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"En Face" Spectral-Domain Optical Coherence Tomography versus Multifocal Electroretinogram in Hydroxychloroquine Retinopathy Screening

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Keywords
Hydroxychloroquine toxicity · Hydroxychloroquine retinopathy · Multifocal electroretinogram · Optical coherence tomography · "En face" optical coherence tomography

Abstract
Introduction: The performance of "en face" optical coherence tomography (OCT) in screening for chloroquine (CQ) or hydroxychloroquine (HCQ) retinopathy has not been largely explored. The aim of this study was to determine the concordance of "en face" OCT with multifocal electroretinography (mfERG) in screening for CQ/HCQ retinopathy. ***Methods:*** This is a prospective cohort study conducted at the Rothschild Foundation Hospital, Paris, between August 2016 and February 2021. Patients taking HCQ were followed up over 2 consecutive years and received an "en face" OCT and a mfERG on each visit. ***Results:*** A total of 91 patients (182 eyes) were analyzed. mfERG and "en face" OCT were concordant in 147 eyes (86.3%). Cohen's kappa coefficient for concordance between mfERG and "en face" OCT was considered weak with a value 0.61 (95% CI: 0.50–0.72). The sensitivity and specificity of "en face" OCT were 70% (95% CI: 59–79%) and 91% (95% CI: 83–96%), respectively, relatively to mfERG. Proportion of abnormal R2/R5 and R3/R5 ratios did not differ between patients with normal and abnormal "en face" OCT ($p = 0.2$). ***Discussion:*** "En face" OCT and mfERG have low concordance and cannot be used interchangeably as each investigation evaluates a different facet of CQ/HCQ retinopathy. "En face" OCT could be used as a complement in screening for CQ/HCQ retinal toxicity if the anomalies detected on "en face" OCT are confirmed by B-scan OCT sections.

Introduction

Long-term exposure to chloroquine (CQ) or hydroxychloroquine (HCQ) can lead to a rare acquired toxic retinopathy [1]. It is characterized by a slowly progressive bilateral retinal damage, causing clinically subtle macular

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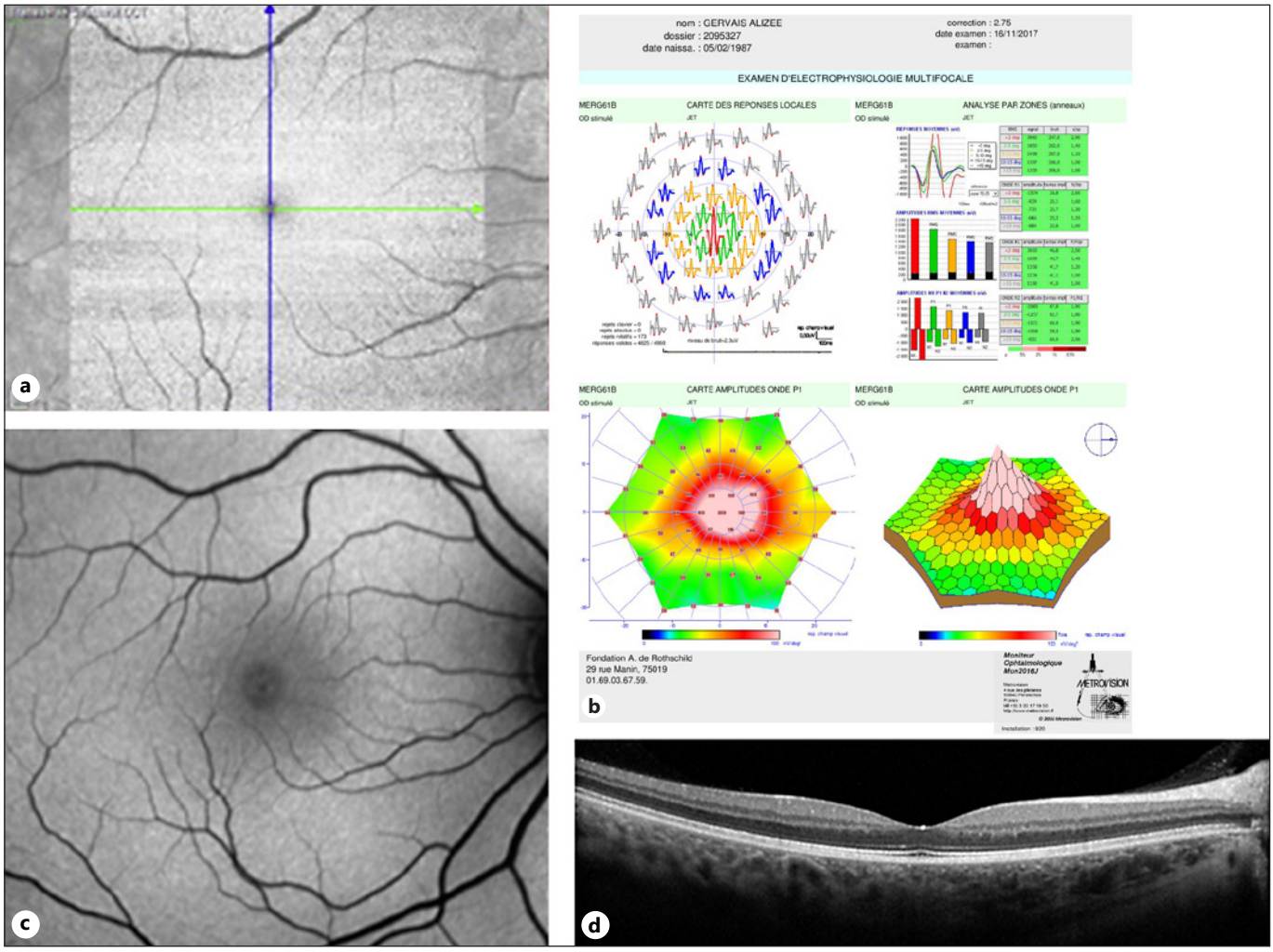

Fig. 1. Normal findings on optical coherence tomography (OCT) and multifocal electroretinography (mfERG). **a** "En face" OCT of the right eye showing uniform hyperreflectivity at the level of EZ. **b** Normal mfERG. **c** Normal fundus autofluorescence (FAF). **d** Horizontal OCT B-scan with no abnormalities.

pigmentary changes that eventually lead to bull's-eye maculopathy [1, 2]. When bull's eye maculopathy is detected on fundoscopy, damage is already severe and irreversible even if CQ/HCQ is stopped [1, 2]. It is therefore important to detect toxic retinopathy secondary to CQ/HCQ at an early subclinical stage [1].

