The Diagnostic Utility of Multifocal Electroretinography in Detecting Chloroquine and Hydroxychloroquine Retinal Toxicity

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• PURPOSE: To evaluate multifocal electroretinography (mfERG) as a screening test for detecting hydroxychloroquine and chloroquine toxicity.
• DESIGN: Diagnostic accuracy study.
• METHODS: Patients referred to the University of Ottawa for hydroxychloroquine or chloroquine retinopathy screening during 2011–2014 underwent 10-2 automated visual field, spectral domain optical coherence tomography, and mfERG testing. Patients with amblyopia, high myopia or hyperopia, coexisting retinal disease, or prior surgery were excluded. Abnormalities in parafoveal ring amplitudes or ring ratios were considered a positive mfERG result. We used the definition for hydroxychloroquine and chloroquine toxicity provided by the 2016 American Academy of Ophthalmology recommendations. Area under the curve (AUC) for each mfERG parameter and the sensitivity and specificity of mfERG were calculated. Logistic regression was used to model the effect of covariates in receiver operating characteristic (ROC) analyses.
• RESULTS: In total, 63 patients (47 female, 16 male) were included. Of 120 eyes, 16 (13.3%) had toxicity according to the American Academy of Ophthalmology guidelines, and 39 (32.5%) had positive mfERG findings. mfERG was found to have a sensitivity of 1.00 (95% CI 0.79–1.00) and a specificity of 0.78 (95% CI 0.69–0.85). Ring 2 amplitude had the best performance among all parameters (AUC 0.97, 95% CI 0.94–1.00). Ring 2 amplitude decreased linearly with increasing cumulative dose and daily dose.
• CONCLUSIONS: The high sensitivity of parafoveal depression on mfERG and its relationship to cumulative and daily dose illustrates an important role for objective functional testing. The high false-positive rate suggests a potential period where physiologic dysfunction is detected objectively on mfERG before structural change on spectral domain optical coherence tomography. (Am J Ophthalmol 2019;206:132–139. © 2019 Elsevier Inc. All rights reserved.)

HYDROXYCHLOROQUINE IS A DISEASE-MODIFYING antirheumatic drug used for the treatment of rheumatologic and dermatologic diseases. This anti-malarial agent has become a mainstay of anti-inflammatory treatment, given its relative low cost and favorable safety profile compared with other disease-modifying antirheumatic drugs. Retinal toxicity remains a well-known side effect of the long-term use of hydroxychloroquine and its predecessor chloroquine. The prevalence of hydroxychloroquine retinopathy has been estimated at 1% after a cumulative dose of 1000 g, but it has been reported to occur at cumulative doses as low as 57 g.[1] Risk factors for the development of retinal toxicity include duration of use (>5 years); excessive daily dose by real body weight (RBW) or ideal body weight; concurrent tamoxifen use; certain cytochrome P450 gene polymorphisms; and pre-existing retinal, hepatic, and renal disease.[2] The current 2016 American Academy of Ophthalmology (AAO) recommendations suggest baseline examination with or without automated visual fields (AVFs) and spectral-domain optical coherence tomography (sdOCT) within the first year of initiating therapy, determining the patient's risk factors, and establishing fundus appearance and functional status.[2] In the absence of risk factors, the current guidelines suggest annual screening after 5 years of exposure with the proper AVF according to race, and sdOCT.[2]

Early detection of disease and cessation of hydroxychloroquine and chloroquine therapy are paramount, particularly before the occurrence of structural retinal pigment epithelium damage.[2,3] Cessation of the drug does not prevent the progression of retinopathy or reverse vision loss, but conservation of the retinal pigment epithelium is a positive prognostic factor for a milder form or limited progression of disease.[2,3] Early stage disease is poorly defined, and whether any signs of retinal toxicity are reversible before permanent structural change occurs remains poorly understood. Although sdOCT can definitively detect retinal toxicity, this test may not be as sensitive as AVF or multifocal electroretinography

AJO.com Supplemental Material available at AJO.com.

Accepted for publication Apr 23, 2019.

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0002-9394/$36.00
https://doi.org/10.1016/j.ajo.2019.04.025

(mfERG).[2,4–8] Determining the validity of these screening tests is critical to the development of evidence-based guidelines for the detection of retinal toxicity during the earliest stages when it may be reversible. To address this gap, we conducted a cross-sectional study of patients undergoing routine screening for hydroxychloroquine and chloroquine toxicity. Our goal was to evaluate the sensitivity and specificity of mfERG in comparison with the current AAO recommended screening tests for retinal toxicity.

