Original Investigation

Analysis of Inner and Outer Retinal Thickness in Patients Using Hydroxychloroquine Prior to Development of Retinopathy

Luis de Sisternes, PhD; Julia Hu, BA; Daniel L. Rubin, MD, MS; Michael F. Marmor, MD

IMPORTANCE Retinopathy is a known risk of long-term use of hydroxychloroquine sulfate. However, whether the inner as well as outer retina are involved before retinopathy develops and whether changes in the retina might signal impending toxic effects during screening remain unknown.

OBJECTIVE To determine the degree of inner and outer retinal involvement in short- and long-term use of hydroxychloroquine before the development of retinopathy.

DESIGN, SETTING, AND PARTICIPANTS This retrospective medical record review of spectral-domain optical coherence tomography (SD-OCT) findings was performed at an academic medical center. Thirty-two patients without retinopathy and with high-quality SD-OCT images were studied. Twenty-seven patients were age matched (49-65 years old) for comparison of retinal layers among patients who used the drug less than 5 years (n = 12) or longer than 15 years (n = 15) at the initial visit. Populations were also defined (without age limitation) for comparison of change during 25 to 52 months of follow-up among patients with initial use of less than 5 years (n = 7) or longer than 15 years (n = 8). Data were collected from 2010 to 2015.

MAIN OUTCOMES AND MEASURES Measurements of inner and outer retinal thickness in SD-OCT images using commercial software and a Stanford pixel-by-pixel segmentation software that also provided topographic maps of thickness dimensions and change.

RESULTS Thirty-two patients (5 men and 27 women) were included in the analysis. Measurements of inner retinal thickness between short- and long-term hydroxychloroquine users (n = 27) in different retinal regions, and during a median 39 months of follow-up (n = 15), showed no statistically significant differences or change. Similarly, no significant differences or changes were identified in outer retinal thickness except for the final visit of 1 patient who developed focal parafoveal thinning, a toxic effect of hydroxychloroquine use. Cirrus ganglion cell analysis measurements were inaccurate in the presence of outer retinal damage.

CONCLUSIONS AND RELEVANCE The inner retina appears not to be involved in hydroxychloroquine-induced retinopathy to any clinically relevant degree within the limitations of our sample size. No clinically apparent warning of outer retinal damage was seen in the SD-OCT images of long-term hydroxychloroquine users until the actual appearance of focal retinopathy. Early detection of hydroxychloroquine-induced retinopathy is known to prevent visual acuity loss and serious progression after the therapy is stopped, and these data suggest that screening should seek distinct new areas of retinopathy (shown by topographic thickness maps) rather than long-term progressive thinning.

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Author Affiliations: Department of Radiology, Stanford University, Stanford, California (de Sisternes, Rubin); Byers Eye Institute at Stanford, Department of Ophthalmology, Stanford University School of Medicine, Palo Alto, California (Hu, Marmor); Department of Medicine (Biomedical Informatics), Stanford University, Stanford, California (Rubin).

Corresponding Author: Michael F. Marmor, MD, Byers Eye Institute at Stanford, Department of Ophthalmology, Stanford University School of Medicine, 2452 Watson Ct, Palo Alto, CA 94303 (marmor@stanford.edu).

The predominant clinical finding in human retinopathy caused by hydroxychloroquine sulfate use is thinning of outer retina and eventual damage to the retinal pigment epithelium.[1] However, long-term hydroxychloroquine exposure in primates causes generalized neuronal damage that also injures the inner retina.[2] This finding has led to concern that the inner retina might be affected by long-term exposure to hydroxychloroquine in humans, and subsequent spectral-domain optical coherence tomography (SD-OCT) studies have suggested that inner retinal changes could be a marker for early toxic effects.[3-5] However, the magnitude of change in these studies was very small, and retinal segmentation was performed with the Cirrus SD-OCT (Carl Zeiss Meditec, Inc) ganglion cell analysis (CGCA) that can be unreliable in the presence of outer retinal disease.[6-8]

We reported recently that inner retinal thickness was identical between mild and severe stages of hydroxychloroquine toxic effects, and it was stable after stopping the therapy.[6] That previous study used a Stanford pixel-by-pixel segmentation paradigm that adjusts for outer retinal disease. However, important questions remain about whether any damage to the inner or outer retina might begin during hydroxychloroquine use before retinopathy develops and, more critically, whether the outer retina shows any gradual changes that would be warning signs of impending retinopathy. This understanding would influence the practice of clinical screening. In the present report, we investigate SD-OCT findings in populations of short- and long-term hydroxychloroquine users without toxic effects and whether the inner or outer retinal thicknesses change during 25 to 52 months of follow-up.

Key Points

Question Does inner and/or outer retinal thickness change with increased exposure to hydroxychloroquine before retinopathy develops?

Findings No clinically relevant changes were identified retrospectively in inner or outer retinal thickness among 27 short- and long-term hydroxychloroquine users or among those followed up sequentially for more than 2 years, except for 1 patient who developed retinopathy.

Meaning Because the inner retina is not involved in retinopathy and outer retina does not show thinning with increased duration of use, screening should look for new signs of retinopathy rather than gradual changes over time.

