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Phase I trial on robot assisted retinal vein cannulation with ocriplasmin infusion for central retinal vein occlusion.

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Complete List of Authors:	Willekens, Koen; UZLeuven, Ophthalmology Gijbels, Andy; KULeuven, Mechanical engineering Smits, Jonas; KULeuven, Mechanical engineering Schoevaertds, Laurent; Katholieke Universiteit Leuven Blanckaert, Johan; UZLeuven, Ophthalmology Feyen, Jean; ThromboGenics NV Reynaerts, Dominiek; Katholieke Universiteit Leuven, Mechanical engineering Stalmans, Peter; UZLeuven, Ophthalmology
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RVC CRVO movie.mov movie 2 RVC CRVO.mov	

ACTA-19-09-1176.R1

Minor Review: Phase I trial on robot assisted retinal vein cannulation with ocriplasmin infusion for central retinal vein occlusion.

Dear chief editor dr. Stefansson,
Dear associate editor dr. Kaarniranta,
Dear colleague,

We thank the reviewers for their valuable comments. Each remark is highlighted below and the adaptations to the manuscript clearly described. Additional video files were added separately.

Reviewer: 1

- Really interesting paper with a good video showing flow from the cannula into the Hemi Retinal Vein. More videos on demand would be helpful-- Show the cannula being removed from the vein (to prove there isn't major hemorrhage after removal) and demonstrate what the robotic structures look like outside the eye.

Reply: a second video showing removal of the cannula is added to the paper. Following was added to the manuscript discussion section explaining the importance of the shape of the needle-tip and movement while retracting from the vein:

"The shape and tip design of the microneedle result in minimal vessel wall damage illustrated by the absence of a severe intra-ocular hemorrhage after removal of the tip from the vein (movie 2)."

- The most significant aspect of this research is the ability to reproducibly cannulate and inject substances into a retinal vein. Please discuss the possibility (or challenges) of BRVO cannulation as BRVO is more prevalent and the occlusion site is visible. Re-established flow could probably be demonstrated immediately.

Reply: We thank the reviewer for this valuable comment and have made following additions to the discussion section of the manuscript:

"This would be of particular interest in branch retinal vein occlusion (BRVO) patients. Here the obstruction site is usually visible and the effect of re-established flow would be an important intra-operative finding determining the time and volume of infusion. As a BRVO is situated more to the periphery and thus in smaller veins or at arterio-venous crossings, cannulating these vessels remains a challenge, even with robotic assistance. Future trials could include BRVO patients with extend macular edema but will also benefit from a larger useful working space of the robot and better visualization outside the macula."

- Delete the radial optic neurotomy references and comments unless you want to state it was a failed therapy with no reprodicible results from Opremcek's original reports

Reply: we agree with the reviewer and made following adaptations to the manuscript:

"Opremcak et al. have reported good results with radial optic neurotomy where an incision in the nasal part of the optic nerve could result in an increased intravenous lumen by providing more space for the CRA and CRV. (Opremcak et al., 2006) However, others were not able to reproduce these results and optic neurotomy has been largely abandoned as therapy for CRVO."

- You might comment on why case 2 was chosen. 0.4 Log MAR with no edema on OCT images provided? And it seemingly was a disaster with 1.3 Log MAR post operatively. Why was the vision decreased? I'm assuming the RVO got worse? Probably future cases should be limited to LogMar 1.0 or worse until the technique is refined.

Reply: We agree with the reviewer that the inclusion and evolution of case 2 needs more explaining. In brief, the patient was referred to our center very rapidly (only experienced symptoms for 1 day) and her fundus did show hemorrhages 360° together with a delayed venous filling time on Fluo-angiography. We believe that she presented too soon to have already developed significant macuar edema like the other patients. Furthermore, during follow up she developed signs of an arteriovenous occlusion (NAION-like) resulting in decreased vision.
To clarify this in the manuscript following as added to the discussion section:

"Although visual acuity did not change overall, the first patient experienced a clinically significant increase in vision and the second case experienced a drop in visual acuity postoperatively. This was most likely due to the development of an arteriovenous anterior ischemia of the optic nerve postoperatively."

- The concept of ischemic and non-ischemic is outdated. All RVOs with edema have to have some ischemia or there wouldn't be VEGF production. And Case 1,3,and 4 were all count fingers so probably these were all "pre-proliferative". Was there an RAPD in these cases? That is the most sensitive and specific predictor of RVOs with a poor visual potential and the likelihood of neovascularization. Please discuss.

