

**Figure 2.** Cross-sectional B-scans of Patient N.2 for SD-OCT (left), PRD-OCT intensity (middle), and PRD-OCT flow (right) at postoperative days one, five, 13, six weeks, and seven months (row-wise top-down). In the SD-OCT images the vessel lumina are marked by *black arrows*, the thickness of the graft under the fovea is marked by a *square bracket*, and the vessel diameter is marked by a *black and white arrow*. In the PRD-OCT images *white arrows* show the presence of flow within the graft; black and white areas indicate blood flow, gray represents no flow. The PRD-OCT images span over roughly half the width of the SD-OCT images as can be seen by matching the retinal structures of the SD-OCT images and the PRD-OCT intensity images.

**A,B,C:** One day after surgery: *imbibition stage*. The vessels are small and the graft is thin. The vessels in the graft (black arrows) are optically more clear than the vessels of the underlying recipient choroid.

**D,E,F:** Five days after surgery. There is a visible increase of the thickness of the graft as well as the number and diameter of the vessels: *afferent stage*. Most large vessels in the graft (black arrows) are optically more clear than the vessels of the underlying recipient choroid. A few small more superiorly located vessels in the graft show a gray shading more alike to the gray shading of the recipient choroidal vessels.

**G,H,I:** 13 days after surgery. There is a reduction of thickness of the graft and of the vessels diameter inside graft compared to image **D**. Also, the vessels (black arrows) in the graft show a gray shading more alike to the gray shading of the recipient choroidal vessels, compared to the more optical clear vessels in the graft in image **2 A** or the large optically clear vessels in **2 D**. A vessel in the periphery of the graft is visible on the PRD-OCT images. The *efferent stage* has started.

**J,K,L:** Six weeks after surgery. A decrease of the thickness as well as diameter of the vessels inside the graft is clearly visible compared to 13 days after surgery. The presence of flow is shown in the PRD-OCT images.

**M,N,O:** Seven months after surgery. The thickness of the graft and diameter of the vessels remain relatively stable compared to six weeks after surgery. Flow in the graft is still present.

horizontal single B-scans were taken at various locations in the graft around the macula. In the presented images (figures 2A) 4 to 10 B-scans were averaged to improve the image quality. Additionally, several volume scans were made in order to evaluate the perfusion of the graft over a large area. The presence or absence of blood flow was registered for every visit and compared to the revascularization steps as observed in the structural imaging with the SD-OCT (Table 2).

## RESULTS

### Patients

The mean age of the seven patients was  $80.1 \pm 13.9$  years (mean  $\pm$  SD). All patients had exudative AMD. Indication for surgery in two patients (N.3 and N.4) was a submacular hemorrhage and an RPE tear; both were on anti-VEGF therapy, and patient N.4 received rtPA in an earlier surgery. Patient N.1 had developed a hemorrhage after one anti-VEGF injection, a fibrotic reaction and posterior uveitis. Patient N.2 presented with a new submacular hemorrhage after anti-VEGF and rtPA surgery. Patient N.5 had a residual hemorrhage and fibrosis after anti-VEGF injections and rtPA surgery. Patient N.6 presented with an RPE tear and macular pucker. Patient N.7 was a non-responder to anti-VEGF therapy. Four patients were on anticoagulants before surgery (three patients on platelet aggregation inhibitors; one patient on anti-coagulants).

**Table 2: Quantitative measurements of Spectral Domain-Optical Coherence Tomography Images**

<b>Patient N. 1</b>							
	Day 1 (I) (-)	Day 4 (I) (NA)	Day 7 (A) (-)	Day 15 (E) (-)	1 Month (E) (+)	3 Months (E) (+)	
Thickness of the Graft ( $\mu\text{m}$ )	234	287	361	337	299	306	
N° of Vessels	14	15	21	22	24.5	27.5	
Diameter of Vessels ( $\mu\text{m}$ )	68	68	171	128	90	94	
Fluid under the Graft ( $\mu\text{m}$ )	0	11	207	444	351	312	
<b>Patient N. 2</b>							
	Day 1 (I) (-)	Day 5 (A) (-)	Day 13 (E) (+)	6 Weeks (E) (+)	3 Months (E) (+)	7 Months (E) (+)	
Thickness of the Graft ( $\mu\text{m}$ )	182	460	350	284	340	320	
N° of Vessels	16.5	33	35.5	38.5	33.5	33	
Diameter of Vessels ( $\mu\text{m}$ )	97	272	143	117	128	125	
Fluid under the Graft ( $\mu\text{m}$ )	0	36	158	269	219	218	
<b>Patient N. 3</b>							
	Day 1 (I) (-)	Day 3 (I) (-)	Day 10 (A) (+)	Day 16 (E) (+)	1 Month (E) (+)	2 Months (E) (NA)	3 Months (E) (+)
Thickness of the Graft ( $\mu\text{m}$ )	161	300	316	277	271	267	259
N° of Vessels	11.5	24	19.5	34.5	34	32.5	35
Diameter of Vessels ( $\mu\text{m}$ )	60	52	137	69	57	66	67
Fluid under the Graft ( $\mu\text{m}$ )	52	10	129	199	163	187	195
<b>Patient N. 4</b>							
	Day 7 (A) (-)	Day 12 (E) (+)	Day 14 (E) (+)	Day 19 (E) (+)	6 Weeks (E) (+)		
Thickness of the Graft ( $\mu\text{m}$ )	200	232	228	230	244		
N° of Vessels	8	20	35	38	40		
Diameter of Vessels ( $\mu\text{m}$ )	178	117	105	98	108		
Fluid under the Graft ( $\mu\text{m}$ )	87	0	0	0	0		
<b>Patient N. 5</b>							
	Day 1 (I) (-)	Day 5 (A) (-)	Day 7 (A) (+)	Day 11 (E) (+)	Day 15 (E) (NA)	6 Weeks (E) (NA)	3 Months (E) (+)
Thickness of the Graft ( $\mu\text{m}$ )	130	242	225	215	223	176	205
N° of Vessels	14	20.5	25	23.5	20.5	23	22
Diameter of Vessels ( $\mu\text{m}$ )	70	117	134	116	109	117	112
Fluid under the Graft ( $\mu\text{m}$ )	435	415	379	286	211	201	201

## Visual Acuity

Visual acuity (VA) at baseline ranged from 20/87 to 1/300 (0.64 – 2.8 logMAR), with a median of 1.26 logMAR (20/364) and a mean of 1.32 logMAR (standard deviation [SD]  $\pm$  0.72). VA after three months ranged from 20/42 to 20/418 (0.32 to 1.32 logMAR) with

**Table 2: Quantitative measurements of Spectral Domain-Optical Coherence Tomography Images**  
(continued)

Patient N. 6													
	Day 1 (I) (-)	Day 3 (A) (-)	Day 4 (A) (-)	Day 7 (A) (-)	Day 8 (A) (-)	Day 10 (A) (+)	Day 11 (E) (+)	Day 14 (E) (+)	Day 16 (E) (+)	Day 18 (E) (+)	Day 22 (E) (+)	7 Weeks (E) (NA)	3 Months (E) (+)
Thickness of the Graft ( $\mu\text{m}$ )	93	166	181	168	176	166	142	153	146	147	143	126	129
N° of Vessels	12	19	23	23	21.5	22	23	23	22	22.5	23	23.5	23.5
Diameter of Vessels ( $\mu\text{m}$ )	62	164	157	143	129	122	101	97	97	98	93	91	88
Fluid under the Graft ( $\mu\text{m}$ )	11	11	8	7	0	0	0	0	0	0	0	0	0

Patient N. 7 (This patient was only measured once with PRD-OCT, at day 365)										
	Day 1 (I) (NA)	Day 2 (I) (NA)	Day 3 (I) (NA)	Day 4 (I) (NA)	Day 6 (I) (NA)	Day 8 (I) (NA)	Day 15 (I) (NA)	1 Month (I) (NA)	6 Months (E) (NA)	1 Year (E) (+)
Thickness of the Graft ( $\mu\text{m}$ )	109	102	104	104	98	94	90	76	159	165
/N° of Vessels	17	17	16	15	17	18	16.5	16.5	27	25.5
Diameter of Vessels ( $\mu\text{m}$ )	89	71	72	63	71	74	74	72	61	69
Fluid under the Graft ( $\mu\text{m}$ )	0	0	0	0	0	0	0	0	0	0

(I) = Imbibition stage; (A) = Afferent stage; (E) = Efferent stage; (-) = No flow detected on PRD-OCT; (+) = First time flow detected on PRD-OCT; (NA) = No PRD-OCT was performed.

a median of 0.84 logMAR (20/138) and a mean of 0.88 logMAR (SD  $\pm$  0.31). VA after six months ranged from 20/42 to 20/276 (0.32-1.14 logMAR) with a median of 0.8 logMAR (20/126) and a mean of 0.73 logMAR (SD  $\pm$  0.26). The characteristics of each patient are summarized in Table 1.

### Spectral-Domain OCT

Quantitative measurements were performed for all seven patients. However, due to poor image quality, analysis was not possible for two sets of OCT data (on postoperative day one, due to vitreous hemorrhage; and on postoperative day 90 due to emulsification of silicone oil) belonging to one patient (N.4). Six out of seven patients were examined at day one. All these six patients showed a very thin graft with few visible vessels with small diameters (imbibition stage). Between day three and day ten, five patients showed an increase in thickness of the graft, in the number of vessels and in the mean diameter of the vessels (afferent stage). Between day 11 and day 16 the thickness of the graft decreased as well as the diameter of the vessels (efferent stage) in these five patients. A slight increase in number of vessels was noted within the same time frame. One patient (N.4) with no suitable images at day one showed an increase in thickness of the graft and in the number of vessels while a decrease in mean diameter was noted between day seven and day 12. Considering the large diameter of the vessels visible at day seven we ranked the first examination (day seven) as the afferent stage and the second examination (day 12) as the efferent stage. Stabilization was then reached for these six

patients; the same values for thickness of the graft, number and diameter of the vessels were maintained till the end of the follow-up. For the data of every individual patient see Table 2.

Patient N.7 had an unusual revascularization pattern observed in a previous study. No clear revascularization steps were found with SD-OCT up to five weeks after treatment, although the VA was relatively good (0.56 logMAR). At six months after surgery the graft had thickened, and both the mean vessel diameter and the number of vessels had increased. This suggested that the graft had become revascularized although this could not be concluded with certainty due to the absence of the normal revascularization stage transitions. At one year's follow-up, the graft's features remained stable compared to the six months scan; the graft was still assumed to be revascularized.

### **Phase-Resolved Doppler OCT**

PRD-OCT images could be obtained from all seven patients. In patient N.4 images from day one were of very poor quality and analysis was not possible, which was also the case for the SD-OCT. Poor quality images, which made the observation of blood flow difficult, were also obtained for patient N.1 at day 15 and day 31. PRD-OCT showed flow in the graft for all seven patients. The first observation of the flow was between day seven and day 31 after surgery for the six patients who were scanned during the first post-operative period. After the first observation all these six grafts showed flow on PRD-OCT until the end of the follow-up. Patient N.7 was only scanned one year after surgery and showed blood flow during this visit.

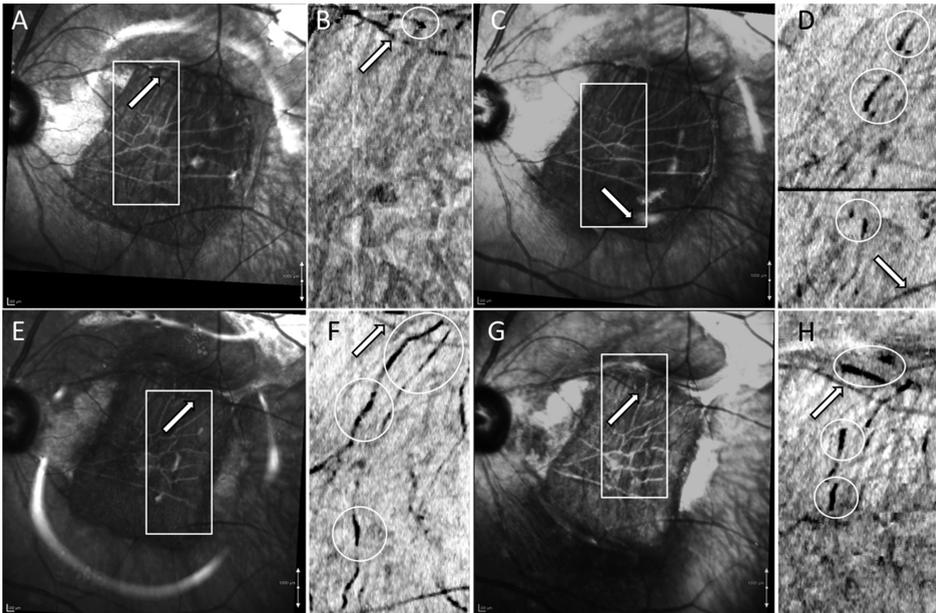
### **Comparison of SD-OCT with Phase-Resolved Doppler OCT**

We compared, in every patient, the observation of flow made with PRD-OCT to the revascularization process seen on SD-OCT. Flow was not detected by PRD-OCT at the days defined as the imbibition stage with SD-OCT in any patient. In three out of six patients, flow was visible on PRD-OCT during the afferent stage as defined by SD-OCT (N.3, N.5 and N.6). For all three patients, the next follow-up visit was considered to be the start of the efferent stage on SD-OCT, as all the parameters showed that the complete graft was revascularized.

In two out of six patients (N.2 and N.4) flow was seen with PRD-OCT at the same day that the start of the efferent stage was detected with SD-OCT.

In patient N.1, based on SD-OCT, the efferent stage started at day 15, however, due to suboptimal image quality, PRD-OCT data could not be obtained that day. At the next follow-up visit of this patient, day 31, flow was detected with PRD-OCT.

One example of the additional use of PRD-OCT was noticed by patient N.7. This patient had uncommon revascularization stages on SD-OCT; a very thin graft with almost no changes visible during the first five weeks. He seemed therefore not to be



**Figure 3.** Infrared images (**A,C,E**, and **G**) and en-face PRD-OCT images (**B,D,F**, and **H**) of patient N.2. at day 13, six weeks, three months and seven months postoperative. The *rectangles* depict the area where the three-dimensional PRD-OCT datasets were taken. *White circles* indicate the presence of flow which is visualized in black in the choroidal vessels of the retinal pigment epithelium choroid-graft, and the *white arrows* indicate flow from retinal vessels.

**A,B:** 13 days after surgery. Inside white circle the black part of the vessel indicates presence of flow in the top of the graft. The white arrow indicates flow in a retinal vessel.

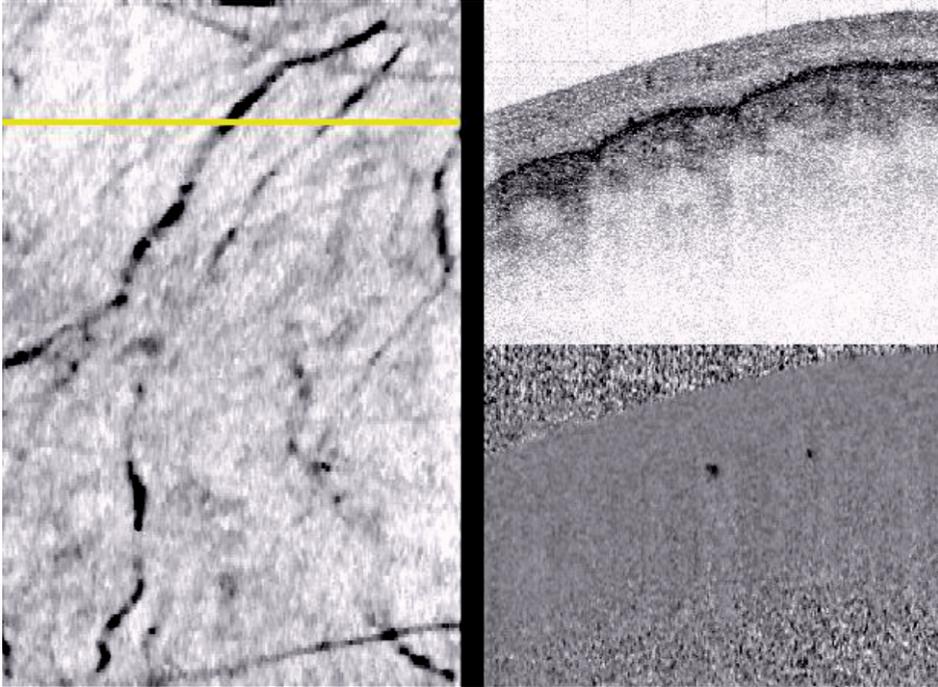
**C,D:** Six weeks after surgery. The PRD-OCT image shows an increased amount of blood flow in the center of the graft. The ladder-like parallel oriented pattern of the vasculature of the graft can now started to be seen.

**E,F:** Three months after surgery. The PRD-OCT image now outlines several complete vessels for which blood flow was detected. The ladder-like parallel oriented pattern of the vasculature of the graft can now more clearly be seen.

**G,H:** Seven months after surgery. The PRD-OCT image shows ongoing revascularization which is also visible at the edges of the graft. Eye movement in the middle of the dataset created a discontinuity in the visualized blood vessel as indicated by the white ovals.

revascularized. At the six months follow-up the graft finally showed changes which made revascularization likely. At one year follow-up the graft's features on SD-OCT resembled the ones at six months. At that time, PRD-OCT became available in our clinic and was able to confirm blood flow in the graft at one year, in agreement with the SD-OCT findings.

An example of the results for the SD-OCT and the PRD-OCT measurements of patient N.2 are shown in figures 2 and 3. These figures show the revascularization stages, visible to the bare eye, on SD-OCT. The presence or absence of flow, detected with PRD-OCT, is shown in the same way as in figure 1. A ladder-like pattern (parallel orientation) of vessels can be clearly seen on ICGA images after a free graft transplantation.<sup>11</sup> This pattern is

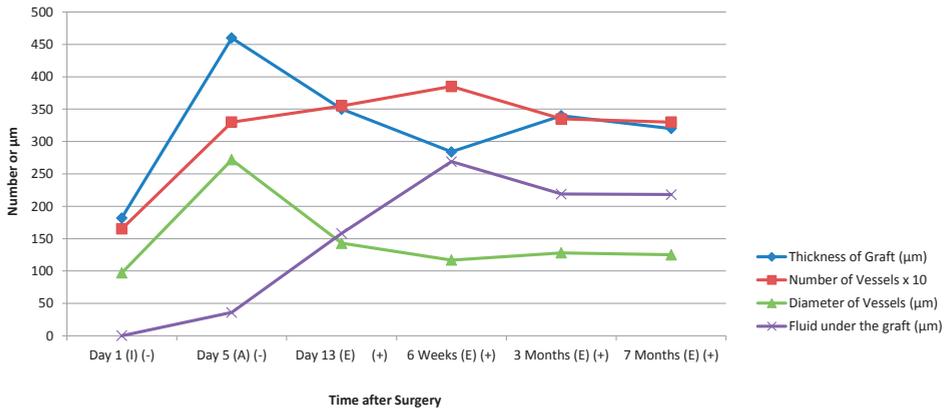


#### Clip 1. Blood flow in a graft

The movie shows an example of the blood flow in the graft in patient N.2, three months after surgery. At the left the en-face PRD-OCT image is shown equal to figure 3F. At the right the PRD-OCT cross-sectional images are shown with the structural image at the top and the bidirectional phase-resolved flow image at the bottom. The yellow line in the en-face image indicates where the cross-sectional images were obtained. This movie shows that throughout the whole imaged volume, blood flow can be seen within the graft tissue, which is hard to appreciate from static cross-sectional and en-face images. (see the e-pub version, available on <http://www.e-pubs.nl/?epub=elsbethvanzeeburg>).

typical for the midperipheral choroid but is atypical for the foveal area. The appearance of this ladder-like pattern on ICGA in the foveal area confirms that the angiogram shows the vessels of the transplanted graft, which is taken from the midperiphery, and not of the underlying choroid. On PRD-OCT, this ladder like pattern can also be appreciated, most clearly, in figures 3 (D) and (F).

Figure 4 shows a graph of the same patient N.2, in which the measured thickness of the graft, the number of vessels, the diameter of vessels and the fluid under the graft are delineated. What is visible to the bare eye on the SD-OCT images in figure 2 can now easily be correlated with the measurements outlined in the graph. Additionally, Clip 1 shows clearly, in a movie, that blood flow is visible throughout the whole graft.



**Figure 4.** Pattern of changes of the graft and fluid under the graft of Patient N.2

(I) Imbibition stage, (A) Afferent vessel stage, (E) Efferent vessel stage (+) presence of flow measured by phase-resolved Doppler OCT. (-) No flow detected on PRD-OCT.

The change in thickness of the graft, diameter of the vessels and fluid under the graft are delineated in micrometers in this graph. The change in number of vessels is also delineated; however the number is multiplied by ten in order to make the changes visible in the same graph. Day one is the imbibition stage; small vessel lumina are measured and the graft is thin. Day five is the afferent stage; the number and mean diameter of the vessels increase and the graft becomes thicker. Day 13 is the start of the efferent vessel stage; the thickness of the graft and the diameter of the vessels decrease while the number of vessels slightly increases. The fluid under the graft in appears in this patient during the afferent stage, increases throughout the early efferent stage and decreases later in the efferent stage. The fluid under the graft does not follow the pattern of changes of the overlying graft.

## DISCUSSION

A previous study demonstrated that with SD-OCT, early perfusion and flow of an RPE-choroid graft can be imaged. Revascularization steps, as well as flow, can be distinguished from structural changes on SD-OCT when a patient is scanned regularly during follow-up.<sup>9</sup> We earlier reported the use of PRD-OCT for the detection of blood flow in an RPE-choroid graft.<sup>20</sup> In this study both OCT methods were used side-by-side. Both SD-OCT and PRD-OCT are less invasive techniques than the currently used techniques such as FA and ICGA,<sup>10, 11</sup> and therefore would be more applicable for frequent follow-up measurements in the evaluation of, for instance, RPE-choroid grafts.

This study was designed to evaluate whether the revascularization steps found on SD-OCT could be confirmed by flow detection with PRD-OCT. We found an overall good agreement; flow was *not* detected during the imbibition stage, and flow was detected for either the afferent or efferent vessel stage. Detection of flow during the afferent vessel stage with PRD-OCT is probably due to the presence of a partially revascularized graft at this stage, as described previously.<sup>9</sup> The localized expansion of revascularization is much more prominently visible on FA and ICGA, but can also be visible on SD-OCT.

A drawback of identification of the revascularization by SD-OCT is that these scans need to be performed repeatedly. The first revascularization steps might be missed, because image quality of the SD-OCT scans is suboptimal if patients are not able to fixate properly, or when media opacities are present. This makes the confirmation of graft revascularization difficult: on a single late scan one cannot conclude whether the graft has slimmed down after the perfusion steps, or is not perfused and becoming atrophic.

PRD-OCT might be a solution to these problems. One single scan could be performed and would be able to demonstrate flow in the graft, in just one single visit when the media are sufficiently clear, as for instance demonstrated by patient N.7. However, the number of visualized vessels with blood flow by PRD-OCT is less than can be expected based on the number of vessels seen with SD-OCT. This suggests that our current implementation of PRD-OCT is not able to detect all the (slow) blood flow that is assumed to exist during the start of the revascularization process. This can be attributed to the steep angle of incidence of the OCT light with retinal surface (Doppler angle), and therefore with the direction of the blood flow in the majority of the vessels. Consequently the detected PRD-OCT phase-difference is small and restricts the observation of blood flow to high flow velocities.<sup>23</sup> As stated above, the minimum detectable flow velocity ranges from 5.6 mm/s for a 70° Doppler angle to 110 mm/s for a 89° Doppler angle. Recently it has been shown that with improved PRD-OCT scan techniques, blood flows with low velocities or with unfavorable Doppler angles can be better observed by increasing the time interval between the compared A-scans.<sup>23-25</sup> It can therefore be expected that the visualization of the graft revascularization with PRD-OCT will significantly improve in the future.

We conclude that PRD-OCT is a useful experimental tool to analyze the presence of flow after RPE and choroid translocation in a single exam. Moreover, PRD-OCT confirmed the interpretation of structural and hemodynamic changes within a free RPE-choroid graft as previously observed with FA, ICGA, and SD-OCT.

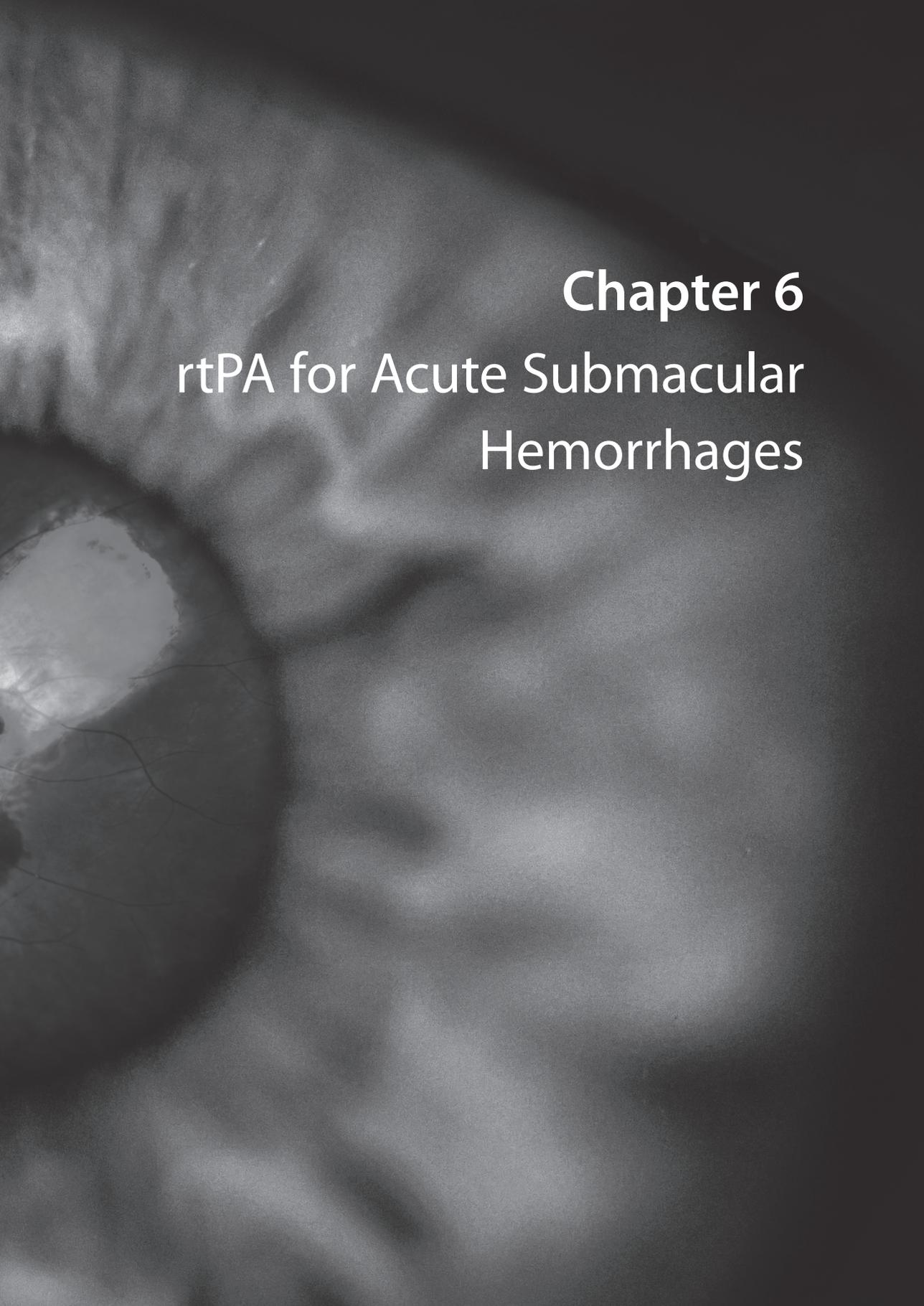
## REFERENCES

1. Augood CA, Vingerling JR, de Jong PT, et al. Prevalence of age-related maculopathy in older Europeans: the European Eye Study (EUREYE). *Arch Ophthalmol*. 2006;124:529-535.
2. Martin DF, Maguire MG, Ying GS, Grunwald JE, Fine SL, Jaffe GJ. Ranibizumab and bevacizumab for neovascular age-related macular degeneration. *N Engl J Med*. 2011;364:1897-1908.
3. Hwang JC, Del Priore LV, Freund KB, Chang S, Iranmanesh R. Development of subretinal fibrosis after anti-VEGF treatment in neovascular age-related macular degeneration. *Ophthalmic Surg Lasers Imaging*. 2011;42:6-11.
4. Gelissen F, Ziemssen F, Voelker M, Bartz-Schmidt KU, Inhoffen W. Retinal pigment epithelial tears after single administration of intravitreal bevacizumab for neovascular age-related macular degeneration. *Eye (Lond)*. 2009;23:694-702.
5. Rosenfeld PJ, Shapiro H, Tuomi L, Webster M, Elledge J, Blodi B. Characteristics of Patients Losing Vision after 2 Years of Monthly Dosing in the Phase III Ranibizumab Clinical Trials. *Ophthalmology*. 2011;118:523-530.
6. Van Zeeburg EJ, van Meurs JC. Literature review of recombinant tissue plasminogen activator used for recent-onset submacular hemorrhage displacement in age-related macular degeneration. *Ophthalmologica*. 2013;229:1-14.
7. Maaijwee K, Heimann H, Missotten T, Mulder P, Jousen A, van Meurs J. Retinal pigment epithelium and choroid translocation in patients with exudative age-related macular degeneration: long-term results. *Graefes Arch Clin Exp Ophthalmol*. 2007;245:1681-1689.
8. Van Zeeburg EJ, Maaijwee KJ, Missotten TO, Heimann H, van Meurs JC. A free retinal pigment epithelium-choroid graft in patients with exudative age-related macular degeneration: results up to 7 years. *Am J Ophthalmol*. 2012;153:120-127.
9. Van Zeeburg EJ, Cereda MG, van der Schoot J, Pertile G, van Meurs JC. Early perfusion of a free RPE-choroid graft in patients with exudative macular degeneration can be imaged with spectral domain-OCT. *Invest Ophthalmol Vis Sci*. 2011;52:5881-5886.
10. Cereda MG, Parolini B, Bellesini E, Pertile G. Surgery for CNV and autologous choroidal RPE patch transplantation: exposing the submacular space. *Graefes Arch Clin Exp Ophthalmol*. 2010;248:37-47.
11. Maaijwee K, van den Biesen PR, Missotten T, van Meurs JC. Angiographic evidence for revascularization of an rpe-choroid graft in patients with age-related macular degeneration. *Retina*. 2008;28:498-503.
12. Yun S, Tearney G, de BJ, Iftimia N, Bouma B. High-speed optical frequency-domain imaging. *Opt Express*. 2003;11:2953-2963.
13. Yun SH, Tearney G, de BJ, Bouma B. Pulsed-source and swept-source spectral-domain optical coherence tomography with reduced motion artifacts. *Opt Express*. 2004;12:5614-5624.
14. Chen Y, Burnes DL, de BM, Mujat M, de Boer JF. Three-dimensional pointwise comparison of human retinal optical property at 845 and 1060 nm using optical frequency domain imaging. *J Biomed Opt*. 2009;14:024016.
15. Unterhuber A, Povazay B, Hermann B, Sattmann H, Chavez-Pirson A, Drexler W. In vivo retinal optical coherence tomography at 1040 nm - enhanced penetration into the choroid. *Opt Express*. 2005;13:3252-3258.

