2014 and December 31, 2016, 17 patients (4.8%, 16 female, 1 male), mean age 53 [26–79] presented with suspected toxicity on the basis of the visual field, were included (Table 1).

Thirteen patients were treated for systemic lupus, two for Gougerot-Sjögren syndrome, one for sclerodermia and one for rheumatoid arthritis.

Among these 17 patients, all had an abnormal mfERG result in at least one eye. In one eye, both mfERG and C-Scan OCT results were unreliable (lens opacity), 16 patients had an abnormal C-Scan OCT in at least one eye, in one patient the C-Scan OCT was normal in both eyes (Table 2). Each eye was also analyzed separately (Table 3).

In three eyes, the mfERG was abnormal with a normal C-Scan OCT. In two eyes of the same patient, only the P1 R3/R5 ratio was abnormal, with a normal P1 R2/R5 ratio. In this patient, the duration of HCQ treatment was only of 9 months with a low theoretical risk of toxicity.

Conversely, in the only case with an abnormal C-Scan OCT finding in both eyes and a normal mfERG in one eye the patient had been treated for 6 years with CQ (a relatively high risk of toxicity). This patient had an abnormal mfERG on the other eye

C-Scan OCT enabled to detect as abnormal 16 patients of the 17 presenting with an abnormal

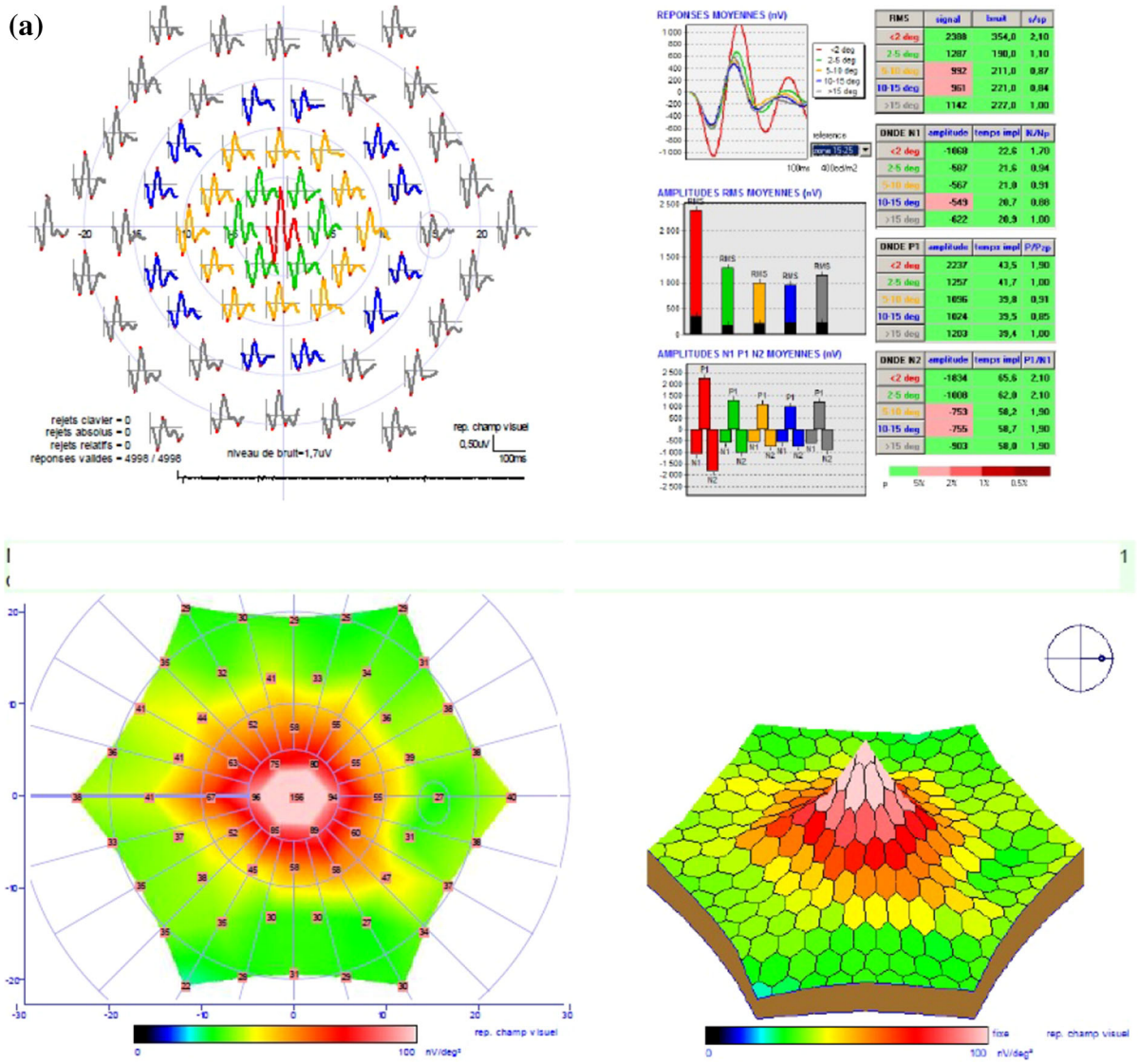

Fig. 4 Twenty-eight-year-old female patient, systemic lupus, hydroxychloroquine 400mg/day for 9 months, 8mg/kg (estimated cumulative dose: 110 g). **a** The multifocal ERG (field view) is abnormal, the R2/R5 ratio is above 1 (1.04), however the R3/R5 ratio is below 1 (0.91). **b** No changes on retinal biomicroscopy were noted, the infrared image was normal. **c** Abnormal C-Scan OCT of the ellipsoid layer with hyporeflective lesions (Arrows). **d** Superposition of the C Scan OCT with the multifocal ERG responses. The local ERG responses did not correlate with the local changes in the ellipsoid layer, although the mean P1 amplitude was reduced on the multifocal ERG (as the R3/R5 ratio was below 1)

mfERG, whereas the B-Scan OCT alone only detected 5 abnormal patients (10 eyes) (Table 4).

The overall concordance between the P1 R2/R5 ratio and an abnormal C-Scan OCT was fair with a kappa coefficient of 0.72 [0.34–1], whereas this coefficient was poor (0.27[− 0.23–0.78]) when comparing the C-Scan OCT outcome with the P1 R3/R5 ratio.

