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TITLE: Association of Antiretinal Antibodies with Hydroxychloroquine Toxicity in SLE

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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.

15. SUBJECT TERMS None listed.					
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1. INTRODUCTION:

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 **hypothesize** 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	50%
Milestone Achieved: samples shipped to OHSU	3	*50%*
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	Ongoing- 50%
Database Management and cleaning	4-8	Ongoing—10%
Perform Anti-retinal Ab testing in longitudinal samples	4-10	Ongoing
Work with statistical core at UCLA to perform analysis	10-11	0%
Review and discuss Aim 1 results	10-12	0%
Dissemination of findings: Prepare and submit abstract for national meeting	11-12	0%
Dissemination of findings: *Prepare manuscript for publication*	12	0%
Milestone Achieved: Aim 1 complete	12	*Ongoing*
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	*Ongoing-10%*
Obtain, process, and store plasma samples from each patient at 0,3, 6, and 9 months	1-12	*Ongoing-10%*
Send samples to OHSU	9-12	*0%*
Perform anti-retinal AAb testing on samples from Aim 2	9-12	*0%*
Isolate PBMCs and T-cells from each patient at *at 0,3, 6, and 9 months*	1-12	*Ongoing-10%*
Perform cell stimulation studies/cytokine measurements	1-12	*0%*
Work with statistical core at UCLA to perform analysis	10-11	*0%*
Review and discuss Aim 1 results	10-12	*0%*
Dissemination of findings: Prepare and submit abstract for national meeting, prepare manuscript for publication	11-12	*0%*
Milestone Achieved: Aim 2 complete	12	

What was accomplished under these goals?

Our research activities were unfortunately delayed over the past year by the pandemic, because we experienced university-wide ramp-downs in both clinical and lab research efforts. As a result, we did not obtain final Human Subjects approval for our study from the Department of Defense until July 13, 2021. During the past two months since study opening, we have prepared and shipped our first batch of 149 SLE samples to OHSU for retinal antibody testing. Results are pending. The baseline disease characteristics of these subjects are detailed in Table 2.

Table 2. Baseline Characteristics of SLE subjects

Baseline Characteristic of SLE subject (n=149)	**Mean ± SD or % (n)**
Age	**42.0 ± 13.0**
BMI	**26.1 ± 6.1**
Disease Duration	**13.0 ± 9.5**
Lifetime hydroxychloroquine dose (g)	**1619.14 ± 1372**
History of confirmed retinopathy	**8.7% (13)**
Race/Ethnicity	
Caucasian	**50.3 (75)**
African American	**11.4%(17)**
Asian	**13.4% (20)**
Mixed/Other	**5.4% (8)**
Hispanic	**19.4% (29)**

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

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report

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

Over the next reporting period we plan to prepare and send the remaining SLE and control samples to OHSU. We will continue to clean baseline and longitudinal cohort data, including confirmation of retinopathy, and calculation of lifetime hydroxychloroquine doses. We will then analyze the associations between anti-retinal antibodies and a) the length of exposure to HCQ and b) relationship with abnormalities on retinal screening. We will also continue to recruit our prospective cohort of SLE patients with new initiation of hydroxychloroquine, to determine whether exposure to HCQ induces anti-retinal antibodies. Finally, we will 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/IFN secretion.

4.IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**
  - *Nothing to Report.*
- **What was the impact on other disciplines?**
  - *Nothing to Report.*
- **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:

- **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 delays as noted above due to pandemic-related research ramp-downs. We are now fully operational, and do not anticipate any further delays.
- **Changes that had a significant impact on expenditures**

- Because of the delays due to the pandemic, we have requested and been approved for a NCTE; no changes in the overall expenditures are anticipated.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  - There are no applicable changes to any of the above. Human subjects approval was obtained on July 13, 2021

6. PRODUCTS:

a. **Publications, conference papers, and presentations**
Nothing to report.

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 will be responsible for the overall oversight and design of the project. She will coordinate between investigators at UCLA and OHSU and will lead monthly phone conferences to review progress. She will also review patient charts to confirm patient data for Aim 1, and will recruit patients for Aim 2. She will also oversee 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 will do 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 will isolate PBMCs and T-cells from patient samples, and will perform the experiments in Aim 2b. He will also be responsible for maintaining the study database. He will participate 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 will help to recruit SLE patients for Aim 2. She will also participate in the clinical data collection in Aim 1. She will participate 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 will help to review the cases of reported HCQ retinal toxicity and confirm the diagnosis. He will also advise the team on issue related to the eye. He will participate 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 will be used to measure the retinal autoantibodies. She will oversee the measurement of anti-retinal antibodies in her lab. She will also advise the team on interpretation of results, and will participate 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 will perform 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