Two-Step versus One-Step Sub-Retinal Injection

STUDY PROTOCOL

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INVESTIGATOR SIGNATURE PAGE

I agree to implement and conduct this study diligently and in compliance with the protocol, good clinical practices and all applicable laws and regulations.

I have read this protocol and I agree to all aspects.

Investigator Printed Name

Signature

Date

(Retain original in investigator site binder)

STUDY SUMMARY

Title	Two-Step versus One-Step Sub-Retinal Injection
Primary Objective	The study is aimed at assessing the reflux and retention of tPA delivered by the one and two-step methods of sub-retinal injection for the management of sub-retinal haemorrhage.
Study Design	Randomised, controlled study in which patients diagnosed with sub-macular haemorrhage and who have been scheduled for sub-retinal tPA and pneumatic displacement will be randomly assigned to either the two-step or one-step group. a) **Two-step injection:** In Step 1, 0.2mL of balanced salt solution will first be used to open the anatomical plane of the sub-retinal space. In Step 2, 50µg/0.1mL of tPA with 0.1mL of fluorescein sodium at a concentration of 0.1mg/mL. b) **One-step injection:** 50µg/0.1mL of tPA with 0.1mL of fluorescein sodium at a concentration of 0.1mg/mL
Sample Size	20 participants with sub-macular haemorrhage
Eligibility	Participants of age 18 years or above and who have a clinical diagnosis of sub-macular haemorrhage of ≤7 days duration.

ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse Event
BCVA	Best Corrected Visual Acuity
eCRF	Electronic Case Report Form
GCP	Good Clinical Practice
ICH	International Conference on Harmonisation
IOP	Intraocular Pressure
HREC	Human Research Ethics Committee
OCT	Optical Coherence Tomography
SAE	Serious Adverse Event
tPA	Tissue Plasminogen Activator

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1. Background

The retina is light sensitive and lines the inside of the eyeball in much the same way that wallpaper lines the inside of a room. If the eye were to be considered analogous to a camera, then the retina is its film. Diseases of the retina, in turn, have a significant impact on visual function.

Emerging treatments for retinal disease involve iatrogenic detachment of the retina for the delivery of therapeutic substances into the sub-retinal space, as well as in the delivery of viral vectors as a means of "gene replacement" in patients with hereditary retinal degenerations caused by insufficiency of proteins vital for visual function (either null mutations or halpoinsufficiency).

There are many different variables in treating retinal conditions via such an approach e.g. surgical variables, the nature and stage of the condition and the viral vector itself (serotype, concentration, total dose etc.). However, of these variables, surgical variables themselves are largely overlooked.

Sub-retinal injection is a surgical manoeuvre used to deliver medications into the sub-retinal space. The sub-retinal space is a potential space and it has been argued that opening of the space should precede injection of therapeutic substances in order to accurately and precisely control the amount of delivered therapeutic substance.

In current clinical practice, the main indication for performing sub-retinal injections is the management of bleeding underneath the macula (sub-macular haemorrhage). To date, two principal approaches of delivering sub-retinal injections have been described, though little is known about the fate of substances injected into this space, beyond theoretical conjecture.

Broadly speaking, the two methods are:

1. A two-step approach, in which a physiologically insert fluid (balanced salt solution) is used to open the potential sub-retinal space, after which the therapeutic substance is injected (Simunovic et al., 2017).
2. A one-step approach, in which the therapeutic substance itself is used to create a limited iatrogenic retinal detachment, thereby opening up the sub-retinal space (Simonelli et al., 2010).

1.1 Rationale for performing the study

Researchers investigating emerging treatments, such as gene therapy, often overlook surgical variables in the delivery of substances in the sub-retinal space. This study will attempt to address this issue by studying the reflux of therapeutic substances delivered by the one- and two-step methods using the sub-retinal injection of tissue plasminogen activator (tPA) for the management of sub-macular haemorrhage.

The advantage of the one-step injection method is that it may be quicker than the two-step injection. The disadvantages are that there may be more wastage of medication, either due to the needle being in the incorrect anatomical plane (retinal layer) or because fluid may be more likely to regurgitate.

In order to compare these two methods, an inert fluorescent salt (fluorescein sodium) will be used to track the passage of the therapeutic drug Alteplase (Actilyse), a tissue plasminogen activator (tPA). In this study, the fluorescein will be directly injected under the retina.