Multifocal electroretinography (mfERG) and optical coherence tomography (OCT) play central roles in screening for CQ/HCQ retinopathy. According to the recommendations of the American Academy of Ophthalmology (AAO) published in 2016 [1], patients taking CQ or HCQ should have a complete baseline ophthalmological examination including a fundus examination within a year of the start of the treatment. This baseline examination can be complemented by a central automated 10-2 visual field (AVF) (or an automated 24-2 visual field in Asian patients) (functional evaluation) and a spectral-domain (SD) or a swept-source (SS) macular OCT (anatomic evaluation). If there is no risk factor for CQ or HCQ toxicity (consisting in HCQ >5 mg/kg of real weight or CQ >2.3 mg/kg of real weight, renal insufficiency, tamoxifen use, other retinopathy), an annual screening visit is recommended 5 years after the beginning of the treatment. These annual visits should include a central AVF and a macular SD- or SS-OCT. Fundus autofluorescence (FAF) can also be added if available. In case of suspicious findings on SD-OCT or visual field, mfERG, by its high sensitivity, can aid in the confirmation of a beginning toxic

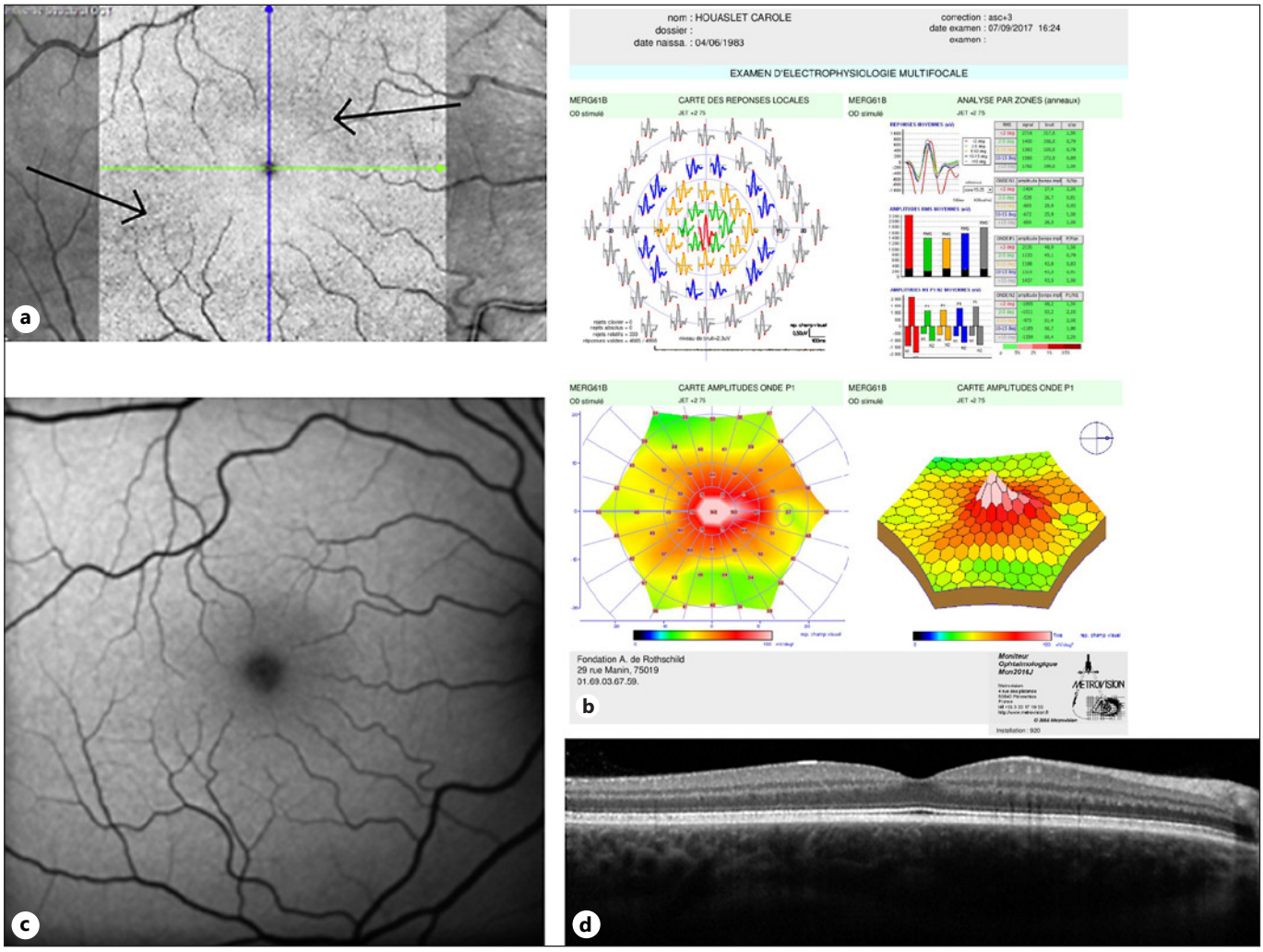

Fig. 2. Moderate toxicity findings with abnormal optical coherence tomography (OCT) and abnormal multifocal electroretinography (mfERG). **a** Moderate retinal anomalies (black arrows) characterized by minor hyporeflectivity around the fovea on "en face" OCT at the level of the ellipsoid zone (EZ). **b** Abnormal mfERG with the RMS analysis findings of histograms representing R2 and R3 lower in amplitude than the histogram representing R4. **c** Fundus autofluorescence (FAF) showing no abnormalities. **d** Horizontal OCT B-scan with no abnormalities.

maculopathy. According to the Royal College of Ophthalmologists guidelines published in 2020 [3], screening of patients should include SD-OCT and FAF and are confirmed by AVF. If AVF shows no anomalies, mfERG is used to confirm toxicity.

OCT images are usually obtained in axial sections (B-scan) and show, in the perifoveal area, a thinning of the outer nuclear layer (ONL) and an interruption of the ellipsoid zone (EZ) in CQ or HCQ retinal toxicity beginning in the inferior macula [4, 5]. OCT images can be analyzed in coronal sections using "en face" OCT or C-scan [6, 7]. These sections are obtained by volumetric reconstruction of consecutive aligned B-scans [6]. Unlike B-scan, "en face" OCT allows a view of the photoreceptor layer in a single image, making it possible to detect any anomaly without having to scroll through several sections [7, 8]. Ahn et al. described the appearance of CQ/HCQ retinopathy on "en face" OCT [8]. "En face" OCT images demonstrate beaten-bronze appearance in the area corresponding to photoreceptor defects on B-scan images and more pronounced hyporeflectivity in the area corresponding to combined photoreceptor and retinal pigment epithelium (RPE) defects on B-scan [8]. The detection value of "en face" OCT in comparison to mfERG has not yet been

