Inflammatory Markers in Glaucoma and the Prevalence of Autoimmune Diseases in Primary Open Angle Glaucoma (POAG) Patients Undergoing Ophthalmic Surgeries

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Scholarly report submitted in partial fulfillment of the MD degree at Harvard Medical School

Date: 25 April 2020

Student Name: Maltish M. Lorenzo, BS, MS

Scholarly Report Title: Inflammatory Markers in Glaucoma and the Prevalence of Autoimmune Diseases in Primary Open Angle Glaucoma (POAG) Patients Undergoing Ophthalmic Surgeries

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Abstract

Title:
Inflammatory Markers in Glaucoma and the Prevalence of Autoimmune Diseases in Primary Open Angle Glaucoma (POAG) Patients Undergoing Ophthalmic Surgeries

Purpose:
To identify inflammatory markers in glaucoma patients undergoing ophthalmic surgeries prospectively. Concurrently, assess the prevalence of autoimmune diseases in patients with primary open angle glaucoma (POAG) undergoing ophthalmic surgery retrospectively.

Methods:
A prospective study is being conducted at the Massachusetts Eye and Ear (MEE). Patients with different subtypes of glaucoma were included if undergoing routine cornea, glaucoma, or cataract surgery. Control subjects were scheduled for cataract surgery and excluded if they carried the diagnosis of glaucoma, glaucoma suspect, or have a family history of glaucoma. Patients with any inflammatory or autoimmune disease were excluded from the prospective study but included in the retrospective analysis. Blood and aqueous humor samples were collected at the time of the surgery and sent to collaborators at the Schepens Eye Research Institute (SERI) of MEE for immune analysis.

A retrospective study was also performed using the same set of patient demographic and ophthalmic information from the prospective study. Patients with POAG and controls without glaucoma undergoing ophthalmic surgery at MEE were included. Similar to the prospective study, control subjects were scheduled for cataract surgery and excluded if they carried the diagnosis of glaucoma, glaucoma suspect, or have a family history of glaucoma. The presence of autoimmune diseases was determined based on all available medical record information. The difference in prevalence of autoimmune diseases between POAG and controls was assessed with chi-square test and these results were adjusted for covariates including age, body mass index (BMI), gender, ethnicity, and type 2 diabetes using multinomial logistic regression.
**Results:**

For the prospective study, patient recruitment and analysis of the peripheral blood and aqueous humor samples are still in process and ongoing. Results will be finalized once patient recruitment is completed.

For the retrospective study, 62 POAG patients and 97 controls were included. The overall prevalence of autoimmune diseases was 27% in the POAG group and 9% in the controls (p=0.003). In the fully adjusted multinomial logistic regression analysis, having an autoimmune disease was associated with 4.61-fold increased odds of POAG relative to controls (95% CI: 1.69-12.5, p=0.003), while age and non-white ethnicity also increased odds of POAG (odds ratio = 1.05, 1.54, respectively, p<0.05 for both).

**Conclusions:**

The retrospective study suggests a higher prevalence of autoimmune diseases in POAG patients compared to control patients undergoing surgery. The presence of an autoimmune disease significantly increased the risk for POAG after adjusting for covariates. This provides further support for the prospective study, which will explore the role of autoimmunity in the pathogenesis of POAG.
Glossary of Abbreviations

+AD..................................................................................with autoimmune disease
-AD..................................................................................without autoimmune disease
BCVA..............................................................................best corrected visual acuity
BMI..................................................................................body mass index
CPC.................................................................................cyclophotocoagulation
dB.........................................................................................decibels
HLA-B27+........................................................................human leukocyte antigen B27 positive
HVF MD...........................................................................Humphrey visual field mean deviation
IOP......................................................................................intraocular pressure
KPro...............................................................................keratoprosthesi
LogMAR...........................................................................logarithm of the minimum angle of resolution
MEE..................................................................................Massachusetts Eye and Ear
MIGS..............................................................................minimally invasive glaucoma surgery
mmHg................................................................................millimeters of mercury
NTG..................................................................................normal-tension glaucoma
OD.................................................................................right eye
OHT..................................................................................ocular hypertension
OS....................................................................................left eye
OU........................................................................................both eyes
PACG..............................................................................primary angle closure glaucoma
POAG..............................................................................primary open angle glaucoma
SERI..................................................................................Schepens eye research institute
TNF-α.............................................................................tumor necrosis factor alpha
RGC..............................................................................retinal ganglion cell
RNFL................................................................................retinal nerve fiber layer
Section 1: Introduction

Glaucoma is a disease that causes progressive degeneration of the optic nerve. Degeneration of the optic nerve results in “cupping”, a characteristic excavated appearance of the nerve disc, that leads to visual loss and ultimately irreversible blindness if left untreated. Laboratory and clinical studies have shown that progressive degeneration and apoptosis of retinal ganglion cells (RGCs) occur in glaucoma, resulting in permanent loss of peripheral or central vision. Glaucoma is expected to affect 79.6 million people worldwide in 2020, making it the leading cause of irreversible blindness in the world. Glaucoma is divided into two classes: open angle and closed angle. Primary open angle glaucoma (POAG) is the most common type of glaucoma in the United States, affecting approximately 3.36 million people in 2020. POAG diagnosis is based on open iridocorneal angles on gonioscopy examination, characteristic appearance of the optic nerve such as an increased cup to disc ratio and/or focal thinning of the neuroretinal rim, the absence of other possible causes of optic neuropathy, and evidence of peripheral vision loss anatomically corresponding to the optic nerve damage.