Reply: We agree with the reviewer that an RAPD is a powerful clinical sign with prognostic value regarding retinal ischemia and corresponding visual field and acuity. Unfortunately this was not adequately tested and reported preoperatively. To highlight the importance of this clinical sign we have added following statement to the discussion section:

"Future trials should include a more profound clinical investigation with testing of a relative afferent pupillary defect (RAPD) because this is a reliable predictor for visual potential and the likelihood of the development of neovascularization"

- Longer term follow-up would be ideal. While 1/3 of cases improve spontaneously, if 3/4 resolve, then this supports further work including a randomized trial. This trial could be enriched by including eyes with recurrent edema after 3-6 months of anti-VEGF therapy but this may be too late to have an effect on the clot. Please discuss your thoughts on timing. All of these cases were acute. Is this critical?

Reply: We agree with the reviewer that longer follow up is indeed needed to explore the clinical potential of this treatment. We however do believe that treating patients as soon as possible would be beneficial when attempting a retinal vein cannulation. The pathogenesis of CRVO is most likely multifactorial and a cannulation with thrombolysis will not change external compression on the CRV form the CRA. What we do believe is that patients developing a CRVO suffer from an acute cessation of blood flow (otherwise collaterals would have formed) and this acute event is most likely due to an acute clogging of the venous lumen. Trying to cannulate after several months probably would not change that much since the natural plasmin would have cleared the cloth by then and the predominant factor in developing the CRVO probably would be external compression or anatomic alterations. To highlight this opinion the follow statement was added to the discussion section:

"Furthermore, the timing of such a procedure could play an important role as well. As BRVO and CRVO exhibit signs of an acute cessation of blood flow, a short treatment time would be beneficial in removing the clot and re-establishing blood flow. However, contributing factors such as CRA compression on the CRV or venous thinning on arteriovenous crossings are not treated with RVC and these anatomic alterations will predispose the patient for future relapses. Secondary prevention thus remains key in the treatment and follow up."

Reviewer: 2

- Introduction: Too redundant, especially 1st paragraph. Please shorten this section.

Reply: we agree with the reviewer and have shortened the first paragraph of the introduction section.

- Methods: Too redundant. The authors did not need to say anything about Optos Wide-field laser camera, because they did not provide any Optos Data in the current study.

Reply: we agree with the reviewer and have omitted the statement about Optos fundus photography.

- Inclusion criteria: The contralateral eye should have a BCVA <1.0 logMAR. 1.0 logMAR VA equals to 20/200 Snellen VA, which is very poor. Is this inclusion criteria appropriate?

Reply: we agree that the required minimal visual acuity in the contralateral eye for inclusion has been set at a low level. This was done in accordance to the inclusion criteria proposed by Van Overdam et al. (2015) that stated that monoftalmia was an exclusion criterium. As a VA below 20/200 is seen as legally blind, the contralateral eye should thus see better than 1.0 logMar.

- Please provide the recruitment period of the participants. Is this study a consecutive case series?

Reply: the recruitment period was added to the methods section:

"The recruitment period ran from December 2016 to August 2017."

- Please provide the method how to decide the punctuation site of the retinal vein for REVS.

Reply: we thank the reviewer for this interesting question as this is a surgeon's decision based on careful pre-operative planning and understanding the range of motion and optimal 'angle of attack' of the angulated microneedle. In brief, the robot is positioned temporally so the best candidate is either the superior or inferior temporal hemivein running temporal. Close the the disc these veins howver have te tendency to run superior and inferior, respectively. Because of the occurrence of vascular tortuositas in CRVO, pre-operative fundus fotography and fluo-angiography help in determining that part of the hemivein that is as close to the optic disc as possible but also is almost parallel to the papillomacular axis. To clarify this in the manuscript the following was added to the discussion section:

"The robot is positioned temporally so the best puncture site lies either on the superior or inferior temporal hemivein running temporal. In view of the larger size, the puncture site is preferably as close as possible to the optic disc. However, the hemiveins usually have the tendency to first run superior and inferior before starting to curve towards the temporal periphery. Because of the occurrence of vascular tortuositas in CRVO, pre-operative fundus photography and fluo-angiography help in determining that part of the hemivein that is as close to the optic disc as possible but also is almost parallel to the papillomacular axis."

Title: Phase I trial on robot assisted retinal vein cannulation with ocriplasmin infusion for central retinal vein occlusion.

