Protocol ID: CVAE 01

ASOCIACIÓN PARA EVITAR LA CEGUERA EN MÉXICO, I.A.P.
HOSPITAL “DR. LUIS SÁNCHEZ BULNES”

Academic Protocol

Vascular changes associated with endophthalmitis.

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1. General Data:

1.1. **Title:** Vascular changes associated with endophthalmitis

1.2. **Area of study**. Diagnostic Methods

1.3. **Probable start and end date of the survey:** July 2024 – March 2026

1.4. **Principal Author:** Dr. Benjamín Aboytes Ríos.

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1.6. Principal investigator: Raúl Vélez Montoya.

1.7. Participating departments: Retina and Vítreous

1.8. Participating institutions: Asociación Para Evitar la Ceguera I.A.P

2. Theoretical Framework

Background: Bacterial endophthalmitis is a serious condition that can lead to irreversible vision loss and, if left untreated, can result in loss of the eye. The most common causes (although not limited to these) are: ocular surgery, invasive procedures, open ocular trauma with or without the presence of an intraocular foreign body. The risk of endophthalmitis after cataract surgery is low, with an incidence of 0.03% to 1.3% (2-6). The incidence of endophthalmitis following intravitreal injection does not vary significantly according to the anti-angiogenic agent used; the MARINA and ANCHOR trials reported an incidence of 0.05% for ranibizumab. In the VIEW trial for ranibizumab, it was 1,5%. (3-6) This explains its probable relationship to the surgical procedure rather than the mechanism of the drug itself. (1) The current treatment paradigm is intravitreal antibiotic injections; however, this is changing towards early vitrectomy. (1-7)

Inflammation and tissue damage are mediated by the host immune response, the etiologic agent, ischemia, and drug toxicity.

The inflammatory response in the posterior chamber leads to protein condensation in the vitreous humor, making it difficult to visualize the fundus and therefore to characterize the degree of damage to the retina and its vasculature.

In 80% of patients, retinal vessels cannot be seen, and ultrasound is necessary to check for the initial presence of tractional membranes or retinal detachment. To this

day, the role that this increase in proteins, increased permeability, and blood stasis (Secondary to significant local inflammation) may have on the retinal vasculature remains unclear.

Chronic degenerative diseases, through their respective pathophysiological processes, condition a systemic pro-inflammatory state. In cases where they exist as an underlying pathology, they can contribute to the presence of incipient vascular damage, making individuals more susceptible to damage to the microcirculation in ocular infectious processes, such as endophthalmitis.

The clinical evolution of endophthalmitis depends on the intravitreal release of inflammatory cytokines, which promote neutrophil aggregation, leading to a variable degree of tissue necrosis and resulting in the presence of vitritis, perivascular exudate, vessel sheathing, diffuse patchy hemorrhages, phlebitis/periphlebitis, and occlusion of arteries proximal to the macula. However, these changes have been described as nonspecific; the main factor related to their presentation is unknown, and there is the possibility of undescribed damage (such as the presence of peripheral vascular lesions)

Imaging techniques have undergone enormous advances in recent years, drastically improving the knowledge of the anatomy, functional, and metabolic state of the retina. (2) Optical coherence tomography (OCT) allows non-invasive cross-sectional imaging of the retina and choroid. Fluorescein angiography is the study of choice for the evaluation of the vascular system, and wide-field fundus photographs provide an overview of the integrity of the retina and its vasculature (7-9)

Nowadays, there are no studies that describe vascular changes associated with endophthalmitis. However, when the previously mentioned techniques are used in combination, they can be used to evaluate the response to treatment and demonstrate ischemic changes or early vascular damage (11-14). Thus opening the possibility to implement early therapies that lead to a better final visual prognosis (12-14)

Justification: The implementation of multimodal imaging studies during the diagnosis and follow-up of patients with endophthalmitis may help to identify, characterize, and differentiate between changes associated with host immune response, drug toxicity, or ischemia, thus opening the possibility of the implementation of early treatments leading to a better final prognosis

Problem statement

Enophthalmitis is a serious condition that can result in both anatomical and functional consequences for the eye. Despite being an inflammatory condition of the eye, little is known about the vascular changes that occur secondary to endophthalmitis. For this reason, it has been decided to investigate whether angiographic changes correlate with visual prognosis in the short or medium term, as well as the comorbidities associated with each case. Do angiographic vascular changes correlate with visual prognosis?

