

NH_SiOP-001 Study Protocol

1. Study Overview

This study is designed as a prospective, single-center, single-blind interventional cohort trial to be conducted at the Department of Ophthalmology, University of Szeged, Hungary, between March 2022 and February 2025.

Following written informed consent, patients requiring intravitreal aflibercept (IVA) treatment will be enrolled and allocated to one of three predefined study groups based on vitreous status and surgical history. All participants will be analyzed according to their assigned cohort.

2. Ethical Approval

The study protocol is reviewed and approved by the Institutional Ethics Committee of the University of Szeged (Protocol No. NH_SiOP-001, Reference No. 168/2022-SZTE RKEB).

All procedures will be conducted in accordance with the Declaration of Helsinki and with applicable national and institutional regulations concerning human research.

3. Study Population

Study Groups

- G-SiO group:
Eyes that have previously undergone pars plana vitrectomy (PPV) with silicone oil (SiO) endotamponade using *Oxane 1300* (Bausch & Lomb, Bridgewater, NJ, USA).
- G-PPV group:
Eyes that have undergone 23G PPV surgery at least 3 months prior to enrollment, without current SiO tamponade or following previous SiO removal.
- G-NVIT group:
Eyes with an intact vitreous body and no history of vitrectomy.

Treatment History

Previous intravitreal injections (IVIs) do not exclude participation. However, all study eyes must be treatment-naïve to antiglaucoma medications, including any temporary intraocular pressure (IOP)-lowering therapy following SiO implantation or PPV.

Eligible eyes must show no clinical or structural evidence of glaucomatous optic neuropathy.

Bilateral Enrollment

If both eyes meet inclusion criteria, both will be included. The treatment method and observation protocol are identical across all groups.

4. Diagnostic and Baseline Procedures

All participants will undergo a comprehensive ophthalmologic evaluation prior to enrollment.

Visual and Anterior Segment Assessment

- Best-Corrected Visual Acuity (BCVA): measured using a standardized ETDRS chart.
- Axial Length (AL), Anterior Chamber Depth (ACD), and Central Corneal Thickness (CCT): measured using the ARGOS® Swept Source OCT Biometer (Movu – Santec Corporation, Santa Clara, CA, USA).
- Lens Status (phakic/pseudophakic): documented for all eyes.

Gonioscopy

- Conducted under topical anesthesia in an undilated dark room using a Magnaview gonio laser lens (Ocular Instruments, Bellevue, WA, USA).
- The modified Shaffer classification system will be used to identify angle structures.
- A Shaffer360 (S360) index will be calculated by summing the four quadrant scores (0–16), where 16 represents a fully open angle (visible ciliary body in all quadrants) and 0 represents complete closure (no Schwalbe’s line visible).
- The extent of iridotrabecular contact (ITC) and presence of emulsified SiO will be recorded in degrees of arc, where applicable.
- Angles will be categorized as open or closed based on ITC >180°.
- Eyes showing angle or iris neovascularization or gonioscopic closure will be excluded

Posterior Segment Assessment

- Fundus Examination: performed to confirm the indication for IVA.
 - Optical Coherence Tomography (OCT): performed with *Heidelberg Spectralis* (Heidelberg Engineering GmbH, Heidelberg, Germany).
-

5. Intraocular Pressure (IOP) Measurement Protocol

IOP will be measured using both rebound tonometry (RBT) followed by Goldmann applanation tonometry (GAT) under standardized conditions.

- Rebound tonometry: *iCare-100* (Icare Finland Oy, Vantaa, Finland)
- Goldmann applanation tonometry: *Haag-Streit* (Köniz, Switzerland)

Measurements will be taken with participants in a seated position.

Two investigators will participate in each measurement session:

- One examiner will perform the measurement while remaining masked to the result.
- A second investigator will record the readings, both remaining blinded to vitreous status.

For RBT, the mean of six rapid consecutive manual-mode readings will be recorded. For GAT, a single reading will be obtained after topical anesthesia.

The primary IOP data for analysis will be derived from GAT, while RBT readings will be used for agreement analysis.

IOP will be measured at the following time points:

- Baseline (BL)
- Immediately after IVA (T1; ≤ 1 min)
- 5, 15, 30, 60, and 180 minutes post-IVA (T5–T180)
- Day 1 (D1) and Week 1 (W1)

All post-injection IOP assessments will be conducted with dilated pupils for consistency.

6. Study Intervention

No IOP-lowering pretreatment will be administered immediately before or after injection.

Indications for IVA

Indications for IVA will follow approved clinical indications, such as diabetic macular edema, retinal vein occlusion, and neovascular age-related macular degeneration.

Injection Technique

Intravitreal aflibercept will be administered in accordance with the European Society of Retina Specialists (EURETINA) recommendations.

- Drug: 0.05 mL (2 mg) aflibercept (*Eylea*®, Bayer AG, Leverkusen, Germany)
- Syringe: 1 mL *BD Luer-Lok Tip* syringe (Becton, Dickinson and Company, Franklin Lakes, NJ, USA)
- Needle: 30G *Medoject hypodermic needle* (CHIRANA T.Injecta, Stará Turá, Slovak Republic)
- Technique:
 - The needle enters the sclera at approximately 30°, forming a tunneled path before being raised to 90° for scleral penetration.
 - The injection is directed toward the center of the vitreous cavity.
 - The needle and syringe are withdrawn along the same 30° path to minimize reflux.
 - A sterile cotton swab is applied for 10 seconds after withdrawal to prevent reflux.

Reflux classification:

The appearance of any non-bloody fluid at the injection site upon withdrawal is classified as reflux. Dimensions will be measured in millimeters using calipers.

Eyes exhibiting reflux will be excluded from statistical analysis but will continue follow-up according to the study protocol.

Rescue Procedure

Rescue intervention will be performed if:

- The patient reports no light perception (NLP) immediately after injection (T1), or
- IOP remains >50 mmHg at 30 minutes (T30) despite observation.

Rescue options include paracentesis and/or intravenous acetazolamide administration.

7. Statistics

Sample Size Calculation

Sample size estimation is based on a two-way repeated-measures ANOVA (group × time interaction) with the following parameters:

- Standardized effect size $f = 0.25$ (medium)
- Three independent groups
- Nine repeated measurements
- Statistical power = 95%
- $\alpha = 0.05$
- Correlation among measures = 0.40
- Greenhouse–Geisser $\epsilon = 0.50$

The minimum required sample size is 51 participants, calculated using G*Power 3.1.9.7.

Principal Investigator

Attila Kovács MD, PhD

Department of Ophthalmology,

University of Szeged

6720 Szeged, Korányi fasor 10-11.

Hungary