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TITLE: Association of Antiretinal Antibodies with Hydroxychloroquine Toxicity in SLE

PRINCIPAL INVESTIGATOR: Maureen McMahon, MD

CONTRACTING ORGANIZATION: University of California Los Angeles

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Maureen McMahon email: mmcmahon@mednet.ucla.edu

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UNIVERSITY OF CALIFORNIA, LOS ANGELES
OFFICE OF RESEARCH ADMINISTRATION
10889 WILSHIRE BLVD STE 700
LOS ANGELES CA 90024-4201

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13. SUPPLEMENTARY NOTES

14. ABSTRACT Hydroxychloroquine (HCQ) is an important treatment for SLE patients because of its ability to reduce flares and prevent accumulation of damage. Recent studies, however, have suggested the risk of HCQ-related retinal toxicity may be higher than previously recognized. Unfortunately, there are currently no methods available to clinicians to identify patients at highest risk for HCQ toxicity. Autoantibodies (AAbs) against multiple retinal proteins have been associated with vision disturbance in both paraneoplastic and non-paraneoplastic autoimmune retinopathies (AR). Given the AAb-producing nature of SLE, it is reasonable to consider that AAbs against retinal antigens may also play a role in SLE retinopathy. In addition, AR and HCQ–related toxicity share many similarities on imaging, raising the possibility that some retinopathy attributed to HCQ could be autoimmune in nature. Our group has preliminary data indicating that 20/22 subjects with a diagnosis of HCQ-induced retinal toxicity had anti-retinal antibodies (91%), compared to 2/6 with normal retinal testing. 83% of these subjects had antibodies to 3 or more retinal antigens. Based on this preliminary data, **we hypothesize that anti-retinal antibodies may be a biomarker for retinal toxicity in SLE patients taking HCQ.** Before we can establish anti-retinal AAbs as a biomarker for SLE, we must more fully understand their typical prevalence in SLE patients. To evaluate our hypothesis, the Specific Aims of our proposal are to:
1. Determine the cross-sectional frequency of anti-retinal AAbs in a cohort of 285 SLE patients and 100 healthy age-matched controls, and to determine the relationship of antibodies with a) the length of exposure to HCQ and b) relationship with abnormalities on retinal screening tests, and. **2.** Prospectively examine the impact of HCQ on anti-retinal antibody formation and conditions leading to antibody formation by testing antibody formation before and after initiation of HCQ.
HCQ is a critically important medication for SLE patients because of its beneficial effects on disease activity and damage accumulation. Identification of novel biomarkers for risk of retinopathy may help us to identify at-risk patients who should consider HCQ dose reductions and more sensitive retina testing. In addition, if retinal AAbs are found to be pathogenic, our work may identify AAb-targeting treatment strategies that could be of use in patients with HCQ-related retinopathy.
In this report, we will describe the progress made on our proposal to date.

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NONE LISTED					
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.* Hydroxychloroquine (HCQ) is a mainstay of treatment for SLE patients because of the body of literature demonstrating that it confers both a reduction in disease flares and protection from damage accumulation (1-3). Recent studies, however, have suggested that the prevalence of HCQ toxicity may be higher than previously recognized (4). Thus, 2016 recommendations from the American Academy of Ophthalmology suggest maximum treatment doses of 5 mg/kg and limitation of cumulative lifetime HCQ dosages (5). Both of these recommendations are at odds with traditional rheumatologic prescribing practices. Unfortunately, there are currently no other methods available to clinicians to identify the patients at highest risk for HCQ toxicity.

   The mechanisms underlying HCQ-induced retinopathy are also unclear. One hypothesis is that the cationic drug binds to polyanionic melanin, which is found at high concentrations in retinal pigment epithelial cells. However, there is no consistently increased incidence in more heavily pigmented individuals (6). In addition, damage progression often continues even after drug cessation, yet there is no clear explanation for this phenomenon (7). Furthermore, although drug dosage is usually linked to HCQ-related retinopathy, patients can present after very brief exposure or low doses of medication (8).