2.1. Objectives and hypotheses

2.1.1.1. **General Objectives:** To describe and investigate vascular changes associated with exogenous endophthalmitis, as well as to create a photo library where they are evidenced

2.1.1.2. **Specific Objectives**

- 1. To demonstrate the presence or absence of perivascular exudate, vessel sheathing, phlebitis/periphlebitis, diffuse or localized hemorrhages, arteriovenous shunts, vascular closure zones, and neovessels, as well as their pattern of location (in the central or peripheral region of the retina) by means of fundus photography, optical coherence tomography, and fluorescein angiography
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- 2. To identify and correlate the presence of incipient vascular damage that could make the microcirculation more susceptible to damage during ocular infectious processes in patients with significant personal pathological history
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- 3. To describe vascular changes according to their probable etiology (ischemic, drug-mediated toxicity, and host immune response) using fundus photography, optical coherence tomography, and fluorescein angiography

2.1.2. Hypothesis

2.1.3. Null hypothesis

-The incidence of vascular changes (exudates, hemorrhages, areas of capillary closure) is greater than 50% of cases, regardless of the pathogen or the treatment indicated

2.1.3.1. Alternate hypothesis

- The incidence of vascular changes (exudates, hemorrhages, areas of capillary closure) will be less than 50% of the cases, regardless of pathogen or treatment indicated.

3. Study design

According to the moment at which the information is collected.

- Prospective - a study where the information will be collected in the future, after the beginning of the research

3.1. According to the number of times the primary variable of interest is measured or determined.

- Longitudinal.- The primary variable of interest is measured more than once over time

3.2. According to the researcher´s interference in the phenomenon being analyzed

- Observational.- The researcher only describes or measures the phenomenon

3.3. According to the analysis of the study population(S)

- Descriptive.- The researcher only has a population and describes it

4. Methodology

4.1. Place and duration

The population will be patients of the retina and vitreous service of Asociación para Evitar la Ceguera en México, attended from July to December 2024, who have a complete clinical record (age, sex, pathological personal history, non-pathological personal history, heredofamilial history, ophthalmologic history, allergies, medications, ophthalmologic examination with visual acuity, intraocular pressure at each visit), signed informed consent and with a history of exogenous or endogenous endophthalmitis with clear resolution criteria and clear means one week and one month after meeting the resolution criteria to be able to perform fluorescein angiography at both visits.

4.2 Description

Patients who meet the inclusion criteria will be invited to participate in the study, who will have a history of endophthalmitis and who have clear media (clear cornea, anterior chamber cellularity + or less, vitritis + or less), one week and one month after meeting the resolution criteria established by the Mexican Endophthalmitis Group.

After signing the informed consent form
A comprehensive ophthalmologic examination will be performed, including assessment of visual acuity and intraocular pressure.
A fluorescein angiography will be performed one week after meeting resolution criteria, and one month later.
Fluorescein 10% (10mg/ml) will be injected intravenously through a peripheral line.
Fundus photographs will be taken with the Clarus 700 equipment
The pictures will be analyzed in different phases and in different areas
The changes will be compared at different times, one week after meeting the resolution criteria and one month later
Consecutive recruitment will be done

The data will be stored in a table in Excel 2007

4.2. Inclusion criteria

- **Patients with a history of endophthalmitis**

- Diagnosis of Endophthalmitis

  - Clinical symptoms: Pain, decrease in visual acuity, anterior chamber cells and/or vitreous turbidity, corneal edema, conjunctival hyperemia
  - Laboratory studies: B-mode USG and or positive culture

- Patients who meet the endophthalmitis resolution criteria of the Mexican Endophthalmitis Study Group
- Patients with previously signed informed consent

4.3. Exclusion criteria

- Patients who have not signed the informed consent
- Patients under 18 years.
- Patients with endophthalmitis who do not meet the resolution criteria of the Mexican Endophthalmitis Study Group

5. Study variables

Variable name	Independent or Dependent	Nominal /ordinal/ dimensional	Unit of measurement	Measurement instrument	Time of measurement
Visual acuity	Independent	Ordinal	logMAR	Snellen Chart	At diagnosis, one week after resolution, and one month after
Vascular changes in FAG (Areas of capillary closure)	Independent	Ordinal	Disc areas	Fluorescein angiography	one week after resolution and one month after
Vascular changes in FAG (Periphlebitis)	Independent	Ordinal	Presence or absence	Fluorescein angiography with fluoroscein	one week after resolution and one month after
Vascular changes in FAG (Hejorrhages on staining)	Independent	Ordinal	Presence or absence	Fluorescein angiography with fluoroscein	one week after resolution and one month after
Vascular changes in FAG (Areas of leakage)	Independent	Ordinal	Disc areas	Fluorescein angiography	one week after resolution and one month after

Isolated body	Independent	Ordinal	Presence or absence of microorgan	Medium Culture	Single. When an organism grows in the culture

			ism in culture		

Age	Independent	Dimensional	Years completed	Interrogation	Visit 1

Sex	Independent	Nominal, dichotomic	Female/ Male	Tab from the collection	Unique

5.1.1. Measuring Instrument

Retinal Imaging and Analysis: Fluorescein angiography will be performed using the **Clarus 700 Zeiss** device (Serial # CL700-73467), with Software version 1.1.2.59261 and Digital Camera Magnification of 133°.

