Surgical Treatment of Acute Submacular Hemorrhages and Advanced Exudative Age-related Macular Degeneration

Elsbeth J.T. van Zeeburg

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Surgical Treatment of Acute Submacular Hemorrhages and Advanced Exudative Age-related Macular Degeneration

Chirurgische behandeling van acute submaculaire bloedingen en van vergevorderde exsudatieve leeftijdsgebonden macula degeneratie

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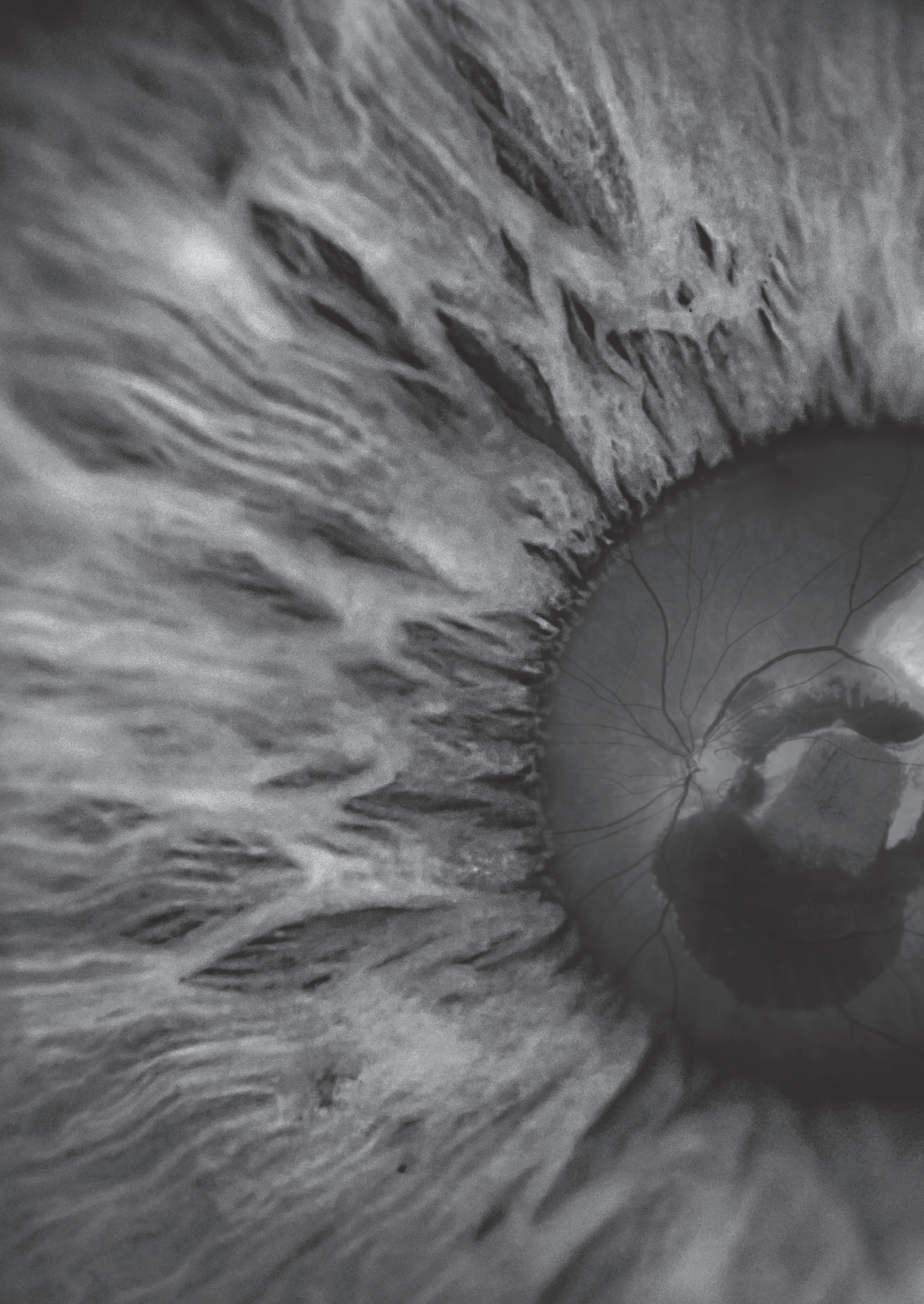

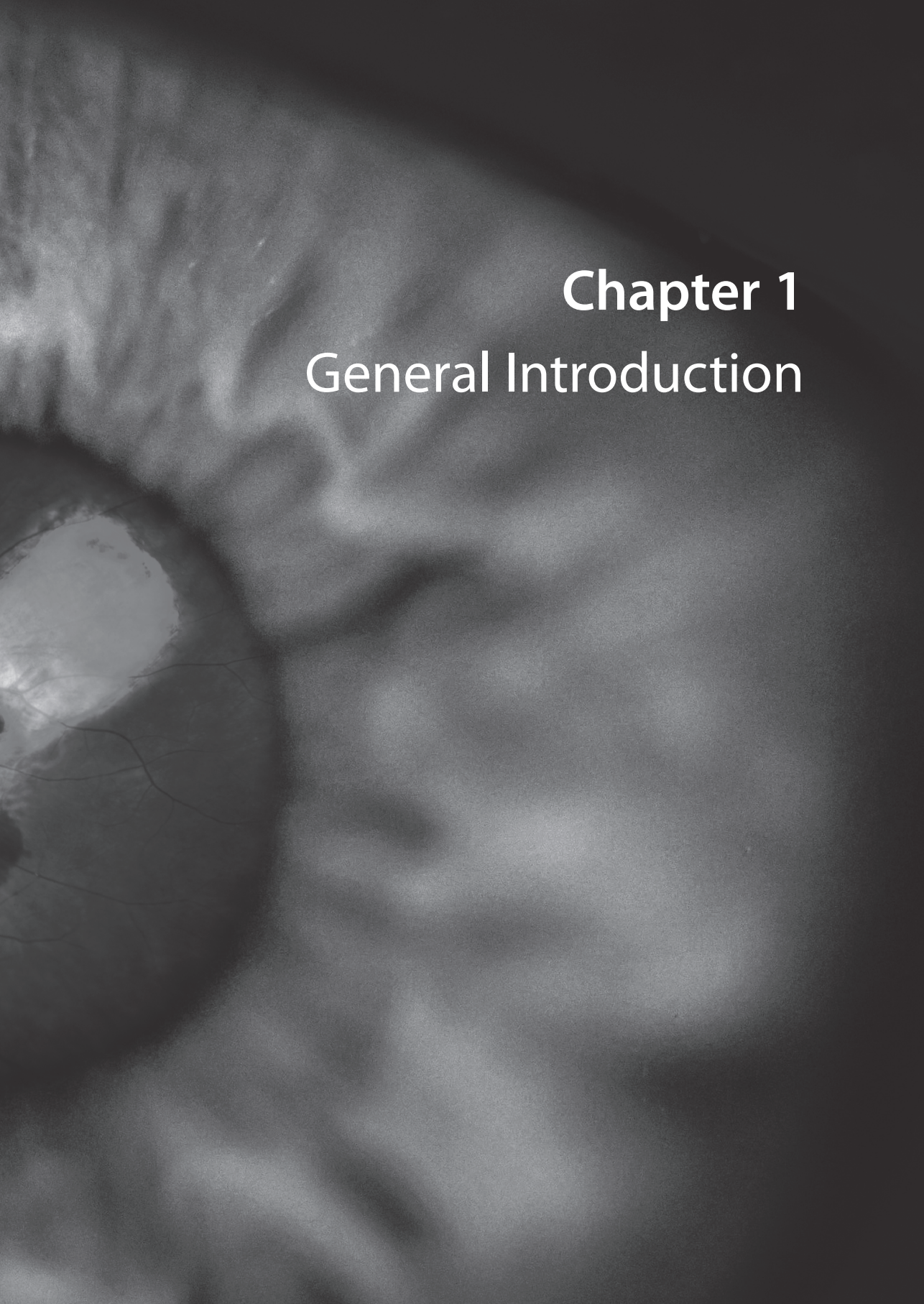

Chapter 1
General Introduction

1.1 The Anatomy of the Eye

Located behind the cornea, iris and lens, the vitreous is a clear gel in the center of the eye (figure 1). The inner surface of the eye is lined with retina, a multi-layered sensory tissue. In the retina the photoreceptors, rods and cones, are capable of phototransduction; light is converted into signals that will stimulate neuronal impulse transmission.[1] The nerve impulses then travel through axons of the retinal ganglion cells, via the optic nerve to the visual cortex of the brain where these signals are processed. Under the retina are the retinal pigment epithelium (RPE) and Bruch's membrane. The RPE is a monolayer of highly pigmented hexagonal cells which forms the outer blood-retinal barrier of the eye. The RPE is highly specialized in nutrient and waste transport, and in the synthesis and secretion of the proteins needed for retinal function.[2] The retina is also protected from light damage by the RPE, as the RPE melanosomes absorb excess incoming light.[1] The basement membrane of the RPE is the innermost layer of the five layers of Bruch's membrane, and the basement membrane of the choriocapillaris forms the bottom layer of Bruch's membrane. Bruch's membrane further consists of fine collagen and elastic fiber layers through which nutrients pass from the choriocapillaris to the RPE, and through which cellular waste products pass from the RPE to the choriocapillaris.[3] The choroid, which contains the choriocapillaris, supplies oxygen to the outer layers of the retina, whereas the retinal vessels supply the inner retina. The outermost layer of the eye is the sclera, which gives support and protects the eye, and to which the eye musculature is attached.

The macula, with a diameter of approximately 5.5 mm, centered at the fovea, contains a higher concentration of photoreceptor cells than the more peripheral part of the retina. The fovea, the center of the macula, approximately 1.5 mm in diameter, has a high density of only cones and its center is devoid of retinal vessels.[4] In the central macula every cone is connected to a single axon, whereas in the peripheral retina several cones or rods are connected to one axon. The macula is used for detailed visual function, such as color vision, reading and face recognition.[5] The anatomy of the eye is visualized in figures 1 and 2.

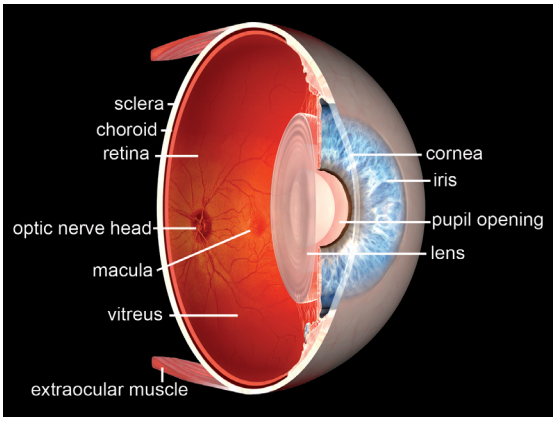

Figure 1: Anatomy of the eye.

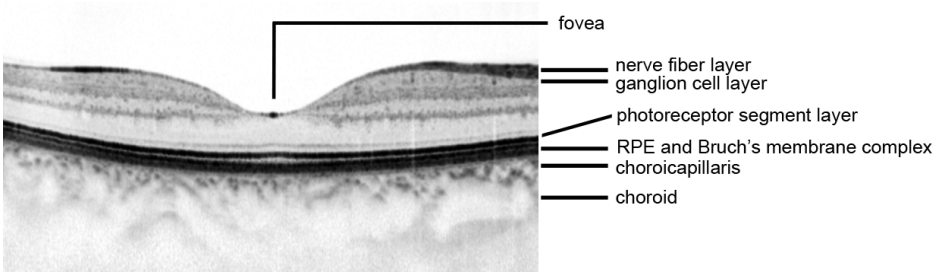

Figure 2: Spectral domain optical coherence tomography scan; cross-section of the posterior segment of the eye.

1.2 Age-related Macular Degeneration

Age-related macular degeneration (AMD) is a progressive disease of the macula. It is the leading cause of visual impairment and irreversible legal blindness in elderly people in industrialized countries and is the third-leading cause of blindness globally.[6-8] Due to the rapidly aging population, prevalence data suggest that the number of persons with AMD will increase by 50%, to three million, in the United States alone by 2020.[9]

There are two distinct types of AMD: dry (atrophic) and wet (exudative) AMD. The dry type is characterized by drusen, small yellow deposits at the level of the basal lamina of the RPE and Bruch's membrane, composed of various lipids and proteins. In advanced stages progressive RPE atrophy may eventually lead to distortion of vision and blind spots in the center of vision. This dry type progresses slowly, but other than antioxidants and zinc to slow the progression there is no therapy available yet.[1;10] Wet AMD however, progresses more rapidly. This may lead to a rapid decrease in visual acuity (VA), due to abnormal, newly formed small choroidal blood vessels, causing fluid leakage or hemorrhages in the retinal layers of the macula, between the retina and the RPE, or between the RPE and the choroid.

The pathology of AMD is multifactorial and not completely understood. Age, gender, race, diet, cigarette smoking and cardiovascular risk factors all seem to modulate the risk of AMD. However, study results were not consistent for all risk factors.[11] Genetic factors may also play a role, around 23% of the AMD cases may be attributed to a genetic component.[12] Most important genetic mutations thus far are CFH and ARMS2.[13]

An association has been found between AMD and alternative complement pathway dysregulation. As with other age-related diseases as atherosclerosis, a major inflammatory component might therefore be involved in the pathogenesis of AMD.[11;14] Also, genes involved in extracellular matrix regulation and mitochondrial oxidative stress play a role in AMD pathogenesis.[15] AMD therefore may be attributable to systemic changes, as well as to local, ocular ones.

Not all of the heritability of AMD has yet been explained. The current identified loci which contribute to genetic susceptibility account for approximately 55% of the heritability of advanced AMD.[16]

The histological changes in AMD are due to a combined malfunction of retinal cells, RPE, Bruch's membrane and choroid. Bruch's membrane generally becomes thicker with increasing age, leading to changes in elasticity and hydraulic permeability, resulting in reduced transport between RPE and choroid. Also, drusen accumulate in and on Bruch's membrane with increasing age.[1;3] The Age-Related Eye Disease Study (AREDS) showed that patients with large drusen have higher risk at developing exudative AMD.[10] The inflammatory processes involved in AMD include the generation of pro-inflammatory molecules in Bruch's membrane, recruitment of macrophages and dendritic cells, and complement activation. Due to the pro-inflammatory toxic milieu, the choriocapillaris becomes dysfunctional or atrophic, and the adjacent RPE cells become hypoxic.[5] Age-related changes of the RPE are changes in the pigmentation, an increase in number of lipofuscin granules due to decreased metabolic and phagocytotic activity and a reduction of melanosomes and cell density. This reduction in cell density may is result of apoptosis, caused by accumulation of toxic substances due to a chronic exposure to oxidative stress.[1] RPE cells secrete growth factors and proteins which support photoreceptor metabolism, the visual cycle, and they help to maintain retinal blood supply. One of the most important proteins is pro-angiogenic vascular endothelial growth factor (VEGF), secreted from the basal surface of the RPE.[2] Dysfunction of the majority of these proteins has direct links to AMD pathogenesis. Increasing age furthermore leads to choroidal thinning,[17] resulting in ischemic RPE. Subsequent hypoxia-induced upregulation of VEGF then stimulates growth of choroidal neovascular membranes in exudative AMD, which grow through Bruch's membrane and spread under the RPE.[1] The damaged Bruch's membrane and RPE complex causes degeneration of RPE and loss of photoreceptor cells,[18] resulting in VA loss.

1.3 Non-Surgical Treatment for Exudative Age-related Macular Degeneration

The current standard treatment for patients with exudative AMD includes intravitreal injections with anti-VEGF agents[19], photodynamic therapy (PDT)[20] or a combination of both. By counteracting the VEGF production, the growth of new vessels and thereby the leakage of fluid from these newly formed vessels may be reduced or stopped. Unfortunately, although many patients benefit from anti-VEGF treatment, some patients do not respond to this therapy.[21;22] Some patients develop a tear of the retinal pigment epithelium[23;24] and yet others develop a large hemorrhage under the macula. For these patients, (continuation of) anti-VEGF treatment will not result in the restoration, maintenance or improvement of visual acuity, and therefore surgical treatment might be an option.

1.4 Surgical Treatment for Age-related Macular degeneration

A relatively common but severe complication of age-related macular degeneration is a submacular hemorrhage, particularly in patients taking anticoagulant medications.[25] In order to limit visual acuity loss due to irreversible damage to the RPE and retina, these acute submacular hemorrhages should be surgically removed, or displaced, as soon as possible, preferably within seven or maximally 14 days.[26-29] The use of recombinant tissue plasminogen activator (rtPA) may liquefy a recent hemorrhage via lysis of the fibrin.[30] This rtPA, together with an intravitreal gas tamponade and gravity may help to displace the hemorrhage from the submacular region.[31]

In patients who do not respond to anti-VEGF therapy, who have developed an RPE tear, who have an old submacular hemorrhage, or who have developed submacular fibrosis,[32] the RPE is (most likely) damaged. However, as described earlier, an active interaction with RPE is necessary for retinal function. Therefore, retinal rotation surgery might be beneficial as the macula is relocated to a part of the RPE which is less damaged.[33] Another method is to transplant RPE tissue, but as only replacing the very thin layer of RPE is extremely difficult, a so called autologous 'graft' of RPE, Bruch's membrane, choriocapillaris and choroid can be transplanted; RPE-choroid graft transplantation surgery.[34;35]

While it is likely that blood-clot displacement surgery should be performed as soon as possible for acute submacular hemorrhages, the indication for RPE-choroid graft surgery is more controversial, as severe complications may occur. When the retina has become severely damaged due to the disease process, surgery may no longer be helpful, as only a relatively intact retina may benefit from an RPE-choroid graft and maintain or improve VA. With a new imaging technique, spectral domain optical coherence tomography (SD-OCT), the preservation of retinal layers can now be visualized and may therefore help in the decision whether and when this surgery should be initiated.

1.5 New Imaging Techniques in Ophthalmology

About a decade ago, retinal imaging techniques in Ophthalmology clinics consisted mainly of fundoscopy, fluorescein angiography (FA) and indocyanine angiography (ICGA). These all yield images in the coronal or *en face* plane. Therefore, the multi-layered retina could only be clearly analyzed by histopathology. With the development of OCT, in vivo ophthalmologic imaging of the retina became available for clinics. OCT is an optical interferometric imaging technique, analogous to ultrasound, measuring the echo time delay of backscattered infrared light using an interferometer and a light source.[36] The OCT technique was revolutionary, as it is a noncontact and noninvasive modality that produces cross-sectional, morphological images of ocular structures. The cross-sectional scans have become highly accurate over the last years due to increased acquisition speed and better eye-tracking mechanisms. Further, 'follow-up' modes

allow the (almost) exact same areas to be scanned at different points in time, so that morphological changes due to progression of ocular diseases may clearly be visualized.

Spectral domain (SD)-OCT is one of the newer OCT devices, with a high resolution (2-15 µm) and the ability to create 3-dimensional area scans, by combining multiple cross-sectional single B-scans. Because of the high resolution, the fovea, the different retinal layers, RPE, Bruch's membrane, choriocapillaris and choroid can be clearly visualized and analyzed (Figure 2).

An even newer OCT technique, optical frequency domain imaging (OFDI), has been developed more recently. With this technique scans can be made over a greater depth compared to SD-OCT, with minimal image quality loss,[37] which is an advantage when scanning the choriocapillaris and choroid.[38;39] The SD-OCT (Spectralis HRA, Heidelberg Engineering, Heidelberg, Germany) uses 850 nm wave lengths, while the recently developed OFDI system uses a swept-source laser in the 1050 nm wavelength range.[40]

Phase-resolved Doppler (PRD)-OCT is an extension of the standard OCT technique, whereby blood flow is detected from phase changes in successive OCT measurements. These phase changes originate from the Doppler effect caused by light reflection from moving particles in the blood flow such as erythrocytes or leukocytes.[41-43] PRD-OCT can therefore be used to identify locations with blood flow in tissues, making it useful in the evaluation of graft revascularization.

1.6 The Outline of This Thesis

The main objective of this thesis was to evaluate the results of RPE-choroid graft transplantation surgery, and to compare this surgery with standard treatment for AMD: anti-VEGF treatment. The second objective was to study graft behavior with new imaging techniques. The third objective was to explore whether the administration of recombinant tissue plasminogen activator for acute submacular hemorrhages, due to AMD or a retinal macroaneurysm, is feasible and safe.

In **chapter 2.1** the long-term results up to seven years of a free RPE-choroid graft are discussed. Additionally, visual acuity outcomes of the graft surgery were compared with data from a study in which only the CNV membrane or CNV membrane with submacular hemorrhage was surgically removed. In **chapter 2.2** the relationship between the location of the donor site after RPE-choroid graft transplantation, and the occurrence of proliferative vitreoretinopathy (PVR), one of the major complications of the RPE-choroid graft surgery, was assessed. Specifically, would an inferiorly located donorsite, in direct contact with the pro-fibrotic and inflammatory milieu, increase the risk of PVR?

Chapter 3 focuses on microperimetry, which is used to assess function of an RPE-choroid graft. Microperimetry is able to test the visual function of a very specific area of the retina, for instance over an RPE-choroid graft. The functional results of the microperimetry may be correlated to the morphological data of the SD-OCT and other

imaging modalities. This chapter details the results obtained from three patients during a long-term follow-up of an RPE-choroid graft.

Chapter 4 discusses the results of a prematurely ended study which was originally set up as a prospective, international multicenter, randomized intervention study. In this study the RPE-choroid graft surgery was compared with (continuation of) anti-VEGF therapy for patients with an RPE tear, submacular hemorrhage or patients who did not respond to anti-VEGF treatment.

In **chapter 5.1** the revascularization pattern of an RPE-choroid graft was analyzed by comparing SD-OCT scans with two other, more invasive, imaging techniques: fluorescein and indocyanine angiography. Subsequently, in **chapter 5.2**, patients with an RPE-choroid graft were scanned with both the SD-OCT and the PRD-OCT shortly after surgery. The established revascularization steps of the SD-OCT and actual blood flow measurements of the graft measured by PRD-OCT were correlated with one another.

In preparation for a randomized controlled trial to compare the most effective and safe administration technique of rtPA and gas for the displacement of acute submacular hemorrhage in AMD, we conducted a literature review (**chapter 6.1**), in which also the results of patients of the Rotterdam Eye Hospital were included. The displacement and visual acuity data of patients with a submacular hemorrhage due to a retinal macroaneurysm, treated with rtPA, were analyzed in **chapter 6.2**. These patients have hemorrhages similar to those seen in AMD, in the absence of preexisting macular pathology. They are therefore of interest in order to demonstrate the potential of displacement of an acute submacular hemorrhage.

Finally, in **chapter 7**, which is the general discussion, the results, limitations and complications of the RPE-choroid graft translocation surgery will be discussed. Also, the future of RPE graft transplantation surgery and possible new patient selection criteria for this surgery will be outlined, as well as potential future developments in cell-based therapies. That rtPA is helpful for patients with a recent-onset submacular hemorrhage is a relatively new, apparently effective but not sufficiently proven concept. The potential of rtPA based therapy, and the help of OFDI-OCT and PRD-OCT in the treatment decision and follow-up of patients with a submacular hemorrhage, is discussed.

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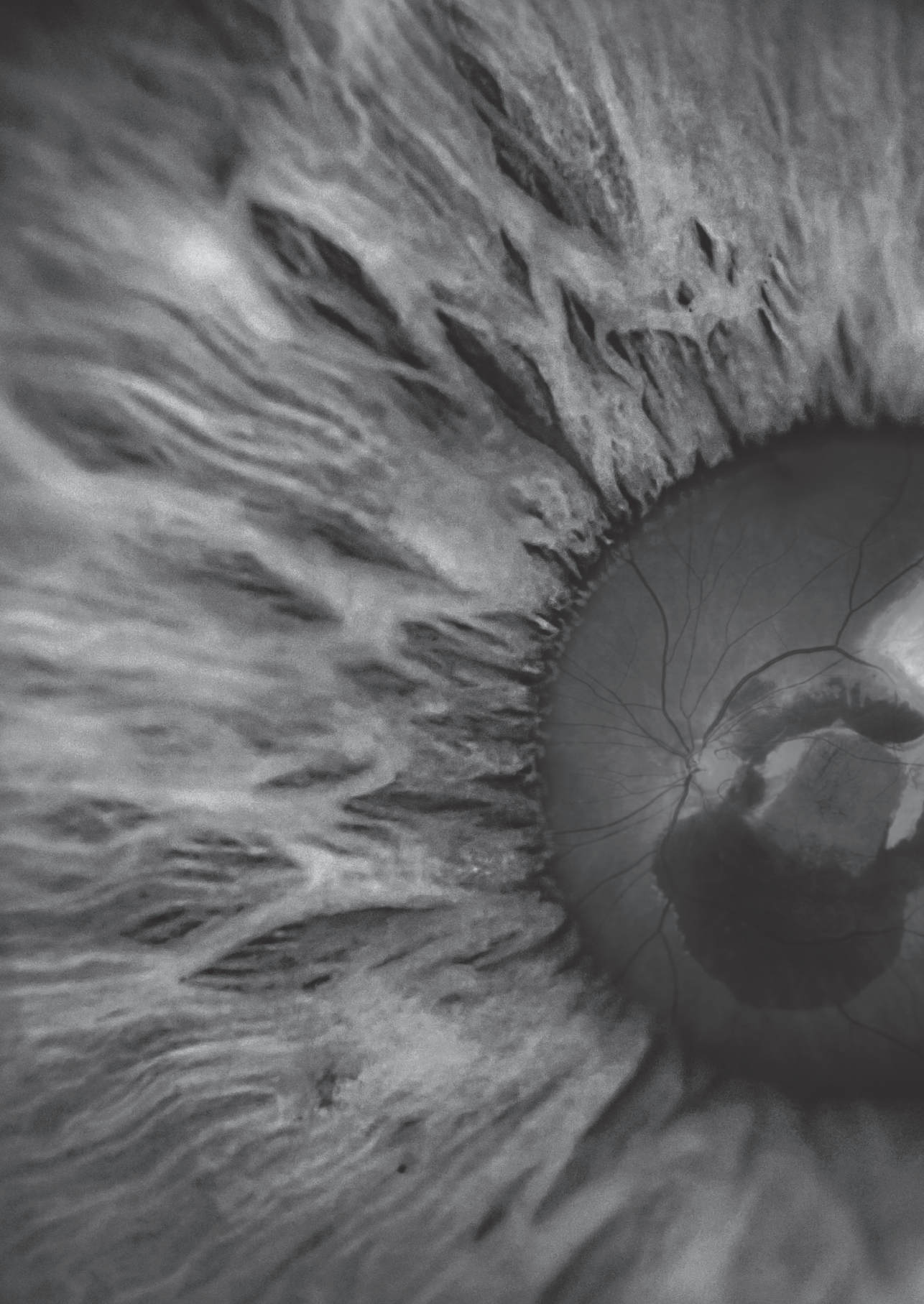

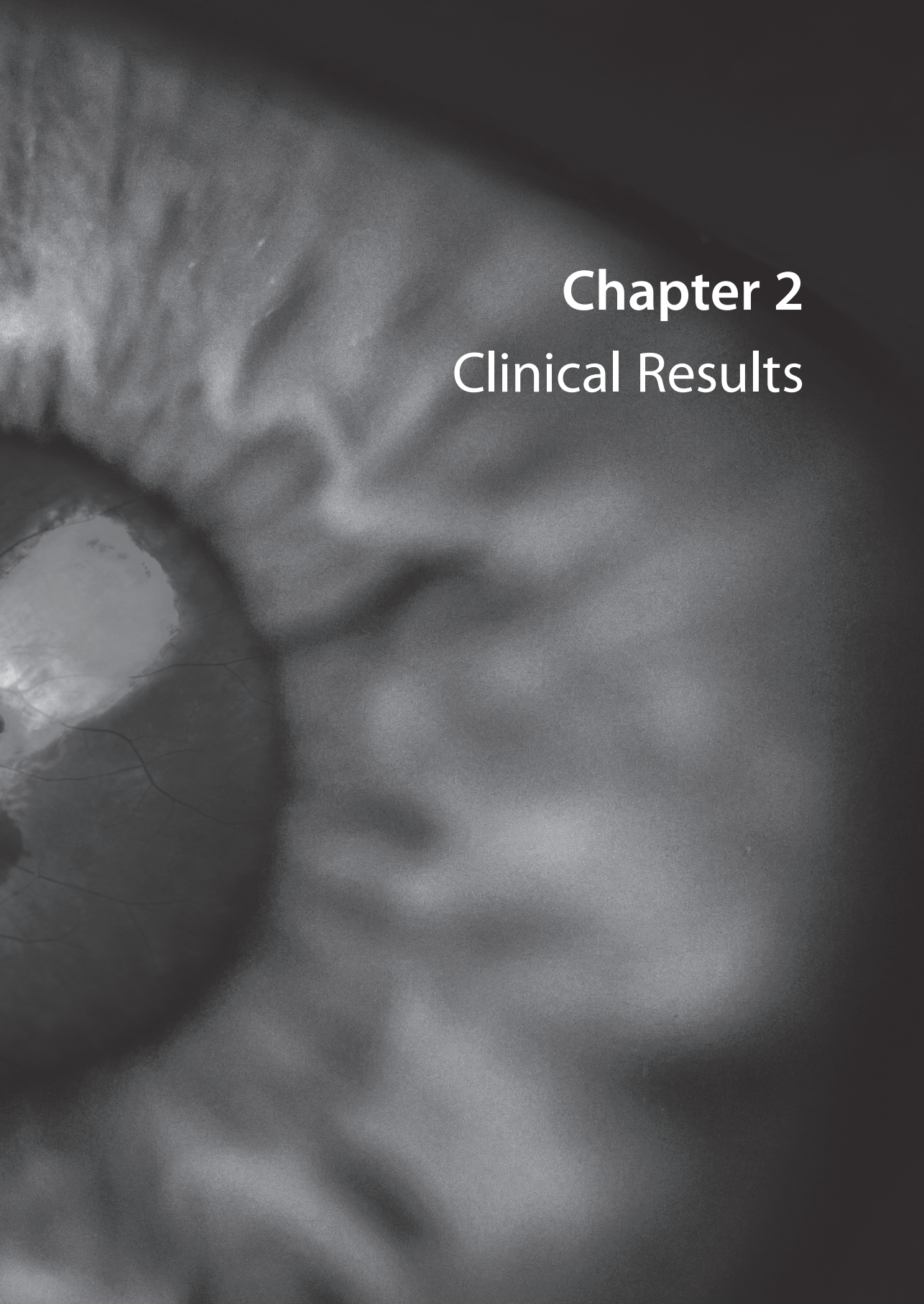

Chapter 2
Clinical Results

Chapter 2.1

A Free Retinal Pigment Epithelium-Choroid Graft in Patients With Exudative Age-Related Macular Degeneration; Results up to 7 Years

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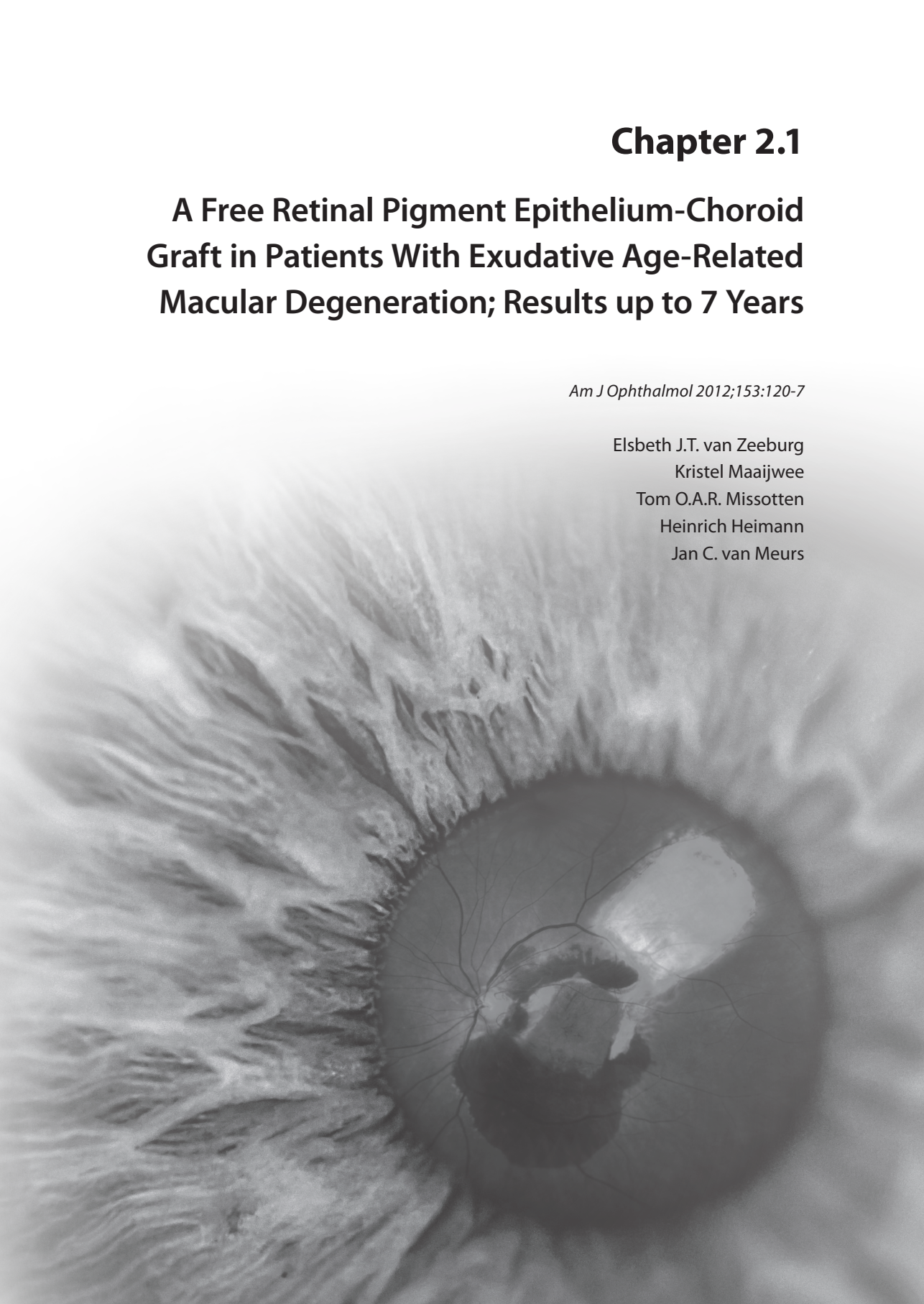

ABSTRACT

Purpose

To report and analyze long-term best-corrected visual acuity (BCVA) outcomes following a free autologous retinal pigment epithelium (RPE)-choroid graft translocation in patients with exudative age-related macular degeneration (AMD).