   Autoantibodies (AAbs) against retinal proteins have been associated with vision disturbance in both paraneoplastic and non-paraneoplastic autoimmune retinopathies (AR)(9). Multiple antibodies against retinal antigens have been identified in non-rheumatic disease patients with AR, including AAbs against glycolytic enzymes such as enolase, aldolase, glyceraldehyde-3-phosphate dehydrogenase, and pyruvate kinase M2 (9). In patients with AR, AAbs persist over time in the circulation and can associate with either a stable or progressive course of vision loss (10). Given the autoantibody-producing nature of SLE and RA, it is reasonable to consider that AAbs against retinal antigens may also play a role in retinal disease in these patients. In addition, autoimmune retinopathy and HCQ–related toxicity share many similarities on imaging, including paracentral loss of ellipsoid on optical coherence tomography, paracentral visual field defects, and a circular ring of hyper-autofluourescence (11), raising the possibility that some retinopathy attributed to HCQ could be autoimmune in nature. We **hypothesized** that anti-retinal antibodies may be a biomarker for retinal toxicity in SLE patients who are taking HCQ.

2. **KEYWORDS:** ***SLE, hydroxychloroquine, anti-retinal antibodies, retinal toxicity***

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

The study Specific Aims are:

1. Determine the cross-sectional frequency of anti-retinal antibodies in a cohort of 285 patients with SLE and 100 healthy age-matched controls, and to determine the relationship of antibodies with a) the length of exposure to HCQ and b) relationship with abnormalities on retinal screening
2. Prospectively examine the impact of HCQ on anti-retinal antibody formation and conditions leading to antibody formation by testing:
   a. whether exposure to HCQ induces anti-retinal antibodies by testing a cohort of 45 SLE patients prior to any HCQ exposure, 3 months, 6 months, and 9 months after exposure, and
   b. Determine possible mechanisms underlying retinopathy in SLE patients taking HCQ by examining PBMCs from patients treated before and after HCQ vs. patients with retinopathy for IL10/IFNγ secretion

Table 1. Major Task 1: Perform Specific Aim 1	**Proposed months to completion**	**Actual percent complete**
Subtask 1: Prepare Regulatory Documents and Research Protocol for Study 1	1	100%
Coordinate with Sites for material transfer agreements (MTAs)	1	100%
Finalize consent form & human subjects protocol	1	100%
Coordinate with Sites for IRB protocol submission	1	100%
Milestone Achieved: Local IRB approval at UCLA	*1*	*100%*
Specific Aim 1: Determine the cross-sectional frequency of anti-retinal antibodies in a cohort of 285 patients with SLE and 100 healthy age-matched controls.		
Subtask 1: Prepare and ship Aim 1 plasma samples for shipment to OHSU	2-3	100%
Milestone Achieved: samples shipped to OHSU	3	*100%*
Subtask 1: Characterize SLE subjects for cross-sectional study with regards to total HCQ length of exposure and lifetime drug dosage, cova	2-4	
Review and confirm cases of reported HCQ retinopathy	1-4	100%
Database Management and cleaning	4-8	100%
Perform Anti-retinal Ab testing in longitudinal samples	4-10	100%
Work with statistical core at UCLA to perform analysis	10-11	100%
Review and discuss Aim 1 results	10-12	100%
Dissemination of findings: Prepare and submit abstract for national meeting	11-12	100%
Dissemination of findings: *Prepare manuscript for publication*	12	*Ongoing*
Milestone Achieved: Aim 1 complete	12	
Specific Aim 2: Prospectively examine the impact of HCQ on anti-retinal antibody formation and conditions leading to antibody formation by testing 45 SLE patients before and after HCQ		
Recruit and consent 25 SLE patients prior to starting HCQ	1-3	*80%*
Obtain, process, and store plasma samples from each patient at 0,3, 6, and 9 months	1-12	*80%*
Send samples to OHSU	9-12	*80%*
Perform anti-retinal AAb testing on samples from Aim 2	9-12	*80%*
Isolate PBMCs and T-cells from each patient at *at 0,3, 6, and 9 months*	1-12	*80%*
Perform cell stimulation studies/cytokine measurements	1-12	*80%*
Work with statistical core at UCLA to perform analysis	10-11	*0%*
Review and discuss Aim 1 results	10-12	*100%*
Dissemination of findings: Prepare and submit abstract for national meeting, prepare manuscript for publication	11-12	*80%*
Milestone Achieved: Aim 2 complete	12	

What was accomplished under these goals?