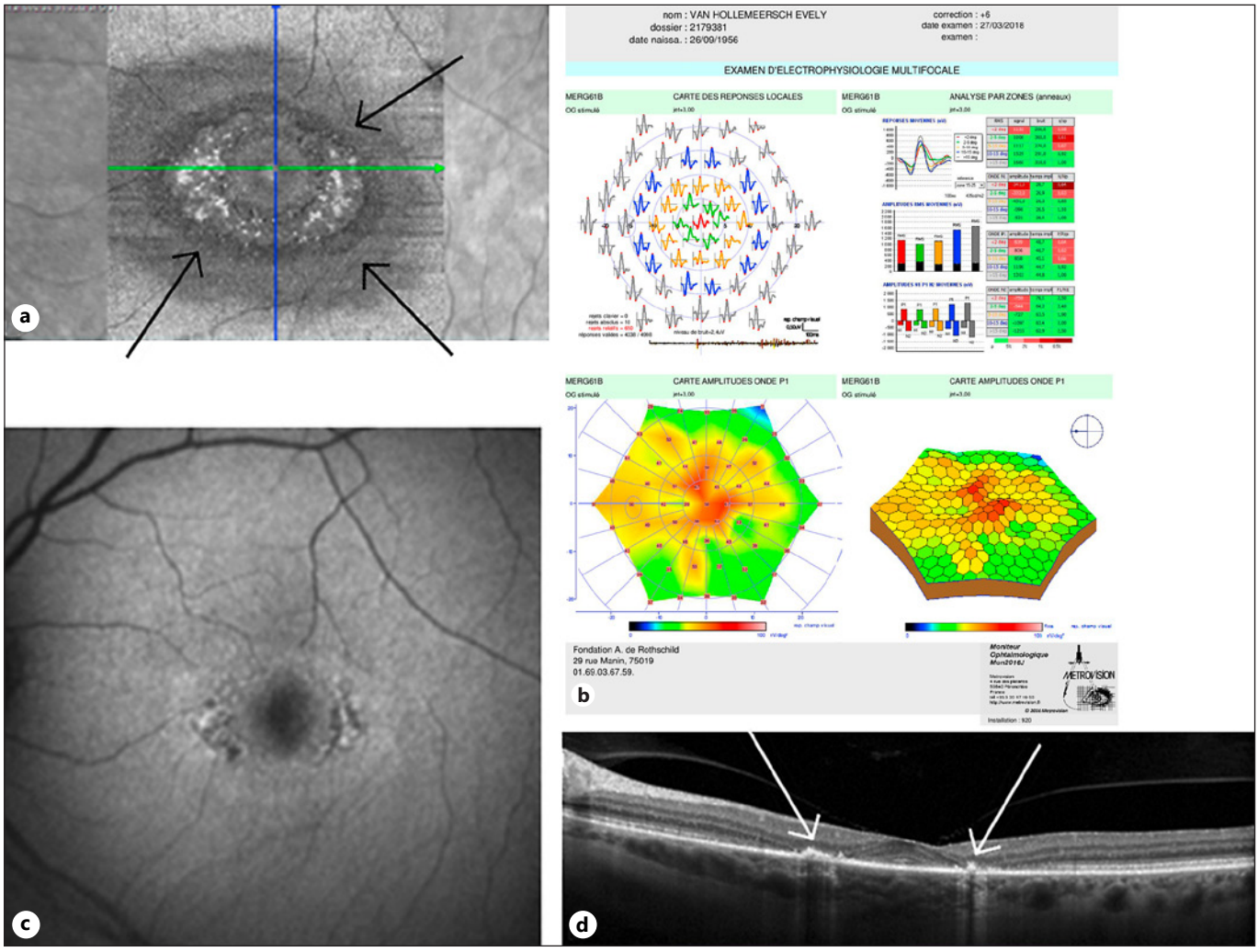

Fig. 3. Severe toxicity findings with abnormal optical coherence tomography (OCT) and abnormal multifocal electroretinography (mfERG). **a** Severe retinal anomalies characterized by major hyporeflectivity surrounding the fovea on "en face" OCT (black arrows) at the level of the ellipsoid zone (EZ). **b** Abnormal mfERG with reduced amplitude of the trace array in the parafoveal R2 and R3 rings and the histogram representing R2 or R3 showing lower in amplitudes than the histogram representing R4 on the RMS analysis representation. **c** Hyper- and hypofluorescence in the perifoveal area on fundus autofluorescence (FAF). **d** Diffuse EZ disruption on B-scan OCT (white arrows).

evaluated. The aim of this prospective study was to compare the role of mfERG and "en face" OCT in the detection of CQ/HCQ toxicity and to determine the concordance of "en face" OCT with mfERG, a screening tool with high sensitivity [9], in screening for CQ/HCQ retinopathy.

Methods

Population and Design

This is a prospective cohort study (National Clinical Trial number: NCT02719002) conducted at the Rothschild Foundation Hospital, Paris, between August 2016 and February 2021. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by an independent Ethics Committee (Certified programs and proofs "Ile de France VIII") on December 21, 2015, and by the French competent authority "Agence nationale de securite du medicament et de ses produits" (ANSM) on December 28, 2015 (NCT02719002).

Informed consent form was signed by all included subjects. Inclusion criteria were age older than 18 years and HCQ treatment for a minimum of 5 years. Exclusion criteria were media opacities (dense cataract, corneal opacity), amblyopia, high myopia or hyperopia (>8 diopters), history of retinal surgery, pregnancy, and breastfeeding. All included subjects consulted on 5 occasions