Running title: robot-assisted RVC with ocriplasmin

Authors: 1) Willekens Koen[1], MD, PhD

2) Andy Gijbels[2], PhD

3) Jonas Smits[2]

4) Laurent Schoevaerdts[2]

5) Johan Blanckaert, MD[1]

6) Jean H.M. Feyen[3], PhD

7) Dominiek Reynaerts[2], PhD

8) Peter Stalmans[1], MD, PhD

Affiliations:

1) Department of Ophthalmology, University Hospitals Leuven, Leuven, Belgium.
2) Department of Mechanical Engineering, University of Leuven, Leuven, Belgium
3) ThromboGenics NV, Leuven, Belgium

Corresponding author:

Peter Stalmans, MD, PhD.

Department of Ophthalmology

University Hospitals Leuven, Campus St Raphaël

Herestraat 49

B-3000 Leuven, Belgium

tel: +32-16-33.26.60; fax: +32-16-33.23.67

e-mail: peter.stalmans@uzleuven.be

Conflict of interest: A. Gijbels, J. Smits, L. Schoevaerdts, and D. Reynaerts are investigating the exploitation potential of the developed robotic technology. Disclosures of Peter Stalmans: AMO – Johnson&Johnson: speaker fee & travel reimbursement, Bausch + Lomb: advisory board + consultancy, DORC: consultancy, Haag-Streit: travel reimbursement, Nano-Retina: consultancy, Ophtec: consultancy, ReNeuron: advisory board, Vitreq: consultancy, Thrombogenics: research grant, Zeiss: consultancy.

Abstract

Purpose:

To evaluate the safety and feasibility of robot-assisted retinal vein cannulation with Ocriplasmin infusion for central retinal vein occlusion.

Methods:

Prospective phase I trial including 4 patients suffering from central retinal vein occlusion (CRVO). Diagnosis was confirmed by pre-operative fluo-angiography and followed by a standard three-port pars plana vitrectomy. Afterwards, a custom-built microneedle was inserted into a branch retinal vein with robotic assistance and infusion of Ocriplasmin started. Primary outcomes were the occurrence of intraoperative complications and success of cannulation. Secondary outcomes were change in visual acuity, central macular thickness (CMT) and venous filling times (VFT) during fluo-angiography two weeks after the intervention.

Results:

Cannulation with infusion of ocriplasmin was successful in all 4 eyes with a mean total infusion time of 355±204sec (range 120 – 600 sec). Best corrected visual acuity (BCVA) remained counting fingers (CF) in case 3 and 4, increased in case 1 from CF to 0.9LogMAR and decreased in case 2 from 0.4 to 1.3 LogMAR. CMT and VFT both showed a trend towards significant decrease comparing pre-operative measurements with two weeks post-intervention (1061±541micron versus 477±376micron, p=0.068) and 24±4sec versus 15±1sec, p=0.068, respectively).

In one eye a needle tip broke and could be removed with an endoforceps. There were no other intervention-related complications.

Conclusion:

Robot-assisted retinal vein cannulation is feasible and safe. Local intravenous infusion with Ocriplasmin led to an improved retinal circulation.

Keywords: robot-assisted intraocular surgery, retinal endovascular surgery, retinal vein cannulation, Ocriplasmin, retinal vein occlusion

Introduction

Central retinal vein occlusion (CRVO) occurs when a blood clot clogs the outflow pathway of the retinal circulation (Vein, 1997). It is hypothesized that the obstruction occurs where the central retinal vein (CRV) enters the lamina cribrosa and that retinal arterial disease could have a significant impact because of the close proximity of the central retinal artery (CRA) and –vein within the optic nerve (Jefferies *et al.*, 1993 & Zhao *et al*., 1993). With ageing, the prevalence and incidence of retinal vein occlusion grow significantly (Rogers *et al.*, 2010, Kolar, 2014). There is currently no widely accepted curative treatment as most patients receive intravitreal injections with antiVEGF or corticosteroids, retinal laser photocoagulation or even vitrectomy to treat secondary complications like macular edema, retinal ischemia and vitreous hemorrhages (Hayreh, 2003 & Berger et al. 2015). Many did investigate the possibility of dislocating or dissolving the blood clot in the central retinal vein. Opremcak et al. have reported good results with radial optic neurotomy where an incision in the nasal part of the optic nerve could result in an increased intravenous lumen by providing more space for the CRA and CRV.