Methods

The study was based on a retrospective review of medical records and SD-OCT images. All patients were under the care of one of us (M.F.M.) for evaluation or screening. Patients were included who underwent evaluation from 2010 to 2015 with good-quality SD-OCT imaging studies (ie, recordings not distorted by movement, artifacts, or low OCT signal, so that an instrument software was able to produce thickness measurements) and who showed no signs of retinal abnormalities at the beginning of the study. Patients were divided into those seen initially less than 5 years of starting hydroxychloroquine therapy (short-term use) and those seen initially after more than 15 years of exposure (long-term use). A mean value was calculated from data from both eyes for each patient. One scan from 1 eye was excluded owing to low OCT quality, in which case only the data from the other eye were considered. This study was approved by the institutional review board of Stanford University School of Medicine, who waived the need for informed consent.

All SD-OCT data were acquired using a Cirrus instrument (Carl Zeiss Meditec, Inc) in the form of scan volumes with a topographic dimension of 6 × 6 mm and scan depth of 2 mm. The scanning pattern had a different resolution in each direction so that the individual voxels were 12 µm horizontally, 47 µm vertically, and 2 µm axially, for a total of 512, 128, and 1024 voxels in the respective directions. The raw data were imported into the OCT proprietary software (Cirrus Research Browser, version 6.2.0.3; Carl Zeiss Meditec, Inc) and analyzed using Cirrus full-thickness and CGCA measurements (Cirrus analysis) and the previously developed pixel-by-pixel analysis tool[6] (Stanford analysis).

Cirrus Analysis Measurements

Full-thickness values were read from the Cirrus Early Treatment Diabetic Retinopathy Study (ETDRS) cube diagrams, and measurements of inner retinal ganglion cell layer thickness were read from the CGCA diagram. The full-thickness ETDRS cube values show the distance between the inner limiting membrane and the retinal pigment epithelium, with the mean measurements calculated within the following 8 nonfoveal topographic regions: parafovea and peripheral fovea in a superior, nasal, inferior, or temporal quadrant. The parafovea is defined as a ring 0.5 to 1.5 mm from the foveal center; the peripheral fovea, as a ring 1.5 to 3.0 mm from the foveal center.

The CGCA records the distance between the outer boundary of the retinal nerve fiber layer and the inner boundary of the inner nuclear layer. The mean values are calculated from 0.5 to 0.6 mm from the center to 2.0 to 2.4 mm from the center (long axis horizontally) within the superior, superonasal, inferonasal, inferior, inferotemporal, and superotemporal nonfoveal sectors. We reviewed the Cirrus segmentation cross-sectional image to confirm the segmentation lines for every case, and demarcation of the ganglion cell layers was correct, except for the final visit of 1 patient who developed signs of retinopathy (described in the Results section).

Stanford Analysis Measurements

The collected SD-OCT volumes were exported to files describing the reflectivity measured at each voxel location with 8-bit precision (limit imposed by Carl Zeiss Meditec, Inc, proprietary software). These data were processed with the Stanford analysis software, which we have developed and validated.[6] This software can accurately demarcate 10 intraretinal boundaries across the entire central macula, whether or not the outer retina has focal damage, and allows topographic and linear analysis of thickness and changes over time. This method has been verified previously,[6,9] but cross sections from every patient were also checked to confirm accurate layer separation.

Table 1. Demographics of Age-Matched Populations With Short- and Long-term Use of Hydroxychloroquine[a]

Patient No./ Sex/Age, y[b]	Duration of Use, y	Mean Daily Hydroxychloroquine Sulfate Dose, mg	Height, cm	Weight, kg	Daily Hydroxychloroquine Sulfate Dose by Weight, mg/kg[c]
Short-term use					
5/F/50	3	400	165.1	92.7	4.30
13/M/60	1.5	400	180.3	88.7	4.48
22/F/54	1	371	302.0	65.3	5.64
27/F/61	1	400	154.9	60.3	6.58
32/M/60	0	400	182.9	84.2	4.72
38/F/52	2	400	166.4	76.5	5.19
90/F/52	3	400	157.5	44.1	9.00
105/F/49	4	400	172.7	77.9	5.10
114/F/65	0.2	200	160.0	54.0	3.67
127/F/52	0.3	200	154.9	45.0	4.41
135/F/49	3	343	160.0	55.8	6.10
140/F/62	0	400	NA	55.4	7.17
Mean (SD)	1.6 (1.3)	359.5 (73.3)	165.9 (9.2)	66.6 (16.1)	5.5 (1.4)
Long-term use					
3/F/58	22	400	162.6	67.1	5.92
7/F/51	15	400	302.0	77.4	5.13
8/F/54	25	200	160.0	65.7	3.50
9/F/65	33	400	170.2	71.1	5.58
23/F/49	15	200	168.9	NA	N/A
29/F/56	19	400	165.1	101.3	3.92
51/F/57	20	400	154.9	81.5	4.87
52/F/50	15.5	200	175.3	NA	N/A
82/F/65	16	400	154.9	101.3	3.92
103/F/65	17	400	172.7	58.5	6.87
104/F/55	31	200	157.5	71.1	2.79
132/F/59	19	288	160.0	60.8	4.70
136/F/58	23	371	174.0	67.5	5.45
137/F/55	30	400	NA	80.1	4.95
139/M/57	18	294	NA	NA	NA
Mean (SD)	21.2 (5.8)	330.2 (86.2)	165.1 (7.0)	74.5 (14.2)	4.8 (1.1)

Abbreviation: NA, not available.

[a] Indicates patients aged 49 to 65 years without toxic effects at the initial examination. Short-term use indicates less than 5 years; long-term use, longer than 15 years.