Risk factors for glaucoma depend on the glaucoma subtype; the risks for POAG include older age, positive family history, African American and Hispanic race, thin central corneal thickness, and possibly systemic diseases such as diabetes and hypertension. The most important risk factor, and the target of all treatments in POAG patients, is elevated intraocular pressure (IOP). Elevated IOP is thought to directly propagate damage to the RGCs. However, glaucoma progression and the corresponding visual field decline can still occur in patients whose IOP is well-controlled by use of IOP-lowering medications (Figure 1), suggesting mechanisms beyond IOP-mediated damage in glaucomatous neurodegeneration. In angle closure glaucomas, increased IOP is caused by impaired aqueous outflow secondary to obstruction of the anterior chamber drainage angle. Angle closure glaucoma can also be primary or secondary to an underlying etiology. In patients undergoing surgery for a type of artificial cornea implantation, known as a Boston keratoprosthesis (KPro), the prevalence of glaucoma ranges from 36 to 76%. De novo glaucoma has been reported to occur in 2-28% of patients after KPro implantation.
Despite extensive research conducted in the field of glaucoma, the exact pathogenesis is poorly understood. Certain patients develop optic nerve damage in the midst of having significantly elevated IOP (i.e. angle closure glaucoma), while other patients’ visual decline progresses despite IOP consistently in the normal range of less than 20 mmHg (i.e. POAG). Despite this heterogeneity, all current treatments used in glaucoma are focused on lowering and controlling IOP. Initial treatments include topical IOP-lowering medications. As optic nerve damage progresses, especially in the setting of persistently elevated IOP, laser procedures or surgeries to install drainage devices may be indicated. IOP alone cannot accurately predict glaucoma progression or development, therefore it is essential that other associated medical risk factors and biomarkers are identified. The inflammatory, and possibly autoimmune, characteristic of glaucoma has been postulated as a new possible treatment strategy. Some of the first evidence of possible autoimmunity in glaucoma were seen by Wax et.al. who observed elevated antibody titers against heat shock protein 60 (HSP60) in glaucoma patients. Other studies have helped further characterize the potential autoimmune nature of glaucoma. Inflammatory markers have been shown to be upregulated in patients with glaucoma, including POAG, providing further evidence of its inflammatory nature. In particular, tumor necrosis factor alpha (TNF-α), a cytokine involved in systemic inflammation, levels has been shown to be elevated in the aqueous humor of POAG patients. This is particularly of interest as TNF-α blockers are already in the market and being used to treat conditions such as rheumatoid arthritis, juvenile arthritis, psoriasis, ulcerative colitis, and Crohn's disease. Recent laboratory studies by Chen et.al. provided new insights about the pathogenesis of glaucoma. They demonstrated that glaucomatous progression in glaucoma animal models can be mediated by T cells specific to heat shock proteins, which are expressed by retinal ganglion cells after initial IOP-related injury. Recent studies revealed that chronic inflammation may also take place in patients with a KPro, whose glaucoma is the most severe and more likely related to angle closure glaucoma. Paschalis et. al. identified inflammatory factors associated with KPro patients. Finding new inflammatory markers has the potential to better characterize glaucoma based on its etiologies and change clinical management of glaucoma for specific subtypes. It can change our perspective from an IOP-focused treatment mindset to one that prioritizes neuroprotection.
While most published studies have focused on the laboratory analysis of inflammation in glaucoma, one prior study has been conducted to assess the relationship of inflammatory diseases with glaucoma in human subjects. The study by Cartwright et al. found that 30% of normal-tension glaucoma (NTG) patients had one or more immune-related diseases compared with 8% of patients in the ocular hypertension (OHT) comparison group. However, this study was not specific to autoimmune diseases, but rather immune-related diseases that included diagnosis such as allergic dermatitis, allergic rhinitis.

The purpose of the prospective study is to identify inflammatory markers in the peripheral blood and aqueous humor of glaucoma patients. In the future, we hope that the information gained from this study will lead to expanded treatment options and identify patients who might benefit from therapy targeting those immune pathways. We hypothesize that different phenotypes of glaucoma, such as POAG patients and KPro patients have distinct inflammatory profiles.