(Opremcak et al., 2006) However, others were not able to reproduce these results and optic neurotomy has been largely abandoned as therapy for CRVO. (Arevalo et al., 2008; Yamamoto et al. 2004; McAllister & Constable, 1995). Some targeted a thrombolytic effect by injecting recombinant tissue plasminogen activator (rtPA) either intravitreally, local (in the CRV) or systemically intravenously with varying success (Glacet-Bernard et al., 2000; Hattenbach et al., 2009; Weiss & Bynoe, 2001). Especially the local intravenous administration of a thrombolytic drug via retinal vein cannulation (RVC) seems the most appealing option to have abundant exposure of the blood clot to the drug while minimizing possible systemic complications. Weiss & Bynoe (2001) reported excellent results using a glass micropipette to inject rtPA into a branch retinal vein. However, their results could not be confirmed by Feltgen et al. who reported having suffered from significant difficulties and complications (Feltgen et al., 2007). They state to have erroneously perforated the vein injecting intra- or subretinal and often needed more than one piercing attempt.

Taking into account the involuntary movements of any well-trained microsurgeon, the task of cannulating a vein with a width of approximately 150µm remains a challenging task (Singhy & Riviere, 2002). Furthermore, holding the tip of the needle inside the vein without strain on the vessel wall or surrounding tissue for several minutes is almost beyond human capabilities. To overcome these technical difficulties linked to retinal endovascular surgery (REVS), a surgical robot was designed to increase the surgeon's precision and stability (Gijbels et al., 2018). Furthermore, a microneedle with an outer diameter of 30 microns and an angulated 500-micron tip was constructed to allow a more stable intravenous position. Both the robot and the needle were tested in a porcine retinal vein occlusion model showing that prolonged RVC with local intravenous medication infusion up until 10 minutes is possible (Willekens et al. 2017).

To further increase the chance of successfully removing the blood clot, the use of active plasmin was preferred over rtPA. As the fibrinolytic action of rtPA depends on the amount of

plasminogen that can be converted to plasmin, direct exposure of the clot to a high dose of active plasmin could, theoretically, improve clot lysis (Gurman et al., 2015). Ocriplasmin is the smaller active portion of the native plasmin molecule and is registered for intra-ocular use to treat vitreomacular tractional disease (Stalmans et al. 2012). Its fibrinolytic activity has been investigated by others showing similar thrombolytic effectiveness in respect to rtPA and an acceptable safety profile when administered intravenously in large doses (Verhamme et al., 2012; Dommke et al., 2010; Thijs et al., 2009).

To overcome the reported technical difficulties related to REVS and maximize the chance of successful clot lysis, this trial thus aims to investigate the safety and feasibility of robot-assisted RVC with ocriplasmin infusion for the treatment of CRVO.

Methods

Investigator-initiated, single-arm, mono-center, prospective, interventional case series phase I trial including patients older than 18 years of age presenting with a recent (symptoms of decrease in vision or metamorphopsia <10 days) CRVO willing to sign informed consent. The recruitment period ran from December 2016 to August 2017. To fulfil inclusion criteria, the affected eye should have a best corrected visual acuity (BCVA) >0.3 LogMAR (logarithm of minimal angle of resolution) and macular edema (central macular thickness (CMT) > 250µm) while the contralateral eye should have a BCVA <1.0 LogMAR. Exclusion criteria consisted of previous intraocular surgery, concomitant eye disease decreasing visual acuity except for cataract, fluorescein allergy, active neovascularization and high myopia (<-10 Dioptres). This study was approved by the ethical review committee (institutional review board) at the University Hospitals Leuven and was conducted in accordance with good clinical practice within the tenets of the Declaration of Helsinki. It was registered on clinicaltrials.gov (NCT

02747030) and the off-label use of Ocriplasmin and the experimental robotic device were approved by the Belgian Federal agency for medicines and health products (FAMHP). Being a phase I study investigating the safety and feasibility of robot-assisted RVC with ocriplasmin infusion, a follow up of 2 weeks postoperatively was determined in the study protocol and approved by all review committees. All records were filed in an electronic study record (Open Clinica (Smeets & Visser, 2011) and exported to perform data analysis. According to Storer, a bi-phasic phase I study design was chosen with an interim analysis of the data of the first 3 included patients by the independent data monitoring committee before permission could be granted to start the second phase of inclusion (Storer, 1989).