Design

Prospective cohort study.

Methods

Setting: Institutional. Study population: One hundred and thirty consecutive patients (133 eyes) with AMD underwent RPE-choroid graft translocation between October, 2001 and February, 2006. All patients had a subfoveal choroidal neovascular membrane with or without hemorrhage and/or an RPE tear. All were either ineligible for, or non responsive to, photodynamic therapy, the standard treatment at the time of surgery. Observation procedures: Data collection included pre- and postoperative visual acuity measurements, fundus photography, fluorescein and indocyanine green angiography, and microperimetry. Main Outcome Measures: Postoperative BCVA.

Results

The mean preoperative BCVA was 20/250. Four years after surgery, 15% of the eyes had a BCVA of >20/200, and 5% had a BCVA of ≥20/40. One patient achieved a BCVA of 20/32, which was maintained at seven years after surgery. Complications consisted of proliferative vitreoretinopathy (n = 13), recurrent neovascularisation (n = 13) and hypotony (n = 2).

Conclusions

RPE-choroid graft transplantation may maintain macular function for up to 7 years after surgery, with relatively low complication and recurrence rates. Retinal sensitivity, BCVA data, and fixation on the graft suggest that the graft, rather than simply the removal of submacular hemorrhage and/or choroidal neovascular membrane, was responsible for the preservation of macular function. This surgery may be an alternative for patients with AMD who cannot undergo other standard treatment.

INTRODUCTION

Age-related macular degeneration (AMD) is the third-leading cause of blindness globally and the leading cause of irreversible legal blindness in elderly people in industrialized countries.[1, 2] The current standard treatments for patients with exudative AMD are anti-vascular endothelial growth factor (anti-VEGF) injections, photodynamic therapy (PDT),[3, 4] or a combination of both modalities. However, anti-VEGF treatment might not be effective in restoring or improving visual acuity (VA) in patients with a retinal pigment epithelium (RPE) tear,[5, 6] or a large submacular hemorrhage and in a small subset of patients with exudative AMD who fail to respond to anti-VEGF treatment.[7] As potential alternative treatments for these patients, several surgical treatment modalities have been described, four of which are discussed below.

The first method, removal of the submacular choroidal neovascular membrane and/or hemorrhage, was studied in the Submacular Surgery Trials (SST). In the subgroup of patients in whom the hemorrhage was surgically removed, the percentage of patients who lost more than 6 lines of VA was statistically smaller compared to untreated controls.[8] A second method is the displacement of an acute hemorrhage by gas and recombinant tissue plasminogen activator, with or without vitrectomy, and with subsequent treatment with PDT or anti-VEGF. Several case series suggest a benefit over natural course.[9-12] The third method, macular translocation after 360° retinectomy, was first reported by Machemer *et al*. in 1993.[13] This method is associated with several vitreoretinal complications such as a macular pucker, retinal detachment (RD), proliferative vitreoretinopathy (PVR), recurrence of choroidal neovascularization (CNV), choroidal hemorrhage, a tilted image, and diplopia.[14-17] Despite an appreciable complication rate, stabilization or improvement of VA of this surgical technique has been demonstrated, with long-term (14-79 months) beneficial results in some patients with exudative AMD.[18, 19] Further, this surgical technique demonstrated superior 2-year VA results than PDT in a randomized controlled trial.[20,21] The fourth method is the transplantation of an autologous graft of RPE, Bruch's membrane, choriocapillaris and choroid. This method was first described by Peyman and associates in 1991.[22] In early studies, the graft was harvested from the edge of the macular RPE defect;[23, 24] later studies used tissue from the mid-peripheral retina.[25, 26] The most frequent complications of transplantation are recurrent CNV, RD, PVR, non-revascularization of the graft, macular pucker, and postoperative hemorrhages.

RPE and choroid graft surgery has been compared with macular translocation surgery.[27] Both techniques seem to lead to better VA outcomes than no intervention.[21, 26] However, although macular translocation surgery might produce better VA outcomes than RPE and choroid graft surgery, it has a greater risk of complications.[18, 26] Moreover, there is a physical limit to the amount of translocation possible (even after 360° retinectomy),

which makes it less suitable for large CNVs. Several centers have reported case series of free RPE and choroid grafts, albeit with relatively short follow-up periods.[25, 26, 28-30] MacLaren and associates have presented patients with up to six years of follow-up, although this applied to only four of the nine patients initially included.[31]

The current study analyzes the primary outcome, defined as best-corrected visual acuity (BCVA), of a large group of 130 consecutive patients (133 eyes) who underwent a free RPE and choroid graft procedure. The follow-up period of these patients varies between one and seven years. In addition, for some of these patients, BCVA results were correlated with macular sensitivity as measured by microperimetry.

PATIENTS & METHODS

At the Rotterdam Eye Hospital, RPE-choroid graft surgery was first performed in October 2001. As of February 2, 2010, 130 consecutive patients (133 eyes) had been included in this institutional prospective cohort study and had undergone this surgery before February 2, 2006. This resulted in registration of follow-up data of at least four years. The main outcome measure of this institutional prospective cohort study was postoperative BCVA up to seven years after surgery. The inclusion criteria for RPE-choroid graft surgery were exudative AMD patients with a VA <20/80 (>0.6 logarithm of minimal angle of resolution [logMAR]) and a subfoveal choroidal neovascular membrane. The patients were not eligible for PDT, as they had a minimally classic lesion larger than four disc diameters and or one of the following findings: a large hemorrhage, an RPE tear, or a 3-line VA loss after PDT. Exclusion criteria were underlying diseases other than AMD or anticoagulant drugs that could not be discontinued. All patients were operated before anti-VEGF therapy was routinely available at the Rotterdam Eye Hospital in February 2006.

Surgery

After the induction of a posterior vitreous detachment, a complete vitrectomy was performed. The macular retina was separated from the RPE and/or blood and/or CNV membrane by injecting a balanced salt solution into the subretinal space through a 28-gauge subretinal cannula. A paramacular temporal retinotomy was made, through which the CNV membrane and/or subretinal hemorrhage was removed from the subretinal space with Thomas subretinal forceps. After circular heavy diathermy in the midperiphery at the 6 or 12 o'clock position, vitreous scissors were used to cut a full-thickness graft of retina, RPE, Bruch membrane, and choroid of approximately 2-3 x 2-3 mm. Initially, the graft was loaded onto an aspiration-reflux spatula (Dutch Ophthalmic Research Center [DORC], Zuidland, The Netherlands), as described by Maaijwee and associates.[32] Later on, the graft was grasped from the choroidal side by fine forceps.

The retina was removed from the graft and was repositioned underneath the macula through the existing paramacular retinotomy. Perfluorocarbon liquid was injected over the macula to hold the graft in position during retraction of the instrument. A vibration device attached to the forceps facilitated the release of the graft.[32] The midperipheral donor site was then encircled by laser photocoagulation, followed by intraocular tamponade with silicone oil.

In a second procedure, approximately three months later, the silicone oil was removed and the inner limiting membrane was peeled. This peeling was performed to prevent formation of a macular pucker. In phakic patients, lensectomy and intraocular lens (IOL) insertion was performed during either the first or second surgery. All surgical procedures were performed by one surgeon (J.v.M.).

Grading of Preoperative Images

Masked readers (H.H. and T.M.) independently assessed the preoperative color fundus photographs, fluorescein angiograms (FA), indocyanine green angiograms (ICGA) and red-free images of all 133 eyes. The images were imported into image analysis software (ImageJ; http://rsweb.nih.gov/ij/, last access April 21, 2010; National Institute of Health, Bethesda, Maryland, U.S.A.) The fundus, FA and ICGA images were used to determine disc area, lesion size (all lesion components taken together, measured in relation to disc area), composition, and size of hemorrhage (in disc area and % of total lesion size). If FA was not available, ICGA images were used in combination with red-free and/or color images for classification. The patients were then classified into three groups by lesion composition (predominantly classic, minimally classic or occult), according to the Macular Photocoagulation Study (MPS) protocol.[33] A fourth group, labeled "hemorrhagic lesion," included all lesions masked for >50% by extensive hemorrhage.

Ophthalmic Examination and Follow-up

Preoperative examination included BCVA in Snellen and logMAR equivalents, dilated fundoscopy, FA and/or ICGA. Postoperative visits were scheduled at 1, 3 and 6 weeks, at 3, 6, 9, 12, 18 months, and subsequently every year. During each visit, BCVA testing was performed. Visual acuity data was converted to logMAR for calculations and statistical analysis. The visual acuity data were reported as the percentage of patients with a BCVA of either >20/200 or ≥20/40.

Microperimetry

After surgery, two subgroups of patients were tested with the microperimeter (MP-1; Nidek Technologies, Padova, Italy) with an automatic eye tracker compensating for eye movements. Due to the inconsistent availability of the MP-1, not every patient could be tested. The first group consisted of nineteen patients who were tested shortly after

surgery.[34] The second subgroup consisted of ten patients who were tested at least four years after surgery. MP-1 software version 1.7.3 was used during the second period. Pupils were dilated with Tropicamide 0.5%, (Chauvin Pharmaceuticals Ltd, Kingston-Upon-Thames, United Kingdom) and phenylephrine 2.5% (Chauvin Pharmaceuticals Ltd). The stimulus size was Goldmann III with white stimuli of 200 ms projection time. A customized Cartesian pattern centered on the graft, with a staircase 4-2 threshold strategy, was tested automatically. The brightness of the test stimuli ranged from 0 to 20 decibels (400 to 4 asb). After performing these tests, a color image of the fundus was made. The infrared images of the microperimetry tests were matched to the color image using reference points, to display the tests on the color fundus image.

RESULTS

Baseline Characteristics

In total, 130 consecutive patients (78 female, 52 male; 133 eyes) underwent RPE-choroid graft surgery. Three patients were operated on both eyes. The mean age was 79 ± 8 years (range: 50-95). The 60 patients who were on anticoagulant therapy before surgery received permission to discontinue this medication two weeks prior to surgery. Before surgery, 87 eyes were phakic and 46 were pseudophakic. The median duration of VA loss prior to surgery, as reported by the patients, was 61 days with a range of seven days to three years. The median duration of VA loss in patients with a hemorrhagic lesion was 39 days, ranging from two days to 183 days. However, in the few patients with a reported VA loss of ≤ four weeks, this loss was due to an aggravation of a pre-existing, hemorrhagic lesion. Nine patients had undergone Argon laser therapy and seven had undergone PDT before surgery.

Follow-up

One-year follow-up BCVA data were available for 130 out of 133 eyes, two-year data were available for 101 eyes, three-year data for 72 eyes, four-year data for 46 eyes, five-year data for 27 eyes, six-year data for ten eyes, and seven-year data were available for nine eyes (Table 1).

In total during seven years of follow-up, 24 patients were lost to follow-up with a last BCVA measurement of >20/200. Nine of these patients died, two patients were physically/mentally unable to come, one wished not to return, one patient did now show up for unknown reasons, and eleven patients did not yet reach the next year follow-up visit (of five or six years after surgery). Of all 130 patients, 17 patients were lost to follow-up because they died from unrelated causes. Others did not return for follow-up because they were physically and/or mentally unable to do so, or for unknown reasons.

Table 1: Visual Acuity Results Preoperatively and Each Year up to 7 Years After Retinal Pigment Epithelium-Choroid Graft Surgery

Years After Surgery	Number of Eyes	Median VA (logMAR)	Range (logMAR)	VA <1.0 logMAR (>20/200) (No. Eyes)
Preoperative	133	1.00	0.40 - 2.20	52
1 year	130	1.16	0.20 - 2.48	46
2 years	101	1.30	0.30 - 2.64	32
3 years	72	1.30	0.10 - 2.90	19
4 years	46	1.10	0.20 - 2.90	20
5 years	27	1.30	0.20 - 2.77	7
6 years	10	1.20	0.40 - 1.78	4
7 years	9	1.30	0.20 - 2.48	4

logMAR = logarithm of minimal angle of resolution
VA = Visual Acuity

Grading of Preoperative Images

Of the 133 eyes, 108 were available and eligible for grading, as the image quality was insufficient to allow grading in 25 eyes. Ninety eyes were graded with fundus images and FA. For 18 eyes no FA was available and grading was carried out based on ICGA, color fundus pictures and/or red-free images. According to MPS criteria, 16 patients had a minimally classic lesion, 11 had a predominantly classic lesion, and 35 had an occult subfoveal neovascularization. Forty-six of the eyes were classified as having a hemorrhagic lesion (>50% blood). Subretinal hemorrhage was present in 99 of the 108 eyes. The median size of the total lesion in disc areas was 6.22, ranging from 1.11 to 73.92 disc areas. The median size of the hemorrhage was 3.76 disc areas, ranging from 0.03 to 73.92 disc areas.

All patients had a subfoveal neovascular membrane before surgery, of which 122 contained blood and 11 did not. Three patients of the 133 had an RPE tear, one with and the other two without blood. The eleven patients without blood were five patients with an occult CNV, two patients with minimally classic CNV, two with predominantly classic CNV, and two patients with an RPE tear.

Visual Acuity

The median preoperative BCVA of all 133 eyes (130 patients) was 1.10 logMAR (20/250). Table 1 shows the median BCVA of the eyes for each postoperative year with their range, while figure 1 shows the percentage of patients with a BCVA of either ≤20/200 or >20/200. Three groups are depicted in this figure. Group 1 consists of the percentage of eyes with a BCVA >20/200 at each time point. Group 2 consists of the percentage of eyes with a BCVA of ≤20/200 at the moment the patients were lost to follow-up. Group 3 consists of eyes lost to follow-up and whose last measured BCVA was >20/200. Only in figure 1 has the last measured data of this third group been carried forward.

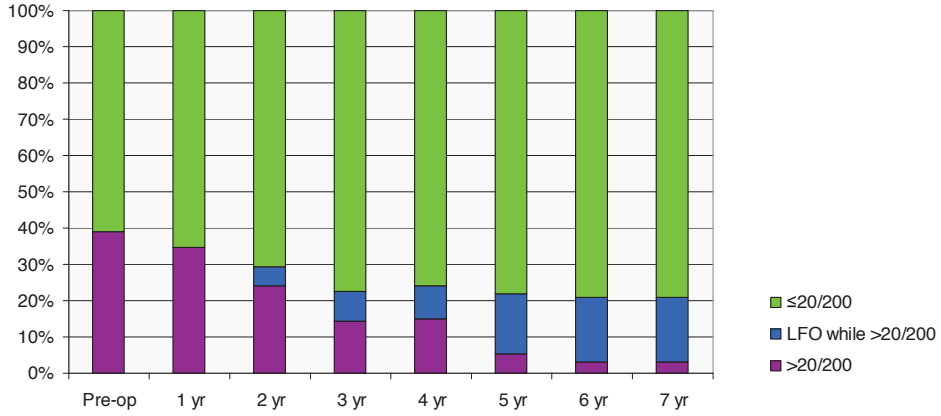

Figure 1: Visual acuity of 130 patients (133 eyes) before retinal pigment epithelium-choroid graft surgery (pre-op) and each year up to 7 years thereafter. Three groups are depicted. Groups 1 and 2 comprise percentages of patients with a visual acuity (VA) of >20/200 or ≤20/200. The third group comprises percentages of patients whose last measured VA had been >20/200 before they were lost to follow–up (LFO), and patients who were not able to reach their next yearly follow-up visit yet, while VA was >20/200 at last measurement (LFO while >20/200). The data of this third group have been carried forward in this figure.

The results were divided into two patient groups (Table 2); AMD patients with surgery for either predominantly classic, minimally classic, or occult neovascular lesions (group N), and AMD patients with surgery for hemorrhagic (> 50% blood) lesions (group B). While no patients in either group N or B had a VA of ≥20/40 at baseline, 3.2% of the patients in group N, and 8.7% of the patients in group B, had a VA of ≥20/40, four years after surgery. Nineteen percent of the patients in group N and 13% of the patients in group B had, four years after surgery, a VA of >20/200.

Of all 133 operated eyes, 20 (15%) had a BCVA of >20/200 after four years. Six of these 20 patients had a BCVA of ≥20/40, which is 5% (6/133) of the total operated eyes. Their median BCVA was 0.56 logMAR (20/73), and the highest measured BCVA was 0.2 logMAR (20/32). Nine patients had a follow-up period of seven years. Four of them had a BCVA of >20/200, with a median of 0.55 logMAR (20/71) and a highest measured BCVA of 0.20 logMAR (20/32).

Visual Acuity Course of a Subgroup

We further examined the VA course of the subgroup of 20 patients whose BCVA was >20/200 at four years after surgery. The median BCVA of these 20 patients was, at three months after surgery, 20/125 (0.80 logMAR, range 0.30-1.53 logMAR); at 6 months 20/80 (0.6 logMAR, range 0.30-1.70 logMAR); at one year 20/89 (0.65 logMAR, range 0.20-2.10 logMAR); and at four years after surgery, 20/73 (0.56 logMAR, range 0.20-0.90 logMAR). In 11 of these 20 patients an improvement in BCVA ranging from one to seven Early Treatment Diabetic Retinopathy Study (ETDRS) lines was found between three and six

Table 2: Percentage of Eyes With a Best-Corrected Visual Acuity of ≥20/40, or >20/200, Shown at Baseline and up to 4 Years After Retinal Pigment Epithelium-Choroid Graft Surgery

	BCVA ≥ 20/40	BCVA ≥ 20/40	BCVA >20/200	BCVA >20/200
	Group N[a] (n[c] = 62)	Group B[b] (n = 46)	Group N (n = 62)	Group B (n = 46)
Baseline	0%	0%	50%	37%
1 year	4.8%	4.3%	45%	28%
2 years	4.8%	4.3%	34%	22%
3 years	1.6%	4.3%	18%	15%
4 years	3.2%	8.7%	19%	13%

BCVA = best-corrected visual acuity.

[a] Group N: Surgery for subfoveal choroidal neovascularization, either minimally classic, predominantly classic or occult, in age related macular degeneration

[b] Group B: Surgery for hemorrhagic (> 50% blood) choroidal neovascular lesions of age-related macular degeneration

[c] n: Number of eyes.

months after surgery. After this initial increase, BCVA stabilized up to four years after surgery. Seven of these 20, however, had a slowly and only slightly increasing BCVA, starting three to six months after surgery, up to four years after surgery. Two of these 20 patients had yet another visual acuity course. One had a slow but progressive increase of seven ETDRS lines between three months and four years after surgery; the other had a more or less sudden increase of six lines at three years and an improvement of two more lines one year later.

In 17 of these 20 patients (85%), fixation on the graft was documented. In 15 of these 20 patients the natural lens was removed and replaced in first surgery (n=9) or at time of silicone oil removal (n = 6). In six of these patients a BCVA improvement was seen after IOL insertion at the time of silicone oil removal, which might also be attributed to this IOL insertion, but subsequent further improvement of BCVA was seen in three of them.

One year after surgery, 46 patients had a BCVA of >20/200. As described above, 20 of those preserved this BCVA up to four years after surgery. Eighty-seven patients had a BCVA of ≤20/200 one year after surgery. Five of these 87 patients' BCVA improved only temporarily to >20/200 during the follow-up period of the study.

Microperimetry

Microperimetry data was available for a subgroup of ten patients who were tested at least four years after surgery. Two of these patients were also included in the first subgroup of 19 patients who were tested shortly after surgery.[34] The patient shown in figure 2 was examined eight months[34] and seven years after surgery. The preoperative BCVA was 20/200 (1.0 logMAR). At eight months after surgery the BCVA was 20/80 (0.6 logMAR) and seven years after surgery it was 20/32 (0.2 logMAR). Figures 3 and 4 show two patients

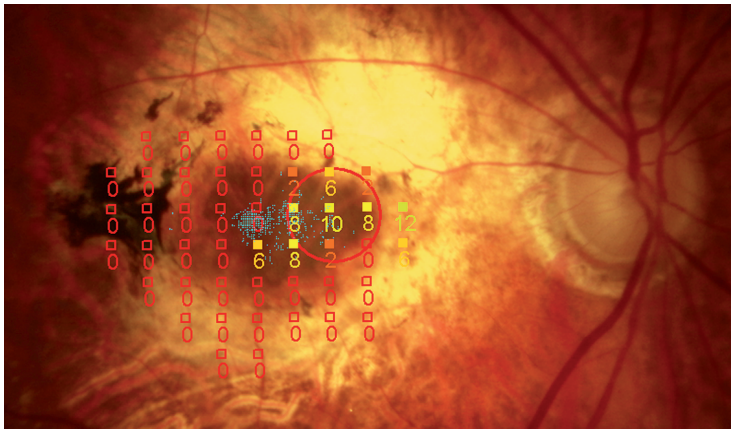

Figure 2: Microperimetry of the macular region of a retinal pigment epithelium-choroid graft patient, 7 years after surgery: visual acuity 20/32, fixation on the graft.

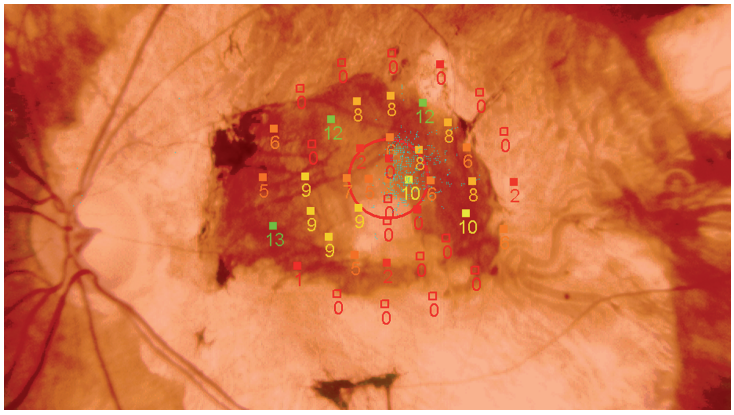

Figure 3: Microperimetry of the macular region of a retinal pigment epithelium-choroid graft patient, 4 years and 10 months after surgery: visual acuity 20/80, fixation on the graft.

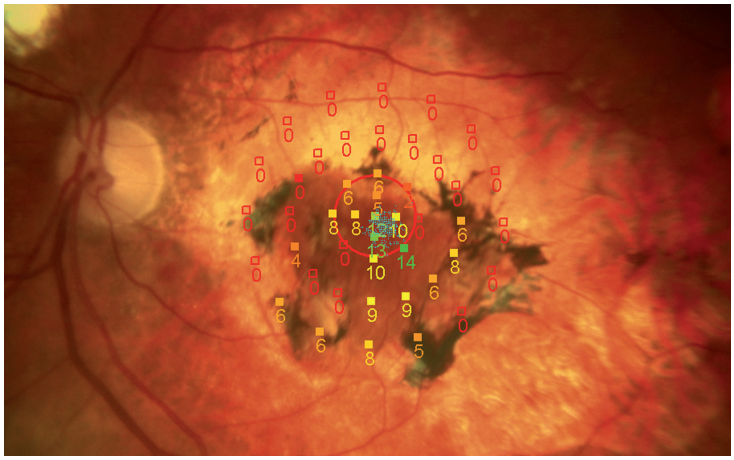

Figure 4: Microperimetry of the macular region of a retinal pigment epithelium-choroid graft patient, 6 years and 7 months after surgery: visual acuity 20/50, fixation on the graft.

who completed only one post-operative microperimetric evaluation. The first had a preoperative BCVA of 20/800 (1.6 logMAR) and a VA of 20/80 (0.6 logMAR) after four years and ten months (Figure 3). The second patient had a preoperative BCVA of 20/2400 (2.1 logMAR) and a VA of 20/50 (0.4 logMAR) after six years and seven months (Figure 4).

Postoperative Complications

Of the 130 patients, 13 developed PVR and 13 developed recurrent CNV. In the subgroup of 20 patients with a BCVA of >20/200 at the four-year follow-up, one patient had additional surgery for PVR in the first postoperative year. Two patients were treated with anti-VEGF injections for a recurrent CNV, starting in their second postoperative year. Silicone oil was removed from 125 of the 133 eyes, two of which later developed hypotony. In the remaining eight eyes, silicone oil was left in situ to prevent the occurrence of hypotony (n = 3) or local retinal detachment in combination with poor retinal function and PVR (n = 4). One patient died between the first operation and the planned oil removal.

DISCUSSION

Our results suggest that RPE-choroid graft surgery may preserve some degree of macular function, and that this improvement may be preserved even in the longer term.

In order to assess whether the RPE-choroid graft, rather than simply the removal of the subfoveal choroidal neovascular membrane or blood, was responsible for preservation of vision, the current results of both N and B groups were compared to the SST studies in which surgical removal of a subfoveal CNV membrane (group N)[35] or surgical removal of a subfoveal membrane with hemorrhage (group B)[8] was compared to natural course. For this comparison, the percentage of patients having a BCVA of >20/200 was calculated for each time point. The VA of 20/200 was selected because it was considered to be a relatively good VA in the SST trials,[8] and a BCVA of ≤20/200 is also specified as 'legal blindness' in several countries.[36] We further specified our comparison to a BCVA of ≥20/40, which is more indicative of macular function. We compared the percentage of patients with a VA of >20/200 and ≥20/40 at a particular time point as a percentage of the initial group size in our study (Table 2) and in both SST studies. The initial group size (133) of the study cohort was used for the indirect comparison with the SST trials, because even among patients with a relatively good VA (BCVA of >20/200 at their last follow-up visit), a significant number of patients were lost to follow-up.

No patient in either study had a BCVA of ≥20/40 at baseline. In the SST study, 45% of the patients in the N (neovascular) group and 36% of the patients of the B (subfoveal hemorrhage) group had a BCVA of >20/200 at baseline, comparable to those of the

current study (50% and 37%, respectively). After three years, 1.6% (N) and 4.3% (B) of the patients in the current study had a BCVA of ≥20/40, compared to 0.4 % (N) and 2.4% (B) of the patients in the SST study. Moreover, after four years, 8.7% of our patients in group B had a VA of ≥20/40. In our study after 3 years, 18% (N) and 15% (B) had a BCVA of >20/200, compared to 8% (N) and 7% (B) in the SST study, suggesting that patients in both subgroups in our study obtained better VA results. However, given the much smaller number of patients in our study, as well as the different study design, statistical comparison was not possible.

One of the limitations of our cohort study is the amount of patients lost to follow-up. Although loss of vision may decrease the motivation to return for follow-up, some of these patients were either lost independent of functional status, or have not yet reached their five- or six-year follow-up point. However, given the relatively stable, or sometimes even improving, BCVA in the patients with a complete follow-up and a good BCVA after four years, it is likely that some patients with a relatively good BCVA who were lost to follow-up may have preserved this BCVA. Therefore, the percentage of patients with documented preserved vision in our study is a conservative result that might underestimate the potential visual improvement.

During this cohort's enrollment period, PDT was available, but anti-VEGF treatment was not. PDT had been shown to produce better results than sham treatment in patients with subfoveal classic and occult neovascular membranes, but not in large minimally classic membranes. Also, a small percentage of patients (2%) sustained heavy visual loss after PDT,[37] and patients with a hemorrhage or an RPE tear had been excluded from the Treatment of Age-Related Macular Degeneration with Photodynamic Therapy investigation, and Verteporfin in Photodynamic Therapy trials.[37-39] There are similarities in eligibility for PDT and the present-day best evidence-based treatment with anti-VEGF. In the Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular AMD (MARINA) and Anti-VEGF Antibody for the Treatment of Predominantly Classic Choroidal Neovascularisation in AMD (ANCHOR) trials, patients with RPE tears and patients with large hemorrhages were excluded.[3, 4] Further, a small percentage of patients was identified with sustained visual loss after 24 months treatment,[7] as well as patients with a 3-line BCVA decrease after three injections, with no later improvement (L. Morse et al, unpublished data, 2008). Patients who were not eligible for PDT share many characteristics with patients now not eligible for anti-VEGF. Therefore, the findings of the patients of our study may be quite applicable to present-day practice. Moreover, Joussen and associates describe the use of an RPE and choroid graft in patients with geographic atrophy.[29]

RPE-choroid translocation surgery is traumatic, with two retinotomy sites and extensive manipulations. In our study PVR was the most severe complication, as it not only reduces macular function but may jeopardize the function of the originally normal peripheral retina.

Although the incidence of PVR in our study compares favorably with that found in other studies,[25, 29] it remains a major concern. Complications during surgery are another concern. We have previously shown that there is a statistically significant effect of the intraoperative course on postoperative visual outcomes.[40] However, given the improvements in surgery techniques in recent years, BCVA results may be expected to improve in the future.