Using this same pool of patient information obtained during screening and enrollment of the prospective study, we aimed to conduct a retrospective study to investigate whether POAG is associated with autoimmune diseases. Finding an association between autoimmune diseases and POAG can provide clinical evidence to support the laboratory findings by Chen et al. which showed that POAG may have an autoimmune component in its pathogenesis.
FIGURE 1. Patient example: Humphrey visual field (HVF) of the left eye (OS) and retinal nerve fiber layer (RNFL) of both eyes (OU) from a 62 year old male with severe POAG and HLA-B27+ juvenile rheumatoid arthritis (JRA; in remission and off steroids/immunosuppressants for 37 years, no history of long-term steroid use). IOP OS range 6-42 mmHg with an average of 14.65 mmHg over 3 years (isolated IOP spike to 42 mmHg after cataract surgery). POAG progression despite average IOP below 20 mmHg while on multiple IOP-lowering medications.

Section 2: Student role

Lucy Q. Shen, MD, the principal investigator, led the study concept and design team. I worked closely with her to draft and submit the necessary IRB documents for both the prospective and retrospective studies. I also worked closely with her to design the logistics and protocols for the peripheral blood and aqueous humor collection, sample transfer and processing. I helped to design the protocols for patient screening and recruitment. I also helped train an international research fellow and an undergraduate student to perform patient screening/recruiting and samples transferring between the operating room and the laboratory.

I helped screen and recruit patients from the Massachusetts Eye and Ear (MEE) Glaucoma, Cornea and Comprehensive ophthalmology services. After getting approval from their primary surgeons, I called patients by telephone prior to their surgery appointment, informed them of the study, and asked if they would like to participate in the study. For patients who agreed verbally, either I or another research assistant obtained informed consent on the day of surgery. The phlebotomy team at MEE collected any peripheral blood samples. Aqueous humor sample was obtained by the surgeons at the start of the surgery. Participating surgeons included five glaucoma surgeons,
three cornea surgeons, and one cataract surgeon. Both the blood and aqueous samples were transferred to Dr. D. F. Chen’s laboratory for storage, processing and future analysis.

For the retrospective study, I was responsible for collecting the patients’ demographic and ophthalmologic data and conducting the analysis. I was responsible for making all of the figures and tables for a research abstract. Statistical analysis was accomplished with the help of Eleftherios Paschalis, PhD. I presented our data as a poster at a national meeting in March 2020.

Section 3: Methods

*Prospective Study Design*

1. Total number of subjects: 80
2. Location: MEE Main Campus and MEE Longwood Glaucoma, Cornea and Comprehensive ophthalmology services.
3. Groups:
   a. Group 1 consists of patients with Boston Keratoprosthesis with or without glaucoma (KPro, n=20)
   b. Group 2 consists of patients with primary angle closure glaucoma (PACG, n=20)
   c. Group 3 consists of patients with primary open angle glaucoma (POAG, n=20)
   d. Group 4 consists of healthy patients without glaucoma or KPro, who are undergoing cataract surgery (n=20)

**Inclusion Criteria for Groups 1-3:**

- Male or female 30 years of age or older
- Ability to perform informed consent
- Patients undergoing any routine intraocular surgery with a diagnosis of KPro with or without glaucoma (Group 1), PACG (Group 2), and POAG (Group 3)

**Inclusion Criteria for Group 4:**

- Male or female 30 years of age or older
• Ability to perform informed consent
• Patients undergoing routine cataract surgery

**Exclusion criteria for Groups 1-4:**

- Significant ocular disease affecting visual acuity and visual field (except for corneal disease in group 1 or glaucoma in groups 2 and 3)
- Patients with prior ocular surgery or emergency visit within 1 month of enrollment
- Patients with ophthalmic laser procedures within 1 month of enrollment
- Significant and unstable systemic disease or surgery within 1 month of enrollment
- Patients with autoimmune, inflammatory or immunodeficiency diseases
- Patients who are systemically treated with systemic immunosuppression at the time of the surgery
- Presence of nystagmus
- Smokers
- BMI exceeding 40 (as it may elevate inflammatory markers in the blood)
- Non-English speakers

**Additional exclusion criteria for Group 4:**

- Patients with family history of glaucoma, any evidence or suspicion of glaucoma, prior glaucoma diagnosis, or ocular hypertension
- Patients with corneal disease

4. All patients in the groups were scheduled to undergo routine intraocular surgeries. Anti-inflammatory eye drops were held prior to surgery. Blood draw was performed using standard protocol on the day of surgery by the phlebotomy team. The anterior chamber paracentesis in the surgery eye was performed by the surgeon, who was to use the same incision for the scheduled surgery. The removal of aqueous humor was followed by replacement with balanced salt solution (BSS) or viscoelastic (a gel-like material) at the
beginning of the surgery, both of which are considered standard protocol. This ensures that the risk of aspirating aqueous humor is minimized.

5. After surgery:
   a. Patients will undergo optical coherence topography imaging (Spectral Domain OCT, swept source OCT), visual field testing (if not performed within the last 12 months), and disc photographs (if not performed within the last 24 months) to document the state of glaucoma.
   b. Blood and aqueous humor samples were sent to Dr. Chen’s laboratory for storage and immune analysis. The results will be analyzed in the future.