The primary endpoints of this trial are 1) technical success to cannulate a retinal vein and infuse Ocriplasmin, 2) the number of intervention-related complications and 3) duration of infusion. Secondary outcomes consisted of change in BCVA, central macular thickness (CMT) and fluo-angiography assessed venous filling time (VFT).

Pre-operative investigations

A comprehensive ophthalmological investigation including medical history taking, measurement of BCVA, intraocular pressure (IOP) and axial length in phakic eyes together with biomicroscopy and fundoscopy was performed. Retinal imaging using a 55° (Topcon TRC-50DX, Topcon Europe Medical NV, Capelle aan den Ijssel, The Netherlands), spectral domain-optical coherence tomography (SD-OCT, Cirrus 5000, Carl Zeiss Meditec AG, Zaventem, Belgium) and fluo-angiography (FA) (Heidelberg Spectralis, Heidelberg, Germany) was done to assess macular edema, measure the central macular thickness and retinal circulation times. VFT was calculated as the difference between the occurrence of fluorescein dye in the retinal arteries and the first moment of complete temporal venous filling, as described elsewhere (Hansen et al., 1989). If indicated, patients were referred to internal medicine colleagues to

screen for hematologic abnormalities in coagulation or fibrinolysis. The experimental nature of the study was explained to all patients and surgery was planned only after signing of the informed consent form and approval of the anesthesiologist for general anesthesia induction.

Surgical intervention

All patients were operated under general anesthesia. In phakic eyes, a cataract surgery preceded a standard three-port23 Gauge pars plana vitrectomy. A twinlight chandelier was installed and a fourth, enlarged sclerotomy was made with a 20G (Gauge) MVR blade (Laseredge 20G MVR blade, Bausch & Lomb Incorporated, Rochester NY, USA). A custom-built 18G trocar was inserted and sealed with a silicone valve (DORC closure valves 20G, Zuidland, The Netherlands). The remote center of motion of the robotic device was aligned with the sclerotomy and the position stored. The alignment tool was exchanged for a microneedle connected to a 3ml syringe (BD, Erembodegem, Belgium) filled with Ocriplasmin 1.25mg/ml. The syringe was installed into a flow controlled pump (UltraMicroPump, World Precision Instruments Inc., Florida, USA) and the microneedle was inserted into the vitreous cavity. The protective hull was retracted to expose the needle tip. A retinal hemi-vein close to the optic disc was approached and an RVC performed (video). Infusion was done in an on-/off pattern allowing refilling of the vessel with blood. This was continued as long as possible for a maximum of 10 minutes. A fluid-air exchange was performed and the sclerotomies closed with vicryl 7-0 suture. A parabulbar injection with a mixture of triamcinolone (Volon) and clindamycin (Lincocin) was done at the end of each surgical intervention.

Postoperative follow-up

Standard postoperative care comprised of a first check of BCVA and IOP together with biomicroscopy and fundoscopy the first postoperative day. This was repeated on day 2 or 3 and

after one to two weeks, depending on how fast the air bubble in the posterior chamber disappeared. At this last postoperative within-study visit, the OCT-scan and fluo-angiography were repeated.

Adhering to the standard post vitrectomy schedule, all patients had an additional visit after 6-8 weeks. Afterwards, they were followed up by our medical retina department or by their own ophthalmologist.

The robotic device and micro-needle

The surgical robot is designed to improve the stability and accuracy of the surgeon. It will always pivot the instrument it is holding around a certain point in space; the remote center of motion (RCM). Using an alignment tool (the tip of this instrument coincides with the RCM) to position the robot relatively to the sclerotomy, the technology assures stabilizing the eye by always pivoting the instrument around the sclerotomy site while movements in 4 dimensions remain possible. To further increase safety and learning curve of the surgeon, the robot was handled in a co-manipulation setting. The instrument entering the eye is held by the surgeon and the robot at the same time. Only by exerting force on the instrument by the surgeon, the instrument will move. The robot is thus to be regarded as a true surgical assistive stabilizer.

The surgeon can lock or unlock the robotic device by using a foot pedal. The robot (and instrument) can be fixated in a certain position, obviating the need for manually holding the instrument. To improve access to and visualization of the cannulation site, the entire robot (and thus the RCM) could be moved during surgery in the horizontal plane with the use of a separate foot pedal.

The glass microneedle has a 30° angulated 0.5mm tip with an outer diameter of 30 microns. To protect the glass shaft a retractable metal hull (18G) was installed around it. A micro-infusion

line connected both the Luer lock of a 3ml syringe and the Luer lock at the back end of the needle. The total dead volume of the complete infusion system (infusion line + needle) was approximately 0.5cc.