The potentially promising research in development of stem cell-derived RPE-grafts grown in culture, rather than an autologous graft,[41] might eventually be able to provide better BCVA outcomes. This would allow for "new tissue" to be used, rather than "old" and/or damaged RPE tissue, as in the current study.

In summary, we have shown that four years after surgery, 15% of our patients achieved a BCVA of >20/200, and 5% a BCVA of ≥20/40. Although these functional results remain modest, this VA was achieved with relatively low complication and recurrence rates, in patients whose vision might otherwise have deteriorated due to their AMD. It is even possible to preserve macular function for up to seven years, as demonstrated by one patient who had a BCVA of 20/32 at the last follow-up visit. In a subgroup of patients, the VA results were correlated with high sensitivity of the macular region on microperimetry, and fixation on the graft was confirmed. Our findings suggest that it is the RPE-choroid graft, rather than simply the removal of the subfoveal choroidal neovascular membrane or blood, that is responsible for the reasonably good long-term BCVA results in some patients. This surgery may be an alternative for patients with AMD who have not benefitted from, or cannot undergo, other standard treatments.

In October 2009, we started a multicenter prospective randomized clinical trial to study the effect of an RPE-choroid graft in AMD patients with an RPE tear, patients who are not responsive to further anti-VEGF treatment, and patients with a large and older hemorrhage, who are no longer eligible for treatment with recombinant tissue plasminogen activator.

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Chapter 2.2

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 Ophthalmol. In press

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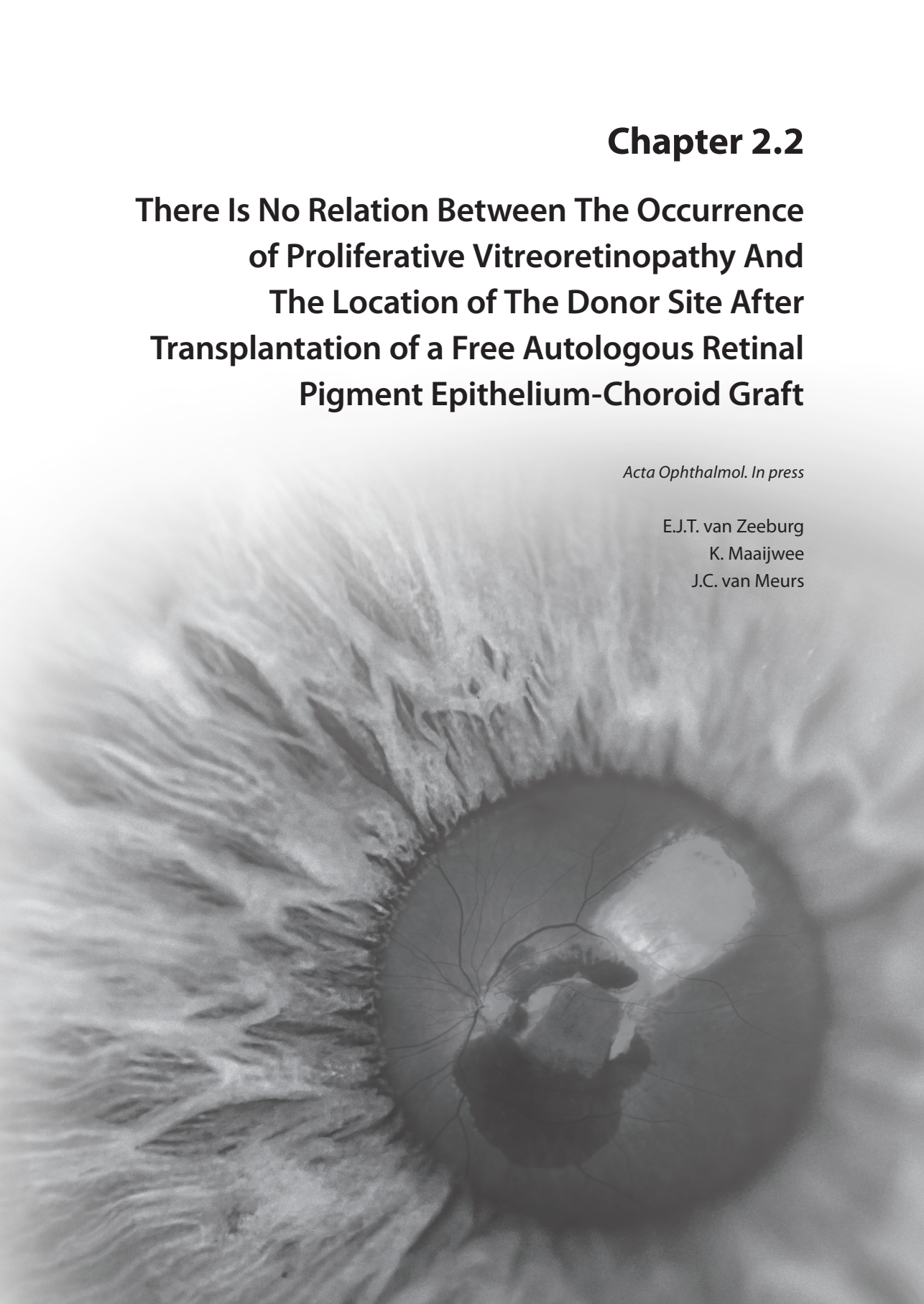

ABSTRACT

Purpose

A free autologous retinal pigment epithelium (RPE)-choroid graft can be harvested during transplantation surgery from a 6 or 12 o'clock site in the midperiphery. This study evaluated whether proliferative vitreoretinopathy (PVR) occurs more frequent in patients with an inferior donor site retinotomy, which is not closed by the tamponade and is in contact with the hydrophilic, pro-inflammatory and fibrotic environment, than in patients with a superior donor site retinotomy.

Methods

Retrospective analysis of a prospective cohort of 246 patients with exudative age-related macular degeneration treated with an RPE-choroid graft transplantation and a lighter-than-water, 5000 centistokes silicone oil endotamponade. The location of the donor site, the presence or absence of PVR development, and the location of PVR were noted. The two-tailed Fisher's Exact test was used for statistical analysis.

Results

Thirty-nine of 246 (15.9%) patients developed PVR, of whom 35 had a superior donor site, and four an inferior donor site. Of the 209 patients without PVR, 155 had a superior donor site and 25 had an inferior one. For 27 patients, no donor site location was explicitly documented in the patient files. We found no difference between the groups with a superior or inferior donor site and the occurrence of PVR ($p=0.8$).

Conclusion

Shifting the inflammatory aqueous milieu away from the graft donor site does not prevent the occurrence of PVR.

INTRODUCTION

Transplantation of free grafts of retinal pigment epithelium (RPE) and choroid after removal of the submacular neovascular complex has previously been shown to be effective in patients with exudative age-related macular degeneration (AMD) not responding to less invasive treatment [1-3]. However, this is a relatively traumatic vitreoretinal surgical procedure that requires a parafoveal retinotomy, a local submacular retinal detachment (RD), and a chorioretinotomy in the midperipheral retina. Retinal detachment due to proliferative vitreoretinopathy (PVR) is the most serious potential complication.

Proliferative vitreoretinopathy is an inflammatory and fibrotic process that may complicate retinal tears, RD, and vitreoretinal trauma. Following the breakdown of the blood-aqueous barrier, a pro-inflammatory and fibrotic milieu with macrophages, cytokines and growth factors develops in the hydrophilic compartment surrounding a posterior segment tamponade.[4;5] Retinal pigment epithelium cells, dispersed by the actual tearing of the retina, are believed to play a pro-fibrotic role in the development of PVR, and they are found in formed PVR membranes.[5;6]

Proliferative vitreoretinopathy has a predilection to occur in the inferior quadrants of the fundus. Gravity favours the deposition of dispersed RPE cells in these inferior quadrants, whereas the choice of tamponade [i.e. lighter or heavier (heavy silicone oil [HSO]) than water] determines whether the aqueous inflammatory milieu is compartmentalized superiorly or inferiorly in the posterior segment.[4;7;8]

The donor site of the RPE-choroid graft is chosen either at the 6 or 12 o'clock position in the midperiphery to avoid destruction of the vortex ampullae and the creation of a visually disturbing scotoma at the 3 and 9 o'clock positions. Superior donor retinotomies are closed by a lighter-than-water tamponade, with the hydrophilic pro-PVR milieu positioned inferiorly under the tamponade, whereas an inferior donor site is not covered by the tamponade but is instead in direct contact with the PVR milieu. Therefore, it was hypothesized that there might be a higher incidence of PVR in patients with an inferior donor site than in patients with a superior one.

We analyzed the occurrence of PVR in relation to the donor site location to test the hypothesis that shifting the pro-PVR milieu away from retinal tears or breaks might decrease the incidence of PVR, a concept that is central to the development of a heavier-than-water semi-permanent tamponade.

PATIENTS AND METHODS

Free RPE-choroid graft transplantations were performed from October 2001 through October 2011 in an institutional prospective cohort study at the Rotterdam Eye Hospital

(REH). This study was approved by the Medical Ethical Committee (REH 2001-26 and MEC 2009-17), Rotterdam, The Netherlands. All patients provided informed consent for the procedure and examinations, in accordance with the tenets of the Declaration of Helsinki. The medical records of all the transplanted patients in this time frame were retrospectively reviewed. Inclusion criteria for this retrospective analysis of occurrence of PVR in this prospective cohort were exudative AMD, a posterior segment tamponade using 5000 centistoke (cSt) silicone oil and a perimacular retinotomy with a midperipheral donor site at the 6 or 12 o'clock position. Exclusion criteria were etiologies other than exudative AMD, other tamponades, such as HSO or gas, or a 180° temporal retinectomy. We excluded all patients with a follow-up time shorter than six months, as most PVR cases develop in that time period.[9] We identified patients with PVR by notations in chart and reoperations for PVR. Proliferative vitreoretinopathy was defined as the presence of epiretinal tissue or shortening of the retina, leading to RD. Proliferative vitreoretinopathy could appear to originate from the donor site or the temporal retinotomy, or from another location entirely. The number and locations of the detached retina were noted.

Surgery

This surgical procedure has been described previously.[2,3] Briefly, the macular retina was separated from the RPE and/or submacular hemorrhage and/or choroidal neovascular membrane (CNV) by injecting a balanced salt solution into the subretinal space and thereby creating a local RD. A paramacular temporal retinotomy was made in the raphe, through which the CNV and, if present, the subretinal hemorrhage was extracted from the subretinal space. Residual blood or debris was removed by flushing the balanced salt solution under the macula. After significant diathermic coagulation or laser photocoagulation in the midperiphery at the 6 or 12 o'clock position, vitreous scissors were used to cut a full-thickness graft of retina, RPE, Bruch's membrane and choroid (RPE-choroid graft). The graft was either loaded onto an aspiration-reflux spatula [Dutch Ophthalmic Research Centre (DORC), Zuidland, the Netherlands],[10] or grasped from the choroidal side using fine forceps. The retina was removed from the graft and the graft was repositioned underneath the macula through the existing paramacular retinotomy. Perfluorocarbon liquid was injected over the macula to hold the graft in position during retraction of the instrument. A vibration device attached to the forceps facilitated the release of the graft.[10] The midperipheral donor site was then encircled by laser photocoagulation. We left a silicone oil tamponade, either after fluid air or Perfluorocarbon air exchange. All eyes were completely filled with silicone oil; no distinction was made between eyes with superior or inferior donor sites. All surgical procedures were performed by one surgeon (JvM). The silicone oil was removed in a second procedure approximately 3 months after the first surgery in all patients. In

phakic patients, lensectomy or phacoemulsification and insertion of an intraocular lens were performed during either the first or second procedure.

RESULTS

In 291 patients, a free RPE-choroid graft transplantation was performed. In 24 patients, a 180° temporal retinectomy (flap-over technique) was used, in six a gas tamponade, and in four an HSO endotamponade. In two patients, the diagnosis was geographic atrophic AMD, and one patient had angiod streaks. Two patients were lost to follow-up because they lived abroad. Six patients had a follow-up shorter than six months. These patients were excluded from analysis.

The data of the remaining cohort of 246 patients with an RPE-choroid graft were analyzed retrospectively. These patients had a follow-up time ranging from six to 112 months, during which 39 patients were found to develop PVR (15.9%). Of the 246 patients, 219 donor site locations were known, including all 39 patients with PVR. These data are summarized in Table 1. Of the patients with a superior donor site, 18.4% (35/190) developed PVR, opposed to 13.8% (4/29) of the patients with an inferior donor site. Using the available data, no significant difference was found between the patients with a superior or inferior donor site and the development of PVR (p=0.8, Two-tailed Fisher's Exact test).

If we would assume that all missing patients had inferior donor sites, we still find no significant difference between the two groups based on the assumed data; p=0.06. If we would assume that all missing 27 donor sites had superior donor sites, we find p=1. The donor site data of the 27 missing patients would thus not influence these results. Based on our data we cannot find a statistically significant difference between the donor site location and the occurrence of PVR.

The 39 patients with PVR had a mean age of 78.2 years (range 57-96). Median preoperative visual acuity was 1.0 logarithm of minimal angle of resolution (logMAR) (range 0.5-2.8), with one patient having only light perception (LP+). Median visual acuity at the last follow up was 1.95 logMAR (range 0.78-2.8), with additionally two patients

Table 1: Location of the donor site and the occurrence of PVR

	PVR +	PVR -	Total
Superior donor site	35	155	190
Inferior donor site	4	25	29
Unknown donor site	0	27	27
Total	39	207	246

PVR = proliferative vitreoretinopathy

Table 2: Location of RD and donor site

Location of RD/donor site location	Inferior RD	Superior RD	Inferior and superior RD	Temporal RD	Total RD	Unknown location RD	All	PVR relation (%)
Superior donor site (n = 190)	22	3	3	1	3	3	35	18.4*
Inferior donor site (n = 29)	2	0	2	0	0	0	4	13.8*
Donor site unknown (n = 27)	0	0	0	0	0	0	0	0
Total (n = 246)	24	3	5	1	3	3	39	15.9

PVR = Proliferative vitreoretinopathy RD = Retinal detachment

*This is a relative percentage, as 27 patients have an unknown donor site and are therefore not included what makes this PVR percentage higher than in reality. The accurate PVR incidence for the total group is 15.9%.

with LP+ and 1 patient with no light perception (LP-). Median follow-up time of the 39 PVR patients was 31 months (range 6-85 months). In 17 of the 39 patients, silicone oil tamponade was still present at last follow-up visit.

Retinal detachment due to PVR developed in the 39 patients between one and 18 months after graft surgery, with a median of 4.5 months. Between one and four quadrants were involved, with a median of two quadrants. Twenty-four patients had a retinal detachment of the inferior quadrants, of whom 22 had a superior donor site and two an inferior donor site. Contraction was noted around the retinotomy in the raphe in two patients with a superior donor site. Three patients had a retinal detachment in the superior quadrants, all with a superior donor site. Two of them had contraction at the superior donor site. In five patients, the RD involved both the superior and inferior quadrants. Three had a superior donor site, two an inferior one. In one of them contraction was noted at the superior donor site. One patient had a RD in the temporal quadrants originating from contraction at the retinotomy at the raphe, whilst having a superior donor site. Three patients had a total RD; all three had a superior donor site. One of these patients showed contraction at the retinotomy in the raphe. In three patients, no information on a specific site of epiretinal contraction could be found. In summary, epiretinal contraction originated from either the donor site (n=3) or the retinotomy in the raphe (n=4) in seven patients. In all other patients, epiretinal tissue developed distant from the donor site. The information about the location of the RD and the donor site is summarized in Table 2.

DISCUSSION

In the described technique of a free RPE-choroid graft translocation, the creation of two retinotomies (a superior or inferior donor site and a temporal paramacular retinotomy) and a local RD in the macula causes blood retina barrier breakdown and may disperse RPE cells. Older hemorrhages and/or peroperative bleeding may further contribute to a pro-inflammatory and pro-fibrotic milieu favouring the development of PVR.

The frequent development of PVR is a major concern of this elective surgical technique.[3;11-13] In this study, in order to try and find ways to decrease the incidence of PVR, we address whether there was a correlation between the incidence of PVR and the location of the donor site.

With the use of lighter-than-water silicone oil, a higher incidence of PVR would be expected in patients with an inferior donor site, as the hydrophilic milieu is located inferiorly, in direct contact with the donor retinotomy. However, the apparent origin of epiretinal contraction was located near either the donor site or the paramacular temporal retinotomy in < 20% of the patients. Furthermore, no correlation between the donor site location and occurrence of PVR was found. Thus, the expected higher incidence of PVR in patients with a donor site retinotomy at 6 o'clock was not apparent.

The findings of the current study are similar to the failure to demonstrate superiority of a heavy tamponade in the treatment of inferior PVR in the HSO study.[14] Both observations show that neither direct closure of retinal breaks by the endotamponade, nor compartmentalizing of the aqueous milieu away from the retinal breaks, prevents PVR. The aqueous milieu appears to contain sufficient cells and agents to produce PVR, regardless of the presence of a closed or open break. Additionally, in preventing fluid movement through a retinal break, actual closure by direct contact with the tamponade may not be necessary; restriction of the fluid phase, as under a lighter-than-water tamponade such as gas or silicone oil, may sufficiently reduce fluid movements.[15] Therefore, the challenge would be to find ways of modulating the inflammatory aqueous milieu.

Shifting the aqueous phase does determine the site of PVR, however.[16] In the current study, PVR manifested itself primarily in the inferior quadrants, where gravity may slow the progression of retinal detachment. In the HSO study, however, PVR developed in the superior quadrants, which is clinically less desirable as the resulting RD tends to be more rapidly progressive.

A drawback of the current study is the lack of sufficient consistent information concerning the preoperative situation of each patient. If extensive macular hemorrhage was present before surgery, the graft was more likely to be taken superiorly, as the inferior retina would be more likely to have been damaged by the hemorrhage. Furthermore, existence of hemorrhage before surgery might have increased the chance

of development of PVR. Thus, patients with an inferior donor site may represent a subgroup of patients with fewer risk factors for PVR.

Nevertheless, this study's findings establish that surgeons may choose the donor site where the RPE appears least damaged, as the results do not support the hypothesis that shifting the hydrophilic pro-inflammatory and fibrotic milieu away from retinal breaks might prevent the occurrence of PVR.

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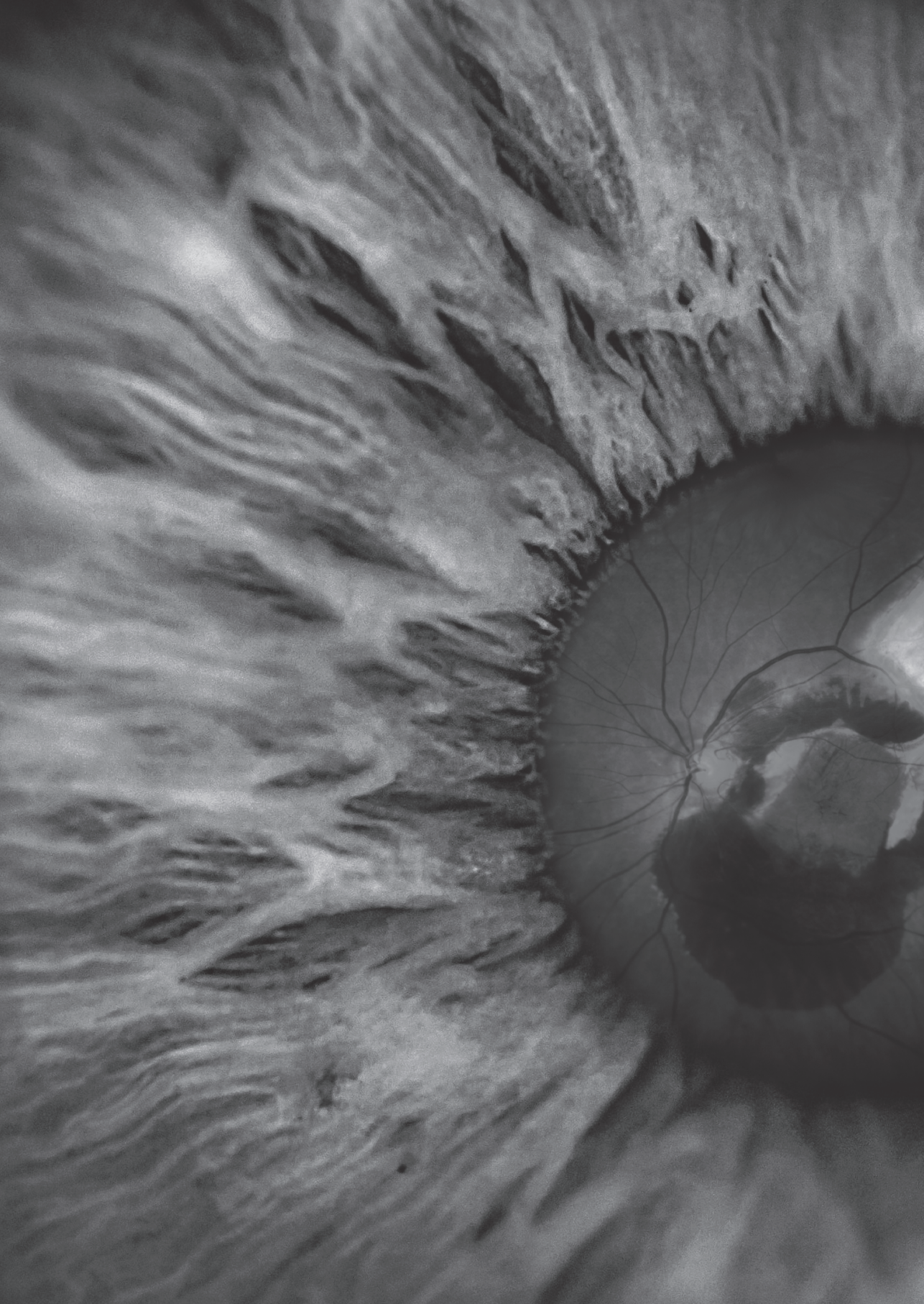

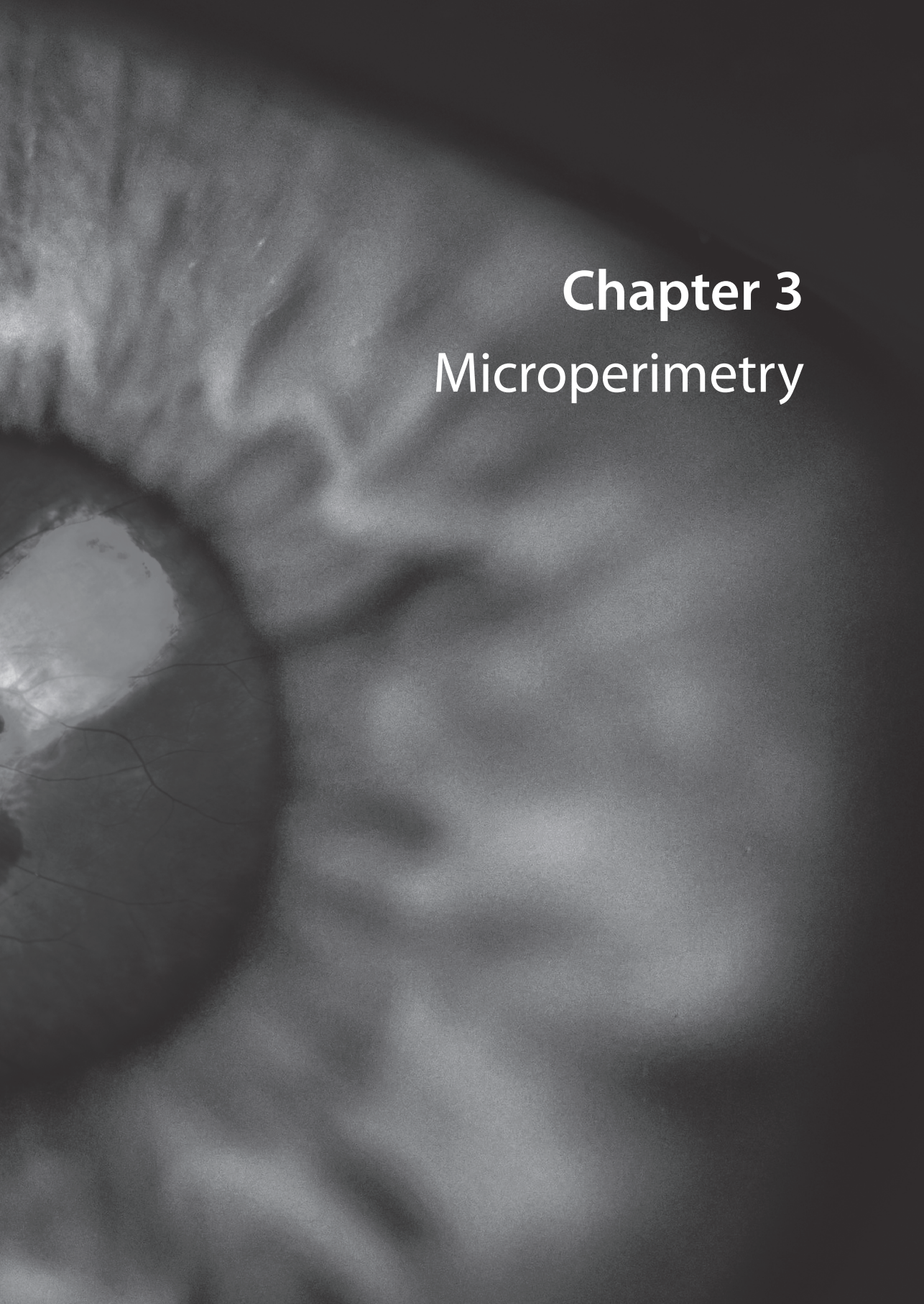

Chapter 3
Microperimetry

Chapter 3.1

Retinal Pigment Epithelium and Choroid Graft

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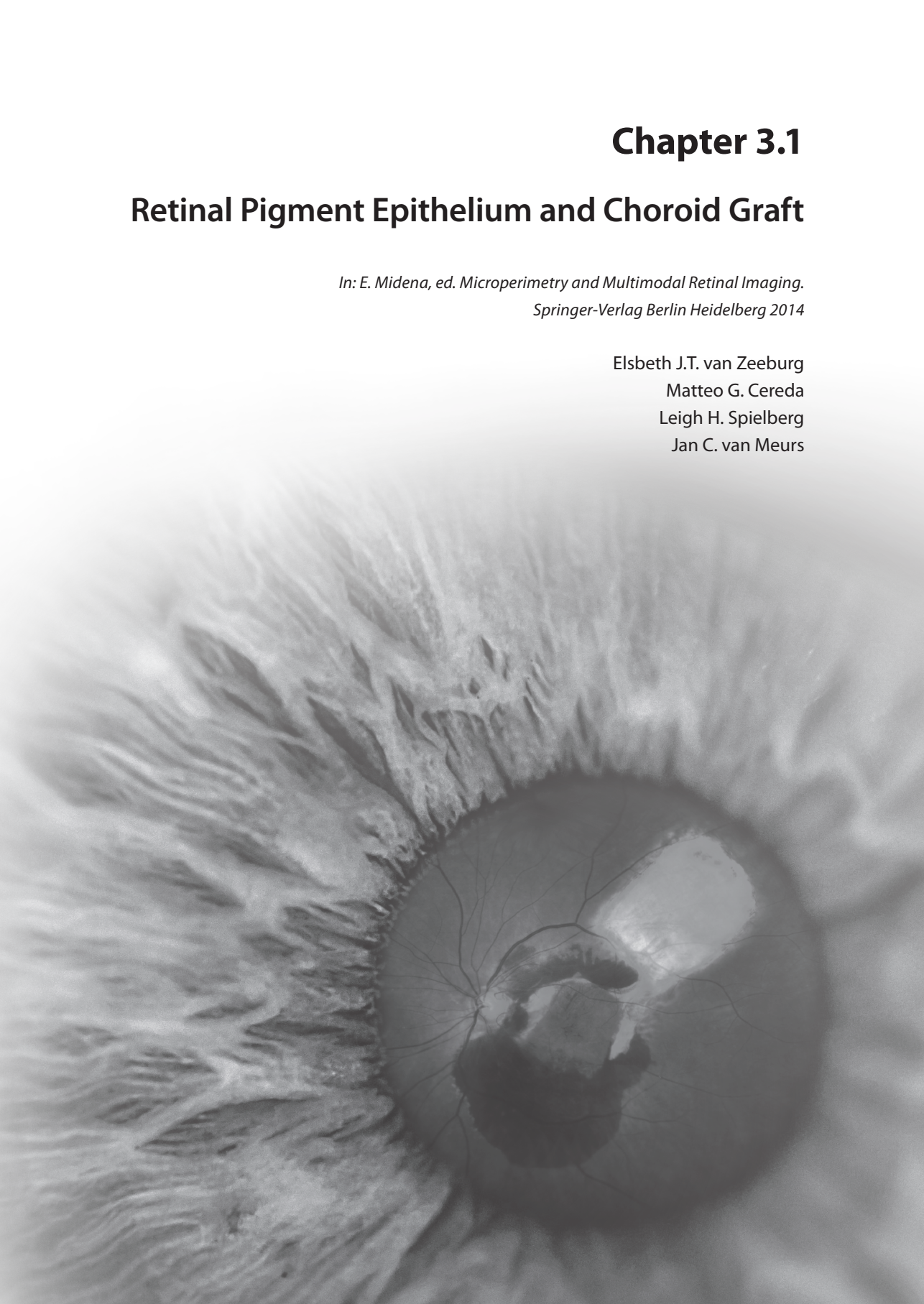

ABSTRACT

Introduction

Following retinal pigment epithelium (RPE)-choroid graft translocation, visual acuity measurement is only one single modality to test postoperative graft function. Microperimetry, alone or combined with other image modalities, may add valuable information and was therefore used for the follow-up of these patients.

Methods

Three examples of microperimetry results in patients with an RPE-choroid graft were evaluated. Microperimetry results were correlated with autofluorescence (AF), fluorescein angiography (FA), and spectral domain optical coherence tomography (SD-OCT) images.

Results

Microperimetry indicated that areas with damaged RPE and/or retina, as detected on AF, FA, and SD-OCT, had decreased retinal sensitivity. Furthermore, microperimetry exams were able to give accurate information concerning location and stability of fixation over the graft while documenting a shift in preferred retinal locus over the graft during follow-up.

Conclusion

Microperimetry may help to interpret anatomical disorders visible on AF, FA, and SD-OCT and the associated effect on visual function, by accurately measuring sensitivity and fixation over specific areas over the graft. In patients with decreased macular function, more intense or longer stimuli might improve the usefulness of the microperimeter. Further studies might explore whether microperimetry data correlate with reading vision, metamorphopsia, or quality of life measurements.

INTRODUCTION

This chapter explores the use of microperimetry in patients with exudative age related macular degeneration (AMD) who have undergone a retinal pigment epithelium (RPE) and choroid graft translocation. Microperimetric data are analyzed and correlated with other more conventional imaging and examination techniques.

The spectral domain optical coherence tomography (SD-OCT) and phase-resolved Doppler OCT have been used to detect early revascularization of an RPE-choroid graft by a noninvasive method.[1,2] Changes in the vascular structure of the graft can be visualized using the SD-OCT, and intravascular flow can be measured in the vessels of the graft using the Doppler OCT. [1,2] This demonstration of graft revascularization is valuable information, as a non-perfused graft will dysfunction and visual acuity will deteriorate.[3]

However, successful functional outcome of RPE-choroid graft surgery depends not only on graft perfusion. Other factors include the extent of preoperative RPE damage and the condition of the overlying retina, with patient selection and the peroperative course weighing as major factors.[4] In patients with a perfused graft, visual acuity (VA) varies widely.[5] Visual acuity alone, however, is only one measure of graft function and does not necessarily correlate with the patient's visual function. Besides microperimetry, other measures, such as metamorphopsia, reading visual acuity, contrast sensitivity, and quality of life might correlate with graft function.