16. de Bruin DM, Burnes DL, Loewenstein J, et al. In vivo three-dimensional imaging of neovascular age-related macular degeneration using optical frequency domain imaging at 1050 nm. *Invest Ophthalmol Vis Sci*. 2008;49:4545-4552.
17. Chen Z, Milner TE, Dave D, Nelson JS. Optical Doppler tomographic imaging of fluid flow velocity in highly scattering media. *Opt Lett*. 1997; 22:64-66.
18. Izatt JA, Kulkarni MD, Yazdanfar S, Barton JK, Welch AJ. In vivo bidirectional color Doppler flow imaging of picoliter blood volumes using optical coherence tomography. *Opt Lett*. 1997;22:1439-1441.
19. Zhao Y, Chen Z, Saxer C, Xiang S, de Boer JF, Nelson JS. Phase-resolved optical coherence tomography and optical Doppler tomography for imaging blood flow in human skin with fast scanning speed and high velocity sensitivity. *Opt Lett*. 2000;25:114-116.
20. Braaf B, Vermeer KA, Sicam VA, van Zeeburg E, van Meurs JC, de Boer JF. Phase-stabilized optical frequency domain imaging at 1-microm for the measurement of blood flow in the human choroid. *Opt Express*. 2011;19:20886-20903.
21. Maaijwee K, van Meurs JC. Retinal Pigment Epithelium and Choroid translocation in patients with exudative age-related macular degeneration. In: Soubrane G (ed), *Les DLMA*s. 9th edition ed. Paris: Masson; 2007:562-567.
22. Holladay JT. Visual acuity measurements. *J Cataract Refract Surg*. 2004;30:287-290.
23. Braaf B, Vermeer KA, Vienola KV, de Boer JF. Angiography of the retina and the choroid with phase-resolved OCT using interval-optimized backstitched B-scans. *Opt Express*. 2012;20:20516-20534.
24. Grulkowski I, Gorczynska I, Szkulmowski M, et al. Scanning protocols dedicated to smart velocity ranging in spectral OCT. *Opt Express*. 2009;17: 23736-23754.
25. Makita S, Jaillon F, Yamanari M, Miura M, Yasuno Y. Comprehensive in vivo micro-vascular imaging of the human eye by dual-beam-scan Doppler optical coherence angiography. *Opt Express*. 2011; 19:1271-1283.







# Chapter 6

## rtPA for Acute Submacular Hemorrhages

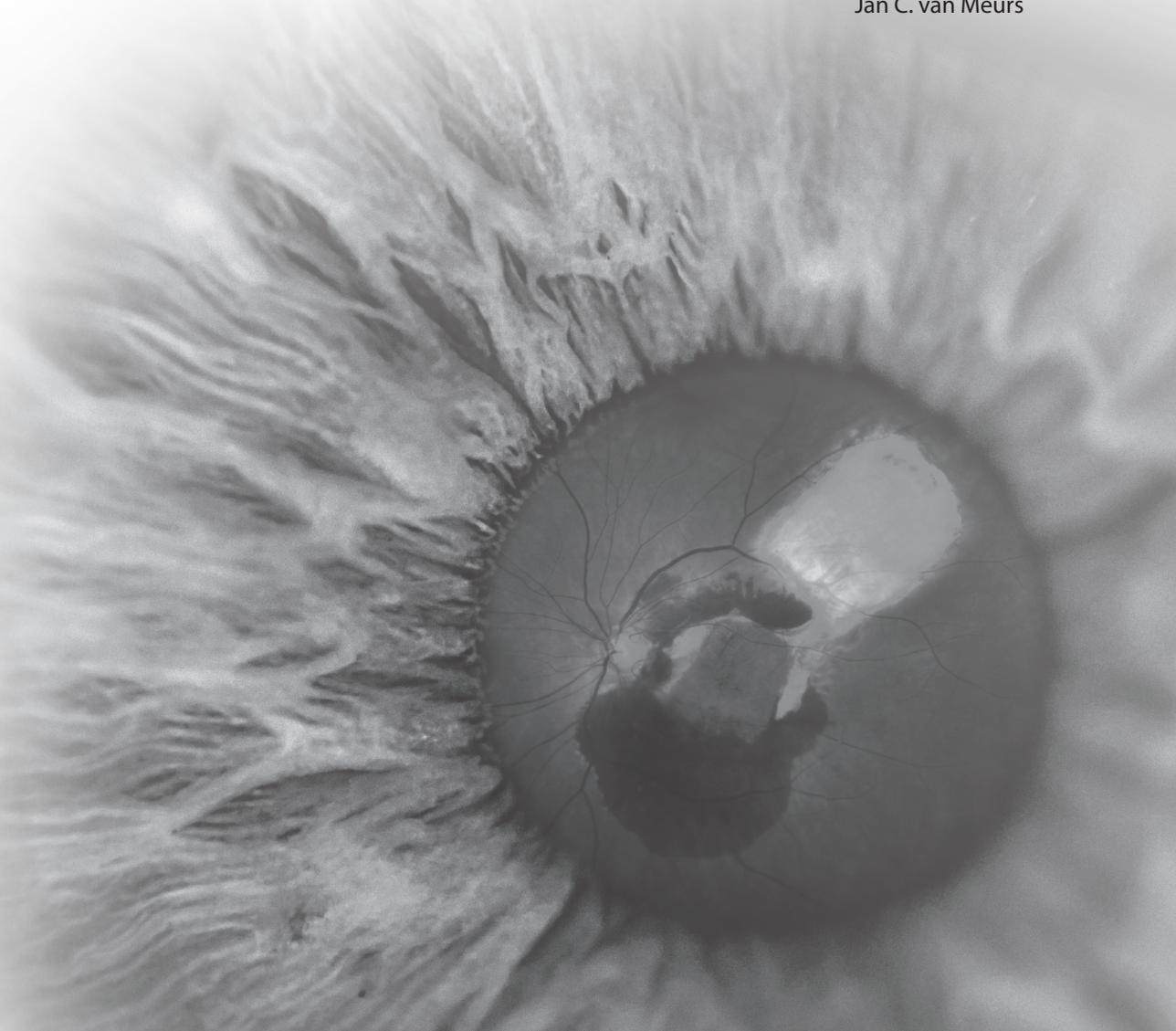


## **Chapter 6.1**

# **Literature Review of Recombinant Tissue Plasminogen Activator Used for Recent-Onset Submacular Hemorrhage Displacement in Age-Related Macular Degeneration**

*Ophthalmologica 2013;229:1-14*

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## ABSTRACT

### Aims

To review and discuss the literature on recombinant tissue plasminogen activator (rtPA) for the treatment of a recent-onset submacular hemorrhage in patients with age-related macular degeneration.

### Methods

The administration technique of rtPA, the use of additional gas and vascular endothelial growth factor inhibitors (anti-VEGF), and the displacement rate of submacular hemorrhage and complications were noted from published reports, and a case series from the Rotterdam Eye Hospital (REH).

### Results

Thirty-eight studies with a total of 1185 patients (1176 eyes), and 28 patients from the REH, were analyzed. Several methods for rtPA administration are available, which can be divided into two groups, submacular rtPA administration with vitrectomy; or intravitreal rtPA administration without vitrectomy. In both groups the administration of gas and/or anti-VEGF agents could be additional. There appears to be no clear difference in complete displacement or complication rate between the more or the less invasive treatment groups.

### Conclusion

Although intravitreal injection of rtPA and gas only was reported to be as effective as subretinal rtPA with vitrectomy and gas, recent studies tend to use vitrectomy. These data underscore the need for a randomized controlled trial to choose the most effective and safe method of rtPA administration.

## INTRODUCTION

Intravitreal injection of vascular endothelial growth factor inhibitors (anti-VEGF) is the standard of care for patients with exudative age-related macular degeneration (AMD). However, anti-VEGF injections are not effective in restoring or improving visual acuity (VA) when a large submacular hemorrhage (SMH) is present. SMH is a relatively common and severe complication of exudative AMD, particularly in patients taking anticoagulant medications.<sup>1</sup> It leads to immediate and extensive, albeit sometimes reversible, loss of visual acuity, and if left untreated, can cause irreversible damage to the retinal pigment epithelium (RPE) cells and retina.<sup>2,3</sup>

Heriot firstly reported a minimally invasive pneumatic displacement method: intravitreal injection of recombinant tissue plasminogen activator (rtPA) and gas, in which the gas bubble and gravity combine to displace the hemorrhage inferiorly and away from the submacular region. This allows for extrafoveal resorption of the hemorrhage which causes less functional damage to the central VA.<sup>4</sup> Another, more invasive, method of rtPA-assisted displacement of a hemorrhage has since been described: vitrectomy, followed by subretinal rtPA injection, and a gas<sup>5,6</sup> or air<sup>7-9</sup> tamponade.

Both minimally invasive and invasive methods were, in later studies, combined with anti-VEGF therapy,<sup>10-14</sup> allowing for simultaneous treatment of the causative choroidal neovascularization.<sup>11</sup>

These displacement methods are primarily intended for hemorrhages of recent onset, i.e. hemorrhages existing for approximately 14 days or less, as the hemorrhage causes progressively severe damage to the retina,<sup>2,3</sup> and older hemorrhages may become more difficult to displace. However, in many studies included in this review, the displacement methods have been used for patients with older hemorrhages as well.

The results of a large number of uncontrolled studies suggest that patients with recent onset submacular hemorrhage, whose prognosis might have been dire in the past, can now be effectively treated with relatively safe surgical interventions. The primary difference between the various studies is whether rtPA and anti-VEGF are administered intravitreally, in which case no further surgical procedure is employed, or subretinally, in which case vitrectomy is required. The effectiveness of the different rtPA approaches used seems to be comparable, but the complication rate is likely to be higher if more invasive methods are used.

Besides the treatment modality, there are several variables that may affect the treatment outcome. These include the thickness<sup>15</sup> and size<sup>16,17</sup> of the hemorrhage, and elevation of the retina by the hemorrhage.<sup>15,17</sup>

The recent trend has been toward the subretinal administration of rtPA after vitrectomy, followed by gas tamponade. This has been shown to be an effective combination. However, it remains uncertain whether the primary mechanism for displacement of

the hemorrhage is a pushing or rolling action of a partial tamponade<sup>4</sup> or whether it is the effect of gravity working upon the hemorrhage in the context of a complete tamponade.<sup>18,19</sup>

Further, a randomized, controlled trial might help to elucidate the relative advantages and disadvantages of the more invasive method for rtPA administration, involving vitrectomy, submacular administration of rtPA and gas tamponade as compared to the less invasive method, involving the simple intravitreal injection of rtPA and gas tamponade. Both arms of the study should then also include injection of an anti-VEGF agent intravitreally preoperatively. As part of the preparation for this controlled trial, we analyzed the feasibility (in terms of hemorrhage displacement) and in especially the safety (complication rates) of the different procedures using information derived from existing clinical reports as well as from data of the Rotterdam Eye Hospital (REH).

## PATIENTS AND METHODS

### Literature Search

Literature searches of the PubMed database were last conducted on January 10th, 2012. The first PubMed search was conducted using the following key words: "recombinant tissue plasminogen activator AND gas AND age related macular degeneration." The second search was conducted with the following key words: "submacular hemorrhage AND gas." The third search was conducted with the following key words: "recombinant tissue plasminogen activator AND age related macular degeneration AND air," and the fourth search string with the key words "tissue plasminogen activator AND age related macular degeneration." The searches were limited to articles published in English and German. No date restrictions were employed. We also searched the reference lists of the studies included in the review for other potential inclusions. We specifically searched for studies that used either rtPA or gas tamponade only, or a combination of both, as a method to remove a submacular hemorrhage for AMD patients. These methods could be combined with vitrectomy and/or the administration of anti-VEGF agents. All studies had to at least include patients with AMD, and the total number of patients in the study had to be five or more. For studies in which rtPA treatment was compared with bevacizumab or gas only or gas and bevacizumab combined, or natural history or manual removal of the hemorrhage, only the patients who were included in the rtPA arm of the study were included in the analysis.

The following data were entered into a database: the number of patients included in the study; the etiology of the submacular hemorrhage; whether treatment included vitrectomy; the type of tamponade used; whether rtPA and/or anti-VEGF agents were used and their method route of administration (submacular or intravitreal); the rate

of hemorrhage displacement and its extent, defined as no (complete displacement) or some (partial displacement) blood left in the foveal area; percentage of eyes with a final VA > 20/200; percentage of eyes which gained two lines (Early Treatment Diabetic Retinopathy Study [ETDRS] or Snellen) or more in VA; and number of complications, such as recurrent subretinal/submacular hemorrhage, (exudative inferior) retinal detachment, proliferative vitreoretinopathy (PVR) or vitreous hemorrhage. When VA results were presented in a table but the authors themselves did not calculate VA improvement rates or percentages, an attempt was made to do so, if the available data allowed. All VA measurements were converted into logarithm of minimal angle of resolution (logMAR) values for statistical analysis in which "finger counting" at 60 cm was transposed into logMAR 2, and "hand motion" into logMAR 3.<sup>20</sup> These same parameters were entered into the table of our own retrospective case review of patients with SMH due to AMD who were treated in the REH between July 2008 and February 2011.

### Patients of the REH

The sole inclusion criterion for our own patient group was neovascular AMD complicated by SMH involving the fovea. The sole exclusion criterion was an SMH whose etiology was a disease other than AMD. Pre- and postoperative examinations consisted of a standard ophthalmologic examination supplemented by fundus photography, optical coherence tomography and, when choroidal neovascularization was suspected, fluorescein and/or indocyanine green angiography. The following surgical technique was employed: after induction of a posterior vitreous detachment, a complete pars plana vitrectomy was performed. We used a 23 G cannula with a 41G tip, connected by tubing to a tuberculin syringe (Dutch Ophthalmic Research Center [DORC], Zuidland, The Netherlands), filled with the rtPA solution. Once the 41 G tip was inserted through the retina, the assistant would inject 0.1 ml of fluid (20µg/0.1 ml rtPA (Actilyse, Boehringer Ingelheim, Ingelheim, Germany) into the subretinal space or clot, creating a local retinal detachment encompassing (a part of) the blood hemorrhage. After rtPA injection, the internal limiting membrane (ILM) was peeled, starting from the injection site, which was most frequently created at the superior edge of the hemorrhage. This peeling was performed to prevent formation of a macular pucker or PVR after surgery. After fluid/air exchange, the vitreous cavity was filled with a 15% sulfur hexafluoride (SF6) or 10% perfluoropropane (C3F8) gas/air mixture. Patients were instructed to maintain an upright, lateral or prone position, depending on the intended direction of hemorrhage displacement.

## RESULTS

### Literature Search

The PubMed first search string, with the key words “recombinant tissue plasminogen activator AND gas AND age related macular degeneration,” retrieved 50 articles, of which 30 were considered to be relevant. The second search string, with the key words “submacular hemorrhage AND gas” retrieved 60 articles, of which four were both relevant and had not been identified by the previous search string. The third search string with the key words “recombinant tissue plasminogen activator AND age related macular degeneration AND air,” retrieved nine articles, of which three were relevant and new. The fourth search string with the key words “tissue plasminogen activator AND age related macular degeneration” retrieved 113 articles, of which one was relevant and new. The reference lists of the studies included in the review for other potential inclusions did not add any new relevant studies. This search resulted in a total of 38 articles that were deemed to be relevant to the topic. These 38 studies had included a total of 1185 eyes (1176 patients) which were deemed eligible for further study according to the inclusion criteria and were entered into the database (Table 1).

### Surgical Technique

Several techniques for the treatment of acute submacular hemorrhage were described and are summarized in Table 1. In three studies, only intravitreal gas injection was used in an attempt to displace the hemorrhage.<sup>21-23</sup> Four studies employed either gas injection or both rtPA and gas injected intravitreally, depending on the study arm.<sup>24-27</sup> One of these studies administered anti-VEGF only postoperatively in eight out of 53 patients.<sup>26</sup> One study employed intravitreal rtPA with and without intravitreal gas injection.<sup>28</sup> Fifteen studies reported intravitreal injection of both rtPA and gas;<sup>29-43</sup> one of these 15 studies incorporated the results of its own, older article.<sup>32,39</sup> Five studies reported intravitreal rtPA and intravitreal anti-VEGF agents and gas injections.<sup>11-13,44,45</sup> In four of these five studies, next to the rtPA, anti-VEGF agents were administered during surgery; in the fifth study, anti-VEGF was administered during the 4-6 week interval after surgery, if clinically indicated.<sup>45</sup> Four other studies reported the combination of vitrectomy, subretinal rtPA application and air tamponade;<sup>7-9,46</sup> in one of these studies, intravitreal anti-VEGF agents were only administered postoperatively, if required by the study protocol.<sup>46</sup> Two studies administered subretinal rtPA combined with vitrectomy and gas tamponade.<sup>5,47</sup> Two studies retrospectively compared subretinal versus intravitreal rtPA administration, both combined with vitrectomy and gas injection;<sup>6,10</sup> patients in one of these two studies also received intravitreal anti-VEGF.<sup>10</sup> Two studies, one of which included long-term results of the patients of the other, earlier study, reported subretinal rtPA and also subretinal anti-VEGF administration during surgery, with a gas tamponade, followed by intravitreal anti-VEGF after surgery<sup>14,48</sup> (Table 1).

### Analysis of the Displacement and Complication Rate in Literature

To describe the difference between administration techniques of rtPA and gas or air tamponade, we clustered only the (parts of) the studies which used rtPA and gas or air, plus our own study, into four groups. As complication or displacement rates were not consistently reported, or not specified between different subgroups in a study, and not all studies used rtPA in each patient, not all applicable studies could be included in either the analysis of displacement or complication rates. Thus, of the 1185 eyes out of the studies from the literature, the data of 221 eyes could not be included in this analysis.

Group 1: no vitrectomy, intravitreal administration of rtPA, intravitreal injection of gas; group 2: no vitrectomy, intravitreal administration of rtPA and anti-VEGF agents, intravitreal injection of gas; group 3: vitrectomy, subretinal administration of rtPA, total gas or air tamponade; group 4: vitrectomy, subretinal administration of rtPA and either subretinal or intravitreal administration of anti-VEGF agents, total gas or air tamponade. As only complete hemorrhage displacement and major complications like retinal detachments were reported consistently, this study focuses on comparison of these data. Other variables of interest, such as preoperative VA, percentage of patients with a final VA >20/200, VA gain, partial displacement and complications other than retinal detachments, vitreous hemorrhages and/or recurrent submacular hemorrhages were too inconsistently described to allow comparison, and are therefore only displayed in the table. Two studies were excluded from comparison as they were earlier studies of later long-term studies and therefore used the same patients.<sup>39,48</sup> Two other studies compared the intravitreal versus subretinal rtPA administration, both combined with vitrectomy and gas tamponade. Twenty-five patients who underwent vitrectomy and intravitreal rtPA administration in these two studies were not included in our analysis as they do not fit in either of the four groups, as they undergo vitrectomy (invasive) but the method of rtPA administration is intravitreal (minimal invasive).<sup>6,10</sup>

The results of the less invasive method of rtPA administration (intravitreal rtPA; groups 1 and 2) were compared to those of the more invasive method (vitrectomy plus subretinal rtPA with gas or air; groups 3 and 4). The less invasive group had a range of 50-100% complete displacement rate ( $n = 467$ )<sup>11-13,24,25,29-35,37,38,40,42,44,45</sup> while the most invasive group had a range of 53-100% displacement rate ( $n = 194$ , including 28 REH patients).<sup>5-10,14,46</sup> The range of recurrence of submacular hemorrhage was 0-27% in both the less invasive and most invasive group. The range of retinal detachments was 0-45% in the less invasive group and 0-11% in the more invasive group. The percentage of vitreous hemorrhages ranged from 0-45% in the less invasive group ( $n = 724$ )<sup>11-13,24,25,27-35,37,38,40-45</sup> and from 0-67% in the more invasive group ( $n = 203$ , including 28 REH patients)<sup>5-10,14,46,47</sup> (Table 2).

Comparison of the complications between groups 1 and 2 (intravitreal rtPA without (group 1;  $n = 607$ ) or with (group 2;  $n = 117$ ) anti-VEGF agents), revealed that in group 1,

**Table 1: Review of 38 studies from the literature and one study from The Rotterdam Eye Hospital, The Netherlands.**

Study Group	Year of Press	Number of eyes Treated with rtPA	Etiology of Hemorrhage	Vitrectomy	Tamponade	rtPA	Anti-VEGF
<b>Gas Only</b>							
Ohji[21]	1998	5 Gas only	AMD (4), RAM (1)	-	Gas (C3F8)	-	-
Gopalakrishan[22]	2007	20 Gas only	AMD (5), Polypoidal choroidal vasculopathy (8), Extramacular CNVM and ruptured macroaneurysm (3), Other (3)	-	Gas (C3F8)	-	-
Ron[23]	2007	24 Gas only	AMD	-	Gas (C3F8: n=11; SF6: n=13)	-	-
<b>Combined studies: Gas only or intravitreal rtPA with Gas</b>							
Yang[27]	2005	8 rtPA and Gas, 16 Gas only	AMD (18) traumatic choroidal rupture (2), RAM (2) IPCV (1) proliferative diabetic retinopathy (1)	-	Gas (C3F8 n=19; SF6: n=5)	rtPA IV / Gas only	-
Fang[25]	2009	28 rtPA and Gas, 25 Gas only	AMD	-	Gas (C3F8: n=5; SF6: n=48)	rtPA IV / Gas only	-
Çakir[24]	2010	7 rtPA and Gas, 14 Gas only	AMD (16), Myopic CNV(2), RAM (2), trauma (1)	-	Gas (C3F8)	rtPA IV / Gas only	7/7 bevacizumab postoperative
Mizutani[26]	2011	40 rtPA and Gas, 13 Gas only	AMD (39), RAM (14)	-	Gas (SF6)	rtPA IV / Gas only	8 eyes postoperative (6 ranibizumab, 2 pegaptanib)
<b>Combined study: intravitreal rtPA only or intravitreal rtPA with Gas</b>							
Tsymanava[28]	2011	64 rtPA and Gas, 46 only rtPA, no Gas	AMD	-	Gas (not specified) / None	IV	-
<b>Intravitreal rtPA and Gas</b>							
Hesse[33]	1999	11	AMD	-	Gas (C3F8: n=3; SF6: n=7, C2F6: n=1)	IV	-
Hassan[31]	1999	15	AMD (13), RAM (1), trauma (1)	-	Gas (C3F8: n=7, SF6: n=8)	IV	-
Meier[35]	1999	22	AMD	-	Gas (SF6)	IV	-

Successful Displacement (%)	Final VA >20/200 (%), Follow-Up	Eyes Gained 2 (ETDRS or Snellen) Lines or More (%), Follow-Up	Complications
3/5 (60%)	3/5 (60%) (one week to 13 months' follow-up)	5/5 2 or more ETDRS lines increase (100%) (one week to 13 months' follow-up)	Vitreous hemorrhage (1), retinal detachment (1)
Complete 10/20 (50%), partial 6/20 (30%)	Final VA 20/63 or better in 12/20 patients (60%), non AMD patients. (median of 6 months, range 3-42 months)	Mean best corrected VA improved from 1.6 to 0.72 logMar (9 ETDRS lines), at three months' follow-up	Mild vitreous hemorrhage (6), nonresolving vitreous hemorrhage (4), one of them developed a scarred choroidal neovascular membrane, retinal detachment and massive subretinal bleeding (1), retinal tear (1)
NI	Best postoperative VA >20/200 10/24 (42%). (In 14 patients achieve by the first month of treatment)	11/24 (46%) 2 or more Snellen line improvement, best postoperative VA.	None.
Total or subtotal in all eyes, not further specified	10/24 (42%) (mean 15.5, range 6-50 months' follow-up)	11/24 (46%) two or more Snellen line improvement, final VA.	Gas only: Recurrent SMH (1), vitreous hemorrhage (7), rtPA IV: recurrent SMH (1)
Gas and rtPA: Complete: 14/28 (50%), partial: 9/28 (32.1%). Gas only: complete 6/25 (24%), partial 7/25 (28%)	Gas and rtPA: 12/28 (43%) final VA. (mean 12.8 months follow-up, SD 0.96). Gas only: 3/25 (12%) final VA, (mean 10.8 months' follow-up, SD 0.68)	Gas and rtPA: 17/28 (60.7%) improvement of 2 or more Snellen lines, best postoperative VA. Gas only: 8/25 (32%) 2 or more line improvement.	Gas and rtPA: Vitreous hemorrhage (1), staphylococcal endophthalmitis (1), increased IOP (2). Gas only: vitreous hemorrhage (2), increased IOP(1)
13/14 (93%) 7/7 (100%)	4/7 (57%) 5/14(36%),at 6 months' follow-up	2 or more ETDRS lines improvement in 6/7(86%) and 12/14(86%) at 6 months	Recurrent submacular hemorrhage (2), in only gas group.
NI	AMD 80%, RMA 84% (two methods taken together)	3 ETDRS lines or more: Best PO VA: AMD:76.9%, last visit AMD: 53.8%. RAM: 92.9% both Best PO VA and last visit VA. Mean 18.4, range 3-61 months' follow-up.	Not specified between groups: recurrent SMH (11) vitreous hemorrhage (3) Increased IOP (5)
NI	NI	At three months without gas 2 or more Snellen lines improvement: 8%, with gas 25%. At six months without gas 13%, with gas 32%.	Endophthalmitis (2), vitreous hemorrhage (10), subretinal hemorrhage (1), increased IOP (3)
9/11 (82%)	6/11 (55%), range 3-12 months' follow-up	Best PO VA: 2 or more Snellen lines improvement in 5/11 (45%), Mean 4.8, range 3-12 months follow-up.	Exudative inferior retinal detachment (5), mild subretinal recurrent hemorrhage (1) vitreous haze (5) postoperative vitreous hemorrhage (5)
15/15 (100%)	6/15 (40%), range 4-19 months' follow-up	Best PO VA increased by 2 Snellen lines or more in 14/15 (93%) (average 4.6 months' follow-up). Final VA: 10/15 (67%), Mean 10.5, range 4-19 months' follow-up.	Breakthrough vitreous hemorrhage (3), endophthalmitis (1), recurrent hemorrhage (4)
22/22 (100%)	16/22 (73%)	No accurate information, only graph	Recurrent submacular hemorrhage (2), retinal detachment (1), vitreous hemorrhage (1), endophthalmitis (1)

**Table 1: Review of 38 studies from the literature and one study from The Rotterdam Eye Hospital, The Netherlands. (continued)**

Study Group	Year of Press	Number of eyes Treated with rtPA	Etiology of Hemorrhage	Vitrectomy	Tamponade	rtPA	Anti-VEGF
Buhl[37]	1999	53	AMD (47), RAM (6)	-	Gas (SF6)	IV	-
Framme[38]	2000	8	AMD	-	Gas (SF6)	IV	-
Krepler[34]	2000	11	AMD	-	Gas (SF6)	IV	-
Handwerger[30]	2001	14	AMD	-	Gas (C3F8)	IV	-
Hattenbach[32]	2001	43	AMD	-	Gas (SF6)	IV	-
Hattenbach[39] *	2002	25	AMD	-	Gas (SF6)	IV	-
Schulze[36]	2002	67	AMD	-	Gas (C3F8/SF6)	IV	-
Tsai[42]	2003	15	AMD	-	Gas (C3F8)	IV	-
Ratanasukon[41]	2005	19	AMD (15) idiopathic choroidal neovascularization (2) traumatic (1) valsalva retinopathy (1)	-	Gas (C3F8: n=7; SF6: n=12)	IV	-
Chen[29]	2007	104 (19 lost to follow-up and therefore excluded)	AMD(88), high myopia (4), RAM (5), trauma(3), IPCV (2), Angioid streaks (1), intraoperative complications (1)	-	Gas (C3F8: n=56, SF6: n=47), 1 air	IV	-
Kung[40]	2010	45	AMD (28), IPCV (11), RAM (2), pathologic myopia (3), trauma (1)	-	Gas (C3F8)	IV	-
Wu[43]	2011	120	AMD (88), IPCV (14), RAM (7), Pathologic myopia (8), trauma (3)	-	Gas (C3F8) either the same day, or in case of elevated IOP after rtPA injection, 1 day after surgery	IV	-

Successful Displacement (%)	Final VA >20/200 (%), Follow-Up	Eyes Gained 2 (ETDRS or Snellen) Lines or More (%), Follow-Up	Complications
45/53 (85%)	10/47 (21%) AMD, 4/6 (67%) RAM, mean 21 weeks follow-up	AMD: 23/47 (49%) 2 Snellen lines or more improvement. RAM 5/6 (83%) 2 Snellen lines or more improvement. Mean of all patients 21 weeks' (+-1 week) follow-up	Vitreous hemorrhage, (4) endophthalmitis (1)
Complete: 1/8 (13%) after 1 week, 5/8 (also absorbed) (63%) at 6 months	3/8 (38%) at 6 months	3/8 (38%) 2 or more ETDRS lines improvement	IOP increase (1), bacterial endophthalmitis (1), vitreous hemorrhage (1)
10/11 (91%)	3/11 (27%)	1 week: 2 or more Snellen lines increase in 8/11 (72%), and 5/11 (45%) at 12 months	Recurrent submacular hemorrhage (2)
Complete: 10/14 (71%) Partial: 3/14 (21%)	4/14 (29%), 1-15 months' follow-up	8/14 (57%) 2 or more Snellen lines improvement at 2 months. At last Follow-up: 2/14 (13%) 2 or more Snellen lines improvement. Mean follow-up 7.7 months, range 1-15 months	Corneal edema(1), vitreous hemorrhage (1), posterior retinal tear within the superotemporal arcade (1), RPE tear (1)
35/43 (81%)	15/43 (35%), 4-18 months' follow-up	Best PO VA: 2 or more Snellen lines increase in 19/43 (44%). Final VA: 2 or more lines increase 13/43 (30%). Range: 4-18 months' follow-up	Recurrent hemorrhage(9), mild breakthrough vitreous hemorrhage(3), IOP increased first day postoperative(2)
Complete 21/25 (84%)	13/25 (52%) last VA, Mean 7.1, range 4-18 months' follow-up	11/25 (44%) 2 or more Snellen lines, best PO VA. Mean best PO VA 4.7 months (range 0.5-12 months)	Recurrent subretinal hemorrhage (7) IOP increased first postoperative day (2)
NI	NI	Mean VA improvement/shift of 1,96 visual steps after 3 months' follow-up for the 47 patients tested at three months	5 patients underwent vitrectomy and subretinal surgery within 3 months, not further specified
Complete 12/15 (80%), partial 3/15 (20%)	Final VA 10/15 (67%) (mean 13 months, range 6-19)	7/15 (47%) 2 or more Snellen lines, best postoperative VA, mean 2.1 months (range 2 weeks-4 months)	Breakthrough vitreous hemorrhage (2)
'Most cases'	11/19 (58%), mean 13 months, range 6-39 months' follow-up	Final VA: 2 or more ETDRS lines improvement in 12/19 (63.2%), 3 lines or more improvement in 10/19 (52.6%) Mean 13 months' follow-up (range 6-39 months)	Breakthrough vitreous hemorrhage (3), retinal detachment (2), with unfavorable outcomes after multiple vitrectomies
63/85 (74%)	30/83 (36%), 12 months' follow-up	49/77 (63%) had a 2 Snellen lines improvement at 3 months, 52/81(64%) at 12 months' follow-up	Breakthrough vitreous hemorrhage(8), retinal detachment(3)
Complete: 40/45 (89%) Partial: 5/45 (11%)	18/45 (40%), 1-60 months' follow-up	Best PO VA: 2 or more Snellen lines improvement 21/45 (46.7%). Final VA 16/45 (36%) 2 or more lines. Mean follow-up 15.6 months, range 1-60	Breakthrough vitreous hemorrhage (9), 1 followed by macular pucker
NI	NI	Mean best postoperative VA: 5 ETDRS lines improvement (4,7 months) Mean final VA 2 Snellen lines improvement (22.6 months)	Breakthrough vitreous hemorrhage (18), retinal detachment (2)

**Table 1: Review of 38 studies from the literature and one study from The Rotterdam Eye Hospital, The Netherlands. (continued)**