Statistical analysis

Data were exported from the electronic case report form (Open clinica) into Microsoft excel. Statistical analysis was done using SPSS 24.0. Because of the small amount of data non-parametric related samples, Wilcoxon signed rank tests were used to analyze changes in continuous data. Statistical significance was considered when the two-sided p-value is below 0.05. Values are depicted as mean ±Standard Deviation (SD).

Results

Four eyes of 4 patients suffering from a clinical significant decrease in BCVA due to a recent CRVO were included. Table 1 depicts the demographic characteristics. Preoperative OCT showed an averaged increased CMT (1061±561μm) and fluo-angiography revealed delayed venous filling times (24±4seconds). Figure 1 depicts the pre-operative color fundus, OCT- and FA image of all 4 cases.

RVC was successful in all eyes with a mean total infusion time of 355±204sec (range 120 – 600 sec). Movie 1 shows the sclerotomy set up and intra-operative effect of thrombolysis. In three eyes the inferior hemivein was cannulated, in the fourth the superior. Table 2 depicts the changes in BCVA, CMT and VFT pre- versus two weeks postoperative. BCVA remained counting fingers (CF) in case 3 and 4, increased in case 1 from CF to 0.9LogMAR and decreased in case 2 from 0.4 to 1.3 LogMAR. OCT data showed a trend towards a significant decrease of

the CMT (1061±541micron versus 477±376micron, p=0.068). The same applies to fluo-angiography assessed VFT (24±4sec versus 15±1sec, p=0.068).

In one eye a needle tip broke and could be removed with endoforceps. There were no other intervention-related complications.

Discussion

Robot-assisted retinal vein cannulation with prolonged (up to 10 minutes) infusion time is feasible in eyes of patients with a CRVO. The robot dampens out the physiologic tremor and significantly increases precision and stability in order to insert the microneedle into a branch retinal vein and hold it in place for several minutes. By infusing Ocriplasmin, the blood column inside the vessel is flushed towards the optic disc (movie 1). This observation confirms the successful puncture and allows the thrombolytic drug to start interacting with the blood clot resulting in its lysis. The angulation of the microneedle results in a directional flow of ocriplasmin according to the flow direction of retinal veins. Injecting in an on-off pattern allows for refilling of the vessel with blood and decreases the likelihood of pushing the ocriplasmin in the opposite direction and thus avoiding iatrogenically increased retinal edema and hemorrhages. The shape and tip design of the microneedle result in minimal vessel wall damage illustrated by the absence of a severe intra-ocular hemorrhage after removal of the tip from the vein (movie 2). Careful pre-operative planning also aids to the success rate of the surgery. The robot is positioned temporally so the best puncture site lies either on the superior or inferior temporal hemivein running temporal. In view of the larger size, the puncture site is preferably as close as possible to the optic disc. However, the hemiveins usually have the tendency to first run superior and inferior before starting to curve towards the temporal periphery. Because of the occurrence of vascular tortuositas in CRVO, pre-operative fundus

photography and fluo-angiography help in determining that part of the hemivein that is as close to the optic disc as possible but also is almost parallel to the papillomacular axis.

The total duration of infusion differed between study subjects and ranged from 2 minutes up to 10 minutes. This is due to the physiologic movements of the retina and head of the patient in view of the cardiac cycle and respiration. The glass tipped microneedle has a certain degree of flexibility and compensates for small movements but the infusion had to be stopped and the needle retracted when too much strain with significant bending of the needle tip was observed during surgery. The impact of tissue movement on the quality of intra-ocular surgery has recently been explored by Brogan et al., 2018. By stabilizing the eye itself at the sclerotomy, larger eye movements are filtered out by the use of the robot. Future research should thus further investigate the possibility of automatic compensation using, for instance, intra-operative and/or intra-ocular OCT measurements of retinal vessel movements. Moreover, a measurement of the venous and arterial blood flow velocity intra-operatively would help to guide the surgeon decision making in view of duration and total dosage of infused thrombolytic drug.

During the trial, there were no technical failures of the robotic device but one needle tip breakage did occur. Due to the coating of the glass tip it remained well visible for the surgeon and removal with endoforceps was possible. The puncture site showed almost no hemorrhage after retraction of the needle (movie 2). During the postoperative follow up no secondary hemorrhage nor a secondary venous obstruction from the puncture site occurred indicating minimal damage to the vessel wall and good retinal tamponade with an air bubble.