Prospective Data analysis
For the prospective study, patients were stratified into 4 study groups (KPro, PACG, POAG, and cataract). In the future, we plan to use analysis of variance for group comparison and linear regression analysis to assess the effect of covariates on the primary comparison results. Power analysis based on current literature suggests that a minimum of 18 patients per group would give 80% power to detect statistically significant differences for our prospective study. We approximated these values based on existing glaucoma studies in literature. For example, Balaiya et. al. obtained an average TNF-α level in the aqueous humor of glaucoma samples of 2.72 ± 1.5 pg/mL, statistically higher compared to their control subjects which had an average TNF-α level of 1.59 ± 0.46 pg/mL. Since we will also be testing TNF-α in aqueous humor as part of our study, we can use Balaiya et. al.’s published literature values and approximate how many glaucoma patients we will need to appreciate a statistically significant result. This corresponds to 18 glaucoma patients per group (multiplied by three groups) and 18 control patients to give at least 80% power. In addition, the laboratory study by Dr. Chen also yielded similar power analysis. Thus, we will aim to recruit at least 60 total glaucoma patients and 20 control patients for this study.

Retrospective Study Design
1. All POAG and cataract patients undergoing surgery by participating surgeons at MEE from April to August 2019. This yielded a total of 62 POAG patients and 97 controls.

2. Presence of autoimmune diseases determined based on all available medical records.

**Inclusion/Exclusion Criteria**

- Male or female 30 years of age or older
- POAG and control patients undergoing ophthalmic surgery MEE
- **Controls patients:**
  - Undergoing cataract surgery
  - No glaucoma diagnosis
  - No family history of glaucoma
  - No glaucoma suspects
- **POAG patients**
  - Undergoing glaucoma surgery, cataract surgery, or both.
- Patients with uveitis were excluded

**Retrospective Data analysis**

The difference in prevalence of autoimmune diseases between POAG and controls was assessed with chi-square test and these results were adjusted for covariates including age, body mass index (BMI), gender, ethnicity, and type 2 diabetes using multinomial logistic regression.

**Section 4: Results from the Retrospective Study**

**Overall POAG vs Controls**

62 POAG patients (Humphrey visual field mean deviation (HVF MD): -11.06 ± 8.00 dB; available and reliable for 78% of patients) and 97 controls were included in the study. POAG group was older than the control group (74.56 ± 7.97 vs 70.92 ± 11.14 years, p=0.027, **Table 1**). The POAG group comprised of 38% male versus 45% in the control group (p=0.380). 42% of POAG patients had a positive family history of glaucoma. Both groups had similar BMI (overall 27.4 ± 4.5, p=0.773) and pre-operative IOP (overall 15.9 ± 4.5 mmHg, p=0.414). The cup to disc ratio was 0.76 ± 0.15 in POAG patients and 0.33 ± 0.13 in controls (p<0.0001). The presence of any history
of systemic steroid use, such as intravenous or oral, overall in POAG patients was 18% versus 14% in controls (p=0.413). The presence of any history of inhaled steroid use overall in POAG patients was 10% versus 20% in controls (p=0.168).

The overall prevalence of autoimmune diseases was 27% in the POAG group and 9% in the controls (p=0.003, Table 1). The most prevalent autoimmune diseases in POAG were polymyalgia rheumatica (4.84%), followed by five diseases at 3.23% including alopecia areata, multiple sclerosis, noninfectious anterior uveitis, optic neuritis, and rheumatoid arthritis. The most prevalent diseases in control patients were psoriasis (3.10%), giant cell arteritis (2.06%), and rheumatoid arthritis (2.06%) (Table 2). In the fully adjusted multinomial logistic regression analysis, having an autoimmune disease was associated with 4.61-fold increased odds of POAG relative to controls (95% confidence interval: 1.69-12.5, p=0.003, Table 3), while age and non-white ethnicity also increased odds of POAG (odds ratio = 1.05, 1.54, respectively, p<0.05 for both).

<table>
<thead>
<tr>
<th>Demographic and Ophthalmic Information</th>
<th>POAG (n = 62)</th>
<th>Controls (n = 97)</th>
<th>p-value</th>
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<tr>
<td>Age (years)</td>
<td>74.56 ± 7.97</td>
<td>70.92 ± 11.14</td>
<td>0.027</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td></td>
<td></td>
<td>0.38</td>
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<tr>
<td>Race (% Caucasian)</td>
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<td></td>
<td>0.003</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.38 ± 4.48</td>
<td>27.62 ± 5.48</td>
<td>0.773</td>
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<td>Type 2 Diabetes (%)</td>
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<td>0.096</td>
</tr>
<tr>
<td>BCVA (LogMAR)</td>
<td>0.36 ± 0.41</td>
<td>0.66 ± 0.87</td>
<td>0.012</td>
</tr>
<tr>
<td>HVF MD (decibels)</td>
<td>-11.06 ± 8.00</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>IOP (mmHg)</td>
<td>15.90 ± 4.50</td>
<td>15.42 ± 2.89</td>
<td>0.414</td>
</tr>
<tr>
<td>Cup to Disc Ratio</td>
<td>0.76 ± 0.15</td>
<td>0.33 ± 0.13</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Any history of systemic steroid use (%)</td>
<td>18%</td>
<td>14%</td>
<td>0.413</td>
</tr>
<tr>
<td>Any history of inhaled steroid use (%)</td>
<td>10%</td>
<td>20%</td>
<td>0.168</td>
</tr>
<tr>
<td>Autoimmune disease (%)</td>
<td>27%</td>
<td>9%</td>
<td>0.003</td>
</tr>
</tbody>
</table>