The microperimeter is able to accurately determine retinal sensitivity, measured in dB, over the graft with high specificity,[5-14] which may help to interpret functional and anatomical changes.

Using three well-documented patients as examples, the potential advantages of microperimetry are discussed below. Limitations and difficulties in patients with low visual acuity will also be discussed, with suggestions of potential improvements of the technique.

PATIENTS AND METHODS

Transplantation of free grafts of RPE and choroid after removal of the submacular neovascular complex has previously been shown to be effective in some patients with advanced exudative AMD.[2;5;12;13;15-17] Patients eligible for this surgery were patients who presented with a large submacular hemorrhage but who were ineligible for treatment with recombinant tissue plasminogen activator,[18] patients with an RPE tear,[8] or macular fibrosis,[19] and patients who have not responded to less invasive treatment such as anti-vascular endothelial growth factor.[20]

During surgery a full-thickness graft of autologous midperipheral RPE, Bruch's membrane, choriocapillaris, and choroid was harvested and placed under the macula

via a retinotomy in the raphe, using either an aspiration-reflux spatula[21] or fine or bent forceps[2;5] (Dutch Ophthalmic Research Center [DORC], Zuidland, The Netherlands). At the end of surgery, silicone oil was used as a tamponade, which was removed approximately three months later. Lensectomy or phacoemulsification and insertion of an intraocular lens was performed in phakic patients during first or second surgery.[2;5;13]

Pre- and/or postoperative examination of RPE-choroid graft patients was performed using the MP-1 microperimeter (Nidek Technologies, Padova, Italy), and the results were integrated with spectral domain optical coherence tomography (SD-OCT) (Spectralis HRA; Heidelberg engineering, Heidelberg, Germany), autofluorescence (AF) (Spectralis HRA; Heidelberg engineering, Heidelberg, Germany, or TOPCON, Tokyo, Japan), and fluorescein angiogram (FA) images (TOPCON, Tokyo, Japan).

RESULTS

Exact Location of Function and Dysfunction

Retinal sensitivity can be determined with the microperimeter with high specificity.[5-14] These data can be used to help interpret functional and anatomical changes.

Patient 1 presented with a submacular hemorrhage and an RPE tear due to age-related macular degeneration (Figures 1 A, B, C). VA was 1.32 logarithm of minimal angle of resolution (logMAR). The patient was unable to perform the Radner reading acuity test,[22] and the Sine Amsler score[23] was 1.

The preoperative microperimetry exam (Figure 1B) showed no detectable retinal sensitivity over the foveal area (Figure 1C). Fixation was unstable and was not located over the fovea. Mean sensitivity of the preoperative tested area with the microperimeter was 0.4 decibel (dB).

After surgery, the location of the preoperative microperimetry turned out not to be centered on the later graft location. Therefore, the same test was repeated six months after surgery, but now centered on the graft (Figure 1E), and one year after surgery a follow-up exam of the six months' exam was performed (Figure 1H). In both the six months' and one year's exam, mean sensitivity had increased to 3.2 dB, and measurable sensitivity was seen on almost the entire graft; however, in the fovea, an area of relative or absolute scotoma was observed (Figures 1E, 1H).

This area of decreased sensitivity corresponded to an RPE defect, visible as a hypoautofluorescent area, rectangular in shape, on the graft on AF (Figures 1D, 1G), and visible on SD-OCT as absence, thinning, or mottling of the highly reflective band of the RPE layer (Figures 1F, 1I). The SD-OCT also revealed foveal retinal atrophy; the outer nuclear layer, external limiting membrane, and inner/outer segment photoreceptor layer were absent, which also contributed to the low sensitivity. (Figures 1F, 1I).

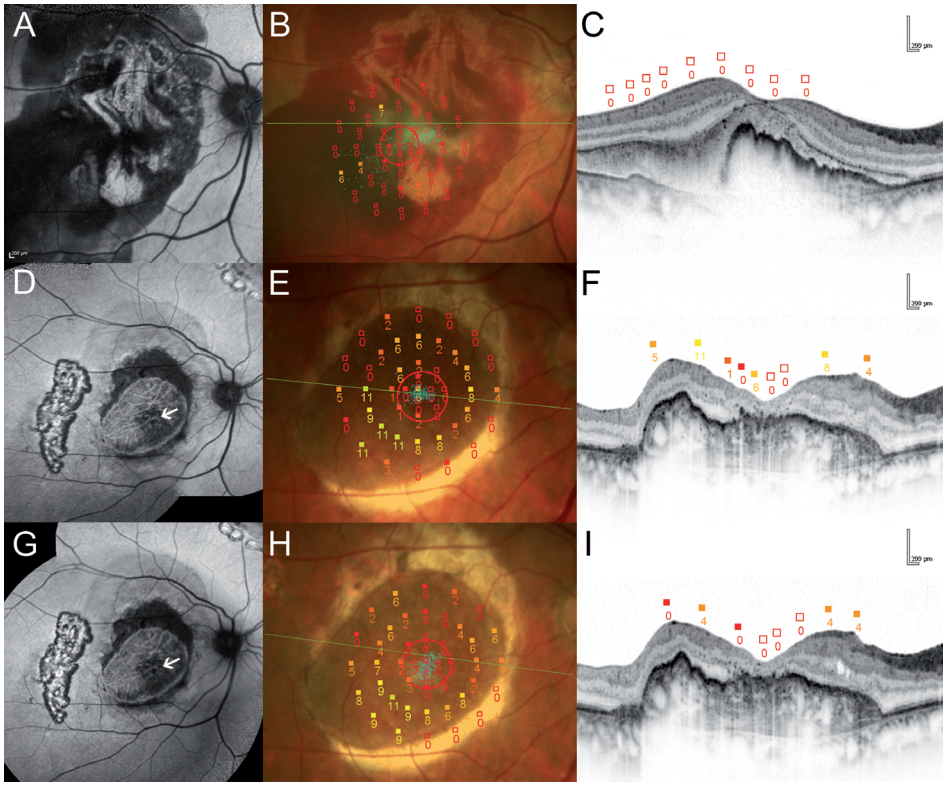

Figure 1. Exact location of function and dysfunction before and after RPE-choroid graft translocation. Autofluorescence (**A, D, G**), microperimetry (**B, E, H**), and SD-OCT (**C, E, F**). Preoperative (**A, B, C**), six months (**D, E, F**), and one year (**G, H, I**) after RPE-choroid graft translocation surgery of patient 1 who presented with a macular hemorrhage and an RPE tear. (**B, E, H**) A macular, 12°, 10 dB pattern, with 45 test loci, with a 2° circle as fixation target, centered on the hemorrhage (**B**) or graft (**E, H**) was tested automatically with a 4-2 staircase threshold strategy, with white Goldmann III stimulus displayed for 200 ms. The brightness of the test stimuli ranged from 0 to 20 dB (400 to 4 asb). Mean sensitivity preoperatively was 0.4 dB with a minimum of 0 dB and a maximum of 7 dB. Mean sensitivity of both six months' and one year's follow-up exam was 3.2 dB, with a minimum of 0 and a maximum of 11 dB. The visual acuity was 1.32 logMAR preoperatively, 0.8 logMAR six months after surgery, and 0.9 logMAR one year postoperatively. (**B, C, E, F, H, I**) The retinal sensitivity values in the SD-OCT images and the green line in the microperimetry image were placed after superimposing the microperimetry results on the matching AF and infrared images. This patient illustrates RPE damage most likely following (excessively) firm gripping of the graft with the bent forceps during surgery, which can be identified as the dark area, rectangular in shape, (*white arrows*) located on the center of the graft and oriented towards the inferonasal edge on the AF images (**D, G**), and disturbances in the highly reflective RPE layer on SD-OCT (**F, I**). In the same macular area, retinal atrophy is visible on SD-OCT (**F, I**). The area with RPE damage and retinal atrophy corresponds to the area with lower sensitivity, as measured with the microperimeter (**E, F, H, I**).

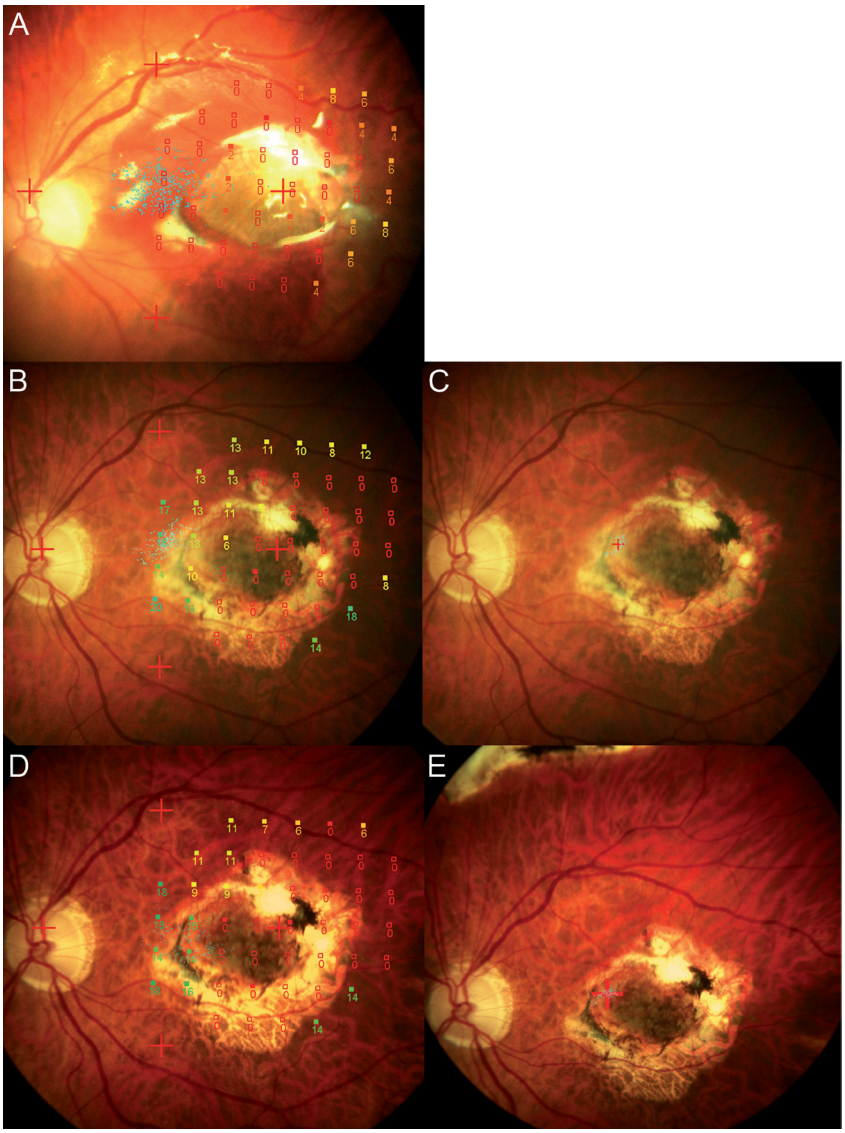

Figure 2. Long-term follow-up: sensitivity, fixation location, and fixation stability over the graft. Figure **B**: *Ophthalmologica* 2012; 228(suppl 1):40. S. Karger AG Basel. Microperimetry sensitivity (**A, B, D**) and fixation (**C, E**) exams at 2 months (**A:** slight reflections due to silicone oil tamponade), 80 months (**B, C**), and 107 months (**D, E**) after RPE-choroid graft translocation surgery of patient 2, who presented with an occult neovascular membrane and a fibrotic scar. A customized pattern (47 test loci, 4-2 staircase threshold strategy, white 200 ms Goldmann III stimulus, fixation target of 4 crosses of 2° at 20° distance) tested a mean sensitivity of 1.6 dB (range 0–8 dB) at 2 months (**A**), 5.6 dB (range 0–20 dB) at 80 months (**B**), and 4.6 dB (range 0–18 dB) at 107 months (**D**) after surgery. Visual acuity at the same time points was 0.7, 0.42, and 0.34 logMAR. Fixation was difficult to interpret in the sensitivity exams (**B, D**), as 4 crosses were used as fixation target. Separate fixation exams at 80 months (single cross, 1°) (**C**) and 107 months (single cross, 3°) (**E**) show stable fixation on the superonasal edge of the graft, corresponding to the area of the graft with the highest measurable sensitivity and, most likely, the foveal area.

One year after surgery, VA had increased to 0.9 logMAR, reading acuity improved to 1.0 logRAD (Reading Acuity Determination), and the Sine Amsler score improved to 0. According to the six months' and the, more pronounced, one-year's microperimetry, SD-OCT, and AF, the macular area was damaged and displayed little retinal sensitivity. However, the patient had a stable fixation during both the six months' and one year's microperimetry exams, and stable fixation on both separate fixation examinations. The fixation target (single cross 3° or circle 2°) had only a luminance of 100 apostilb [asb] (6 dB). Thus, despite retinal and RPE damage, fixation could still be measured over the macular area using only a dim stimulus; furthermore, the patient had reading acuity. Therefore, we hypothesize that there might be more (areas with some) retinal sensitivity in the macula than the microperimeter could detect. Our hypothesis is that, even though the macular area was (partly) damaged, a brighter stimulus, or a stimulus of a longer duration than 200 ms, might have been visible. This hypothesis will be further addressed later in this chapter.

Long-term Follow-up and Fixation Location and Stability over the Graft

Locating (foveal) fixation is another measure of retinal function over the graft. This can be performed with a slit lamp, or more accurately, with the microperimeter.

Patient 2 presented with an occult neovascular membrane and a fibrotic scar and a visual acuity of 0.7 logMAR. Two months after surgery, VA was still 0.7 logMAR, and the mean sensitivity of the microperimetry exam was 1.6 dB (Figure 2A). As the MP1 was momentarily available in our hospital, a regular follow-up exam could not be performed. Therefore, the same pattern as was used on the first exam was manually custom-made on the microperimeter for the second test of this patient at 80 months (Figure 2B). Mean sensitivity of this second test was 5.6 dB, with most sensitivity displayed on the superonasal edge of the graft, which most likely represents the location of the preoperative fovea. A comparable distribution of sensitivity was found at the third exam (Figure 2D) at 107 months (this was a follow-up exam of the 80 months' exam), while mean sensitivity had slightly decreased to 4.6 dB.

Just as the mean microperimetric sensitivity increased at the second exam, so did the visual acuity increase, to 0.42 logMAR. The Sine Amsler score at the second exam was 3 and reading acuity was 0.92 logRAD.

Although the mean sensitivity decreased slightly at the third exam, VA increased to 0.34 logMAR; reading acuity improved to 0.71 logRAD; and the Sine Amsler score improved to 0.

Despite these relatively good sensitivity results over a long follow-up period, fixation was difficult to interpret in these microperimetry sensitivity exams, as the patient was specifically asked to look in between the four crosses, and thus no clear fixation point could be determined. Fixation was ranked as relatively unstable and as unstable at the second and third visit, respectively. Additionally, fixation was also tested in a separate

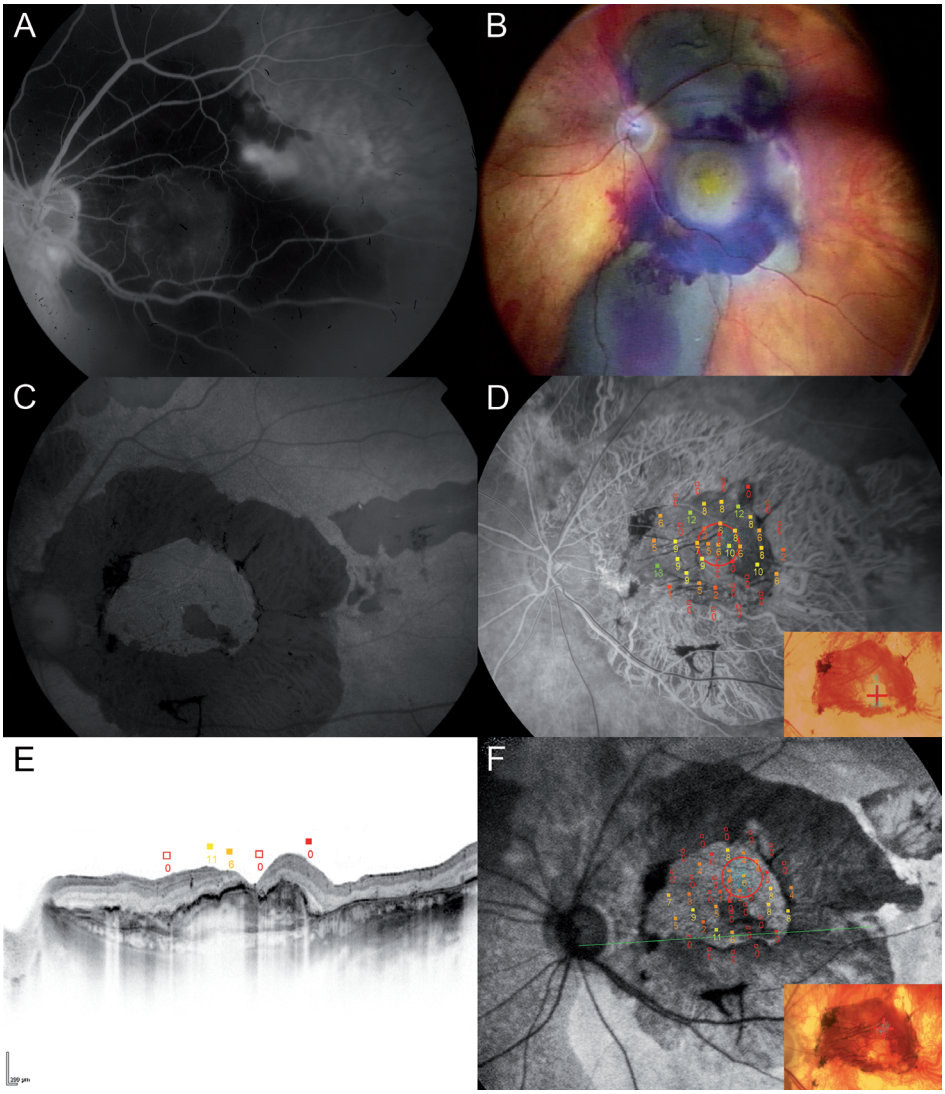

Figure 3. Combining information about function/dysfunction, anatomical changes, and fixation over time as a help for reinterpretation of earlier imaging. Preoperative fluorescein angiogram of patient 3 showing a hemorrhagic pigment epithelium detachment (**A**). Still image taken from video recording of RPE-choroid graft translocation surgery (**B**), on which a large hemorrhage with fibrotic component can be distinguished. Preoperative VA was 1.6 logMAR. At 18 months after surgery, VA was 0.1 logMAR, and on AF (**C**) a clearly demarcated area of RPE damage on the inferotemporal edge of the graft is visible. At 58 months' follow-up, VA was 0.6 logMAR; on the microperimetry sensitivity exam (macula 12°, 10 dB pattern, 200 ms Goldmann III stimulus, 45 test loci centered on the graft, with a 2° circle as fixation target) transposed onto a FA image (**D**), an area with lower sensitivity can be distinguished, corresponding to the RPE damaged area imaged on (**C**), which also seems to correspond with a window defect area visible on FA. Mean sensitivity was 4.4 dB (range 0–13 dB). Fixation exam (single cross, 3°) of 58 months (*inset* in **D**) shows a relatively unstable inferotemporal fixation. (**E**) A B-scan of the macular area of the graft with an SD-OCT at 86 months' follow-up with a VA of 0.76 logMAR. According to the integrated microperimetry results, (part of) the macular

fixation exam, and at both 80 months' follow-up and the 107 months' follow-up, fixation was stable on the edge of the graft (Figures 2 C, E). Therefore, a separate fixation test, especially when the four crosses are used as a fixation target in the sensitivity exam, is mandatory. We could now clearly correlate the fixation and the area with sensitivity on the superonasal edge (macular area) of the graft, which most likely explains the relatively good VA and the reading ability of the patient.

Combining Information about Function/Dysfunction, Anatomical Changes, and Fixation Over Time Can Help to Reinterpret Earlier Imaging

With the lessons learned from patients 1 and 2 about function in relation to anatomical changes, fixation, and long-term follow-up, we have combined microperimetry results with SD-OCT, AF, and FA images over time in patient 3. Because the information gathered from all images together can provide insights into the course of anatomical changes and function, existing RPE damage could at a later time point be correlated with decreased sensitivity and loss of function, which was followed by a shift in fixation location.

This third patient presented with a hemorrhagic pigment epithelium detachment, a fibrotic scar, and a preoperative VA of 1.6 logMAR (Figures 3 A,B). RPE damage can be distinguished at the same area at the 18-month AF (Figure 3C) and on the 58-month FA (Figure 3D). On FA a window defect due to the atrophy of the RPE is visible as a whiter area on the graft, which corresponds with an area with less retinal sensitivity measured with the microperimeter (Figure 3D). The 86-month microperimetry exam transposed onto the AF exam (Figure 3F) confirms that the area with less sensitivity and decreased autofluorescence can be correlated with each other, although now more areas of the graft have lost retinal sensitivity. Also, the SD-OCT B-scan through the macula was now able to pinpoint very accurately the area with hypoautofluorescence and decreased sensitivity. In these areas, the hyperreflective RPE layer is disturbed or absent. At the mid-nasal side of the graft, intact retinal layers and a continuous RPE layer correlate to presence of retinal sensitivity on microperimetry (6 and 11 dB), while at the nasal edge of the graft an area without RPE corresponds to a retinal sensitivity of 0 dB. In the fovea and at the temporal side of the graft no outer retinal layers are visible on SD-OCT, corresponding to a retinal sensitivity of 0 dB.

(Legends figure 3, *continued*)
area does not have retinal sensitivity. This area corresponds to the areas where the highly reflective RPE band is distorted or absent. The areas with intact RPE and intact retinal layers do show sensitivity. On the microperimetry exam transposed onto an AF image (**F**), the RPE damaged area with less sensitivity is well demarcated. The *green line* is the location where the B-scan (**E**) was taken. Fixation exam of 86 months (*inset* in **F**) shows a stable superotemporal fixation. The fovea is located at the inferior edge of the graft, and the new fixation location measured with the microperimeter at the superotemporal edge of the graft is therefore a new preferred retinal locus.

At 58 months, VA had increased to 0.6 logMar, the mean sensitivity of the microperimetry exam was 4.4 dB, and a relatively unstable fixation was measured both during the sensitivity exam as on a separate fixation exam. However, during the fixation examination, fixation was located over the foveal area with the RPE damage, instead of over the superotemporal location revealed during the sensitivity exam (4D).

At 86 months' follow-up, the patient was able to read (1.155 logRAD), with a slightly decreased VA of 0.76 logMAR and a Sine Amsler of 0. Mean sensitivity at 86 months (a follow-up exam of the first microperimetry exam was performed) had decreased to 2.5 dB, with a relatively unstable, superotemporal, fixation. But now the separate fixation exam showed stable fixation also over the superotemporal side of the graft. Clearly it can be seen that (at least) part of the fovea is located in the RPE damaged area (Fig 3E, F), so fixation was no longer in the fovea.

Therefore, fixation had shifted from an unstable, foveal (inferotemporal) fixation at 58 months, to a stable fixation at a macular superotemporal location; a new preferred retinal locus (PRL),[24] where RPE was not damaged and retinal sensitivity over the graft could be measured. The slight VA decrease between the two exams might also correlate with the shifted preferred location of fixation of the patient to a new preferred visual locus as the foveal area seems no longer to be used for fixation.

DISCUSSION

Visual Acuity and Its Correlation with Sensitivity Results over the Graft

Visual acuity and microperimetry results may correlate,[11;17] but not consistently so. Patient 1's VA improved significantly from 1.32 logMAR preoperatively to 0.8 logMAR after six months. The mean sensitivity also increased from 0.4 to 3.2 dB. The slight decrease in VA from 0.8 to 0.9 logMAR from six months to one year after surgery was not mirrored by a decrease in sensitivity, which remained stable at 3.2 dB. For patient 2 also an increase in VA from 0.7 logMAR two months after surgery to 0.42 logMAR 80 months after surgery, corresponded to an increase in dB from 1.6 dB to 5.6 dB. However, the examination at 107 months revealed a slightly better VA, 0.34 logMAR, but the sensitivity had slightly decreased to 4.6 dB. Patient 3 had a VA of 0.6 logMAR at 58 months and of 0.76 logMAR at 86 months of follow-up. This decrease in VA was also visible in a decrease in sensitivity from 4.4 to 2.5 dB.

Therefore, a marked difference in VA corresponds to a marked change in dB, but small changes in VA are harder to correlate with the changes in sensitivity. Larger studies will be needed to be able to clearly correlate VA and sensitivity changes in grafts.

Correlation Between Damage of the Retinal Pigment Epithelium and Retinal Layers, and Sensitivity Measurements over the Graft

In two of the cases described, a correlation was demonstrated between structural change, represented by RPE damage and retinal layer disturbance on AF, FA, and SD-OCT, and functional change, represented by a decrease in retinal sensitivity on microperimetry. Correlation between areas with RPE defect and retinal sensitivity loss over an RPE-choroid graft have been described.[9;10] Also, correlation has previously been found between intact retinal structures over a graft and areas with retinal sensitivity.[7-9] In our first patient, the foveal area at six months' and one year's follow-up shows less retinal sensitivity than other areas of the graft (Fig 1E and 1H). The SD-OCT reveals a damaged retina at the six-month scan and a very thin and sometimes absent RPE (Fig 1 F). At the one-year scan, the foveal retinal layers are also damaged, and the RPE is very thin or absent in some locations. Here, minimal microperimetric sensitivity could be detected (Fig 1 I). Although most retinal layers over the graft in patient 3 at the 86-month SD-OCT scan (Fig 3E) seem to be quite intact, areas with less or absent RPE are visible. These areas correspond to the areas with less retinal sensitivity. At the areas with an intact retina and an intact layer of RPE, retinal sensitivity was indeed measured with the microperimeter. Therefore, it is clear that damage of retinal layers and damage of RPE decreases sensitivity and therefore VA.

The intraoperative course has already been found to play a major role in graft function.[4] For patient 1, the RPE damage, likely a result of excessively firm gripping of the graft during surgery, visible on AF and SD-OCT, correlated with the decreased retinal sensitivity. Further, patient 3 clearly shows that RPE damage alone can already have a devastating effect on retinal sensitivity. Retinal damage may have occurred earlier due to the pathology before surgery but may also occur during surgery, when, for instance, fibrotic components, strongly attached to the retina, need to be peeled off. These examples underscore our need to search for more delicate instruments and techniques for this surgery.

Improvements and Future Possibilities

Although very detailed information of retinal sensitivity over the graft can be obtained with the MP-1, we think more information about RPE-choroid graft patients could be gained if stimuli brighter than 0 dB could be displayed. A clear example is patient 1: at one year, this patient was able to read nearby, had a relatively good VA of 0.9 logMar, was able to see the fixation target of 6 dB, and had a stable fixation. Although her RPE and retinal layers were damaged in the fovea, and foveal microperimetric sensitivity was severely decreased, fixation was located over the fovea. We think it would be very useful to test the function over that area with brighter stimuli. We hypothesize that the patient experienced difficulty concentrating on the fixation ring and was unable

to simultaneously distinguish the projected stimuli in the same area. It is possible that a brighter stimulus would have allowed this patient detect the stimuli, and thus demonstrate residual sensitivity. Also, for patients with very low VA, who now cannot be tested with the MP-1, brighter stimuli might give the option to test if there is any retinal sensitivity over the graft.

Another idea would be to project a stimulus of a longer duration; up to 2000 ms is possible with the MP-1. We have tested this option in another patient with an RPE-choroid graft with relatively good VA, and this allowed the patient to see less bright stimuli. Unfortunately, in this setting, local defect maps, and therefore mean sensitivity and mean defect values, are unavailable. Thus, comparison with other reports, which almost exclusively use the 200 ms as default, is difficult. Further, with long stimulus duration, the interval between projected stimuli becomes shorter, which, in our experience, was not beneficial for the accuracy of the test. Therefore, brighter stimuli with the same duration of 200 ms, longer-duration stimuli with standard interstimulus intervals, and a set of normative data so local defect maps can be obtained, would all be very useful.

CONCLUSION

This study suggests that microperimetry can provide useful information regarding retinal sensitivity over an RPE-choroid graft, by correlating function with anatomical changes on FA, AF, and SD-OCT. Furthermore, microperimetry can provide accurate information on fixation location and its stability over the graft over time.

It is not yet clear whether microperimetric sensitivity information provides additional advantage for diagnosis and follow-up of an RPE-choroid graft. Future studies should address whether microperimetry results correlate with visual acuity, reading acuity, metamorphopsia, and/or quality of life.