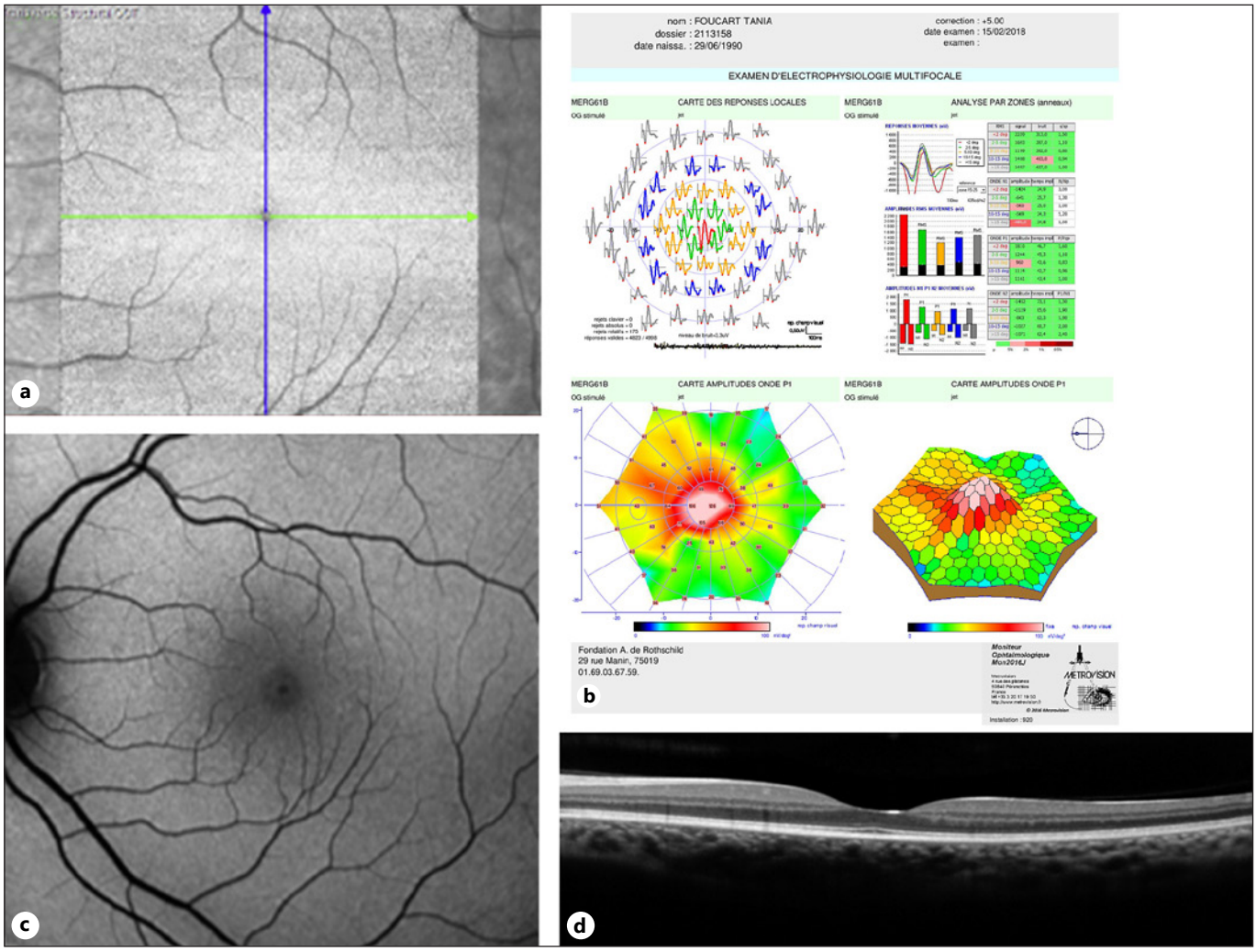

Fig. 4. Discrepancy between normal optical coherence tomography (OCT) and abnormal multifocal electroretinography (mfERG). **a** "En face" OCT of the left eye showing uniform hyperreflectivity at the level of the ellipsoid zone (EZ). **b** Abnormal mfERG with the histogram representing R3 lower in amplitude than the histogram representing R4 on the RMS analysis representation. **c** Normal fundus autofluorescence (FAF). **d** Horizontal OCT B-scan with no abnormalities.

spanning over 2 consecutive years: at inclusion (baseline), at 6 months, at 12 months, at 18 months, and at 24 months. On each visit, every patient underwent a complete ophthalmological examination including macular SD-OCT B-scan, "en face" OCT, and a mfERG. mfERG was considered the reference for the detection of CQ/HCQ drug toxicity; hence, subjects showing mfERG anomalies stopped taking part in the study.

Imaging Modalities

OCT was performed on the SPECTRALIS OCT machine (Heidelberg Engineering, Heidelberg, Germany). The examination included 2 B-scan sections of each eye (Line ART 100, 30°, HR at 180° and 90°) and a macular volume for the C-scan ("en face") analysis of the posterior poles of each eye (macular volume 15° × 15°, 30 µm between 2 sections, 145 sections, ART 16, HR). The segmentation for the "en face" images was automatically performed from the inner border of the EZ to that of the RPE-Bruch's membrane complex using the software provided by the manufacturer. Adequacy of automated segmentation was confirmed by the readers before imaging analysis was done. "En face" OCT was considered normal if the EZ of the retina was regularly hyperreflective with a central hyporeflective zone corresponding to the fovea. It was considered abnormal in the presence of beaten-bronze or hyporeflective spots or zones around the fovea [8]. Of note, in the presence of anomalies on "en face" imaging, B-scans were checked to rule out the presence of artifacts or other macular anomalies unrelated to CQ or HCQ toxicity. Figures 1–4 show examples of normal and abnormal OCT findings.

Table 1. Epidemiological and clinical characteristics of study subjects

Subjects' characteristics	
Total number of included subjects	109
Female gender, *n* (%)	103 (94.5)
Age (mean ± SD), years	47.5±14.5
Weight (mean ± SD), kg	67.8±14.4
Length (mean ± SD), cm	165±7.18
Asian subjects	0
Reason for antimalarial use, *n* (%)	
Systemic lupus erythematosus	89 (81.7)
Rheumatoid arthritis	7 (6.4)
Scleroderma	3 (2.8)
Sjogren syndrome	3 (2.8)
Sarcoidosis	3 (2.8)
ITP	1 (0.9)
Antiphospholipid syndrome	1 (0.9)
Other	2 (1.8)
HCQ dose and duration	
Daily dose (mean ± SD), mg	360±89.3
Dosing (mean ± SD), mg/kg	5.4±1.9
Duration of treatment (mean ± SD), years	14±6.8
Antimalarial retinopathy risk factors, *n* (%)	
Renal insufficiency	15 (13.8)
Liver insufficiency	3 (2.8)
Tamoxifen use	0

ITP, idiopathic thrombocytopenic purpura.

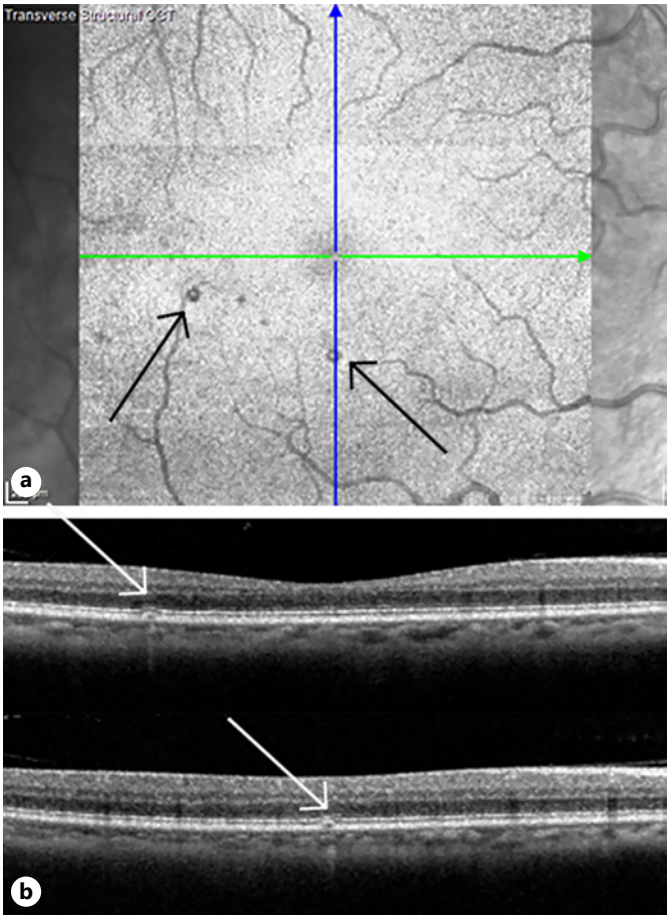

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Fig. 5. Drusen and pseudodrusen artifacts as seen on optical coherence tomography (OCT). Hyporeflective small spots (black arrows) depicted on the "en face" OCT at the ellipsoid level (**a**) corresponding to small pseudodrusen on B-scan OCT (white arrows) (**b**).

mfERG was performed according to the International Society for Clinical Electrophysiology of Vision (ISCEV) recommendations by the Vision Monitor device (Metrovision, Perenchies, France) [10]. Refractive error was compensated for by adjusting the focus with the system's eyepiece. Multiple retinal areas were stimulated using a stimulus array of 61 hexagons and a binary m-sequence. The stimulus pattern subtended an angle of 25° on either side of fixation and was displayed on a cathode ray tube monitor with a frame frequency of 75 Hz. Retinal signals were filtered with low-frequency and high-frequency cutoffs of 5 and 200 Hz, respectively. The mean amplitudes of the first negative, the first positive, and the second negative waves were measured in 5 concentric areas (ring 1 to ring 5). mfERG was considered unreliable if the global noise level exceeded 5 microvolts. Up to this moment, no clear-cut criteria were used to define a particular mfERG tracing as abnormal. In previous studies, some authors relied on the change in the shape of the traces [11], others on the amplitude of the waves of each ring compared to the manufacturer's standards [11–14], and others on the analysis of the ring ratios [7, 14–16]. In the present study, mfERG was considered abnormal if the trace array was considered of reduced amplitude in the parafoveal 2nd ring (R2: 2–5°) or 3rd ring (R3: 5–10°) or if the histogram representing R2 or R3 was lower in amplitude than the histogram representing the 4th ring (R4: 10–15°) on the RMS analysis representation. We particularly looked at the second and third ring because these rings are the most affected in CQ/HCQ retinopathy [7, 11, 15, 17, 18].