METHODS

THIS STUDY WAS A DIAGNOSTIC ACCURACY STUDY OF ALL the patients referred for hydroxychloroquine and chloroquine toxicity screening to The University of Ottawa Eye Institute during 2011–2014. The data collected on 63 patients included sex, age, best-corrected visual acuity, refractive status, medications, duration of hydroxychloroquine or chloroquine therapy (in days), body weight, daily dose (in mg/kg), and history of systemic disease, including hepatic and renal impairment. Lean body weight (LBW) was calculated according to the Acute Respiratory Distress Syndrome Network guidelines.[9] Eyes with amblyopia, myopia, or hyperopia >8 diopters; coexisting retinal disease precluding appropriate evaluation of the retina; and history of retinal surgery were excluded. We obtained informed consent from all patients to collect their information. This cross-sectional study protocol was approved prospectively by The Ottawa Health Science Network Research Ethics Board.

Each patient underwent a detailed ophthalmic examination with fundus photographs (TRC-50DX; Topcon Medical Systems Inc, Paramus, New Jersey, USA). The exam and diagnosis were documented before ancillary testing. Each patient underwent 10-2 AVF (Humphrey Field Analyzer II; Carl Zeiss Meditec Inc, Dublin, California, USA), sdOCT (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany), and mfERG (Espion Profile Multifocal System; Diagnosys LLC, Lowell, Massachusetts, USA) within 1 month of the initial exam. We conducted 10-2 AVF using white SITA (Swedish Interactive Thresholding Algorithm) testing with pattern deviation plots. AVF results were graded independently by 2 assessors. A 10-2 AVF with >3 abnormal points ($p < 2\%$) anywhere in the pattern deviation plot was considered a positive AVF test result.[6] Patients with a full or partial ring scotoma or presence of 3 contiguous abnormal points ($p < 2\%$) on AVF 10-2 were classified as having definite retinal toxicity. sdOCT central fovea cross-sectional images were reviewed for abnormalities characteristic of toxicity. Disruption of photoreceptor outer segmental structural lines (ellipsoid zone line) and thinning of the photoreceptor layers in the foveal and parafoveal regions were classified as evidence of toxicity.[2] The enrolled Asian patients underwent additional 24-2 AVF testing, and there were additional considerations made for changes in their retinal periphery on sdOCT.[2] Toxicity was defined as the presence of a 10-2 AVF defect as assessed by either examiner and any characteristic finding of toxicity on sdOCT. For patients on chloroquine, adjusted cumulative dose was calculated by using a chloroquine-to-hydroxychloroquine ratio of 2.3 mg:5 mg, in accordance with the 2016 AAO guidelines.[2]

We performed mfERGs according to the International Society for Clinical Electrophysiology of Vision standards. Patients underwent correction of their refractive error before testing. With a liquid-crystal display monitor having a luminance of 1000 cd/m^2, we projected a stimulus containing 61 hexagonal elements onto the central 30-degree area surrounding the fovea in the light-adapted eyes of study subjects. Microconductive DTL thread electrodes (Diagnosys LLC, Lowell, Massachusetts, USA) were draped on the conjunctiva at the inferior limbus. ERG signals were extracted using the fast m-transform algorithm ($m = 14$) in eight 30-second epochs. The results were read by S.C., who was blinded to clinical examination, AVF, and sdOCT findings. Individual waveforms composed of trace arrays were assessed for abnormally reduced amplitude or prolonged implicit times, and ring-average analysis was assessed by using age-matched normative data established at our testing center. The lower limit of test reliability was set at 98%. Trace arrays, ring averages, and response density topographic maps were evaluated. Differences of 2 SDs or more were classified as abnormal. Ring ratios were computed as the ratio of rings 1–4:5.

Nonparametric methods were used to fit and compare receiver operating characteristic (ROC) and area under the curve (AUC) analyses. ROC analyses were used to investigate the diagnostic performance of each mfERG measure. Logistic regression was used to model the effect of covariates in the ROC analyses. AUC, sensitivity, and specificity were the accuracy parameters used for inference. Without a true gold standard test, a false-positive case might reflect toxicity that was not detected by AVF or sdOCT. To explore this hypothesis, subgroups divided according to daily dose per kilogram RBW and cumulative dose were compared with a group of age-matched controls who had no exposure to chloroquine or hydroxychloroquine (Table 1). Stata 15.1 software (StataCorp, College Station, Texas, USA) was used to perform the ROC analyses, compare groups by *t* test, and to compute correlation coefficients between continuous variables.

