ISRCTN60434145 https://doi.org/10.1186/ISRCTN60434145

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

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

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
gmtosi18@gmail.com

Contact information

Type(s)
Scientific

Contact name
Prof Gian Marco Tosi

Contact details
Ophthalmology Unit
Department of Medicine, Surgery and Neuroscience
University of Siena
Viale Bracci, 1
Siena
Italy
53100
+39 0577 586162
gmtosi18@gmail.com

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-β1, TGF-β2, and TGF-β3 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)

The Ethics Committee Regione Toscana Area Great South East (CEAVSE), 20/04/2015, ref: HTRA1_GDF6_2015

Study design
Observational single-centre case-control study

Primary study design
Observational

Study type(s)
Other

Health condition(s) or problem(s) studied
Age-related macular degeneration (AMD)

Interventions
We measure the concentrations of active TGF-β1, TGF-β2, and TGF-β3 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 costitutive HSV-TK promoter, is co-transfected as a normalizer for transfection efficiency. Cells are then treated with 10 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-β1, TGF-β2 (Quantikine ELISA kits #DB100B and #DB250, respectively; R&D Systems, Minneapolis, USA) and TGF-β3 (#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
Surgery and Neuroscience
Viale Bracci, 1
Siena
Italy
53100

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
Department of Biotechnology Chemistry and Pharmacy
University of Siena
via Aldo Moro, 53100 Siena, Italy
e-mail: federico.galvagni@unisi.it

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