VEGF Levels in RVO

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Study Title: Correlation of Vascular Endothelial Growth Factor Levels in Anterior Chamber Fluid to Disease State in Patients with Retinal Vein Occlusion Receiving Standard of Care Treatment

Principal Investigator - Mark H. Nelson, MD MBA, Assistant Professor of Ophthalmology, Wake Forest Baptist Health
Co-Investigator: Rebecca Sappington, PhD, Associate Professor of Neurobiology and Anatomy, Vice Chair of Research, Department of Ophthalmology, Wake Forest Baptist Health.
Co-investigator: Sally S. Ong, MD Assistant Professor of Ophthalmology, Wake Forest Baptist Health
Co-investigator: Joseph Rigdon, PhD, Department of Biostatistics and Data-Science, Wake Forest Baptist Health

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Background, Rationale and Context

Retinal vein occlusion (RVO) is the second most common retinal vascular disease after diabetic retinopathy. It presents as a Branch retinal vein occlusion (BRVO) or a Central retinal vein occlusion (CRVO). The BRVO is four times more common as a CRVO. The pathophysiology of RVO involves the intraluminal thrombus formation, which may be associated with systemic conditions such as hypertension, hyperlipidemia, diabetes and abnormal thrombophilia. The blockage of venous circulation causes an elevation of intraluminal pressure in the capillaries, leading to hemorrhages and leakage of fluid within the retina. There is associated reduction of retinal perfusion as well, which leads to the secretion of vascular endothelial growth factor (VEGF). VEGF therefore has a leading role in RVO and leads to the clinical finding of macular edema which limits vision. The current treatment for RVO

is anti-VEGF therapy, injected into the vitreous, which has largely replaced macular photocoagulation. Randomized controlled trials (VIBRANT for BRVO (1) and GALILEO/COPERNICUS (2, 3) for CRVO) have shown efficacy and safety with aflibercept (Eylea) injections. It would be advantageous to be able to measure VEGF since our RVO treatment paradigms depend on initiating therapy with anti-VEGF drugs, as well as maintenance therapy which continues to suppress its production.

The purpose of this project is to treat patients with RVO with standard of care anti-VEGF drug Eylea (aflibercept) and to then correlate levels of VEGF in the ocular fluids to disease state manifested by retinal, specifically macular, thickening, which can be objectively measured by Optical Coherent Tomography (OCT). The VEGF levels will be measured by ELISA, a laboratory test which will be performed by Dr. Sappington. i.e.In addition, further evaluation into the cohort of patients (10%) who have persistent exudation or who require monthly Eylea will be studied. ELISA multiplex will be run simultaneously with the VEGF analysis to evaluate other proteins in the ocular fluids of patients with RVO in order to determine if other classes of cytokines are simultaneously responsible for the pathophysiologic exudative process found in RVO.

Study Goal and Objective

The study hypothesis is that measuring VEGF levels from anterior chamber samples from paracentesis prior to Eylea (aflibercept) injections will create a improved paradigm for treatment which currently looks at OCT measurements of foveal thickness and the number of injections.

Overview of Study Design

Patients will receive standard of care intravitreal injections of Eylea (aflibercept) over the course of 52 weeks while undergoing a pre-treatment paracentesis at each study visit. Anterior chamber fluid removed from the eye during the paracentesis will be used to measure VEGF levels as well as other cytokines.

The anterior chamber fluid taken at each visit will be divided for both the ELISA VEGF analysis immediately and a portion to be frozen for further cytokine analysis in the future.

All study activities will occur at Wake Forest Baptist Health Highland Oaks, an outpatient clinic that is part of the Department of Surgery Ophthalmology, expect for the ELISA which will be done in a lab located at the Medical Center by Dr. Sappington.

Methods and Study Design

1. Recruitment and Consent

Recruitment will occur in an outpatient Ophthalmology clinic as patients are receiving eye exams for ocular related issues. Patients that are diagnosed with BRVO or CRVO will be asked by the Principal Investigator if they are interested in learning more about a research study related to their diagnosis. Patients that show interest will be presented with the study consent and the study will be reviewed with the patients. During this time patients will be provided time to review the consent and discuss the study with their families, friends or health care providers. Questions will be answered and any concerns will be addressed prior to obtaining consent. Patients will be informed that if they decide not to participate they will still receive treatment for any eye related issues. Other treatment options will be discussed with the patient by the Principal Investigator.

Patient data will not be collected for study related purposes until informed consent is obtained. Any data used to contact potential subjects prior to obtaining consent will be maintained in the electronic medical record.

2. Screening

Informed consent will be administered prior to any study related activities being performed.