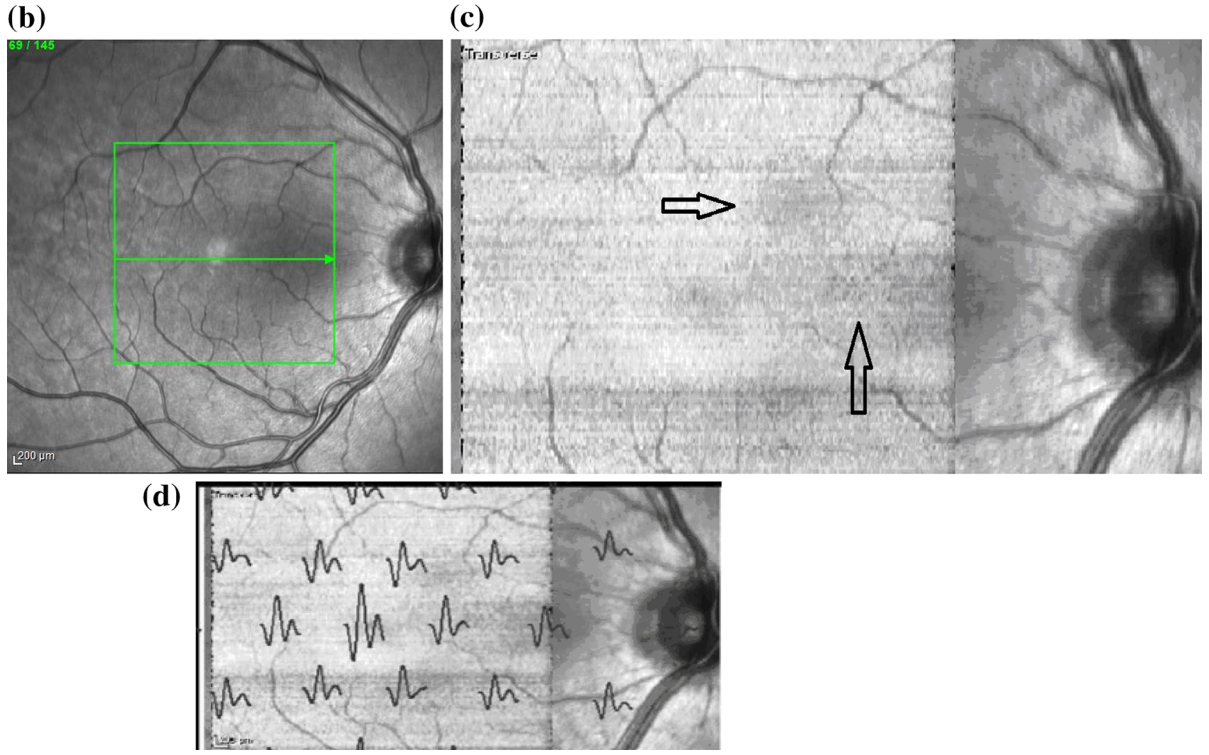

Fig. 4 continued

In addition, in 4 out of 5 eyes in which the C-Scan OCT was considered as normal, the P1 R2/R5 and P1 R3/R5 ratios were both significantly higher (respectively, $p = 0.0057$ and $p = 0.0067$) than in those with an abnormal C-Scan OCT result, although the group of patients with a normal C-OCT scan result was relatively small ($n = 4$) (Fig. 2).

Besides the overall concordance, the spatial correlation between C-Scan OCT changes detected in the ellipsoid layer and the local ERG responses was variable. In case of major lesions detected clinically on the retinal biomicroscopy (Fig. 3), a perfect concordance between the local ERG responses and the retinal changes on the C-Scan OCT was observed (10 eyes). However, when the lesions were only detectable on the C-Scan OCT (with an unremarkable retinal biomicroscopy and a normal standard B-Scan OCT), a poor spatial concordance (Figs. 4, 5) with the local ERG responses was observed. In one case (Fig. 6), a relatively large hyporeflective area was associated with a reduced amplitude of the local ERG response.

Discussion

A fair relationship between functional and anatomic abnormalities could be found in the majority of the patients. However, a spatial correlation between C-Scan OCT changes and mfERG was only observed in severe cases of toxicity. In the other cases, in case of diffuse mottled changes within the ellipsoid layer, they were not correlated with local ERG responses, the extension of each lesion being probably too small to be linked with changes in local retinal function.