All investigations were analyzed by two independent retina specialists (MMF and VV) in a double-blind fashion. In case of disagreement between the two readers, the readers rediscussed the case to decide whether CQ/HCQ drug toxicity signs were present or not.

Statistical Analysis

Statistical analysis was performed using the R software (version 4.0.3, The R Foundation for Statistical Computing). Performance of "en face" OCT in detecting HCQ toxicity signs was compared to mfERG (considered as the reference in the present study). Concordance between mfERG and "en face" OCT was assessed using Cohen's kappa coefficient. Sensitivity and specificity of "en face" OCT were determined relatively to mfERG. The mfERG tracing taken into consideration in the analysis was either the one done at the time the subject was withdrawn from the study because of abnormal mfERG or the one done at the end of the follow-up in the event mfERG remained normal all along (at 24 months). Post hoc analysis comparing the proportion of abnormal R2/R5 and R3/R5 ratios of the first positive wave between

Table 2. Multifocal electroretinography (mfERG) and "en face" optical coherence tomography ("en face" OCT) distribution of the 182 eyes of the study population

		"En face" OCT (number of eyes (%))	
mfERG (number of eyes (%))		Normal	Abnormal
	Normal	86/182 (47.3%)	9/182 (4.9%)
	Abnormal	26/182 (14.3%)	61/182 (33.5%)

Table 3. Comparison of the proportion of abnormal R2/R5 and R3/R5 ratios between patients with normal and abnormal "en face" optical coherence tomography (OCT)

	Abnormal OCT (n = 70)	Normal OCT (n = 112)	p value
R2/R5, mean (SD)	0.98 (0.24)	1.08 (0.18)	–
R2/R5 <1	33/70 (47%)	43/112 (38%)	0.2
R3/R5, mean (SD)	0.91 (0.13)	0.96 (0.12)	–
R3/R5 <1	56/70 (80%)	79/112 (70%)	0.2

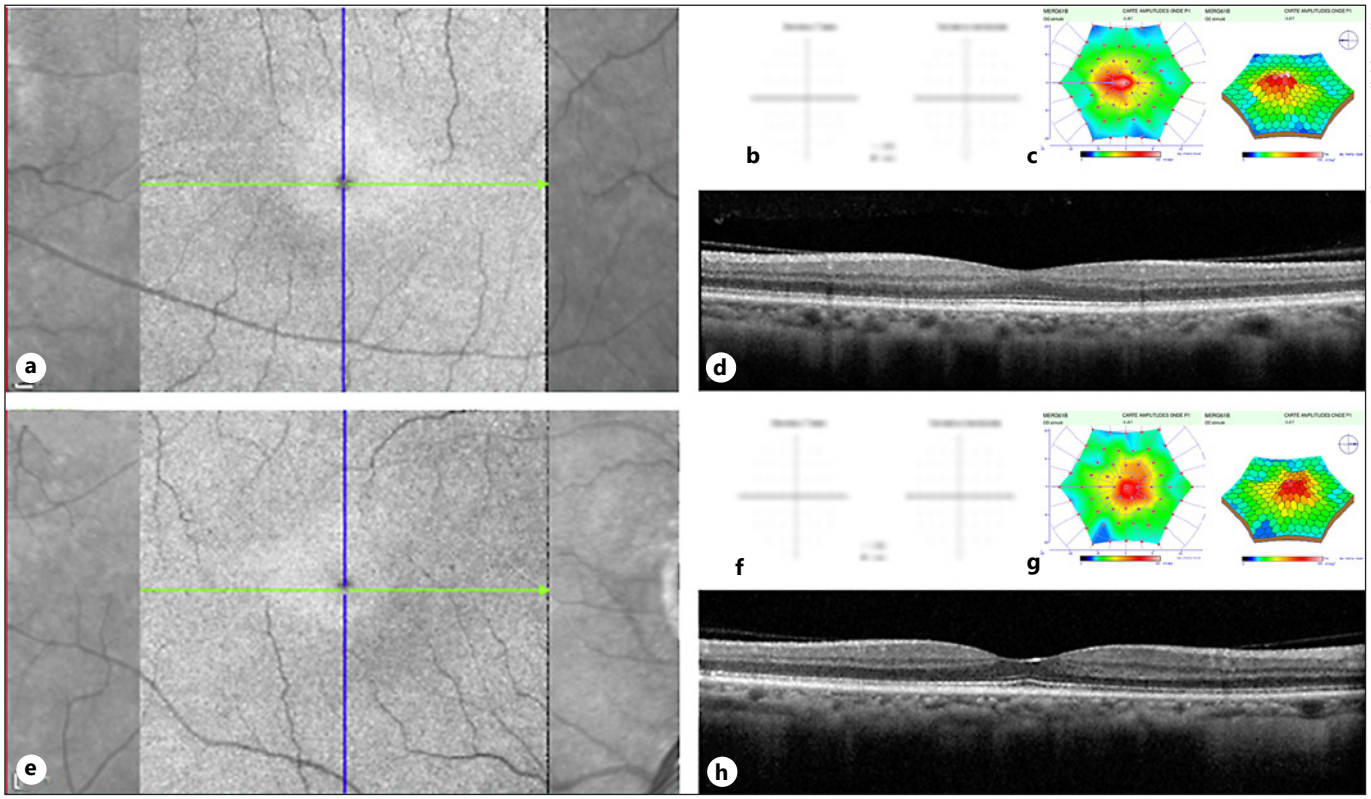

Fig. 6. Stiles-Crawford artifact as seen on optical coherence tomography (OCT). All images are taken in the same myopic woman. Upper row represents left-eye findings, and lower row represents right-eye findings. **a**, **e** Bilateral "en face" OCT with juxtafoveal inferonasal symmetrical hyporeflectivity area at the ellipsoid zone (EZ) level. **b**, **f** Visual field is normal bilaterally. **c**, **g** Multifocal electroretinogram shows uniform reduction of the amplitude of the trace arrays corresponding to myopia. **d**, **h** B-scan OCT showing a thickened hyperreflective outer plexiform layer at the area corresponding to the hyporeflectivity seen on the "en face" OCT.

patients with normal and abnormal OCT was done using the χ^2 test. These ratios were considered abnormal if their value was less than 1 [7, 9]. A p value of less than 0.05 was considered statistically significant.

Results

A total of 109 patients (218 eyes) were included in the study. Most patients were females (94.5%) with a mean age of 47.5 (±14.5) years (range: 24–80 years) and were currently on HCQ for systemic lupus erythematosus (81.7%). The mean duration of treatment was 14 years (±6.8) (range: 1–35 years) with a mean dosing of 5.4 (±1.9) mg/kg (range: 0.5–11 mg/kg). Characteristics of the study population are summarized in Table 1. Eighteen subjects were excluded from the final analysis because their mfERG or "en face" OCT was deemed unreliable or because of concomitant retinopathies (epiretinal membrane, degenerative myopia, dome-shaped macula), leaving 91 patients (182 eyes) analyzed.