Study Group	Year of Press	Number of eyes Treated with rtPA	Etiology ofHhemorrhage	Vitrectomy	Tamponade	rtPA	Anti-VEGF
<b>Intravitreal rtPA and anti-VEGF agents and Gas</b>							
Meyer[11]	2008	19	AMD	-	Gas (SF6)	IV	IV during surgery (bevacizumab)
Sacu[13]	2009	20	AMD	-	Gas (SF6)	IV	IV (only in 3 eyes during same session as rtPA injection, 17 within 3 days after rtPA. On average; mean 1,6 injections during 4 months' follow-up) (16 bevacizumab, 4 ranibizumab)
Matt[12]	2010	10	AMD	-	Gas (SF6)	IV	IV (and at 4-week intervals after surgery if active CNV present or in case of persistent submacular hemorrhage) (ranibizumab)
Guthoff[44]	2011	38	AMD	-	Gas (SF6)	IV	IV (and at 4-week or 8-week intervals after surgery according to CNV activity, in both groups) (during surgery bevacizumab, after surgery either ranibizumab or bevacizumab)
Mayer[45]	2011	32 (9 patients adverse events and left out of follow-up and therefore excluded)	AMD	-	Gas (SF6)	IV	4-6 week interval intravitreal injections in case of persistent sub-/intraretinal edema, new hemorrhage or active lesion (bevacizumab)
<b>Vitrectomy, subretinal rtPA and Air</b>							
Olivier[7]	2004	29	AMD	Vitrectomy	Air	SR	-
Thompson[9]	2005	15	AMD	Vitrectomy	Air	SR	-
Singh[8]	2006	17	AMD	Vitrectomy	Air	SR	-
Sandhu[46]	2010	16	AMD	Vitrectomy	Air	SR	Only postoperative, IV (12). Mean number of injections at 6 months: 3.7, mean number at 12 months: 4.5 (ranibizumab)
<b>Vitrectomy, subretinal rtPA and Gas</b>							
Hauptert[5]	2001	11	AMD	Vitrectomy	Gas SF6 (n=8)/ Air (n=3)	SR	-

Successful Displacement (%)	Final VA >20/200 (%), Follow-Up	Eyes Gained 2 (ETDRS or Snellen) Lines or More (%), Follow-Up	Complications
17/19 (89%)	NI	Mean VA 2.1 lines ETDRS improvement at 1 month, 3.7 lines at 3 months' follow-up	Increased IOP or reduced retinal perfusion with therefore corneal paracentesis (12)
20/20 (100%)	Mean VA 0,27 +0,2, 4 months' follow-up	2 or more Snellen lines increase, 6/20 (30%), 4 months' follow-up	Vitreous hemorrhage (1)
10/10 (100%)	4/10 (40%)	Final VA: 7 eyes 1 Snellen line or more VA line improvement (Mean 6.4 months, range 3-13 months' follow-up)	Increased IOP (1), corneal erosion with IOP increase postoperative (1)
19/38 (50%)	NI	rtPA/gas:3 Snellen lines or more improvement: 8/26 (31%), rtPA/gas/bevacizumab: 3 lines or more improvement: 5/12 (42%), both at 7 months' follow-up	No intra- or postsurgical complications
13/23 (56%)	10/23(44%), 3 months 14/21(67%), 12 months' follow-up	12 letters (2.4 ETDRS lines) improvement 6 months.17 letters (3.4 ETDRS lines) improvement, 12 months' follow-up	Vitreous hemorrhages (10) of which 9 were for that reason excluded from analysis
Complete: 25/29 (86%) Partial 4/29 (14%)	12/29 (41%), range 3 – 6 months' follow-up	2 ETDRS lines or more gain in 17/29 (59%), 3 months' follow-up	Recurrent submacular hemorrhage(1), vitreous hemorrhage(2)
14/15 (93%)	NI	3/15 (20%) at 3 months 3 or more ETDRS lines improvement, 13% at 1 year 3 or more lines	Second vitrectomy for inadequate displacement of hemorrhage; removal of neovascular membrane/ hemorrhagic complex surgically(1), recurrent submacular hemorrhage(1)
Complete: 9/17 (53%) Partial 8/17 (47%)	13/17 (76%) Best postop VA >20/200 (3-48 months)	Improvement of VA, Snellen, (not further specified) in 12/17 patients (71%). Mean follow-up 17.2 months, range 3-48 months	Recurrent hemorrhage(3), retinal detachment(1)
Complete : 11/16 (69%) Partial: 5/16 (31%)	NI (Only graph)	10/16 (63%) 2 or more ETDRS lines improvement, at 6 months' follow-up, mean 3,4 lines	Recurrent SMH (2), one was mild and received anti-VEGF agent, the other, a major SMH, underwent SMH removal and RPE-patch procedure
11/11 (100%)	(4/11) 36% (1-15 months' follow-up)	Final VA: 2 ETDRS lines improvement in 6/11 (55%), Mean 6.5, range 1-15 months' follow-up	Recurrent hemorrhage (3)

**Table 1: Review of 38 studies from the literature and one study from The Rotterdam Eye Hospital, The Netherlands. (continued)**

Study Group	Year of Press	Number of eyes Treated with rtPA	Etiology of Hemorrhage	Vitreotomy	Tamponade	rtPA	Anti-VEGF
Fine[47]	2010	9 (with waiting period of 30-45 minutes. Patients with retinotomy of 180-360 degrees excluded)	AMD (massive SMH)	Vitreotomy	Gas (long-acting, not further specified)	SR	-
Hillenkamp[6]	2010	47	29 SR: AMD (26), RAM (2), trauma (1). 18 IV: AMD (15), RAM (3)	Vitreotomy	Gas (SF6)	SR (29) / IV (18)	-
<b>Vitreotomy, subretinal rtPA, intravitreal/subretinal anti-VEGF agent and Gas</b>							
Treumer[48]*	2010	12	AMD	Vitreotomy	Gas (SF6)	SR	SR during surgery, IV 4 and 8 weeks after surgery (1 patient had an additional anti-VEGF injection during second rtPA application after SMH recurrence) (bevacizumab)
Arias[10]	2010	15	AMD	Vitreotomy	Gas (SF6)	SR (8) / IV (7)	IV during surgery, bevacizumab. During follow-up 'as usual' (2 ranibizumab and 1 bevacizumab) in case of CNV activity in the rtPA IV group
Treumer[14]	2011	41	AMD	Vitreotomy	Gas (SF6)	SR	SR (bevacizumab SR during surgery and IV 4 and 8 weeks after surgery. Thereafter flexible, predominantly visual acuity-driven treatment regimen with either bevacizumab or ranibizumab)
Rotterdam Eye Hospital, The Netherlands	2012	28	AMD	Vitreotomy	Gas (SF6: n=18; SR C3F8: n=10)		IV (n=27 during surgery, bevacizumab and n=27 after surgery, according to standard neovascular AMD treatment regimen ranibizumab or bevacizumab)

rtPA = Recombinant tissue plasminogen activator; Anti-VEGF = anti-vascular endothelial growth factor; VA = Visual acuity; RAM = retinal macro aneurysm; AMD = age-related macular degeneration; NI: No (clear) information provided by the authors; BCVA = best-corrected visual acuity; ETDRS = Early Treatment Diabetic Retinopathy Study; POHS = presumed ocular histoplasmosis syndrome; IPCV = Idiopathic polypoidal choroidal vasculopathy; CNV = choroidal neovascularization; SR = subretinal; IV = intravitreal; IOP= intraocular pressure; RPE tear = retinal pigment epithelium tear; PVR = proliferative vitreoretinopathy.

\* Studies which included the same study patients in two reports.

Successful Displacement (%)	Final VA >20/200 (%), Follow-Up	Eyes Gained 2 (ETDRS or Snellen) Lines or More (%), Follow-Up	Complications
NI	2/9 22% (12 months' follow-up)	4/9 2 or more ETDRS lines improvement (44%), 12 months' follow-up	Retinal detachment (1), hyphaema and/or vitreous hemorrhage (6), glaucoma (1)
IV:4/18 (22%)/ SR 16/29 (55%)	IV: 8/18 (44%) SR:18/29 (62%),1-3 months' follow-up, BCVA	IV: 8 improved - NI how many lines- (44%), SR: 19 improved -NI how many lines- (65%), Snellen. Final VA:1-3 months' follow-up	IV: Vitreous hemorrhage(1), recurrent submacular hemorrhage(1). SR: retinal detachment(3), vitreous hemorrhage(3; 1 associated with RD), recurrent submacular hemorrhage(2). RPE-tear(1)
Complete: 9/12 (75%) Incomplete 3/12 (25%)	5/12 (42%), 12 weeks' follow-up	2 or more ETDRS lines improvement in 10/12 (83%), 12 weeks' follow-up	RPE tear due to inadvertent injection of rtPA and bevacizumab (1), secondary macular hole during subretinal injection(1), SMH recurrence(2)
Complete: 8/8 and 7/7 (100%)	SR 0/8 (0%), IV 2/7 (29%), 3-24 months' follow-up	Final VA: 2 ETDRS lines or more improvement in 7/8 (88%), 7/7 (100%), Mean 11.8 months, range 3-24 months' follow-up	SR: RPE-tear(1), vitreous hemorrhage(3) IV: none
Complete: 35/41(85%)	11/26 (42%) 12-32 months' follow-up	3 months: 30/41 (73%) 2 ETDRS lines or more improvement, final VA (12-32 months): 20/26 (77%)	RPE tear due to inadvertent injection of rtPA and bevacizumab (1), secondary macular hole during subretinal injection (1), retinal detachment (1), recurrence of SMH (8)
Complete:16/28 (57%), Partial: 11/28 (39%) None: 1/28 (4%)	11/28 (39%) 1-26 months' follow-up	14/28 (50%) at 1 month 3 or more ETDRS lines improvement, Final VA: 19/28 (68%) 3 or more lines improvement, range1-26 months' follow-up	Macular hole(1), small pucker(2), recurrent submacular hemorrhage (2); in 1 causing retinal detachment, followed by PVR. Vitreous hemorrhage followed by recurrent retinal detachment and PVR (1), RPE-tear (1), hyphaema + hematocornea followed by vitreous hemorrhage (1)

the percentage of recurrent submacular hemorrhage ranged from 0-27%, No studies in group 2 reported any cases of recurrent submacular hemorrhage. Vitreous hemorrhage rate ranged from 0-45% in group 1,<sup>25,27-35,37,38,40-43</sup> and 0-43% in group 2<sup>11-13,24,44,45</sup>(Table 3).

Comparison of the complications between groups 3 (vitrectomy, subretinal rtPA, gas or air tamponade (n = 110)<sup>5-9,47</sup> and 4 (vitrectomy, subretinal rtPA and anti-VEGF agents with gas or air tamponade (n = 93, including 28 REH patients)),<sup>10,14,46</sup> revealed that in group 3, the rate of recurrent submacular hemorrhage ranged from 0-27%, versus 0-20% in group 4. Vitreous hemorrhage rates ranged from 0-67%<sup>5-9,47</sup> in group 3, to 0-38%<sup>10,14,46</sup> in group 4 (Table 3).

### Patients of the REH

At the REH, 28 patients with submacular hemorrhage due to AMD underwent vitrectomy, submacular rtPA injection and intravitreal gas injection between July 2008 and February 2011. The mean age was 83 years (standard deviation (SD)  $\pm$  6.8); 18 patients were on systemic anticoagulation therapy before surgery, and 16 patients had received one or more intravitreal anti-VEGF injections at some point before surgery. Symptoms of the submacular hemorrhage had been present for a mean of 8.5 days (SD  $\pm$  6.3), with a range of 1-22 days. Seventeen eyes were pseudophakic before surgery, and 11 were phakic; three of the latter underwent phacoemulsification and implantation of an intraocular lens during their follow-up time. Median follow up-time was 6.5 months (range 1-26 months). Twenty-seven patients received an intravitreal anti-VEGF injection during surgery, and 27 (not the same) patients received a mean of four (range 1-15) anti-VEGF injections after surgery, according to standard treatment regimen for exudative AMD.

The submacular hemorrhage was successfully displaced from the fovea in 16 patients (57%). In those patients in whom the hemorrhage was successfully displaced, the hemorrhage had been present for a mean of 8.1 days (SD  $\pm$  6.4). In 11 patients (39%), the hemorrhage was only partially displaced, and in one patient (4%), no displacement occurred. There was no relationship between the duration of hemorrhage and successful displacement.

Median preoperative best-corrected visual acuity (BCVA) was 1.8 logMAR (range 0.3-2.8). The BCVA improved significantly to a median BCVA of 1.2 (range 0.35-2.8) at 1 month postoperatively. Median BCVA at the last follow-up visit further improved 1.14 logMAR (range 0.15-2.1). The best median BCVA was found at a median of two months postoperatively (range 0.5 -13 months). This best median BCVA was 0.8 logMAR (range 0.1-2.3).

At one month after surgery 14/28 (50%) of the patients had improved two or more ETDRS lines. At the last follow-up visit, 19/28 (68%) had improved two or more ETDRS lines as compared to preoperative baseline BCVA.

**Table 2.** Comparison between minimally invasive and more invasive rtPA treatment methods

	Groups 1 and 2	Groups 3 and 4
<b>Number</b>	467	194
<b>Complete displacement, %</b>	50-100	53-100
<b>Number</b>	724	203
<b>Recurrent submacular hemorrhage, %</b>	0-27	0-27
<b>Retinal detachment, %</b>	0-45	0-11
<b>Vitreous hemorrhage, %</b>	0-45	0-67

Group 1: No vitrectomy, administration of rtPA intravitreally; group 2: No vitrectomy, administration of rtPA and anti-VEGF intravitreally; group 3: Vitrectomy, administration of rtPA subretinally; group 4: Vitrectomy, administration of rtPA subretinally, and anti-VEGF either subretinally or intravitreally. Anti-VEGF = anti-vascular endothelial growth factor inhibitors.

### Complications in the Patients of the REH

Eight of the 28 patients experienced complications after surgery. Two patients experienced a recurrent hemorrhage. One patient developed a macular hole. One patient developed an RPE tear after several anti-VEGF injections. Two patients developed a small macular pucker without further treatment. One patient developed a vitreous hemorrhage. One patient developed a hyphaema with hematocornea, followed by a vitreous hemorrhage.

Of these eight patients who experienced complications, two developed PVR. One of the patients with a recurrent submacular hemorrhage had subsequently a retinal detachment followed by PVR. The other patient was the one who developed a vitreous hemorrhage first; which was followed by a retinal detachment, subsequently followed by PVR.

## DISCUSSION

### Review of Case Series

There were several obvious difficulties in comparing the results of these 38 reports. Because all 38 reports were case series with varying sizes and protocols, there was no consistent, uniform definition or reporting of the size of the hemorrhage, initial visual acuity, time between hemorrhage and treatment, total or partial displacement of the hemorrhage, VA gain or loss, or complications. Therefore, comparisons could not be statistically analyzed, and can thus only be descriptive. It was determined that it would be most useful to describe only four groups of studies. Additionally, only the rates of the hemorrhage displacement and the major complications of vitreous hemorrhage, retinal detachment and recurrent submacular hemorrhage were compared, as only these data were retrievable in a majority (32 of 38) of the studies.

**Table 3.** Comparison between minimally invasive and more invasive rtPA treatment methods with or without additional anti-VEGF agents

	Group 1	Group 2	Group 3	Group 4
<b>Number</b>	350	117	101	93
<b>Complete displacement, %</b>	50-100	50-100	53-100	57-100
	Group 1	Group 2	Group 3	Group 4
<b>Number</b>	607	117	110	93
<b>Recurrent submacular hemorrhage, %</b>	0-27	0	0-27	0-20
<b>Vitreous hemorrhage, %</b>	0-45	0-43	0-67	0-38

Group 1: No vitrectomy, administration of rtPA intravitreally; group 2: No vitrectomy, administration of rtPA and anti-VEGF intravitreally; group 3: Vitrectomy, administration of rtPA subretinally; group 4: Vitrectomy, administration of rtPA subretinally, and anti-VEGF either subretinally or intravitreally. Anti-VEGF: vascular endothelial growth factor inhibitors.

Because of these limitations it was not possible to perform a meta-analysis and to draw firm conclusions. However, it was noted that a 100% displacement rate could be achieved in both the less invasive (injections only) and the more invasive (vitrectomy) groups. Further, complication rates were relatively consistent between groups. It is notable that no recurrent submacular hemorrhages were reported in any of the studies with intravitreal rtPA plus anti-VEGF agent.<sup>11-13;44;45</sup> Whether this was because the anti-VEGF agent helped to prevent the recurrence of submacular hemorrhage or because the recurrent submacular hemorrhages were not reported as a complication, but rather a retreatment criterion, is unclear. However, this difference is not clearly seen when vitrectomy was used in combination with anti-VEGF. Without recurrent submacular hemorrhage and treatment of choroidal neovascularization more VA gain might then also be likely, but such conclusions could not be drawn from the available data.

There are several considerations on the safe and effective use of rtPA and anti-VEGF. Regarding rtPA: retinal penetration of the molecule, its potential toxicity and the best possible timing of its administration, both in terms of survival of the overlying retina and the ability to lyse the clotted blood. Regarding the anti-VEGF: the potential side-effects of anti-VEGF agents and its potential interaction with rtPA.

### Liquefaction and Diffusion Capacities of rtPA

rtPA is a thrombolytic agent that activates plasminogen into plasmin, which has the ability to enzymatically liquefy recent hemorrhage via lysis of the fibrin. In the context of a submacular hemorrhage, this fibrin is located between the photoreceptors. Lysis of the fibrin allows for the detachment of the hemorrhage from the outer retina and RPE and its relocation, thereby minimizing shearing damage to the outer segments of the photoreceptors. The rtPA action is believed to be rapid in onset, within minutes. However, this action requires direct contact with fibrin.<sup>49</sup>

rtPA has a molecular weight of 70 kilodaltons (kDa) which prevents its diffusion across intact biological membranes.<sup>50</sup> This includes retina, as the pore size of the outer limiting membrane is between 30 and 36 angstrom, corresponding to molecules around 50 to 60 kDa.<sup>49,51</sup> However, although rtPA theoretically should not diffuse through the retina, Kamei et al. demonstrated a signal of rtPA in the neural retina in some rabbit eyes, which they hypothesize to be due to microscopic retinal tears secondary to the subretinal hemorrhage.<sup>52</sup> This might be the reason that rtPA seems to diffuse into the subretinal space in AMD patients with SMH.

### Toxicity of rtPA

rtPA might be toxic to the RPE and retina, and this has been studied in rabbits and cats.<sup>53,54</sup> In a tolerance study, a concentration of 20 µg/ml was found to be safe for the treatment of SMH, in combination with 0.25mg/ml bevacizumab.<sup>55</sup> Hesse et al. suspect a toxic effect on the retina when 100 µg is given intravitreally, as all patients treated with 100 µg experienced exudative inferior retinal detachments, while no complications were found in the patients who received 50 µg.<sup>33</sup> A case report described toxicity with 50 µg/ml rtPA.<sup>56</sup> In other studies doses up to 100 µg have been given with good outcomes and without side effects due to toxicity.<sup>29,31,44</sup> In other reports, functional improvement in the majority of patients which mainly received up to 50 µg/0.1ml rtPA, suggests the absence of direct retinal toxicity at this concentration of rtPA, administered either subretinally,<sup>5,6,48</sup> or intravitreally.<sup>6,11,32,36</sup> Based on previous reports' conclusions, namely: equal efficacy over the range of 25 µg to 100 µg intravitreally<sup>31</sup> the possibility of toxicity at doses of 50 µg or higher,<sup>33,56</sup> and the lack of adverse events reported in the toxicity tolerance study in which a concentration of 20 µg/ml was applied subretinally in combination with 0.25 mg/ml bevacizumab,<sup>55</sup> we conclude that a dose of 25 µg/0.1ml of rtPA, either administered subretinally or intravitreally, either alone or in combination with a 0.25 mg/ml concentration of bevacizumab administered intravitreally seems likely to be a safe and effective concentration.

### Toxicity of Anti-VEGF

Clinical and dose relation studies have not shown any direct retinal toxicity due to the standard dose of 1.25 mg/0.05 ml bevacizumab and 0.50 mg/0.05 ml ranibizumab. Further, no signs of toxicity were found in safety studies of intravitreal injections in rabbit,<sup>57-59</sup> murine<sup>60</sup> or bovine<sup>55</sup> models. Indeed, no difference in ERG parameters was found in mouse retina after the application of 1.0 ul of a 25 mg/ml solution of bevacizumab.<sup>60</sup> A retinal tolerance study of intravitreal injections of bevacizumab in combination with rtPA application in bovine eyes found that a concentration of 0.25mg/ml (equivalent to 1,25 mg per human eye) was safe, even when combined with the administration of 20 ug/ml rtPA.<sup>55</sup> Most convincingly, several clinical studies involved the administration of

standard doses (up to 1.5 mg Avastin) into the posterior segment after partial or total gas tamponade.<sup>10,44</sup> Given the small fluid phase left in such eyes, a high concentration of anti-VEGF might be reached. However, no toxicity was reported.<sup>10,44</sup> Indeed, Treumer<sup>14,48</sup> injected 1.25 mg/0.05ml bevacizumab subretinally, which might be expected to result in considerably higher concentrations. Also here, no apparent toxicity was noted.

### **Effectiveness of Anti-VEGF**

Severe closure of normal capillaries has been demonstrated after intravitreal injection of 1.25 mg/0.05 ml bevacizumab. When patients with severe CNV are treated with bevacizumab, ischemia of retinal capillaries and choroidal atrophy might occur.<sup>61</sup> However, injections with anti-VEGF have become standard treatment of exudative AMD, because of their effectiveness in treating choroidal neovascularization.<sup>62,63</sup> Nevertheless, certain patient groups do not respond well to anti-VEGF therapy, among whom patients with a large hemorrhage.<sup>64</sup> Other patients may exhibit over-expression of tumor necrosis factor (TNF)- $\alpha$ . This over-expression of TNF- $\alpha$  reduces the expression of FcRN, the receptor responsible for immunoglobulin transmembrane transport. Because the ocular pharmacology of anti-VEGF agents seems to depend on the expression of FcRN, some patients with a large CNV membrane due to AMD might have a low level of FcRN expression, and might therefore derive little therapeutic benefit from anti-VEGF agents.<sup>65</sup>

Subretinal rtPA and bevacizumab can be co-applied; no cleavage or functional inactivation of bevacizumab by rtPA was found *in vitro*, and significant VA improvement in the short and long term were observed *in vivo*.<sup>14,66</sup>

### **Toxicity of a Hemorrhage and Time Frame for Removal of a Hemorrhage**

Damage to the sensory retinal tissue due to SMH is caused by a limitation of nutrient passage to the retina, shrinkage of the outer retinal layers due to clot formation<sup>67</sup> and the release of retinotoxic substances such as fibrin,<sup>3</sup> iron<sup>68,69</sup> and hemosiderin.<sup>70</sup> Toxic effects of the SMH can be observed as early as 24 hours after the hemorrhage.<sup>67</sup> Later, the resolution of the hemorrhage is followed by the formation of a macular scar or fibrous tissue proliferation.<sup>71</sup> The prognosis of large, untreated subretinal hemorrhages is very poor.<sup>16,17</sup> Animal models have shown progressive, irreversible degeneration of the outer retina and RPE. As early as one day after the initiation of the hemorrhage, damage of the photoreceptor cells, characterized by edema and disintegration of the photoreceptors is observed.<sup>67</sup> At seven days, significant destruction of the outer retinal elements is seen, as well as severe degeneration, or even absence of photoreceptor outer and inner segments.<sup>3,67</sup> The RPE cells show shortened apical microvilli, and distortion of mitochondria.<sup>3</sup> At 14 days, extensive and severe destruction of the outer retinal layers is found,<sup>2,3</sup> photoreceptor damage and increasing numbers of phagocytic cells immediately overlying the RPE layer are observed. Atrophy and disorganization of

the outer retinal layers, with proliferation of fibrolytic cells, occurs in the most severely damaged areas of the retina. The inner retinal layer shows severe vacuolization. In the RPE, vacuoles can be observed, along with disorganization of the cytoplasm. The progressive, focally severe injury of the retina seems to occur between seven and 14 days after hemorrhage.<sup>3</sup> It has been reported that if SMH is removed the latest at day seven after the initial hemorrhage, retinal architectural integrity is better maintained.<sup>2</sup>

### Time-Window of Liquefaction of rtPA

Several clinical studies have been performed to investigate the importance of the interval between the initiation of hemorrhage and the surgical treatment. An rtPA-assisted surgical drainage pilot study showed that subretinal blood liquefied in 88% of the cases when injected within seven days or less, while only 37.5% demonstrated blood liquefaction if the SMH had existed for eight to fourteen days.<sup>72</sup> VA improved in all 16 eyes with a SMH of seven days or less, with half of them having a VA of 20/200 or better, while VA improved in only half of the patients with a hemorrhage of eight to 14 days, and none of these patients achieved a 20/200 VA postoperatively.<sup>72</sup>

Morse et al.<sup>73</sup> injected rtPA in four-day-old SMH. They did not find therapeutic benefit from the rtPA injection without surgical removal of the hemorrhage in a cat model. Moreover, they found a second focus of retinal degeneration at a gravity-dependent inferior site to which the blood had migrated. Hesse et al.<sup>33</sup> treated eleven patients with intravitreal rtPA, followed by a gas tamponade. Nine were treated between 12h and 72 hours of the hemorrhage; two were treated more than 14 days after the hemorrhage occurred. There was a clear advantage in those treated early as compared to those treated late. Hattenbach et al.,<sup>32</sup> who also treated with intravitreal rtPA and gas tamponade, showed that eyes with symptoms less than 14 days' duration seem to benefit most. Indeed, VA improved two or more Snellen lines in 67%, as compared with only 29% of eyes with duration of > 14 days.

It thus appears to be best to treat patients within 14 days after the initiation of the hemorrhage, because liquefaction and displacement of the clot is most likely; moreover, irreversible retinal damage occurs after that period.

## CONCLUSIONS

Intravitreal rtPA application with an intravitreal gas injection only, or subretinal rtPA application with vitrectomy and gas tamponade, can achieve successful displacement of a recent submacular hemorrhage, frequently resulting in improvement of VA. Our review of available clinical reports suggests that intravitreal rtPA and gas, without vitrectomy, might be as effective as submacular rtPA with vitrectomy, while most likely

being associated with fewer complications. Nevertheless, recent reports tend to favor the use of vitrectomy. Additional intraoperative or postoperative anti-VEGF treatment shortly after surgery might help to prevent a recurrent submacular hemorrhage. Also, it is likely that treatment within two weeks is most effective in displacing blood and increasing the chances of long-term improvement of visual acuity.

A prospective, randomized study is needed in order to investigate how the combination of intravitreal rtPA, intravitreal bevacizumab and gas tamponade of the posterior segment, without vitrectomy, compares with the combination of vitrectomy, subretinal injection of rtPA, gas tamponade and intravitreal injection of bevacizumab, in terms of safety and efficacy.

## REFERENCES

1. Kiernan DF, Hariprasad SM, Rusu IM, Mehta SV, Mieler WF, Jager RD. Epidemiology of the association between anticoagulants and intraocular hemorrhage in patients with neovascular age-related macular degeneration. *Retina*. 2010;30:1573-1578.
2. Benner JD, Hay A, Landers MB, III, Hjelmeland LM, Morse LS. Fibrinolytic-assisted removal of experimental subretinal hemorrhage within seven days reduces outer retinal degeneration. *Ophthalmology*. 1994;101:672-681.
3. Toth CA, Morse LS, Hjelmeland LM, Landers MB, III. Fibrin directs early retinal damage after experimental subretinal hemorrhage. *Arch Ophthalmol*. 1991;109:723-729.
4. Heriot WJ. Intravitreal gas and TPA; an outpatient procedure for submacular hemorrhage. In: *American Academy of Ophthalmology Annual Vitreoretinal Update*; 1996 B.C.
5. Hauptert CL, McCuen BW, Jaffe GJ, et al. Pars plana vitrectomy, subretinal injection of tissue plasminogen activator, and fluid-gas exchange for displacement of thick submacular hemorrhage in age-related macular degeneration. *Am J Ophthalmol*. 2001;131:208-215.
6. Hillenkamp J, Surguch V, Framme C, Gabel VP, Sachs HG. Management of submacular hemorrhage with intravitreal versus subretinal injection of recombinant tissue plasminogen activator. *Graefes Arch Clin Exp Ophthalmol*. 2010; 248:5-11.
7. Olivier S, Chow DR, Packo KH, MacCumber MW, Awh CC. Subretinal recombinant tissue plasminogen activator injection and pneumatic displacement of thick submacular hemorrhage in Age-Related macular degeneration. *Ophthalmology*. 2004;111:1201-1208.
8. Singh RP, Patel C, Sears JE. Management of subretinal macular haemorrhage by direct administration of tissue plasminogen activator. *Br J Ophthalmol*. 2006;90:429-431.
9. Thompson JT, Sjaarda RN. Vitrectomy for the treatment of submacular hemorrhages from macular degeneration: a comparison of submacular hemorrhage/membrane removal and submacular tissue plasminogen activator-assisted pneumatic displacement. *Trans Am Ophthalmol Soc*. 2005;103:98-107.
10. Arias L, Mones J. Transconjunctival sutureless vitrectomy with tissue plasminogen activator, gas and intravitreal bevacizumab in the management of predominantly hemorrhagic age-related macular degeneration. *Clin Ophthalmol*. 2010;4: 67-72.
11. Meyer CH, Scholl HP, Eter N, Helb HM, Holz FG. Combined treatment of acute subretinal haemorrhages with intravitreal recombinant tissue plasminogen activator, expansile gas and bevacizumab: a retrospective pilot study. *Acta Ophthalmol*. 2008;86:490-494.
12. Matt G, Sacu S, Stifter E, Prunte C, Schmidt-Erfurth U. [Combination of Intravitreal rTPA, gas and ranibizumab for extensive subfoveal haemorrhages secondary to neovascular age-related macular degeneration]. *Klin Monbl Augenheilkd*. 2010;227:221-225.
13. Sacu S, Stifter E, Vecsei-Marlovits PV, et al. Management of extensive subfoveal haemorrhage secondary to neovascular age-related macular degeneration. *Eye (Lond)*. 2009;23:1404-1410.
14. Treumer F, Roeder J, Hillenkamp J. Long-term outcome of subretinal coapplication of rtPA and bevacizumab followed by repeated intravitreal anti-VEGF injections for neovascular AMD with submacular haemorrhage. *Br J Ophthalmol*. 2011.