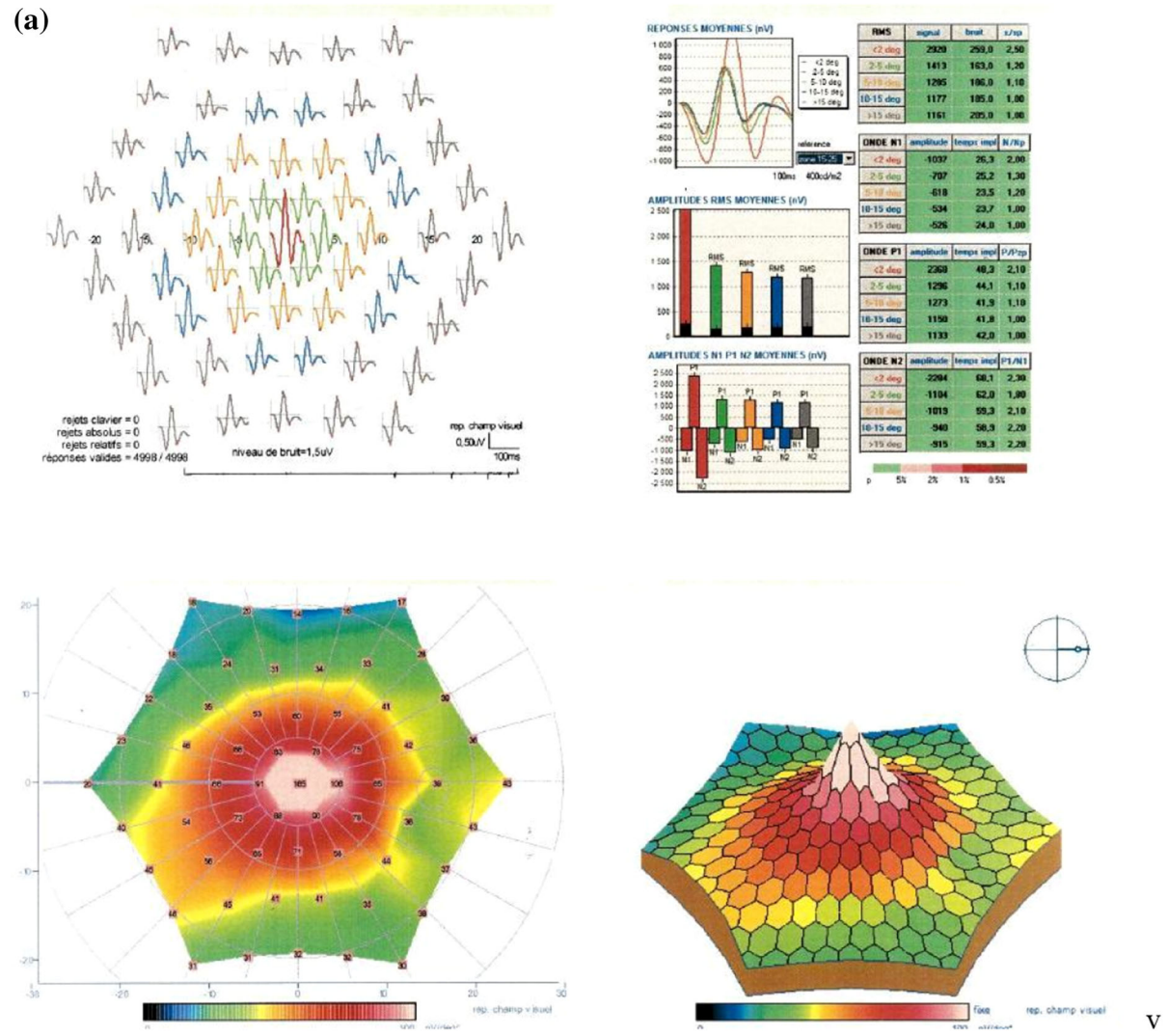

Fig. 5 Fifty-four-year-old female, systemic lupus, chloroquine for 6 years (malaria prophylaxis) 100mg/day and then hydroxychloroquine (HCQ) for 6 years (systemic lupus), 200mg/day, 4mg/kg/day, (estimated cumulative dose of HCQ: 438g). **a** Normal multifocal ERG (field view) R2/R5 = 1.14, R3/R5 = 1.12. **b** No changes on retinal biomicroscopy were noted, the infrared image was normal. **c** C-Scan OCT: several hyporeflective changes were observed on the ellipsoid layer (arrows). **d** Superposition of the C Scan OCT with the multifocal ERG responses.No spatial correlation between the local responses of the multifocal ERG and the changes detected on the C-Scan OCT were found

However, when mottled changes in the pericentral area were observed then the ERG response at the same eccentricity was reduced, as the mean amplitude of the second ring responses was lower than the mean amplitude of 5th ring responses (represented by the mean P1 R2/R5 ratio).