TABLE 1. Comparison between POAG patients and controls. Significant p-values are bolded. Abbreviations: BCVA, best corrected visual acuity; BMI, body mass index; HVF MD, Humphrey visual field mean deviation; IOP, intraocular pressure; LogMAR, logarithm of the minimum angle of resolution.
TABLE 2. Autoimmune diseases present in POAG and control patients undergoing ophthalmic surgery. Note % autoimmune diseases (n) adds up to higher % than the overall prevalence as some patients had multiple autoimmune diseases.

<table>
<thead>
<tr>
<th>POAG (n = 62)</th>
<th>n</th>
<th>% of 62</th>
<th>Controls (n = 97)</th>
<th>n</th>
<th>% of 97</th>
</tr>
</thead>
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<td>Polymyalgia rheumatica</td>
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<td>4.84</td>
<td>Psoriasis</td>
<td>3</td>
<td>3.10</td>
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<tr>
<td>Alopecia areata</td>
<td>2</td>
<td>3.23</td>
<td>Giant Cell Arteritis</td>
<td>2</td>
<td>2.06</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>2</td>
<td>3.23</td>
<td>Rheumatoid arthritis</td>
<td>2</td>
<td>2.06</td>
</tr>
<tr>
<td>Noninfectious anterior uveitis</td>
<td>2</td>
<td>3.23</td>
<td>Crohn’s disease</td>
<td>1</td>
<td>1.03</td>
</tr>
<tr>
<td>Optic neuritis</td>
<td>2</td>
<td>3.23</td>
<td>Hashimoto’s thyroiditis</td>
<td>1</td>
<td>1.03</td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>2</td>
<td>3.23</td>
<td>Polymyalgia rheumatica</td>
<td>1</td>
<td>1.03</td>
</tr>
<tr>
<td>Autoimmune thyroiditis</td>
<td>1</td>
<td>1.61</td>
<td>Sjogren’s syndrome</td>
<td>1</td>
<td>1.03</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>1</td>
<td>1.61</td>
<td>Systemic lupus erythematosus</td>
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<td>1.03</td>
</tr>
<tr>
<td>Giant Cell Arteritis</td>
<td>1</td>
<td>1.61</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grave’s disease</td>
<td>1</td>
<td>1.61</td>
<td></td>
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<td></td>
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<tr>
<td>Hashimoto’s thyroiditis</td>
<td>1</td>
<td>1.61</td>
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<td></td>
<td></td>
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<tr>
<td>Immune thrombocytopenia purpura</td>
<td>1</td>
<td>1.61</td>
<td></td>
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<tr>
<td>Juvenile Rheumatoid Arthritis</td>
<td>1</td>
<td>1.61</td>
<td></td>
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<tr>
<td>Psoriasis</td>
<td>1</td>
<td>1.61</td>
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</table>

TABLE 3. Multinomial logistic regression analysis with covariates in all subjects. Significant p-values are bolded. Abbreviations: BMI, body mass index; OR, odds ratio; POAG, primary open angle glaucoma.

<table>
<thead>
<tr>
<th>Dependent variable: POAG</th>
<th>OR</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
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<td>1.000</td>
<td>1.092</td>
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<td>BMI (kg/m2)</td>
<td>0.952</td>
<td>0.877</td>
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<td>Gender (female: ref)</td>
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<td>0.710</td>
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<td>Ethnicity (Caucasian: ref)</td>
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<td>Type 2 Diabetes (no/yes, no: ref)</td>
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<tr>
<td>Autoimmune Disease (no/yes, yes: ref)</td>
<td>4.61</td>
<td>1.69</td>
<td>12.50</td>
<td>0.003</td>
</tr>
</tbody>
</table>

TABLE 4. Subgroup Analysis Among POAG Patients

A total of 17 POAG patients had the presence of an autoimmune disease while 45 patients did not (Table 4). POAG with autoimmune diseases (+AD) had similar HVF MD in the operative eye compared to POAG without autoimmune disease (-AD) (-10.36 ± 7.86 vs -11.31 ± 8.15, p=0.681, available and reliable for 78% of POAG patients). POAG +AD and POAG -AD also had similar age (72.05 ± 6.35 vs 75.51 ± 8.38 years, p=0.129), BMI (overall 27.76 ± 4.92 kg/m², p=0.198), preoperative IOP (overall p=15.97 ± 4.31 mmHg, p=0.817), and up to disc ratio (overall 0.76 ± 0.14, p=1.000). POAG +AD group comprised of 35% male versus 49% in the POAG -AD group (p=0.337). 41% of POAG +AD patients had a positive family history of glaucoma versus 42% in the POAG -
AD group (p=0.941). The presence of any history of systemic steroid use (either intravenous or oral) in POAG +AD was 35% versus 13% in POAG -AD (p=0.051). The presence of any history of inhaled steroid use in POAG +AD was 12% versus 11% in POAG -AD (p=0.942).