[b] The mean (SD) ages were 55.5 (5.5) years for patients with short-term use and 56.9 (4.9) years for those with long-term use.

[c] Doses relative to real weight.

As in previous work,[6] thickness measurements were generated in a pixel-by-pixel basis between the inner limiting membrane and inner nuclear layer–outer plexiform layer junction for *the inner retina* and between the inner nuclear layer–outer plexiform layer junction and the outer border of the retinal pigment epithelium for *the outer retina*. We also generated topographic maps that include the parafovea and peripheral fovea, with data from the right and left eyes combined in proper nasal and temporal orientation.

When analyzing longitudinal data (multiple visits from the same patient), we also computed the rate of thickness change by determining the slope of a linear fit over time of the recorded thickness values at each pixel location. To reduce variability in these progression maps owing to noise in the scans and possible misalignments in the longitudinal images, the thickness maps were aligned with the center of the image corresponding to the center fovea pit location and passed through a median filter with a circular kernel of a 0.115-mm radius, in a manner similar to what we have reported previously.[6]

Results

Demographics

A total of 32 patients were studied (5 men and 27 women). All patients in this report were judged to be free of hydroxychloroquine toxic effects at their initial visit based on results of at least 2 screening tests, including 10-2 visual fields, SD-OCT, fundus autofluorescence, and multifocal electroretinography (mfERG). We stratified the patients into 2 groups with short-term (<5 years) and long-term (>15 years) hydroxychloroquine exposure at the initial visit.

Because retinal thickness may change with age,[10,11] we restricted the comparison of short- vs long-term users to those aged 49 to 65 years. **Table 1** shows the patient characteristics of these populations, including the daily hydroxychloroquine dosage in milligrams per kilogram of real weight (a more reliable predictor of risk than ideal weight).[12] The short- (n = 12; mean [SD] age, 55.5 [5.5] years) and long-term (n = 15; mean

Table 2. Demographics of Short- and Long-term Users of Hydroxychloroquine With Follow-up of 25 to 52 Months[a]

Patient No./ Sex/Age, y[b]	Duration of Use at Start, y	Follow-up, mo	Mean Hydroxychloroquine Sulfate Dose, mg/d	Height, cm	Weight, kg	Daily Hydroxychloroquine Sulfate Dose by Weight, mg/kg[c]
Short-term use						
22/F/54	1	29	371	170.2	65.3	5.64
88/M/66	1	32	400	170.2	90.0	4.41
95/F/15[d]	4	46	300	157.5	40.5	7.35
105/F/49	4	51	400	172.7	77.9	5.10
113/F/36[e]	4	52	400	22.8	65.3	6.08
127/F/52	0.25	39	200	154.9	45.0	4.41
135/F/49[f]	3	47	343	160.0	55.8	6.10
Mean (SD)	2.5 (1.7)	42.3 (8.5)	344.9 (68.5)	161.8 (8.8)	62.8 (16.2)	5.6 (1.0)[g]
Long-term use						
8/F/54	25	28	200	160.0	56.7	3.50
82/F/65	16	41	400	154.9	101.3	3.92
104/F/55	31	48	200	157.5	71.1	2.79
116/M/42	20	25	400	165.1	NA	N/A
121/F/44	21	37	400	167.6	76.5	5.19
132/F/59	19	39	288	160.0	60.8	4.70
136/F/58	23	50	371	174.0	67.5	5.45
137/F/55	30	34	400	NA	80.1	4.95
Mean (SD)	23.1 (4.9)	37.8 (8.2)	332.4 (84.3)	162.7 (6.1)	73.4 (13.7)	4.4 (0.9)[g]

Abbreviation: NA, not available.

[a] Indicates patients followed up for longer than 24 months without toxic effects at the initial examination. Short-term use indicates less than 5 years; long-term use, longer than 15 years.

[b] The mean (SD) ages were 45.9 (15.0) years for patients with short-term use and 54.0 (7.1) years for those with long-term use.

[c] Doses relative to real weight.

[d] High daily use was maintained because of medical instability.

[e] After the initial visit, the dose was reduced to 200 mg/d (3.04 mg/kg).

[f] After the initial visit, the dose was reduced to 200 mg/d (3.56 mg/kg).

[g] Indicates values for which the difference was statistically significant.

[SD] age, 56.9 [4.9] years) populations were similar in all clinical characteristics other than duration of exposure ($P > .19$, paired t test).

Table 2 shows the characteristics of short-term (n = 7) and long-term (n = 8) hydroxychloroquine users who were followed up for at least 24 (median, 39; maximum, 52) months after their initial visit. These groups were not age matched because progression of retinopathy is independent of age, and only partial overlap with the Table 1 data occurred. The only significant difference between these groups was in the mean initial daily hydroxychloroquine sulfate dosage per kilogram, which was 5.6 mg for the short-term users and 1.2 mg less for the long-term users ($P = .04$). The lower average dose in the long-term group came mostly from 3 individuals and may have contributed to their treatment longevity, but the long-term users were still at much greater risk to show toxic effect than patients in the short-term group.[12] The difference in doses was not statistically significant during the follow-up period because some high initial hydroxychloroquine doses were lowered (Table 2).