In 86 of 182 eyes (47.3%), both mfERG and "en face" OCT were normal; in 26 of 182 eyes (14.3%), mfERG was abnormal, while "en face" OCT was normal; in 9 of 182 eyes (4.9%), mfERG was normal, while "en face" OCT was abnormal; and in 61 of 182 eyes (33.5%), both mfERG and "en face" OCT were abnormal. Hence, mfERG and "en face" OCT were concordant in 147 eyes (86.3%). These results are summarized in Table 2.

Cohen's kappa coefficient for concordance between mfERG and "en face" OCT was considered weak with a value 0.61 (95% CI: 0.50–0.72). Relative to mfERG, the sensitivity and specificity of "en face" OCT were 70% (95% CI: 59–79%) and 91% (95% CI: 83–96%), respectively. The proportion of abnormal R2/R5 and R3/R5 ratios did not differ between patients with normal and abnormal "en face" OCT ($p = 0.2$) (Table 3). In the 13 patients who presented abnormal mfERG and normal en face OCT, HCQ was stopped, and patients were controlled 6 months later. mfERG was considered normal in all patients 6 months after HCQ withdrawal.

Discussion

CQ and HCQ are medications with a good safety profile that are currently used in the treatment of multiple systemic disease. Hence, the ophthalmologist should use reliable, reproducible, and objective investigations to detect with certainty any early damage caused by these drugs before reducing the dose or stopping the treatment. A single technique cannot meet all these criteria: for retinal structural evaluation, SD- or SS-OCT or FAF is used, while for retinal functional evaluation, an AVF or a mfERG is used [1, 3].

mfERG is an objective examination that allows recording of electrical signals from multiple discrete areas across the posterior pole [10]. Electrical signals of each tested zone of the retina are then represented as traces and as numerical values (amplitude and peak time measures) that can be compared to reference values [10]. In addition, a 3D density plot (signal strength per unit area of the retina) and ring averages (groups of responses from the trace arrays can be averaged for 5 successive rings from the center to the periphery) can be obtained [10]. According to multiple reports, the second and third rings are the most affected in CQ/HCQ retinopathy [7, 11, 15, 17, 18]. No firm explanation exists for the preferential involvement of the parafovea by CQ/HCQ toxicity. Light absorption or cone metabolism may play a role, but this remains a speculation [1, 11]. Despite these findings, there are no standard criteria for defining an mfERG as abnormal or to differentiate HCQ impregnation from early toxicity signs. It can be interpreted according to the shape of the traces [11], the amplitude of the waves of each ring compared to the manufacturer's standards [11–14], or the analysis of the ring ratios [7, 14–16].

In CQ/HCQ drug toxicity evaluation, mfERG seems to have a high false-positive rate (abnormal mfERG with no anomalies on AVF) as reported by Tsang et al. in 2015 [9] and early discontinuation of HCQ in patients showing recent alterations on mfERG allows reversibility of damage over a period of 6 months [13, 19–21]. This was confirmed in our study where 13 subjects with an abnormal mfERG and a normal "en face" OCT normalized their mfERG 6 months after treatment withdrawal. These changes observed on mfERG were reported to be an early sign of toxicity and were recommended to be used to confirm HCQ toxicity and therefore stop the medication [9, 17]. We argue that these mfERG changes can be a representation of CQ/HCQ impregnation without necessarily being a sign of toxicity. This is explained by the fact that CQ/HCQ inhibits the transporter of the all-trans-retinol molecules (organic anion transporting polypeptide 1A2) in the RPE and thus interrupts the renewal cycle of the visual pigments responsible of phototransduction [22]. The inhibition of visual pigment renewal can explain mfERG changes seen in patients taking CQ/HCQ without necessarily being related to toxicity that needs medication withdrawal. Furthermore, mfERG has limited accessibility

and requires good patient cooperation (stable fixation, long duration of the examination, no dry eye, etc.) for it to be recorded appropriately. It also seems to have high variability when repeated in patients taking CQ/HCQ [23]. Hence, difficult interpretation and acquisition of mfERG, its limited accessibility, and the possibility of false-positive results (mfERG changes reflecting CQ/HCQ impregnation) make mfERG seem unreliable as a screening tool alone to decide whether to stop or continue the medication.

OCT is, on the other hand, fast, objective, and easily accessible [4, 5]. It can detect on B-scans thinning of the ONL or interruption of the EZ as early signs of CQ or HCQ retinopathy [4, 5]. "En face" OCT has the same advantages as B-scan with the additional possibility of visualizing the retina on a single plane [6]. Ahn et al. reported that all the anomalies visible on B-scan are detected by "en face" OCT as beaten-bronze or hyporeflective zones [8]. In addition, "en face" OCT made it possible to follow the progression of the disease by calculating the area of the hyporeflective zone, something that is difficult to obtain by scrolling through OCT B-scan sections [8]. We therefore tried to explore the role "en face" OCT can have in screening and monitoring for HCQ retinopathy. In our study, sensitivity and specificity of "en face" OCT for the detection of HCQ-related maculopathy were 70% and 91%, respectively. Sensitivity of B-scan OCT was around 79% [24], and sensitivity of mfERG was around 90–100% [9, 17]. This shows that "en face" and B-scan OCTs have close sensitivity values. Disadvantages of "en face" OCT are its low specificity in comparison to B-scan (98%) [24] and its difficult interpretation in the presence of other retinopathies. In fact, some of the subjects that were excluded from analysis had "en face" OCTs that were difficult to interpret because of other retinopathies or artifacts such as pseudodrusen (shown Fig. 5), pattern dystrophy, macular edema, Stiles-Crawford effect (shown in Fig. 6), mask effect from vitreous opacities, and epiretinal membranes. Hence, abnormal findings on "en face" OCT should always be confirmed by B-scan findings (ONL thinning and EZ disruption) before any conclusion is drawn on the presence of a retinal toxicity secondary to CQ/HCQ. If a doubt persists (for instance in case of Stiles-Crawford effect), it will be mandatory to add the interpretation of AVF and/or mfERG before planning CQ/HCQ discontinuation. Other disadvantages include the necessity to confirm that automated segmentation was done reliably by the software [8]. Hence, no firm conclusion can be drawn on the use of "en face" OCT alone as a screening and monitoring tool. It seems useful as a complement for other imaging modalities, especially SD-OCT.

Concordance between mfERG and "en face" OCT was weak with a kappa value of 0.61. Our results slightly differ from previously reported results by Arndt et al. who state a better agreement between mfERG and abnormal "en face" OCT [7]. In addition, abnormal "en face" OCT did not show a higher proportion of abnormal R2/R5 and R3/R5 ratios. These conflicting findings show that mfERG and "en face" OCT cannot be used interchangeably because they assess different facets of the same disease: mfERG assesses retinal function, while "en face" OCT assesses retinal structure.

One limitation of this study is the small number of subjects, especially after many subjects were secondarily excluded because of uninterpretable imaging results. Another limitation is the fact that interobserver agreement was not calculated. This is because when discordance occurred, the two readers reanalyzed the results together and decided on the final judgment.