<table>
<thead>
<tr>
<th>Demographic and Ophthalmic Information</th>
<th>POAG with autoimmune disease (n = 17)</th>
<th>POAG without autoimmune disease (n = 45)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>72.05 ± 6.35</td>
<td>75.51 ± 8.38</td>
<td>0.129</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>35%</td>
<td>49%</td>
<td>0.337</td>
</tr>
<tr>
<td>Race (% Caucasian)</td>
<td>76%</td>
<td>53%</td>
<td>0.098</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.59 ± 6.13</td>
<td>26.93 ± 3.70</td>
<td>0.198</td>
</tr>
<tr>
<td>Type 2 Diabetes (%)</td>
<td>24%</td>
<td>42%</td>
<td>0.174</td>
</tr>
<tr>
<td>BCVA (LogMAR)</td>
<td>0.27 ± 0.24</td>
<td>0.39 ± 0.45</td>
<td>0.302</td>
</tr>
<tr>
<td>HVF MD (decibels)</td>
<td>-10.36 ± 7.86</td>
<td>-11.31 ± 8.15</td>
<td>0.681</td>
</tr>
<tr>
<td>IOP (mmHg)</td>
<td>16.12 ± 3.84</td>
<td>15.82 ± 4.77</td>
<td>0.817</td>
</tr>
<tr>
<td>Cup to Disc Ratio</td>
<td>0.76 ± 0.11</td>
<td>0.76 ± 0.16</td>
<td>1.000</td>
</tr>
<tr>
<td>Any history of systemic steroid use (%)</td>
<td>35%</td>
<td>13%</td>
<td>0.051</td>
</tr>
<tr>
<td>Any history of inhaled steroid use (%)</td>
<td>12%</td>
<td>11%</td>
<td>0.942</td>
</tr>
</tbody>
</table>

TABLE 4. Comparison between POAG patients with versus without autoimmune diseases. **Abbreviations:** BCVA, best corrected visual acuity; BMI, body mass index; HVF MD, Humphrey visual field mean deviation; IOP, intraocular pressure; LogMAR, logarithm of the minimum angle of resolution.

Section 5: Discussion, Limitations, Conclusions, and Suggestions for Future Work

**Discussion**

Chen et. al. previously analyzed the role of the adaptive immune system in glaucoma.20 2-weeks after transient IOP elevation, CD4+ T-cell were found to infiltrate into the retina. The T-cell response also led to RCG degeneration, which persisted despite IOP returning to normal levels. Bacterial and human heat shock proteins (HSPs) were found to be the target antigens of these T-cells. Their results revealed a possible role of autoimmunity in glaucoma. Generally, autoimmune diseases are characterized mechanistically as either T-cell or autoantibody-mediated. In our study, diseases were deemed autoimmune if there was published evidence supporting the autoimmune mechanism. **Table 5** lists the autoimmune diseases observed in all of our patients and are classified by either T-cell or autoantibody-driven mechanisms.23-36 However, the interplay between the humoral, cell mediated, and innate immunity makes it difficult to characterize each disease as purely one entity. For example, immune thrombocytopenia has emerging evidence that it can be either T-cell or autoantibody. In our study, POAG and control groups had a mix of
autoimmune diseases which have T-cell or autoantibody-mediated mechanisms. While, the results of this study do not validate the work conducted by Chen et.al., it does, however, support the possibility that a T-cell mediated process may also be at play in human POAG subjects.

<table>
<thead>
<tr>
<th>POAG</th>
<th>Mechanism</th>
<th>Controls</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymyalgia rheumatica</td>
<td>T-cell</td>
<td>Psoriasis</td>
<td>T-cell</td>
</tr>
<tr>
<td>Alopecia areata</td>
<td>T-cell</td>
<td>Giant cell arteritis</td>
<td>T-cell</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>T-cell</td>
<td>Rheumatoid arthritis</td>
<td>T-cell</td>
</tr>
<tr>
<td>Noninfectious anterior uveitis</td>
<td>T-cell</td>
<td>Crohn's disease</td>
<td>T-cell</td>
</tr>
<tr>
<td>Optic neuritis</td>
<td>T-cell</td>
<td>Hashimoto's thyroiditis</td>
<td>T-cell</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>T-cell</td>
<td>Polymyalgia rheumatica</td>
<td>T-cell</td>
</tr>
<tr>
<td>Autoimmune thyroiditis</td>
<td>T-cell</td>
<td>Sjogren's syndrome</td>
<td>Autoantibody</td>
</tr>
<tr>
<td>Crohn's disease</td>
<td>T-cell</td>
<td>Systemic lupus erythematosus</td>
<td>Autoantibody</td>
</tr>
<tr>
<td>Giant cell arteritis</td>
<td>T-cell</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grave's disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hashimoto's thyroiditis</td>
<td>T-cell</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immune thrombocytopenia</td>
<td>Autoantibody</td>
<td></td>
<td></td>
</tr>
<tr>
<td>purpura</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juvenile rheumatoid arthritis</td>
<td>T-cell</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psoriasis</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Autoimmune diseases in all patients classified as by mechanisms.