15. Bennett SR, Folk JC, Blodi CF, Klugman M. Factors prognostic of visual outcome in patients with subretinal hemorrhage. *Am J Ophthalmol.* 1990;109:33-37.
16. Scupola A, Coscas G, Soubrane G, Balestrazzi E. Natural history of macular subretinal hemorrhage in age-related macular degeneration. *Ophthalmologica.* 1999;213:97-102.
17. Avery RL, Fekrat S, Hawkins BS, Bressler NM. Natural history of subfoveal subretinal hemorrhage in age-related macular degeneration. *Retina.* 1996;16:183-189.
18. Lincoff H, Kreissig I, Stopa M, Uram D. A 40 degrees gaze down position for pneumatic displacement of submacular hemorrhage: clinical application and results. *Retina.* 2008;28:56-59.
19. Stopa M, Lincoff A, Lincoff H. Analysis of forces acting upon submacular hemorrhage in pneumatic displacement. *Retina.* 2007;27:370-374.
20. Holladay JT. Visual acuity measurements. *J Cataract Refract Surg.* 2004;30:287-290.
21. Ohji M, Saito Y, Hayashi A, Lewis JM, Tano Y. Pneumatic displacement of subretinal hemorrhage without tissue plasminogen activator. *Arch Ophthalmol.* 1998;116:1326-1332.
22. Gopalakrishnan M, Giridhar A, Bhat S, Saikumar SJ, Elias A, N S. Pneumatic displacement of submacular hemorrhage: safety, efficacy, and patient selection. *Retina.* 2007;27:329-334.
23. Ron Y, Ehrlich R, xer-Siegel R, Rosenblatt I, Weinberger D. Pneumatic displacement of submacular hemorrhage due to age-related macular degeneration. *Ophthalmologica.* 2007;221:57-61.
24. Cakir M, Cekic O, Yilmaz OF. Pneumatic displacement of acute submacular hemorrhage with and without the use of tissue plasminogen activator. *Eur J Ophthalmol.* 2010;20:565-571.
25. Fang IM, Lin YC, Yang CH, Yang CM, Chen MS. Effects of intravitreal gas with or without tissue plasminogen activator on submacular haemorrhage in age-related macular degeneration. *Eye (Lond).* 2009;23:397-406.
26. Mizutani T, Yasukawa T, Ito Y, et al. Pneumatic displacement of submacular hemorrhage with or without tissue plasminogen activator. *Graefes Arch Clin Exp Ophthalmol.* 2011;249:1153-1157.
27. Yang PM, Kuo HK, Kao ML, Chen YJ, Tsai HH. Pneumatic displacement of a dense submacular hemorrhage with or without tissue plasminogen activator. *Chang Gung Med J.* 2005;28:852-859.
28. Tsymanava A, Uhlig CE. Intravitreal recombinant tissue plasminogen activator without and with additional gas injection in patients with submacular haemorrhage associated with age-related macular degeneration. *Acta Ophthalmol.* 2011.
29. Chen CY, Hooper C, Chiu D, Chamberlain M, Karia N, Heriot WJ. Management of submacular hemorrhage with intravitreal injection of tissue plasminogen activator and expansile gas. *Retina.* 2007;27:321-328.
30. Handwerger BA, Blodi BA, Chandra SR, Olsen TW, Stevens TS. Treatment of submacular hemorrhage with low-dose intravitreal tissue plasminogen activator injection and pneumatic displacement. *Arch Ophthalmol.* 2001;119:28-32.
31. Hassan AS, Johnson MW, Schneiderman TE, et al. Management of submacular hemorrhage with intravitreal tissue plasminogen activator injection and pneumatic displacement. *Ophthalmology.* 1999;106:1900-1906.
32. Hattenbach LO, Klais C, Koch FH, Gumbel HO. Intravitreal injection of tissue plasminogen activator and gas in the treatment of submacular hemorrhage under various conditions. *Ophthalmology.* 2001;108:1485-1492.

- 33.** Hesse L, Schmidt J, Kroll P. Management of acute submacular hemorrhage using recombinant tissue plasminogen activator and gas. *Graefes Arch Clin Exp Ophthalmol*. 1999;237:273-277.
- 34.** Krepler K, Kruger A, Tittl M, Stur M, Wedrich A. Intravitreal injection of tissue plasminogen activator and gas in subretinal hemorrhage caused by age-related macular degeneration. *Retina*. 2000;20:251-256.
- 35.** Meier P, Zeumer C, Jochmann C, Wiedemann P. [Management of submacular hemorrhage by tissue plasminogen activator and SF(6) gas injection]. *Ophthalmologe*. 1999;96:643-647.
- 36.** Schulze SD, Hesse L. Tissue plasminogen activator plus gas injection in patients with subretinal hemorrhage caused by age-related macular degeneration: predictive variables for visual outcome. *Graefes Arch Clin Exp Ophthalmol*. 2002;240:717-720.
- 37.** Buhl M, Scheider A, Schonfeld CL, Kampik A. [Intra-vitreous rt-PA and gas introduction in submacular hemorrhage]. *Ophthalmologe*. 1999;96:792-796.
- 38.** Framme C, Toukhy HE, Sachs HG, et al. [Clinical results of intravitreal administration of tissue-type plasminogen activator (tPA) and gas for removal of subretinal hemorrhage in senile macular degeneration]. *Klin Monbl Augenheilkd*. 2000;216:33-39.
- 39.** Hattenbach LO, Brieden M, Koch F, Gumbel H. [Intravitreal injection of rt-PA and gas in the management of minor submacular haemorrhages secondary to age-related macular degeneration]. *Klin Monbl Augenheilkd*. 2002;219:512-518.
- 40.** Kung YH, Wu TT, Hong MC, Sheu SJ. Intravitreal tissue plasminogen activator and pneumatic displacement of submacular hemorrhage. *J Ocul Pharmacol Ther*. 2010;26:469-474.
- 41.** Ratanasukon M, Kittantong A. Results of intravitreal tissue plasminogen activator and expansile gas injection for submacular haemorrhage in Thais. *Eye (Lond)*. 2005;19:1328-1332.
- 42.** Tsai SC, Lin JM, Chen HY. Intravitreal recombinant tissue plasminogen activator and gas to treat submacular hemorrhage in age-related macular degeneration. *Kaohsiung J Med Sci*. 2003;19:608-616.
- 43.** Wu TT, Kung YH, Hong MC. Vitreous hemorrhage complicating intravitreal tissue plasminogen activator and pneumatic displacement of submacular hemorrhage. *Retina*. 2011;31:2071-2077.
- 44.** Guthoff R, Guthoff T, Meigen T, Goebel W. Intravitreal injection of bevacizumab, tissue plasminogen activator, and gas in the treatment of submacular hemorrhage in age-related macular degeneration. *Retina*. 2011;31:36-40.
- 45.** Mayer WJ, Hakim I, Haritoglou C, et al. Efficacy and safety of recombinant tissue plasminogen activator and gas versus bevacizumab and gas for subretinal haemorrhage. *Acta Ophthalmol*. 2011.
- 46.** Sandhu SS, Manvikar S, Steel DH. Displacement of submacular hemorrhage associated with age-related macular degeneration using vitrectomy and submacular tPA injection followed by intravitreal ranibizumab. *Clin Ophthalmol*. 2010;4:637-642.
- 47.** Fine HF, Iranmanesh R, Del Priore LV, et al. Surgical outcomes after massive subretinal hemorrhage secondary to age-related macular degeneration. *Retina*. 2010;30:1588-1594.
- 48.** Treumer F, Klatt C, Roeder J, Hillenkamp J. Subretinal coapplication of recombinant tissue plasminogen activator and bevacizumab for neovascular age-related macular degeneration

- with submacular haemorrhage. *Br J Ophthalmol*. 2010;94:48-53.
- 49.** Kamei M, Estafanous M, Lewis H. Tissue plasminogen activator in the treatment of vitreoretinal diseases. *Semin Ophthalmol*. 2000;15:44-50.
- 50.** Ranby M, Bergsdorf N, Nilsson T. Enzymatic properties of the one- and two-chain form of tissue plasminogen activator. *Thromb Res*. 1982;27:175-183.
- 51.** Bunt-Milam AH, Saari JC, Klock IB, Garwin GG. Zonulae adherentes pore size in the external limiting membrane of the rabbit retina. *Invest Ophthalmol Vis Sci*. 1985;26:1377-1380.
- 52.** Kamei M, Misono K, Lewis H. A study of the ability of tissue plasminogen activator to diffuse into the subretinal space after intravitreal injection in rabbits. *Am J Ophthalmol*. 1999;128:739-746.
- 53.** Hrach CJ, Johnson MW, Hassan AS, Lei B, Sieving PA, Elner VM. Retinal toxicity of commercial intravitreal tissue plasminogen activator solution in cat eyes. *Arch Ophthalmol*. 2000;118:659-663.
- 54.** Johnson MW, Olsen KR, Hernandez E, Irvine WD, Johnson RN. Retinal toxicity of recombinant tissue plasminogen activator in the rabbit. *Arch Ophthalmol*. 1990;108:259-263.
- 55.** Luke M, Januschowski K, Warga M, et al. The retinal tolerance to bevacizumab in co-application with a recombinant tissue plasminogen activator. *Br J Ophthalmol*. 2007;91:1077-1082.
- 56.** Chen SN, Yang TC, Ho CL, Kuo YH, Yip Y, Chao AN. Retinal toxicity of intravitreal tissue plasminogen activator: case report and literature review. *Ophthalmology*. 2003;110:704-708.
- 57.** Feiner L, Barr EE, Shui YB, Holekamp NM, Brantley MA, Jr. Safety of intravitreal injection of bevacizumab in rabbit eyes. *Retina*. 2006;26:882-888.
- 58.** Manzano RP, Peyman GA, Khan P, Kivilcim M. Testing intravitreal toxicity of bevacizumab (Avastin). *Retina*. 2006;26:257-261.
- 59.** Sari A, Adiguzel U, Canankatan N, Yilmaz N, Dinc E, Oz O. Effects of intravitreal bevacizumab in repeated doses: an experimental study. *Retina*. 2009;29:1346-1355.
- 60.** Heiduschka P, Julien S, Hofmeister S, Bartz-Schmidt KU, Schraermeyer U. Bevacizumab (avastin) does not harm retinal function after intravitreal injection as shown by electroretinography in adult mice. *Retina*. 2008;28:46-55.
- 61.** Ameri H, Chader GJ, Kim JG, Satta SR, Rao NA, Humayun MS. The effects of intravitreal bevacizumab on retinal neovascular membrane and normal capillaries in rabbits. *Invest Ophthalmol Vis Sci*. 2007;48:5708-5715.
- 62.** Brown DM, Michels M, Kaiser PK, Heier JS, Sy JP, Ianchulev T. Ranibizumab versus verteporfin photodynamic therapy for neovascular age-related macular degeneration: Two-year results of the ANCHOR study. *Ophthalmology*. 2009;116:57-65.
- 63.** Rosenfeld PJ, Brown DM, Heier JS, et al. Ranibizumab for neovascular age-related macular degeneration. *N Engl J Med*. 2006;355:1419-1431.
- 64.** Lux A, Llacer H, Heussen FM, Jousseaume AM. Non-responders to bevacizumab (Avastin) therapy of choroidal neovascular lesions. *Br J Ophthalmol*. 2007;91:1318-1322.
- 65.** van Bilsen K, van Hagen PM, Bastiaans J, et al. The neonatal Fc receptor is expressed by human retinal pigment epithelial cells and is downregulated by tumour necrosis factor- $\alpha$ . *Br J Ophthalmol*. 2011;95:864-868.

66. Klettner A, Puls S, Treumer F, Roeder J, Hillenkamp J. Compatibility of Recombinant Tissue Plasminogen Activator and Bevacizumab Co-Applied for Neovascular Age-Related Macular Degeneration With Submacular Hemorrhage. In: 2012.
67. Glatt H, Machemer R. Experimental subretinal hemorrhage in rabbits. *Am J Ophthalmol.* 1982;94:762-773.
68. Bhisitkul RB, Winn BJ, Lee OT, et al. Neuroprotective effect of intravitreal triamcinolone acetonide against photoreceptor apoptosis in a rabbit model of subretinal hemorrhage. *Invest Ophthalmol Vis Sci.* 2008;49:4071-4077.
69. Gillies A, Lahav M. Absorption of retinal and subretinal hemorrhages. *Ann Ophthalmol.* 1983; 15:1068-1074.
70. el Baba F, Jarrett WH, Harbin TS, Jr., et al. Massive hemorrhage complicating age-related macular degeneration. Clinicopathologic correlation and role of anticoagulants. *Ophthalmology.* 1986;93: 1581-1592.
71. Scupola A, Coscas G, Soubrane G, Balestrazzi E. Natural history of macular subretinal hemorrhage in age-related macular degeneration. *Ophthalmologica.* 1999;213:97-102.
72. Lewis H. Intraoperative fibrinolysis of submacular hemorrhage with tissue plasminogen activator and surgical drainage. *Am J Ophthalmol.* 1994;118:559-568.
73. Morse LS, Benner JD, Hjelmeland LM, Landers MB, III. Fibrinolysis of experimental subretinal haemorrhage without removal using tissue plasminogen activator. *Br J Ophthalmol.* 1996;80: 658-662.

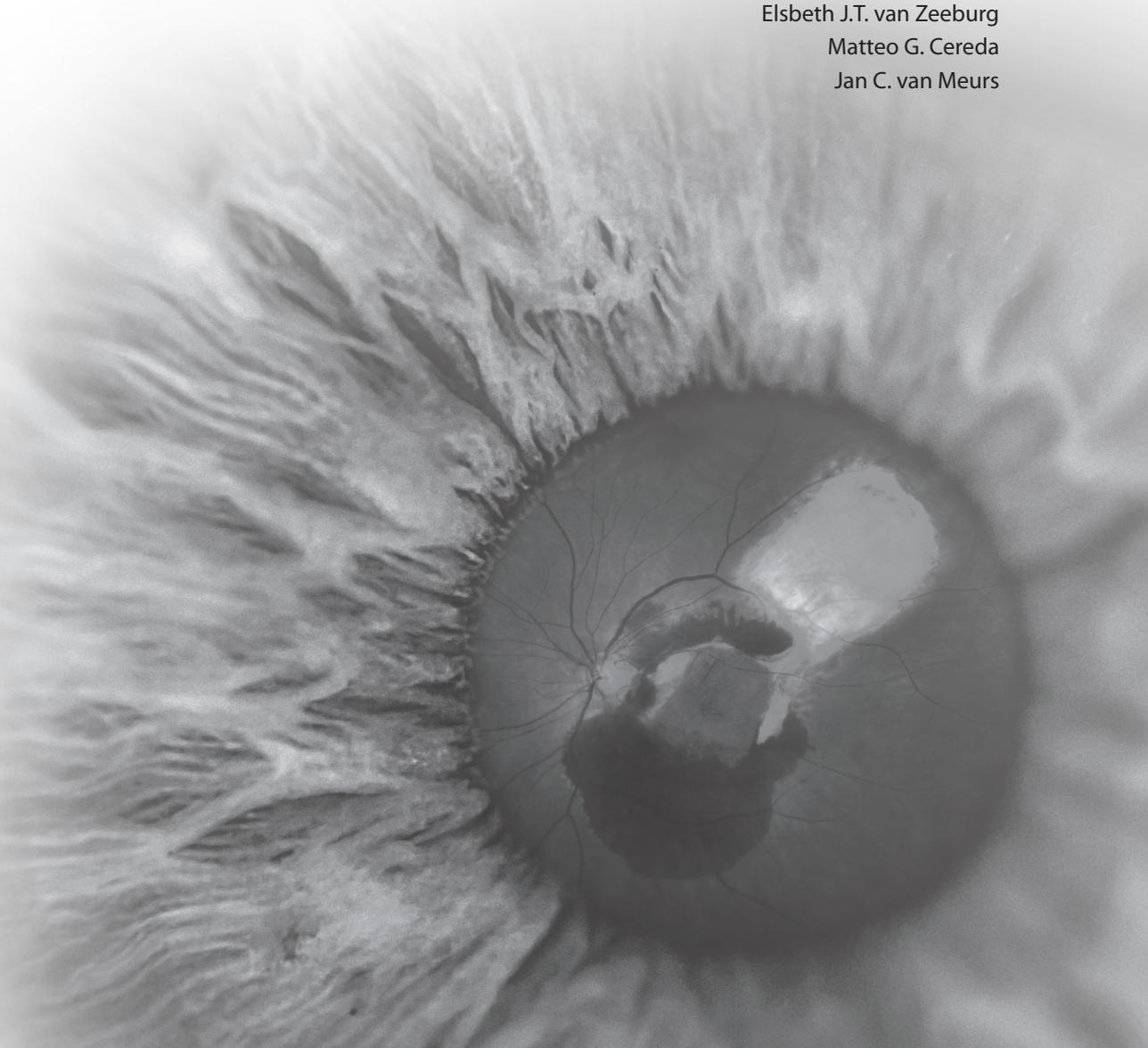


## **Chapter 6.2**

# **Recombinant Tissue Plasminogen Activator, Vitrectomy, and Gas for Recent Submacular Hemorrhage Displacement Due To Retinal Macroaneurysm**

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## ABSTRACT

### Background

The visual prognosis of submacular hemorrhages caused by a retinal arterial macroaneurysm (RAM) is poor if left untreated. The use of recombinant tissue plasminogen activator (rtPA) has frequently been reported to displace submacular hemorrhages from the foveal area in patients with age-related macular degeneration. This study aims to investigate the results of displacement of recent-onset submacular hemorrhages due to RAM.

### Methods

Institutional retrospective interventional case series of 12 patients with macular hemorrhage due to RAM, who underwent pars plana vitrectomy (PPV); followed in 11 by submacular injection of rtPA and gas tamponade. Main outcome measures were displacement of the hemorrhage, complication rate and visual acuity at one month after surgery and at the last follow-up visit.

### Results

One month after surgery, the hemorrhage had been successfully displaced in ten out of 11 patients. In these ten patients, visual acuity (VA) increased by a mean of 1.2 logMAR at one month after surgery. At the last follow-up visit the mean increase was 1.5 logMAR. Complications consisted of a vitreous hemorrhage and hyphaema, retinal detachment, a new submacular hemorrhage, and vitreous hemorrhage after argon laser retinal photocoagulation of the RAM.

### Conclusions

PPV with submacular rtPA and gas injection may successfully displace a recently developed submacular hemorrhage in patients with RAM, with a marked improvement in VA that is likely to be greater than if left untreated.

## INTRODUCTION

A retinal arterial macroaneurysm (RAM) is a localized dilatation of a retinal artery. It is a condition most frequently encountered in elderly patients and is associated with hypertension and vascular diseases.<sup>1</sup> Women in the sixth to eight decades of life with systemic hypertension are most frequently affected.<sup>1</sup> The precise incidence of RAM remains unknown, as most reports concern only symptomatic patients. Visual symptoms can be caused by extravasation of fluid and lipids, or by hemorrhage, which may dissect into the vitreous, under the hyaloid, under the internal limiting membrane (ILM), within the retina, or under the retina. The visual prognosis of RAM patients can be poor; approximately half of the patients end up with a VA of 20/100 or less, (Schatz et al. 1990) although the final VA strongly depends on the location of the hemorrhage. A subretinal hemorrhage rapidly causes damage to the overlying retina.<sup>2,3</sup> As patients with a submacular hemorrhage have the poorest visual outcome, with a median final VA of 20/200,<sup>4</sup> these patients merit consideration of early displacement.

Recombinant tissue plasminogen activator (rtPA) has been reported to liquefy the hemorrhage and, in combination with gas tamponade, to displace it from the macula to a site where it is less harmful to central visual acuity.<sup>5</sup> Previous submacular hemorrhage treatment modalities, with or without rtPA, have primarily concerned patients with age-related macular degeneration (AMD), and only a few studies have included or report only about RAM patients.<sup>6-19</sup> Although these hemorrhages are analogous, the underlying pathology is very different. In patients with AMD, functional outcome after hemorrhage displacement is largely determined by the extent of damage to the retina due to choroidal neovascularization and atrophy, whereas the fovea in patients with RAM is often otherwise normal. The goal of this study was to evaluate the results of 12 RAM patients with a recent submacular hemorrhage, of whom 11 underwent subretinal injection of rtPA and vitrectomy with gas tamponade.

## PATIENTS & METHODS

### Patients

In this retrospective institutional consecutive case series, 12 patients with a recent submacular hemorrhage and a RAM were included. Diagnosis of both submacular hemorrhage and RAM was based on fundus examination. Eleven patients underwent vitrectomy with subretinal rtPA injection and gas tamponade between December 2006 and February 2012 at the Rotterdam Eye Hospital, Rotterdam, The Netherlands. One patient underwent vitrectomy and ILM removal only. The only exclusion criterion was submacular hemorrhage of non-RAM etiology.

The main outcome measures were displacement of hemorrhage and complications after surgery. Secondary outcome measures were best-corrected visual acuity (BCVA) one month after surgery, and BCVA at the last follow-up visit. Patients were typically examined on the first postoperative day, at one or two weeks, and at one and/or two months. Thereafter, patients were followed up based on need, as determined by the treating retinal specialist. Follow-up examinations consisted of a comprehensive ophthalmological examination, including BCVA, applanation tonometry measurements, fundus examination and fundus photography.

Visual acuity was measured in decimal fraction using a projector or in Snellen notations using an Early Treatment of Diabetic Retinopathy Study (ETDRS) chart. All measurements were transposed into logarithm of minimal angle of resolution (logMAR) values before analysis in which "finger counting" was transposed into logMAR 2, and "hand movement" into logMAR 3.<sup>20</sup>

The hemorrhage size was estimated from preoperative photographs or angiograms, perioperative video or drawings of the hemorrhage in the patients chart and calculated in number of disk area (DA), as not all patients were imaged before surgery. The location (i.e. preretinal, intraretinal or subretinal) of the hemorrhage before surgery was determined either from the chart, from fundus photographs, perioperative videos or from the surgical notes, ultrasound was not performed in these cases. Displacement of the hemorrhage was evaluated on fundus examination and categorized as follows: complete: the macular area is completely free from hemorrhage; partial: hemorrhage was still present in the macular area but the volume of blood was considerably reduced; absent: hemorrhage was still present in the macular area, and the volume of blood did not reduce considerably.

For each patient the following data were entered into a database: duration of symptoms, VA before surgery, size and location of the hemorrhage, use of anti-coagulant drugs, presence of hypertension or diabetes, displacement of the hemorrhage at two weeks and one month, VA one month postoperatively, VA at time of last follow-up, positioning advice after surgery, and complications.

All patients provided informed consent for the surgical procedure and for preoperative and postoperative examinations, in accordance to the tenets of the Declaration of Helsinki. The Scientific Review Board of The Rotterdam Eye Hospital, The Netherlands waived approval for the retrospective study.

### **Surgery With Recombinant Tissue Plasminogen Activator and Gas**

After the induction of a posterior vitreous detachment, a complete pars plana vitrectomy (PPV) was performed. We used a 23 G cannula with a 41-G tip, connected by tubing to a tuberculin syringe (Dutch Ophthalmic Research Center [DORC], Zuidland, The Netherlands), filled with the rtPA solution. Once the 41-G tip was inserted through

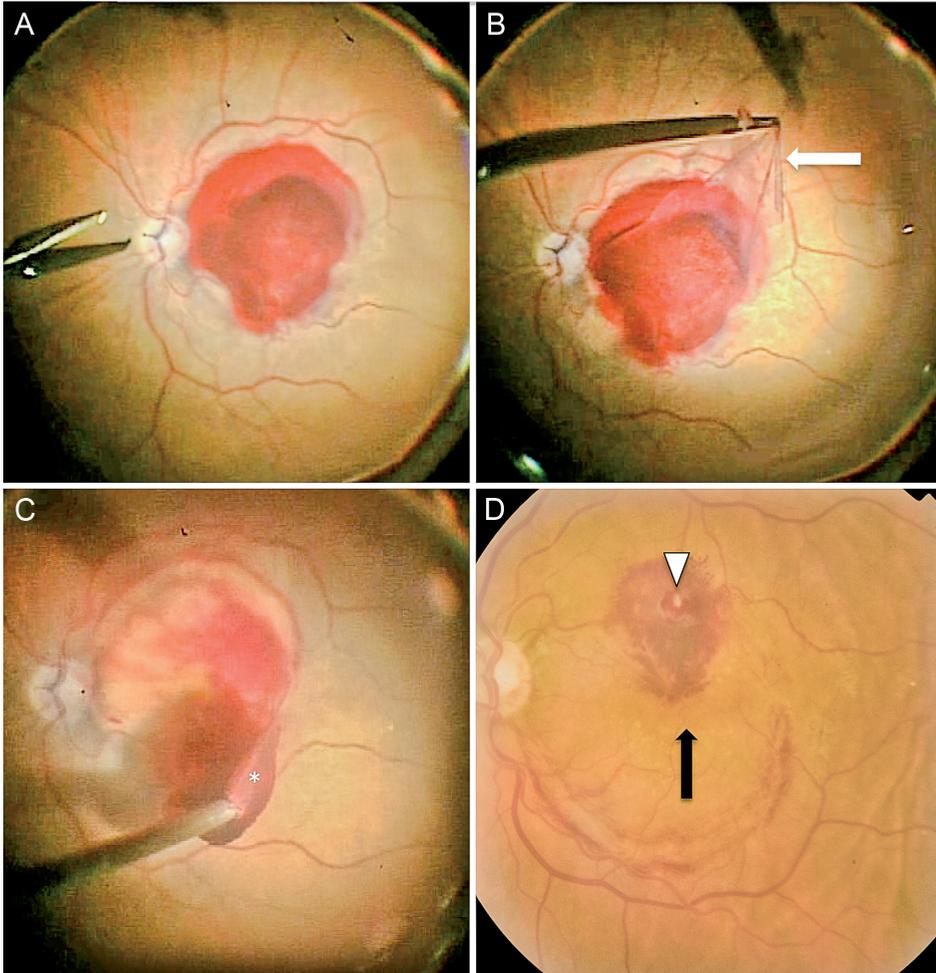
the retina, the assistant would inject 0.1 ml of fluid (20 µg/0.1 ml, Actilyse, Boehringer Ingelheim, Ingelheim, Germany), into the subretinal space or clot, creating a local retinal detachment encompassing (a part of) the blood clot. The injection hole was most frequently created at the superior edge of the hemorrhage. The ILM was peeled, starting from the injection site. This peeling was performed to prevent formation of a macular pucker or proliferative vitreoretinopathy (PVR) after surgery. After fluid/air exchange, the vitreous cavity was filled with a 15% hexafluoride (SF<sub>6</sub>) gas/air mixture. Patients were instructed to maintain an upright, a lateral or a prone position. Argon laser retinal photocoagulation was performed on and/or around the RAM after surgery, when activity such as hard exudates, macular edema, subretinal fluid or new hemorrhage was observed.

## RESULTS

### Patients

Twelve patients with RAM were operated. The diagnosis of the RAM was made before surgery in seven patients, during surgery in four patients and after surgery in one patient. One patient turned out to have a primarily premacular hemorrhage. In this case, removal of the ILM and sub-ILM blood allowed visualization of the posterior pole, revealing that the subretinal hemorrhage did not extend into the fovea. The patient did not receive any rtPA (Fig 1).

The mean age of the remaining 11 patients who received rtPA during surgery was 79.9 years (standard deviation [SD] 10.7). Seven patients were on anticoagulant therapy before surgery (three platelet aggregator inhibitor and four anticoagulant therapy). Seven patients used anti-hypertensive drugs. One patient had diabetes which was well controlled by diet alone. The mean duration of symptoms before surgery was 5.9 days (SD 2.6, range 3-10 days). All patients had a subretinal hemorrhage. The mean size of the subretinal hemorrhage before surgery was 6.6 DA (SD 2.5, range 3-10 DA). In addition to the subretinal hemorrhage, pre-retinal hemorrhage was present in two patients, preretinal and sub-ILM hemorrhages in one patient, a sub-ILM hemorrhage in two patients, an intravitreal hemorrhage in one patient, and an intraretinal hemorrhage in three patients. Of the 11 patients who received rtPA during surgery, four patients were pseudophakic before surgery and seven patients underwent cataract extraction and intraocular lens implantation during the surgery (n = 1) or shortly thereafter (n = 6) (Table 1).



**Figure 1.** Removal of a sub-internal limiting membrane hemorrhage due to retinal arterial macroaneurysm. Color fundus photography of only sub-internal limiting membrane (ILM) hemorrhage removal obtained during surgery (**a**, **b**, **c**) and at last follow up (**d**). The patient initially presented to the clinic with visual acuity of logMAR 2. **a** As soon as surgery started a pre-retinal hemorrhage was visible at the posterior pole. No vascular abnormalities were visible. **b** Peeling of the ILM (*white arrow*) was performed. **c** After peeling, the clot (*white asterisks*) was easily removable from the posterior pole, exposing a preserved retina. **d** At last follow-up, the fovea (*black arrow*) appeared completely free of blood. The macroaneurysm was clearly visible (*white arrow head*). A new hemorrhage close to the retinal arterial macroaneurysm was observed. Visual acuity had increased to 0.7 logMAR.

### Visual Acuity

Median pre-operative BCVA of the 11 patients who received rtPA during surgery was 1.8 LogMAR (range 0.52-2.8). Median follow-up time was eight months (range 3-48 months). The median BCVA one month after surgery was 0.8 LogMAR (Range 0.16-1.8). Median BCVA at the last follow-up visit was 0.46 LogMAR (range 0.18-1.8).



**Figure 2.** Pre- and postoperative color fundus photography of an initially incomplete displacement of a submacular hemorrhage due to retinal arterial macroaneurysm. Color fundus photography of patient 3: at presentation (**a**), 1 month (**b**) and 3 months (**c**) after surgery. **a** Patient 3 presented with a suddenly decrease of visual acuity in his left eye. Visual acuity was 1.3 logMAR. A subretinal hemorrhage (white asterisks) involving the macula and fovea area was present. Co-existence of a preretinal hemorrhage (black arrow showing the level of the blood) obscured the macroaneurysm. **b** One month after surgery the subretinal hemorrhage was partially displaced but still present (white asterisks). Visual acuity had increased up to 0.82 logMAR. The macroaneurysm (white arrow) was clearly visible. **c** Three months after surgery the subretinal hemorrhage had almost completely disappeared. Visual acuity reached 0.7 logMAR. At this moment argon laser retinal coagulation was applied directly to the macroaneurysm (white arrow) and around it (black asterisks). The whitening of the retinal arterial macroaneurysm and of the retinal tissue around it clearly shows that the color fundus photography was obtained a few minutes after laser treatment.

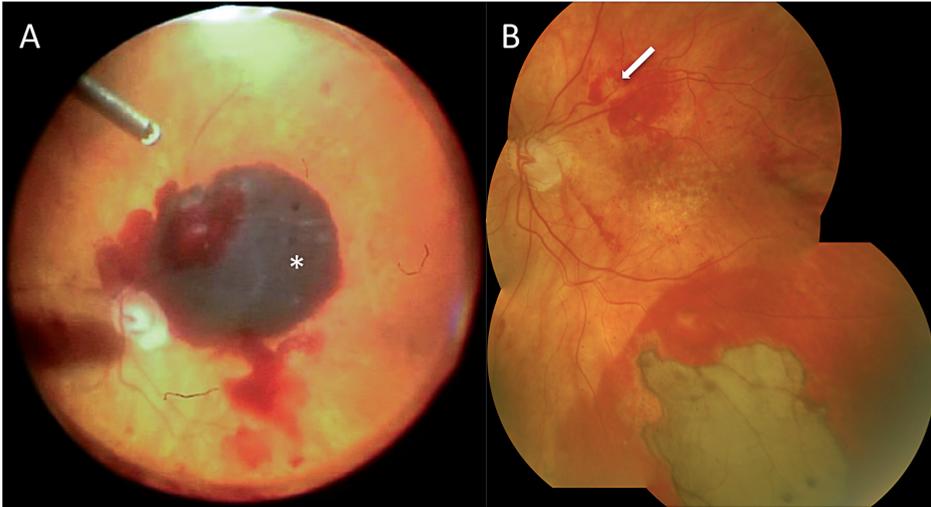
**Table 1: Patient characteristics**

Patient	Age	Gender	Eye	Time Before Surgery (Days)	Hemorrhage Size (DA)	Hemorrhage Location	Head Position	Displacement After 2 Weeks	Displacement After 1 Month	Preop VA (logMAR)	1 month VA (logMAR)	Last stop VA (logMAR)	Time Point Last Measured VA (months)	Laser After Surgery	Complications
1	71	F	OS	3	7	Subretinal and sub-ILM	None	partial	complete	2.8	1	0.7	19	yes	no
2	79	M	OD	10	4	Subretinal and intravitreal	Straight up, right side	partial	complete	2.8	0.52	0.3	48	no	no
3	88	F	OS	6	10	Subretinal and preretinal	Straight up	partial	partial at 1 month, complete at 3 months	1.3	0.82	0.8	8	yes, twice	hyphema and vitreous hemorrhage
4	87	F	OD	3	5	Subretinal and intraretinal	Right side	partial	partial	1.8	0.8	0.22	7	no	no
5	85	F	OD	10	8	Subretinal and preretinal and sub-ILM	Straight up	complete	complete	2.1	0.8	0.46	12	no	retinal detachment
6	60	F	OD	6	10	Subretinal and sub-ILM	Straight up	partial	complete	2.3	1.5	0.7	18	yes	no
7	64	M	OD	4	3	Subretinal and intraretinal	Straight up	complete	complete	2.8	0.28	0.4	8	yes, twice	no
8	88	F	OD	5	6	Subretinal	Right side	complete	complete	0.52	0.16	0.18	8	no	no
9	81	F	OS	8	4	Subretinal and preretinal	Prone posturing	partial	complete	1.5	0.7	0.52	5	yes	vitreous hemorrhage after argon laser

**Table 1: Patient characteristics** (continued)

Patient	Age	Gender	Eye	Time Before Surgery (Days)	Hemorrhage Size (DA)	Hemorrhage Location	Head Position	Displacement After 2 Weeks	Displacement After 1 Month	Preop VA (logMAR)	1 month VA (logMAR)	Last Postop VA (logMAR)	Time Point Last Measured VA (months)	Laser After Surgery	Complications
10	82	F	OS	3	7	Subretinal and intraretinal	Prone and posturing	partial	complete	1.8	1.8	1.8	3	no	new subretinal hemorrhage
11	94	F	OS	7	9	Subretinal	Left side	partial	complete	1.3	0.4	0.4	3	no	no

DA = Disc area; VA = visual acuity; logMAR = logarithm of the minimum angle of resolution.