Although the visual acuity did not change overall, the first patient experienced a clinically significant increase in vision and the second case experienced a drop in visual acuity postoperatively. This was most likely due to the development of an arteriovenous anterior

ischemia of the optic nerve postoperatively. Future trials should include a more profound clinical investigation with testing of a relative afferent pupillary defect (RAPD) because this is a reliable predictor for visual potential and the likelihood of the development of neovascularization. (Rong et al., 2019) The anatomy did change favorably with a trend towards a significant decrease in CMT and VFT. As vitrectomy alone could have a significant effect on the presence of inflammatory factors in the vitreous cavity and a significant change in the oxygen distribution in the posterior segment of the eye (Eliasdottir et al. 2015 & Stefansson, 2001), a decrease in macular edema is to be expected by vitrectomy alone (Tachi et al., 1999). The trend towards a significant decrease in VFT does, however, support the additional benefit of retinal vein cannulation indicating a restored blood flow after the intervention. Future research should focus on the intra-operative assessment of blood flow as a guide for treatment duration and success.

The particular and located thrombolytic effect of intravenous ocriplasmin is shown in case 3. The inferior hemivein was cannulated and ocriplasmin infused in the direction of the optic disc. Post-surgery the color fundus photographs showed a significant decrease in retinal hemorrhages of the superior hemisphere in respect to the inferior part of the retina (figure 2). Furthermore, FA filling times were lower for the superior hemivein with respect to the inferior retinal circulation. These findings indicate a localized thrombolytic effect effectively removing the clots from the central retinal vein and the superior vein, but not the cloth expanding more peripheral from the puncture site. Future treatments should be guided by intra-operative blood flow measurement in order to determine if a second cannulation site is necessary to remove as much thrombus as possible. This would be of particular interest in branch retinal vein occlusion (BRVO) patients. Here the obstruction site is usually visible and the effect of re-established flow would be an important intra-operative finding determining

the time and volume of infusion. As a BRVO is situated more to the periphery and thus in smaller veins or at arterio-venous crossings, cannulating these vessels remains a challenge, even with robotic assistance. Future trials could include BRVO patients with extend macular edema but will also benefit from a larger useful working space of the robot and better visualization outside the macula. Furthermore, the timing of such a procedure could play an important role as well. As BRVO and CRVO exhibit signs of an acute cessation of blood flow, a short treatment time would be beneficial in removing the clot and re-establishing blood flow. However, contributing factors such as CRA compression on the CRV or venous thinning on arteriovenous crossings are not treated with RVC and these anatomic alterations will predispose the patient for future relapses. Secondary prevention thus remains key in the treatment and follow up.

Visualization of the desired puncture site proved to be fairly difficult, especially when working at maximal zoom with the operating microscope, reducing the amount of light emitted through the oculars. Future studies should use the most modern microscopes possibly equipped with a 3D heads up surgical camera system allowing for digital enhancement of the intra-operative images. Possibly this approach could be combined with a high definition intra-ocular endoscope allowing for detailed visualization of the targeted blood vessel, an approach described by Hattenbach et al. (2012).

Limitations of this trial consist of the small number of patients and the lack of direct measurement of the treatment effectiveness. This effectiveness was shown by the decrease in CMT and VFT, but these measurements were only possible post hoc. Therefore, a future trial should aim at real-time blood flow assessment together with improved visualization of the puncture site in combination with a wider range of the robotic device.

Conclusion

Robot-assisted retinal vein cannulation with ocriplasmin infusion as treatment for central retinal vein occlusion is feasible and safe. Both CMT and VFT showed a trend towards significant decrease two weeks after surgery indicating an effective thrombolysis. Future trials should incorporate real-time blood flow measurement to guide the treatment and improve visualization of the puncture site.

Acknowledgements

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Figure legend

Figure 1: preoperative color fundus image, optical coherence tomography image of the macula and venous phase fluo-angiography image.

Figure 2: Reduction in hemorrhages and a difference in venous filling time for the upper and lower part of the retina in case 3. The superior peripapillary hemorrhages decreased significantly as well as the VFT for the superior hemivein and the capillary filling of the superior hemisphere of the retina.