RESULTS

IN TOTAL, 120 EYES OF 63 PATIENTS (47 FEMALE AND 16 MALE) were included in the analysis (Table 1). Six individual eyes were excluded for amblyopia (1 eye), unilateral unreliable

TABLE 1. Demographics and Characteristics of the Subject and Control Population

Population	Characteristic	Value
Patients n = 63 (120 eyes)	Male, no. (%)	16 (25.4)
	Female, no. (%)	47 (74.6)
	No. patients (eyes) exposed to CQ	6 (12)
	Mean daily dose per RBW of CQ (range) (mg/kg)	6.17 (4.05–9.3)
	Mean cumulative dose of CQ (range) (g)	1380 (456–2114)
	No. patients (eyes) exposed to HCQ	57 (108)
	Mean daily dose per RBW of HCQ (range) (mg/kg)	7.5 (3.25–12.75)
	Mean cumulative dose (range) (g)	1199 (12–3212)
	Mean age (range) (y)	60.6 (34–80)
	Median duration of treatment (range) (y)	10.37 (0.16–30)
Controls n = 28 (56 eyes)	Male, no. (%)	8 (40%)
	Female, no. (%)	20 (60%)
	Mean age (range) (y)	56.3 (32–84)

CQ = chloroquine; HCQ = hydroxychloroquine; RBW = real body weight.

AVF test results (2 eyes), extensive choroidal neovascularization (1 eye), retinal surgery for epiretinal membrane (1 eye), and retinal detachment (1 eye). The mean age of participants was 60.6 ± 11.6 (1 SD) years (range 34–80 years), and their mean refractive error was –0.25 ± 2.5 (1 SD) diopters (range –8.0 to +8.0 diopters). Twelve eyes of 6 patients were exposed to chloroquine therapy, and 108 eyes of 57 patients were exposed to hydroxychloroquine. Twenty-six (41.2%) patients were taking hydroxychloroquine and chloroquine for rheumatoid arthritis, 18 (28.6%) were being treated for systemic lupus erythematous, and the remaining patients were being treated for polymyalgia rheumatica, mixed connective tissue disease, Sjögren syndrome, or other connective tissue disorders.

Sixteen eyes (13.3%) had signs of toxicity on both AVF and sdOCT, and 39 eyes (32.5%) met mfERG criteria for toxicity. There was 100% agreement between accessors when grading sdOCT and 86.7% agreement between accessors when grading AVF ($\kappa = 0.77$). All 16 eyes with an abnormal sdOCT were found to have an abnormal AVF by both graders. Seven patients had evidence of definite toxicity on 10-2 AVF. The patients that had both a positive AVF and sdOCT had an average daily dose of 6.5 (SD 0.2) mg/kg and average cumulative dose of 1989 (SD 1019) g compared with an average daily dose of 5.1 (SD 0.5) mg/kg and average cumulative dose of 1259 (SD 1109) g in patients without toxicity. The difference in both daily dose ($P = .009$) and cumulative dose ($P = .015$) reached statistical significance. The cumulative dose was highly correlated with treatment duration (r = 0.84) and moderately correlated with daily dose per kilogram (r = 0.47). The characteristics of the patients with toxicity diagnoses are outlined in Table 2. The minimum cumulative dose associated with retinal toxicity was 456 g chloroquine and 876 g of hydroxychloroquine. Four patients (7/16 eyes) were >65 years of age. Seven patients were taking an unsafe daily dose according to RBW (>5.0 mg/kg), and 6 patients were taking an unsafe daily dose according to LBW (>6.5 mg/kg).