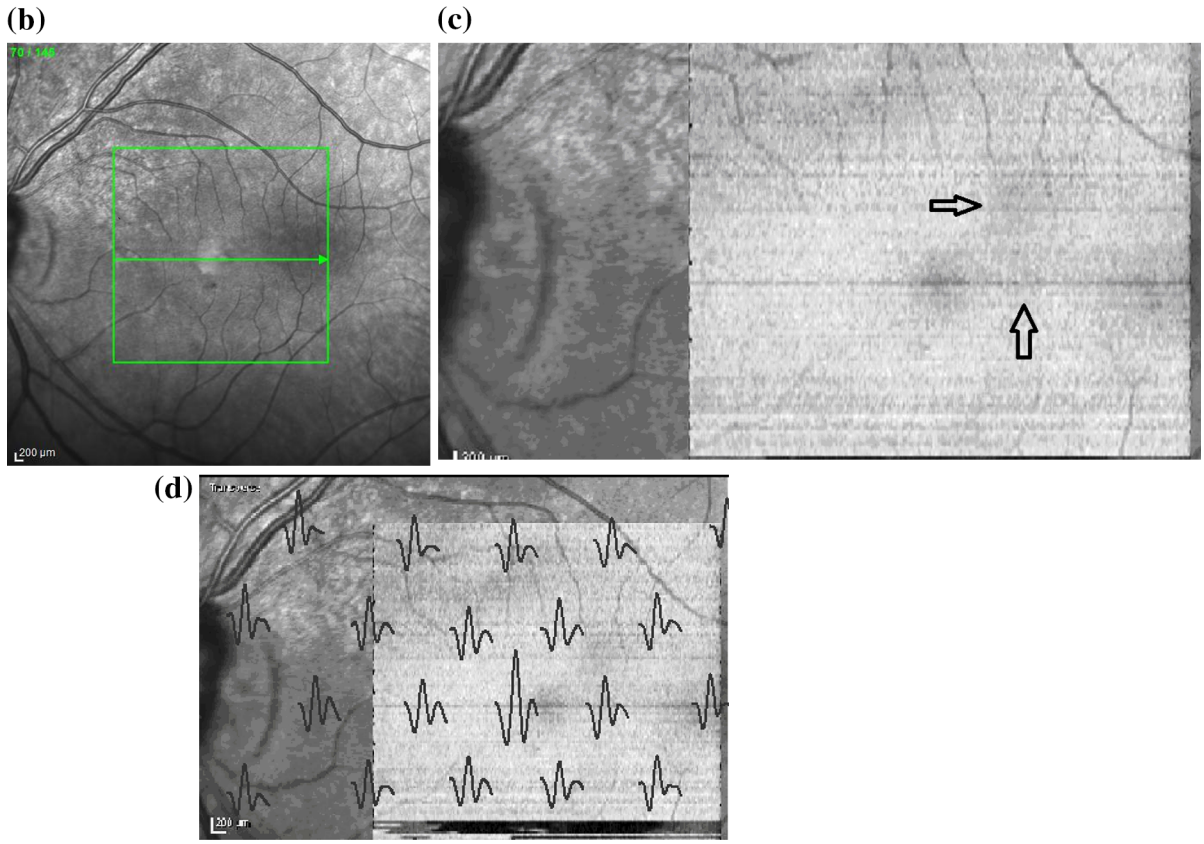

Fig. 5 continued

In one case with an abnormal P1 R3/R5 ratio and a normal P1 R2/R5 ratio, the C-Scan OCT was also considered to be normal. Except in some Asian patients [5], the lesions usually occur in the pericentral ring superimposing with the P1 R2/R5 ratio. Thus, the reliability of P1 R3/R5 ratio as an indicator of AM toxicity appears to be questionable at least in Caucasian patients in whom paracentral (ring 2) loss appears to be the earliest affected region with toxicity. Pericentral (ring 3) loss appears to occur later.

In a large amount of publications, the high proportion of mfERG abnormalities remains difficult to interpret, as this indicator of early toxicity may not be detected with other screening tools [7]. Besides toxicity, another interpretation would be that mfERG changes are simply false positives indicating AM-induced acute and possibly reversible electrophysiologic changes [11]. The choice of the P1 R2/R5 and P1 R3/R5 ratios as indicators of toxicity was based on the fact that the P1 amplitude in the fifth ring was considered to be not significantly different when