Participants in this study were collected from the longitudinal Biomarkers of Atherosclerosis in SLE cohort study conducted at the University of California, Los Angeles (UCLA) and Cedars Sinai Medical Center in Los Angeles from February 2004 to January 2019. We performed antiretinal antibody testing on plasma samples collected at baseline from SLE subjects and retrospectively reviewed charts of those patients for data extraction. Our primary outcome was frequency of specific antiretinal antibodies in SLE patients with HCQ retinal toxicity compared to SLE patients with no retinal toxicity. Patients included in the study met criteria for SLE based on the 1997 revised American College of Rheumatology (ACR) criteria for classification (9). All patients in this study also required a rheumatologist to have documented hydroxychloroquine administration in their medical record. Patients also were required to have documentation of testing for hydroxychloroquine retinal toxicity. This study was conducted with the approval of an institutional review board (IRB) and in accordance with Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

We tested a panel of antibodies to retinal antigens that were previously identified in non-SLE autoimmune retinopathy patients by our collaborator Dr. Adamus. These included antibodies to aldolase, enolase, arrestin, tubulin, pyruvate kinase M2 (PKM2), glyceraldehyde-3-phophate dehydrogenase (GAPDH), heat shock protein (HSP) and carbonic anhydrase II (CAII). Antiretinal antibody testing was conducted at Oregon Health Sciences University via ELISA.

Overall, 270 SLE patients in our cohort had documentation of previous hydroxychloroquine use and had documentation of eye exams. 28 SLE patients in this group had a diagnosis of HCQ retinal toxicity. Baseline characteristics of the cohort are presented in Table 1. Patients with retinal toxicity were more likely to be older and were more likely to have higher lifetime cumulative hydroxychloroquine doses.

Table 2. Baseline Characteristics of SLE subjects

Baseline Characteristic	SLE patients with eye toxicity (n=28) Mean ± SD or % (n)	SLE patients without eye toxicity (n=242) Mean ± SD or % (n)	p-value
Age	48.1 ± 15.7	42.6 ± 12.6	0.04
Gender	7.1% (2)	3.7% (9)	ns
BMI	27.0 ± 7.0	26.4 ± 6.2	ns

Disease Duration	**14.4 ± 11.7**	**12.2 ± 8.6**	**0.10**
Lifetime hydroxychloroquine dose (g)	**2082.4 ± 1629.7**	**1513.8 ± 1220.9**	**0.05**
Lifetime hydroxychloroquine dose (g) per year disease duration	**88.3 ± 46.8**	**79.5 ± 53.7**	**ns**
Lupus Severity Index	**5.9 ± 1.5**	**6.2 ± 1.8**	**ns**
Any Antiphospholipid Antibody	**39.3% (11)**	**41.3% (100)**	**ns**
Active Renal Disease	**3.5% (1)**	**3.7% (9)**	**ns**
Race/Ethnicity			
Caucasian	**60.7% (17)**	**46.7% (113)**	**ns**
African American	**14.3%(4)**	**12.8% (31)**	**ns**
Asian	**3.5% (1)**	**14.0% (34)**	**0.14**
Mixed/Other	**3.5% (1)**	**7.4% (18)**	**ns**
Hispanic	**17.9% (5)**	**19.0% (46)**	**ns**

Patients with retinal toxicity had a higher likelihood of testing positive for anti-arrestin antibodies (60.7% of patients vs. 30.7% of patients, p=0.001) and PKM2 antibodies (46.4% of patients vs. 28.2% of patients, p=0.047) compared to patients without a diagnosis of retinal toxicity (see table 1). Patients with a history of HCQ eye toxicity also had a trend towards a higher number of anti-retinal antibodies, with a mean of 3.0 ± 2.40 vs. 2.04 ± 1.7 (p=0.013) in the group with no history of toxicity.