Supplemental Table presents each of the diagnostic categories when mfERG is compared with AVF and sdOCT. Forty-nine eyes (40.8%) had an abnormal AVF and a normal sdOCT test result. All eyes with abnormal sdOCT findings (n = 16, 13.3%) had abnormal AVF findings. Compared with the 2016 AAO guidelines for toxicity, mfERG was found to have a sensitivity of 1.00 (95% CI 0.79–1.00) and a specificity of 0.78 (95% CI 0.69–0.85). Out of 104 eyes without toxicity detected by AVF or sdOCT, 23 were positive by mfERG. For all cases with positive reference tests, mfERG showed abnormalities. Table 3 presents the AUC values from the ROC analysis for each of the mfERG parameters. The ring ratio R2:R5 showed good diagnostic performance, with an AUC of 0.81 (95% CI 0.66–0.96). Ring 2 P1 amplitude had the best performance among all parameters (AUC 0.97, 95% CI 0.94–1.00) followed by Ring 3 P1 amplitude (AUC 0.86, 95% CI 0.77–0.96). mfERG latency and other mfERG ring ratios and P1 amplitudes were poor markers of toxicity (Table 3). When only cases of definite toxicity on AVF were used in the reference standard, AUC values decreased overall, but R2:R5 remained the best parameter among all ring ratios (AUC 0.78, 95% CI 0.58–0.97).

The Figure depicts the R2:R5 ring ratios and Ring 2 P1 amplitudes of each group relative to drug exposure. The Ring 2 P1 amplitude decreases markedly for any use (patients vs controls) and decreases linearly with increasing adjusted cumulative dose (<500 g, 500–1000 g, <2000 g) and increasing daily dose per kilogram

TABLE 2. Risk Factors and Test Results of Patients with a Positive sdOCT and 10-2 AVF

Patient	Disease	Sex	Age (y)	Fundus exam	AVF	mfERG	sdOCT	FAF	Weight (kg)	Height (cm)	BMI (kg/m^2)	LBW (kg)	Daily dose per LBW (mg/kg)	Daily dose per RBW (mg/kg)	Duration (y)	Cumulative dose (g)
Patient 1 (HCQ)	RA	F	62	+	+[a]	+	+	+	49.9	160	19.5	52.4	7.6	8	9	1314
Patient 2 (HCQ)	Sjögren	F	78	NA	+[a]	+	+	NA	74.8	161	28.9	53.3	7.5 (4 y) 3.75 (4 y)	5.3 (4 y) 2.7 (4 y)	8	876
Patient 3 (HCQ)	RA	F	61	NA	+	+	+	NA	53.5	170	18.5	61.5	3.3	3.7	12.5	912.5
Patient 4 (HCQ,1 eye)	SLE	F	65	NA	+[a]	+	+	NA	80.2	167	28.8	58.8	3.4	2.5	12	876
Patient 5 (CQ)	RA	F	46	+	+[a]	+	+	+	61.7	158	24.7	50.6	10.7	8.7	5	456 (CQ)
Patient 6 (CQ)	SLE	F	59	+	+[a]	+	+	+	59.4	158	23.8	50.6	10.7	9.1	20	1825 (CQ)
Patient 7 (HCQ)	SLE	F	73	+	+[a]	+	+	+	56.2	173	18.8	64.2	6.2	7.1	16.8	2448
Patient 8 (HCQ,1 eye)	SLE	F	74	NA	+	+	+	NA	56.7	163	21.3	55.1	7.3	7.1	20	2920
Patient 9 (HCQ)	RA	F	68	+	+[a]	+	+	+	54.5	169	19.1	60.6	6.6	7.3	20	2920

AVF = automated visual field; BMI = body mass index; CQ = chloroquine; FAF = fundus autofluorescence; HCQ = hydroxychloroquine; LBW = lean body weight; mfERG = multifocal electroretinogram; NA = not applicable; RA = rheumatoid arthritis; RBW = real body weight; sdOCT = spectral-domain optical coherence tomography; SLE = systemic lupus erythematous.

A "+" sign indicates an abnormal test result suggestive of probable toxicity.

[a]Finding of definite toxicity on AVF.

TABLE 3. Area Under the Curve and Associated 95% CIs of Different Multifocal Electroretinography Parameters

Ring	P1 amplitude (nV/2 degrees)	Latency (ms)	Ratio to Ring 5
1	0.75 (0.61–0.89)	0.54 (0.36–0.72)	0.52 (0.32–0.72)
2	0.97 (0.94–1.00)	0.78 (0.62–0.95)	0.81 (0.66–0.96)
3	0.86 (0.77–0.96)	0.76 (0.60–0.92)	0.72 (0.56–0.87)
4	0.70 (0.57–0.83)	0.67 (0.53–0.81)	0.58 (0.42–0.75)
5	0.65 (0.49–0.80)	0.69 (0.54–0.85)	Reference

RBW (quartiles). The R2:R5 ring ratio increased markedly between controls and the low cumulative dose (<500 g) and then showed a progressive decrease with increasing cumulative dose. A similar but less variable pattern in the R2:R5 ring ratio was observed in comparison with daily dose per kilogram RBW.