Optical Coherence Tomography (OCT) will be performed using the **HRA Spectralis Heidelberg**, Serial # Spec-CAM-15095-S3610, with a 30° magnification and software version 7.0.6.0.

5.1.2. Time of measurement.

5.2. Sample size. Description of the main maneuver(s)

Best-Corrected Visual Acuity (BCVA) will be assessed according to the **ETDRS guidelines**.

The test must be performed with the patient positioned **4 meters from the backlit panel**, which must be calibrated to emit **85 cd/m²**.

Testing will be conducted in **both eyes** (including both the randomized and non-randomized eye). Each eye must be tested using a chart with a different character layout to **prevent patient memorization** when such a chart variation is possible. If not, testing must begin with the **study eye**.

The **total number of letters correctly identified by the patient must be recorded**, along with the **Snellen equivalent** and **LogMAR score**. The test must be conducted under **best-corrected visual conditions**.

The following conversion chart will be used to record **best-corrected visual acuity**.

Valor LogMAR	Snellen (pies)	Snellen (metros)
1.6	20/800	20/240
1.5	20/640	6/190
1.4	20/500	6/150
1.3	20/400	6/120
1.2	20/320	6/96
1.1	20/250	6/75
1.0	20/200	6/60
0.9	20/160	6/48
0.8	20/125	6/38
0.7	20/100	6/30
0.6	20/80	6/24
0.5	20/63	6/19
0.4	20/50	6/15
0.3	20/40	6/12

Valor LogMAR	Snellen (pies)	Snellen (metros)
0.2	20/32	6/9.5
0.1	20/25	6/7.5
0.0	20/20	6/6
-0.1	20/15	6/4.5
-0.2	20/12	6/3.6

To measure **Best-Corrected Visual Acuity (BCVA)**, the patient will be asked to identify the letters they can distinguish until they reach the line where they are unable to locate **at least three optotypes correctly**. The number of letters correctly identified will be recorded.

Additionally, if using vision charts that **do not begin at the 20/800 line** (the point from which letter counting for ETDRS equivalence begins), the following adjustment will be made: a specific number of letters will be **added to the patient's result**. This procedure will be carried out by the **retina fellow assigned to the case**. The assessment will be performed at **each visit** (7 days and 1 month after meeting the inactivation criteria for endophthalmitis).

Indirect Ophthalmoscopy

A detailed examination of the retina will be performed. After **pupillary dilation** with **TP Ofteno® (tropicamide 5% / phenylephrine 0.8%)**, an **indirect ophthalmoscope** and a

20D lens will be used for a comprehensive retinal evaluation. The brand and model of the instruments used will be at the **discretion of the principal investigator**.

The structures to be evaluated include the **optic nerve**, **retina**, **retinal vasculature**, and transparency of the **posterior segment media**, among others. This procedure will be performed **at all study visits** by the designated clinical team.

Spectral-Domain Optical Coherence Tomography (SD-OCT)

OCT imaging will be performed using the HRA Spectralis Heidelberg, Serial # Spec-CAM-15095-S3610, with a Magnification of 30° and Software version 7.0.6.0. This will aid in evaluating the retinal condition, including central macular thickness and central retinal volume (commonly referred to as macular cube volume in OCT terminology).

A technician or delegated personnel may carry out this procedure, and will be performed at all study visits.

Fundus Photography and Fluorescein Angiography

Fundus imaging will be performed using the **Clarus 700 Zeiss**, Serial #CL700-73467, Software version 1.1.2.59261, Digital Camera Magnification 133°. Images of the **posterior pole and retinal periphery** will be captured, providing a comprehensive view of retinal integrity, the optic nerve, and its vasculature.

Fluorescein angiography will be performed subsequently to evaluate **associated vascular changes**. The patient must undergo an **8-hour fast** and have **pupillary dilation with TP Ofteno® (tropicamide 5% / phenylephrine 0.8%)**. The procedure involves preparing the patient with aseptic technique for an **intravenous injection of fluorescein dye** in an upper limb, followed by imaging with a fundus camera.