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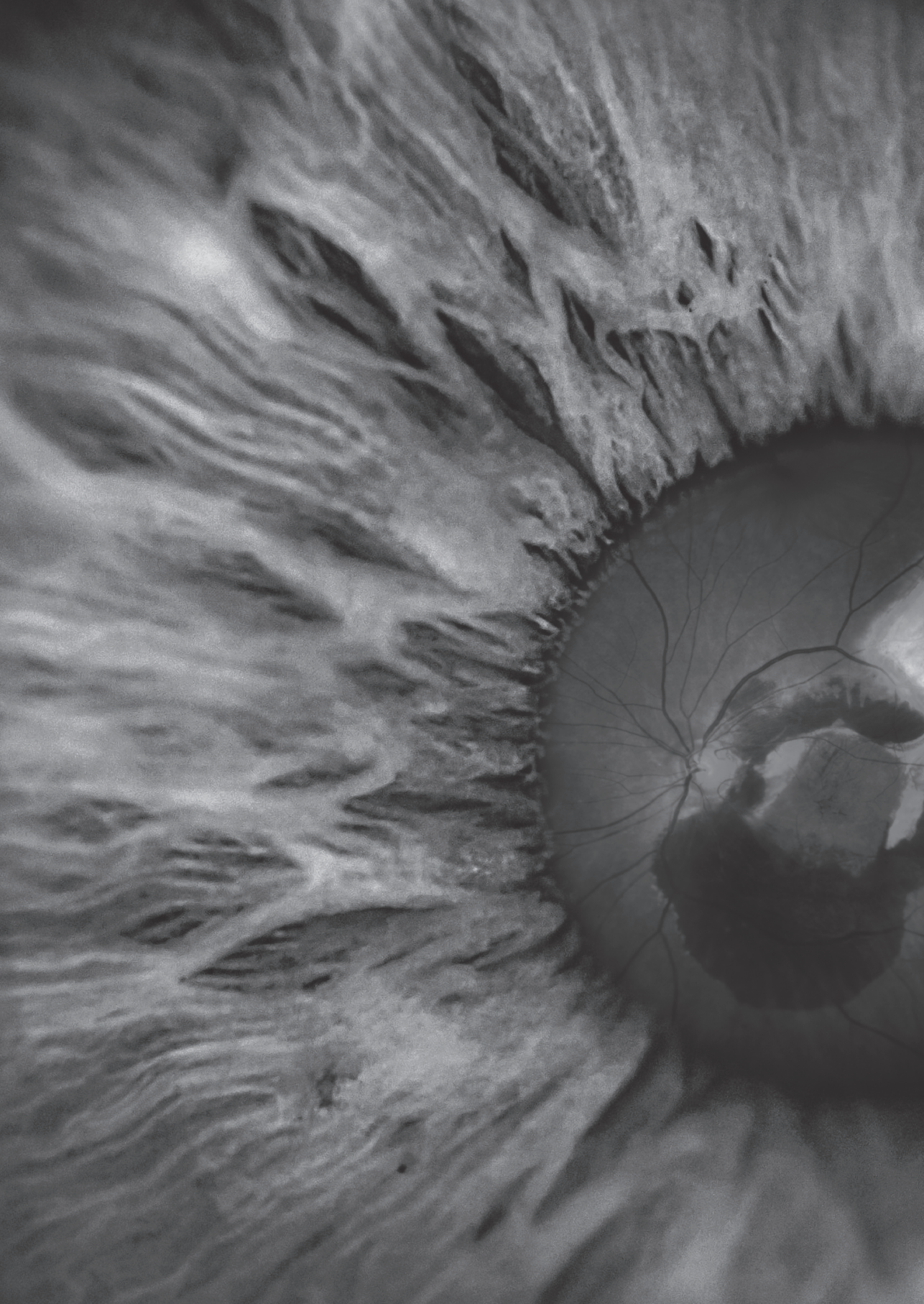

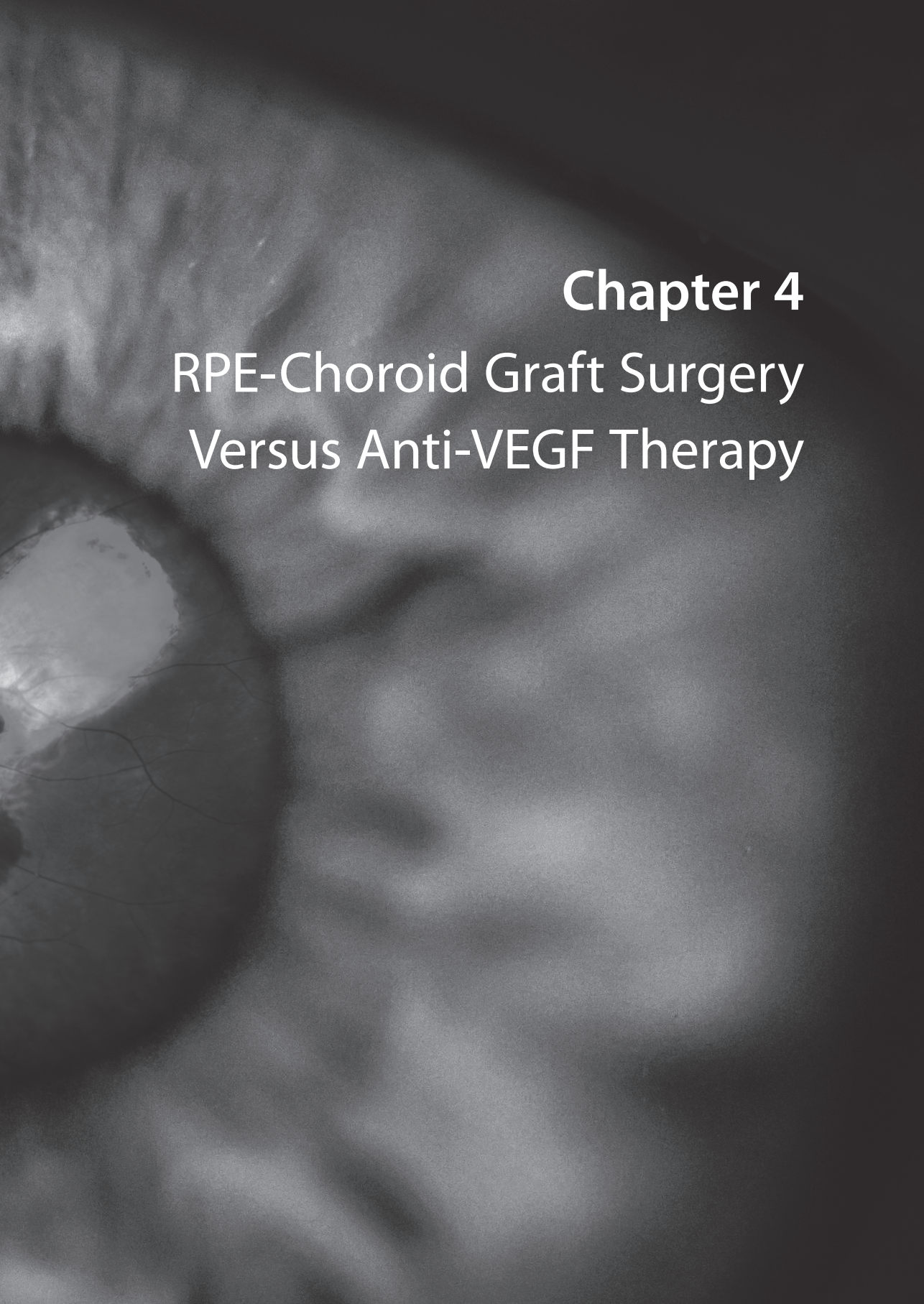

Chapter 4
RPE-Choroid Graft Surgery Versus Anti-VEGF Therapy

Chapter 4.1

Prospective, Randomized Intervention Study Comparing Retinal Pigment Epithelium-Choroid Graft Surgery and Anti-VEGF Therapy in Patients With Exudative Age-Related Macular Degeneration

Submitted

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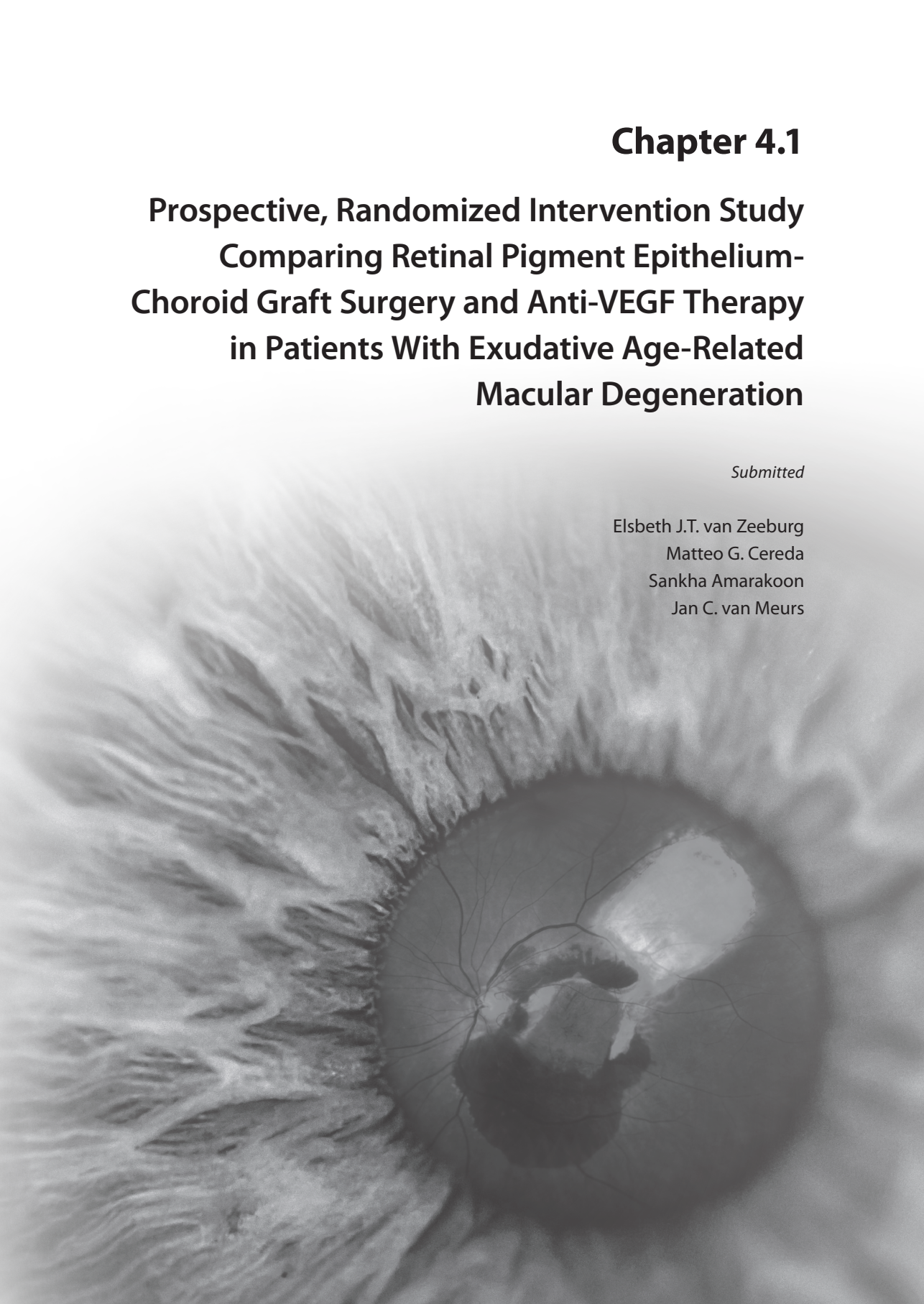

ABSTRACT

Purpose

To investigate whether patients with exudative age-related macular degeneration and a submacular hemorrhage (SMH), retinal pigment epithelium (RPE) tear, or non-responders to anti-vascular endothelial growth factor (VEGF) benefit more from a free RPE-choroid graft transplantation surgery than from (continuation of) anti-VEGF treatment.

Design

A prospective, international multicenter, randomized intervention study.

Participants

Twenty patients were included, ten in each study arm, all in Rotterdam.

Methods

Patients either received anti-VEGF treatment, or underwent RPE-choroid graft surgery. If existent, the SMH was removed first.

Main outcome measures

The primary study objective was to compare visual acuity (distance and reading acuity) and foveal fixation of RPE-choroid graft translocation versus intravitreal anti-VEGF therapy. The secondary objective was to compare safety of both treatments.

Results

The greatest improvement in the graft group (non-responder) was an increase in visual acuity (VA) from 0.66 logMAR preoperatively to 0.14 logMAR one year after surgery. The patient who experienced the least benefit in the graft group (SMH) had a VA of 0.62 logMAR before surgery but developed proliferative vitreoretinopathy (PVR) and hypotony after surgery. VA decreased to 2.3 logMAR. The greatest improvement in the anti-VEGF group (RPE tear) was an increase from 0.58 to 0.44 logMAR after one year of anti-VEGF therapy. The patient who experienced the least benefit in the anti-VEGF group (SMH) decreased from 0.9 logMAR to 1.42 logMAR. Complications: Graft group: PVR (3 patients), recurrent submacular hemorrhage (3), hypotony (2), cerebral-vascular accident (1). Anti-VEGF group: PVR (1), transient ischemic attack (2), minor stroke (1).

Conclusions

The multicenter study was stopped because inclusion failed in other centers. The included patient group is far too small to draw conclusions whether an RPE-choroid graft or anti-VEGF treatment is more successful. Also, this surgical group suffered an

unprecedented run of complications. Both gain and loss of VA may be experienced by patients undergoing either treatment method; more gain might be possible for patients with a graft, however, in the absence of complications.

INTRODUCTION

Transplantation of an autologous free retinal pigment epithelium (RPE) and choroid graft is a surgical technique which was first described by Peyman and associates in 1991.[1] Although anti-vascular endothelial growth factor (anti-VEGF) treatment has become the current therapy of choice for patients with exudative age-related macular degeneration (AMD), it is not always effective. The RPE-choroid graft surgery may possibly be a suitable alternative for at least three subgroups of patients with exudative AMD. This procedure has been performed at the Rotterdam Eye Hospital (REH) for patients with severe visual loss due to advanced AMD since October 2001.

The first group of patients who might possibly benefit from an RPE-choroid graft surgery are patients with an old submacular hemorrhage. These patients have a poor prognosis with anti-VEGF monotherapy (even for relatively shallow submacular hemorrhages);[2-4] simple removal of the hemorrhage without further therapy;[5] or if left untreated.[6;7] The second group consists of patients with AMD who do not respond to extensive anti-VEGF therapy.[8;9] The third group of patients are those with RPE tears involving the macula. Preliminary reports of RPE tears treated with an RPE-choroid graft showed promising results.[10] We have shown in previous publications that an RPE-choroid graft can be successful for up to seven years in patients with various etiologies.[11;12]

This study was set up to compare the efficacy of the RPE-choroid graft surgery to (continuation of) anti-VEGF therapy in a controlled manner, for these three subgroups of patients with exudative AMD. Sample size calculation for a randomized intervention study with three groups and two possible treatments, required at least 240 patients. We expected that one or two eligible patients would present per month at the REH. We therefore set up a prospective, international multicenter, randomized intervention study: "Anti-VEGF (bevacizumab/ranibizumab) versus RPE-choroid graft in the treatment of 1) non-responders to three intravitreal anti-VEGF injections, or 2) patients with AMD and pigment epithelium rip, or 3) patients with AMD and massive hemorrhage. A randomized trial."

We included 20 patients at the REH in the period between October 2009 and March 2011. Enrollment at others sites however failed to start; therefore the study was prematurely ended as it was unlikely that we would reach the inclusion target within a reasonable time span. In the course of the treatment of the 20 patients that did enter the study, we gathered valuable experience and knowledge about RPE-choroid grafts,

anti-VEGF treatment and submacular hemorrhage removal surgery. In this paper we will describe the results of anti-VEGF treatment versus RPE-choroid graft surgery in a limited cohort.

PATIENTS AND METHODS

Patients

Inclusion criteria were: Visual acuity (VA) loss of ≥15 letters on the Early Treatment of Diabetic Retinopathy Study (ETDRS) chart after at least three anti-VEGF injections (referred to as 'non-responder'); or subfoveal RPE tear; or a massive submacular hemorrhage (i.e., extending beyond the vascular arcades and/or a subfoveal thickness of at least 1.0 mm and no longer eligible for rtPA treatment);[13] visual acuity between 20/63 and 20/800; age 50 years or older.

Exclusion criteria were: the use of anticoagulant drugs which could not be discontinued for six weeks (two weeks before and four weeks after the RPE-choroid graft surgery); hemorrhage or choroidal neovascularization (CNV); secondary pathology other than AMD (such as retinal angiomatous proliferation, retinal macro aneurysm, CNV associated with high myopia, or polypoidal choroidopathy); current acute ocular or peri-ocular infection; any major surgical procedure (scheduled) within one month of study entry, not related to this study (except cataract surgery); known allergy for fluorescein angiography (FA) or indocyanine green angiography (ICGA); significant other ocular disorders affecting visual acuity, other than glaucoma.

Study Design

This study was started as a prospective, international, multicenter, randomized intervention study. This study is registered at www.trialregister.nl: "Anti-VEGF (bevacizumab/ranibizumab) versus RPE-choroid graft in the treatment of 1) non-responders to three intravitreal anti-VEGF injections, or 2) patients with AMD and pigment epithelium rip, or 3) patients with AMD and massive hemorrhage. A randomized trial."

As it was anticipated that the RPE-choroid graft would provide better results than anti-VEGF therapy, a single-sided test was chosen for the analysis to demonstrate significance. Sample size therefore was calculated for each subgroup separately, with $\alpha=0.05$ and $P=0.80$. Group size for the non-responders had to be 76, RPE tears 60 and hemorrhages 76, for a total of 212 patients. Allowing about 10% withdrawal during the study, the total number of patients needed was set at 240.

All patients were randomized after determination of eligibility and immediately after written consent of the patient was received. For each separate subgroup, patients were

assigned to either the RPE-choroid graft translocation or (continuation of) anti-VEGF treatment at a 1/1 ratio. The anti-VEGF treatment followed the regimen as described in the Prospective Optical Coherence Tomography Imaging of Patients with Neovascular AMD Treated with Intraocular Ranibizumab (PrONTO) study.[14] Per subgroup, lots of six envelopes were prepared (3x RPE-choroid graft and 3x anti-VEGF), one of which was randomly drawn after inclusion of a patient.

It has been shown that patients with a large subretinal hemorrhage experience greater benefit from removal of the hemorrhage than no treatment (Bressler et al. reported less risk of >6 lines visual acuity loss).[5] This subgroup of patients was therefore randomized between hemorrhage removal by forceps after vitrectomy, with subsequent anti-VEGF treatments according to the PrONTO protocol, and removal of the hemorrhage combined with an RPE-choroid graft transplantation.

Subsequently, there were six treatment groups. Group 1: Submacular hemorrhage: RPE-choroid graft. Group 2: RPE tear: RPE-choroid graft. Group 3: Non-responder: RPE-Choroid graft. Group 4: Submacular hemorrhage: Hemorrhage removal surgery and anti-VEGF therapy if necessary. Group 5: RPE tear: Anti-VEGF therapy. Group 6: Non-responder: Anti-VEGF therapy.

All examinations and surgical procedures were performed at the Rotterdam Eye Hospital, The Netherlands. All patients provided informed consent for the surgical procedure and the preoperative and postoperative examinations, in accordance with the tenets of the Declaration of Helsinki. The study was approved by the Medical Ethical Committee of the Erasmus University, The Netherlands.

Study Objectives

The primary study objective was to compare visual acuity (distance and reading acuity) and foveal fixation of RPE-choroid graft translocation versus intravitreal anti-VEGF therapy, at 12 and 24 months' follow-up. The secondary objective was to compare safety of both treatments and quality of life.

Examinations and Treatment Regimens

Visual acuity (VA) was measured in logMAR (logarithm of minimal angle of resolution) with an ETDRS chart, and reading acuity was tested in logRAD (Reading Acuity Determination), with the Radner visual acuity chart.[15] Conversions to logMAR were done according to Holladay's conversion method in which finger counting at 60 cm was transposed to logMAR 2 and hand motion to logMAR 3.[16] Fixation was tested with a microperimeter (MP-1; Nidek Technologies, Padova, Italy). Sine Amsler charts (SAC) were used to evaluate metamorphopsia.[17] Other imaging was performed using spectral domain optical coherence tomography (SD-OCT) (Spectralis HRA; Heidelberg Engineering, Heidelberg), fourier domain optical coherence tomography (RTVue-100,

Optovue Inc., Frement, CA) fundus photography (TRC 50 DX, Type 1A, TOPCON, Tokyo, Japan), Microperimeter (MP-1), autofluorescence imaging (AF) (TRC 50 DX, Type 1A, TOPCON, Tokyo, Japan or Spectralis HRA; Heidelberg Engineering, Heidelberg), FA and/or ICGA (TRC 50 DX, Type 1A, TOPCON, Tokyo, Japan), multifocal electroretinogram (mfERG) (Espion E2, Diagnosys LLC, Lowell, USA) and ultrasound (Xario, Toshiba Medical Systems Corporation, Tochigi, Japan). These other examination techniques were performed to evaluate retinal sensitivity and to analyze different features of patients receiving an RPE-choroid graft transplantation or intravitreal anti-VEGF injections.

At the baseline visit, patient eligibility was assessed and all patients were tested for near and distance VA, fixation ability and grade of metamorphopsia. During the same examination, fundoscopy and SD-OCT images were obtained. When possible, microperimetry, color fundus photography, mfERG, AF, FA and ICGA were performed. Patients with submacular hemorrhage were also examined using ultrasound. Visual Function Questionnaire-25 (VFQ-25) forms were completed, as part of a quality of life assessment.

Patients who had received an RPE-choroid graft (groups 1, 2 and 3) were examined regularly during the first weeks after surgery, during which interval between visits was established at the surgeon's (J.v.M.) discretion. Each postoperative examination included slit lamp examination, tonometry, fundoscopy and SD-OCT. At 6 months, distance visual acuity was measured and, when possible, microperimetry, fixation, fundus photography, AF and SD-OCT were performed.

Patients who were randomized into the anti-VEGF group (groups 4, 5 and 6) were treated according to the PrONTO protocol.[14] Patients in the RPE tear: anti-VEGF (group 5) and non-responder: anti-VEGF groups (group 6) received the first three monthly injections of 1.25 mg bevacizumab (Avastin®, Genentech Inc., San Francisco, CA) in 0.05 ml. shortly after inclusion into the study. After those three injections, the patients were examined monthly with fundoscopy and fourier domain OCT and received an anti-VEGF injection at the same visit when indicated. Patients in the submacular hemorrhage: anti-VEGF group (group 4) were examined each month after submacular hemorrhage removal surgery and received three monthly intravitreal injections when one or more of the PrONTO criteria were met for the first time after surgery. At 6 months, a VA (distance) and, if possible, microperimetry and fixation test were performed.

For all patients, at one year after surgery, VA (distance and reading), SD-OCT, AF, microperimetry, fixation test, mfERG, SAC score, VFQ-25 and fundus photography were repeated if possible. If recurrent choroidal neovascularization or other choroidal or retinal pathology was suspected any time after inclusion in either the RPE-choroid graft or the anti-VEGF group, an additional FA and/or ICGA exam was performed.

Surgery

For the patients included in the RPE-choroid graft group, a full-thickness translocation of autologous midperipheral RPE, Bruch's membrane, choriocapillaris, and choroid was performed. As described previously,[18;19] two surgical procedures were possible. In short, the first consisted of the creation of a small retinectomy at the raphe followed by the positioning of a midperipheral graft under the macula using bent forceps; the second consisted of the creation of a large (180°) peripheral temporal retinotomy followed by the placement of a free graft of RPE and choroid over the macula area. At the end of the surgery, 5000 centistokes (cSt) silicone oil was used as tamponade. The silicone oil was removed approximately three months after the first surgery, and during this procedure the internal limiting membrane (ILM) was peeled to prevent formation of a macular pucker. Lensectomy or phacoemulsification was performed during the first procedure in phakic patients. Insertion of the intraocular lens was performed during first or second surgery.

Patients with a submacular hemorrhage who were randomized to the anti-VEGF treatment group were scheduled for hemorrhage removal surgery prior to the start of the PrONTO regimen. The surgical technique for these patients included the induction of a posterior vitreous detachment and a complete vitrectomy. A small paramacular temporal retinotomy was made to inject a balanced salt solution into the subretinal space through a 28-gauge subretinal cannula. The macular retina was thereby separated from the RPE and blood/CNV membrane, and the subretinal hemorrhage and CNV membrane were removed from the subretinal space with Thomas subretinal forceps (Dutch Ophthalmic Research Center [DORC], Zuidland, the Netherlands). The ILM was peeled and silicone oil (5000 cSt) or gas (C3F8) was used as intraocular tamponade. Phacoemulsification or lensectomy, followed by the insertion of an intraocular lens were performed during either the first or the second procedure (silicone oil removal) in case of phakic patients. All surgical procedures were performed by one surgeon (J.v.M.).

Intravitreal Anti-VEGF Injection

All patients in the anti-VEGF study group received the following therapy: intravitreal injection of 1.25 mg bevacizumab (Avastin®, Genentech Inc., San Francisco, CA) in 0.05 ml. Indications for injections were based on the PrONTO protocol: vision loss of five letters or more, associated with fluid detected by OCT examination; increase in central retinal thickness of 100 microns or more as compared to baseline; new-onset hemorrhage; new classic CNV; or persistent fluid following last injection.[14]

RESULTS

Patients

Twenty patients met the inclusion criteria and were randomized to the following groups: Group 1: Submacular hemorrhage: graft (n = 6). Group 2: RPE tear: graft (n = 3). Group 3: Non-responder: graft (n = 1). Group 4: Submacular hemorrhage: anti-VEGF (n = 5). Group 5: RPE tear: anti-VEGF (n = 3). Group 6: Non-responder: anti-VEGF (n = 2).

RPE-Choroid Graft Groups Patient Characteristics

The mean age of the ten patients in the three graft groups was 78.6 years (standard deviation [SD] 10.4). Median duration of vision loss as reported by the patient was 86 days (range 18-365). Median preoperative VA was 0.75 logMAR (range 0.46 - 2.8). Four patients were able to perform the Radner reading acuity test, with a mean of 0.68 logRAD (SD 0.18). Three patients used intra-ocular pressure-lowering eye drops. Two patients were insulin-dependent diabetics, one patient was a tablet-dependent diabetic. Four patients were on anti-coagulant therapy (three on platelet aggregation inhibitors; one on coumarin therapy).

All but one patient underwent the surgical technique with a temporal raphe retinotomy. The only patient who underwent the 180° peripheral temporal retinotomy was in the submacular hemorrhage group. Five patients were phakic before surgery: a phacoemulsification or lensectomy was performed during the first surgery. The intraocular lens was inserted in three patients during the first operation and in two patients during the second one.

One year postoperatively, one patient was lost to follow-up due to a cerebral-vascular accident and consequent inability to adhere to the study protocol. Median VA of the remaining nine patients was 1.48 logMAR (range 0.14-2.8). In two patients the VA increased more than two ETDRS lines. In Table 1 the mean visual acuity change expressed in visual acuity score (VAS) over one year of treatment are displayed. Two patients were able to perform the Radner test, with a mean of 0.775 logRAD (SD 0.78). Three patients required anti-VEGF injections after surgery, with a median of 1 injection (range 1-2). More detailed patient characteristics can be found in Table 2.

Anti-VEGF Groups Patient Characteristics

The mean age of the ten patients in the three anti-VEGF groups was 83.8 years (SD 8.2). Median duration of vision loss was 64 days (range 28-365). Median VA at inclusion was 1.36 logMAR (range 0.58-1.6). Six patients were able to perform the Radner reading acuity test, with a mean of 1.16 logRAD (SD 0.37). Six patients were on anti-coagulant therapy (all platelet aggregator inhibitors). Eight patients required anti-VEGF injections, receiving a median of 8.5 injections (range 3-12). The two patients who did not receive injections

Table 1: Mean visual acuity change

	Inclusion VA (VAS)	1 Year follow-up VA (VAS)	Mean number of ETDRS letters gain or loss	Number of patients with >10 ETDRS letters gain/loss or stable after 1 year
RPE-choroid Graft	36	18	-15 (range -54 to +26)	Gain: 2 Loss: 4 Stable: 3 LFO: 1
Anti-VEGF	24	18	-8 (range -26 to +6)	Gain: 0 Loss: 3 Stable: 6 LFO: 1

VA = visual acuity; VAS = visual acuity score; ETDRS = Early Treatment Diabetic Retinopathy Study; RPE =Retinal pigment epithelium; LFO = Lost to Follow-up; Anti-VEGF = anti-vascular endothelial growth factor

were two patients from the hemorrhage group; after removal of the hemorrhage there was no sign of new hemorrhage or macular edema for which anti-VEGF was indicated.

After one year, one patient was lost to follow-up (deceased due to unrelated causes). Median VA of the remaining nine patients was 1.42 logMAR (range 0.44-1.66). No patients experienced VA improvement of two ETDRS lines or more. The visual acuity expressed in VAS and the mean number of ETDRS letters and patients with gain or loss over the year are displayed in Table 1. Five patients were able to perform the Radner test, with a mean of 1.28 logRAD (SD 0.43). More detailed patient characteristics can be found in Table 2.

Visual Function Tests

Only ten patients could perform the Radner test and 14 could perform the SAC test at baseline, one-year follow-up, or both. The VFQ-25 test was performed both at baseline and at one year by 14 patients, four of which were incomplete. For six patients, either the first or second test was not completed. Multifocal ERG was performed both at baseline and at one year for five patients. For 11 patients, the test was performed only once, and in four the test was not or could not be performed at any time. Because of the inconsistent completion of these evaluations, neither the VFQ-25 nor the multifocal ERG tests were further analyzed.

For the ten patients for whom reading acuity and grade of metamorphopsia (Radner and SAC) could be evaluated, the correlation between SAC and reading acuity was weak.

A distance VA increase of at least one ETDRS line resulted in SAC improvement in two patients, and VA decrease resulted in SAC score worsening in four patients. A VA increase and SAC score decrease, however, occurred in two patients, and a VA decrease and SAC score increase in one patient. In five patients, VA remained stable but SAC score changed, of which three worsened and two improved.

Table 2. Patient Characteristics

Patiënt Number	Male/ Female	Age at intervention	Eye	Days loss of vision	Lens	Anticoagulants	Relevant interventions before inclusion	Relevant comorbidities	logMAR at inclusion
Group 1: Submacular Haemorrhage: Graft									
2	F	82	OD	35	Phakia	platelet aggregator inhibitor	None	Insulin dependent diabetic	2,8
5	F	79	OD	26	Phakia	anticoagulant therapy	Trabeculectomy for glaucoma	Glaucoma	1,16
7	F	82	OD	60	Pseudophakia	no	3 anti-VEGF injections	Glaucoma	0,8
9	M	75	OS	28	Phakia	no	4 anti-VEGF injections and photodynamic therapy	No	0,62
12	F	79	OD	129	Pseudophakia	no	3 anti-VEGF injections	No	1,44
21	F	90	OS	18	Pseudophakia	no	None	No	1,56
Group 2: RPE-TEAR: Graft									
1	F	85	OD	365	Pseudophakia	no	7 anti-VEGF injections	Glaucoma	0,46
13	F	86	OS	153	Pseudophakia	no	2 anti-VEGF injections	Tablet dependent diabetic	0,58
17	F	76	OD	112	Phakia	platelet aggregator inhibitor	3 anti-VEGF injections	Thyroid medication	0,7
Group 3: Non-Responder: Graft									
10	M	52	OD	213	Phakia	platelet aggregator inhibitor	5 anti-VEGF injections	Insulin dependent diabetic	0,66
Group 4: Submacular Haemorrhage: Anti-VEGF									
4	M	70	OS	28	Phakia	platelet aggregator inhibitor	None	No	0,9
14	M	93	OD	51	Pseudophakia	platelet aggregator inhibitor	12 anti-VEGF injections, followed by a rtPA injection for a hemorrhage which did not replace.	No	1,46
15	F	86	OS	76	Phakia	platelet aggregator inhibitor	None	No	1,6
16	M	86	OS	40	Pseudophakia	no	21 anti-VEGF injections, atrophia and 9 months before surgery a submacular hemorrhage developed which displaced partially without intervention; followed by an increase/ new hemorrhage.	No	1,16
20	M	90	OD	108	Pseudophakia	no	None	No	1,58
Group 5: RPE-TEAR: Anti-VEGF									
3	F	85	OS	153	Phakia	no	5 anti-VEGF injections	No	0,58
8	F	82	OS	42	Phakia	no	11 anti-VEGF injections	No	1,36

logMAR 6 months	logMAR 1 year	VAS at inclusion	VAS 6 months	VAS 1 year	Radner at inclusion	Radner 1 year	Sine Amsler Score at baseline	Sine Amsler Score 1 year	Foveal fixation baseline	Foveal fixation 6 months	Foveal fixation 1 year	Number of anti-VEGF injections after 1 year
LP+	2,8	0	0	0	CG	CG	CG	CG	Yes	CG	CG	2
1,4	0,9	26	NI	38	CG	1,325	0	2	Yes	Yes	Yes	None
1,5	2,3	43	10	0	CG	CG	CG	CG	No	Yes	NI	None
1,5	2,3	54	13	0	CG	CG	0	CG	Yes	CG	Yes	1
NI	1,46	13	NI	12	CG	CG	1	0	No	NI	Yes	None
1,68	1,48	7	1	11	CG	CG	CG	CG	Yes	Yes	no	None
0,8	LFO	60	NI	LFO	0,445	LFO	CG	LFO	Yes	Yes	LFO	None
1,32	1,1	55	19	26	0,7	CG	1	3	Yes	No	No	None
2,8	1,8	50	0	0	0,695	CG	5	CG	No	CG	No	1
0,32	0,14	52	66	78	0,89	0,225	5	2	Yes	Yes	Yes	None
1,22	1,42	40	21	14	0,985	1,305	0	4	No	No	No	None
1,5	LFO	11	0	LFO	1,5	LFO	4	LFO	No	CG	LFO	None
1,66	1,48	5	2	10	CG	CG	CG	2	CG	No	No	3
1,62	1,66	25	4	2	CG	CG	2	0	No	No	No	8
1,8	1,66	6	3	2	CG	CG	1	2	No	CG	CG	3
0,8	0,44	56	43	62	0,82	0,53	1	2	No	No	No	10
1,16	1,4	17	27	15	1,47	1,5	3	1	Yes	Yes	Yes	12

Table 2. Patient Characteristics *(continued)*

Patiënt Number	Male/ Female	Age at intervention	Eye	Days loss of vision	Lens	Anticoagulants	Relevant interventions before inclusion	Relevant comorbidities	logMAR at inclusion
18	M	83	OS	365	Phakia	platelet aggregator inhibitor	3 anti-VEGF injections	No	0,62
Group 6: Non-Responder: Anti-VEGF									
11	F	70	OD	49	Pseudophakia	platelet aggregator inhibitor	7 anti-VEGF injections	Graves's Orbitopathy	1,36
19	F	93	OD	335	Pseudophakia	platelet aggregator inhibitor	1 anti-VEGF injection, followed by rtPA injection for a new hemorrhage, followed by 3 anti-VEGF injections.	No	1,58

VAS = visual acuity score; CG = Cannot Grade; NI = No information; LFO = lost to follow-up; LP+ = light perception positive; logMAR = logarithm of minimal angle of resolution; Anti-VEGF = anti-vascular endothelial growth factor. Patient 6 was excluded after randomization but before inclusion as the patient was found to be emotionally and physically unable to participate in the study.