DISCUSSION

THIS IS THE FIRST DIAGNOSTIC ACCURACY STUDY EVALUating the mfERG against the 2016 AAO recommendations for chloroquine and hydroxychloroquine retinopathy screening. This model estimates the sensitivity and specificity of mfERG to be 100% and 78%, respectively. These results are greater than the previous estimates of 84.9% sensitivity and 63.38% specificity when mfERG was compared with AVF alone, but fall below estimates of 96.55% sensitivity and 91.30% specificity when compared with a combination of 2 of 3 tests (AVF, fundus autofluorescence, or sdOCT) in a previously published systematic review.[4] AVF is a subjective screening test that has a prominent role in clinical practice because of its availability, but previous studies have suggested that AVF may be less sensitive than mfERG and underestimate cases of true toxicity.[5,10] Two of the 9 patients with retinopathy did not have evidence of "definite" toxicity on AVF and were only found to have scattered abnormal points. This properly reflects the clinical norm, where a recommendation to stop hydroxychloroquine treatment would rarely be based on an inconclusive AVF result alone. These patients had clear evidence of toxicity on sdOCT and mfERG. Diagnosing hydroxychloroquine toxicity involves a balance between detecting disease before visual disability occurs and ensuring that an

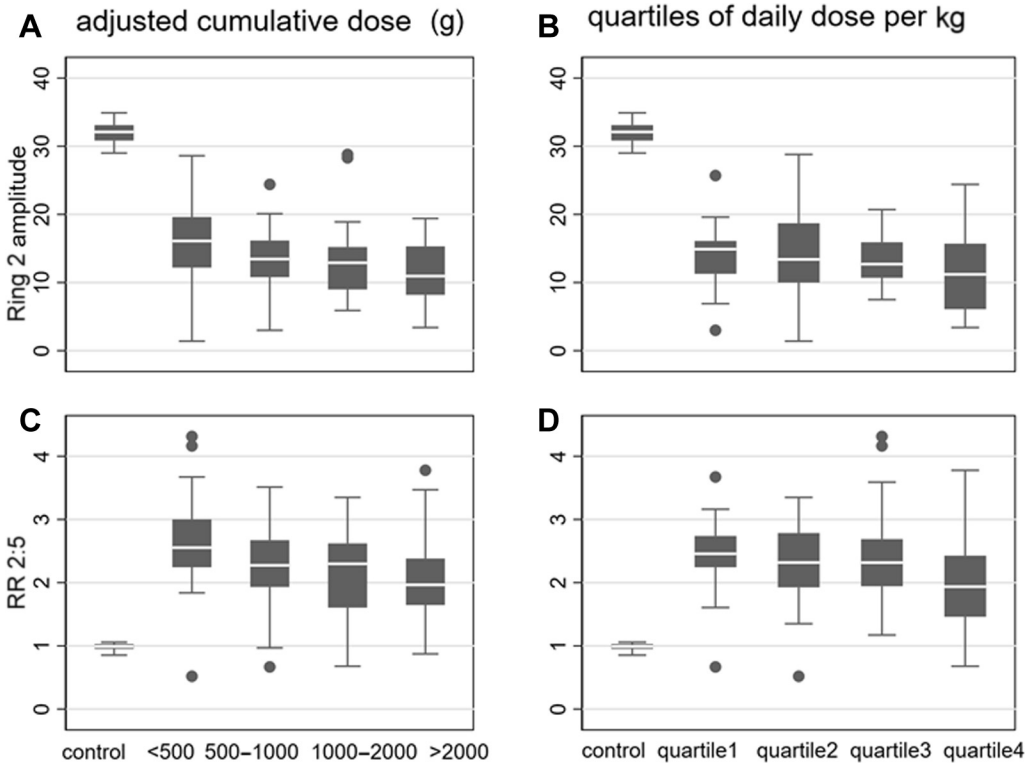

FIGURE. Ring 2 P1 amplitudes (nV/2 degrees) and R2:R5 ring ratios according to hydroxychloroquine dose groups. Controls (no exposure) were compared against subgroups of increasing adjusted cumulative dose (>500 g, 500–1000 g, <2000 g) and increasing daily dose per kilogram of real body weight (RBW) (quartiles). A. Ring 2 P1 amplitudes for subgroups of increasing adjusted cumulative dose. B. Ring 2 P1 amplitudes for subgroups of increasing daily dose per kilogram RBW. C. R2:R5 ring ratios for subgroups of increasing adjusted cumulative dose. D. R2:R5 ring ratios for subgroups of increasing daily dose per kilogram RBW. RR = ring ratio.