**Figure 3.** Pre- and postoperative color fundus photography of a complete displacement of a submacular hemorrhage due to retinal arterial macroaneurysm. Color fundus photography of patient 11: at presentation (**a**), and at 7 weeks (**b**) after surgery. **a** Patient 11 presented with a sudden decrease of visual acuity and flashes of light in her left eye. Visual acuity was 1.3 logMAR. A subretinal hemorrhage (*white asterisks*) involving the macula and fovea area was present. **b** Seven weeks after surgery the subretinal hemorrhage had completely displaced from the foveal area to inferior. The *white arrow* points at the macroaneurysm. Visual acuity had increased to 0.4 logMAR.

At one month after surgery nine out of 11 patients (82%) experienced improvement in VA to 1.0 logMAR or better. One patient had no change in VA up until last follow-up visit. Mean VA improvement, of the ten patients who improved at one month, was 1.2 logMAR. Mean VA improvement of all 11 patients at one month was 1.1 logMAR. At last follow-up visit, ten out of 11 patients (91%) improved in VA to a logMAR of 0.8 or better. Mean improvement in VA at last follow-up visit of these ten improved patients was 1.5 logMAR. Mean improvement of all 11 patients at last visit was 1.3 logMAR.

### Displacement of Hemorrhage

Two weeks after surgery the hemorrhage was completely displaced from the submacular retina in three out of 11 patients. At one month after surgery the hemorrhage was completely displaced in nine patients. In the remaining two patients, one had a very thin layer of blood still visible under the macula but VA improved significantly at three months (patient 4). The displacement of the second patient (patient 3), partial at one month and complete at three months, is shown in Figure 2. In Figure 3, the complete displacement of a subretinal hemorrhage only (patient 11) is shown.

## Laser Therapy

After surgery five patients received retinal argon laser photocoagulation therapy on and/or around the RAM at least once (at median two months, range 2-13 months), of whom two twice (second time at four and ten months postoperatively). One patient had a vitreous hemorrhage after argon laser treatment, which required a secondary vitrectomy one month thereafter without further complications.

## Postoperative Complications

One patient developed a macula-off retinal detachment, which was successfully repaired with subsequent vitrectomy and gas tamponade. One patient developed a vitreous hemorrhage and a hyphema due to a recurrent hemorrhage from the RAM, which cleared sufficiently to undergo laser treatment two and four months after surgery. One patient developed a new submacular hemorrhage 20 days after initial surgery while the older submacular blood had only partially displaced at that time. This patient underwent a second operation with submacular rtPA administration and gas filling one day after the new hemorrhage, after which a macular hole developed. The hemorrhage displaced completely after second surgery. This was the only patient who did not experience any visual improvement.

## DISCUSSION

We retrospectively studied 12 patients with a macular hemorrhage due to a RAM, of whom 11 had a submacular hemorrhage and underwent pars plana vitrectomy with submacular rtPA and intravitreal gas injection. We intended to intervene early enough (mean duration of the hemorrhage was 5.9 days (SD 2.6)) to prevent irreversible damage due to the presence of blood, and to allow liquefaction by rtPA.<sup>2,3,21,22</sup>

We intended to displace the hemorrhage from the submacular area without undue delay. It was not always possible to determine, preoperatively, whether the hemorrhage was due to AMD or RAM and whether the observed hemorrhage represented sub-ILM blood only or also intraretinal or submacular blood.<sup>16</sup>

Indeed, in one patient (fig 1), ophthalmoscopy alone did not suffice to localize the hemorrhage, and the use of spectral domain optical coherence tomography, but not fluorescein or indocyanine green angiography could have helped to exclude subhyaloid/sub-ILM hemorrhage only.<sup>23,24</sup> We could have considered neodymium-doped yttrium aluminum garnet (YAG) laser opening of the subhyaloid hemorrhage,<sup>25,26</sup> if we would have been certain there was no subretinal component. Because we felt that the preretinal hemorrhage was too thick to allow optical coherence tomography to exclude the presence of subretinal hemorrhage, we choose to proceed to vitrectomy directly, without further imaging.

In AMD patients, the rates of complete displacement of a submacular hemorrhage with vitrectomy, submacular rtPA administration and air or gas as a tamponade vary between 53% and 100%.<sup>8,27-33</sup> However, successful displacement is not always associated with VA improvement.<sup>27,31</sup> As expected from the different underlying pathology, it appears that patients with RAM enjoy a better functional outcome after hemorrhage displacement, than those with AMD.<sup>9,12</sup>

More invasive rtPA assisted surgery has been described for both AMD and RAM patients, with removal of older hemorrhages by forceps or surgical drainage, with subsequent poorer results and more complications.<sup>9,14,34,35</sup>

Less invasive treatment options have been described for patients with an acute submacular hemorrhage due to RAM and AMD. In particular, intravitreal injection of rtPA and gas tamponade without vitrectomy was performed, assuming sufficient diffusion through the retina through microscopic retinal tears secondary to the subretinal hemorrhage.<sup>36</sup> This was reported in a case series of six patients with RAM,<sup>12</sup> as well as in five and 14 RAM patients included in two case series of submacular hemorrhages due to various etiologies.<sup>6,15</sup> Only one report included five patients with RAM which underwent a vitrectomy and an intravitreal ( $n = 2$ ) or a subretinal rtPA injection ( $n = 3$ ) and a gas tamponade.<sup>8</sup>

The current study of 11 patients, the largest series describing subretinal rtPA administration for RAM, reports good functional results with vitrectomy, submacular rtPA injection and gas tamponade. Ten out of 11 patients had a remarkable VA improvement (mean improvement of 1.5 logMAR), and ten out of 11 had a VA of  $> 20/200$  at last follow-up visit after surgery. Mean VA improvement of all 11 patients at last visit was 1.3 logMAR. These results were obtained despite several significant postoperative complications, including one retinal detachment, one vitreous hemorrhage and one recurrent submacular hemorrhage, and suggests outcomes better than natural history.

It remains uncertain whether a pushing or rolling effect<sup>10</sup> of a partial fill gas bubble after intravitreal gas injection,<sup>5,10</sup> or gravity,<sup>37,38</sup> in a more complete gas fill after vitrectomy, is the major mechanism for blood displacement after liquefaction by rtPA. Intravitreal injection of rtPA and gas would rely more on the former mechanism of blood displacement, and may be a safer method, whereas subretinal injection of rtPA during vitrectomy with gas tamponade would rely on the latter displacement mechanism. These assumptions must be investigated in a controlled trial, firstly in AMD patients as there are too few RAM patients to perform such a trial, in which intravitreal injections of rtPA and gas would be compared to vitrectomy, submacular rtPA injection and gas.

From our results we can conclude that vitrectomy followed by submacular rtPA injection and SF6 gas tamponade can successfully displace a recent hemorrhage secondary to RAM. Although there are complications associated with this surgery, and preoperative evaluation of the exact location of the hemorrhage is valuable to prevent

unnecessary surgery, this surgery may lead to a marked VA improvement that is likely be superior to that experienced by natural progression of untreated hemorrhage.

## REFERENCES

1. Rabb MF, Gagliano DA, Teske MP. Retinal arterial macroaneurysms. *Surv Ophthalmol*. 1988;33:73-96.
2. Glatt H, Macherer R. Experimental subretinal hemorrhage in rabbits. *Am J Ophthalmol*. 1982;94:762-773.
3. Toth CA, Morse LS, Hjelmeland LM, Landers MB, III. Fibrin directs early retinal damage after experimental subretinal hemorrhage. *Arch Ophthalmol*. 1991;109:723-729.
4. Tonotsuka T, Imai M, Saito K, Iijima H. Visual prognosis for symptomatic retinal arterial macroaneurysm. *Jpn J Ophthalmol*. 2003;47:498-502.
5. Heriot WJ. Intravitreal gas and TPA; an outpatient procedure for submacular hemorrhage. *American Academy of Ophthalmology Annual Vitreoretinal Update*; 1996. Ref Type: Conference Proceeding
6. Chen CY, Hooper C, Chiu D, Chamberlain M, Karia N, Heriot WJ. Management of submacular hemorrhage with intravitreal injection of tissue plasminogen activator and expansile gas. *Retina*. 2007;27:321-328.
7. Hassan AS, Johnson MW, Schneiderman TE, et al. Management of submacular hemorrhage with intravitreal tissue plasminogen activator injection and pneumatic displacement. *Ophthalmology*. 1999;106:1900-1906.
8. Hillenkamp J, Surguch V, Framme C, Gabel VP, Sachs HG. Management of submacular hemorrhage with intravitreal versus subretinal injection of recombinant tissue plasminogen activator. *Graefes Arch Clin Exp Ophthalmol*. 2010;248:5-11.
9. Humayun M, Lewis H, Flynn HW, Jr., Sternberg P, Jr., Blumenkranz MS. Management of submacular hemorrhage associated with retinal arterial macroaneurysms. *Am J Ophthalmol*. 1998;126:358-361.
10. Ohji M, Saito Y, Hayashi A, Lewis JM, Tano Y. Pneumatic displacement of subretinal hemorrhage without tissue plasminogen activator. *Arch Ophthalmol*. 1998;116:1326-1332.
11. Cakir M, Cekic O, Yilmaz OF. Pneumatic displacement of acute submacular hemorrhage with and without the use of tissue plasminogen activator. *Eur J Ophthalmol*. 2010;20:565-571.
12. Wu TT, Sheu SJ. Intravitreal tissue plasminogen activator and pneumatic displacement of submacular hemorrhage secondary to retinal artery macroaneurysm. *J Ocul Pharmacol Ther*. 2005;21:62-67.
13. Peyman GA, Nelson NC, Jr., Alturki W, et al. Tissue plasminogen activating factor assisted removal of subretinal hemorrhage. *Ophthalmic Surg*. 1991;22:575-582.
14. Nakamura H, Hayakawa K, Sawaguchi S, Gaja T, Nagamine N, Medoruma K. Visual outcome after vitreous, sub-internal limiting membrane, and/or submacular hemorrhage removal associated with ruptured retinal arterial macroaneurysms. *Graefes Arch Clin Exp Ophthalmol*. 2008;246:661-669.
15. Mizutani T, Yasukawa T, Ito Y, et al. Pneumatic displacement of submacular hemorrhage with or without tissue plasminogen activator. *Graefes Arch Clin Exp Ophthalmol*. 2011;249:1153-1157.
16. Zhao P, Hayashi H, Oshima K, Nakagawa N, Ohsato M. Vitrectomy for macular hemorrhage associated with retinal arterial macroaneurysm. *Ophthalmology*. 2000;107:613-617.
17. Buhl M, Scheider A, Schonfeld CL, Kampik A. [Intra-vitreous rt-PA and gas introduction in

- submacular hemorrhage]. *Ophthalmologie*. 1999; 96:792-796.
- 18.** Gopalakrishnan M, Giridhar A, Bhat S, Saikumar SJ, Elias A, N S. Pneumatic displacement of submacular hemorrhage: safety, efficacy, and patient selection. *Retina*. 2007;27:329-334.
- 19.** Yang PM, Kuo HK, Kao ML, Chen YJ, Tsai HH. Pneumatic displacement of a dense submacular hemorrhage with or without tissue plasminogen activator. *Chang Gung Med J*. 2005;28:852-859.
- 20.** Holladay JT. Visual acuity measurements. *J Cataract Refract Surg*. 2004;30:287-290.
- 21.** Benner JD, Hay A, Landers MB, III, Hjelmeland LM, Morse LS. Fibrinolytic-assisted removal of experimental subretinal hemorrhage within seven days reduces outer retinal degeneration. *Ophthalmology*. 1994;101:672-681.
- 22.** Kamei M, Estafanous M, Lewis H. Tissue plasminogen activator in the treatment of vitreoretinal diseases. *Semin Ophthalmol*. 2000;15: 44-50.
- 23.** Sampangi R, Chandrakumar HV, Somashekar SE, Joshi GR, Ganesh S. SD-OCT to differentiate traumatic submacular hemorrhage types using automatic three-dimensional segmentation analysis. *Ophthalmic Surg Lasers Imaging*. 2011;42 Online:e32-e36.
- 24.** Shah VA, Poulouse A, Cassell M, Sabates NR. Spectral Domain Optical Coherence Tomography Findings of Retinal Macroaneurysm. *Ophthalmic Surg Lasers Imaging*. 2010:1-4.
- 25.** Iijima H, Satoh S, Tsukahara S. Nd:YAG laser photodisruption for preretinal hemorrhage due to retinal macroaneurysm. *Retina*. 1998;18:430-434.
- 26.** Rennie CA, Newman DK, Snead MP, Flanagan DW. Nd:YAG laser treatment for premacular subhyaloid haemorrhage. *Eye (Lond)*. 2001;15: 519-524.
- 27.** Arias L, Mones J. Transconjunctival sutureless vitrectomy with tissue plasminogen activator, gas and intravitreal bevacizumab in the management of predominantly hemorrhagic age-related macular degeneration. *Clin Ophthalmol*. 2010;4: 67-72.
- 28.** Hauptert CL, McCuen BW, Jaffe GJ, et al. Pars plana vitrectomy, subretinal injection of tissue plasminogen activator, and fluid-gas exchange for displacement of thick submacular hemorrhage in age-related macular degeneration. *Am J Ophthalmol*. 2001;131:208-215.
- 29.** Olivier S, Chow DR, Packo KH, MacCumber MW, Awh CC. Subretinal recombinant tissue plasminogen activator injection and pneumatic displacement of thick submacular hemorrhage in Age-Related macular degeneration. *Ophthalmology*. 2004;111:1201-1208.
- 30.** Singh RP, Patel C, Sears JE. Management of subretinal macular haemorrhage by direct administration of tissue plasminogen activator. *Br J Ophthalmol*. 2006;90:429-431.
- 31.** Thompson JT, Sjaarda RN. Vitrectomy for the treatment of submacular hemorrhages from macular degeneration: a comparison of submacular hemorrhage/membrane removal and submacular tissue plasminogen activator-assisted pneumatic displacement. *Trans Am Ophthalmol Soc*. 2005;103:98-107.
- 32.** Treumer F, Klatt C, Roeder J, Hillenkamp J. Subretinal coapplication of recombinant tissue plasminogen activator and bevacizumab for neovascular age-related macular degeneration with submacular haemorrhage. *Br J Ophthalmol*. 2010;94:48-53.
- 33.** Sandhu SS, Manvikar S, Steel DH. Displacement of submacular hemorrhage associated with age-

related macular degeneration using vitrectomy and submacular tPA injection followed by intravitreal ranibizumab. *Clin Ophthalmol.* 2010;4: 637-642.

**34.** Lewis H. Intraoperative fibrinolysis of submacular hemorrhage with tissue plasminogen activator and surgical drainage. *Am J Ophthalmol.* 1994;118:559-568.

**35.** Bopp S. Retinal Arterial Macroaneurysms. In: A.M.Joussen, T.W.Gardner, B.Kirchhof, S.J.Ryan (eds), *Retinal Vascular Disease*: Springer; 2007: 543-560.

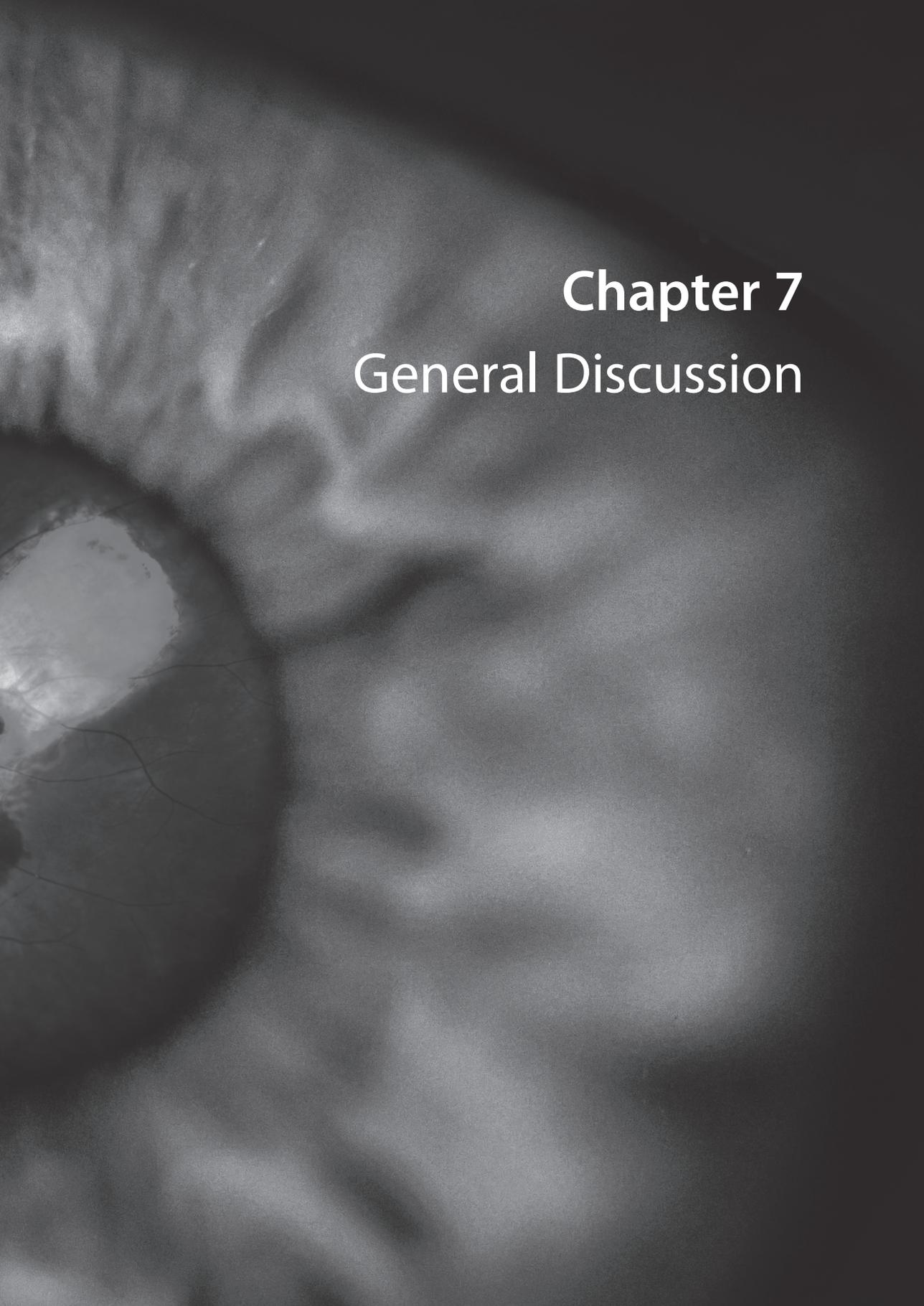
**36.** Kamei M, Misono K, Lewis H. A study of the ability of tissue plasminogen activator to diffuse into the subretinal space after intravitreal injection in rabbits. *Am J Ophthalmol.* 1999;128:739-746.

**37.** Lincoff H, Kreissig I, Stopa M, Uram D. A 40 degrees gaze down position for pneumatic displacement of submacular hemorrhage: clinical application and results. *Retina.* 2008;28:56-59.

**38.** Stopa M, Lincoff A, Lincoff H. Analysis of forces acting upon submacular hemorrhage in pneumatic displacement. *Retina.* 2007;27:370-374.







**Chapter 7**  
General Discussion



Age-related macular degeneration (AMD) is a visually impairing disease whose pathogenesis involves degeneration of the choriocapillaris, Bruch's membrane, retinal pigment epithelium (RPE) and retinal layers.

The more common subtype, dry AMD, is characterized by drusen and slow progressive atrophy of the RPE and outer retinal layers, with eventually loss of the choriocapillaris. The inner retinal layers frequently maintain a relatively normal appearance.<sup>1</sup>

Exudative AMD is seen in 15-20% of all AMD patients.<sup>1,2</sup> Due to changes in Bruch's membrane resulting in reduced transport between choroid and RPE, and a progressive dysfunction or atrophy of the choriocapillaris, RPE cells become hypoxic. The RPE has a high level of oxygen consumption and metabolic demand due to exposure to lipid peroxidation products derived from the photoreceptor outer segments and constant presence of light stimuli, which further induces hypoxia.<sup>2</sup> The RPE subsequently produces excessive amounts of vascular endothelial growth factor (VEGF). VEGF initiates growth of neovascular membranes, which then spread under the RPE.<sup>1</sup> The RPE, outer retinal layers and eventually also the inner retinal layers are damaged in advanced exudative AMD, more rapidly as compared to dry AMD.<sup>2</sup>

The RPE is a monolayer of cells crucial for metabolic transport to and from the retina, which is vital for the function of the overlying photoreceptors.<sup>3</sup> Because both RPE and photoreceptor cells divide and differentiate before birth, they are incapable of regeneration.<sup>4</sup> Hence, loss or damage of the RPE and/or photoreceptors in both dry and exudative AMD may lead to irreversible loss of function of the overlying neuroretina, resulting in visual acuity deterioration.

New treatment options for dry AMD therefore focuses mainly on surgical replacement of RPE cells by suspensions or sheets of RPE (stem cell derived) cells, thereby maintaining or improving outer retinal layer function.<sup>4,5</sup> In exudative AMD, the mainstay of therapy is intravitreal injections of anti-VEGF,<sup>6-8</sup> with a more marginal role for cell-based therapy.

The first and main objective of this thesis was to evaluate the results of a free autologous RPE-choroid graft transplantation for patients with advanced exudative AMD. In this chapter we will discuss the potential of RPE and choroid transplantation and macular rotation methods, and the future possibilities of cell based therapy, as well as the use of electronic subretinal or epiretinal implants.

As a second objective of this thesis, the revascularization pattern of an RPE-choroid graft was analyzed with new imaging techniques such as spectral domain optical coherence tomography (SD-OCT), optical frequency domain imaging (OFDI)-OCT and phase resolved Doppler (PRD)-OCT. The results and future possibilities of these OCT devices in patient selection before, and follow-up after RPE-choroid graft surgery, are discussed.

Third, we explored the feasibility and safety of administration of recombinant tissue plasminogen activator (rtPA) for acute submacular hemorrhages (SMH), a severe

complication of several retinal diseases including exudative AMD. This includes discussion of a recently initiated controlled trial to find the optimal administration method.

### 7.1 Autologous Retinal Pigment Epithelium Replacement Methods

In patients with exudative AMD, choroidal neovascular membranes (CNV) grow through Bruch's membrane and spread out under the RPE. Therefore, surgical removal of the CNV, can lead to damage or removal of the RPE, especially when the CNV is associated with submacular hemorrhage or subretinal fibrosis. The induced RPE defect is expected to persist after surgery, and a bare area of (damaged) Bruch's membrane remains, which may in turn lead to progressive atrophy of the subfoveal choriocapillaris.<sup>9</sup> As a result, submacular surgery with removal of CNV and/or hemorrhage was found not beneficial in patients with exudative AMD in a Cochrane review,<sup>10</sup> although earlier reports suggested less severe visual acuity (VA) loss for patients with only hemorrhage removal compared to no treatment.<sup>11</sup> Therefore, several other methods have been developed in the last 20 years to replace the absent or damaged RPE after CNV and/or hemorrhage/subretinal fibrosis removal; these are discussed.

Transplantation of a suspension of autologous RPE cells harvested from the periphery during submacular surgery for removal of the hemorrhage/CNV has shown limited benefits.<sup>12-14</sup> Cells in suspension have difficulties attaching to the diseased Bruch's membrane<sup>15</sup> and often do not form the desired monolayer required for optimal RPE function. Instead, they form rosettes, or undergo a form of apoptosis.<sup>16,17</sup>

In 1993; Machemer and Steinhorst<sup>18</sup> reported a method of translocation of the macula after a 360° peripheral retinectomy. This procedure rotates the entire retina around the optic disk, relocating the macula to an area with less severely damaged RPE. To prevent diplopia, counter-rotation of the eye by muscle surgery was then performed in a subsequent surgery. This macular translocation or rotation technique is associated with some (severe) complications, such as retinal detachment, proliferative vitreoretinopathy (PVR), choroidal hemorrhage, macular pucker, a tilted image and, as mentioned, diplopia. Nevertheless, long-term visual acuity improvement has been demonstrated in some patients with exudative AMD.<sup>19-24</sup>

An autologous RPE-choroid graft transplantation with a pedicle to preserve vascularization, was reported in one patient by Peyman in 1991.<sup>25</sup> Aylward introduced the concept of a free graft in six patients, taken from an area directly adjacent to the excised choroidal membrane.<sup>26</sup> Van Meurs modified the technique by harvesting the graft from the midperiphery.<sup>27</sup> This autologous free RPE-choroid graft transplantation technique (also named RPE choroid sheet or RPE patch graft translocation) is described in this thesis.

Transplantation of an autologous free RPE-choroid graft has been compared to autologous RPE cell suspension transplantation. However, although anatomical and

functional outcomes seem comparable, an RPE-choroid graft may provide a better cell source with more chance of regaining normal retinal structures and thus visual acuity.<sup>28</sup>

RPE-choroid graft transplantation surgery and macular rotation have also been compared to each other, and both techniques seem to lead to better VA outcomes than no intervention.<sup>29</sup> Although macular translocation lead to slightly better VA results than the RPE-choroid graft transplantation, it was associated with a greater risk of various complications than transplantation, especially recurrent CNV (3-56%), retinal detachment (17-24%), PVR (16%-26%) and hypotony (2-28%).<sup>20-22, 30-34</sup> Conversely, our studies have found a rate of 10% for CNV, 10%-15.9% for PVR and 1.5% hypotony as major complications in RPE-choroid graft transplantation studies described in this thesis (chapters 2.1 and 2.2).<sup>35</sup> Macular translocation also has an increased risk of cystoid macular edema (13-29%), secondary RPE atrophy underneath the new foveal location(33-72%), diplopia (6-46%) and macular pucker (5-23%), complications which are all rare in transplantation.<sup>20-22, 30-34</sup> Furthermore, in macular rotation surgery, the degree of rotation is limited, which might pose difficulties in patients with a large CNV in which healthy RPE might not be located within a few degrees of the original site of the macula. This is a problem that can be overcome with the transplantation of a free RPE-choroid graft.

Another variant of RPE-choroid graft surgery was recently described by Han et al.<sup>36</sup> Dr. Zhizong Ma transplanted an autologous sheet of RPE only patients with a pigment epithelial detachment and a large submacular hemorrhage due to AMD.<sup>36</sup> The possibility of harvesting a single autologous layer of RPE, which is difficult in Caucasian patients, is thought to be related to the high prevalence of choroidal polypoid degeneration subtype of exudative AMD in Asian patients, in which a cleavage plane exists within Bruch's membrane. Two complications of the RPE-choroid graft surgery are the leakage of hemorrhage from the donor site retinotomy (when taken superiorly) to the graft; and new macular hemorrhage, due to either manipulation of choroid during the relocation of the graft, or removal of the old hemorrhage/CNV/fibrosis. With Dr. Ma's method, a choroidal incision is avoided, as the RPE is taken from the retina at the location of the already existing pigment epithelium detachment. Less complications might therefore occur due to the less invasive surgery. This technique is also relevant to the issue whether laboratory elaborated RPE should be RPE alone or with choroid attached.

## 7.2 Electronic Implants

Electronic prosthetic devices have been developed for patients with very severe, end-stage retinitis pigmentosa. These devices are very far from being conceivably useful for patients with AMD who still have a useful peripheral visual field. Two subtypes of implants have been tested in a small number of patients; a subretinal implant with photodiodes stimulated by light through the pupil,<sup>37</sup> and an epiretinal subtype, in which electrodes

are activated by a video camera mounted on glasses.<sup>38</sup> Only when technology advances considerably, they might be of some use in patients with dry or exudative AMD.

At this point in time, it is not clear whether an increased pixel density might lead to an improvement in discrimination, as the pixel density of the subretinal implant is twenty times greater than that of the epiretinal device. Another hurdle is the external camera in the epi-retinal system, which eliminates the use of natural eye movements, and may require continuous head movements. Theoretically, the subretinal light-sensitive implant may perceive the shape of objects more naturally, because natural microsaccades refresh the image and the cells stimulated are the bipolar cells. This more closely resembles the normal functioning of the retina, than does the stimulation of the ganglion cells, as with the epiretinal device.<sup>37</sup> Color vision is not possible with either device. For both devices clinical trials and developments are ongoing.<sup>37,38</sup>

### 7.3 Cell-based Therapy

The surgical treatment methods for exudative AMD described above, utilize autologous RPE already damaged by AMD. Furthermore, only submacular RPE can be transplanted or replaced, so the potential for improvement in the central visual field immediately adjacent the graft remains limited.

To overcome these size limits, a sheet of adult human RPE harvested from a donor was cultured on gelatin. This allogeneic RPE sheet was transplanted into 12 eyes of patients with AMD under immunosuppression; but without success.<sup>39,40</sup>

Transplantation of a suspension of human fetal RPE was found to be feasible, although there were concerns about immune rejection.<sup>41,42</sup> Transplantation of human fetal RPE patches<sup>43,44</sup> and of fetal neural retinal cell layer sheets with RPE have both been shown to result in VA improvement,<sup>45</sup> but mainly due to the source of the transplanted tissue, these techniques remain controversial.

An alternative source of replacement tissue for the RPE monolayer is the use of human embryonic stem cells (HESCs) and induced pluripotent stem (iPS) cells, which differentiate into cells that are functionally and morphologically similar to RPE cells.<sup>46</sup> HESC-derived RPE cells seem to have a gene and protein expression that is similar to that of human fetal RPE cells,<sup>47</sup> and experiments in rat eyes showed phagocytosis of photoreceptor material by both HESC and iPS cells, which may indicate that these cells can function as RPE.<sup>16,46</sup> Some VA improvement was found in rats with transplanted iPS-RPE cells,<sup>48</sup> and also in a preliminary report of one of the first clinical trials in which HESC-derived RPE cell suspensions were transplanted in humans.<sup>5</sup>

At least four stem cell-based therapies are currently being investigated in patients in three medical centers in Japan, the USA and the United Kingdom. In Japan and the USA, ESC-derived cells are transplanted. In the United Kingdom, iPS cells are used for both autologous and for human leukocyte antigen (HLA)-matched transplantation.<sup>4,5</sup>

Human umbilical tissue also harbors multipotent stem cells that have been classified as adult stem cells. After successful subretinal injection of human umbilical tissue derived stem cells (hUTSCs) in rats,<sup>49</sup> the first clinical trial in patients with dry AMD was recently started in the USA.<sup>17</sup> The beneficial effect would be derived from trophic factors, such as pigment epithelium-derived factor (PEDF), and ciliary neurotrophic factor (CNTF), which are agents that stimulate differentiation and survival of cells.<sup>50</sup>

#### **7.4 Clinical Results of Retinal Pigment Epithelium and Choroid Graft Transplantation**

In this thesis, we describe the results of long-term follow-up of the RPE-choroid graft transplantation surgery.<sup>35</sup> We have found that up until four years after surgery, useful vision (> 20/200) could be obtained in 20 of 130 patients (133 eyes). Four patients achieved this best-corrected visual acuity (BCVA) seven years after surgery; one of them achieved a BCVA of 20/32. Graft function was confirmed by microperimetry. The percentage of patients obtaining this relatively good VA is low, but it may be better than the natural course of the disease. After seven years, most (121) patients were lost to follow-up, probably because of inability to come to the hospital due to their age, or because they had died. Among those patients, there was a substantial group (24 patients) with a VA of > 20/200 at their last follow up (Chapter 2.1, Figure 1), so the actual rate with useful VA might have been higher.