Population Data

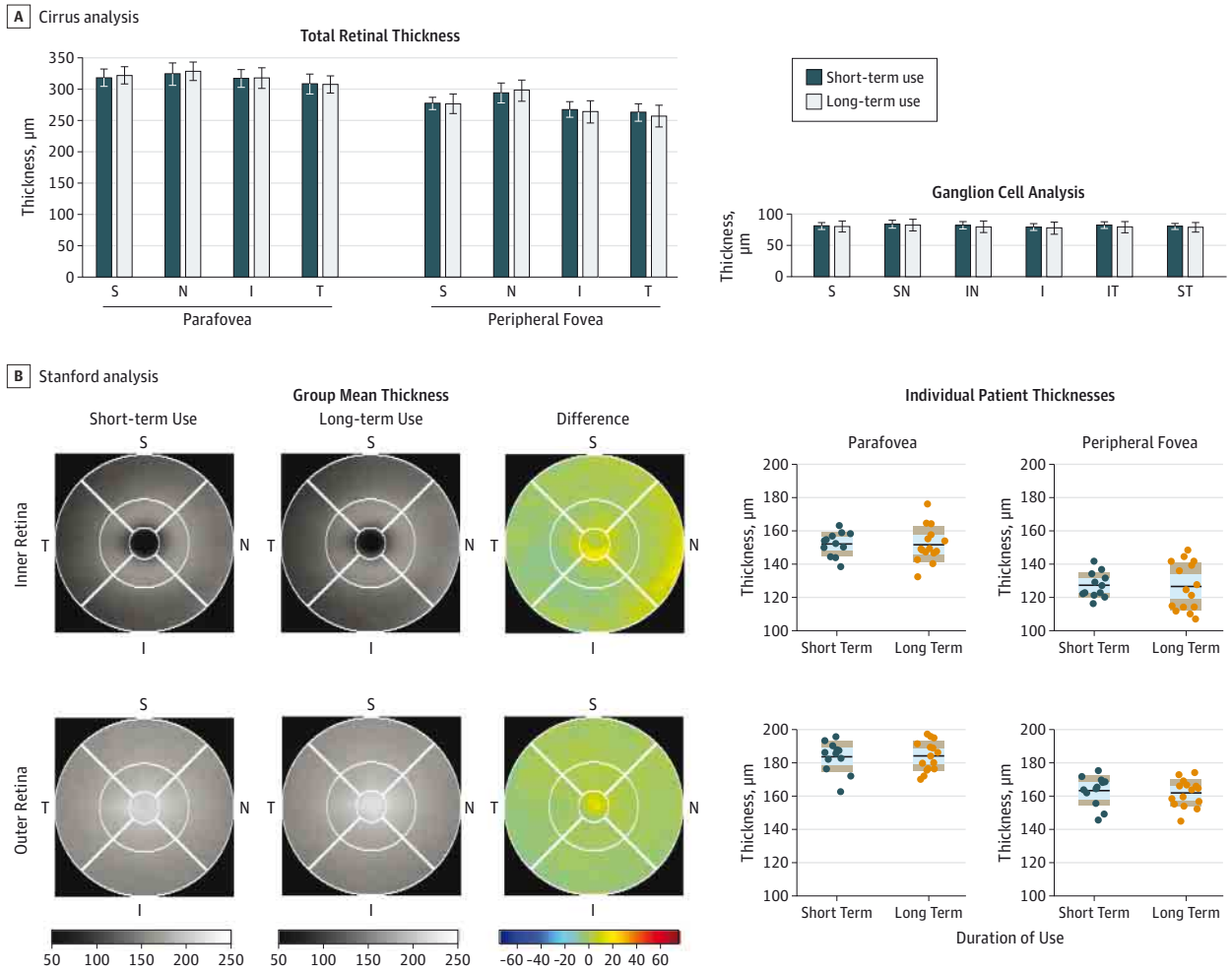
Figure 1 compares the inner and outer retinal thickness between the age-matched short- (<5 years) and long-term (>15 years) exposure groups. Measurements were obtained at the initial visit. Figure 1A shows the Cirrus analysis data for full retinal thickness in each parafoveal and peripheral sector as well as CGCA thickness in each sector. Mean differences between exposure groups were less than 5 µm (within scan reproducibility error) for full-thickness or CGCA sectors, and none of the differences were statistically significant ($P > .26$, paired t test). Thinning was not localized to the inferior and temporal parafoveal regions, which are typical sites of initial retinopathy.[1,6] The CGCA measurements were slightly lower in all sectors of the group with use longer than 15 years, but the SDs were also greater.

Figure 1B shows Stanford analysis with data based on pixel-by-pixel retinal segmentation of the inner and outer retina. The topographic plots show the mean inner and outer retinal thickness for the short- and long-term users in gray scale and differences between the short- and long-term groups in color maps. The difference plots indicated no major thickness differences between short- and long-term users in the inner or outer retina. The scattergrams depict similar mean inner and outer retinal thickness values for the individual patients between the short- and long-term users, with no major outliers.

Progression Data

Because any 2 populations may have slightly different mean thickness values, we also investigated whether inner or outer retinal thickness changed over time in short- or long-term users. **Figure 2**A shows the Cirrus analysis measurements. The

Figure 1. Comparison of Inner and Outer Retinal Thickness in Short- and Long-term Users of Hydroxychloroquine



A, Cirrus analysis of total retinal thickness and ganglion cell analysis in different regions of the retina. Error bars indicate SD. B, Stanford analysis of inner and outer retinal thickness as population means topographically and for individuals graphically. Short-term use indicates less than 5 years; long-term use, longer than 15 years. I indicates inferior; IN, inferonasal; IT, inferotemporal; N, nasal; S, superior; SN, superonasal; ST, superotemporal; T, temporal; dots, individual data; light blue–shaded boxes, SD; and warm gray–shaded boxes, 95% CI.