The Radner score correlated slightly better to VA change; Radner score improved with improvement of VA in three patients, decreased with decrease of VA in four patients and VA and Radner both remained stable in one. In two patients VA remained stable, while Radner improved in one and decreased in the other.

Five Patients of Interest Highlighted

Three RPE-choroid Graft Patients

Patient 10 presented as a non-responder to anti-VEGF treatment, with a preoperative VA of 0.66 logMAR. Six weeks after uncomplicated RPE-choroid graft surgery, VA increased slightly to 0.56 logMAR. Six months after surgery, VA had increased to 0.32 logMAR, and after one year to 0.14 logMAR. Sine Amsler score improved from 5 to 2. Microperimetry sensitivity values clearly increased after surgery, especially in the macular area (Figure 1). No postoperative complications occurred and anti-VEGF therapy was no longer required.

Patient 13 presented with an RPE tear and a visual acuity of 0.58 logMAR (Figure 2). After an apparently uncomplicated surgery and despite a well revascularized graft (as confirmed by SD-OCT),[18] visual acuity worsened. SD-OCT revealed sub-foveal perfluorocarbon which had probably moved unnoticed under the graft during surgery (Figure 2 E). VA decreased to 1.1 logMAR one year postoperatively. Preoperative microperimetry showed low sensitivity values in the area of the RPE tear temporal to the macula. One year after surgery, microperimetric sensitivity values were low in the

logMAR 6 months	logMAR 1 year	VAS at inclusion	VAS 6 months	VAS 1 year	Radner at inclusion	Radner 1 year	Sine Amsler Score at baseline	Sine Amsler Score 1 year	Foveal fixation baseline	Foveal fixation 6 months	Foveal fixation 1 year	Number of anti-VEGF injections after 1 year
0,84	0,8	54	36	35	0,685	1,525	5	5	Yes	No	No	9
1,38	1,38	17	16	16	CG	1,515	0	5	No	No	No	7
1,54	1,64	6	8	2	1,5	CG	0	3	No	No	No	9

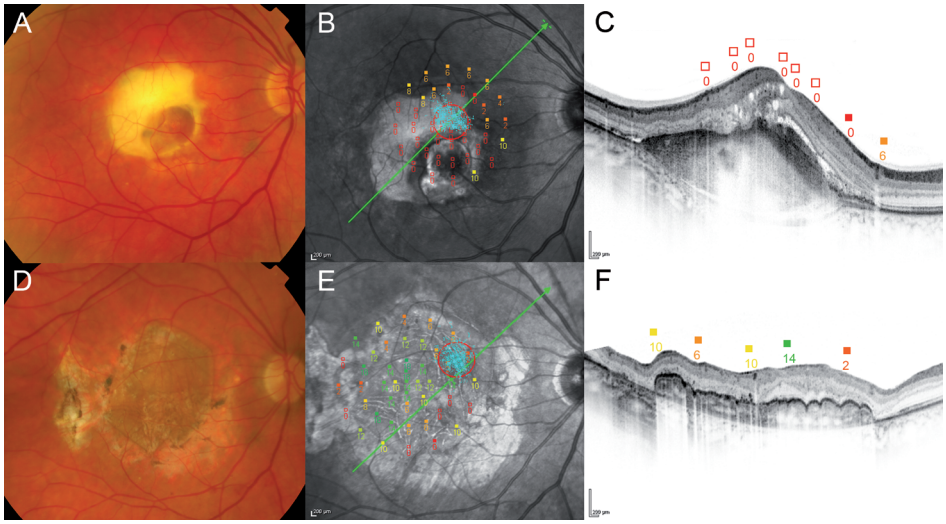

Figure 1. Successful RPE-choroid graft surgery for a non-responder to Anti-VEGF therapy. Color fundus photographs (**A, D**), infrared (IR), fundus photographs with microperimetry sensitivity values (**B, E**), and spectral domain optical coherence tomography (SD-OCT) (**C,F**) images of patient 10. Pre-operative (**A, B, C**), and one year after RPE-choroid graft translocation surgery (**D, E, F**). This patient presented as a non-responder to anti-VEGF treatment. **B, E**: A macular, 12°, 10-dB pattern, with 45 test loci and a 2° circle as fixation target, centered on the macula was tested automatically with a 4-2 staircase threshold strategy, with white Goldman III stimulus displayed for 2000 milliseconds. The brightness of the test stimuli ranged from 0 to 20 decibels (400 to 4 asb). Mean sensitivity preoperatively was 1.8 dB, with a minimum of 0 dB and a maximum of 10 dB. Mean sensitivity postoperatively was 8.5 dB, with a minimum of 0 dB and a maximum of 16 dB. The visual acuity was 0.66 logMAR preoperatively and 0.14 logMAR one year postoperatively. **B, C, E, F**: The retinal sensitivity values and the green line on the IR image were placed after superimposing the microperimetry results on the matching IR images with the Nidek MP-1. The retinal sensitivity values in the SD-OCT images were placed manually after superimposing the microperimetry results on the matching SD-OCT, AF and IR images for location reference. Microperimetry sensitivity values increased markedly after surgery, especially in the macular area. The retinal layers, including in the fovea, appear to be intact.

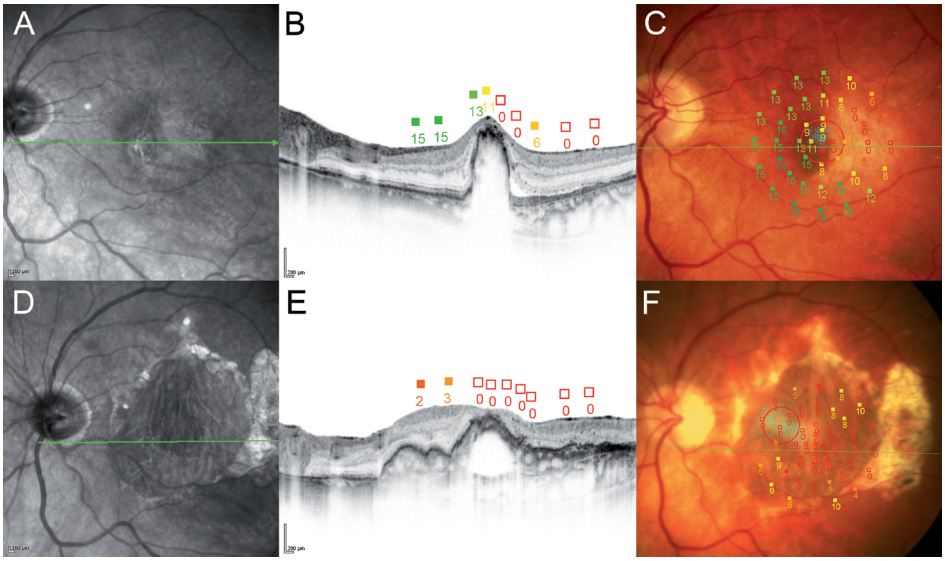

Figure 2. RPE-choroid graft surgery for an RPE- tear, complicated by perfluorocarbon bubble under the graft. Infrared (IR) (**A,D**), spectral domain optical coherence tomography (SD-OCT) (**B, E**), and microperimetry images (**C, F**), of patient 13, pre-operatively (**A, B, C)**, and one year after graft surgery (**D, E, F**). Patient 13 presented with an RPE-tear and a visual acuity of 0.58 logMAR. VA did not increase as expected, despite apparently uncomplicated surgery and a well revascularized graft postoperatively. Unfortunately, as visualized by SD-OCT, a perfluorocarbon bubble had gone unnoticed under the graft during surgery (**E**). VA one year postoperative was 1.1 logMAR.

C, F: A macular, 12°, 10-dB pattern, with 45 test loci and a 2° circle as fixation target, centered on the macula was tested automatically with a 4-2 staircase threshold strategy, with white Goldman III stimulus displayed for 200 milliseconds. The brightness of the test stimuli ranged from 0 to 20 decibels (400 to 4 asb). Mean sensitivity preoperatively was 9.8 dB, with a minimum of 0 dB and a maximum of 19 dB. Mean sensitivity postoperatively decreased to 2.1 dB, with a minimum of 0 dB and a maximum of 10 dB. **B, C, E, F**: The green line in the microperimetry image and the retinal sensitivity values in the SD-OCT images were placed manually after superimposing microperimetry results on the matching SD-OCT and IR image for location reference. Before surgery the microperimetric sensitivity values were low in the area of the RPE-tear; the temporal side of the macula. After surgery, the microperimetric sensitivity values decreased especially in the macular area where the perfluorocarbon bubble was located. The inferonasal and superotemporal side of the graft did display retinal function, as detected by microperimetry. Pre-operatively fixation was located in the fovea while one year postoperatively fixation had shifted from macular to a new preferred retinal locus superior and nasal to the macula, most likely because of the decreased function of the macula due to the perfluorocarbon bubble.

macular area where the perfluorocarbon bubble was located, while the inferonasal and superotemporal side of the graft demonstrated some retinal sensitivity. This patient also showed a shift of fixation, most likely due to the perfluorocarbon bubble under the macula: pre-operatively the fixation was centered in the fovea, one year postoperatively it had shifted to a new preferred retinal locus[20] of fixation more superior and nasal to the macula.

Patient 17 presented with an RPE tear and a visual acuity of 0.7 logMAR. After uncomplicated surgery, the graft was located under the fovea and appeared normal on SD-OCT at the two-week follow-up (Figure 3). At four weeks, the graft was well revascularized with an intact fovea as visible on SD-OCT. During the oil removal surgery at three months, a new submacular hemorrhage was found. Recombinant tissue plasminogen activator and anti-VEGF were administered during the same procedure. Nevertheless, hemorrhage and fluid were still present five months after the primary surgery. At the one year follow-up visit, SD-OCT showed a damaged neurosensory retina, with fibrosis under the graft. Visual acuity had decreased to 1.8 logMAR.

Two anti-VEGF Patients

Patient 3 presented with an RPE tear and a visual acuity of 0.58 logMAR. On SD-OCT the fovea appeared to be anatomically well preserved. However, there was a persistent detachment of the neurosensory retina, (Figure 4). The patient received 10 anti-VEGF injections during the first year of follow-up, and VA improved to 0.44 logMAR. However, Sine Amsler score deteriorated from 1 to 2. The submacular fluid completely disappeared but intraretinal cysts located at the fovea were visible at last follow-up.

Patient 15 presented with a large submacular hemorrhage and a VA of 1.6 logMAR. The hemorrhage was removed during surgery and was followed by three anti-VEGF injections for a micro hemorrhage and VA decline seven months after surgery, according to PrONTO criteria.[14] One year postoperatively the VA was 1.48 logMAR. After surgery almost no sensitivity could be detected with microperimetry. The RPE of the area where the hemorrhage was removed disappeared and the overlying retina became atrophic (Figure 5).

Complications

Severe postoperative complications occurred with an unexpectedly high frequency. In the RPE-choroid graft group, recurrent retinal detachment due to PVR developed in three out of ten patients, severe hypotony in two (one without PVR) and recurrent submacular hemorrhage in three patients (two without PVR). In earlier reports, we experienced a 10-15,9% incidence of PVR, and 10% incidence of recurrent CNV.[12;21] A cerebral-vascular accident occurred in one patient. In the anti-VEGF group, a recurrent retinal detachment due to PVR developed in one out of ten patients, a transient ischemic attack was experienced in two, and a minor stroke occurred in one patient (Table 3).

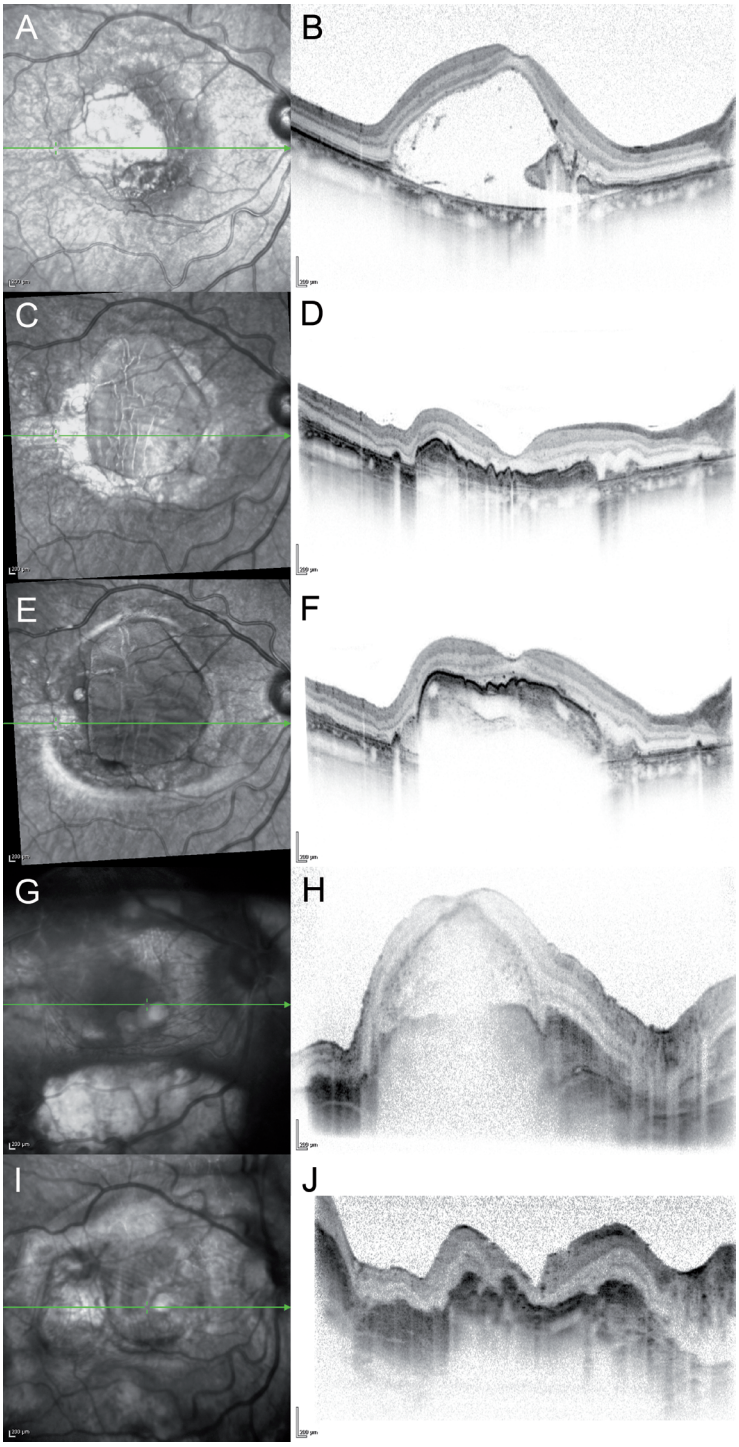

Figure 3.

Table 3. Complications

Patient Number	Ocular complications	Non-ocular complications
Group 1: Submacular Hemorrhage: Graft		
2	Recurrent SMH, new CNV, PVR, recurrent RD due to PVR.	None
5	None	None
7	PVR, recurrent RD due to PVR twice. Permanent silicone oil tamponade.	None
9	Hypotony. RD with PVR. Permanent silicone oil tamponade.	None
12	Recurrent SMH.	None
21	Vitreous hemorrhage and pucker. Hypotony. Permanent silicone oil tamponade.	None
Group 2: RPE-Tear: Graft		
1	None	Cerebral Vascular Accident
13	Small Perfluorocarbon bubble under the graft.	None
17	Recurrent SMH.	None
Group 3: Non-Responder: Graft		
10	None	None
Group 4: Submacular Hemorrhage: Anti-VEGF		
4	None	None
14	PVR. Recurrent RD twice.	None
15	None	TIA
16	None	Minor stroke
20	None	None
Group 5: RPE-Tear: Anti-VEGF		
3	None	None
8	None	TIA
18	None	None
Group 6: Non-Responder: Anti-VEGF		
11	None	None
19	None	None

SMH = submacular hemorrhage; CNV = choroidal neovascularization; RD = retinal detachment; PVR = proliferative vitreoretinopathy; RPE = retinal pigment epithelium; Anti-VEGF = anti-vascular endothelial growth factor; TIA = transient ischemic attack. Patient 6 was excluded after randomization but before inclusion as the patient was found to be emotionally and physically unable to participate in the study.

Figure 3. RPE choroid Graft surgery for RPE-tear, complicated by new submacular hemorrhage. Infrared (**A, C, E, G, I**), and spectral domain optical coherence tomography (SD-OCT) (**B, D, F, H, J**) images of patient 17, pre-operatively (**A, B**), two weeks (**C, D**), four weeks (**E, F**), five months (**G, H**) and one year after graft surgery (**I, J**). This patient presented with an RPE-tear and a visual acuity of 0.7 logMAR. No complications occurred during surgery. Two weeks after surgery the graft was well centered at the posterior pole and the fovea appeared anatomically normal on SD-OCT (**D**)**.** At four weeks, on SD-OCT the graft appeared revascularized and the fovea apparently well preserved (**F**). During the oil removal surgery at three months, a new submacular hemorrhage was discovered. Recombinant tissue plasminogen activator and anti-VEGF were administered during the procedure but hemorrhage and fluid were still found at five months follow-up (**H**). One year after surgery the retina appeared damaged with fibrosis under the graft (**J**). VA decreased to 1.8 logMAR.

DISCUSSION

This international multicenter randomized intervention study was initiated in order to investigate whether patients with AMD and a submacular hemorrhage or a RPE tear or non-responders to anti-VEGF would benefit more from a free RPE-choroid graft transplantation surgery, than from (continuation of) anti-VEGF treatment. A sample size of 240 patients was needed to be able to detect potential significant difference between the graft and anti-VEGF groups. As we estimated that the recruitment rate would be at best 20 patients per year, we invited other centers to participate. Initially, the study group deemed this possible. During the study, we came to the realization that centers other than Rotterdam were unable to recruit patients, possibly because patients were reluctant to be randomized, the indication for surgery became less common, or that the surgical technique was not yet sufficiently convincing or familiar to surgeons in all the centers involved. We may conclude that this multicenter trial was started too early and expectations were unrealistic.

As the sample size would not be reached in a reasonable time span in the REH alone, the study was stopped. As a result, only 20 patients were included; ten patients in the RPE-choroid graft group, and ten in the anti-VEGF group, with each group subdivided

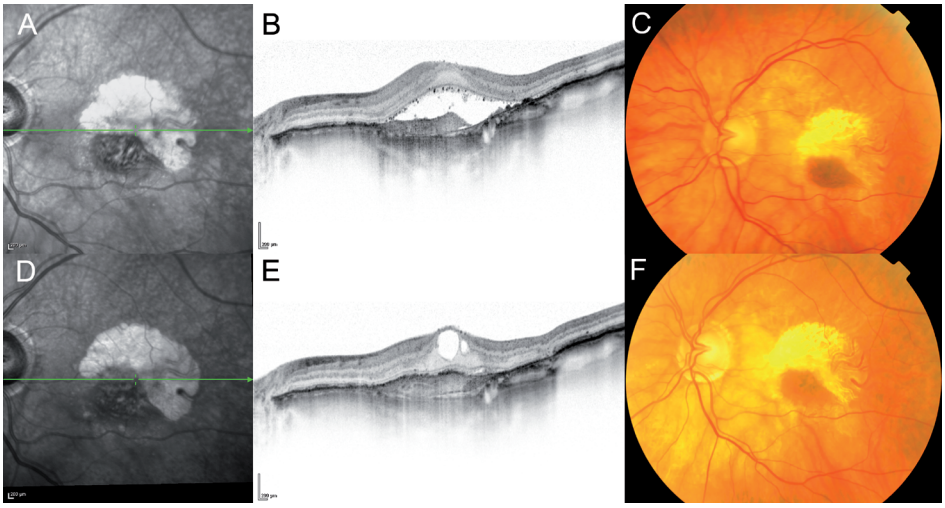

Figure 4. One year of anti-VEGF therapy. Infrared (**A, D**), spectral domain optical coherence tomography (SD-OCT) (**B, E**), and fundus images (**C, F**) of patient 3, pre-operatively (**A, B,C**), and one year after graft surgery (**D, E, F**). This patient initially presented with an RPE-tear and a visual acuity of 0.58 logMAR. On SD-OCT the fovea appeared to be still intact, (preserved external limiting membrane and probably internal segment outer segment junction) despite the presence of a neurosensory retinal detachment. (**B**). The patient received 10 anti-VEGF injections over a year, VA improved to 0.44 logMAR. The submacular fluid disappeared while some new intraretinal cysts were now visible. On a careful examination of this SD-OCT scan, some new RPE remnants located precisely under the fovea can be noticed.

into three subgroups: RPE tears, submacular hemorrhages, or non-responders to anti-VEGF. Because of the small size of these groups, only descriptive statistics are provided.

We have decided to report the single-center, one-year results of this randomized intervention study because it may illustrate the complications and shortcomings this surgery may have, even in this relative small group of patients. Unfortunately, the patients who underwent RPE-choroid graft experienced an unprecedentedly high number of complications, including unusual complications, compared to our earlier patient groups.[12;21]

Nevertheless, some remarkable results were found. One patient in the RPE-choroid graft group presented with an RPE tear and a VA of 0.66 logMAR. At the one-year postoperative follow-up visit, visual acuity had increased to 0.14 logMAR after one year, near reading acuity showed a marked increase, and improvements were seen in the SAC score and microperimetry sensitivity, especially in the foveal area (Figure 1).

Surgical hemorrhage removal without RPE-choroid graft transplantation is associated with complete RPE atrophy, absent retinal sensitivity and very low VA, as observed in patient 15 (Figure 5). VA however increased slightly from 1.6 logMAR preoperatively to 1.48 logMAR after one year. This correlates with the findings of the SST trial for submacular hemorrhages; the percentage of patients with a more than 6 lines VA

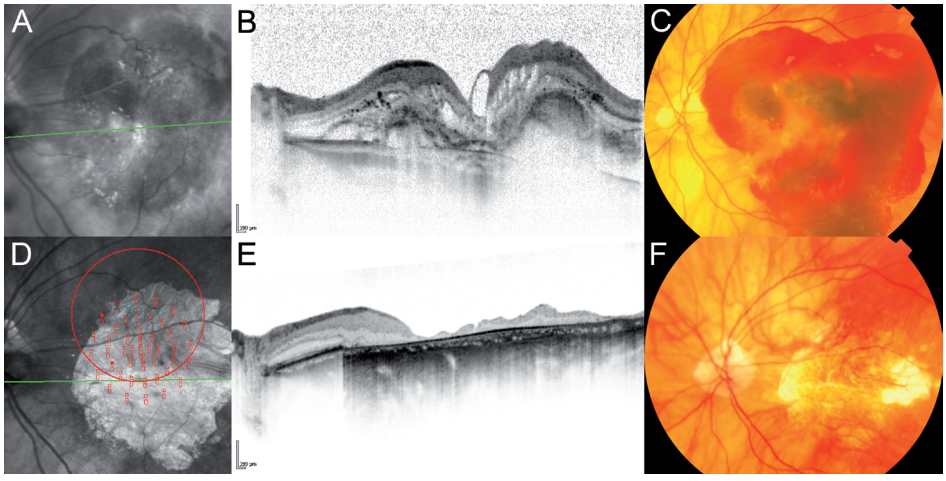

Figure 5. Atrophy after hemorrhage removal surgery and anti-VEGF therapy. Infrared (**A**), spectral domain optical coherence tomography (SD-OCT) (**B, E**), fundus (**C, F**) and infrared with microperimetry sensitivity values (**D**) images of patient 15, who presented with a large submacular hemorrhage. Pre-operatively (**A, B, C**) and one year after hemorrhage removal surgery and three subsequent anti-VEGF injections for a micro hemorrhage and VA decline (**C, D, E**). **D**: A macular, 12°, 10-dB pattern, with 45 test loci and a 8° circle as fixation target was tested automatically with a 4-2 staircase threshold strategy, with white Goldman III stimulus displayed for 200 milliseconds. The brightness of the test stimuli ranged from 0 to 20 decibels (400 to 4 asb). Mean sensitivity after one year was 0.0 dB. The visual acuity was 1.6 logMAR preoperatively and 1.48 logMAR one year postoperatively. After surgery almost no microperimetry sensitivity values could be detected. **E**: One year after hemorrhage removal the RPE had completely disappeared and the retina had become completely atrophic.

decrease was smaller in the group where the hemorrhage was surgically removed, compared to untreated controls.[5]

Combining imaging techniques may give valuable additional information; the perfluorocarbon bubble under the macula, visualized on SD-OCT in patient 13, corresponded to an area with less retinal sensitivity, and was associated with a shift in preferred retinal locus (Figure 2).

Several other intended functional outcome measures proved to be impractical or too difficult to perform. Multifocal ERG proved to be a very time-consuming and difficult examination technique for patients to complete, and it yielded no reproducible data when performed. The VFQ-25 questionnaires were inconsistently returned and/or incomplete.

Reading ability (Radner) and metamorphopsia grading (SAC) could be tested in ten patients, in whom reading score correlated slightly better than metamorphopsia grading with ETDRS scores.

We may conclude that the group of patients is too small to draw conclusions about whether anti-VEGF or an RPE-choroid graft is indicated for one of the three patient groups. Patients in the RPE-choroid graft group suffered more complications than usual. VA gain and loss may be obtained by both treatment methods. We suggest that greater VA gain might be possible for patients who undergo an RPE-choroid graft transplantation surgery than those who continue with anti-VEGF treatment alone. However, this may come at the risk of some severe complications.