Fluid samples will be taken from the eye at the completion of surgery to compare how much fluid has leaked during the surgical procedure. The samples are taken from the fluid-air exchange, a standard step

in vitrectomy surgery. Instead of discarding the fluid extracted, it will be instead collected. The total volume of fluid will be measured, as well as the concentration of fluorescein in this solution, using a fluorophotometer. Once measurements are taken, the fluid will be destroyed.

The procedure will be video-recorded in order to provide a means of subjectively assessing reflux at the time of injection of the tPA solution.

The results of this study may help determine the optimum approach in the delivery of treatments in retinal disease via sub-retinal injection in future studies.

1.2 Hypothesis

We hypothesise that the two-step approach will result in less reflux at the time of initiation of iatrogenic retinal detachment and during subsequent surgical steps.

2. Study Objectives

The overall objective of this study is to assess the reflux and retention of a therapeutic substance (tPA) delivered via two different techniques into the sub-retinal space to determine the optimum approach for sub-retinal injections.

3. Study Design

This is a randomised, controlled study in which patients diagnosed with sub-macular haemorrhage and who have been scheduled for sub-retinal tPA and pneumatic displacement will be randomly assigned (1:1) to either the two-step or the one-step group.

The target recruitment is 20 subjects. The sample size is based on the assumption that there is 10% (0.01mL) reflux of sodium fluorescein from blebs in both groups, and that there is a 60% wastage of fluid in the one-step procedure. The calculated sample size for a power of 0.80 and significance level of 0.05 is therefore 20 subjects.

We will review the results after the first 10 subjects. The trial will be terminated early if the appropriate statistical significance has been achieved (or in the event of a serious adverse event due to the study protocol).

Patients scheduled to undergo surgery for the displacement of sub-macular haemorrhage and who satisfy the eligibility criteria will be approached to participate in the study.

3.1 Study Group

a) Two-step injection: In Step 1, 0.2mL of balanced salt solution will first be used to open the anatomical plane of the sub-retinal space. In Step 2, 50µg/0.1mL of tPA with 0.1mL of fluorescein sodium at a concentration of 0.1mg/mL.

b) One-step injection: 50µg/0.1mL of tPA with 0.1mL of fluorescein sodium at a concentration of 0.1mg/mL

3.2 Endpoints

Primary Outcome

- The primary endpoint is the amount of refluxed fluorescein sodium between the two-step and one-step approach.

Secondary Outcomes

- Time taken to perform the sub-retinal injection.
- Change in visual acuity at 3 and 12 months

3.3 Involved Drugs

Two drugs will be used in this study – Alteplase (Actilyse) and Fluorescein Sodium (Fluorescite injection).

3.4 Quality Assurance

Standardised protocols have been developed for sub-retinal injection and assessment of sub-macular haemorrhage.

The major quality assurance features of the study are:

- Standardised eligibility and exclusion criteria
- Adherence to treatment protocol and follow-up
- Monthly meetings of personnel at the study centre to review methods and discuss problems

3.5 Organisational Structure of the Study Group

The centre will have trained BCVA and OCT examiners, a study co-ordinator and an ophthalmologist performing the surgery and sub-retinal injection.

4. Participant Eligibility

4.1 Participant Consent

Participants identified as being suitable will be asked if they are interested in participating in this study. The study requires that written informed consent is obtained from each participant prior to their enrolment in the study. The participant will be asked to sign the consent form only after an investigator has explained the purpose of the study, the participant has had time to read and understand the information sheet, has been given sufficient time to ask questions and agrees to participate voluntarily. The patient will be given a copy of the signed consent form to take home.

4.2 Eligibility of Participants into the Study

Patients will be approached at the time of consent for surgery. To participate in this study, the patient must meet all the following criteria:

4.2.1 Inclusion Criteria

- Participants will be patients with either a new or an established diagnosis of age-related macular degeneration and with a ≤ 7-day history of vision loss secondary to sub-macular haemorrhage.
- Age ≥ 18 years (Participants with sub-macular haemorrhage or retinal arteriolar macroaneurysm are typically over 60 years of age.)
- Area of haemorrhage of greater than 1 disc area but not meeting the criteria for massive sub-retinal haemorrhage (haemorrhage extending beyond the temporal vascular arcades).
- Density of haemorrhage sufficient to obscure the RPE and choroidal detail.
- Haemorrhage involving the fovea.
- BCVA ≤ 6/60
- Written informed consent has been obtained