With this cohort of 130 patients, we reported the longest follow-up period of RPE-choroid graft patients, presenting patients with useful vision up to seven years.<sup>51-53</sup> This group included the first RPE-choroid graft surgeries ever performed in our hospital. Patients were those in which other therapies such as photodynamic therapy were no longer helpful (anti-VEGF treatment was not yet available for this cohort at the time of surgery) and RPE-choroid graft surgery was the last treatment option. Due to previous loss of their RPE and/or damage to their retina/photoreceptors secondary to the AMD disease process, CNV and/or large and old submacular hemorrhage, and/or subretinal fibrosis, many of these patients had little or no chance of spontaneous improvement.

The results have not improved significantly over the last few years. One might assume that the next cohort of patients would have better results with more experience and development of the technique, as the intraoperative course has been proven to be one of the major factors of the outcome after RPE-choroid graft surgery.<sup>54</sup> Although changes and developments have been applied in the technique over the years, including the use of new different shaped forceps to insert the graft, a new version of the vibration device, or even switching to a 180° retinectomy, surgical complications continued to occur. These included curling of the graft; graft damage due to manipulation; and donorsite or submacular hemorrhage.

Besides the lack of improvement of surgical technique, less patients were eligible for surgery. In October 2006, anti-VEGF therapy was introduced, which proved beneficial in many patients who had not derived benefit from previous other treatments and who might have been treated with RPE-choroid graft surgery before. In the four years and four months following the first RPE-choroid graft surgery 130 patients were included, while in the following five years and nine months, the total amount of patients included was 116. These data exclude an additional 24 patients who underwent 180° retinectomy surgery between 2001 and 2011. This technique was more frequently performed in the latter years.

Thus, the only patients who remained for surgical intervention were those who were not responding to anti-VEGF therapy, often late after initial presentation. An interesting point is that the results achieved in the group of patients who were included prior to the anti-VEGF era are roughly comparable to those obtained in the patients included since the introduction of anti-VEGF treatment, as seen in the results of our discontinued randomized controlled trial.

In this thesis we have described the SD-OCT in the follow-up of RPE-choroid graft patients. This technique revealed that RPE-choroid graft surgery might not have benefited many of the patients we treated, due to pre-existing, irreversible retinal damage. The status of the inner and outer photoreceptor layer (IS/OS) junction reflects the photoreceptor integrity and was found to be associated with VA and retinal sensitivity on microperimetry.<sup>55</sup> Furthermore, the status of the external limiting membrane (ELM) might also be a good indicator of retinal/visual function, in eyes with AMD.<sup>56, 57</sup> We therefore initially wished to study the relationship between the status of the IS/OS and ELM and outcomes after RPE-choroid graft surgery in the patients we enrolled in the last study comparing the RPE-choroid graft versus anti-VEGF therapy (Chapter 4). Although we extensively tried to interpret the SD-OCT results and correlated them, for instance, with microperimetric data; we had too few patients without complications to draw firm conclusions. Nevertheless, two examples may be illustrative. Patient 10 had an intact IS/OS junction and an uninterrupted ELM layer before and after RPE-choroid graft surgery, and this patient experienced a VA improvement from 0.66 to 0.14 logMAR (logarithm of the minimum angle of resolution) one year postoperatively. In another patient (7), the ELM layer and IS/OS junction were absent prior to surgery. Postoperatively, the retina was thin, ELM layer and IS/OS junction remained absent. VA decreased over the year, however probably also partly caused by the complications. Selection of patients for surgery, based on the presence of IS/OS and ELM, may be the best method to improve the results of the RPE-choroid graft transplantation.

PVR and recurrent CNV are the major complications of RPE-choroid graft surgery. Recurrent CNV occurs in 10%-37% of the patients after RPE-choroid graft transplantation

surgery.<sup>35, 52, 58, 59</sup> It forms at the edge of the patch in area's devoid of RPE. Anti-VEGF therapy can then be initiated to maintain VA, but a slow functional decline generally occurs.

PVR is the most severe potential complication of RPE-choroid graft surgery. In an attempt to prevent the development of PVR after surgery, we studied whether PVR occurred more often in patients with a donor site located in the superior or inferior quadrants (Chapter 2.2). Since the inferior donorsite after surgery is in direct contact with the pro-fibrotic inflammatory milieu, a higher prevalence of PVR development might be expected in these patients. This thought is key to the hypothesis that heavy silicone oil tamponade for retinal detachments of the inferior quadrants may diminish the chance of developing PVR, by shifting the inflammatory milieu away from the retinal break.<sup>60, 61</sup> However, we found no statistically significant difference in the occurrence of PVR in patients with either a superior (18.4%) or inferior (13.8%) donor site. Therefore, one might assume that also the use of heavy tamponades for inferior retinal breaks to prevent PVR development, might not be necessary. With heavy silicone oil, PVR might still develop but may instead displace superiorly, due to the shifted pro-inflammatory milieu, where it might even possibly harm the patients' visual field more than it would inferiorly, since patients' inferior field of vision is more useful than the superior field.

For RPE-choroid graft surgery, this finding suggests that the graft can be taken either superiorly or inferiorly, at the surgeon's discretion or based on the aspect of the patients' RPE. However, no solution has yet been found to decrease the likelihood of PVR development. Research is currently being conducted to try and modulate the intraocular inflammatory milieu.<sup>62</sup>

We initiated a multicenter, randomized controlled trial, to compare RPE-choroid graft surgery with anti-VEGF therapy, which has been available since 2006 (Chapter 4). Even though the study was terminated prematurely, 20 patients had already been included in our own hospital. The VA improvement was limited by the unexpected number and degree of complications, both ocular and systemic, that occurred in both treatment groups. The most severe ocular problems occurred in the graft group. One patient in the RPE-choroid graft group was, however, a great success. This patient had VA of 0.14 logMAR one year after surgery, which is an improvement of more than five Early Treatment Diabetic Retinopathy Study (ETDRS) lines. The patient also experienced improved reading acuity and decreased metamorphopsia.

Nevertheless, due to the prospective setup of this study, we were able to gather a large amount of data on these patients. We therefore were able to detect the three revascularization steps of the RPE-choroid graft in vivo on SD-OCT with the help of fluorescein angiography (FA) and indocyanine angiography (ICGA) (Chapter 5.1). These three steps are the serum imbibition phase, the afferent vessel phase and the efferent vessel phase.<sup>63</sup> This evaluation of the revascularization process with the SD-OCT limits

the need for more invasive exams such as FA and ICGA, which were the methods previously used to observe graft revascularization.<sup>64, 65</sup> These revascularization steps were subsequently tested with the help of PRD-OCT, which confirmed flow in the graft in the efferent phase. (chapter 5.2).

Using the SD-OCT, we were also able to correlate structural images to functional results of the microperimetric exams (chapters 2.1 and chapter 3). This gave us new insight into what might be the cause of poor function after relatively uncomplicated RPE-choroid graft transplantation, such as RPE defects due to excessively firm gripping of the forceps during graft insertion. Although microperimetry provides specific functional extra information, this time-consuming examination might not be entirely necessary in clinical practice, as the exam is at present difficult for patients with low macular function.

We conclude that RPE-choroid graft transplantation surgery may be a feasible technique, although further technical developments are needed and difficulties remain. With the current use of the SD-OCT and the future use of PRD-OCT, patients can be followed after surgery more easily and accurately. More importantly, accurate patient selection using the SD-OCT might breathe new life into RPE-choroid graft surgery.

The role of RPE replacement surgery in AMD will not only depend on advancements in surgical and imaging techniques but also on those in stem cell therapy, and on the developments in anti-VEGF therapy, including VEGF trap-eye,<sup>66</sup> anti-PEDF therapy,<sup>67</sup> and other so-called biologicals, or (adenoviral vector-mediated) ocular gene transfer therapy.<sup>68</sup>

### **7.5 Recombinant Tissue Plasminogen Activator and Gas for Submacular Hemorrhages**

Submacular hemorrhage is a relatively common and severe complication of AMD, with a prevalence ranging from 0.06% for small lesions up to 40% for large occult CNVs after intravitreal anti-VEGF injections.<sup>69</sup> Unfortunately, a substantial number of AMD patients use systemic anti-coagulant therapy, increasing their risk of developing intraocular hemorrhage (submacular or vitreous) due to AMD. Furthermore, most of these patients develop AMD in both eyes, and bilateral AMD increases the risk of developing an intraocular hemorrhage.<sup>70</sup>

As a method to treat these hemorrhages, Heriot described in 1996 a less invasive technique, compared to former surgical removal by forceps: pneumatic displacement of the hemorrhage by injection of gas and rtPA. rtPA serves to liquefy the hemorrhage, while the gas bubble and gravity combine to displace the hemorrhage inferiorly, where it causes less functional damage.<sup>71</sup> Based on rtPA liquefaction and displacement with gas, there are two treatment strategies; minimally invasive treatments such as simple gas injection,<sup>72</sup> intravitreal injection of both rtPA and gas,<sup>71, 73-75</sup> and more invasive treatments, such as vitrectomy with subretinal rtPA administration and air<sup>76</sup> or gas

tamponade.<sup>77</sup> In patients with AMD, both treatments may be combined with anti-VEGF treatment to treat the underlying cause, choroidal neovascularization.<sup>78-81</sup>

Our reviewing of the relevant publications found no difference in the displacement rate between the less and more invasive surgeries, and complication rates were relatively similar (Chapter 6.1).<sup>82</sup> Thus, the recent trend towards more invasive surgery has not yet been supported by better results. Furthermore, we found that these patients are best treated within 14 days after occurrence of the hemorrhage, to limit the damage to the RPE and photoreceptors by the retinotoxic blood. Irreversible damage after 14 days may prevent any improvement in VA, even after complete displacement of the hemorrhage.<sup>83-86</sup>

AMD is not the only cause of submacular hemorrhage. Patients with a retinal arterial macroaneurysm (RAM) may also develop a submacular hemorrhage, leading to loss of VA.<sup>87</sup> However, because the underlying disease does not involve the macula, these patients have a better VA prognosis after hemorrhage displacement.<sup>88,89</sup> In the 11 patients with a RAM we treated with rtPA and gas, the hemorrhage had completely displaced in 82% of the patients, and 91% of the patients had a marked VA improvement, mean improvement was 1.3 logMAR (Chapter 6.2).<sup>90</sup> This compared favorably to the 28 patients we treated with AMD, 57% experienced a complete displacement and another 39% experienced partial displacement of the hemorrhage. VA improved two or more ETDRS lines in 68% of these patients. Therefore rtPA-based therapy may be a good treatment option for submacular hemorrhages due to AMD, but an even better VA improvement is expected in patients with a RAM.

With our recently started randomized controlled trial, the results of which do not constitute part of this thesis, we may demonstrate that more invasive rtPA surgery does not lead to superior VA or safety outcomes than the less invasive surgery. This is of importance because less invasive surgery is easier to schedule and therefore may be applied sooner after the initiation of the hemorrhage, with better VA expectation due to the shorter time allowed for toxic damage to develop. In the literature, the successes of rtPA treatment were described mainly in terms of displacement (none, partial or complete). In our recently initiated study comparing subretinal and intravitreal rtPA and gas, both functional and anatomical results will be studied with VA measurements, fundus photography, autofluorescence (AF) and SD-OCT. Further, using the SD-OCT will allow us to also calculate the pre- and postoperative volumes of the subretinal and sub-RPE hemorrhage and macular scar and/or CNV, to give more precise, quantitative, information of the treatment effect. Moreover, more precise and quantitative data may allow us to draw more definite conclusions with fewer patients.

Newer OCT devices allow us to measure large hemorrhages due to the greater depth range of imaging capture of the OFDI-OCT compared to SD-OCT. With the additional use of Doppler OFDI, we might better be able to discern where the submacular or sub-RPE

hemorrhage ends and the choroid starts in cases this is still hard to visualize by SD-OCT and OFDI-OCT. The Doppler OFDI can indicate choroidal flow, making this distinction possible.

Currently, in approximately the first two weeks after surgery, treatment effect of rtPA cannot be judged with conventional tools due to the presence of an intraocular gas bubble tamponade. The new possibility of producing an OCT scan through a gas bubble<sup>91</sup> may be a great advantage in the early follow-up of these rtPA treated patients. Early adjuvant treatment may be considered in the future, based on OFDI images through gas, when a hemorrhage has not displaced in the first days after intravitreal rtPA treatment. Possibly, the relatively simple submacular administration of a balanced salt solution (BSS) might further facilitate the displacement.

Therefore, the SD-OCT, OFDI-OCT and Doppler-OCT may be of great help in the treatment decision and follow-up of patients with an acute submacular hemorrhage.

## 7.6 Conclusions

While cell-based therapy, although associated with immune rejection problems, might be a future treatment for patients with dry AMD, developments in anti-VEGF and PEDF therapy may hold most promise for patients with exudative AMD. RPE-choroid graft transplantation and macular rotation will play a limited role for a small number of patients with severe exudative AMD and a relatively intact macular retina for whom no other therapies are available.

Advancements in OCT technology will continue to allow more accurate patient selection and more informative postoperative follow-up after both RPE-choroid graft surgery for advanced AMD and rtPA and gas treatment for patients with a submacular hemorrhage.

Currently, rtPA treatment seems the most promising already available therapy of all those described. Although its use is not yet widespread, it may best improve the disease course and VA of patients with a submacular hemorrhage due to AMD or RMA with a relatively simple procedure.

## REFERENCES

1. Bhutto I, Luty G. Understanding age-related macular degeneration (AMD): relationships between the photoreceptor/retinal pigment epithelium/Bruch's membrane/choriocapillaris complex. *Mol Aspects Med.* 2012;33:295-317.
2. Klettner A, Kauppinen A, Blasiak J, Roeder J, Salminen A, Kaarniranta K. Cellular and molecular mechanisms of age-related macular degeneration: From impaired autophagy to neovascularization. *Int J Biochem Cell Biol.* 2013;45:1457-1467.
3. Kay P, Yang YC, Paraoan L. Directional protein secretion by the retinal pigment epithelium: roles in retinal health and the development of age-related macular degeneration. *J Cell Mol Med.* 2013.
4. Carr AJ, Smart MJ, Ramsden CM, Powner MB, Da Cruz L, Coffey PJ. Development of human embryonic stem cell therapies for age-related macular degeneration. *Trends Neurosci.* 2013.
5. Schwartz SD, Hubschman JP, Heilwell G, et al. Embryonic stem cell trials for macular degeneration: a preliminary report. *Lancet.* 2012; 379:713-720.
6. Brown DM, Michels M, Kaiser PK, Heier JS, Sy JP, Ianchulev T. Ranibizumab versus verteporfin photodynamic therapy for neovascular age-related macular degeneration: Two-year results of the ANCHOR study. *Ophthalmology.* 2009;116: 57-65.
7. Martin DF, Maguire MG, Ying GS, Grunwald JE, Fine SL, Jaffe GJ. Ranibizumab and bevacizumab for neovascular age-related macular degeneration. *N Engl J Med.* 2011;364:1897-1908.
8. Rosenfeld PJ, Brown DM, Heier JS, et al. Ranibizumab for neovascular age-related macular degeneration. *N Engl J Med.* 2006;355:1419-1431.
9. Del Priore LV, Kaplan HJ, Magrath GN, Tezel TH. Cellular transplantation for advanced age-related macular degeneration. In: Lin JM (ed), *Age-related macular degeneration.* Third ed.: CRC press; 2012: 334-353.
10. Giansanti F, Eandi CM, Virgili G. Submacular surgery for choroidal neovascularisation secondary to age-related macular degeneration. *Cochrane Database Syst Rev.* 2009:CD006931.
11. Bressler NM, Bressler SB, Childs AL, et al. Surgery for hemorrhagic choroidal neovascular lesions of age-related macular degeneration: ophthalmic findings: SST report no. 13. *Ophthalmology.* 2004; 111:1993-2006.
12. Binder S, Stolba U, Krebs I, et al. Transplantation of autologous retinal pigment epithelium in eyes with foveal neovascularization resulting from age-related macular degeneration: a pilot study. *Am J Ophthalmol.* 2002;133:215-225.
13. van Meurs JC, ter Averst E, Hofland LJ, et al. Autologous peripheral retinal pigment epithelium translocation in patients with subfoveal neovascular membranes. *Br J Ophthalmol.* 2004; 88:110-113.
14. Binder S, Krebs I, Hilgers RD, et al. Outcome of transplantation of autologous retinal pigment epithelium in age-related macular degeneration: a prospective trial. *Invest Ophthalmol Vis Sci.* 2004; 45:4151-4160.
15. Tsukahara I, Ninomiya S, Castellarin A, Yagi F, Sugino IK, Zarbin MA. Early attachment of uncultured retinal pigment epithelium from aged donors onto Bruch's membrane explants. *Exp Eye Res.* 2002;74:255-266.
16. Vugler A, Carr AJ, Lawrence J, et al. Elucidating the phenomenon of HESC-derived RPE: anatomy of

- cell genesis, expansion and retinal transplantation. *Exp Neurol*. 2008;214:347-361.
17. Ramsden CM, Powner MB, Carr AJ, Smart MJ, Da Cruz L, Coffey PJ. Stem cells in retinal regeneration: past, present and future. *Development*. 2013;140:2576-2585.
  18. Macherer R, Steinhilber UH. Retinal separation, retinotomy, and macular relocation: II. A surgical approach for age-related macular degeneration? *Graefes Arch Clin Exp Ophthalmol*. 1993;31:635-641.
  19. Aisenbrey S, Bartz-Schmidt KU, Walter P, et al. Long-term follow-up of macular translocation with 360 degrees retinotomy for exudative age-related macular degeneration. *Arch Ophthalmol*. 2007;125:1367-1372.
  20. Abdel-Meguid A, Lappas A, Hartmann K, et al. One year follow up of macular translocation with 360 degree retinotomy in patients with age related macular degeneration. *Br J Ophthalmol*. 2003;87:615-621.
  21. Eckardt C, Eckardt U, Conrad HG. Macular rotation with and without counter-rotation of the globe in patients with age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol*. 1999;237:313-325.
  22. Pertile G, Claes C. Macular translocation with 360 degree retinotomy for management of age-related macular degeneration with subfoveal choroidal neovascularization. *Am J Ophthalmol*. 2002;134:560-565.
  23. Polito A, Cereda M, Romanelli F, Pertile G. Macular translocation with 360(degrees) retinotomy for management of retinal pigment epithelial tear: long-term results. *Br J Ophthalmol*. 2010.
  24. Wolf S, Lappas A, Weinberger AW, Kirchhof B. Macular translocation for surgical management of subfoveal choroidal neovascularizations in patients with AMD: first results. *Graefes Arch Clin Exp Ophthalmol*. 1999;237:51-57.
  25. Peyman GA, Blinder KJ, Paris CL, Alturki W, Nelson NC, Jr., Desai U. A technique for retinal pigment epithelium transplantation for age-related macular degeneration secondary to extensive subfoveal scarring. *Ophthalmic Surg*. 1991;22:102-108.
  26. Stanga PE, Kychenthal A, Fitzke FW, et al. Retinal pigment epithelium translocation and central visual function in age related macular degeneration: preliminary results. *Int Ophthalmol*. 2001;23:297-307.
  27. van Meurs JC, van den Biesen PR. Autologous retinal pigment epithelium and choroid translocation in patients with exudative age-related macular degeneration: short-term follow-up. *Am J Ophthalmol*. 2003;136:688-695.
  28. Falkner-Radler CI, Krebs I, Glittenberg C, et al. Human retinal pigment epithelium (RPE) transplantation: outcome after autologous RPE-choroid sheet and RPE cell-suspension in a randomised clinical study. *Br J Ophthalmol*. 2011; 95:370-375.
  29. Chen FK, Patel PJ, Uppal GS, et al. A comparison of macular translocation with patch graft in neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2009;50:1848-1855.
  30. Chen FK, Patel PJ, Uppal GS, Tufail A, Coffey PJ, Da CL. Long-term outcomes following full macular translocation surgery in neovascular age-related macular degeneration. *Br J Ophthalmol*. 2010;94: 1337-1343.
  31. Yamada Y, Miyamura N, Suzuma K, Kitaoka T. Long-term follow-up of full macular translocation for choroidal neovascularization. *Am J Ophthalmol*. 2010;149:453-457.

- 32.** Degenring RF, Cordes A, Schrage NF. Autologous translocation of the retinal pigment epithelium and choroid in the treatment of neovascular age-related macular degeneration. *Acta Ophthalmol.* 2011;89:654-659.
- 33.** Aisenbrey S, Lafaut BA, Szurman P, et al. Macular translocation with 360 degrees retinotomy for exudative age-related macular degeneration. *Arch Ophthalmol.* 2002;120:451-459.
- 34.** Gelisken F, Voelker M, Schwabe R, et al. Full macular translocation versus photodynamic therapy with verteporfin in the treatment of neovascular age-related macular degeneration: 1-year results of a prospective, controlled, randomised pilot trial (FMT-PDT). *Graefes Arch Clin Exp Ophthalmol.* 2007;245:1085-1095.
- 35.** Van Zeeburg EJ, Maaijwee KJ, Missotten TO, Heimann H, van Meurs JC. A free retinal pigment epithelium-choroid graft in patients with exudative age-related macular degeneration: results up to 7 years. *Am J Ophthalmol.* 2012;153:120-127.
- 36.** Han L, Ma Z, Wang C, et al. Autologous Transplantation of Simple Retinal Pigment Epithelium Sheet for Massive Submacular Hemorrhage Associated with Pigment Epithelium Detachment. *Invest Ophthalmol Vis Sci.* 2013.
- 37.** Stingl K, Bartz-Schmidt KU, Besch D, et al. Artificial vision with wirelessly powered subretinal electronic implant alpha-IMS. *Proc Biol Sci.* 2013; 280:20130077.
- 38.** Humayun MS, Dorn JD, Ahuja AK, et al. Preliminary 6 month results from the Argus II epiretinal prosthesis feasibility study. *Conf Proc IEEE Eng Med Biol Soc.* 2009;2009:4566-4568.
- 39.** Del Priore LV, Kaplan HJ, Tezel TH, Hayashi N, Berger AS, Green WR. Retinal pigment epithelial cell transplantation after subfoveal membranectomy in age-related macular degeneration: clinicopathologic correlation. *Am J Ophthalmol.* 2001;131:472-480.
- 40.** Tezel TH, Del Priore LV, Berger AS, Kaplan HJ. Adult retinal pigment epithelial transplantation in exudative age-related macular degeneration. *Am J Ophthalmol.* 2007;143:584-595.
- 41.** Weisz JM, Humayun MS, De Juan E Jr, et al. Allogenic fetal retinal pigment epithelial cell transplant in a patient with geographic atrophy. *Retina.* 1999;19:540-545.
- 42.** Algvere PV, Gouras P, Dafgard KE. Long-term outcome of RPE allografts in non-immunosuppressed patients with AMD. *Eur J Ophthalmol.* 1999;9:217-230.
- 43.** Algvere PV, Berglin L, Gouras P, Sheng Y. Transplantation of fetal retinal pigment epithelium in age-related macular degeneration with subfoveal neovascularization. *Graefes Arch Clin Exp Ophthalmol.* 1994;232:707-716.
- 44.** Algvere PV, Berglin L, Gouras P, Sheng Y, Kopp ED. Transplantation of RPE in age-related macular degeneration: observations in disciform lesions and dry RPE atrophy. *Graefes Arch Clin Exp Ophthalmol.* 1997;235:149-158.
- 45.** Radtke ND, Aramant RB, Petry HM, Green PT, Pidwell DJ, Seiler MJ. Vision improvement in retinal degeneration patients by implantation of retina together with retinal pigment epithelium. *Am J Ophthalmol.* 2008;146:172-182.
- 46.** Carr AJ, Vugler AA, Hikita ST, et al. Protective effects of human iPSC-derived retinal pigment epithelium cell transplantation in the retinal dystrophic rat. *PLoS One.* 2009;4:e8152.
- 47.** Klimanskaya I, Hipp J, Rezai KA, West M, Atala A, Lanza R. Derivation and comparative assessment of retinal pigment epithelium from human embryonic stem cells using transcriptomics. *Cloning Stem Cells.* 2004;6:217-245.

48. Buchholz DE, Hikita ST, Rowland TJ, et al. Derivation of functional retinal pigmented epithelium from induced pluripotent stem cells. *Stem Cells*. 2009;27:2427-2434.
49. Lund RD, Wang S, Lu B, et al. Cells isolated from umbilical cord tissue rescue photoreceptors and visual functions in a rodent model of retinal disease. *Stem Cells*. 2007;25:602-611.
50. Tibbetts MD, Samuel MA, Chang TS, Ho AC. Stem cell therapy for retinal disease. *Curr Opin Ophthalmol*. 2012;23:226-234.
51. MacLaren RE, Bird AC, Sathia PJ, Aylward GW. Long-term results of submacular surgery combined with macular translocation of the retinal pigment epithelium in neovascular age-related macular degeneration. *Ophthalmology*. 2005;112:2081-2087.
52. Chen FK, Uppal GS, MacLaren RE, et al. Long-term visual and microperimetry outcomes following autologous retinal pigment epithelium choroid graft for neovascular age-related macular degeneration. *Clin Experiment Ophthalmol*. 2009;37:275-285.
53. Jousen AM, Heussen FM, Joeres S, et al. Autologous translocation of the choroid and retinal pigment epithelium in age-related macular degeneration. *Am J Ophthalmol*. 2006;142:17-30.
54. Maaijwee K, Missotten T, Mulder P, van Meurs JC. Influence of intraoperative course on visual outcome after an RPE-choroid translocation. *Invest Ophthalmol Vis Sci*. 2008;49:758-761.
55. Landa G, Su E, Garcia PM, Seiple WH, Rosen RB. Inner segment-outer segment junctional layer integrity and corresponding retinal sensitivity in dry and wet forms of age-related macular degeneration. *Retina*. 2011;31:364-370.
56. kagi-Kurashige Y, Tsujikawa A, Oishi A, et al. Relationship between retinal morphological findings and visual function in age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol*. 2012;250:1129-1136.
57. Oishi A, Hata M, Shimoazono M, Mandai M, Nishida A, Kurimoto Y. The significance of external limiting membrane status for visual acuity in age-related macular degeneration. *Am J Ophthalmol*. 2010;150:27-32.
58. Maaijwee K, Heimann H, Missotten T, Mulder P, Jousen A, van Meurs J. Retinal pigment epithelium and choroid translocation in patients with exudative age-related macular degeneration: long-term results. *Graefes Arch Clin Exp Ophthalmol*. 2007;245:1681-1689.
59. Heussen FM, Fawzy NF, Joeres S, et al. Autologous translocation of the choroid and RPE in age-related macular degeneration: 1-year follow-up in 30 patients and recommendations for patient selection. *Eye (Lond)*. 2008;22:799-807.
60. Asaria RH, Kon CH, Bunce C, et al. Silicone oil concentrates fibrogenic growth factors in the retro-oil fluid. *Br J Ophthalmol*. 2004;88:1439-1442.
61. Heimann H, Stappler T, Wong D. Heavy tamponade 1: a review of indications, use, and complications. *Eye (Lond)*. 2008;22:1342-1359.
62. Bastiaans J, van Meurs JC, van Holten-Neelen C, et al. Factor Xa and thrombin stimulate proinflammatory and profibrotic mediator production by retinal pigment epithelial cells: a role in vitreoretinal disorders? *Graefes Arch Clin Exp Ophthalmol*. 2013;251:1723-1733.
63. Van Zeeburg EJ, Cereda MG, van der Schoot J, Pertile G, van Meurs JC. Early perfusion of a free RPE-choroid graft in patients with exudative macular degeneration can be imaged with spectral domain-OCT. *Invest Ophthalmol Vis Sci*. 2011;52:5881-5886.
64. Cereda MG, Parolini B, Bellesini E, Pertile G.