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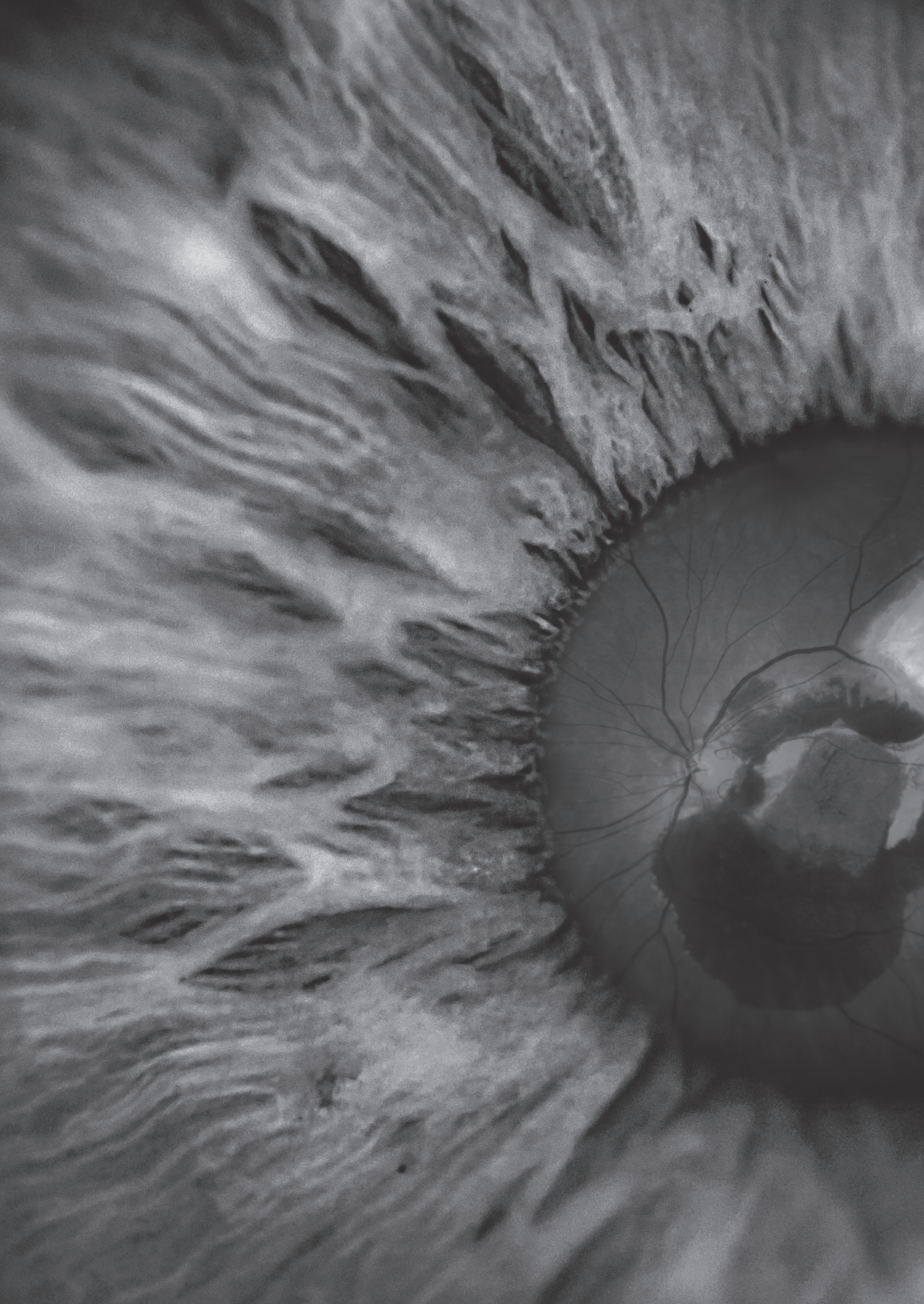

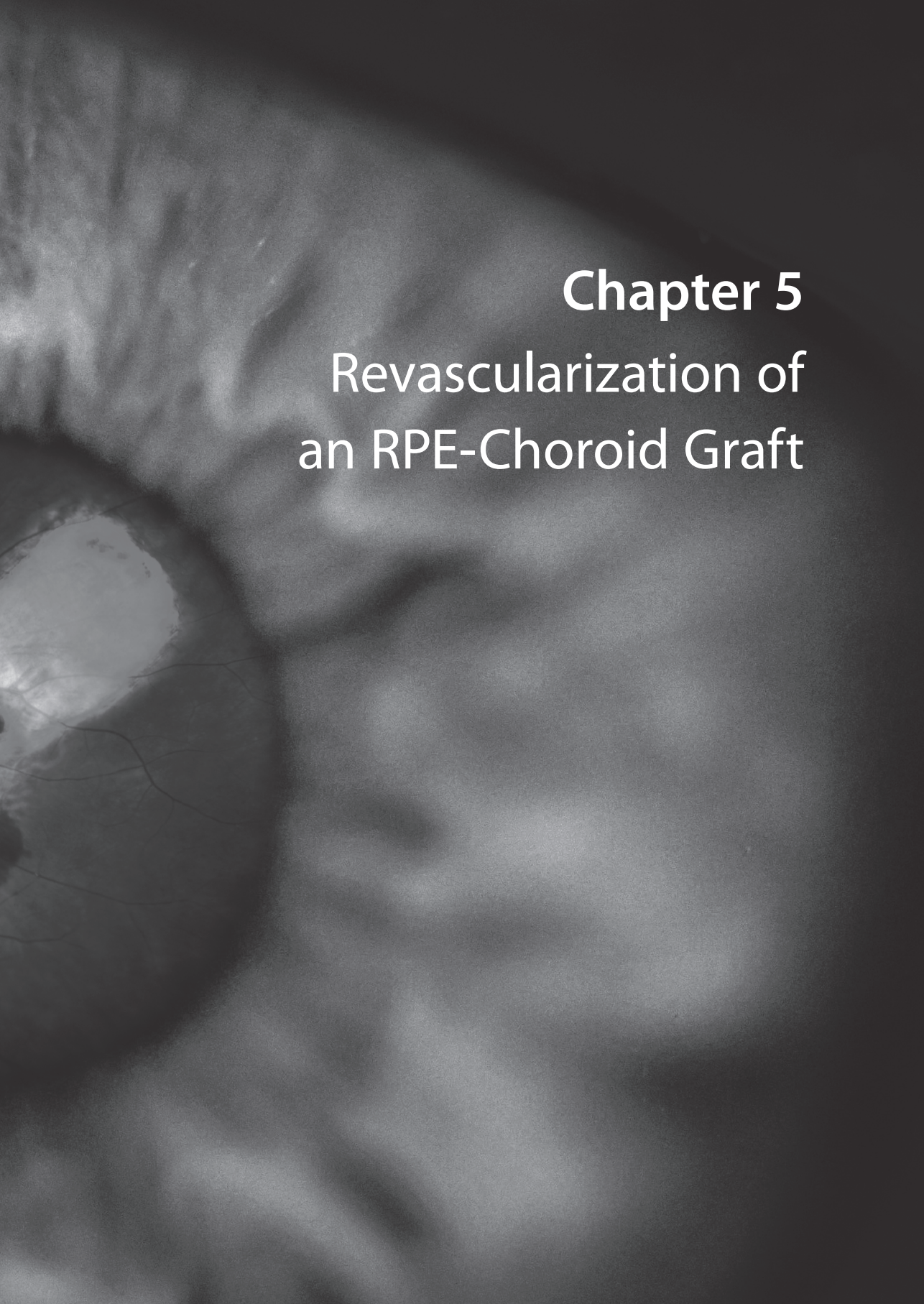

Chapter 5
Revascularization of an RPE-Choroid Graft

Chapter 5.1

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;52:5881-6

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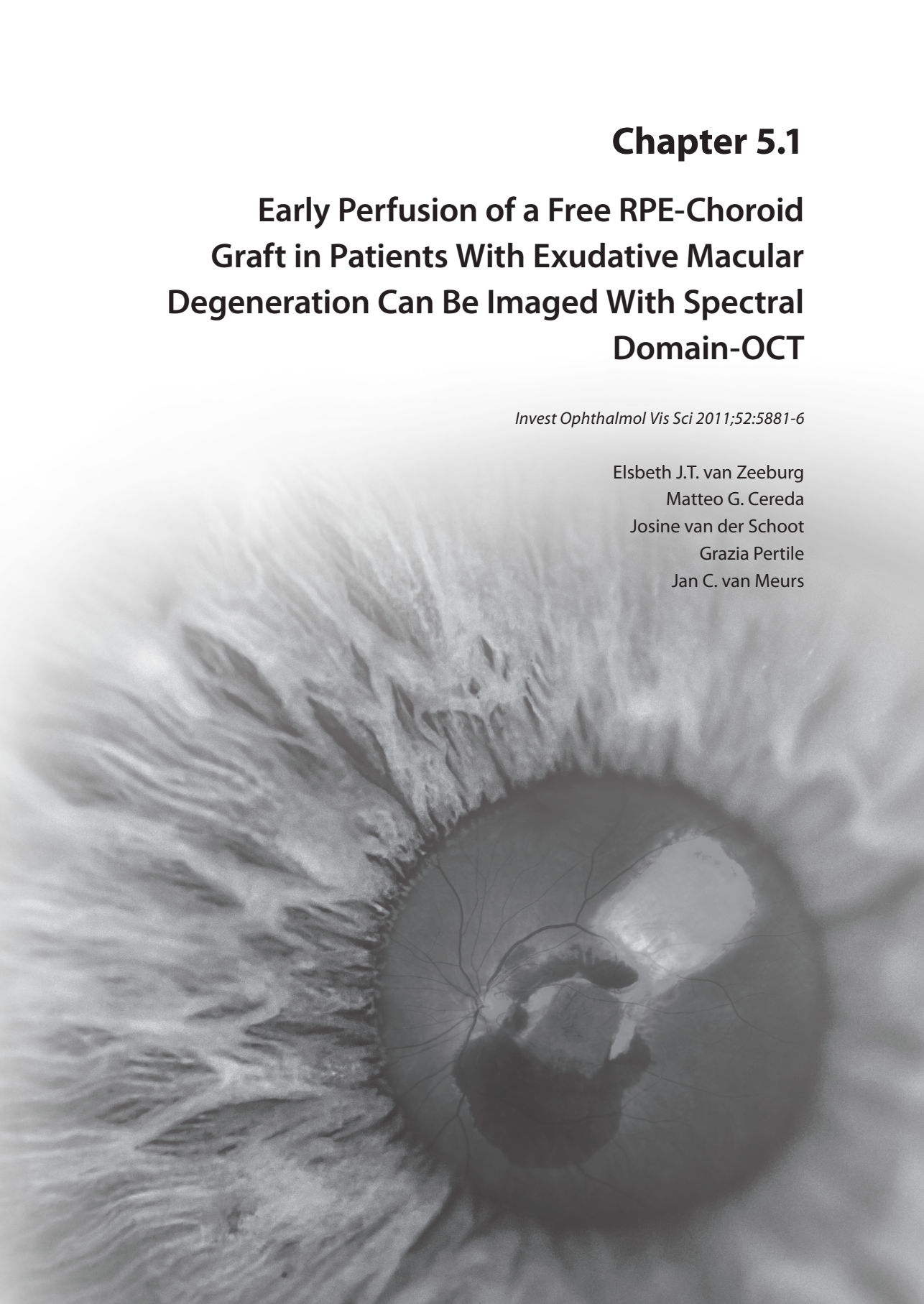

ABSTRACT

Purpose

To study early flow and revascularization in a free, autologous, retinal pigment epithelium (RPE)-choroid graft.

Methods

This prospective cohort study used spectral domain optical coherence tomography (SD-OCT) after RPE-choroid graft surgery in 12 patients. This SD-OCT was combined with fluorescein angiography (FA) and indocyanine green angiography (ICGA) in 5 patients.

Results

SD-OCT revealed that vessel diameter, number of vessels, and graft thickness increased in 10 of 12 patients, starting between 3 and 10 days after surgery. A subsequent decrease in thickness was found in all 10 patients, beginning as early as 8 days after surgery. Initially, the graft vessels were optically clearer than the underlying choroidal recipient vessels. Between 8 days and 30 days after surgery, the optically clear vessels became gray, similar to the recipient choroid. FA and ICGA revealed perfusion in 4 of 5 patients between postoperative days 6 and 15. Between postoperative days 12 and 60, the entire choroidal structure of the graft was visible on ICGA.

Conclusions

These data suggest that enlargement of vessel diameter, increase in the number of choroidal vessels, and graft thickening visualized by SD-OCT correspond with the ingrowth of afferent vessels, as demonstrated by ICGA. The subsequent establishment of efferent vessels results in flow, imaged as a change in color of the graft's vessels from optically clear to gray, graft thinning on SD-OCT, and complete revascularization on ICGA. SD-OCT, a non-invasive examination, can be used to demonstrate early graft perfusion in patients.

INTRODUCTION

The leading cause of irreversible legal blindness among senior citizens in the industrialized world is age-related macular degeneration (AMD). AMD is also the third most common cause of blindness worldwide.[1, 2] Exudative AMD involves choroidal neovascularization, whereby new choroidal blood vessels cross Bruch's membrane and grow into the space underneath the retinal pigment epithelium (RPE) and/or retina.[3, 4] These newly formed vessels are prone to leakage and bleeding into the submacular area; if left untreated, the condition can ultimately lead to irreversible damage to RPE cells and the retina.[5, 6]

In randomized controlled trials, intravitreal injections of anti-vacular endothelial growth factor (anti-VEGF) have been shown to be effective in patients with exudative AMD with classic, occult, and mixed choroidal neovascular membranes.[7, 8] However, patients with large hemorrhages and RPE tears were excluded from these studies. Additionally, these studies identified a small percentage of non-responders: patients who had lost ≥15 letters after 24 months of ranibizumab treatment[9] and patients with a 3-line drop after three injections, with no later improvement (unpublished data: Morse L, et al. 2-Year ANCHOR Study, World Ophthalmology Congress 2008, Hongkong). Other methods of treatment may be indicated for these three patient groups.[10-13]

In patients with a large hemorrhage, the submacular hemorrhage alone can be removed. Although results from the Submacular Surgery Trial Hemorrhage group showed no overall better outcome of blood removal in AMD patients compared with untreated patients, the percentage of patients with > six lines of visual acuity (VA) loss was statistically significantly smaller in the surgery group.[14] Another method is rotation of the macula, after a 360° retinectomy, to an area with less damaged RPE.[15] A third method is the transplantation of an autologous free graft of RPE, Bruch's membrane, choroicapillaris, and choroid.[16]

In free grafts, the best functional outcome is likely to relate to preserved neuroretina in the macula combined with a functioning RPE-choroid graft. For neuroretina and graft alike, surgery with minimal trauma would limit damage to these tissues. Furthermore, patient selection is important for a preserved neuroretina, and early revascularization is likely to help minimize ischemic damage to the graft.

Most studies regarding the revascularization of a free graft show results no earlier than one week after surgery. Due to the invasive nature of such studies (histology in animals[17] and angiography in human patients[18-20]), such data were neither early nor consecutive.

In this study, we further analyzed early revascularization by a non-invasive method: spectral domain optical coherence tomography (SD-OCT) (Spectralis HRA; Heidelberg Engineering, Heidelberg, Germany). SD-OCT was chosen because it allows visualization of the choroid in a repeatable manner thanks to its eye-tracking facility. We combined SD-OCT images with indocyanine green angiography (ICGA) and fluorescein angiography

(FA) images obtained at the same time points as the SD-OCT scans. We studied revascularization in patients after RPE-choroid graft translocation with invasive and non-invasive techniques, and tried to correlate data between these different imaging tools.

MATERIALS AND METHODS

Patients

Twelve consecutive patients with exudative AMD who were ineligible for (further) anti-VEGF or any other treatment were included in this study. Patients could be included if they were non-responders to anti-VEGF treatment (i.e., if they had a visual loss of ≥ 15 letters on the Early Treatment Diabetic Retinopathy Study [ETDRS] chart after at least three anti-VEGF injections). Other indications were a massive submacular hemorrhage (>1.5mm thickness on ultrasound) that was no longer eligible for recombinant tissue plasminogen activator (rtPA) injection (i.e., existing for > two weeks[21, 22]), or an RPE tear. Seven of the included patients were at the Rotterdam Eye Hospital, The Netherlands (REH), and five were at the Ospedale Sacro Cuore, Negrar, Verona, Italy (OSC).

All patients provided informed consent for the surgical procedure and for preoperative and postoperative examinations, in accordance with the tenets of the Declaration of Helsinki. The study was also approved by the Medical Ethical Committee of the Erasmus University, The Netherlands. In accordance with institutional guidelines, the surgical procedure and examinations are considered part of standard care in the OSC.

Surgery

At two centers, the REH and the OSC, we examined twelve patients who underwent a full-thickness translocation of autologous midperipheral RPE, Bruch's membrane, choriocapillaris, and choroid (RPE-choroid graft), as described previously.[19, 23, 24] At the REH for patients 1 to 6, a retinotomy was made in the raphe, and the midperipheral graft was placed under the retina using a bent forceps. At the OSC and for patient 7 at the REH, a 180° temporal retinotomy was made to permit folding over the temporal retina (and therefore exposure of the macula). The graft could then be dragged over the macular area from its donor site. Before harvesting the graft, we applied linear diathermia or laser to the RPE and choroid in a rectangular or oval shape. The graft was then cut out within the diathermia or laser borders, avoiding diathermized or lasered tissue in the graft itself. At the end of surgery, silicone oil was used as a tamponade. All surgical procedures were performed by one surgeon at each site (JCvM at REH, GP at OSC). The silicone oil was removed in a second procedure approximately three months later. Lensectomy or phacoemulsification and insertion of an intraocular lens were performed during the first or second surgery in the eight phakic patients, as shown in Table 1. We examined the

Table 1. Patient Characteristics

Patient	Indication Surgery	Age (y)	Eye	M/F	Baseline VA (logMAR)	SD-OCT Timing	Silicone Oil Removal	IOL Insertion	FA/ICGA	VA 3 Months (logMAR)
1	RPE tear	85	OD	F	0.46	Preoperatively. Days 1, 2, and 8, and 2 months PO.	6 weeks PO	Pseudophakia	N/A	0.7
2	Submacular hemorrhage	79	OD	F	1.2	Preoperatively. Days 1-4 and 60 PO.	15 weeks PO	15 weeks PO, lensectomy at graft surgery.	N/A	1.5
3	Submacular hemorrhage	82	OD	F	0.8	Preoperatively. Day 1-4 and weeks 3 and 6 PO.	9 weeks PO	Pseudophakia	N/A	1.5
4	Non-responsive to anti-VEGF	52	OD	M	0.66	Preoperatively. Days 1-4, 6, and 8, and weeks 2 and 5 PO.	20 weeks PO	20 weeks PO, lensectomy at graft surgery.	N/A	0.32
5	Submacular hemorrhage	79	OD	F	1.4	Preoperatively. Days 1, 3, 4, 8, and 9 PO. (New hemorrhage developed at day 9 PO.)	10 days PO, new submacular hemorrhage.	10 days PO, lensectomy at graft surgery.	N/A	2.1
6	RPE tear	86	OS	F	0.58	Preoperatively. Days 1, 4, 6, 8, 11, 14, and 20 and 60 PO.	12 weeks PO	Pseudophakia	N/A	1.5
7	Fibrotic scar after anti-VEGF	68	OD	M	1.58	Preoperatively. Days 1, 4-8, 11, 13, 15, 19, 30, and 60 PO.	11 weeks PO	Pseudophakia	N/A	1.4
8	Non-responsive to anti-VEGF	68	OS	M	0.9	Preoperatively. Days 1, 3, 5, 7, 10, 15, and 21 post operatively. 1, 2, 3, and 5 months PO.	3 months PO	Lensectomy and IOL insertion at graft surgery.	Preoperatively. Days 7, 10, 15, and 21 PO. 1 and 3 months PO.	1.3

Table 1. Patient Characteristics *(continued)*

Patient	Indication Surgery	Age (y)	Eye	M/F	Baseline VA (logMAR)	SD-OCT Timing	Silicone Oil Removal	IOL Insertion	FA/ICGA	VA 3 Months (logMAR)
9	Non-responsive to anti-VEGF	68	OS	F	0.8	Preoperatively. Days 1, 3, 7, 10, 14, and 21 PO. 1, 2, 3, and 4 months PO.	3 months PO	Lensectomy and IOL insertion at graft surgery.	Preoperatively. Days 3, 7, 10, 14, and 21 PO. 1 and 2 months PO.	0.5
10	Submacular hemorrhage and RPE tear	75	OS	F	1.5	Preoperatively. Days 1, 2, 6, 9, 13, 18, and 25 PO. 1, 2, 3, and 4 months PO.	3 months PO	Lensectomy and IOL insertion at graft surgery.	Preoperatively. Days 2, 6, 9, 13, 18, and 25 PO. 1, 2, and 4 months PO.	1.5
11	Submacular hemorrhage	57	OD	M	1.0	Preoperatively. Days 1-3, 6, 13, and 20 PO. 1, 2, and 3 months PO.	3 months PO	Lensectomy and IOL insertion at graft surgery.	Preoperatively. Days 6, 13, and 20 PO. 1 and 2 months PO.	0.4
12	Non-responsive to anti-VEGF	64	OS	M	0.5	Preoperatively. Days 1, 2, 5, 7, 12, and 20 PO. 1, 2, and 3 months PO.	2 months PO	Lensectomy and IOL insertion at graft surgery.	Preoperatively. Days 5, 7, 12, and 20 PO. 1, 2, and 3 months PO.	0.6

VA = Visual Acuity; SD-OCT = Spectral-Domain Optical Coherence Tomography; IOL = Intraocular lens; FA = Fluorescein Angiography; ICGA = Indocyanine Green Angiography; PO = Postoperatively, N/A = Not applicable; OD = Oculus Dexter (right eye); OS = Oculus Sinister (left eye)

status of the graft shortly after the RPE-graft translocation operation in patients with Heidelberg SD-OCT at the REH, and with Heidelberg SD-OCT, FA, and ICGA at the OSC.

Spectral Domain-Optical Coherence Tomography

Twelve patients with exudative AMD treated with an RPE-choroid graft were scanned by SD-OCT pre- and postoperatively (timing of examination for each patient summarized in Table 1). For SD-OCT scanning, the software provides an automatic real time (ART) function to increase image quality and reduce noise. Multiple frames (B-scans) of the same scanning location are performed during the scanning process with ART activated, and images are averaged for noise reduction. In this study, 51 frames were acquired for each single B-scan. Preoperative single B-scans were made through the macula, and follow-up scans were performed postoperatively. Four types of single B-scans were performed on each patient: horizontal, vertical, 45° and 135°.

SD-OCT Analysis

For quantitative analysis the number and diameter of the vessel lumina, as well as thickness of the graft and fluid under the graft, were measured in the horizontal and vertical OCT images of every patient at each visit. The thickness of the graft and subgraft fluid was measured both precisely under the fovea and at 500 µm on either side of the fovea. These six measurements were averaged. The intragraft vessels visualized on the vertical and horizontal OCT scans were counted and averaged. The maximal vessel diameter was measured in each of the three largest vessels of each graft. Six such diameter measurements per graft were averaged. ImageJ software (developed by Wayne Rasband, National Institutes of Health, Bethesda, MD; available at http://rsbweb.nih.gov/ij/index.html) was used for the measurements.

Two masked observers (EJTvZ and MCG) independently graded changes in gray shading of the vessel lumina in the graft compared with the gray shading of the vessels in the underlying choroid.

Fluorescein Angiography and Indocyanine Green Angiography

Both FA and ICGA (HRA Spectralis, Heidelberg Engineering) were performed pre- and postoperatively on the patients at the OSC. In FA, capillary flush (diffuse filling of the choriocapillaris at the early arterial phase) was regarded as a sign of revasularization. In ICGA, video-angiography in the first 40 seconds was used to identify parallel-oriented, ladder-like choroidal vessels to recognize graft perfusion.[20] Stereo images were made to confirm that the parallel-oriented, ladder-like vessels were located between the graft and the recipient choroid. The perfused area of the graft on FA and ICGA was measured with ImageJ software.

RESULTS

Patients

The mean age of the patients was 71.9 ± 3.1 years (mean ± standard deviation [SD]) years. Indication for surgery in five patients was a submacular hemorrhage; one of these five patients with a submacular hemorrhage also had an RPE tear. Two other patients had an RPE tear only. Five patients were non-responders to anti-VEGF treatment. These patients had a visual loss of ≥ 15 letters on the ETDRS chart after at least three anti-VEGF injections. One of these patients had developed a fibrotic scar.

VA at baseline ranged from 20/58 to 20/760 (0.46-1.58 logarithm of minimal angle of resolution [LogMAR]), with a median of 0.85 logMAR (20/142) and mean 0.94 logMAR (SD ± 0.11). VA after three months ranged from 20/42 to 20/2518 (0.32-2.1 logMAR), with a median of 1.35 logMAR (20/448) and mean 1.11 logMAR (SD ± 0.16). The characteristics of each patient are summarized in Table 1. SD-OCT, FA, and ICGA images from patient 9 are shown in Figure 1. SD-OCT images from patient 7 are shown in Figure 2.

Changes on SD-OCT, FA and ICGA: Days after Surgery and Measurements

Qualitative and quantitative measurements could be performed for all 12 patients (Table 2). However, the data from only nine patients were used for the qualitative description of the process of revascularization, because two patients did not show revascularization and one patient developed a hemorrhage nine days after surgery. When the mean number of days or the mean change of a feature is given in the text and Table 2, it is the mean of these nine patients (unless the mean is otherwise specified with an n).

Immediately after surgery, small vessels are seen in the graft (n = 12) (Figs. 1, 2). The diameter of these small vessels in the graft increased (mean, 94.5-178.3 µm) between postoperative days three and ten (mean, 5.6 days). During the same time period (mean, 5.8 days, n = 8), the number of vessels increased (mean, 19.8-26.0) and graft thickness (mean, 5.4 days) also increased (mean, 156.2-243.2 µm) in most patients (n = 10). After the rapid initial increase, the number of vessels increased progressively over time (mean, from 26.0 to 35.8 at mean 60 days). Among the ten patients who experienced graft thickening, fluid and/or material under the graft accumulated in six patients. Measurements could be performed for five of these patients. The first fluid appeared in each patient between postoperative day one and 21 (mean, seven days), and had a mean thickness of 182 µm. The maximum fluid thickness under the graft (mean, 228.2 µm) of these patients was measured between days one and 30 (mean, 14 days). The excluded patient was the one with hemorrhage described previously.

Between postoperative days eight and 30 (mean, 16 days, n = 8), the gray shading of the vessel lumina changed from clearly more white, compared with the underlying choroid, to a gray shading similar to that of the underlying choroidal vessels (n = 10; Figs.

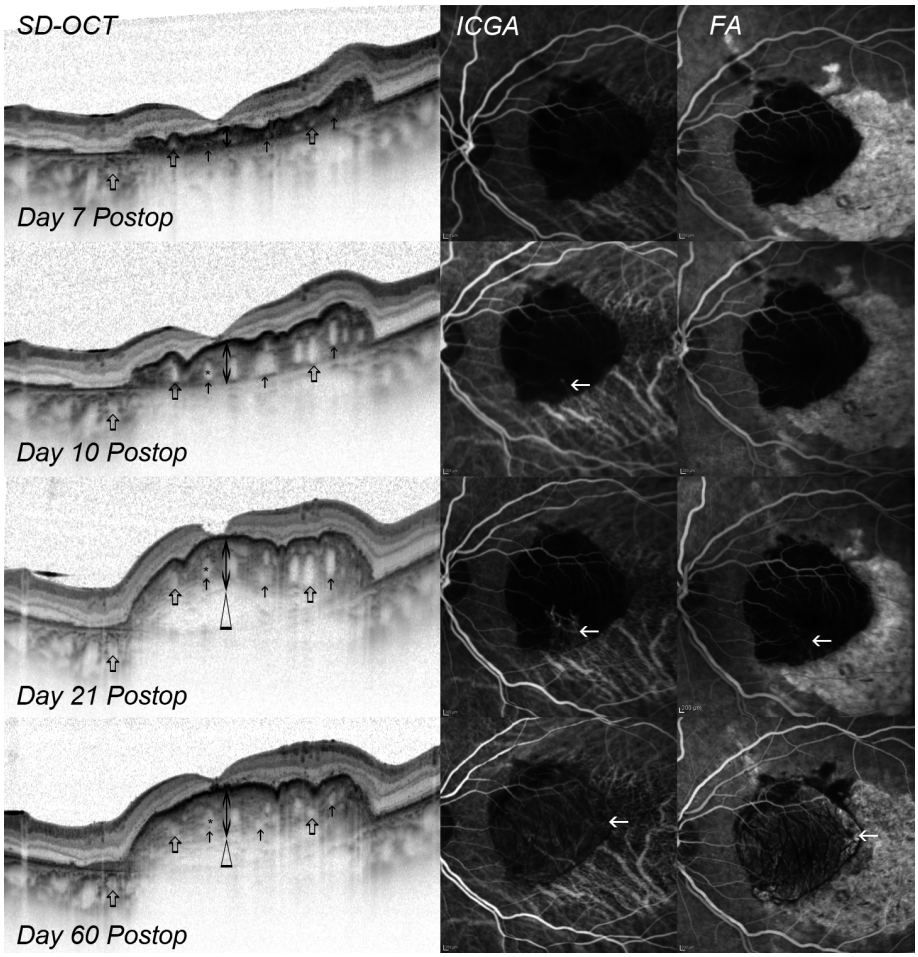

Figure 1. Patient 9: Spectral Domain Optical Coherence Tomography (SD-OCT), Indocyanine Green Angiography (ICGA) and Fluorescein Angiography (FA) images at postoperative (postop) days 7, 10, 21, and 60. The SD-OCT images are vertical B-scans. The vessel lumina are marked by an *asterisk* (*) and the number of these vessel lumina are shown by *small arrows* (↑). The graft thickness is depicted by a *double-pointed arrow* (↕), and the fluid under the graft is shown by a *delta* (△). *Large arrows* (⇨) point at the vessel lumina of the graft and the vessel lumina of the underlying choroid to show the difference or likeness in gray shading between these lumina. *White arrows* (←) point at the capillary flush in FA and the ladder-like choroidal vessels of the graft in the ICGA images. At postop day 10, there was a significant increase in vessel diameter, number of vessels, and graft thickness, combined with first ICGA lines visible at the inferior edge of the graft. At postoperative day 14, there was a further increase in vessel diameter and graft thickness, and more ICGA lines were visible. At postop day 21, there was a significant increase in fluid under the graft. Optically clearer vessels in the graft compared to vessels of the choroid could be seen until postop day 21. The SD-OCT at postop day 21 shows the same gray shading in the vessel lumina as the underlying choroid vessels in the left half of the graft, while the right half of the graft has optically clearer vessels compared to the underlying choroid vessels. This gray shading on the left half of the graft coincides with the inferior part of the graft, in which FA flush and ICGA lines are visible. At postop day 60, the vessel lumina had gray shading comparable to that of the choroid, the diameter of the vessels had decreased, thickness and fluid under the graft had decreased, all the vessels of the graft were visible on ICGA, and FA showed fluorescence comparable to the surrounding choroid.

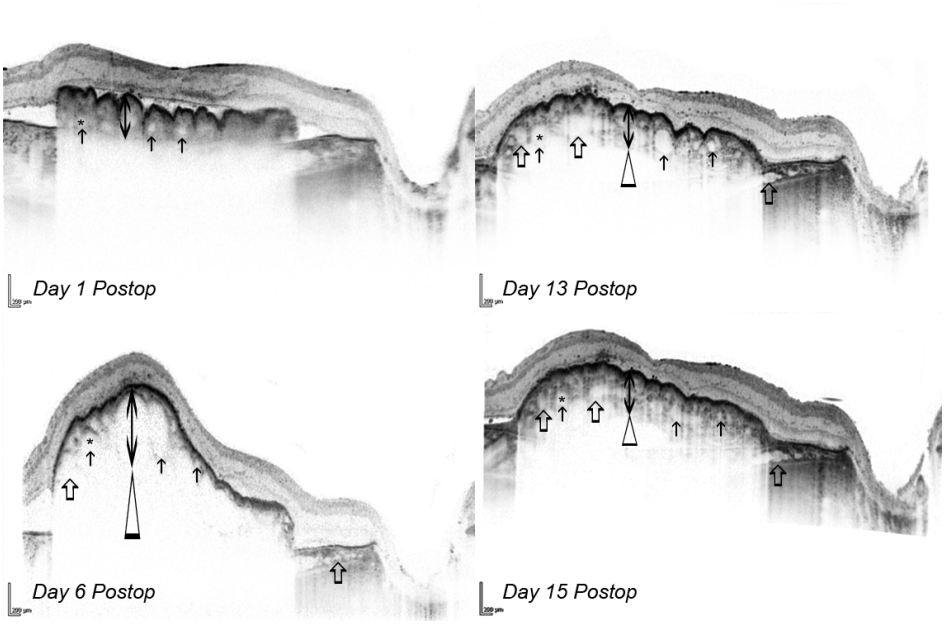

Figure 2. Patient 7: Spectral Domain Optical Coherence Tomography (SD-OCT) images at postoperative (postop) days 1,6,13, and 15. The SD-OCT images are horizontal B-scans. The vessel lumina are marked by an *asterisk* (*) and the number of these vessel lumina are shown by *small arrows* (↑). The graft thickness is depicted by a *double-pointed arrow* (↕), and the fluid under the graft is shown by a *delta* (△). *Large arrows* (⇨)point at the vessel lumina of the graft and the vessel lumina of the underlying choroid to show the difference or likeness in gray shading between these lumina. At postop day 6, there was a significant increase in vessel diameter and number, the graft thickness increased, and fluid accumulated under the graft. The vessels of the graft are optically clearer than the vessel lumina of the underlying choroid. At postop day 13, the diameter of the vessels decreased in size, together with thinning of the graft and decrease of fluid under the graft. The lumina of the vessels in the middle of the graft are optically clearer than the lumina of the choroidal vessels; only some vessels on the very edges of the graft show the same gray shading as the vessels of the underlying choroid. At postop day 15, only a few lumina of the vessels in the middle of the graft are still optically clearer than the lumina of the vessels of the underlying choroid. The graft vessel lumina at both sides of the graft show gray shading more similar to the underlying choroid vessel lumina.

1, 2). This change in gray shading of the graft coincided with the early choriocapillary flush[20] and ICGA lines of the graft.

Early choriocapillary flush (mean, 4.8 mm^2) could be seen on FA in four of five patients between postoperative days six and 15 (mean, 10.5 days). The first vessels of the graft also were visible on ICGA (mean: 2.9 mm^2) between postoperative days five and 15 (mean, nine days) in the same four patients.

A second increase in graft thickness (mean, 243.2-316.2 μm) was observed in six patients, between postoperative days nine and 15 (mean, 11.6 days). A subsequent significant decrease (mean,178.3-146.3 μm and later to mean of 136 μm at mean 60 days) in vessel diameter began as early as eight, ranging to 40 days (mean 25.6 days) after surgery (n = 7 of 12). This was combined in these seven patients with an initial

Table 2: Quantitative Measurements of OCT Images

		Days (mean)	Value (mean)	Range (min-max Values)
Diameter of the vessels in the graft (µm)	Start	1.0	94.6	77-113
	Increase	5.6	178.3	105-352
	Decrease	25.6	146.3	115-153
	Last data	60.0	136.3	107-153
Number of vessels in the graft (n)	Start	1.0	19.8	15-27.5
	Increase	5.8	26.0	19-36.5
	Last data	60.0	35.8	25-54.5
Thickness of the graft (µm)	Start	1.0	156.2	79-233
	Increase	5.4	243.2	155-463
	Increase 2	11.6	316.2	242-511
	Decrease	24.6	244.9	167-394
	Last data	60	231.0	167-356
Fluid under the graft (µm)	Start	7.0	182.0	35-304
	Maximum	14.0	228.2	138-314
	Decrease	25.0	137.2	16-218
	Last data	60.0	58.6	0-200
Perfused area of the graft on FA* (mm^2)	Start	10.5	4.8	0.6-11.8
	Last data	60.0	18.2	14.2-25.0
Perfused area of the graft on ICGA† (mm^2)	Start	9.0	2.9	0.2-5.0
	Last data	60.0	17.9	14.1-24.0

Values are mean and range of 9 patients.
FA = Fluorescein Angiography; ICGA = Indocyanine Green Angiography

decrease (mean, 316.2-244.8 µm) in thickness (mean, 24.6 days) of the graft, in the same time frame. A decrease of fluid/material under the graft (mean, from 228.2-137.2 µm) started between days two and 60 (mean, 25 days). This decrease in thickness was progressive and lasted through the first weeks to months after surgery (mean, 58.6 µm at mean 60 days) . The decrease in graft thickness was also initially progressive, but later stabilized at mean 60 days (mean thickness, 231 µm).