Cartwright et. al. previously analyzed the relationship between inflammatory diseases and normal-tension glaucoma (NTG) in human subjects. NTG is a common form of POAG. It is defined also by the presence of open iridocorneal angles on clinical exam, but absence of any history of IOP elevation above 21 mmHg. Clinically, NTG is also similar to POAG: NTG results in chronic, progressive optic nerve degeneration that results in “cupping”, retinal nerve fiber layer (RFNL) thinning and visual field loss. Despite the lack of an observed IOP elevation, the current medical and surgical treatment of NTG continues to be aimed at lowering IOP, just as is done in other forms of POAG. Similar to our study design, the Cartwright et. al. study conducted a retrospective chart review. They include 67 patients with a diagnosis of NTG from the Bascom Palmer Eye Institute. NTG patients were matched with respect to age, race, and sex to a control patient with ocular hypertension (OHT; IOP above 21 mmHg but no signs of glaucomatous optic disc or visual field changes). This resulted in 22 matched pairs with at least one patient in the pair having an immune-related disease and 45 matched pairs without any immune-related diseases. They found that 20 of the 67 patients (30%) with NTG had one or more immune-related diseases.
compared to 5 of the 67 patients (8%) in the OHT comparison group (p=0.00134). In our study, we aimed to assess the prevalence of autoimmune diseases in POAG patients undergoing ophthalmic surgery. We found that the overall prevalence of autoimmune diseases is higher in POAG patients compared to cataract controls: 27% vs 9% (p=0.003).

While the overall prevalence was similar to the Cartwright study, we measured prevalence without the use of matching. In their study, 137 total patients were included: 67 NTG patients matched to 67 OHT patients. Our study included 159 total patients: 62 POAG and 97 cataract controls. We included only surgical patients, while they analyzed all patients in their database; this inherently limits our generalizability. There were several additional differences between the Cartwright study and ours. Their study used patients with OHT as the comparison group. This is a notable difference as patients with OHT are at a higher risk of progressing to POAG. The 5-year cumulative probability of developing POAG in untreated OHT patients was found to be 9.5% (95% CI: 9.25-9.75) in the ocular hypertension treatment study (OHTS). The progression from OHT to POAG has been reported to be even higher (18.1%) in black populations. The prevalence of immune-related diseases from both groups could possibly be different if patients with OHT developed glaucoma in the years following the study. This dilemma was avoided in our study since we used healthy patients undergoing routine cataract surgery without a family history of glaucoma, evidence or suspicion of glaucoma, prior glaucoma diagnosis, or ocular hypertension. Their study also included all patients with a history of unspecified arthritis. They do not distinguish between the etiology of arthritis, such as osteoarthritis versus rheumatoid arthritis. However, when they removed the diagnosis of arthritis from both groups, the results remained statistically significant. Unspecified hypothyroidism was also included in their study. This is another important limitation as hypothyroidism can be due to several etiologies. Common causes of hypothyroidism can include autoimmune etiologies (such as Hashimoto's thyroiditis). However, additional non-inflammatory causes are also possible including history of thyroid surgery, radiation therapy, use of thyroid-stimulating medications, or pregnancy. Diabetes, which has evidence for being inflammatory in nature, was not also not included in their tabulation of immune-related diseases. They postulated that their use of matched controls could have possibly eliminated bias resulting from difficulties of disease classification. Other diseases found
in their NTG group, but not in our POAG group, included rheumatic fever, ulcerative colitis, and pernicious anemia. Larger studies might help expand the scope of immune-related or autoimmune diseases associated with glaucoma.

Further analysis of the POAG group in our study was also conducted. A total of 17 POAG patients had the presence of an autoimmune disease of the 62 total POAG patients (Table 4). Interestingly, POAG with autoimmune diseases (+AD) had similar HVF MD to POAG without autoimmune disease (-AD) (-10.36 ± 7.86 vs -11.31 ± 8.15, p=0.681), suggesting no difference in the severity of glaucoma despite the presence of an autoimmune disease. However, the operative eye may not fully reflect the glaucoma severity, as glaucoma may present asymmetrically in each patient and either the more severe or less severe eye may be the operative eye. In addition, we are unable to assess the rate of progression, which could possibly be different between POAG +AD and POAG -AD. Of the total POAG patients, we were able to obtain reliable HVFs (<33% fixation loss, <20% false positives or false negatives) in 78% of patients. Thus, it would likely be even more difficult to trace the progression of their glaucoma through serial HVFs. A larger patient sample size and a prospective study would be needed.