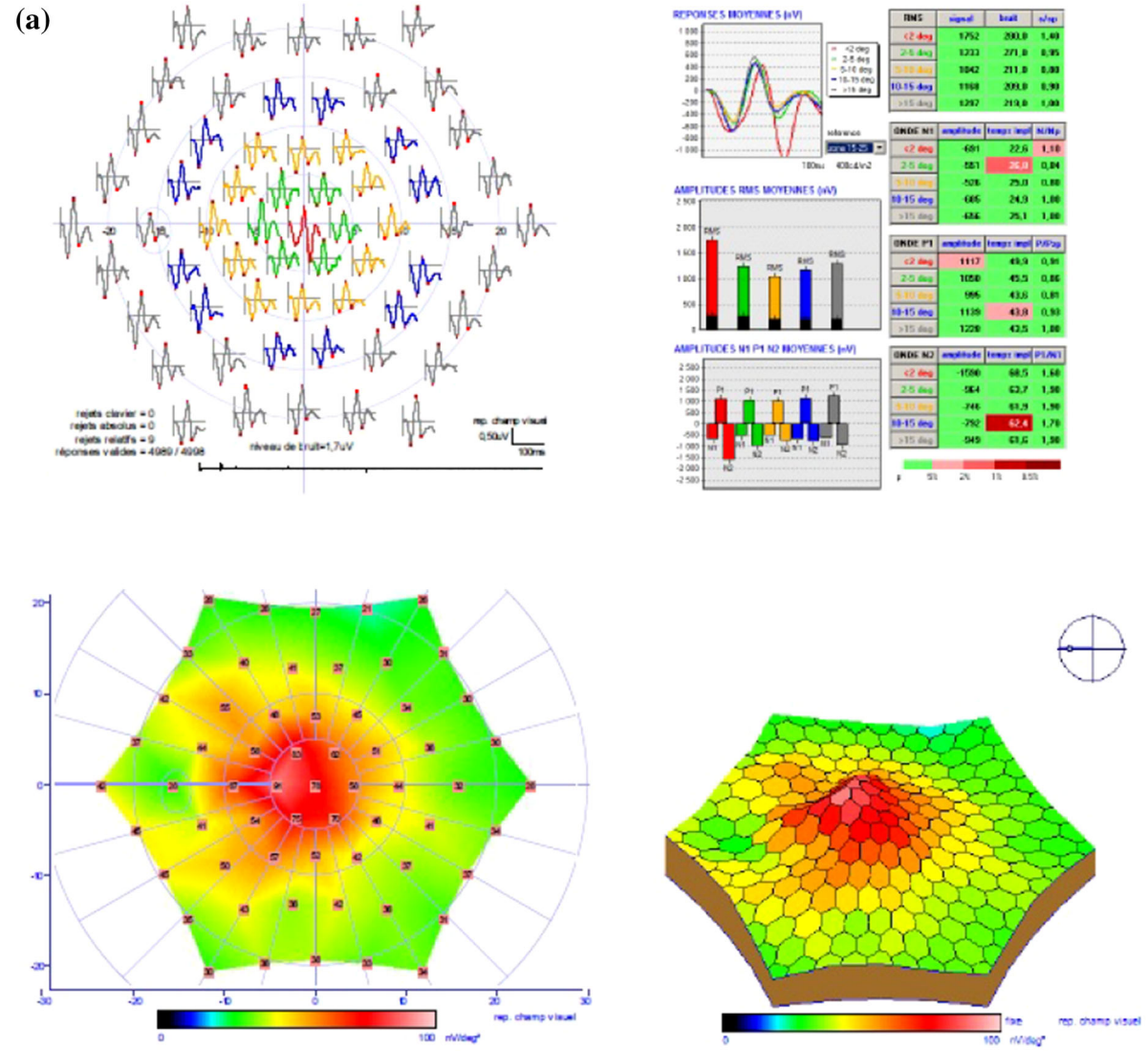

Fig. 6 Fifty-three-year-old female, systemic lupus, 10 years hydroxychloroquine, 400mg/day, 4.04 mg/kg (estimated cumulative dose: 1461g). **a** The multifocal ERG (field view) is abnormal, both the R2/R5 ratio (0.86) and the R3/R5 ratio (0.81) were below 1. **b** No changes on retinal biomicroscopy were noted, the infrared image was normal. **c** Abnormal C-Scan OCT of the ellipsoid layer: several hyporeflective changes (arrows). **d** Superposition of the C Scan OCT with the multifocal ERG responses. The local ERG responses were reduced in the large temporal hyporeflective zone (circle), however beyond ellipsoid changes on the C-Scan the local ERG responses were also altered (arrows). In addition, no abnormal local ERG response could be recorded in the area of the second small hyporeflective area (*). Again in this case, the mean amplitude of the P1 was reduced on multifocal ERG (both the R2/R5 and R3/R5 ratio were below 1)

comparing normals and hydroxychloroquine-treated patients [11]. The coexistence of subtle C-Scan OCT abnormalities with the reduced pericentral mfERG responses could then be rather interpreted as indicators of early toxicity, both changes occurring at the same retinal eccentricity although they were not always localized in the same retinal area.