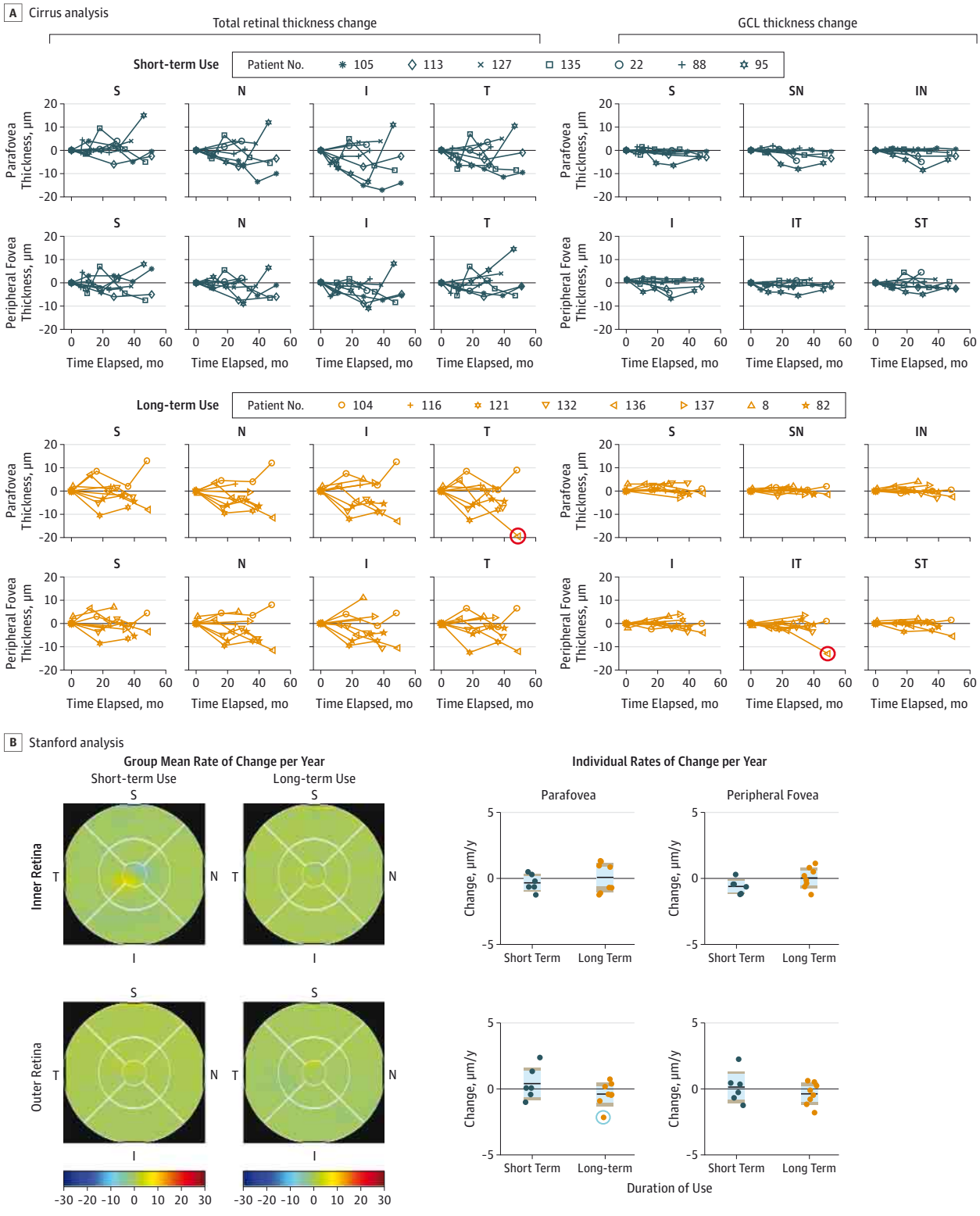
plots show the difference in thickness from baseline for every patient to give an overview of measurement variability, to show trends of change, and to identify outliers. The full-thickness ETDRS cube measurements (Figure 2A, left) show a fair amount of individual variability, but no consistent gain or loss in any region, for the short- or the long-term hydroxychloroquine users. One outlying temporal parafoveal point was found in the long-term exposure group at the final visit of patient 136, who showed evidence of retinopathy at this visit. The CGCA measurements (Figure 2A, right) showed extremely stable values over time in all regions, except for 1 outlying point at the final visit of patient 136. However, this value is actually an error resulting from incorrect retinal segmentation by the CGCA software (below).

Figure 2B shows the rates of change over time (micrometers per year) between baseline and the final visit for inner and outer retinal thickness using the Stanford analysis. The color topographic plots show no consistent change among short- or long-term hydroxychloroquine users. The small focal lesion in patient 136 was not large enough to alter the mean rate of change substantially from that of all patients combined. The scattergrams (Figure 2B, right) display the annual rates of thickness change in individual patients to show consistency and outliers. The rate of inner retinal thickness change was essentially minimal during the follow-up interval for short- and long-term users, including patient 136. Outer retinal thickness change was also minimal, save for 1 outlier among short-term users with increased thickness and 1 negative outlying point among long-term users for patient 136 (see below).