4.2.2 Exclusion Criteria

- Symptoms suggestive of sub-macular haemorrhage for > 7 days.
- Amblyopia in the study eye.
- Presence of another ocular disease affecting vision in the study eye.
- Known allergy to fluorescein
- Women who are pregnant, nursing or planning pregnancy or who are of childbearing potential and not using reliable means of contraception. (A woman is considered of childbearing potential unless she is post-menopausal and without menses for 12 months or is surgically sterilised)
- Participants unable to adhere to post-operative posturing.
- BCVA < HM
- Known allergy to agents used in the study e.g. fluorescein sodium
- Known history of significant cardio-pulmonary disease
- Multiple concomitant drug therapies – in particular, beta-blockers (including eye drops)
- Patients who, in the opinion of the treating surgeon and anaesthetist, are unfit for a surgical procedure, with a condition that may put the patient at considerable risk, may confound the study results or may interfere significantly with the patient's participation in the study.

5. Entry into the Study

5.1 Patient Orientation

At presentation, patients are examined to determine whether they are eligible for the study.

A complete baseline examination is conducted (as part of standard of care) which includes the following:

a) BCVA
b) IOP measurement
c) Slit lamp examination
d) Fundus examination
e) Central macular thickness by OCT

If they are deemed eligible, the investigator will discuss the study with the patient. They are informed of the possible risks and benefits of the study treatments. The patient either reads the detailed informed consent, or it is read to them. Any questions related to the study will be answered. The patient is allowed some time to consider the study prior to the consent form being signed.

5.2 Randomisation

After determining patient eligibility and signing the informed consent form (if the patient has agreed and is willing to participate in the study), each patient will be randomised to receive either two-step or one-step treatment. Randomisation will be performed using a random number generator in bit output (i.e. 0 vs 1). Patients will be sequentially assigned to either group according to the sequence of bits randomly generated (i.e. 0= 1-step group, 1= 2-step group). It is not anticipated that there will be a significant difference in outcome between the two arms of the trial. There is no placebo group.

6 Treatment

The surgical procedure will be identical to that undertaken in the routine management of significant sub-macular haemorrhage. The only difference between standard of care in the current study is that fluorescein sodium will be used to track flow of the tPA.

Following regional anaesthesia with sedation, the eye undergoing surgery and the periocular skin will be prepared with povidone iodine, after which a fenestrated sterile drape will be placed over the patient. Thereafter, a pars-plana vitrectomy will be undertaken in a routine fashion: after placement of a sterile lid speculum, 3 trans pars plana 25G ports will be placed infero-temporally, supero-temporally and supero-nasally. A core and limited peripheral vitrectomy will then be undertaken using the Alcon Constellation Vision System. At the completion of vitrectomy, sub-retinal injection of tPA will be undertaken as follows (either a. or b.), according to the group allocation. In each case, sub-retinal injection will be performed using the viscous fluid control mechanism of the Alcon Constellation Vision System and will be injected using viscous fluid control via a 25/38g teflon-tipped cannula. The site of initiation will be within an area of attached retina close to the region of sub-retinal clot. In each instance, the bleb will unite with the sub-retinal clot with the aim of its dissolution and displacement for therapeutic purposes.

Following peripheral search for retinal breaks, a fluid-air exchange will then be undertaken using a soft-tipped extrusion cannula. This is a standard step in this procedure, however, instead of discarding the fluid extracted in this manoeuvre, it will be collected. The total volume of fluid will be measured as well as the concentration of fluorescein in this solution using a fluorophotometer. Once measurements are performed, the fluid will be discarded.

The procedure will be video-recorded in order to provide a means of subjectively assessing reflux at the time of injection of the tPA solution. The video will be stored on an encrypted portable USB drive. The footage will be anonymous and will be deleted at the completion of the study.

6.1 Non-Study Eye

The non-study eye will be treated as per the standard of care at the investigator's discretion.

7 Patient Visits and Examination

7.1 Introduction

Patients will be assessed with respect to safety outcomes for which standardised procedures have been developed:

- BCVA by a trained technician will be undertaken
- Adverse event assessment
- Dilated fundal examination
- Central macular thickness by OCT

7.2 Patient Follow-up

Standard of care post-surgery visits will be conducted as per normal through the Outpatient Department, Sydney Eye Hospital. There will be no additional study-specific visits post-surgery. The Principal Investigator and/or Sub-Investigator will collect adverse events during these follow-up visits throughout the post-operative period (to twelve months).