effective medication is not stopped prematurely. Guidance from the AAO advocates that AVF should be interpreted with a high index of suspicion and verified with an objective test, such as sdOCT or mfERG. Thirty-nine eyes in this study had abnormal mfERG results, but less than half of these would have received hydroxychloroquine retinopathy diagnoses when using 2016 AAO guidelines. The number of false-positives and poor specificity performance of mfERG against the 2016 AAO guidelines emphasizes the importance of distinguishing between hydroxychloroquine-induced acute electrophysiologic changes and clinically relevant toxicity.

Historically, the introduction of the age-corrected R1:R2 ring ratio analysis significantly reduced interindividual variations and increased confidence in the interpretation of mfERG for chloroquine and hydroxychloroquine retinal toxicity.[11] Further studies showed that normalization to Ring 5, which is made up of multiple hexagons as opposed to a single central hexagon (Ring 1), could increase the sensitivity of mfERG and reduce reliance on age correction.[12] The R2:R5 ring ratio was found to be a strong indicator of disease and showed an inverse relationship to cumulative dose and increasing quartiles of dose by RBW. The initial marked increase in R2:R5 between the control and low-exposure groups is the result of global mfERG depression with hydroxychloroquine exposure.[13] While nonspecific mfERG depression has been previously reported, analysis by R5-based ring ratios revealed the impairment is more severe in the characteristic parafoveal Ring 2 region.[13] Consistent with previous studies, Ring 2 P1 amplitude and Ring 3 P1 amplitude in this study were shown to be strong indicators of disease.[12] There was an inverse relationship between reduced Ring 2 P1 amplitudes and both cumulative dose and dose per kilogram of RBW. Reduction in both R2:R5 ring ratio and Ring 2 P1 amplitude shown in this study is consistent with the geographic predilection of hydroxychloroquine retinopathy to the parafoveal area.

Primary prevention, namely proper hydroxychloroquine dosing and attention to known risk factors to determine proper screening intervals, is paramount to reducing the prevalence of hydroxychloroquine retinopathy. Patients with a combination of age >65 years, daily dose greater than the recommended safe dose, and a high cumulative dose had the greatest chance of developing toxicity within this cohort. Despite frequent recommendations in the literature, prescription of unsafe daily doses is still a common practice. The bulk of current data indicates that in most cases of retinal toxicity doses of medication have exceeded 6.5 mg/kg of hydroxychloroquine and 3.0 mg/kg of chloroquine.[7,14,15] Our findings reinforce those presented in the 2016 AAO guidelines suggesting that doses beyond 5.0 mg/kg for hydroxychloroquine or 2.3 mg/kg for chloroquine can significantly increase the risk of toxicity.[2,8] The increased prevalence of hydroxychloroquine and chloroquine retinopathy (13.3%) in our study may be due to the high mean daily dose in our cohort. This finding is consistent with a previously estimated 10% risk of retinal toxicity within 10 years in patients with a daily dose exceeding 5.0 mg/kg RBW.[8] It has been suggested that dosing by RBW is simpler than performing LBW calculations, and rationale for these recommendations is based on a population study that showed that patients with a dose of hydroxychloroquine <5.0 mg/kg have <1% risk in the first 5 years of therapy.[2,8] Of the 9 patients with toxicity, 1 additional patient was found to have an unsafe dose under RBW guidelines (n = 7) than under LBW guidelines (n = 6). However, the calculated daily dose by LBW is often greater than the calculated dose by RBW in patients with a high body mass index, and thus may be more likely to raise clinical suspicion. The converse was true in patients with toxicity and low body mass index (<19 kg/m^2). Reliance on RBW calculations may be safe for most patients and necessary in the case of thin individuals, but this builds on the evidence suggesting that obese patients still require dosing adjusted to LBW.[16] Ultimately, physicians prescribing hydroxychloroquine and ophthalmologists play an important role in reducing a patient's risk, and new technology may further assist clinical decision-making with respect to determining a safe dose specific to each patient.[17]

The interpretation of these results is limited by the cross-sectional single-center design and the small sample size. Furthermore, 25.4% of the patients in this study were male, who are known to have lower incidences of hydroxychloroquine and chloroquine retinopathy. The low reported incidence of chloroquine and hydroxychloroquine toxicity can hinder prospective trials, and the low number of cases limits the power of statistical analyses. This study was carried out at a tertiary academic hospital, inducing a selection bias resulting in a higher prevalence of cases in our study population. Only 2 patients in this cohort were of Asian descent, and signs of peripheral scotoma or extra macular pathology were not found in these individuals with any of the testing modalities. None of the patients enrolled in this study had taken tamoxifen, and thus, the results of this study cannot be applied to this patient group.