Certain features have been studied in POAG patients that are more common in those who develop rapid worsening or progression in glaucoma, despite having normal IOP levels. These features include the presence of optic disc hemorrhages on clinical exam or defects in the lamina cribrosa, the supportive structure of the optic nerve.\textsuperscript{40,41}

The presence of any history of steroid use was also assessed. The use of steroids can lead to IOP elevation; both the duration of steroid treatment and the potency of steroid therapy play a role. IOP elevation can occur when steroids are administered via topical, periocular, intraarticular, systemic or inhalational routes.\textsuperscript{42} Systemic steroid use is reportedly the least likely route to cause IOP elevation.\textsuperscript{43} In steroid responsive patients, IOP elevation may develop within the first few weeks of steroid use. However, it has been noted that IOP elevation can occur within an hour or years after chronic steroid use. We found no difference in the overall history of any systemic steroid use: POAG 18% and controls 14% (p=0.413). An association between inhaled corticosteroid use, such as those used for treatment of asthma, and ocular hypertension has also
been studied. The risk increased with higher doses and higher number of puffs in patients with family history of glaucoma. We also found no difference in the overall history of inhaled steroid use: POAG 10% and controls 20% (p=0.168).

**Limitations**

Our study limitations include those inherent to retrospective studies. Namely, retrospective studies carry lower level of evidence compared with prospective studies and are limited to establishing association and not causation. Related to the retrospective nature is the age difference between POAG (74.56 ± 7.97) and control (70.92 ± 11.14 years) patients and the inherent difficulty in adjusting this age gap between the two groups. Retrospective studies are also subject to bias, especially bias related to the collection of medical information. This type of bias likely limits the extent of the control group’s documented records as many patients were referred to MEE by outside providers solely for cataract surgery. Thus, an extensive medication, past medical or surgical history was often lacking. This was less so in the POAG group given that many have received care at MEE for years and thus have a more extensive medical record. This study may also have limitations on the generalizability given the studied patients population all underwent surgery. The study was designed as a branch off the prospective study, which only aimed to recruit surgical patients. One concern is we could potentially have enriched for glaucoma patients with more severe disease, given that we only included glaucoma patients undergoing surgery (and not those in clinic). However, this may not be entirely true as some glaucoma patients were also undergoing cataract surgery only (Table 6). Additionally, only the eye undergoing surgery was analyzed for each subject in this study and does not uniformly represent the least/most severe eye. The length and dosing of steroid use could not be uniformly assessed, and we therefore only analyzed the presence of any systemic or inhaled steroid use. We were therefore limited by analyzing the presence of a prescription in patients’ active medication lists. This is especially important as IOP elevation had been noted to be correlated with higher doses and higher number of puffs in patients with a family history of glaucoma. Other forms of steroid use could not be accurately assessed including periocular steroid use.
**TABLE 6.** The breakdown of surgery types in all POAG patients, POAG patients with and without an autoimmune disease. **Abbreviations:** CPC, cyclophotocoagulation; MIGS, minimally invasive glaucoma surgery; POAG, primary open angle glaucoma.

<table>
<thead>
<tr>
<th>Surgery Type</th>
<th>n</th>
<th>% out of 62</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cataract surgery only</td>
<td>15</td>
<td>24.19%</td>
</tr>
<tr>
<td>MIGS ± cataract surgery</td>
<td>21</td>
<td>33.87%</td>
</tr>
<tr>
<td>Penetrating glaucoma surgery</td>
<td>19</td>
<td>30.65%</td>
</tr>
<tr>
<td>CPC only</td>
<td>7</td>
<td>11.29%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>62</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Conclusions**

We conducted an extensive retrospective study to assess the prevalence of autoimmune disease in POAG patients undergoing intraocular surgery. We saw a higher prevalence of autoimmune diseases in POAG patients versus control subjects undergoing cataract surgery. Additionally, the presence of an autoimmune disease is associated with an increased the risk for POAG of 4.61-fold after adjusting for covariates. Many of the autoimmune diseases in the POAG patients are mediated by T-cells. There were no significant differences in severity of glaucoma in the operative eye indicated by HVF MD, IOP, and cup to disc ratio between POAG patients with versus without autoimmune diseases. While there are limitations to this retrospective study, autoimmunity should be explored further in the pathogenesis of POAG.

The prospective portion of this study will establish a foundation for discovering new immunologic markers. With the peripheral blood and aqueous humor samples collected, we hope to evaluate the activity of both innate and adaptive immune cells in glaucoma patients. While still in process and ongoing, the end results of this study will help to explain the pathogenesis and progression of glaucoma, including amongst KPro patients.

**Suggestion for future work**

The results of this retrospective study support the need for additional prospective studies. Elucidation of the glaucomatous mechanism via prospective studies may open the door for treatment customization and targeted immunotherapies based on immunologic markers and not on IOP alone. Future work should also be conducted towards utilizing immunologic markers to improve glaucoma screening, diagnosis, and patient identification for neuroprotective
therapies. Furthermore, neuroprotective agents could be especially effective for patients with IOP-independent glaucoma progression. These neuroprotective agents may target immune cells, such as such as HSP-specific T-cells, or novel inflammatory cytokines. Future research could result in neuroprotective interventions which supplement IOP control and create a more effective means to prevent glaucomatous damage.
References


37. The Ocular Hypertension Treatment Study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of p... - PubMed - NCBI.