8 Safety

8.1 Study Procedure Risks

The risks of this study are identical to the standard management for sub-macular bleeding except for one difference: the use of fluorescein sodium, a yellow dye, to track the movement of fluid under the retina and within the vitreous cavity. Fluorescein is used routinely to study the ocular surface and in performing ocular angiography (where it is injected intravenously, typically in concentrations of up to 10%). In conditions that cause bleeding underneath the retina, fluorescein will leak into the sub-retinal space in concentrations approaching 10% without any known detrimental effects.

We will be using a lower concentration to study the flow of fluid within the eye during the proposed procedure. This concentration has previously been shown to have no detrimental effect on nerve cells (Kato et al., 1983) or on human embryonic kidney cells (Salvetti et al., 2017). Higher concentrations have been reported for visually tracking tPA in routine care (Khan et al., 2017). There is a small risk of allergy to fluorescein and patients with a history of such allergy will be excluded from this study.

8.2 Adverse Event Reporting

Adverse Event definition: any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of a medicinal product, whether or not related to the medicinal product.

Examples of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition, including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after investigational product administration, even though it may have been present prior to the study.
- Signs, symptoms or the clinical sequelae of a suspected interaction.
- Signs, symptoms or the clinical sequelae of a suspected overdose of either investigation product, or of a concurrent medication (overdose per se should not be reported as an AE/ SAE).

This trial is being carried out in the facilities of a primary ophthalmic centre, with extensive experience in vitrectomy surgery. The medical staff is well-experienced in dealing with all adverse events associated with vitrectomy surgery.

Adverse events will be collected from the date of surgery. The incidence of adverse events will otherwise be recorded throughout the duration of the study. These will be graded by the Principal Investigator as mild, moderate or severe.

Serious Adverse Event definition: any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect

For serious adverse events (SAEs) the relationship to the study drug will be graded as unrelated, possibly related, probably related or definitely related. SAEs will also be graded as expected or unexpected.

SAEs as defined by the International Conference on Harmonisation, Good Clinical Practice (ICH-GCP) as per above, will be reported as SAEs to the site's approving HREC according to local requirements.

The following events are to be considered AEs, but not serious AEs and therefore reporting as SAEs is not required:

- Hospitalisations for elective surgery
- Hospitalisations for admissions of less than 24 hours in duration
- Hospitalisations to the emergency department not requiring admission within the hospital
- Care delivered as "hospital in the home" or similar in-home service (unless other SAE criteria are met, such as persistent disability)

8.2.1 Infectious Endophthalmitis

Infectious endophthalmitis can be an acute complication of vitrectomy surgery. Study patients are informed in detail about the symptoms of a developing infection and are advised to contact the study staff if they have any doubt about their eyes after surgery.

If patients present with infectious endophthalmitis, they will be treated according to the standard treatment. In general, this includes vitreous aspiration for gram stain, microbiology and resistance testing, followed by an injection of Vancomycin 1mg/ 0.1mL and Ceftazidime 2.25mg/ 0.1mL. Depending on the general situation, patients will be admitted to hospital or follow very closely as outpatients.

8.2.2 Retinal Detachment and Vitreous Haemorrhage

Patients presenting with a rhegmatogenous retinal detachment or massive vitreous haemorrhage following the surgery will be referred to the vitreoretinal service at Sydney Eye Hospital.

8.2.3 Damage to the Crystalline Lens

Acute opacification of the crystalline lens due to lens damage during surgery will be observed and treated surgically as appropriate.

8.2.4 Elevation of Intraocular Pressure

Patients with IOP measured ≥ 30mmHg may be commenced on first line glaucoma medication.

8.2.5 Cataract Formation

The decision to perform cataract surgery will be made along conventional lines in consultation with the patient, taking into account the level of visual acuity in both eyes, the degree of lens opacification and the extent of functional disability.

8.2.6 Contraindication to use of Fluorescite

- Hypersensitivity to the active substance or to any of the excipients

Fluorescein sodium has been routinely used to perform ocular angiograms and study retinal disease for more than 50 years and is known to enter into the sub-retinal space of patients with common retinal pathology to act as a marker. It is not known to be associated with ocular toxicity, and has been previously shown to be non-toxic in chick neurones (Kato et al., 1983) and human embryonic kidney cells (Salvetti et al., 2017). It has been used previously in humans to track sub-retinal tPA delivery visually (Khan et al., 2017).