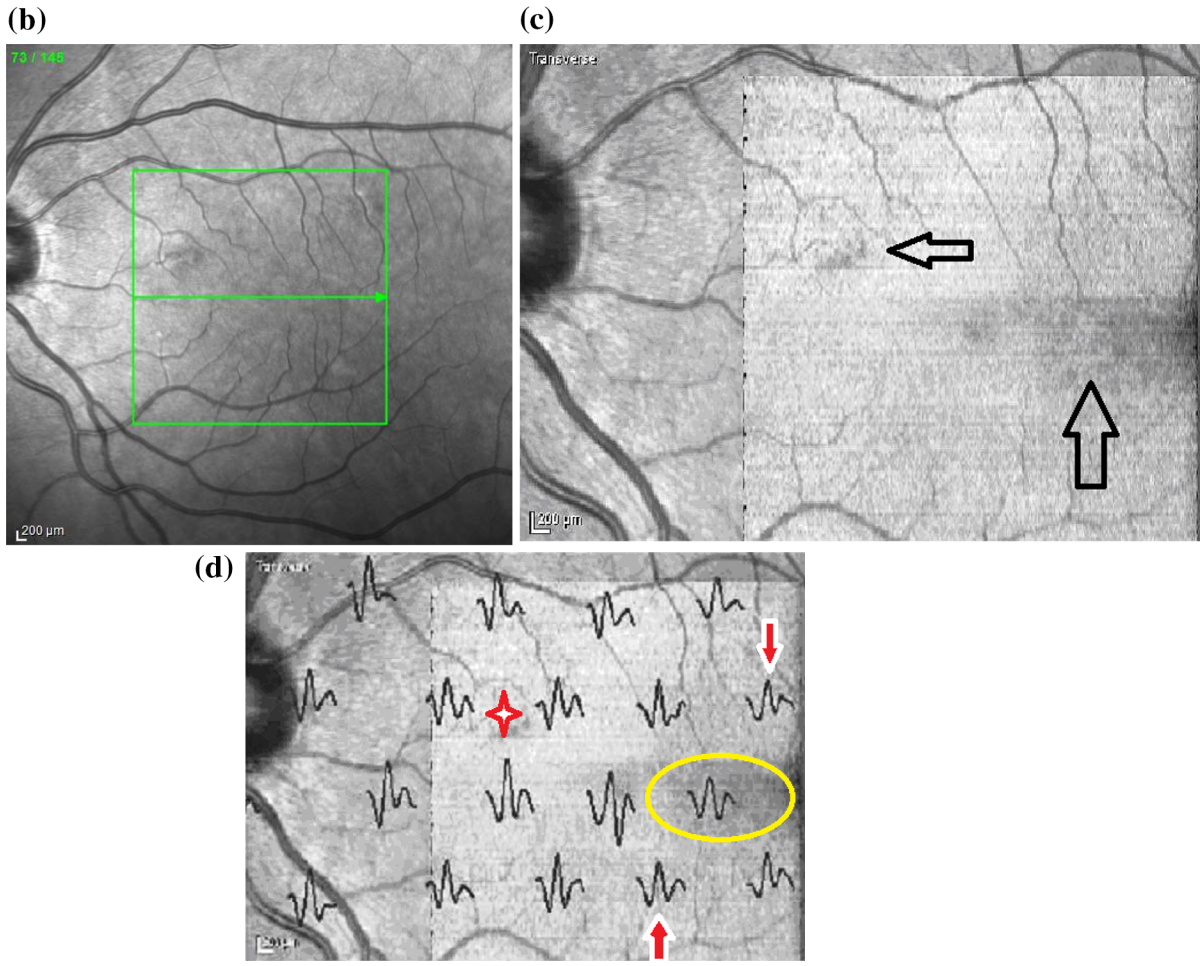

Fig. 6 continued

We acknowledge many limitations to this preliminary study. These included patients had a high probability of potential lesions due to AM toxicity, as in all patients on inclusion, AM treatment was discontinued on the basis of abnormal visual fields.

Notwithstanding, this study suggested the superiority of the C-Scan OCT compared to the B-Scan OCT recommended in the screening for patients taking AM treatment [5]. It raises the question if C-Scan OCT may replace the classical B-Scan for detecting early signs of toxicity. A prospective multicenter study (NCT 02805686) is currently in progress to confirm the association between hyporeflective lesions on the C-Scan OCT and local retinal dysfunction on mfERG.

Compliance with ethical standards

Conflict of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakera's bureaus; membership; employment; consultancies; stock ownership; or other equity interest; and expert testimony or patent-licensing arrangements) or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research

committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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