It is not essential to use a device of a specific brand or model, as long as a system with **equivalent specifications** is used consistently for the **same patient throughout the study**. The procedure may be performed by a **technician or**

delegated personnel, and will be conducted **at all study visits** (7 days and 1 month after meeting the inactivation criteria for endophthalmitis).

Sample size

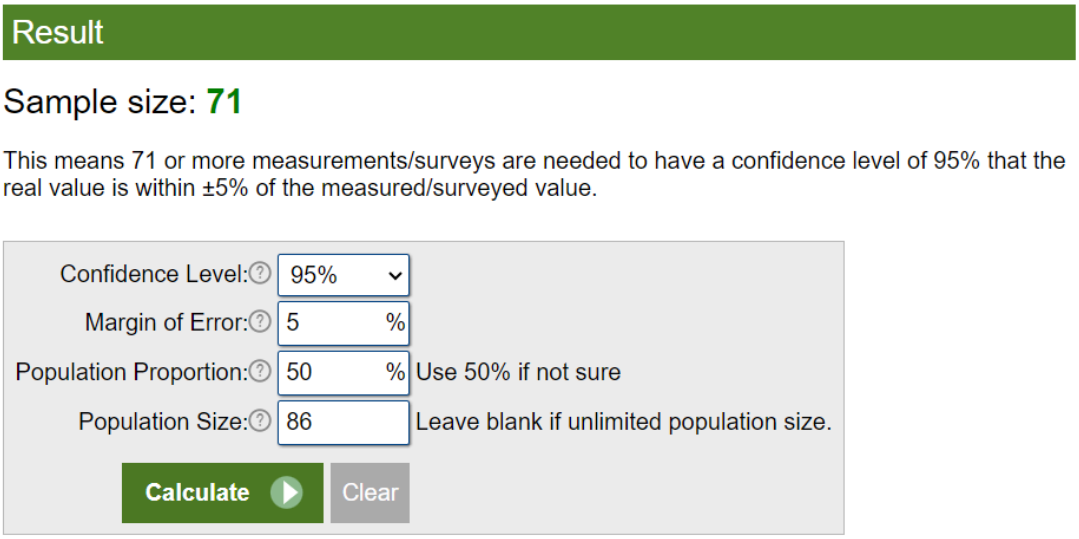
A review of hospital medical records was conducted to identify patients diagnosed with **endophthalmitis** from **February 2024 to September 2024**, resulting in a total of **57 patients**.

Considering this data was collected over 8 months, a **proportional extrapolation** was made to estimate the number of cases in a **12-month calendar year**, yielding approximately **86 patients**.

Based on this projection, we decided to use the **sample size formula for proportion estimation**, as our working hypothesis suggests that the **incidence of vascular changes** will be **greater than 50%**.

The sample size was calculated using an **online proportion estimation calculator**:

https://www.calculator.net/sample-size-calculator.html?type=1&cl=95&ci=5&pp=50&ps=86&x=Calculate:



A total of **71 patients** were included to achieve a **95% confidence interval**, with a **5% margin of error**, a **population proportion of 50%**, and a **total population size of 86 patients**.

We will calculate **measures of central tendency** (mean, median, and mode). **Chi-square tests** will be used for qualitative variables.

Data will be analyzed using the **SPSS software, version 25**.

Organization

Human Resources:

- Retina specialists affiliated with the Retina Service
- Medical intern completing social service
- Retina fellows
- Nursing staff

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Material Resources:

- Computer
- **HRA Spectralis Heidelberg OCT**, Serial #Spec-CAM-15095-S3610, Magnification 30°, Software version 7.0.6.0
- **Clarus 700 Zeiss**, Serial #CL700-73467, Software version 1.1.2.59261, Digital Camera Magnification 133°
- Dye
- Needles
- Plastic catheters
- Vials of fluorescein

5.3. Ethical Aspects

This study was designed in accordance with the principles of the Declaration of Helsinki and in compliance with the guidelines for good clinical practice. All patients must sign an informed consent form prior to their inclusion in the study. Patient privacy will be protected at all costs, and only data relevant to this research will be collected.

5.4. Schedule of activities

Activities	September 2024	October 2024	November 2024	December 2024	January 2024
Search for files	X	X			
Collation of inclusion criteria		X	X		
Excel database			X	X	

Analysis of data				X	
Interpretation Data interpretation and delivery of results					X

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