Findings in the Patient Who Developed Retinopathy

Patient 136 in our series developed signs of hydroxychloroquine retinopathy during the follow-up period, and her findings over time are shown in **Figure 3**A. This white woman in her late 50s at the initial visit had mixed connective tissue disease and used a daily dose of hydroxychloroquine sulfate,

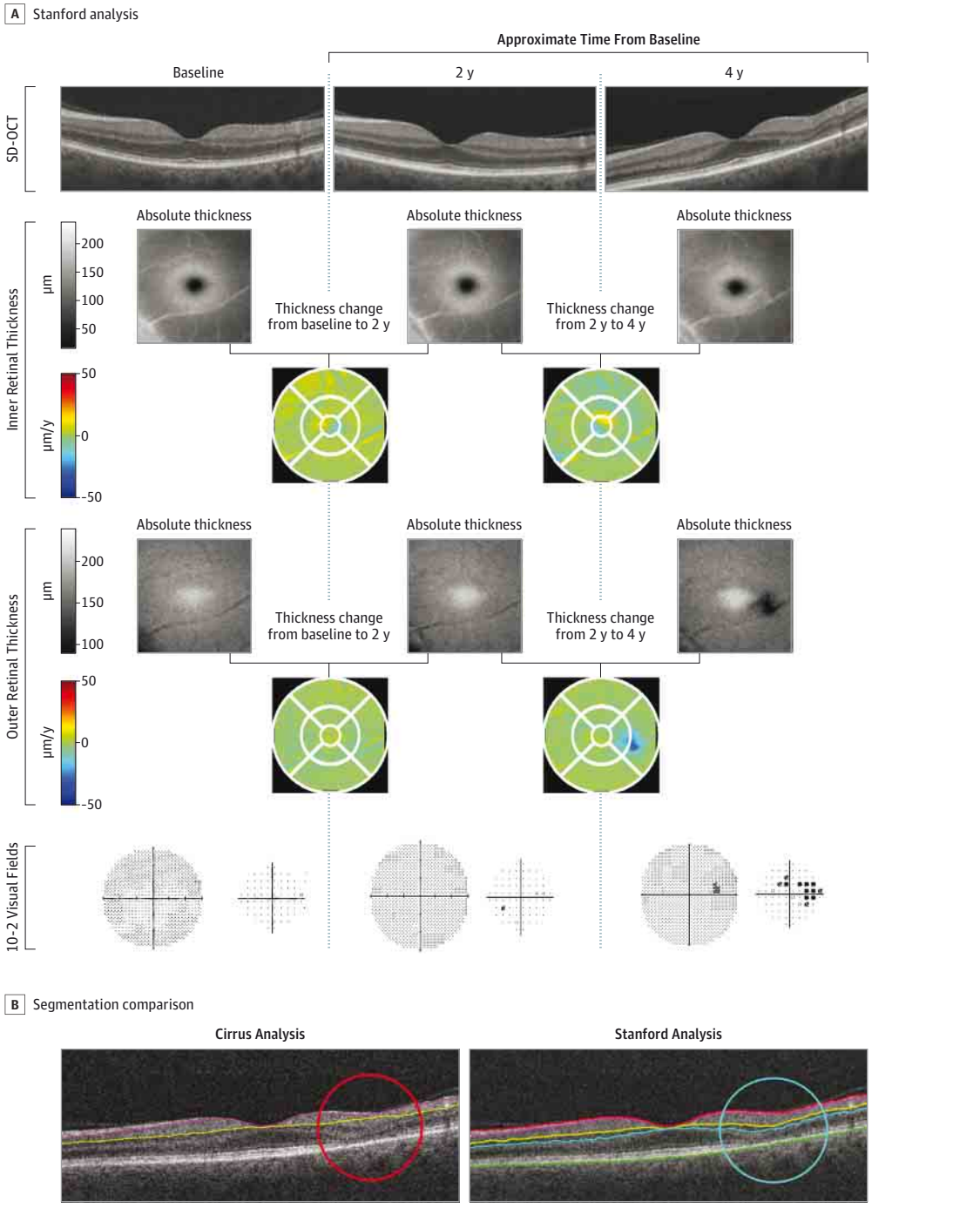
Figure 2. Comparison of Inner and Outer Retinal Thickness Changes Over Time in Short- and Long-term Users of Hydroxychloroquine



A, Cirrus analysis for total retinal thickness (left) and ganglion cell analysis (right) in each patient over time. Numbers in the keys identify the individual patients. The 2 outlying values (circles) are from patient 136; the total thickness value is valid, but the ganglion cell layer (GCL) thickness value is erroneous.

B, Stanford analysis data for rates of inner and outer retinal thickness change during follow-up, as group means topographically and for individuals graphically. The outlying point (circle) is patient 136, which shows that thinning only occurred in the outer retina. For abbreviations and symbols, see Figure 1.

Figure 3. Evolution of Findings in a Patient Who Developed Toxic Effects



A, Spectral-domain optical coherence tomography (SD-OCT) cross sections, gray scale topographic plots, color maps of change, and 10-2 visual fields (gray scale and pattern deviation plots) in the left eye of patient 136. These images were normal at baseline and at approximately 2 years. However, parafoveal damage was evident at the (approximate) 4-year visit. B, Cirrus segmentation error with outer retinal thinning (circle) is seen. Cirrus demarcation of inner retina (yellow line) fails to follow the retinal depression, whereas Stanford analysis demarcation is correct.