Table legend

Table 1: Demographic and pre-operative data of the 4 patients. BCVA=best correct visual acuity, IOP=intra-ocular pressure, CMT= central macular thickness, VFT= venous filling time, CRVO= central retinal vein occlusion, CF= counting fingers, LogMar= Logarithm of minimal angle of resolution

Table 2: Comparison of the pre- and postoperative Visual Acuity (VA), Central Macular Thickness (CMT) and Fluo-angiography (FA) filling time. LogMar= Logarithm of minimal angle of resolution, sec=seconds

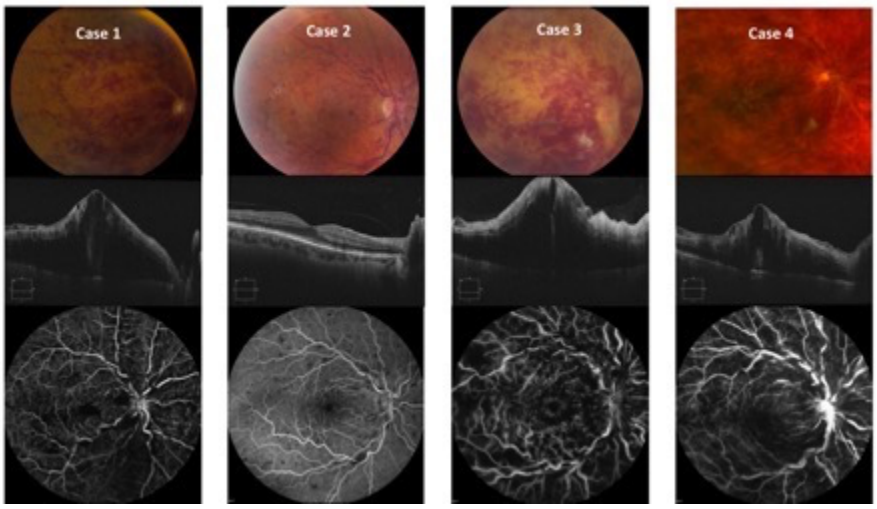

Figure 1

38x21mm (300 x 300 DPI)

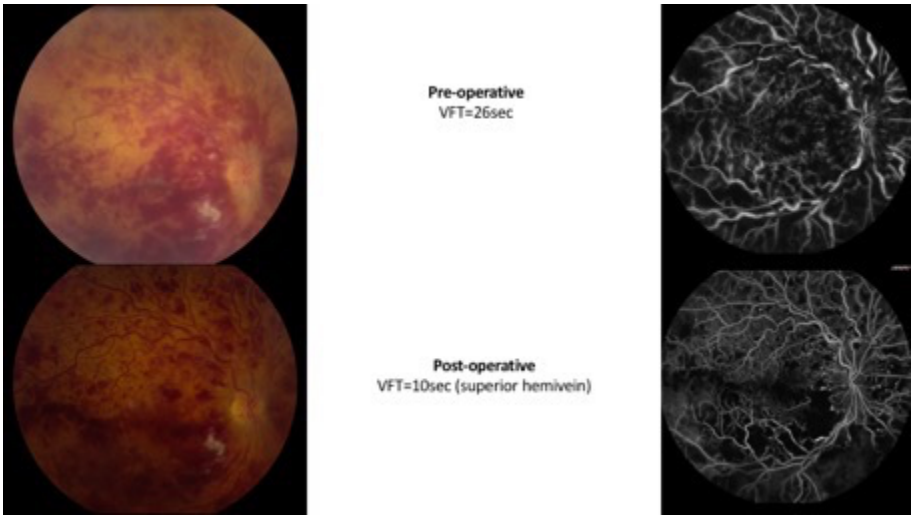

Figure 2

38x21mm (300 x 300 DPI)

table 1. Demographic and pre-operative data

	age	gender	duration of symptoms (days)	concomitant ocular disease	previous intraocular surgery	BCVA (LogMAR)	IOP (mmHg)	CMT (μm)	VFT (seconds)	ischemic CRVO	neovascularization
case 1	82	male	5	glaucoma	cataract	CF	25	1338	29	no	no
case 2	64	female	1	none	none	0.4	17	262	20	no	no
case 3	57	male	5	none	none	CF	17	1204	23	yes	no
case 4	78	male	3	none	none	CF	12	1440	22	yes	no

Table 2.

	Case 1		Case 2		Case 3		Case 4	
	pre	post	pre	post	pre	post	pre	post
VA (LogMAR)	CF	0,9	0,4	1,3	CF	CF	CF	CF
CMT (µm)	1338	178	262	161	1204	632	1440	936
FA filling time (sec)	29	15	27	11	26	10	23	19