In conclusion, mfERG has controversial interpretation, difficult acquisition, and limited accessibility and seems to have high numbers of false-positive results which makes its role questionable in screening and monitoring for CQ/HCQ toxicity. "En face" OCT could be used as a complement in screening for CQ/HCQ retinal toxicity since it is easily accessible and allows a rapid evaluation of the entire retinal surface. It seems more prudent to confirm anomalies detected on "en face" OCT by B-scan OCT sections to confirm that retinal anomalies are in fact secondary to CQ/HCQ toxicity. "En face" OCT and mfERG have low concordance and cannot be used interchangeably as each investigation evaluates a different facet of CQ/HCQ retinal toxicity. Improvement in OCT imaging will improve and ease the detection of CQ/HCQ toxicity. The upcoming era of artificial intelligence-coupled analysis of OCT and mfERG will also surely allow a better detection of this potentially blinding retinopathy.

Statement of Ethics

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by an independent Ethics Committee (Certified programs and proofs "Ile de France VIII" on December 21, 2015) and by the French competent authority ANSM (Agence nationale de securite du medicament et de ses produits) on December 28, 2015 (NCT02719002). Written informed consent was obtained from all the participants in this study.

Conflict of Interest Statement

The authors declare no conflict of interest.

Funding Sources

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Author Contributions

Martine Mauget-Faysse, Georges Sukkarieh, Vivien Vasseur, and Nathalie Costedoat-Chalumean conceived the design of the study, participated in data acquisition and interpretation, helped revise the manuscript, and gave their final approval and their accountability for the final form of the manuscript. Hajar Jebrane, Sabine Derrien, Veronique Le Guern, and Francine Behar-Cohen participated in data acquisition and interpretation, wrote the first draft of the paper, helped revise the manuscript, and gave their final approval and their accountability for the final form of the manuscript. Justine Lafolie, Anne Sophie Alonso, Elsa Laumonier, Sophine Thevenin, Emmanul Augé, and Jessica Guillaume participated in data acquisition, data analysis and interpretation. They also helped in revising the manuscript and gave their final approval and their accountability for the final form of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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NEW PROPOSAL FOR A MULTIMODAL IMAGING APPROACH FOR THE SUBCLINICAL DETECTION OF HYDROXYCHLOROQUINE-INDUCED RETINAL TOXICITY IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

SUPPLEMENTARY DATA

Supplementary Table S1. Characteristics of patients with hydroxychloroquine-induced retinal toxicity.

	Case 1	Case 2	Case 3	Case 4
Age (years)	77	50	48	40
Sex	F	F	F	F
Risk factors HCQ toxicity				
Real body weight (Kg)	61	50	52	117.4
Body mass index (Kg/m2)	27.11	20.55	N/S	66.00
Chronic kidney disease	No	No	No	No
Chloroquine	Yes	Yes	No	No
Tamoxifen	No	No	No	No
Hydroxychloroquine				
Average daily dose (mg/d)	200	200	200	200
Daily dose RBW (mg/d)	3.28	4.00	3.85	1.70
Cumulative dose hydroxychloroquine (g)	1,512	792	120	132
Duration (years)	21	11	1.67	1.83
Chloroquine				
Average daily dose (mg/d)	250	250	0	0
Daily dose RBW (mg/d)	4.1	5	-	-
Cumulative dose chloroquine (g)	630	540	-	-
Duration (years)	7	6	-	-
Glucocorticoids				
Average daily dose (mg/d)	0	5	5	2.50
Cumulative dose (g)	-	30.60	3.30	1.05
Laboratory features				
Anti dsDNA antibodies > 20 UI/mL	Yes	No	Yes	Yes
Low complement	Yes	No	Yes	Yes
Antiphospholipid antibodies	Yes	Yes	No	Yes
Antiphospholipid syndrome	No	No	No	Yes
Disease activity and damage index				
SLEDAI-2K ≥ 4	Yes	No	Yes	Yes
SDI > 0	Yes	Yes	No	Yes
Ophthalmologic examination				
Visual Acuity RE;LE	20/20;20/25	20/20;20/20	20/20;20/20	20/20;20/20
Macular Thickness RE/LE	259/254	177/175	274/273	265/253
R2/R5 RE/LE	2.42/2.87	1.92/1.61	2.93/3.25	2.21/2.35
R3/R5 RE/LE	1.87/1.56	1.93/2.32	2.15/1.80	1.42/1.54

FAZ Circularity RE/LE	0.60/0.50	0.71/0.69	0.74/0.80	0.80/0.70
VD (mm/mm^2) RE/LE	16.4/15.7	11.6/8.9	18/18	19/18
VP (%) RE/LE	0.30/0.29	0.22/0.18	0.45/0.46	0.45/0.45
en face OCT altered	Yes	Yes	Yes	Yes
OCTA altered	Yes	Yes	Yes	Yes
VF altered	Yes	Yes	Yes	Yes
FAF altered	Yes	Yes	Yes	Yes

Abbreviations: FAF: fundus autofluorescence; FAZ: foveal avascular zone; LE: left eye; OCT: Optical coherence tomography; OCTA: Optical coherence tomography angiography; R2/R5: Ratio between R2 and fifth sector; R3/R5: Ratio between R3 and the fifth sector; RBW: real body weight; RE: right eye; SDI: Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity; VD: vascular density; VP: vascular perfusion; VF: visual fields.

Supplementary Table S2. *En face* optical coherence tomography (*en face* OCT) and *en face* optical coherence tomography angiography (*en face* OCTA) distribution of the entire series.

	***en face* OCT**		***en face* OCTA**	
	RE	**LE**	**RE**	**LE**
Normal, no. (%)	55 (83.3)	55 (83.3)	52 (80)	55 (84.6)
Altered, no. (%)	11 (16.7)	11 (16.7)	13 (20)	10 (15.4)

Abbreviations: LE: Left eye; RE: Right eye.

Supplementary Table S3. Distribution of changes in functional and structural ophthalmological tests by hydroxychloroquine treatment duration

	Total N 66	**HCQ <5 years N 34**	**HCQ ≥5 years N 32**	**P value**
OCT *en face* altered (N 66) no.(%)	16 (24.2)	8 (23.5)	8 (25)	0.889
OCTA altered (N 65) no.(%)	16 (24.6)	8 (24.2)	8 (25)	0.943
FAF altered (N 63) no.(%)	5 (7.9)	3 (9.7)	2 (6.3)	0.615
VF altered (N 66) no. (%)	36 (54.5)	16 (47.1)	20 (62.5)	0.208
mfERG altered (N 65) no.(%)	4 (6.2)	2 (6.1)	2 (6.3)	0.975

Abbreviations: FAF: fundus autofluorescence; HCQ: Hydroxychloroquine; mfERG: multifocal electroretinography; OCT: Optical coherence tomography; OCTA: Optical coherence tomography angiography; VF: visual field.

Supplementary Table S4. Spectral-domain (SD) optical coherence tomography (OCT) and OCT angiography (OCTA) analysis: Morphological parameters* and relationship with the duration of hydroxychloroquine treatment.