Table 3. Preliminary results of anti-retinal antibody testing in SLE subjects

Antibody to retinal antigen	**Diagnosis of HCQ retinal toxicity n=28**	**No retinal toxicity n=241**	**P value**
Aldolase	39.3% (11)	33.1% (80)	ns
Enolase	57.1% (16)	41.3% (100)	0.11

Arrestin	**60.7 (17)**	**30.6% (74)**	**0.001**
Tubulin	17.9% (5)	16.1% (39)	ns
PKM2	**46.4% (13)**	**28.1% (68)**	**0.04**
GAPDH	21.4% (6)	14.9% (36)	ns
HSP	17.9% (5)	13.6% (33)	0.23
CAII	35.7% (10)	27.3% (66)	ns

Table 2: results of anti-retinal antibody testing in SLE subjects.

We next used logistic regression to determine whether the association between the presence of ant-arrestin antibodies and retinal toxicity, even after accounting for other potential confounding risk factors for hydroxychloroquine induced eye toxicity. Even after accounting for active renal disease, age, BMI, average HCQ dose per year of disease duration, and Lupus disease severity (using the Lupus Severity Index), we found that the presence of arrestin antibodies was associated with an odds ratio (OR) of 3.6 for developing HCQ toxicity (95% CI 1.4-9.1, p=0.007). Age was also a significant predictor (1.05, 95% C.I. 1.008-1.085, p=0.016). All other antibodies did not reach statistical significance for odds of developing HCQ eye toxicity.

We are continuing recruitment of our prospective cohort of SLE patients with new initiation of hydroxychloroquine (to determine whether exposure to HCQ induces anti-retinal antibodies). This recruitment has been slower than anticipated due to the continuing number of patients who participate in video visits. Studies of PBMCs and cytokine secretion from this prospective cohort of patients are also ongoing.

What opportunities for training and professional development has the project provided?

Two of our UCLA Rheumatology fellows, Dr. Jordan Jacquez and Dr. Samuel Good have been working on this project to help calculate lifetime hydroxychloroquine doses and to confirm cases of retinopathy. Dr. Good was the lead author and abstract presenter at the American College of Rheumatology meeting in San Diego, November 2023, and won a "Up and coming researcher" award for his work. Dr. Good will also be the lead author on the manuscript describing our findings (in preparation).

How were the results disseminated to communities of interest?

Abstract presentation at American College of Rheumatology Covergence 2023:

A manuscript detailing our findings is currently being prepared. We also presented our findings at the UCLA Department of Medicine Research Day in November 2023.

What do you plan to do during the next reporting period to accomplish the goals?

Nothing to report.

4.IMPACT: *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

- **What was the impact on the development of the principal discipline(s) of the project?**

  Our cross-sectional findings demonstrate that even when controlling for other risk factors associated with HCQ eye toxicity, the presence of anti-arrestin AAbs was associated with increased odds for the development of eye toxicity. Although the pathogenesis and clinical significance of these autoantibodies are not clear, the cross-sectional association suggests a potential role for antiretinal AAbs as a biomarker of HCQ eye toxicity risk. Plans are currently under way to develop prospective studies to further evaluate the risk of developing HCQ retinal toxicity in patients with known circulating antiretinal AAbs.

- **What was the impact on other disciplines?**
  - *Further studies will be required to determine whether anti-retinal antibodies can be used to identify patients at high risk for retinal toxicity and in need of close ophthalmology follow-up.*
- **What was the impact on technology transfer?**
  - *Nothing to Report..*
- **What was the impact on society beyond science and technology?**
  - *Nothing to Report.*
  -

5.CHANGES/PROBLEMS: *The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

- **Changes in approach and reasons for change**
  - *No changes to report*
- **Actual or anticipated problems or delays and actions or plans to resolve them**
  - We experienced some delays as noted previously due to pandemic-related research ramp-downs and the frequency of video visits. Due to these delays, manuscript submissions and analysis of some data in Aim 2 will occur after the close of the grant period.
- **Changes that had a significant impact on expenditures**

  Nothing to report

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  - *Nothing to report*

6. PRODUCTS:

Publications, conference papers, and presentations

Abstract #1062. Good S, Adamus G, Gorin M, Jacquez, J, Grossman J, Skaggs, B, and McMahon M. Antiretinal antibodies in hydroxychloroquine eye toxicity *Arthritis Rheumatol.* 2023; 75 (suppl 9). https://acrabstracts.org/abstract/antiretinal-autoantibodies-in-hydroxychloroquine-eye-toxicity/.