5.45 mg/kg, for 23 years. She had no renal disease and did not use tamoxifen citrate. Her SD-OCT scans and 10-2 fields were normal at baseline and 1 and 2 years later. The mfERG was normal at baseline and not repeated. At the examination 4 years after baseline (she missed the 3-year visit), she had no visual complaints but her tests showed evidence of early retinopathy (Figure 3). Her 10-2 fields revealed bilateral nasal parafoveal scotomas with some superior extension, and a new mfERG showed relative signal reduction in a ring 5° to 6° from the foveal center. The SD-OCT cross sections and

ETDRS cube thicknesses were still normal in the right eye but showed a distinct focal region of temporal thinning in the left eye (Figure 3) with loss of outer segment marker lines. Stanford analysis topographic maps of retinal thickness and thickness change showed no abnormalities or warning signs until the appearance at 4 years of focal outer retinal thinning.

Figure 3B compared Cirrus and Stanford OCT segmentation of the inner vs outer retina at this patient's final visit. The Cirrus program incorrectly localized the outer edge of the ganglion cell layer because the segmentation line failed to dip into the region of photoreceptor loss. This failure led to the false reporting of inner retinal thinning, which was shown in Figure 2A. The Stanford analysis followed the contour of outer retinal damage and showed no thinning in the inner retina.

Discussion

Our data show 2 major findings based on SD-OCT data. First, the inner retina was not affected by hydroxychloroquine exposure to any clinically relevant degree. Second, we could not identify any chronic or progressive thinning of the outer retina during hydroxychloroquine use before toxic effects. When retinopathy was identified in 1 patient, it appeared in areas with normal thickness 2 years earlier.

Inner retinal thickness was essentially identical between our short- and long-term users of hydroxychloroquine. The small decrease in users for longer than 15 years with Cirrus data could result from greater variability or represent a small population difference. We also could not identify inner retinal thinning during 25 to 52 months of follow-up of short- or long-term users, including the patient who developed retinopathy. Cirrus data from other studies of the inner retina[3-5,13] may have been influenced by errors in CGCA measurements when the outer retina was abnormal,[6-8] as shown in Figure 3B. One report[13] found major inner retinal loss in select cases with severe toxic effects, but the investigators also used Cirrus analysis. Our own prior data using the Stanford analysis in patients with severe toxic effects[6] showed no loss of inner retinal thickness. We checked the segmentation lines for every eye in this study, and the CGCA appeared to be highly reliable in eyes without outer retinal damage. However, CGCA should not be used for screening hydroxychloroquine retinopathy or evaluating other disorders that affect the outer retina.

None of our patients showed measurable outer retinal thinning (or any focal warning signs of toxic effects in the parafovea) while taking hydroxychloroquine before the appearance of toxic effects, despite the fact that the groups with long-term use had a mean duration of use that exceeded 20 years and an approximately 20% risk for toxic effects.[12] The findings in our patient who developed hydroxychloroquine retinopathy during follow-up are instructive. Her imaging studies showed no evidence that toxic effects were developing during the initial 2 years of follow-up, when her retinal thickness measurements and visual fields were normal. When she returned after a 2-year gap, she had partial ring scotomas in both eyes (evident in visual fields and mfERG), and the SD-OCT scan showed a focal zone of rather severe parafoveal thinning in 1 eye, presumably because some threshold of cellular decompensation was reached. The fields and mfERG showed more widespread and bilateral functional loss than was evident in the SD-OCT imaging. This disparity has been observed before,[14,15] and it reinforces our belief that annual screening for hydroxychloroquine toxic effects should ideally include visual fields (which may not be reliable for all patients) and SD-OCT.

One limitation of this report is the modest size of our short- and long-term user groups. Although population differences between these groups cannot be entirely ruled out, no differences in retinal thickness were found during follow-up (that would be independent of population norms). The follow-up periods had a median duration of only about 3 years, but few data are available for longer follow-up because SD-OCT is a relatively recent innovation. Finally, we have only 1 patient who developed toxic effects during follow-up, so conclusions about the pattern of initial toxic effects cannot be generalized. However, the critical finding is that no warning signs of toxic effects occurred in any of the cases. We calculated a mean value for data from both eyes, so a very small change in only 1 eye might have been obscured in this presentation, but this process avoided the issue that different eyes would not be independent variables. We were looking for consistent change rather than minimal unilateral changes. Our data show how topographic SD-OCT thickness maps can be an important tool for hydroxychloroquine retinopathy screening because focal damage might be missed by some cross-sectional scans. Focal damage can affect mean thickness in an ETDRS sector, but topographic assessment shows the location and the extent of the damage. Although our single case shows how focal thinning on SD-OCT can be quite dramatic, parafoveal changes in visual fields, mfERG, and fundus autofluorescence are often more diffuse[1,15,16] and may begin outside the parafovea in Asian patients.[17] This study was based on routine SD-OCT scans that do not extend beyond the vascular arcades (although none of our patients who had wider-angle testing with other modalities showed abnormality). We cannot say whether early toxic damage in Asian eyes, which is often outside the arcades, would behave in an identical manner.