During the screening phase medical history will be reviewed to determine eligibility. All eligibility criteria must be met before a patient can move forward with study treatment and testing of VEGF levels.

Patients that do not meet enrollment criteria (screen-failed patients) will be offered standard of care treatment.

3.Treatment Period and Visit Schedule

Study assessments, procedures and standard of care treatment will be started on Day 1 for patients successfully enrolled into the clinical trial and continue through week 52.

If a patient is experiencing an ocular issue they will be instructed to contact the study doctor to determine if an additional follow up visit is needed.

The study will end at week 52. There will a need to continue treatment since anti-VEGF drugs are not a cure. This indefinite period where Eylea is continued will not be paired with saving anterior chamber samples.

4. Subject Selection Criteria

This is a non-randomized exploratory pilot study that will enroll approximately 20 subjects; 10 subjects with BRVO and 10 subjects with CRVO.

Inclusion Criteria:

1. **Willingness and ability to provide written informed consent**
2. **Age ≥18 years**
3. **Diagnosis of Retinal Vein Occlusion with macular edema and central foveal thickness of ≥300 microns confirmed by intravenous fluorescein angiography and Optical Coherence Tomography**
4. **Visual Acuity between 20/25 and 5/200**

Exclusion Criteria:

1. **Bilateral Retinal Vein Occlusion**
2. **Vision worse than 5/200 in study eye.**
3. **History of myocardial infarction, ischemia, or cerebrovascular accident within 6 weeks of screening**
4. **Concurrent Proliferative Diabetic Retinopathy and/or Maculopathy**
5. **Concurrent Exudative Age-related Macular Degeneration**

6. **Concurrent optic neuropathy with the presence of an afferent pupillary defect**
7. **Previous vitrectomy in the study eye**
8. **Currently pregnant or planning to become pregnant during the duration of the study. Women currently breastfeeding are also excluded**
9. **Previous treatment for retinal vein occlusion in the study eye**
10. **Any current medical condition which, in the opinion of the investigator is considered to be uncontrolled**
11. **History of allergy or hypersensitivity to study treatment, fluorescein, or any study procedure and treatment related ingredients (e.g. topical anesthetics, betadine, etc.)**
12. **If any of these conditions start after the initiation of the study, the patient will not be allowed to participate in the study, however, will be offered the treatment as described above.**

5. Study Treatment

The recommended dose of aflibercept for the treatment of RVO is 2mg (0.05 mL). All subjects will be administered an intravitreal injection of 2mg (0.05 mL) aflibercept in a single-dose pre-filled syringe. All intravitreal injections will be performed by the Principal Investigator.

6. Study Assessments and Procedures

During the screening phase, after consent has been obtained, a complete medical history, including clinically significant diseases, chronic and ongoing conditions, and all current medications will be collected.

Blood pressure will be collected prior to the paracentesis and intravitreal injection.

Ocular assessments will be performed on both eyes at each study visit and will include:

1. **Best Corrected Visual Acuity (BCVA) will be assessed using Snellen charts. If a subject's visual acuity is so poor that the subject can't see**

any letters on the charts, finger counting and/or hand motions will be checked.

2. **Intraocular Pressure (IOP) will be measured prior to the paracentesis. IOP will be measured in both eyes with the results in mmHg.**
3. **Slit lamp biomicroscopy will be performed to examine the eye structures for both eyes. The slit lamp exam will be performed prior to the paracentesis, and will evaluate lids, lashes, lens, conjunctiva, cornea, anterior chamber, pupils, cataract status and vitreous.**
4. **Dilated indirect ophthalmoscopy will be performed to examine the retina of each eye after the pupils have been sufficiently dilated. The dilated indirect exam will occur prior to the paracentesis, and will evaluate the retina vessels, macula, fovea, periphery, optic nerve and vitreous.**
5. **Optical Coherence Tomography will be performed prior to the paracentesis on both eyes, and will be assessed to determine the central foveal thickness.**

A paracentesis will be performed at each study visit prior to anti-VEGF treatment. A topical anesthetic such at lidocaine, will be applied to the eye prior to the paracentesis. A lid speculum will be inserted under the eyelids to hold the eye open during the paracentesis and the intravitreal injection. An application of betadine solution will be applied to the inferior sclera where a 30 gauge needle attached to a syringe will be injected into the eye to remove 0.1mL of anterior chamber fluid. Following the paracentesis, while the speculum is still in place, the intravitreal injection of aflibercept will occur. Directly following the intravitreal injection, betadine will be applied to the injection site to minimize the risk of infection. The samples will be divided in the clinic. All anterior chamber fluid samples will be stored until ELISA analysis has been completed.