- Surgery for CNV and autologous choroidal RPE patch transplantation: exposing the submacular space. *Graefes Arch Clin Exp Ophthalmol.* 2010;248:37-47.
- 65.** Maaijwee K, van den Biesen PR, Missotten T, van Meurs JC. Angiographic evidence for revascularization of an rpe-choroid graft in patients with age-related macular degeneration. *Retina.* 2008;28:498-503.
- 66.** Heier JS, Boyer D, Nguyen QD, et al. The 1-year results of CLEAR-IT 2, a phase 2 study of vascular endothelial growth factor trap-eye dosed as-needed after 12-week fixed dosing. *Ophthalmology.* 2011;118:1098-1106.
- 67.** Dawson DW, Volpert OV, Gillis P, et al. Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. *Science.* 1999;285:245-248.
- 68.** Campochiaro PA, Nguyen QD, Shah SM, et al. Adenoviral vector-delivered pigment epithelium-derived factor for neovascular age-related macular degeneration: results of a phase I clinical trial. *Hum Gene Ther.* 2006;17:167-176.
- 69.** Goverdhan SV, Lochhead J. Submacular haemorrhages after intravitreal bevacizumab for large occult choroidal neovascularisation in age-related macular degeneration. *Br J Ophthalmol.* 2008;92:210-212.
- 70.** Kiernan DF, Hariprasad SM, Rusu IM, Mehta SV, Mieler WF, Jager RD. Epidemiology of the association between anticoagulants and intraocular hemorrhage in patients with neovascular age-related macular degeneration. *Retina.* 2010;30:1573-1578.
- 71.** Heriot WJ. Intravitreal gas and TPA; an outpatient procedure for submacular hemorrhage. *American Academy of Ophthalmology Annual Vitreoretinal Update;* 1996. Ref Type: Conference Proceeding
- 72.** Ohji M, Saito Y, Hayashi A, Lewis JM, Tano Y. Pneumatic displacement of subretinal hemorrhage without tissue plasminogen activator. *Arch Ophthalmol.* 1998;116:1326-1332.
- 73.** Chen CY, Hooper C, Chiu D, Chamberlain M, Karia N, Heriot WJ. Management of submacular hemorrhage with intravitreal injection of tissue plasminogen activator and expansile gas. *Retina.* 2007;27:321-328.
- 74.** Hesse L, Schmidt J, Kroll P. Management of acute submacular hemorrhage using recombinant tissue plasminogen activator and gas. *Graefes Arch Clin Exp Ophthalmol.* 1999;237:273-277.
- 75.** Wu TT, Kung YH, Hong MC. Vitreous hemorrhage complicating intravitreal tissue plasminogen activator and pneumatic displacement of submacular hemorrhage. *Retina.* 2011;31:2071-2077.
- 76.** Olivier S, Chow DR, Packo KH, MacCumber MW, Awh CC. Subretinal recombinant tissue plasminogen activator injection and pneumatic displacement of thick submacular hemorrhage in Age-Related macular degeneration. *Ophthalmology.* 2004;111:1201-1208.
- 77.** Hauptert CL, McCuen BW, Jaffe GJ, et al. Pars plana vitrectomy, subretinal injection of tissue plasminogen activator, and fluid-gas exchange for displacement of thick submacular hemorrhage in age-related macular degeneration. *Am J Ophthalmol.* 2001;131:208-215.
- 78.** Arias L, Mones J. Transconjunctival sutureless vitrectomy with tissue plasminogen activator, gas and intravitreal bevacizumab in the management of predominantly hemorrhagic age-related macular degeneration. *Clin Ophthalmol.* 2010;4:67-72.
- 79.** Meyer CH, Scholl HP, Eter N, Helb HM, Holz FG. Combined treatment of acute subretinal haemorrhages with intravitreal recombined

- tissue plasminogen activator, expansile gas and bevacizumab: a retrospective pilot study. *Acta Ophthalmol.* 2008;86:490-494.
- 80.** Treumer F, Roider J, Hillenkamp J. Long-term outcome of subretinal coapplication of rtPA and bevacizumab followed by repeated intravitreal anti-VEGF injections for neovascular AMD with submacular haemorrhage. *Br J Ophthalmol.* 2011.
- 81.** Sacu S, Stifter E, Vecsei-Marlovits PV, et al. Management of extensive subfoveal haemorrhage secondary to neovascular age-related macular degeneration. *Eye (Lond).* 2009;23:1404-1410.
- 82.** van Zeeburg EJ, van Meurs JC. Literature Review of Recombinant Tissue Plasminogen Activator Used for Recent-Onset Submacular Hemorrhage Displacement in Age-Related Macular Degeneration. *Ophthalmologica.* 2012.
- 83.** Benner JD, Hay A, Landers MB, III, Hjelmeland LM, Morse LS. Fibrinolytic-assisted removal of experimental subretinal hemorrhage within seven days reduces outer retinal degeneration. *Ophthalmology.* 1994;101:672-681.
- 84.** Toth CA, Morse LS, Hjelmeland LM, Landers MB, III. Fibrin directs early retinal damage after experimental subretinal hemorrhage. *Arch Ophthalmol.* 1991;109:723-729.
- 85.** Hattenbach LO, Klais C, Koch FH, Gumbel HO. Intravitreal injection of tissue plasminogen activator and gas in the treatment of submacular hemorrhage under various conditions. *Ophthalmology.* 2001;108:1485-1492.
- 86.** Lewis H. Intraoperative fibrinolysis of submacular hemorrhage with tissue plasminogen activator and surgical drainage. *Am J Ophthalmol.* 1994;118:559-568.
- 87.** Tonotsuka T, Imai M, Saito K, Iijima H. Visual prognosis for symptomatic retinal arterial macroaneurysm. *Jpn J Ophthalmol.* 2003;47:498-502.
- 88.** Wu TT, Sheu SJ. Intravitreal tissue plasminogen activator and pneumatic displacement of submacular hemorrhage secondary to retinal artery macroaneurysm. *J Ocul Pharmacol Ther.* 2005;21:62-67.
- 89.** Humayun M, Lewis H, Flynn HW, Jr., Sternberg P, Jr., Blumenkranz MS. Management of submacular hemorrhage associated with retinal arterial macroaneurysms. *Am J Ophthalmol.* 1998;126:358-361.
- 90.** van Zeeburg EJ, Cereda MG, van Meurs JC. Recombinant tissue plasminogen activator, vitrectomy, and gas for recent submacular hemorrhage displacement due to retinal macroaneurysm. *Graefes Arch Clin Exp Ophthalmol.* 2013;251:733-740.
- 91.** Maruko I, Sugano Y, Oyamada H, Sekiryu T, Lida T, Akiba M. Choroidal imaging in gas-filled eye using swept source optical coherence tomography. ARVO annual meeting Seattle; 2013. Ref Type: Conference Proceeding

# Summary





## SUMMARY

This thesis describes a surgical treatment for late exudative age-related macular degeneration (AMD): the retinal pigment epithelium (RPE) and choroid graft transplantation surgery. This treatment may, by exception, be considered when the standard treatment with anti-vascular endothelial growth factors (anti-VEGF) is ineffective. In addition, this thesis describes the revascularization of a graft, analyzed with the help of new imaging techniques. Finally, a technique is described to which, in our view, not enough attention is paid to; the injection of recombinant tissue plasminogen activator (rtPA) and gas as a treatment for submacular hemorrhages, one of the complications of AMD.

**Chapter 1** is a general introduction on the disease process of AMD; the causes and possible treatment strategies are discussed. Furthermore, an introduction is given on new imaging techniques which are used in a number of chapters of this thesis: spectral domain optical coherence tomography (SD-OCT), optical frequency domain (OFDI)-OCT and the phase resolved Doppler (PRD)-OCT.

During an RPE-choroid graft transplantation surgery, after vitrectomy, the subretinal neovascular membrane, subretinal fibrosis and/or submacular hemorrhage is removed through a paramacular temporal retinotomy. Then an RPE-choroid graft of approximately 3x4 millimeter is prepared, from a superior or inferior midperipheral donor site location. Subsequently, this free graft is transplanted underneath the macula. At the end of the RPE-choroid graft surgery, a tamponade of silicone oil is left in the eye; an oil which is lighter than water. The oil does not fill the eye completely, and therefore inflammatory cells end up in the intraocular fluid under the oil bubble; a fibrotic, pro-inflammatory milieu. These inflammatory cells are released by trauma of the retina and subretinal layers (the creation of the donor site and the temporal retinotomy) and eventually may lead to proliferative vitreoretinopathy (PVR), one of the most frequent complications of the RPE-choroid graft transplantation surgery.

In **Chapter 2.1** the long-term results of the RPE-choroid graft transplantation surgery are described. In this study we have compared the visual acuity at one, two and three years after surgery of 130 patients with a minimum of four years and a maximum of seven years of follow-up, with the visual acuity (VA) of patients from an American study in which the old submacular hemorrhage ( $n = 168$ ) or the neovascular membrane ( $n = 226$ ) surgically was removed. Three years after surgery a VA of 20/40 or more was achieved by 4.3% of the graft patients with a preoperative hemorrhage (B), and by 1.6% of the graft patients with a preoperative neovascular membrane (N). In the studies where only the hemorrhage or the neovascular membrane was removed, respectively 2.4% and 0.4% of the patients achieved this VA after three years. A VA of  $> 20/200$  was achieved after three years in 15% of the patients in graft group B, and 18% in graft group N. This was better

compared to the 7% and 8% respectively, which was achieved by the patients in which only the hemorrhage or neovascular membrane was removed. After four years, 15% of all patients with a graft achieved a VA of  $> 20/200$ , and 5% achieved a VA of  $\geq 20/40$ . Four patients out of this study group achieved a VA of  $> 20/200$  up to seven years after surgery, with a median of 20/71; the best patient achieved a VA of 20/32. In this patient with the best VA seven years postoperatively, sensitivity was measured with microperimetry over the macular area of the graft, and fixation was location in the macula. This confirmed that the RPE-choroid graft was functioning. A relatively low complication rate was found in the group of RPE-choroid graft patients; 10% of the patients developed PVR, and 10% developed a recurrent choroidal neovascular membrane.

In **chapter 2.2** we studied whether there was a higher incidence of PVR in patients with a superior or an inferior donorsite. The hypothesis was that for patients with an inferior donorsite, PVR would occur more often, as the defect (the donorsite) is in direct contact with the inflammatory environment. However, no relation between the incidence of PVR and the location of the donorsite was found. Therefore, a donorsite may be chosen superiorly or inferiorly, as it does not affect the occurrence PVR.

In **chapter 3** we analyzed three patients with an RPE-choroid graft with microperimetry. This is a functional test in which the sensitivity of the retina for light stimuli is assessed for a very small part of the visual field. In this chapter we correlated the results of the microperimeter with morphological data, as visualized with the SD-OCT, and other imaging techniques, such as fluorescein angiography (FA) and autofluorescence (AF). Areas with RPE and/or retinal damage, as visualized on AF, FA, and SD-OCT, could be correlated to areas with diminished retinal sensitivity. Furthermore, we were able to document a shift of the preferred retinal locus (eccentric fixation) of a patient with foveal damage. With microperimetry, extra information can be gathered about graft function in addition to morphological and anatomical imaging.

In **chapter 4** the results of a randomized controlled trial were described. Anti-VEGF therapy was compared with the RPE-choroid transplantation surgery for three patient groups: 1) non-responders for anti-VEGF therapy, 2) submacular hemorrhages, 3) RPE tears. This trial was prematurely ended, as the number of patients needed for statistical significance would not be reached within a reasonable time span, (because other participating centers were not including patients) and because there was an unprecedented high number of (unusual) complications. However, of the 20 patients who were included in our hospital, we gathered interesting results. In contrary to the anti-VEGF patient group ( $n = 10$ ), where VA stabilized or declined, two patients in the RPE-choroid graft group ( $n = 10$ ) showed marked gain in VA. The best patient improved 26 letters to a VA of 0.14 logarithm of minimal angle of resolution (logMAR) one year after surgery. The reading acuity improved and there was a marked improvement in the degree of metamorphopsia. But, due to the high number of complications in the

graft group such as hypotony and PVR, this group also experienced the highest loss in visual acuity. In the anti-VEGF group also unforeseen complications occurred; one patient developed PVR after only hemorrhage removal surgery. Though, most of the complications in this group were extra-ocular/systemic. It is interesting that the results from this patient group which were included after the introduction of anti-VEGF therapy, are comparable to those from the long-term follow-up study in chapter 2.1, in which patients were included before anti-VEGF therapy was regularly available.

Revascularization is important for RPE-choroid graft function. In **chapter 5.1** we analyzed the revascularization steps of a graft in 12 patients with invasive imaging techniques (FA and indocyanine green angiography [ICGA]), and SD-OCT; a new, non-invasive technique. Immediately after surgery a thin graft was visualized with small, optically clear vessels: the imbibition phase. Several days after surgery, the thickness of the graft and the diameter and the number of vessels of the graft increased, but the vessel lumina in the graft remained optically clear. These events correlated with the local appearance of ICGA (hyper-fluorescence); an afferent choroidal vessel connecting to the edge of the graft. The graft was pumped up by this connection to an afferent vessel in the absence of an efferent vessel, as blood may flow into the vessels but there is no flow through the graft. The diameter of the vessels of the graft and the thickness of the graft therefore increase: the afferent phase. Subsequently, the number of vessels progressively increased and the color of the vessel lumina changed from optically clear to a gray shading (minimal hyper-reflectivity), which was comparable to the color of the recipient choroidal vessels. Furthermore, the thickness of the graft slowly decreased, and the diameter of the vessels of the graft decreased. These last events on SD-OCT correlated with the perfusion of the entire graft visible on FA and ICGA. Seemingly, the establishment of an efferent vessel connection facilitated blood flow through the graft and a subsequent decrease in thickness of the graft: the efferent phase. By the establishment of these revascularization steps, invasive techniques such as FA and ICGA are no longer necessary after RPE-choroid graft surgery. A series of SD-OCT scans shortly after surgery is sufficient to confirm revascularization.

After the establishment of the revascularization steps with the SD-OCT, a more conventional imaging technique, the findings were correlated with flow measurements of the graft by PRD-OCT; an OCT scan which simultaneously measures Doppler signals. In **chapter 5.2** we scanned seven patients with an RPE-choroid graft with SD-OCT and PRD-OCT, one year after surgery ( $n = 1$ ) or several times shortly after surgery ( $n = 6$ ). In none of the six patients which were scanned regularly, flow was measured during the imbibition phase. In three out of six patients, flow was detected in the afferent phase, which can be explained by the fact that the graft may already be partially revascularized in this phase. In all patients flow was measured in the efferent phase. The revascularization steps, established by SD-OCT could therefore be confirmed by

the measurements of blood flow in the graft by PRD-OCT. With PRD-OCT also structural images can be obtained as with the conventional SD-OCT. Therefore, in the future, when the PRD-OCT is allowed to be used in clinics, it is possible that in one single non-invasive measurement graft revascularization can be analyzed.

**Chapter 6.1** is a review of the literature describing different administration methods of rtPA for the displacement of acute submacular hemorrhages. Several studies demonstrate the possibility to liquefy and displace a hemorrhage up to 14 days after the initiation. Photoreceptor damage, as a result of the first toxic effects of the hemorrhage, can be observed as early as after 24 hours. After seven days, a progressive, irreversible degeneration of the outer retinal layers and the RPE is visible. Between seven and 14 days, extensive destruction of retina and the photoreceptor cells eventually leads to atrophy of the retina. Therefore we conclude that a hemorrhage should be treated with rtPA preferably within seven days, but at most within 14 days; as then displacement of the hemorrhage and visual improvement are most likely. The results were compared of 38 studies with a total of 1.185 patients, and an additional 28 patients of the Rotterdam Eye Hospital, in whom rtPA was administered to displace a submacular hemorrhage. Two methods of rtPA administration can be distinguished. Subretinal rtPA administration with vitrectomy, or intravitreal rtPA administration, without vitrectomy. Variations of these two methods consist of tamponades of air or gas, or no tamponade, as well as the administration of anti-VEGF during or after surgery. We found no difference in safety or efficacy between the two administration methods. More recent studies, however, tend to favor the use of vitrectomy, while this method can possibly cause more complications, and is a longer procedure than the intravitreal method. Based on our review we suggest to treat patients with a submacular hemorrhage within 14 days after onset with 25  $\mu$ g rtPA, either subretinal or intravitreal. A randomized controlled trial (which is not part of this thesis) was started in our hospital, to compare the complications and results of the intravitreal and the subretinal method of administration of 25  $\mu$ g rtPA, within 14 days after onset. During the rtPA administration procedure, anti-VEGF medications are administered, to treat the exudative AMD, the underlying cause of the hemorrhage. Anti-VEGF treatment will be continued at one and two months after surgery. The results will be analyzed with pre- and postoperative volume scans of the submacular hemorrhage by SD-OCT.

For patients with a submacular hemorrhage due to a retinal macro aneurysm (RMA), the hemorrhage can be displaced by rtPA and gas as well. This patient group has a remarkably better prognosis compared to patients with AMD, as these patients mostly have no pre-existent macular damage. Therefore, a marked visual acuity improvement may be expected after displacement of the hemorrhage, provided that the hemorrhage caused none, or little, toxic damage to the retina.

In **chapter 6.2** we have described the results of 12 patients with a hemorrhage due to RMA, with 11 of them receiving a submacular rtPA injection and gas tamponade. In ten of the 11 patients the hemorrhage had (almost) completely displaced and visual acuity had significantly increased with a mean of 1.5 logMAR.

In **chapter 7**, the general discussion, the results of the chapters of this thesis are discussed in a broader scientific view. The future of the RPE-choroid graft surgery is discussed; better patient selection by the relatively new imaging techniques as the SD-OCT may play a role. In the laboratory, work has been done on stem cell-based RPE transplantation and the first phase I studies have been started. Also electronical sub- and epiretinal implants might play a role in the future. Furthermore, a preview is given about the additional clinical value OFDI-OCT and PRD-OCT; as an example, in the treatment decision and follow-up of patients with submacular hemorrhages.



# Samenvatting





## SAMENVATTING

Dit proefschrift beschrijft een chirurgische behandeling voor vergevorderde exsudatieve leeftijdsgebonden macula degeneratie (AMD): de retinaal pigment eptiheel (RPE) en choroidea transplantatie operatie. Deze behandeling kan bij uitzondering worden overwogen als de standaard behandeling met vaatgroeiremmers tekort schiet. Daarnaast wordt beschreven hoe met behulp van nieuwe beeldvormende technieken de revascularisatie van een transplantaat kan worden geanalyseerd. Tenslotte wordt een ons inziens onderbelichte behandeling voor acute submaculaire bloedingen, één van de complicaties bij AMD, beschreven: injectie van recombinant tissue plasminogen activator (rtPA) en gas.

**Hoofdstuk 1** is een algemene inleiding op het gebied van AMD, waarin de oorzaken en mogelijke behandelingen voor deze aandoening worden besproken. Daarnaast wordt een inleiding gegeven op nieuwe beeldvormende technieken die gebruikt zijn in enkele hoofdstukken van dit proefschrift: spectral domain optical coherence tomography (SD-OCT), optical frequency domain (OFDI)-OCT en phase resolved Doppler (PRD)-OCT.

Bij een RPE-choroidea transplantatie operatie wordt, na vitrectomie, het subretinale neovasculaire membraan, eventuele subretinale fibrose en/of submaculaire bloed verwijderd via een paramaculaire temporale retinotomie. Vervolgens wordt een RPE-choroidea transplantaat ter grootte van ongeveer 3 bij 4 millimeter vrijgeprepareerd, vanuit een superieure of inferieure midperifere donor locatie. Dit vrije transplantaat wordt vervolgens ter hoogte van de macula onder het netvlies geplaatst. Aan het einde van de RPE-choroidea transplantatie operatie, wordt het oog getamponneerd met siliconen olie, een olie die lichter is dan water. De olie vult niet het gehele oog en daardoor kunnen eventuele ontstekingscellen in het oogvocht onder de oliebel terechtkomen; een fibrotisch, inflammatoir milieu. Deze ontstekingscellen komen vrij bij schade aan het netvlies en subretinale lagen (het creëren van de donor locatie en een temporale retinotomie), wat uiteindelijk tot proliferatieve vitreoretinopathie (PVR) kan leiden, een van de meest voorkomende complicaties van de RPE-choroidea transplantatie operatie.

In **hoofdstuk 2.1** worden de lange termijn resultaten van deze RPE-choroidea transplantatie operatie beschreven. In deze studie hebben we de visus op één, twee en drie jaar na de operatie van 130 patiënten die minimaal vier jaar tot maximaal zeven jaar follow-up hadden, vergeleken met de visus van patiënten uit een Amerikaanse studie waarin bij patiënten alleen de oude submaculaire bloeding met membraan ( $n = 168$ ) chirurgisch werd verwijderd, of alleen een neovasculair membraan zonder bloeding ( $n = 226$ ) werd verwijderd. Na drie jaar zag 4.3 % van de RPE-choroidea transplantatie patiënten met een preoperatieve bloeding (B), en 1.6% van de RPE-choroidea transplantatie patiënten met een preoperatief neovasculair membraan (N)  $\geq 20/40$ . In de studies waarin alleen de bloeding of alleen het neovasculair membraan werd verwijderd

zag respectievelijk 2.4% en 0.4% van de patiënten  $\geq 20/40$  na 3 jaar. Een visus van  $>20/200$  werd bereikt na 3 jaar in 15% van de patiënten in de RPE-choroidea transplantatie groep B en 18% in de RPE-choroidea transplantatie groep N, wat beter is dan de 7% en 8% die werden bereikt door alleen de bloeding of het membraan te verwijderen. Na vier jaar zag 15% van alle patiënten met een RPE-choroidea transplantaat  $>20/200$  en 5% zag  $\geq 20/40$ . Vier patiënten uit deze studiegroep bereikten zeven jaar na de operatie een visus van  $>20/200$ , met een mediaan van 20/71; de best ziende patiënt zag 20/32. In deze best ziende patiënt zeven jaar na de operatie was met microperimetrie sensitiviteit meetbaar over het maculaire gedeelte van het transplantaat en was de fixatie in de macula gelokaliseerd, waarmee aangetoond werd dat het RPE-choroidea transplantaat functioneerde. In deze groep van RPE-choroidea transplantatie patiënten traden relatief weinig complicaties op, 10% ontwikkelde PVR, en 10% ontwikkelde een recidiverend choroïdaal neovasculair membraan.

In **hoofdstuk 2.2** is vergeleken of PVR vaker voorkwam bij patiënten met een superieure of inferieure donor locatie. De hypothese was dat bij patiënten met een inferieure donor site vaker PVR zou optreden omdat dan het defect (de donorsite) in direct contact staat met het inflammatoire milieu. Dit bleek echter niet het geval te zijn. Het maakt daarom niet uit, wat betreft de kans op PVR, waar een donor locatie gekozen wordt.

In **hoofdstuk 3** hebben we drie patiënten met een RPE-choroidea transplantaat uitgebreid geanalyseerd met behulp van microperimetrie. Dit is een functionele test waarbij de gevoeligheid van het netvlies voor lichtstimuli wordt bepaald in een zeer klein gedeelte van het gezichtsveld. In dit hoofdstuk hebben we de resultaten van de microperimetrie gecorreleerd met morfologische data, zoals zichtbaar met behulp van SD-OCT, en met andere beeldvormende technieken, zoals fluorescentie angiografie (FA) en autofluorescentie (AF). Gebieden met RPE en/of retina schade, zoals gezien met AF, FA en SD-OCT, konden worden gecorreleerd aan gebieden met een verminderde retinale sensitiviteit. Daarnaast konden we een verschuiving van de preferred retinal locus (excentrische fixatie) vaststellen bij een patiënt met schade in de fovea. Met behulp van microperimetrie kan extra informatie worden verkregen over het functioneren van een RPE-choroidea graft, als aanvulling op morfologische/anatomische beeldvorming.

In **hoofdstuk 4** werden de resultaten beschreven van een gerandomiseerde interventie studie waarin anti-vascular endothelial growth factor (anti-VEGF) therapie werd vergeleken met RPE-choroidea transplantatie chirurgie voor drie patiëntengroepen: 1) Non-responders voor anti-VEGF, 2) submaculaire bloedingen, 3) RPE scheuren. Deze trial werd vroegtijdig gestopt omdat het beoogde patiëntenaantal welke nodig was om statistische significantie te kunnen bereiken, niet binnen afzienbare tijd kon worden gehaald (omdat in de andere deelnemende centra geen patiënten werden geïncludeerd), en omdat er onevenredig veel en ongebruikelijke complicaties optraden.

Toch waren er interessante bevindingen in de 20 door ons geïnccludeerde patiënten. In tegenstelling tot de anti-VEGF groep (n = 10) waar de visus van de patiënten stabiel bleef of verslechterde, hadden twee patiënten in de RPE-choroidea transplantatie groep (n = 10) significante visuswinst. De beste patiënt verbeterde met 26 letters naar een visus van 0.14 logarithm of minimal angle of resolution (logMAR) één jaar na de operatie, de leesvisus verbeterde en er was een duidelijke afname van de metamorfopsie. Echter door aanzienlijke complicaties in de graft groep zoals hypotonie en PVR, trad in deze groep ook het grootste visusverlies op. Ook in de anti-VEGF groep traden onvoorziene complicaties op, waaronder één patiënt die PVR ontwikkelde na enkel het verwijderen van de bloeding; maar het merendeel van deze complicaties in de gehele groep waren extra-oculair of systemisch van aard. Interessant is dat deze resultaten van een groep patiënten uit het anti-VEGF tijdperk, vergelijkbaar blijven met de resultaten die werden gevonden in de lange termijn studie in hoofdstuk 2.1, waarbij de patiënten werden geïnccludeerd voordat anti-VEGF beschikbaar was.

Het is van belang voor het functioneren van een vrij RPE-choroidea transplantaat dat deze wordt gerevasculariseerd. In **hoofdstuk 5.1** hebben we de revascularisatie stappen van een graft geanalyseerd in 12 patiënten met behulp van invasieve beeldvormende technieken (FA en indocyanine groen angiografie (ICGA)) en de SD-OCT; een relatief nieuwe, niet-invasieve techniek. Direct na de operatie werd een dunne graft gezien met kleine vaten die optisch 'leeg' waren op de SD-OCT scans: de imbibitiefase. Binnen enkele dagen na de operatie werd de diameter van de vaten in de graft snel groter en nam het aantal vaten toe. Tegelijkertijd werd de graft dikker maar de vaten zelf bleven optisch leeg. We zagen wel dat er heel plaatselijke ICGA aankleuring (hyperfluorescentie) was van een aanvoerend choroïdaal vat aan de rand van de graft waardoor waarschijnlijk bloed wel in de vaten kon stromen maar nog niet eruit, waardoor de dikte van de vaten en het transplantaat zelf toenamen: de afferente fase. Daarna nam het aantal vaten langzaam verder toe en veranderde de kleur van de vaten van 'optisch leeg' naar een grijze waas (minimale hyperreflectiviteit), vergelijkbaar met wat zichtbaar was in de onderliggende choroïdale vaten. Daarnaast nam de dikte van het transplantaat langzaam af en werd de diameter van de vaten weer kleiner. Deze laatste stappen correspondeerden met het tijdstip van het zien van complete perfusie van het transplantaat op FA en ICGA. De afname van dikte van de graft en vaten leek veroorzaakt omdat er bloedstroom aanwezig was; de afvoerende vaten waren aangesloten op het transplantaat waardoor bloed kon stromen: de efferente fase. Door het aantonen van deze revascularisatie stappen zijn de invasieve FA en ICGA onderzoeken na een RPE-choroidea transplantatie operatie niet meer nodig, maar kan er worden volstaan met seriële SD-OCT scans om revascularisatie vast te stellen.

Na het analyseren van deze revascularisatie stappen met de SD-OCT, de meer conventionele beeldvormende techniek, hebben we deze bevindingen gecorrigeerd

aan doorbloedingsmetingen in het transplantaat met behulp van de PRD-OCT; een OCT scan waarbij gelijktijdig een Doppler signaal meting wordt gedaan. In **hoofdstuk 5.2** hebben we zeven patiënten met een RPE-choroidea transplantaat met SD-OCT én de PRD-OCT gescand, op één moment, één jaar na de operatie ( $n = 1$ ), of enkele tijdstippen kort na de operatie ( $n = 6$ ). Bij geen enkele van de 6 regelmatig gemeten patiënten was bloedstroom te meten in de imbibitie fase. Bij drie van de zes patiënten was al bloedstroom te meten in de afferente fase, hetgeen te verklaren is door het feit dat het transplantaat al gedeeltelijk gerevasculariseerd kan zijn in deze fase. Bij alle zeven patiënten was bloedstroom te meten in de efferente fase. De revascularisatie stappen aangetoond met behulp van SD-OCT konden dus door middel van het meten van de doorbloeding van de vaten in de graft met de PRD-OCT bevestigd worden. Aangezien met behulp van PRD-OCT ook structurele informatie kan worden verkregen zoals met de conventionele SD-OCT, is het mogelijk dat in de toekomst, als de PRD-OCT klinisch toegepast mag worden, in één enkele niet-invasieve meting geanalyseerd kan worden of een graft gerevasculariseerd is.

**Hoofdstuk 6.1** is een overzicht van de literatuur over de methodes van toediening van rtPA voor de verplaatsing van acute submaculaire bloedingen. Uit verschillende studies blijkt dat het vloeibaar maken en verplaatsen van een bloeding mogelijk is tot uiterlijk 14 dagen na het ontstaan van de bloeding. Echter, al 24 uur na het ontstaan van de bloeding treden de eerste toxische effecten als gevolg van de bloeding op, waarbij de fotoreceptoren beschadigen. Na zeven dagen wordt al progressieve, onomkeerbare degeneratie van de buitenste retinale lagen en het RPE zichtbaar. Tussen zeven en 14 dagen na het ontstaan van de bloeding wordt de schade aan de retina en de fotoreceptoren zo groot dat atrofie van de retina ontstaat. Daarom concludeerden wij dat een bloeding het liefst binnen zeven maar uiterlijk binnen 14 dagen behandeld moet worden met rtPA zodat de kans op verplaatsing van de bloeding en visus verbetering het grootst is. De resultaten uit 38 studies met in totaal 1.185 patiënten en 28 patiënten van het Oogziekenhuis bij wie rtPA werd toegediend voor een submaculaire bloeding, zijn vervolgens met elkaar vergeleken. De toedieningmethodes van rtPA konden opgedeeld te worden in twee groepen. Subretinale rtPA toediening na vitrectomie, of intravitreale toediening van rtPA, zonder vitrectomie. Varianten op deze twee toedieningsmethoden waren lucht of gas tamponade of geen tamponade, en het wel of niet toedienen van anti-VEGF tijdens of na de ingreep. Wij vonden geen verschil in veiligheid of effectiviteit van een van beide toedieningmethodes. De meer recente artikelen in de literatuur neigen daarentegen naar de subretinale rtPA techniek. Dit terwijl deze techniek mogelijk meer complicaties kan veroorzaken, en een langere procedure is dan intravitreale methode. Op basis van deze review bevelen wij aan patiënten met een submaculaire bloeding bij AMD binnen 14 dagen te behandelen met 25 µg rtPA, subretinaal of intravitreaal. Een reeds door ons gestarte gerandomiseerde gecontroleerde studie (welke buiten het

bestek van dit proefschrift valt) zal vergelijken of de complicaties en de resultaten van intravitreale of subretinale toediening van 25 µg rtPA, binnen 14 dagen na het ontstaan van de bloeding, vergelijkbaar zijn. Tijdens deze rtPA behandelingen wordt tevens intravitreaal anti-VEGF medicatie achtergelaten om de onderliggende oorzaak van de bloeding, de exudatieve AMD, te blijven behandelen. De anti-VEGF behandeling wordt poliklinisch herhaald op respectievelijk één en twee maanden na de ingreep. Een van de belangrijke uitkomstmaten zullen pre- en postoperatieve volumemetingen van de submaculaire bloeding zijn, die worden gemaakt met de SD-OCT.

Bij patiënten met een submaculaire bloeding met als oorzaak een retinaal macroaneurysma (RMA) kan ook de bloeding verplaatst worden met behulp van rtPA en gas. Deze patiëntengroep heeft echter een aanzienlijk betere prognosis dan patiënten met AMD, omdat er bij patiënten met RMA meestal geen pre-existente maculaire schade is, en daarom na verplaatsing van het bloed grote visusverbetering te verwachten is, mits er nog geen of geringe toxische schade door het submaculaire bloed aan de retina is aangericht.

In **hoofdstuk 6.2** hebben we de resultaten beschreven van 12 patiënten met een bloeding bij RMA waarvan 11 een submaculaire rtPA injectie en gas tamponade ondergingen. Bij tien van de 11 patiënten bleek de bloeding (bijna) geheel te zijn verplaatst en was de visus significant (gemiddeld 1.5 logMAR [logarithm of minimal angle of resolution]) gestegen.

In **hoofdstuk 7**, de algemene discussie, worden de resultaten uit de hoofdstukken van dit proefschrift belicht vanuit een breder wetenschappelijk perspectief. De toekomst van RPE-choroidea transplantatie chirurgie wordt besproken, waarbij onder andere betere patiëntselectie door de relatief nieuwe beeldvormende technieken zoals de SD-OCT een rol speelt. In het laboratorium wordt gewerkt aan op stamcel technologie gebaseerde RPE transplantatie, waarmee ook de eerste klinische fase van start is gegaan. Ook de elektronische sub- en epiretinale implantaten krijgen mogelijk een plaats. Daarnaast wordt vooruitgeblikt wat de OFDI-OCT en PRD-OCT aan meerwaarde kunnen opleveren in de kliniek; bijvoorbeeld bij de behandelbeslissing en de follow-up van patiënten met submaculaire bloedingen.