Early detection of hydroxychloroquine retinopathy can prevent visual acuity loss and serious progression after the drug is stopped.[18] The implication of this study relative to hydroxychloroquine retinopathy screening with SD-OCT is that health care professionals need to be alert at each visit for the development of new functional or anatomic loss rather than seek measures of gradual or continual loss in the photoreceptor layers. Nonetheless, the development of confirmable signs of toxic effects seems to require 1 to 2 years, which supports recommendations for annual routine screening and retesting borderline abnormalities before stopping treatment with a useful medication.[12,19] Patient 136 was diagnosed as having retinopathy at a relatively early stage before any retinal pigment epithelium damage and thus is not likely to be at risk for foveal damage or a loss of visual acuity.[18]

Conclusions

Our data suggest that the inner retina is not involved in hydroxychloroquine retinopathy to any clinically relevant degree, within the limitations of our sample size. No clinically apparent warning of outer retinal damage in the SD-OCT scans of long-term hydroxychloroquine users was seen until the actual appearance of focal retinopathy. These data may guide ophthalmologists to look for distinct new areas of retinopathy rather than long-term progressive thinning, and they demonstrate the value of topographic thickness maps for this purpose.

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Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: de Sisternes, Rubin, Marmor.
Critical revision of the manuscript for important intellectual content: All authors.
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REFERENCES

1. Marmor MF. Comparison of screening procedures in hydroxychloroquine toxicity. *Arch Ophthalmol*. 2012;130(4):461-469.

2. Rosenthal AR, Kolb H, Bergsma D, Huxsoll D, Hopkins JL. Chloroquine retinopathy in the Rhesus monkey. *Invest Ophthalmol Vis Sci*. 1978;17(12):1158-1175.

3. Pasadhika S, Fishman GA. Effects of chronic exposure to hydroxychloroquine or chloroquine on inner retinal structures. *Eye (Lond)*. 2010;24(2):340-346.

4. Pasadhika S, Fishman GA, Choi D, Shahidi M. Selective thinning of the perifoveal inner retina as an early sign of hydroxychloroquine retinal toxicity. *Eye (Lond)*. 2010;24(5):756-762.

5. Ulviye Y, Betul T, Nur TH, Selda C. Spectral domain optical coherence tomography for early detection of retinal alterations in patients using hydroxychloroquine. *Indian J Ophthalmol*. 2013;61(4):168-171.

6. de Sisternes L, Hu J, Rubin DL, Marmor MF. Localization of damage in progressive hydroxychloroquine retinopathy on and off the drug: inner versus outer retina, parafovea versus peripheral fovea. *Invest Ophthalmol Vis Sci*. 2015;56(5):3415-3426.

7. Lee HJ, Kim MS, Jo YJ, Kim JY. Thickness of the macula, retinal nerve fiber layer, and ganglion cell layer in the epiretinal membrane: the Repeatability Study of Optical Coherence Tomography. *Invest Ophthalmol Vis Sci*. 2015;56(8):4554-4559.

8. Lee HJ, Kim MS, Jo YJ, Kim JY. Ganglion cell–inner plexiform layer thickness in retinal diseases: Repeatability Study of Spectral-Domain Optical Coherence Tomography. *Am J Ophthalmol*. 2015;160(2):283-289.e1.

9. de Sisternes L, Hu J, Rubin DL, Leng T. Visual prognosis of eyes recovering from macular hole surgery through automated quantitative analysis of spectral-domain optical coherence tomography (SD-OCT) scans. *Invest Ophthalmol Vis Sci*. 2015;56(8):4631-4643.

10. Song WK, Lee SC, Lee ES, Kim CY, Kim SS. Macular thickness variations with sex, age, and axial length in healthy subjects: a spectral domain-optical coherence tomography study. *Invest Ophthalmol Vis Sci*. 2010;51(8):3913-3918.

11. Liu T, Hu AY, Kaines A, Yu F, Schwartz SD, Hubschman JP. A pilot study of normative data for macular thickness and volume measurements using Cirrus high-definition optical coherence tomography. *Retina*. 2011;31(9):1944-1950.

12. Melles RB, Marmor MF. The risk of toxic retinopathy in patients on long-term hydroxychloroquine therapy. *JAMA Ophthalmol*. 2014;132(12):1453-1460.

13. Lee MG, Kim SJ, Ham DI, et al. Macular retinal ganglion cell-inner plexiform layer thickness in patients on hydroxychloroquine therapy. *Invest Ophthalmol Vis Sci*. 2015;56(1):396-402.

14. Greenstein VC, Amaro-Quireza L, Abraham ES, Ramachandran R, Tsang SH, Hood DC. A comparison of structural and functional changes in patients screened for hydroxychloroquine retinopathy. *Doc Ophthalmol*. 2015;130(1):13-23.

15. Marmor MF, Melles RB. Disparity between visual fields and optical coherence tomography in hydroxychloroquine retinopathy. *Ophthalmology*. 2014;121(6):1257-1262.

16. 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.

17. Melles RB, Marmor MF. Pericentral retinopathy and racial differences in hydroxychloroquine toxicity. *Ophthalmology*. 2015;122(1):110-116.

18. Marmor MF, Hu J. Effect of disease stage on progression of hydroxychloroquine retinopathy. *JAMA Ophthalmol*. 2014;132(9):1105-1112.

19. Marmor MF, Kellner U, Lai TY, Melles RB, Meiler WF. AAO statement: recommendations on screening for chloroquine and hydroxychloroquine retinopathy ophthalomology (2016 revision). *Ophthalmology*. In press.