A manuscript is currently in preparation.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

a. **What individuals have worked on the project?**

Name:	*Maureen McMahon*
Project Role:	*PI; no change*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	Dr. McMahon was responsible for the overall oversight and design of the project. She coordinated between investigators at UCLA and OHSU and led monthly phone conferences to review progress. She also reviewed patient charts to confirm patient data. She also oversaw the analysis of the data and preparation of abstracts and manuscripts.
Funding Support:	

b.

Name:	*Brian Skaggs*
Project Role:	*Co-Investigator; no change*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*2*

Contribution to Project:	Dr. Skaggs performed most of the lab work for Aims 1 and 2, including preparation and shipment of stored samples, and collection and preparation of prospectively collected samples in Aim 2. He isolated the PBMCs and T-cells from patient samples, and will perform the experiments in Aim 2b. He has also been responsible for maintaining the study database. He also participated in study group meetings, data analysis, and abstract and manuscript preparation
Funding Support:	

Name:	*Jennifer Grossman*
Project Role:	*Co-Investigator; no change*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*.2*
Contribution to Project:	She helped to recruit SLE patients for Aim 2. She also participated in the clinical data collection in Aim 1. She participated in study group meetings, data analysis and abstract and manuscript preparation.
Funding Support:	

Name:	*Michael Gorin*
Project Role:	*Co-Investigator; no change*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*0.2*
Contribution to Project:	Dr. Gorin is an ophthalmologist and helped to review the cases of reported HCQ retinal toxicity and confirm the diagnosis. He also advised the team on issue related to the eye. He participated in study group meetings, data analysis and abstract and manuscript preparation.

Funding Support:	

Name:	*Grazyna Adamus*
Project Role:	*Co-Investigator; no change*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	Dr. Adamus is the head of the Occular Immunology Lab at OHSU and helped to develop the technology that was used to measure the retinal autoantibodies. She oversaw the measurement of anti-retinal antibodies in her lab. She also advised the team on interpretation of results, and participated in study group meetings, data analysis and abstract and manuscript preparation.
Funding Support:	

Name:	Sufang Yang
Project Role:	*Technician; no change*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Dr. Yang performed the autoantibody measurements at OHSU.
Funding Support:	

c. **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to Report

d. **What other organizations were involved as partners?**

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

Nothing to Report

AWARD NUMBER: W81XWH-20-1-0674:P00001

Award Title: Association of Anti-Retinal Antibodies with Hydroxychloroquine Toxicity in SLE

PI:Maureen McMahon, UCLA , CA **Budget: $200,000**

Topic Area: Lupus Research Program **Mechanism: Concept Award**

Research Area(s): 0505 **Award Status:** 9/01/2020-8/31/2022

Study Goals:

Hydroxychloroquine (HCQ) is a mainstay of treatment for Systemic Lupus Erythematosus because it both reduces disease flares and protects from damage accrual; however, there has been growing concern that retinal toxicity due to HCQ may be more common than previously realized. We hypothesize that anti-retinal antibodies may be a biomarker for retinal toxicity in SLE patients who are taking HCQ. The goal of these studies is to give us a more thorough insight into the potential role of anti-retinal antibodies as predictors of HCQ-related toxicity in SLE., and to explore whether measurement of these antibodies can provide a simple laboratory test to identify SLE and RA patients at greater risk of retinopathy.

Specific Aims:

1. Determine the cross-sectional frequency of anti-retinal antibodies in a cohort of 285 patients with SLE and 100 healthy age-matched controls, and to determine the relationship of antibodies with a) the length of exposure to HCQ and b) relationship with abnormalities on retinal screening
2. Prospectively examine the impact of HCQ on anti-retinal antibody formation and conditions leading to antibody formation by testing: a. whether exposure to HCQ induces anti-retinal antibodies by testing a cohort of 45 SLE patients prior to any HCQ exposure, 3 months, 6 months, and 9 months after exposure, and
b. Determine possible mechanisms underlying retinopathy in SLE patients taking HCQ by examining PBMCs from patients treated before and after HCQ vs. patients with retinopathy for IL10/IFNg secretion

Key Accomplishments and Outcomes:

Publications: none to date

Patents: none to date

Funding Obtained: none to date