

TGF- β s in the eye of patients with neovascular age-related macular degeneration

Submission date 08/01/2018	Recruitment status No longer recruiting	☐ Prospectively registered ☐ Protocol
Registration date 12/01/2018	Overall study status Completed	☐ Statistical analysis plan ☒ Results
Last Edited 10/12/2018	Condition category Eye Diseases	☐ Individual participant data

Plain English summary of protocol

Background and study aim

Wet age-related macular degeneration (AMD) is the commonest cause of blindness in developed countries. Many proteins (called cytokines) are thought to be responsible for this disease. One of these (VEGF-A) is the target of the current therapy. With this study, we want to clarify the role of the cytokines called TGF-beta in the disease to better understand the causes and to develop a more effective therapy.

Who can participate?

Patients aged 50 and over with AMD and cataract patients as controls.

What does the study involve?

Participants affected by AMD receive, as standard treatment, a loading dose of three injections over a three month period. Before each treatment a small sample of aqueous humor (the eyeball is filled with this liquid) is collected by a syringe from the anterior chamber of the eye. Cataract controls are subjected to sample collection just before the planned cataract surgery.

What are the possible benefits and risks of participating?

Study participation may not directly help patients but the information obtained from the study may help to improve the knowledge of the disease and consequently the treatment of people with wet AMD in the future. This is a study embedded in the routine clinical procedures without any additional treatment (except the sample collection), therefore there should be no risks or disadvantages to patients taking part. All participants receive the same treatment as prescribed by their physicians.

Where is the study run from?

Ophthalmology Unit of the Department of Medicine, Surgery and Neuroscience, Siena University Hospital (Italy)

When is the study starting and how long is it expected to run for?

June 2016 to December 2016

Who is funding the study?
University of Siena

Who is the main contact?
Prof. Gian Marco Tosi
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Contact information

Type(s)
Scientific

Contact name
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Additional identifiers

Protocol serial number
AMD001.30

Study information

Scientific Title
TGF- β concentrations and activity in the aqueous humor of patients with neovascular age-related macular degeneration before and after ranibizumab treatment

Study objectives
The aim of this study is to evaluate the protein concentration of active TGF- β 1, TGF- β 2, and TGF- β 3 in the aqueous humor of patients affected by naïve nAMD, at baseline and after intravitreal anti-VEGF-A injection by performing a luciferase-based reporter assay to test the TGF- β pathway activation by aqueous humor samples of the same patients, with the aim to clarify the role of the TGF- β family members in CNV.

Ethics approval required
Old ethics approval format

Ethics approval(s)

Primary study design

Observational

Study design

Observational single-centre case-control study

Study type(s)

Other

Health condition(s) or problem(s) studied

Age-related macular degeneration (AMD)

Interventions

We measure the concentrations of active TGF- β 1, TGF- β 2, and TGF- β 3 by ELISA in the aqueous humor of patients affected by nAMD, who received 3 consecutive monthly intravitreal injections of ranibizumab according to the routine clinical practice management. Age-matched cataract patients serve as controls. Anterior chamber taps are performed in the operating room prior to each intravitreal injection (patients) and before cataract surgery (controls). A 30-gauge needle is inserted into the anterior chamber and 0.16-0.2 mL of aqueous is collected, centrifuged at 1500 g for 20 minutes to remove cells and debris, aliquoted and frozen at -80°C until analysis. Samples are collected at baseline (before the first injection), before the second injection, and before the third injection. The same samples are used in a luciferase-based reporter assay to test the TGF- β pathway activation. To perform the latter, Lenti-X 293T cells are transfected with a plasmid carrying the NanoLuc® gene under the transcriptional control of three copies of the SMAD binding element. The plasmid pGL4.54[luc2/TK] (Promega Corp., Madison, WI), carrying the reporter luc2 gene coding firefly luciferase under the control of the constitutive HSV-TK promoter, is co-transfected as a normalizer for transfection efficiency. Cells are then treated with 10 μ l of aqueous humor of the nAMD patients, naïve or treated, or of the control samples. After 3 hours Firefly and NanoLuc luciferase activities are measured for each sample.

Intervention Type

Drug

Phase

Not Applicable

Drug/device/biological/vaccine name(s)

Ranibizumab

Primary outcome(s)

1. TGF-beta concentration is measured using ELISA kits for human TGF- β 1, TGF- β 2 (Quantikine ELISA kits #DB100B and #DB250, respectively; R&D Systems, Minneapolis, USA) and TGF- β 3 (#SEB949Hu; Cloud-Clone Corp., Houston, TX) at baseline, 1 month and 2 months (Only baseline for controls)
2. TGF- β pathway activation is measured using the transfection of Lenti-X 293T cells and the Nano-Glo® Dual-Luciferase® Reporter Assay System (#N1610; Promega Corp., Madison, WI) at baseline, 1 month and 2 months (only baseline for controls)

Key secondary outcome(s)

There were no secondary outcomes of interest.

Completion date

21/06/2017

Eligibility**Key inclusion criteria**

Case inclusion criteria:

1. Both males and females
2. Patients aged 50 and over
3. Able to provide written, informed consent to the study
4. Active neovascular AMD in study eye with evidence of leakage from CNV on fluorescein angiography.

Control inclusion criteria:

1. Both males and females
2. Patients aged 50 and over, the age of the controls is chosen not to have statistical differences with the cases
3. Able to provide written, informed consent to the study
4. Patients in the waiting list for cataract surgery

Participant type(s)

Patient

Healthy volunteers allowed

No

Age group

Senior

Sex

All

Key exclusion criteria

CASE exclusion criteria:

1. Prior treatment for neovascular age-related macular degeneration
2. Age less than 50 years old
3. Inability to comply with the study or follow up
4. Inability to perform fluorescein angiography
5. Axial length greater than 26 mm
6. Any previous ophthalmic surgery except cataract removal (cataract surgery had to have been performed at least 9 months prior to inclusion)
7. Any other ocular disease other than neovascular age-related macular degeneration
8. Diabetes mellitus, use of immunosuppressive drugs and a malignant tumor in any location

Control exclusion criteria:

1. Any other ocular disease other than cataract
2. Any previous ophthalmic surgery

3. Age less than 50 years old
4. Axial length greater than 26 mm
5. Diabetes mellitus, use of immunosuppressive drugs and a malignant tumor in any location

Date of first enrolment

01/06/2016

Date of final enrolment

21/12/2016

Locations

Countries of recruitment

Italy

Study participating centre**Siena University Hospital**

Ophthalmology Unit of the Department of Medicine

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Sponsor information

Organisation

University of Siena

ROR

<https://ror.org/01tevnk56>

Funder(s)

Funder type

University/education

Funder Name

University of Siena

Results and Publications

Individual participant data (IPD) sharing plan

The datasets generated during and/or analysed during the current study are/will be available upon request from Dr. Galvagni Federico

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IPD sharing plan summary

Available on request

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
Results article	results	23/05/2018		Yes	No