After the initial choriocapillary flush visible on FA (mean: 10.5 days) and the first vessels visible on the graft with ICGA (mean, nine days) , a progressively increasing area of choriocapillary flush was measured (mean, 4.8-18.2 mm^2) on FA (n = 4 of 5, between mean days 10.5 and 28.7) and a progressive area of vessels in the graft (mean, from 2.9 mm^2 to 17.9 mm^2) was measured on ICGA (between mean days nine and 28.7). The whole choroidal structure of the graft was evident on FA, and ICGA revealed that the entire graft was perfused in four out of five patients, both between postoperative days 12 and 60 (mean, 28.7 days).

In two patients, patient 4 and patient 10, no significant changes in vessel number and/or diameter or graft thickness were found throughout the follow-up period. Their SD-OCT showed a thin layer of few small-diameter vessels with very small lumina lying under the RPE. In patient 10, the graft also did not become visible on ICGA; however, the original choroidal vessels underlying the graft became weakly visible starting at postoperative day nine, and were clearly visible four months after surgery.

Stereo images confirmed that the choroidal vessels were part of the graft in all patients in which a ladder-like vasculature could be seen (n = 4 of 5).

DISCUSSION

After surgery, a free RPE and choroid graft is very likely to need revascularization for survival. In this study, we found that the revascularization of an RPE graft can be observed by SD-OCT, as the SD-OCT findings corresponded with those of FA and ICGA.

Studies in free skin transplants (in animals) reveal that, in an early phase, plasma exudes from the recipient site's damaged arteries and veins, fills the lumina of the graft vessels, and supposedly supports graft-tissue metabolism (imbibition phase).[25] Later processes involved in graft survival are neovascularization (vascular ingrowth from the recipient bed) and replacement and/or reconnection of the graft vasculature by endothelial and endothelial-progenitor cells from the recipient bed.[20] Young[26] demonstrated this neovascular ingrowth at the third or fourth day after surgery in the pedicle skin flaps of pigs. This time sequence was also found for wound healing in skin flaps, free skin grafts, and linear incisions.[26]

Revascularization of RPE-choroid grafts has been confirmed in pigs, with connecting vessels between recipient and graft present at one week and three months after surgery.[17] To study revascularization of a free RPE-choroid graft in patients, the early phase of FA and or ICGA is recommended.[19, 20] However, angiography remains an invasive technique that is not readily suitable for the repeated study of revascularization in patients. The advent of an improved, more advanced imaging device (Spectralis HRA SD-OCT; Heidelberg Engineering), with better definition of the choroid and an eye-tracking device, allowed repeated, serial, noninvasive studies. It would therefore be desirable to be able to correlate angiographic data with non-invasive imaging, with the subsequent ability to rely on the non-invasive modality alone.

In our series of patients, we found a consistent pattern of changes at the level of the graft on SD-OCT that coincided with specific changes observed on ICGA and FA. The first days after surgery, choroidal vessel lumina of the graft were relatively small and optically clear on SD-OCT. No filling of the graft vessels was visible on angiography. We suggest that this phase corresponds with serum imbibition, before any revascularization. Several

days after surgery, observed on SD-OCT, the thickness of the graft increased and the vessels of the graft started to enlarge in diameter and to increase in number. The vessel lumina in the graft remained optically clear. These events correlated with the appearance on ICGA of a chorioidal vessel connecting to the graft. We suggest that the graft was pumped up by this connection of an afferent vessel without the presence of an efferent vessel. Several days later, on SD-OCT, the graft became thinner, the diameter of the vessels decreased slightly, and a gray shading appeared inside the vessels, comparable to that of the recipient choroid. These events on SD-OCT coincided with the perfusion of the entire graft on FA and ICGA. We suggest that these observations were caused by the establishment of an efferent vessel connection, facilitating flow through the graft and a subsiding of the graft tissue swelling.

Fluid under the graft does not follow the pattern of changes of the overlying graft. Early fluid may be related to trapped fluid after placement of the graft or an inflammatory exudate after surgical trauma. Later after surgery, subgraft fluid may be secondary to the connection of the afferent vessel before the ingrowth of efferent vessels.

Fortunately, most patients with exudative AMD respond well to anti-VEGF treatment. However, an RPE-choroid graft may be considered in three patient categories: non-responders to anti-VEGF treatment; massive submacular hemorrhage, no longer eligible for rtPA injection;[21, 22] or an RPE tear.

A successful functional outcome of RPE-choroid graft surgery is dependent not only on graft perfusion, but also on the amount of damage to the RPE during insertion and to the condition of the overlying retina, with patient selection and the preoperative course as major factors. We found that VA increased the first year after surgery, mainly after three to six months, and stabilized thereafter.[27] Therefore, VA at three months after surgery in the present study is probably not the best VA that the patient may eventually achieve. At this moment, the small number of patients and short follow-up preclude analysis of a correlation between perfusion and functional outcome.

We demonstrate in this study that the revascularization of a free RPE and choroid graft follows well-established steps of revascularization, as reported in other free grafts. At the same time, this study demonstrates that non-invasive SD-OCT findings correlate with FA and ICGA findings. Therefore, SD-OCT can be used to monitor the postoperative process of revascularization in a free RPE-choroid graft without the need for invasive imaging techniques.

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Chapter 5.2

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

Submitted

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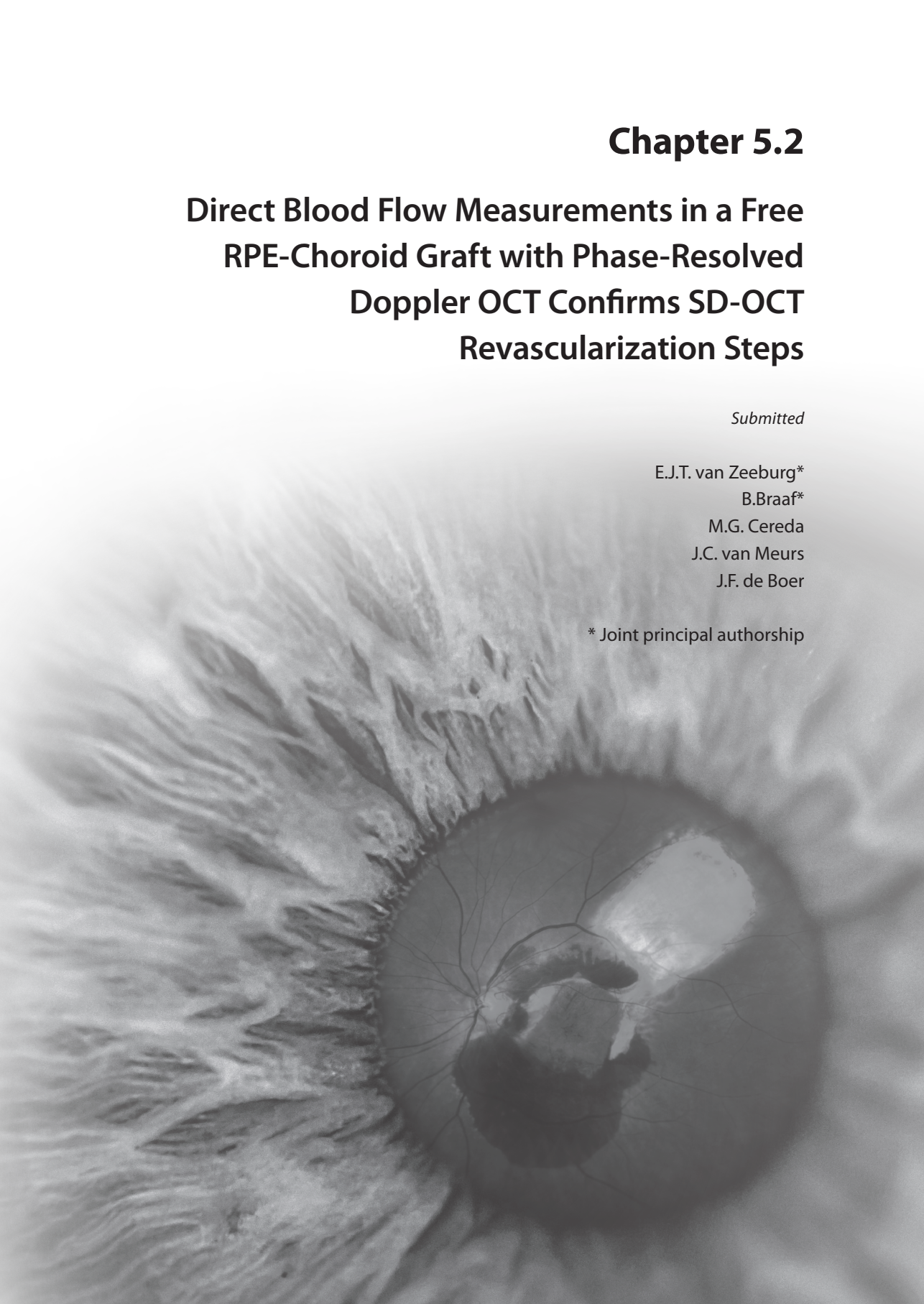

ABSTRACT

Purpose

To correlate the revascularization steps in a free retinal pigment epithelium (RPE)-choroid graft as observed from tissue structure changes by commercially available spectral domain optical coherence tomography (SD-OCT) with direct blood flow detection by experimental phase-resolved Doppler optical coherence tomography (PRD-OCT).

Design

Prospective institutional cohort study.

Methods

Seven patients with age-related macular degeneration underwent an RPE-choroid graft translocation. Multiple postoperative SD-OCT scans were made and structural changes as the thickness of the graft, number and diameter of the vessels, and thickness of the fluid under the graft were measured to monitor the revascularization process of the graft. In addition, the presence of blood flow was evaluated post-operatively with PRD-OCT to directly detect the presence of flow. One patient from a previous study was only measured one year postoperatively with PRD-OCT to confirm blood flow in an unusual revascularization process.

Results

SD-OCT documented both the afferent and efferent vessel ingrowth stage in six out of seven patients. Only the efferent stage was seen in the seventh patient who was measured only once. PRD-OCT confirmed flow in three out of six patients when SD-OCT indicated the afferent vessel ingrowth stage, and in all seven patients when the SD-OCT indicated the efferent vessel ingrowth stage.

Conclusions

PRD-OCT was able to detect the presence of blood flow in a free RPE-choroid graft. PRD-OCT findings confirmed the determination of the revascularization steps based on structural changes in SD-OCT images.

INTRODUCTION

Exudative age-related macular degeneration (AMD) is the leading cause of irreversible legal blindness in elderly patients in the industrialized world.[1] Exudative AMD is predominantly treated with anti-vascular endothelial growth factor (anti-VEGF), which has proven to preserve or improve visual acuity in the vast majority of patients.[2] There are patients, however, who do not benefit from this treatment. These include patients with fibrosis under the macula,[3] retinal pigment epithelium (RPE) tears,[4] patients who do not respond to anti-VEGF therapy,[5] or patients with (large) older hemorrhages which cannot be treated with recombinant tissue plasminogen activator (rtPA).[6] For these patients, an RPE-choroid graft surgery is an alternative treatment option. During this surgery neovascular membranes and fibrotic tissue located beneath the macula are removed and replaced by a free autologous RPE and choroid graft obtained from a location in the midperiphery.[7, 8]

We hypothesize that best functional outcome for this treatment relates to a preserved neuroretina in the macula and revascularization of the graft. As previously described, spectral domain optical coherence tomography (SD-OCT) allows the visualization of structural tissue changes during three well-established revascularization stages in an RPE-choroid graft shortly after surgery.[9] The first step is the serum imbibition stage: small vessel lumina are visible inside the graft, and the graft appears to be very thin. The second step is the afferent vessel stage: the number and the mean diameter of the vessels increase and the graft becomes thicker. The third step is the efferent vessel stage: the thickness of the graft and the diameter of the vessels may slightly decrease while the number of vessels may slightly increase. This is accompanied by a change in gray shading of the vessel lumina, suggesting blood flow.[9] This observation of the three revascularization stages with SD-OCT was confirmed previously by fluorescein angiography (FA) and indocyanine green angiography (ICGA).[9] Postoperative follow-up by SD-OCT reduces the need for more invasive techniques as FA and ICGA.[10, 11]However, SD-OCT is an indirect method to detect blood flow while a direct detection is desirable. We therefore analyzed the graft perfusion during the revascularization process with a new non-invasive but direct method: phase-resolved Doppler optical coherence tomography (PRD-OCT).

Recently, OCT technology has been improved with the introduction of optical frequency domain imaging (OFDI), which is also known as swept-source OCT.[12] The advantage of OFDI over SD-OCT is the lower signal decay at increased imaging depth, and a reduced susceptibility to motion artifacts.[13] In practice, this provides a large imaging depth range, which is especially interesting for the imaging of thick tissue samples. Further, the introduction of broadband light sources at wavelengths around 1 µm have tremendously improved the visualization of choroidal structures due to the lower light

scattering in the retina, compared to traditional 850 nm light sources.[14, 15] The deep tissue penetration of 1 µm OCT is of particular interest for the investigation of diseases, such as exudative AMD, whose pathogenesis begins in the subretinal vascular network.[16] The imaging of vascular networks with OCT is further strengthened by the use of PRD-OCT techniques. In this functional extension of the standard OCT technique, blood flow is detected from phase changes in the OCT signals within successive measurements. These phase changes are caused by the Doppler effect of moving intravascular particles, such as erythrocytes and leukocytes.[17-19] We hypothesized that PRD-OCT, based on OFDI equipped with a 1 µm light source, is an ideal tool for the investigation of blood flows that are deeply embedded within RPE-choroid grafts. An experimental prototype of such an instrument was therefore constructed in our lab, and it has already been used to demonstrate the possibility of detecting blood flow within RPE-choroid grafts.[20]

In this study, seven patients were examined during an extended postoperative follow-up in order to investigate the validity of PRD-OCT for the detection of blood flow after RPE-choroid transplantation surgery, and to directly confirm the revascularization steps that have been detected from tissue structure changes in a previous SD-OCT study.[9]

METHODS

Patients

Seven patients with exudative AMD who were ineligible for (further) anti-VEGF or other treatment were included in this prospective cohort study. Patients could be included if they were non-responders to anti-VEGF treatment (i.e., if they had a visual loss of ≥ 15 letters on the Early Treatment Diabetic Retinopathy Study [ETDRS] chart after at least three anti-VEGF injections), if they had a massive submacular hemorrhage that was no longer eligible for rtPA injection (i.e., existing for ≥ two weeks),[6] a fibrotic macular scar, or an RPE tear. All examinations and surgical procedures were performed at the Rotterdam Eye Hospital, The Netherlands (REH). All patients provided informed consent for the surgical procedure and for pre- and postoperative examinations, in accordance with the tenets of the Declaration of Helsinki. The study was approved by the Medical Ethical Committee of the Erasmus University, the Netherlands.

Surgery

All patients underwent a full-thickness translocation of autologous midperipheral RPE, Bruch's membrane, choriocapillaris, and choroid (RPE–choroid graft). As described previously,[9] one of two possible surgical procedures were performed: one with the creation of a small retinectomy in the raphe followed by the positioning of a midperipheral graft under the macula using a bent forceps[21] (n = 5); or another in

which the free graft of RPE and choroid could be dragged over the macula area after the creation of a large (180°) peripheral temporal retinotomy[10] (n = 2). At the end of the surgery, silicone oil (5000 centistokes [cSt]) was used as tamponade. All surgical procedures were performed by one surgeon (J.v.M.). The silicone oil was removed in a second procedure approximately three months after the first surgery. Lensectomy or phacoemulsification was performed during the first procedure in phakic patients (n = 3). Insertion of the intraocular lens was performed during second surgery.

Visual Acuity

To test visual function, the best corrected visual acuity was measured preoperatively and at three and six months postoperatively on an Early Treatment Diabetic Retinopathy Study (ETDRS) chart. Conversions to logarithm of minimal angle of resolution (logMAR) were done according to Holladay's conversion method in which finger counting at 60 cm was transposed to logMAR 2 and hand motion to logMAR 3.[22]

Spectral-Domain OCT

To image the tissue structure of the graft, all patients were scanned on a regular basis by SD-OCT pre- and postoperatively; five patients up to three months, one patient up to seven months and one patient for one year (timing of examination for each patient summarized in Table 1). For SD-OCT scanning, the Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany) was used, which provides an automatic real time (ART) function to increase the image quality by averaging multiple frames (B-scans) of the same scanning location. In this study, at least 51 frames were acquired which were compiled into a single averaged B-scan. Preoperative B-scans were made through the macula, and postoperative follow-up scans were made at the same scanning locations. B-scans were made for each patient over two different axes: horizontal (0°) and vertical (90°).

After image acquisition, a quantitative analysis on tissue structure changes was performed in which the number and diameter of the vessel lumina, as well as thickness of the graft and fluid under the graft, were measured in the OCT images of every patient at each visit.[9] This analysis was performed with built-in software of the Spectralis OCT. Briefly, the thickness of the graft and subgraft fluid were both measured precisely under the fovea and at 500 µm on either side of the fovea. The number of intragraft vessels visualized on the OCT scans were counted and averaged and the maximal vessel diameter was measured in each of the three largest vessels of each scan. The six measurement results for every parameter were averaged for every patient. In order to identify the different graft revascularization stages, the above described parameters were measured in each consecutive examination for every patient. In case the measurements remained stable in two consecutive controls (no significant changes in the number of vessels, the

Table 1: Patient Characteristics

Patient	Indication Surgery	Age (years)	Eye	M/F	Baseline VA (logMAR)	SD-OCT[c] Timing	PRD-OCT[d] Timing	Silicone Oil Removal	IOL[e] Insertion	VA[f] 3 Months (logMAR[g])	VA 6 Months (logMAR)	Complications
1	Submacular hemorrhage and fibrotic reaction due to uveitis	72	OD[a]	F	1.42	Pre-operatively. Days 1,3,4,7,9,11,15, and 1 and 3 months PO[h].	Preoperatively. Days 1,7,15, and 1 and 3 months PO.	6 weeks PO	6 weeks PO	0.84	0.6	Macular hole, during surgery a fibrotic string was attached to the macula.
2	Submacular hemorrhage	86	OS[b]	F	2.8	Days 1,5,8,13, and 6 weeks, 3 and 7 months PO.	Days 1,5,13, and 6 weeks, and 3 and 7 months PO.	3 months PO oil out and in again, out at 7 months PO	7 months PO	0.82	0.58	New submacular hemorrhage under the graft, with inferior retinal detachment, macula on. Intraretinal fluid after 3 months, disappeared after anti-VEGF[i] injection.
3	Submacular hemorrhage and RPE tear[j]	83	OS	F	1.16	Pre-operatively. Days 1,3,10,16, and 1, 2, and 3 months PO.	Days 1, 3,10,16, and 1 and 3 months PO.	3 months PO oil out and at 5 months PO in again	N/A[k]	0.98	0.82	PVR with retinal detachment outside of the macula, oil in again
4	Submacular hemorrhage and RPE tear	89	OD	F	1.32	Pre-operatively. Days 7,12,14,19, and 6 weeks, and 3 months PO.	Days 7,12,14,19, and 3 months PO.	3 months PO	N/A	1.32	0.8	1st 7 days corneal edema and hazy anterior chamber. Oil emulsification 3 months after surgery
5	Submacular hemorrhage and fibrosis	88	OS	M	1.26	Pre-operatively. Days 1,5,7,11,15, and 6 weeks, and 3 months PO.	Days 1,5,7,11 and 3 months PO.	3 months PO	N/A	1.08	1.14	None

Table 1: Patient Characteristics *(continued)*

Patient	Indication Surgery	Age (years)	Eye	M/F	Baseline VA (logMAR)	SD-OCT[c] Timing	PRD-OCT[d] Timing	Silicone Oil Removal	IOL[e] Insertion	VA[f] 3 Months (logMAR[g])	VA 6 Months (logMAR)	Complications
6	RPE-tear + pucker	91	OD	M	0.64	Pre-operatively; 1, 3,4,7,8,10,11,14,16, 18, 22, week 7, 2 months, and 3 months PO.	Pre-operatively; 1, 3,4,7,8,10,11,14,16, 18, 22 and 3 months PO.	2 and 4 months PO oil out en in again, out at 5 months PO.	N/A	0.82	0.86	Macular hole, which closed shortly after first surgery. PVR 6 months PO.
7	Non-Responder	52	OD	M	0.66	Pre-operatively. Days 1-4, 6 and 8, 15 and 1 and 6 months, and 1 year PO.	1 year PO.	2 weeks PO	20 weeks PO	0.32	0.32 at 6 months, 0.14 at 1 year	None

[a]OD = Oculus Dexter (right eye); [b]OS =Oculus Sinister (left eye); [c]SD-OCT = Spectral Domain-Optical Coherence Tomography; [d]PRD-OCT = phase-resolved Doppler-Optical Coherence Tomography; [e]IOL = Intraocular lens; [f]VA = Visual Acuity; [g]logMAR = logarithm of minimal angle of resolution; [h]PO = Postoperative; [i]Anti-VEGF = anti-vascular endothelial growth factor; [j]RPE tear = Retinal pigment epithelium tear; [k]N/A = Not applicable.

diameter of the vessel lumina, or in the thickness of the graft), they were considered part of the same stage. When a change was observed, a revascularization stage transition was assumed.

After surgery the first stage is the imbibition stage; a thin graft with small vessel lumina is visible. These vessel lumina in the graft are optically clear compared to the recipient choroidal vessels. The following afferent stage is marked by an increase in number and mean diameter of the vessels and the graft becomes thicker. Some vessels in the graft may obtain a gray shading compared to the recipient choroid, while other vessels still appear optically more clear. The start of the efferent vessel stage is marked by a slight decrease in the thickness of the graft and diameter of the vessels while the number of vessels slightly increases. The vessels of the graft all have a gray shading comparable to the underlying choroid of the recipient. The fluid under the graft however does not follow the pattern of changes of the overlying graft.[9]

During the follow-up period for each patient, at every single visit, compared to the previous one, we looked at the presence of a change in one or more of the analyzed parameters (thickness of the graft and subgraft fluid, diameter and number of vessels or a change in gray shading). These changes are generally visible to the bare eye of a trained examiner. If the examiner observed discernable changes, the patient would also be tested with PRD-OCT.

Phase-Resolved Doppler OCT

To detect blood flow in the graft, all patients were imaged with PRD-OCT.

Our group recently developed an experimental OFDI system to perform clinical studies in ophthalmology.[20] The system uses a swept-source laser in the 1 µm wavelength range (Axsun Technologies Inc, MA, USA) which operates with a 100 kHz A-scan rate over a bandwidth 106 nm. The axial resolution was measured to be 6.5 µm in air (4.7 µm in tissue) and the lateral resolution was 10 µm. PRD-OCT imaging of the blood flow was achieved by measuring a calibration signal in parallel with the retinal imaging in order to correct hardware phase-instabilities in post-processing. PRD-OCT images of the blood flow were created by calculating the phase-difference on the interference between sample and reference arm light for succeeding A-scans with an 83% spot-overlap. In this study two PRD-OCT measurement protocols were used. The first protocol consisted of a single B-scan measurement over a line of 2.2 mm in width on the retina for which 2000 A-scans were acquired. The acquisition time for this protocol was 20 ms. The second protocol measured a three-dimensional data volume consisting of 250 single B-scans with 2000 A-scans/B-scan over a retinal area of 2.2 mm in width and 4.1 mm in length. The acquisition time for a three-dimensional volume was 5.0 s. During a single patient visit several single B-scans and three-dimensional volume datasets were acquired for which the total measurement duration was never more than 30 minutes. The three-

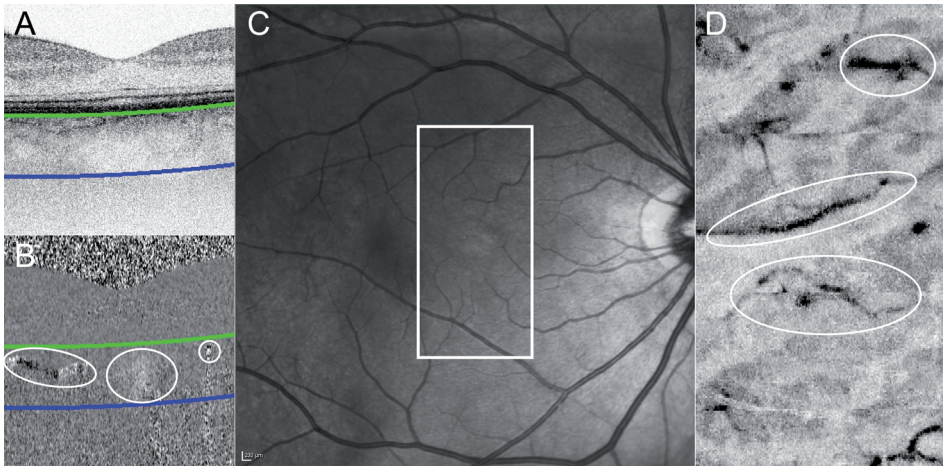

Figure 1. PRD-OCT measurements of the choroid in a healthy subject. A three-dimensional dataset was acquired from the center of the macula including the fovea. **A** A PRD-OCT intensity B-scan showing the retinal and choroidal structures. The choroidal depth over which flow was evaluated is outlined by blue and green lines. **B** A PRD-OCT bidirectional flow B-scan from the location of (**A**). Blood flow is visualized as white and black areas for flow directions that are respectively towards and away from the OCT instrument. Tissue without blood flow is displayed in gray and locations above the inner limiting membrane (i.e., in the transparent vitreous) shows random noise. In the choroid several vessels can be distinguished. **C** An infrared image of the retina of the healthy subject showing the surface area where the three-dimensional dataset was obtained demarcated with a white box. **D** En face PRD-OCT flow image of the choroid. White ovals indicate strong blood flow *which is visualized in black* in several choroidal blood vessels.

dimensional datasets were processed afterwards into flow en-face images by integrating the absolute phase-difference values over depth in order to visualize the distribution of the sparse flow signals in the vascular network of the graft.

In this study the phase-difference for PRD-OCT was calculated from successive A-scans for which the time interval was 10 µs. In our previous study a phase-noise of 0.32 rad was reported for this PRD-OCT time interval in combination with the same lateral sampling density of 2000 A-scans / 2.2 mm.[20] Considering a relatively steep angle of incidence of the OCT light with the blood flow direction (Doppler angle),[23] the minimum detectable flow velocity ranged from 5.6 mm/s for a 70° Doppler angle to 110 mm/s for a 89° Doppler angle. The minimal vessel size for which blood flow could be detected was 60 µm in diameter. Small blood vessels of the choroidal (micro-) vasculature are therefore not detected. The visualization of blood flow is limited to the larger vessels with high blood flow velocity. In figure 1 an example is given of PRD-OCT of the choroid in a healthy subject. This figure shows that, although PRD-OCT gives sparse information on the flow, large sections of several blood vessels can be clearly visualized.

All patients were repeatedly measured post-operatively with PRD-OCT, the last measurement ranging from three months up to one year after surgery (timing of examination for each patient is summarized in Table 1). During each visit several

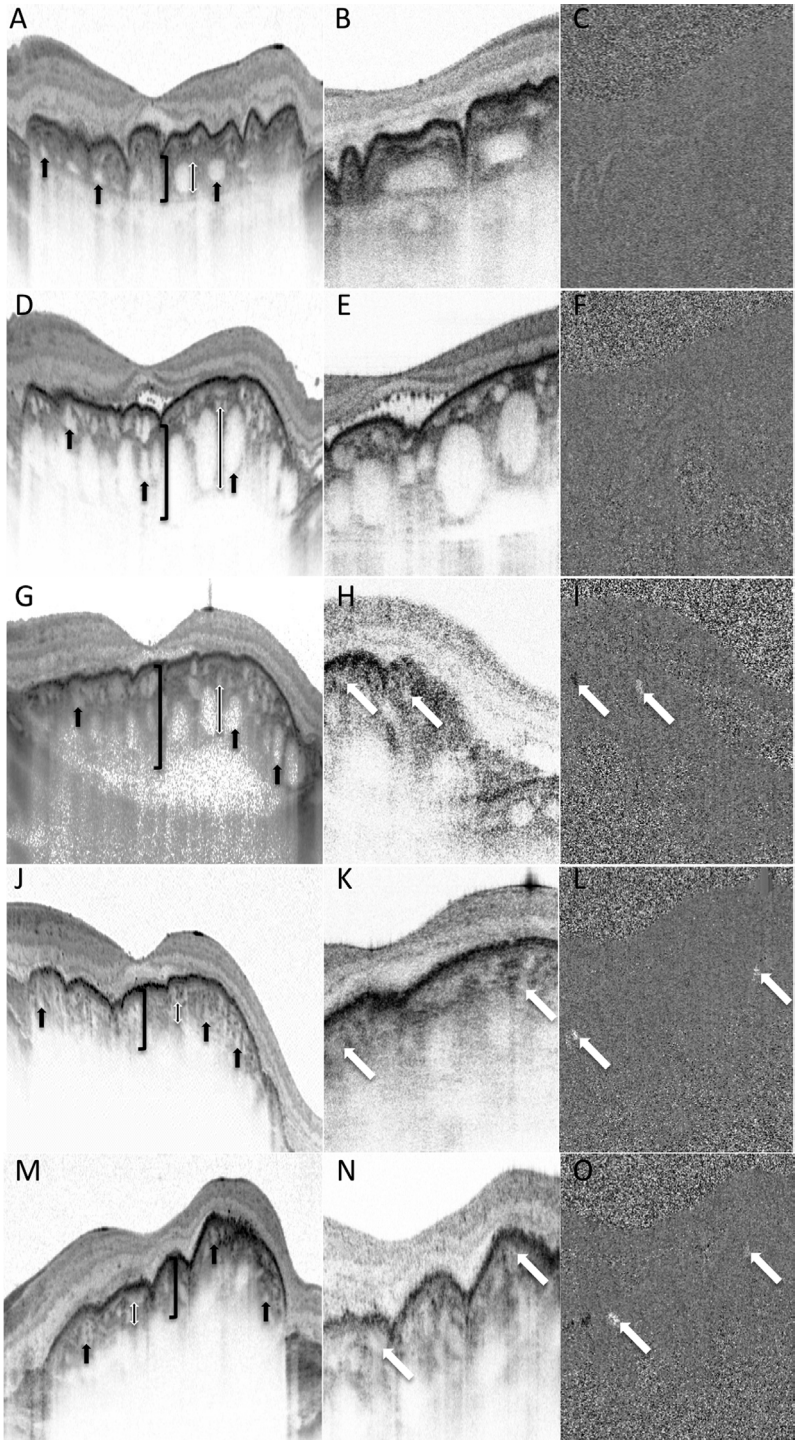

Figure 2.