# List of Abbreviations



**ABBREVIATIONS**

AF	Autofluorescence
Alpha-IMS implant	Electronic subretinal implant
AMD	Age-related macular degeneration
ANCHOR	Anti-VEGF Antibody for the Treatment of Predominantly Classic Choroidal Neovascularisation in AMD
ARED	Age-Related Eye Disease Study
Argus prosthesis	Electronic epiretinal implant
ARMS2	Age-related maculopathy susceptibility 2
ART	Automatic real time
Asb	Apostilb
BCVA	Best-corrected visual acuity
BSS	Balanced salt solution
C3F8	Perfluoropropane
CFH	Complement factor H
CG	Cannot grade
CNTF	Ciliary neurotrophic factor
CNV	Choroidal neovascular membrane
cSt	Centistokes
DA	Disk area
DB	Decibel
DORC	Dutch ophthalmic research center
EDI	Enhanced depth imaging
ELM	External limiting membrane
ESC	Embryonic stem cell
ETDRS	Early Treatment Diabetic Retinopathy Study
FA	Fluorescein angiography
Group B	AMD patients with surgery for either predominantly classic, minimally classic, or occult neovascular lesions
Group N	AMD patients with surgery for hemorrhagic (> 50% blood) lesions
HESC	Human embryonic stem cell
HLA	Human leukocyte antigen
HSO	Heavy silicone oil
hUTSC	Human umbilical tissue derived stem cells
ICGA	Indocyanine green angiography
ILM	Internal limiting membrane
IOL	Intraocular lens
IOP	Intraocular pressure

IPCV	Idiopathic polypoidal choroidal vasculopathy
iPSC	Induced pluripotent stem cell
IR	Infrared
IS/OS junction	Inner and outer photoreceptor layer junction
IV	Intravitreal
kDa	kilodaltons
LFO	Lost to follow-up
logMAR	Logarithm of minimal angle of resolution
logRAD	Reading acuity determination
LP	Light perception
MARINA	Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular AMD
mfERG	Multifocal electroretinogram
MP-1	Microperimeter
MPS	Macular Photocoagulation Study
N/A	Not applicable
NI	No (clear) information (provided by the authors)
OCT	Optical coherence tomography
OD	Oculus Dexter
OFDI	Optical frequency domain imaging
OS	Oculus Sinister
OSC	Ospedale Sacro Cuore
PEDF	Pigment epithelium-derived factor
PDT	Photodynamic therapy
PO	Postoperative
POHS	Presumed ocular histoplasmosis syndrome
Postop	Postoperative
PPV	Pars plana vitrectomy
Preop	Preoperative
PRD-OCT	Phase resolved Doppler-optical coherence tomography
PRL	Preferred retinal locus
PrONTO	Prospective Optical Coherence Tomography Imaging of Patients with Neovascular AMD Treated with Intraocular Ranibizumab
PVR	Proliferative vitreoretinopathy
RAM	Retinal arterial macroaneurysm
RD	Retinal detachment
REH	Rotterdam Eye Hospital
RPE	Retinal pigment epithelium
RPE-choroid graft	Retinal pigment epithelium and choroid graft

RPE-sheet	RPE-choroid graft
rtPA	Recombinant tissue plasminogen activator
SAC	Sine Amsler chart
SD	Standard deviation
SD-OCT	Spectral domain-optical coherence tomography
SF6	Sulfur hexafluoride
SMH	Submacular hemorrhage
SR	Subretinal
SST	Submacular Surgery Trials
TIA	Transient ischemic attack
TNF- $\alpha$	Tumor necrosis factor- $\alpha$
VA	Visual acuity
VAS	Visual acuity score
VEGF	Vascular endothelial growth factor
VFQ-25	25-item visual function questionnaire
YAG	Neodymium-doped yttrium aluminum garnet



## PHD PORTFOLIO

### Summary of PhD training and teaching

PhD student: E.J.T. van Zeeburg	Student research project Boston: March 2008 - March 2009
Institution: The Rotterdam Eye Hospital & The Rotterdam Ophthalmic Institute	PhD period: September 2009 – October 2013
	Promotor(s): Dr. J.C. van Meurs

#### 1. PhD training

	Year	Workload
<b>General courses</b>		
- Good Clinical Practice course, ICH training en advice	2009/10	8 hours
- Biomedical English Writing and Communication	2010/08-11	112 hours
- BROK ('Basiscursus Regelgeving Klinisch Onderzoek')	2010/10	28 hours
<b>Specific courses (e.g. Research school, Medical Training)</b>		
- ARVO USA Fort Lauderdale Additional Course: Eye cancer: new insight from developmental and stem cell biology	2008/05	6 hours
- Molecular Bases for Eye Diseases Fall Course. Schepens Eye Research Institute, Harvard Medical School, Boston, MA, USA.	2008/09-11	10 hours
- Neuro-Ophthalmology Course; Case Method and Evidence-Based Approach. Department of continuing education, Harvard Medical School, Boston, USA.	2008/11	6 hours
- ARVO 1 <sup>st</sup> Research course: Immunology	2010/02	6 hours
- ARVO 2 <sup>nd</sup> Research course: Visual Signals	2010/10	6 hours
<b>Seminars and workshops</b>		
- Eye and orbitatrauma Boerhaave Leiden	2009/09	6 hours
- Scientific day - Rotterdam Ophthalmic Institute	2009/10	6 hours
- Lecture Tour Rotterdam Eye Hospital	2009/11	2 hours
- Fysica lecture day Rotterdam Ophthalmic Institute	2010/06	6 hours
- Scientific day - Rotterdam Ophthalmic Institute	2011/01	6 hours
- Scientific day Rotterdam Eye Hospital (oral presentation)	2011/10	12 hours
- Resident's class Erasmus part 1: Orbita	2012/01	2 hours
- Scientific day Rotterdam Eye Hospital (oral presentation)	2012/10	12 hours
- Scientific day - Rotterdam Ophthalmic Institute	2012/10	6 hours
- Monthly scientific Seminars, Rotterdam Eye Hospital	2009-2013	64 hours
- Weekly Ophthalmology seminars, Rotterdam Eye Hospital	2009-2013	112 hours
- Monthly scientific seminars, Rotterdam Ophthalmic Institute (including 2 oral presentations)	2009-2013	36 hours
<b>(Inter)national conferences</b>		
<b>Oral presentations/poster contributions</b>		
- LIMSC Leiden (Poster contribution)	2009/03	40 hours
- ARVO USA Fort Lauderdale (Oral presentation)	2009/05	40 hours
- ARVO NED Rotterdam (Oral Presentation)	2009/11	24 hours

- ARVO USA Fort Lauderdale (Oral Presentation)	2010/05	64 hours
- NOG Maastricht (Oral Presentation)	2011/03	40 hours
- Euretina Londen (Oral Presentation)	2011/05	40 hours
- ARVO-NED Amsterdam (Oral Presentation)	2011/11	16 hours
- NOG Groningen (Oral Presentation)	2012/03	24 hours
- ARVO USA Fort Lauderdale (Poster contribution)	2012/05	40 hours
- Euretina Milan (Oral Presentation)	2012/09	40 hours
- NOG Groningen (Oral Presentation)	2013/03	24 hours

#### Other (Inter)national conferences

- ARVO USA Fort Lauderdale	2008/05	24 hours
- Third Biennial Military Vision Research Symposium: Traumatic Eye and Brain Injury. TATRC and Schepens Eye Research Institute, Harvard Medical School, Boston, USA	2008/11	10 hours
- Update on Ophthalmology Symposium. Harvard Medical School, Department of Ophthalmology, Boston, USA	2009/02	6 hours
- BEAVRS Amsterdam	2009/11	6 hours
- HEA-TAM Amsterdam	2009/11	6 hours
- Euretina Paris	2010/09	30 hours
- Genetics in Retinal Disease Rotterdam	2010/09	12 hours
- Amsterdam Retina Debate	2010/12	5 hours
- NOG Maastricht	2010/03	18 hours
- 5e Oogheelkundige Fotografie Nederland voorjaarssymposium OZR Rotterdam	2012/02	4 hours
- DOPS Conference Nijmegen	2012/04	10 hours
- Amsterdam Retina Debate	2012/12	5 hours
- ARVO USA Seattle	2013/05	30 hours

## 2. Teaching

	Year	Workload (Hours)
<b>Lecturing</b>		
- Oral presentation Hogeschool Rotterdam	2011/03	6 hours
- Oral presentation Erasmus University, department of Ophthalmology	2011/12	5 hours
- Oral presentation Singapore National Eye Centre, SingHealth	2013/09	6 hours
<b>Supervising Master's theses</b>		
- Lab work and assistance on thesis and manuscript publication of TU Delft student	2009/09-2011/05	30 hours



# List of Publications



## LIST OF PUBLICATIONS

**Van Zeeburg EJ**, Cereda MG, Amarakoon S, van Meurs JC.

Prospective, randomized intervention study comparing retinal pigment epithelium-choroid graft surgery and anti-VEGF therapy in patients with exudative age-related macular degeneration.

*Submitted*

**Van Zeeburg EJ**, Braaf B, Cereda MG, van Meurs JC, de Boer J.F.

Direct Blood Flow Measurements in a Free RPE-Choroid Graft with Phase-Resolved Doppler OCT Confirms SD-OCT Revascularization Steps

*Submitted*

**Van Zeeburg EJT**, Maaijwee K, van Meurs JC.

There is no relation between the occurrence of proliferative vitreoretinopathy and the location of the donor site after transplantation of a free autologous retinal pigment epithelium choroid graft.

*Acta Ophthalmologica*. 2013. In press

**Van Zeeburg EJ**, Cereda MG, van Meurs JC.

Retinal Pigment Epithelium and Choroid Graft.

In: E. Midena, ed. *Microperimetry and Multimodal Retinal Imaging*. Springer-Verlag Berlin Heidelberg 2014

**Van Zeeburg EJ**, van Meurs JC.

Literature review of recombinant tissue plasminogen activator used for recent-onset submacular hemorrhage displacement in age-related macular degeneration.

*Ophthalmologica*. 2013;229:1-14.

Tukker AA, Knulst AJ, Maaijwee KJ, Schutte S, **van Zeeburg EJ**, van Meurs JC, Dankelman, J. Single-sided and small-scaled grasping of delicate tissues: effectiveness of indirect heat-induced attachment and detachment.

*Minim Invasive Ther Allied Technol*. 2012;21:388-395.

**Van Zeeburg EJ**, Cereda MG, van Meurs JC.

Recombinant tissue plasminogen activator, vitrectomy, and gas for recent submacular hemorrhage displacement due to retinal macroaneurysm.

*Graefes Arch Clin Exp Ophthalmol*. 2012;251:733-740.

**Van Zeeburg EJ**, Maaijwee KJ, Missotten TO, Heimann H, van Meurs JC.

A free retinal pigment epithelium-choroid graft in patients with exudative age-related macular degeneration: results up to 7 years.

*Am J Ophthalmol.* 2012;153:120-127.

Braaf B, Vermeer KA, Sicam VA, **van Zeeburg EJ**, van Meurs JC, de Boer JF.

Phase-stabilized optical frequency domain imaging at 1-microm for the measurement of blood flow in the human choroid.

*Opt Express.* 2011;19:20886-20903.

**Van Zeeburg EJ**, Cereda MG, van der Schoot J, Pertile G, van Meurs JC.

Early perfusion of a free RPE-choroid graft in patients with exudative macular degeneration can be imaged with spectral domain-OCT.

*Invest Ophthalmol Vis Sci.* 2011;52:5881-5886.

**Van Zeeburg EJT**, Kolovou PE, Jager MJ, Murray JC, O'Brien JM, Colons D, Bosch JJ, Ostrand-Rosenberg S, Gregory MS, Ksander BR.

Creation of a Retinoblastoma tumor cell vaccine.

*Abstract JIAMSSE, IAMSSE 2009;19 2S:105.*



# Dankwoord





## DANKWOORD

*“Go confidently in the direction of your dreams! Live the life you’ve imagined.” – Thoreau*

Vanaf kleins af aan wist ik al dat ik arts wilde worden. Gelukkig heb ik de hulp en stimulans van zoveel mensen om me heen gekregen dat ik met veel plezier en succes niet alleen dokter, maar nu ook doctor ben geworden! Daarom wil ik graag iedereen bedanken die mij op deze weg heeft geholpen. Een aantal personen wil ik echter graag in het bijzonder bedanken.

Allereerst mijn promotor, Prof.dr. Jan van Meurs. Beste prof, beste Jan. Toen ik voor de laatste keer alle artikelen van dit proefschrift doornam voordat het echt naar de drukker kon, overviel mij een vreemd gevoel van verbazing en trots. Een gevoel dat we eigenlijk echt best wel goed onderzoek hebben gedaan, en uiteindelijk de ontelbaar veel ideeën kort en krachtig in een aantal mooie artikelen hebben opgeschreven. Wat was het in het begin moeilijk om de hoofdlijn uit alle kleine zijlijnen te ontwaren, want wat waren er veel plannen en ideeën. En wat was er veel data. Met name het lange termijn artikel en het PVR artikel behelsden een grote schat aan informatie: statussen, dia’s, operatiefilms, foto’s, ICG’s, FA’s, en oude archieven in de kelder van het ROI. En als alles net doorgenomen was bedachten we dat we eigenlijk nog één factor niet hadden bekeken. En kon het hele circus van lijsten opvragen opnieuw beginnen. Soms was dan even het zicht op bruikbare gegevens voor een artikel oneindig ver weg. Maar de voortdurende vraag naar de status van de dataverwerking van uw kant, de nooit aflatende snelle antwoorden op vragen, alle bezoeken aan het ROI om onduidelijkheden qua handschrift op te lossen of te brainstormen over de inhoud van een artikel, en het ontzettend snel en kundig reageren op een wederom nieuwe versie van een manuscript, maakte in ieder geval dat ik me gelukkig nooit helemaal alleen voor die berg data heb voelen staan. Dank ook voor al uw vertrouwen in mij. Nog maar net begonnen en ik mocht assisteren bij de RPE-choroidea graft operaties, hoe bijzonder! In een continue zoektocht naar verbetering van de techniek van deze operatie van uw kant, brainstormden we over wat er aan de pincet waarmee de graft onder de macula wordt geplaatst, kon worden veranderd. Verbaasd was ik, dat na het maken en wat verbeteren van enkele schetsen van wat ik in mijn hoofd had, u de opdracht gaf aan de instrumentenmaker een prototype van dat pincet te maken, en ik een tijdje later de Elsbeth pincet, en nog wat later de Elsbeth pincet 2 op de instrumententafel mocht ontwaren. Maar ook uw vertrouwen in mij in wat ik dacht te zien en geleerd te hebben over onder andere de SD-OCT en daarop mijn mening over een diagnose of behandelplan (uiteraard na de nodige vragen en onderbouwingen) in uw beslissing mee te nemen, heeft mij ontzettend gestimuleerd om enkel nog meer te willen weten en leren. Ontzettend dank hiervoor!

Beste Netty, Seerp en Willem. Dank jullie wel voor jullie (praktische) hulp en steun de afgelopen vier jaren. Jullie hebben me geleerd dat je niet altijd zelf het wiel hoeft uit te vinden, zeker niet op het ROI. Dank voor het aansturen, helpen zoeken naar oplossingen, relativering, luisterend oor, en de nodige gezelligheid en ontspanning tijdens de koffie of een vrijdagmiddagwiltje.

Johannes, Koen, Boy, het was ontzettend leuk om met jullie samen te werken! Dank voor al jullie tijd en jullie onophoudelijke enthousiasme om iets nog een keer uit te leggen als ik weer eens iets niet (meer) begreep. En de data voor het volgende artikel komt nu écht bijna jouw kant op Koen!

Kristel, dank voor je dataset die je zo perfect geordend aan me hebt doorgegeven. Dank ook voor je handige tips, kordate antwoorden op vragen of edits op een manuscript. Het was best lastig in jouw voetsporen te treden, want wat heb jij een hoop werk verzet en een mooi boekje gemaakt! Ik vond het erg leuk je steeds beter te leren kennen, waardeer de goede gesprekken met je en ik hoop dat we contact houden!

Mirjam, dank voor je hulp en gezelligheid! Of het nu interpreteren van een moeilijk SD-OCT plaatje was, brainstormen over hoe we het verwerken van de data van de rtPA studie aan moeten pakken, of de samenvatting van mijn boekje doornemen op het moment dat de stress echt het hoogst was; jij kwam met rustige en goede ideeën, en leerde me weer een hoop bij. Leuk ook om naast deze gedeelde interesses, na ARVO even samen te kunnen shoppen of heerlijk ontspannen in de zon of tijdens een etentje/drankje. En tijdens een van die gezellig etentjes heb ik zo ook jouw copromotor Dr. F. Verbraak leren kennen. Frank, dank je dat je hebt willen plaatsnemen in mijn kleine commissie, ik waardeer het erg!

Beste Martine, jouw enthousiasme was ongekend. Ik wilde graag een wetenschapsstage in de oogheelkunde doen en jij wist wel wat voor me. Wat schrok ik toen je voorstelde niet een paar maanden naar Afrika te gaan (dat komt later wel zei je, als je écht wat kan doen daar) maar een jaar naar Harvard. Ik weet nog dat ik toen zei dat Engeland me echt niet trok. Ik had voor het gemak Harvard en Oxford even door elkaar gehaald. Gelukkig wist je me snel te corrigeren en me te overtuigen dat Boston een geweldige stad was, en een jaar voorbij zou zijn voordat ik het wist. Je regelde dat ik onderzoek kon gaan doen aan het Schepens Eye Research Institute, naar de ontwikkeling van een tumorcel vaccin voor retinoblastoom; een kinderoogheelkundige tumor. Daarnaast, omdat kinderoogheelkunde me echt heel leuk leek, regelde je dat ik mee kon lopen op de afdeling oogheelkunde in het Childrens' Hospital Boston. Wat ben ik blij dat je me gestimuleerd hebt te gaan en me hebt begeleid, vanuit Nederland via de email

of op bezoek in Boston, zodat alles gesmeerd bleef lopen. Dank ook voor je nimmer aflatende enthousiasme over het onderzoek wat ik toen heb uitgevoerd en dank dat je je nog steeds inzet om de laatste data te vergaren. Ik hoop echt dat we dit jaar alles bij elkaar hebben; ik kan niet wachten om die mooie data eindelijk te gaan publiceren! En wellicht het mooiste, dank voor het overdragen van je enthousiasme voor onderzoek. Zonder deze fantastische ervaring was ik wellicht nooit aan een PhD project begonnen; ontzettend leuk dat jij nu in mijn grote commissie zit!

Dear Dr. Hunter. I do still remember the first time we met in the lobby of Childrens'. I was reading the lonely planet of Boston, waiting for a doctor, the chief of the department of pediatric ophthalmology, to arrive. You were looking for a Dutch girl, without further description. I wasn't expecting someone without a white coat, so even though someone had looked at me several times, I didn't think it was you. Luckily you came to me, seeing my Lonely Planet, and after a good laugh we went for lunch. You immediately offered me your bike, as I was Dutch, you said, and could not possibly do without a bike. You were totally right! I have so much enjoyed your generous gesture, and even biked regularly to Waltham to attend your surgeries over there, following the gorgeous bike path near the Charles river. What amazed me most is the enthusiasm you have for your job. It rubs off on everyone in the department! It was great working with you and the department, I really felt welcome to attend clinics, surgeries, journal clubs, luncheons, lectures, conferences, the Christmas event and even a match of the Boston Red Sox! One thing I best remember is your quote that doing surgery is as being on vacation. You are doing something you like best, and you have no worries about what's going on outside. The idea of time indeed vanished during your surgeries (although sometimes our stomach did remind us of it). Thank you so much for all you taught me and your warm welcome all year long. I really hope I'll get the chance to come back one time!

Bruce and Meredith, thank you so much for the opportunity to work in your lab, providing the many necessary antibodies, western blot cases, and helping me being inventive in so many ways; i.e., running four blots in two blot cases (one borrowed from the neighbours) in one day, with numerous alarms going off; and allowing me to combine the labwork with clinics and/or lectures in Childrens' Hospital. Thank you also for my first ARVO experiences, including my first international oral presentation about the retinoblastoma tumor cell vaccine development. I really hope we'll now soon be able to finish all the great work we've done and get it published!

Beste collega's van het ROI, dank jullie wel voor jullie gezelligheid, hulp en steun. Heerlijk om even een snelle vraag te kunnen stellen, of te ontspannen bij een kopje thee, lunch of borrel. Beste Aline, Aletta, Annemiek, Arni, Caroline, Eva, Gijs, Henk, Jelena, Jetty,

Joziëna, Kari, Kediri, Magda, Marja, Robin, Sietske, Stijn, Verena en Wout, het is waardevol zo'n mooie groep collega's om je heen te hebben want een PhD doe je niet alleen. Daarom alle dank aan jullie!

Dirk en Ellen, m'n eerste kamergenootjes op het ROI. Dank voor het me wegwijs maken binnen een PhD traject, het aanhoren van al mijn enthousiaste en minder enthousiaste verhalen en vragen, het halen van grote hoeveelheden thee, en het delen van elkaars levenslessen. Regelmatig keerde de opmerking terug dat we bijna meer tijd met elkaar doorbrachten dan we soms onze partners thuis zagen. Gelukkig maar dat we het zo goed met elkaar konden vinden, en ondertussen zo hard konden doorwerken! Jullie hebben me zeker geleerd gefocust te werken, en daarna weer tijd te vinden voor ontspanning. Dirk, dank je wel dat ik dan ook juist bij jou en Andrien in Singapore de ontspanning na mijn PhD traject mocht vinden! Wat een gastvrijheid heb ik mogen ervaren; ik heb van jullie gezelschap en van Singapore genoten.

Rene and Susan, my last roommates at ROI. It for sure was not the easiest year of my PhD I've spent with you both, but it probably also was the most funny one! Rene, één van jouw mooie uitspraken was typerend voor stressvolle dagen waarop onze computers het moesten ontgelden: 'Op een gegeven moment zitten hier twee mensen in dezelfde kamer te praten zonder dat er een gesprek plaatsvindt'. And Susan, how often haven't we stared at each other without seeing the other until one of us did and started laughing, because we were both deep in thoughts? Luckily, at other moments we all three did see each other and we could chat and share our successes and sorrows of (for example) our articles. Thanks both for your patience, laughter and support, I greatly appreciate!

Beste collega's van het OZR, alle arts-assistenten, dank voor jullie geduld en begrip toen ik nota bene tijdens de 1e dag van mijn opleiding ineens zelf in het ziekenhuis werd opgenomen en daarna nog een tijdje thuis zat om te herstellen. Rustig aan doen en stil zitten is niet echt mijn ding; dank dat jullie me hebben gestimuleerd in proberen dat vooral wel te doen, zodat ik straks samen met jullie volop kan genieten van de opleiding! Leigh, dank je wel dat je (vaak op het laatste moment) nog snel even naar een manuscript van ons wilde kijken. Het was, naast ontzettend hulpvol, ook erg leuk om zo'n meestal erg enthousiaste reactie erop van je te mogen ontvangen! Josine, Maartje, Myrthe, Sankha en Toine, wat is het leuk om jullie eindelijk te mogen volgen 'naar de overkant', ik zie ernaar uit me weer bij jullie te voegen; wat hebben we een goede tijd gehad samen op het ROI!

Afdeling fotografie, wat heb ik de vloer bij jullie platgelopen. Dank voor alle enthousiasme en flexibiliteit! Wat is het waardevol als je een studie doet waarvoor veel data verzameld

moet worden op één dag voor één patiënt, (soms ook nog per se dezelfde dag nog) dat jullie altijd een gaatje mogelijk wisten te maken, zodat alles meestal op rolletjes liep. Gerard, Cees en Fabienne, dank voor jullie fantastische plaatjes. Gerard, wellicht herken je een gedeelte van de cover? Jouw enthousiasme voor alle mogelijke opties in de fotografie, van stereo tot deze widefield opname, is zeker op mij overgeslagen! Dank ook voor alle compilaties die je vaak zonder te vragen al had gemaakt – ik waardeer je hulp en vakkennis enorm.

Quispel, van origine een gezelschapsspelletjes gezelschap opgericht binnen NSL. Ondertussen is het zoveel meer! Een ontzettend mooie, hechte vriendengroep waar ik heel blij ben deel van te mogen uitmaken. Het speet me ook enorm dat ik het laatste jaar zoveel heb moeten afzeggen omdat het boekje nu echt af moest! Lieve Jan en Esther, Peter en Salomé, Jelle en Renate, Esther en Pieter, Jonathan en Sylvia, Irma, Marieke, Jochem, en alle lieve kleine koters die er ondertussen bijgekomen zijn; dank voor jullie begrip, steun, warmte en gezelligheid in alle mooie en moeilijke tijden de afgelopen jaren. Een zomer zonder BBQispel is geen zomer (net zoals een winter zonder SinterQuispel geen winter is); dank dat ik altijd bij iedereen welkom was een overheerlijk hapje mee te eten zodat we in ieder geval weer even konden bijkletsen voordat de Quispelstrijd begon! En vanaf nu ga ik weer winnen! :D Hoe mooi dat Quispel zelfs buiten landsgrenzen verder gaat; Jelle en Renate, ontzettend bedankt voor jullie onmetelijke gastvrijheid en het regelen van ongeveer alles voor me in Bangladesh, zodat ik werkelijk het allermooiste van jullie land en werkplek heb mogen zien. Dank ook dat ik een poging heb mogen doen een heel klein beetje mee te werken aan het goede werk wat jullie daar doen. Ik heb, ondanks die monsterlijk grote kakkerlakken, ontzettend genoten!

Lieve ooms en tantes, neven en nichten, dank voor jullie interesse en gezelligheid! Heerlijk ook, die eerste neven en nichten dag van de Kamp's kant, wat een kabaal kunnen we met z'n allen maken! Aan enthousiasme en mooie verhalen geen gebrek! Johan en Betty, dank voor het organiseren daarvan, en dank voor de ontelbare telefoontjes de afgelopen jaren, gewoon om even te vragen hoe het ging.

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doorgemaakt, en de tijd gaat altijd te snel als we aan kletsen of shoppen zijn! Dank voor al jullie gezelligheid, bezorgdheid, hulp, spontane etentjes, bezoeken, belletjes en natuurlijk de hulp bij het uitzoeken van mijn promotiejurk! To be continued!

Dear Matteo, already by email we started discussing, exploring ideas and figuring out the best settings for the Microperimeter to work for RPE-choroid graft patients. When you came to the Rotterdam Eye Hospital for a fellowship of a year, we continued what we had started by email and we've spent so many hours on debating, discussing data, working on so many numbers and calculations, and ideas for the next articles. You have taught me so much, and best of all, you did it with so much enthusiasm and new (impossible?) ideas, that we had a lot of fun while learning and working like crazy. Running around on conferences to see the posters we really thought the other should see, or discussing with excitement all our new ideas and inspirations; it was so much fun. But also the time outside of work; dinners with your Italian friends in Milan (really, I've not learned a language so quickly as during that evening), showing me around through the beautiful countryside of Milan, sharing our photography hobby, and the good talks about anything. Thank you for being such an amazing colleague, tutor and friend. I really hope we'll keep up our contact and friendship and will continue working on more new and unimaginable ideas for another great paper! It is just great that you and Paola are coming over for my PhD to celebrate all the hard work we have done, and thank you for being my paranymph! How is the Dutch pronunciation of my 'stellingen' going? ;)

Mariëlle en Ernst, wat vind ik het fijn dat jullie erbij zijn! Mariëlle, jouw eerste dag op het ROI als co-assistent was mijn eerste dag daar als arts-onderzoeker. Een supergezellige tijd hebben we gehad, en nadat je weer terug ging naar het EMC om jouw promotie af te ronden zijn we samen gaan racefietsen, fitnessen, en hebben we zelfs een blauwe maandag een poging gedaan tot zwemmen. Gelukkig beviel gewoon lekker samen eten of theedrinken en bijkletsen ook prima, wat we dan ook de laatste (drukkere) jaren steeds vaker deden; waarop de fietsen het helaas moest ontgelden. We houden de moed erin – komende zomer gaan we weer samen fietsen, echt! Daarnaast ben ik ontzettend blij met je praktische hulp, nuchterheid en je prachtige voorbeeld van een boekje, waardoor ik telkens even van je hoorde waar ik nog op moest letten. Daardoor had ik heerlijk op tijd de locaties al geregeld, is mijn adressenbestand langzaam maar zeker aangevuld en kwam mijn naam gelukkig toch nog onder mijn stellingen te staan. Een betere (en gezelligere) paranymph had ik me niet kunnen wensen! Dank hiervoor! Leuk dat we elkaar op het OZR nu weer meer gaan zien, maar ik zie ook uit naar de daarnaast vast nog wel komende heerlijke thee- en fietsmomenten en etentjes! En vooruit, de mannen mogen er soms ook wel bij ;) Ernst, dank je voor je gezelligheid, adviezen en heerlijke wijnen; proost op twee boekjes!

Dear Martin and Meg, thanks for your support, the good times when you were here, and your interest in and tips for the printing of this book. I really appreciate you are both here today, and we are looking forward to visit you in Massachusetts in the near future!

Kim en Sander, dank voor jullie gezelligheid en interesse in het wel en wee van m'n promotie. Volgens mij was ik vaak vrij enthousiast aan het vertellen en was het soms lastig om te volgen wat ik weer aan het schrijven was. Hopelijk maakt dit boekje alles een stuk duidelijker! Wat was het overigens heerlijk om juist tijdens de laatste loodjes even lekker te ontspannen met Sophia of gezellig en knus op jullie bed een glaasje champagne ter ere van de geboorte van Olivia te drinken! Op naar meer van die heerlijke gezellige momenten!

Lieve Em, dank voor je medeleven, steun en enthousiasme tijdens mijn promotie. Ik heb genoten van je lieve bezoeken, je lekkere (in drukke tijden voor ons meegebrachte) maaltijden, of de lol om Dokter Tinus. Wat was het ook heerlijk even te kunnen ontspannen met mooi weer bij je aan het Waaltje – dat hoop ik nog vaak te mogen komen herhalen!

Rogier, broer, dank je voor je altijd direct klaarstaande computerhulp, of het nu m'n hele computer opnieuw installeren was of me helpen als ik weer eens 'iets doms' had gedaan, plaatjes of praatjes converteren, je mini laptopje even langsbrengen zodat ik die nog mee kon nemen op congres; je hebt me regelmatig uit de brand geholpen! Kim, wat een gezelligheid breng jij met je mee! Leuk dat je erbij bent gekomen; grappig hoe we onze sinterklaas en blond passie al zo lekker delen!

Lieve Hester en Arjen. Zus, wat lijken we op elkaar ondanks dat we zo hard beweren van niet. Dank voor je altijd directe begrip als ik het weer eens even had 'gehad' met bijvoorbeeld een artikel. En heerlijk hoe we op allerhoogste snelheid door elkaar heen kunnen praten van enthousiasme, in een voor onze mannen soms onbegrijpelijke taal en vaart, en elkaar dan nog prima verstaan! Ik heb genoten van de, helaas te zeldzame, dagjes winkelen, museums, wandelingen en etentjes met jullie; waar tussen neus en lippen door weer even de belangrijkste zaken werden besproken. Nu allebei de boekjes af zijn zouden we dit toch veel vaker moeten kunnen doen? Ik hoop het, want ik kan niet wachten om de allerleukste tante van de hele wereld te worden!

Lieve pa, dank voor je oneindige vertrouwen in mijn weten en kunnen, en het altijd stimuleren en leren door te zetten – van de eerste schaatslessen op houtjes of tussen je knieën in de skilift omdat ik er nog te klein voor was, tot later mijn studie, deze promotie, de heerlijke grotere schaatstochten, mijn reizen naar Brazilië en Bangladesh of het jaar in

Boston; jij was soms bijna nog enthousiaster en trotser dan ikzelf! De nimmer aflatende steun en warmte van jou en mama is onbeschrijfelijk.

Lieve mam, wat hadden we je graag nog bij ons gehad vandaag. En wat zou jij hier graag geweest zijn. Zo trots als je op me zijn kon, zo stimulerend, je gezelligheid, je bijna verstikkende knuffels en je lach; de liefste moeder ooit. Wat mis ik je! Maar eigenlijk heb je nu wel de mooiste plek om alles te kunnen zien vandaag, daar, boven. Hopelijk geniet je ervan, samen met oma en oma!

Albert, liefste, wat ben ik blij met jou! Jouw eeuwige optimisme en relativiseringsvermogen zijn echt mooie eigenschappen die soms goed van pas kwamen tijdens mijn promotie! Samen met jou wil ik nu nog meer van de grote én kleine dingen gaan genieten; as for one day you realize they were the big things! Love you!

## About the Author





## ABOUT THE AUTHOR

Elsbeth van Zeeburg was born on August 20, 1983 in Doornspijk, the Netherlands. After graduating from secondary school at the Lambert Franckens College in Elburg in 2001, she obtained her propedeutics in Biology at Utrecht University 2002. Subsequently she started medical school at Leiden University. In 2005, during medical school, she completed a subspecialty rotation in Dermatology in Marilia, Brazil. In 2008 she worked on a student research project, the development of a retinoblastoma tumor cell vaccine, at Schepens Eye Research Institute, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, USA. During that year she additionally completed an observership at Boston Children's Hospital of Harvard Medical School. After obtaining her medical degree, she started the PhD project as described in this thesis in September 2009 under the supervision of Prof. dr. Jan C. van Meurs, at The Rotterdam Eye Hospital. In September 2013 she travelled to Bangladesh to experience (the medical health care in) a developing country. In October 2013 she started her residency training in Ophthalmology (head: Prof. dr. Jan C. van Meurs) at The Rotterdam Eye Hospital.