Lab personnel will be present in the clinic at the time of collection. Upon collection, samples will be immediately placed on dry ice in an appropriate transport container. Samples will be immediately transported to 4087 Gray Building on WFBMC campus by the on-site lab personnel. Samples will be transferred from the storage container to the -80 degree freezer located in Room 4087. Samples will be stored in this location until the requisite number of samples are obtained for Luminex multi-plex ELISA.

All subjects will receive aflibercept starting on Day 1.

9. Adverse Events and Serious Adverse Events

The investigator will be responsible for the monitoring of the data and safety of study participants. The investigator will be assisted by other members of the study staff.

Adverse events (AEs) will be collected at each study visit by the study doctor from the subject. The study doctor will review all relevant information related to the AE to determine causality and severity, and if follow up care is needed.

Severe Adverse Events (SAEs) will be reported to the IRB within 24 hours of the study doctor being made aware or identifying the SAE. Adverse events will also be reported to government agencies as appropriate.

All AEs, regardless of severity will be collected following the initiation of study procedures and until the subject has completed the study. Each AE will be followed until resolution or until the AE is stable.

11. Outcomes

Primary

1. **Change in macular edema from baseline through Week 52 measured by central retinal thickness using optical coherence tomography.**
2. **Change in macular volume from baseline through Week 52 measured by central retinal thickness using optical coherence tomography.**
3. **Changes in VEGF levels in the anterior chamber from baseline through Week 52 measured by ELISA.**

Secondary

1. **Change in best corrected visual acuity using Snellen Visual Acuity Charts from baseline through Week 52.**
2. **Change in intraocular pressure from baseline through Week 52.**
3. **Number of intravitreal injections from baseline through Week 52.**
4. **Changes in analytes from baseline through week 52 will be studied at a later time. Analytes analysis will include:**
    - **CCL2/JE/MCP-1**
    - **CCL11/Eotaxin**
    - **CXCL1/GRO alpha/KC/CINC-1**
    - **CXCL2/GRO beta/MIP-s/CINC-3**
    - **EGF**
    - **FGF basic/FGF2/bFGF**
    - **IFN-alpha 2/IFNA2**
    - **IFN-beta**
    - **IFN-gamma**
    - **IL-1 alpha/IL-1F1**
    - **IL-1 beta/IL-1F2**
    - **IL-1ra/IL-1F3**
    - **IL-2**
    - **IL-3**
    - **IL-4**
    - **IL-5**
    - **IL-6**
    - **IL-7**
    - **IL-8/CXCL8**
    - **IL-10**
    - **IL-12 p70**
    - **IL-13**
    - **IL-15**
    - **IL-17/IL-17A**
    - **IL-17E/IL-25**
    - **IL-33**
    - **PD-L1/B7-H1**
    - **PDGF-AA**
    - **PDGF-AB/BB**
    - **TGF-alpha**

12.Analytical Plan

Changes from baseline to week 52 in main study outcomes (retinal thickness and volume) will be evaluated using a paired t-test, supposing change outcomes for the n=10 study participants are normally distributed. Linear mixed effect models, including fixed effects for time and type of vein occlusion (BRVO vs. CRVO) and a random effect for study participant, will be employed to study longitudinal changes in primary and secondary outcomes. The relationship between VEGF and retinal thickness (or volume) will be characterized at each time point using scatterplots and Pearson (or Spearman) correlations.

Confidentiality and Privacy

Confidentiality will be protected by collecting only information needed to assess study outcomes, minimizing to the fullest extent possible the collection of any information that could directly identify subjects, and maintaining all study information in a secure manner. To help ensure subject privacy and confidentiality, only a unique study identifier will appear on the data collection form. Any collected patient identifying information corresponding to the unique study identifier will be maintained on a linkage file, store separately from the data. The linkage file will be kept secure, with access limited to designated study personnel. Following data collection subject identifying information will be destroyed (state the anticipated time the data will be destroyed, e.g. three years after closure of the study, and the method of destruction), consistent with data validation and study design, producing an anonymous analytical data set. Data access will be limited to study staff. Data and records will be kept locked and secured, with any computer data password protected. No reference to any individual participant will appear in reports, presentations, or publications that may arise from the study. The data will be stored on an Excel spreadsheet in two places – Dr. Sappington's office and in Dr. Nelson's office, both protected by Wake security procedures.

References

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3. Heier, J., et al. “Intravitreal Aflibercept Injection for Macular Edema Secondary to Central Retinal Vein Occlusion”. Ophthalmology 2014 121:1414-1420.
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