The progressive and irreversible course of the disease warrants the development of universally accepted clinical criteria for risk reduction in patients on hydroxychloroquine therapy. Without a gold standard, the past 3 iterations of the AAO guidelines on hydroxychloroquine and chloroquine retinopathy published in 2002, 2011, and 2016 have successively touted the importance of various screening tests above others.[2,18,19] The 2002 guidelines called for screening with AVF, fundus exam, and Amsler grid and confirmation testing with mfERG.[18] The 2011 guidelines recognized that changes on fundus exam and Amsler grid represented advanced retinopathy and shifted the focus toward AVF verified by at least 1 objective screening modality, such as mfERG and sdOCT.[19] This vacillation in recommendations is the result of there being a scarcity of evidence showing that one screening test is

superior to another during the critical period when some minor but permanent visual loss might be present. Any single test is imperfect for detection of toxicity. The interpretation of AVF can be highly variable, and no specific definition for a hydroxychloroquine-related AVF defect is available.[2,6] Two of our patients with retinopathy demonstrated that even scattered changes on AVF should alert the clinician to conduct additional testing. When a less sensitive definition of abnormal AVF is used or when mfERG is evaluated against sdOCT or AVF alone, the diagnostic accuracy of mfERG was decreased in this study. These reference standards resulted in a lower true-positive and a higher false-positive rate for mfERG.

The 2016 revision shifts its focus primarily to subjective functional testing in AVF and objective structural testing in sdOCT.[2] Objective functional testing, such as mfERG, is now only recommended for use as an adjunct, in part due to its lack of availability.[2] Interpretation and execution of mfERG testing can also be variable between centers, limiting its accessibility and generalizability. This shift away from objective functional testing was based primarily on a single study that evaluated patients with only 10-2 AVF and sdOCT and did not consider mfERG.[8] 10-2 AVF verified by sdOCT are the 2 most common recommended tests used in clinical practice. When attempting to establish true preclinical hydroxychloroquine toxicity, this combination of tests would be expected to have high specificity given the confirmatory structural change that is present on sdOCT. In this context, mfERG is less likely to be negative in a patient with a positive reference test than in a patient with true early hydroxychloroquine toxicity. This introduces an imperfect gold standard bias, and the reference standard of 10-2 AVF verified by sdOCT may overstate the estimated sensitivity. In contrast, the high false-positive rate suggests that cases detected by mfERG may be subclinical cases of retinopathy at risk of progression to structurally detectable disease. The relationship between mfERG parameters and cumulative dose shown in this study was repeated in a previous meta-analysis and suggests that mfERG may be more sensitive than the combination of AVF and sdOCT.[4] The consistent validation of mfERG findings by sdOCT in the literature and the predilection for pathologic findings of hydroxychloroquine retinopathy to affect the pericentral area strongly suggest a role for mfERG in detecting hydroxychloroquine toxicity.[13,20–22] Our findings show that a reduction in Ring 2 P1 amplitude, Ring 3 P1 amplitude, and R2:R5 ring ratio may be a preclinical sign of hydroxychloroquine or chloroquine toxicity that is more sensitive than the reference test of sdOCT and AVF put forth in the 2016 AAO guidelines. Based on these observations, mfERG provides objective documentation of visual function and can play an important role in screening for hydroxychloroquine retinopathy. This study is a step toward defining the relationship and time course of physiologic and structural abnormalities detected by mfERG in this disease.

THE AUTHORS INDICATE NO FINANCIAL SUPPORT OR FINANCIAL CONFLICT OF INTEREST. ALL AUTHORS ATTEST THAT THEY meet the current ICMJE requirements to qualify as authors.