8.2.7 Contraindication to use of Actilyse

Actilyse is used routinely for the management of occlusive retinal vascular events, such as acute myocardial infarction and cerebral vascular accidents. It has been used for more than a decade in the management of sub-macular haemorrhage. Doses of up to 50 micrograms have been demonstrated to be safe in animal models (McAllister, 2006).

The investigator does not believe that fluorescein sodium will react adversely with sodium chloride or Actilyse. Its pH at 7.8 (5% solution) is within the ideal range for tPA activity and a combination has previously been reported as being used for sub-retinal injection in 6 patients in a study at Will Eye Hospital, Philadelphia (Khan, 2017). Any adverse outcome will be logged, though these are anticipated to be related to the disease process and/or the surgical procedure, rather than due to the study protocol itself.

8.3 Recording of AEs and SAEs

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g. hospital progress notes, laboratory, and diagnostic reports) relative to the event. The investigator/ site staff will then record all relevant information regarding an AE/SAE in to an electronic case report form (eCRF). Any SAE documentation forwarded to the TGA/HREC will be de-identified, maintaining patient anonymity. For each AE, start and stop dates, action taken, outcome, intensity (see Section 8.2) and the relationship to the study treatment and causality must be documented.

The investigator will attempt to establish a diagnosis of the event, based on signs, symptoms, and/or other clinical information. In the absence of diagnosis, the individual signs/ symptoms should be documented.

9 Statistical Considerations and Safety Monitoring

Although the small sample size will make statistical comparisons difficult, if at any point there is a suggestion of one method being clearly superior to the other ($P = 0.01$), the trial will be terminated early.

9.1 Electronic Case Report Form (eCRF)

An eCRF will be completed for each patient for each study visit, summarising all of the screening and study data. Patients will be referred to by their study identification code, only to retain confidentiality.

10. Storage and Archiving of Study Documents

10.1 Storage and Identification

Data will be stored in re-identifiable form so that pertinent clinical data (baseline vision, diagnosis, final visual outcome) can be checked against hospital records. Data will be anonymised and pooled with

summary statistics presented. The unique participant code will be accessible and known only to the Principal Investigator, Sub-Investigator/s and study co-ordinator.

To ensure the security of information and avoid misuse, loss or unauthorised access to stored information during and after the study, printed information and paper case report forms will be stored in a locked office and computer files will be encrypted and stored on a password protected server.

10.1.1 Withdrawal

Should the participant wish to withdraw from the study, they will be free to do so and to decide what happens to their data. However, some analyses will be conducted on pooled samples, and if this is the case, that portion of the data will not be able to be destroyed.

10.2 Archiving

Information will be stored after the completion of the study for 5 years in order to allow for appropriate analysis and publication/dissemination of results.

10.3 Disposal

Data pertaining to the study would continue to be treated under the same protocols as described above in the event that the principal investigator ceases to be engaged at the planned organisation site. Information will be securely destroyed 5 years following cessation from any cause.

11. Administration Procedures

11.1 Ethical Considerations

This study will adhere to the tenets of the Declaration of Helsinki, the NHMRC National Statement on Ethical Conduct in Research Involving Humans (2007) and the Notes for Guidance on Good Clinical Practice as adopted by the Australia Therapeutic Good Administration (2000) and the ICH GCP Guidelines.

11.2 Human Research Ethics Committee

The protocol will be submitted for approval by the study site's Human Research Ethics Committee (HREC) and written approval obtained before volunteers are recruited and patients are enrolled. It is the responsibility of the investigator to report study progress to HREC as required or at intervals not greater than one year. The Principal Investigator or his nominee will be responsible for reporting any SAEs to HREC as soon as possible and in accordance with local guidelines.

11.3 Protocol Amendments

Protocol modifications that impact on patient safety or the validity of the study will be approved by HREC. If such amendments necessitate a change to the Informed Consent Form, the revised form prepared by the investigator must be approved by HREC.

The original signed copy of amendments will be kept in the study file with the original protocol. It should be noted that where an amendment to the protocol substantially alters the study design or the potential risks to the patients, each patient's consent to continue participation should be obtained.

11.4 Protocol Compliance

The instructions and procedures specified in this protocol require diligent attention to their execution. If any patient is treated in a manner that deviates from protocol, the nature and reasons for the protocol violations shall be recorded as a note to file and kept in the study site study files. The investigator and designees will comply with all applicable federal, state and local laws.

12 References

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