		Total N=66	HCQ <5 years n=34	HCQ ≥ 5 years n=32	P value
MT (μm)	RE	257 (247; 271)	261.5 (247; 274)	253 (241.5; 268)	0.166
	LE	254 (244; 268)	260.5 (249; 272)	253.5 (238 ; 266.5)	0.109
RNFLT (μm)	RE	94 (89; 103)	95 (89; 103)	93 (89.5; 103)	0.480
	LE	94 (88; 102)	94 (91; 101)	93.5 (86; 102)	0.411
CT (μm)	RE	280 (259; 311)	277.5 (257; 308)	286.5 (260; 317)	0.585
	LE	280 (252; 303)	280 (249; 310)	277.5 (257; 297)	0.640
FAZ Circularity	RE	0.68 (0.64; 0.72)	0.69 (0.64; 0.74)	0.66 (0.60; 0.72)	0.361
	LE	0.69 (0.65; 0.75)	0.69 (0.65; 0.74)	0.69 (0.64; 0.75)	0.667
VD (mm/mm^2)	RE	19.6 (17.9; 21.0)	19.8 (18.2; 21.4)	19.6 (17.6; 20.5)	0.230
	LE	19.8 (18.7; 21.2)	20.0 (19.1; 21.4)	19.7 (17.5; 21.2)	0.295
VP (%)	RE	0.36 (0.33; 0.39)	0.37 (0.34; 0.40)	0.36 (0.32; 0.38)	0.054
	LE	0.36 (0.35; 0.39)	0.37 (0.36; 0.39)	0.36 (0.33; 0.38)	0.029

Abbreviations: MT: Macular thickness; RNFLT: Retinal nerve fiber layer thickness; CT: Choroidal thickness; FAZ: Foveal avascular zone; VD: Vascular density VP: Vascular perfusion; HCQ: Hydroxychloroquine; LE: left eye; RE: right eye.

* All parameters are expressed as medians (IQR).

Supplementary Table S5. Quantitative data of multifocal electroretinography (mfERG) in both eyes: second-sector pseudo-response amplitude (R2); third-sector pseudo-response amplitude (R3); R2 to fifth sector ratio (R2/R5); and R3 to fifth sector ratio (R3/R5).

	RE				LE			
	R2/R5	**R3/R5**	**R2**	**R3**	**R2/R5**	**R3/R5**	**R2**	**R3**
Mean (SD)	3.37 (0.70)	2.05 (0.28)	58.30 (15.31)	36.21 (10.58)	3.32 (0.84)	2.03 (0.34)	53.28 (15.63)	32.89 (9.87)
Range (Min; Max)	(1.92; 5.28)	(1.54; 2.79)	(26.30; 102.00)	(14.60; 70.10)	(1.61; 6.44)	(1.52; 2.94)	(17.39; 95.90)	(18.90; 68.40)

Abbreviations: LE: Left eye; RE: Right eye.

Supplementary Table S6. Multifocal electroretinogram (mfERG) and *en face* optical coherence tomography (*en face* OCT) distribution in 130 eyes and mfERG and *en face* optical coherence tomography angiography (OCTA) distribution in 128 eyes of the study population.

		mfERG	
		Normal	Altered
en face OCT	Normal	107/130 (82.3%)	1/130 (0.8%)
	Altered	16/130 (12.3%)	6/130 (4.6%)
OCTA	Normal	104 /128 (81.2%)	1 /128 (0.8%)
	Altered	17 /128 (13.3%)	6 /128 (4.7%)

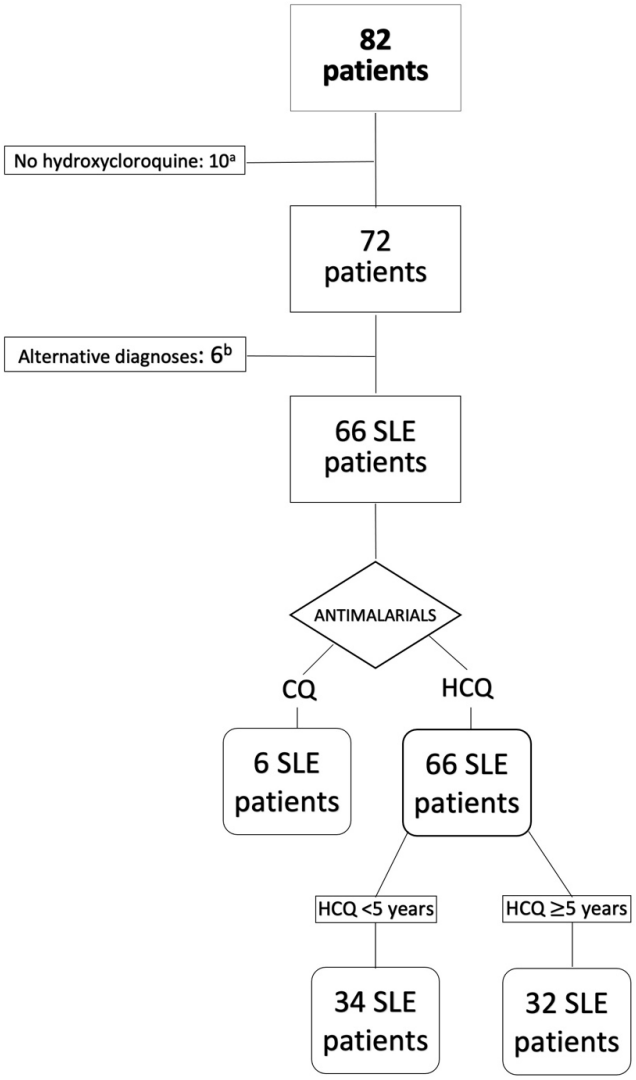
Supplementary Table S7. Concordance between different tests for detecting changes in *en face* OCT, OCTA, AF, VF and mfERG. The table shows the number and proportion of the valid results for each test combination.

	Agreement[a]	**Weighted Kappa**[b]	**95% CI**	**P value**[c]
mfERG - *en face* OCT	52/64 (81.2%)	0.333	0.080-0.587	<0.001
mfERG - en face OCTA	51/63 (80.9%)	0.332	0.079-0.585	<0.001
mfERG - VF	32/64 (50%)	0.099	0.003-0.194	0.069
mfERG - FAF	**60/61 (98.4%)**	**0.880**	0.649-1.111	<0.001
en face OCT - en face OCTA	**62/65 (95.4%)**	**0.873**	0.733-1.013	<0.001
en face OCT - VF	40/66 (60.6%)	0.247	0.061-0.433	0.014
en face OCT - FAF	53/63 (84.1%)	0.432	0.167-0.698	<0.001
en face OCTA - VF	41/65 (63.1%)	0.300	0.117-0.482	0.003
en face OCTA - FAF	52/63 (82.5%)	0.404	0.147-0.661	<0.001
VF - FAF	32/63 (50.8%)	0.121	0.016-0.227	0.044

Abbreviations: FAF: fundus autofluorescence; mfERG: multifocal electroretinography; OCT: optical coherence tomography; OCTA: optical coherence tomography angiography; VF: visual field.

[a] Agreement represents the number and proportion of valid results for each test combination.
[b] Weighted kappa index measures the agreement between two tests.
[c] P-value indicates statistical significance of the agreement.

Supplementary Figure S1. Flowchart of patient selection.



Abbreviations: CQ: chloroquine; HCQ: hydroxychloroquine; SLE: systemic lupus erythematosus.
[a] Reasons for withdrawal of hydroxychloroquine: previous macular toxicity (n=7); patients' choice (n=2); adverse effects (n=1).
[b] Alternative diagnoses of SLE: chronic cutaneous lupus (n=3); Sjögren's syndrome (n=1); mixed connective tissue disease (n=1); palindromic rheumatism (n=1).