REFERENCES

1. Rüther K, Foerster J, Berndt S, Schroeter J. Chloroquine/hydroxychloroquine: variability of retinotoxic cumulative doses. *Ophthalmologe* 2007;104(10):875–879.
2. Marmor MF, Kellner U, Lai TYY, et al. Recommendations on screening for chloroquine and hydroxychloroquine retinopathy (2016 revision). *Ophthalmology* 2016;123(6):1386–1394.
3. Marmor MF, Hu J. Effect of disease stage on progression of hydroxychloroquine retinopathy. *JAMA Ophthalmol* 2014;132(9):1105–1112.
4. Tsang AC, Ahmadi Pirshahid S, Virgili G, et al. Hydroxychloroquine and chloroquine retinopathy: a systematic review evaluating the multifocal electroretinogram as a screening test. *Ophthalmology* 2015;122(6):1239–1251.
5. Chang WH, Katz BJ, Warner JE, et al. A novel method for screening the multifocal electroretinogram in patients using hydroxychloroquine. *Retina* 2008;28(10):1478–1486.
6. Marmor MF, Melles RB. Disparity between visual fields and optical coherence tomography in hydroxychloroquine retinopathy. *Ophthalmology* 2014;121(6):1257–1262.
7. Michaelides M, Stover NB, Francis PJ, Weleber RG. Retinal toxicity associated with hydroxychloroquine and chloroquine: risk factors, screening, and progression despite cessation of therapy. *Arch Ophthalmol* 2011;129(1):30–39.
8. Melles RB, Marmor MF. The risk of toxic retinopathy in patients on long-term hydroxychloroquine therapy. *JAMA Ophthalmol* 2014;132(12):1453–1460.
9. Acute Respiratory Distress Syndrome Network. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med* 2000;342(18):1301–1308.
10. Lyons JS, Severns ML. Using multifocal ERG ring ratios to detect and follow plaquenil retinal toxicity: a review. *Doc Ophthalmol* 2009;118(1):29–36.
11. Lyons JS, Severns ML. Detection of early hydroxychloroquine retinal toxicity enhanced by ring ratio analysis of multifocal electroretinography. *Am J Ophthalmol* 2007;143(5):801–809.
12. Adam MK, Covert DJ, Stepien KE, Han DP. Quantitative assessment of the 103-hexagon multifocal electroretinogram in detection of hydroxychloroquine retinal toxicity. *Br J Ophthalmol* 2012;96(5):723–729.

13. Lai TYY, Chan WM, Li H, et al. Multifocal electroretinographic changes in patients receiving hydroxychloroquine therapy. *Am J Ophthalmol* 2005;140(5):794–807.
14. Payne JF, Hubbard GB, Aaberg TM, Yan J. Clinical characteristics of hydroxychloroquine retinopathy. *Br J Ophthalmol* 2011;95(2):245–250.
15. Walvick MD, Walvick MP, Tongson E, Ngo CH. Hydroxychloroquine: lean body weight dosing. *Ophthalmology* 2011; 118(10):2100.
16. Browning DJ, Lee C. Somatotype, the risk of hydroxychloroquine retinopathy, and safe daily dosing guidelines. *Clin Ophthalmol* 2018;12:811–818.
17. Perlman EM, Greenberg PB, Browning D, et al. Solving the hydroxychloroquine dosing dilemma with a Smartphone app. *JAMA Ophthalmol* 2018;136(2):218.
18. Marmor MF, Carr RE, Easterbrook M, et al. Recommendations on screening for chloroquine and hydroxychloroquine retinopathy: a report by the American Academy of Ophthalmology. *Ophthalmology* 2002;109(7):1377–1382.
19. Marmor MF, Kellner U, Lai TYY, et al. Revised recommendations on screening for chloroquine and hydroxychloroquine retinopathy. *Ophthalmology* 2011;118(2):415–422.
20. Cukras C, Huynh N, Vitale S, et al. Subjective and objective screening tests for hydroxychloroquine toxicity. *Ophthalmology* 2015;122(2):356–366.
21. Kellner S, Weinitz S, Kellner U. Spectral domain optical coherence tomography detects early stages of chloroquine retinopathy similar to multifocal electroretinography, fundus autofluorescence and near-infrared autofluorescence. *Br J Ophthalmol* 2009;93(11):1444–1447.
22. Kellner U, Kellner S, Weinitz S. Chloroquine retinopathy: lipofuscin- and melanin-related fundus autofluorescence, optical coherence tomography and multifocal electroretinography. *Doc Ophthalmol* 2008;116(2):119–127.