LONG-TERM RESULTS OF AUTOLOGOUS RETINAL PIGMENT EPITHELIUM AND CHOROID TRANSPLANTATION FOR THE TREATMENT OF EXUDATIVE AND ATROPHIC MACULOPATHIES

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Purpose: To evaluate the long-term results of autologous retinal pigment epithelium (RPE) and choroid transplantation (RPE–choroid patch) for exudative and atrophic maculopathies.

Methods: Consecutive chart review of 120 eyes, which underwent RPE–choroid patch, from 2007 to 2017 for RPE atrophy or choroidal neovascular membrane secondary to exudative and hemorrhagic age-related macular degeneration, myopia, angioid streaks, and laser. Eyes were tested with best-corrected visual acuity (BCVA), reading ability, optical coherence tomography, fluorescein angiography and indocyanine green angiography, autofluorescence, and microperimetry.

Results: Eighty-eight eyes of 84 patients had complete data, with 2- to 10-year follow-up. Mean age was 71.9 ± 9.06 years. Mean preoperative and postoperative BCVA was 20/320 (1.2 ± 0.2 logMAR) and 20/200 (0.94 ± 0.36 logMAR), respectively ($P = 0.009$). Reading ability recovered in 43% of cases. Microperimetry showed central fixation. A gain of at least 15 letters was obtained in 40% of eyes. Integrity ($P = 0.009$) of external limiting membrane and higher preoperative BCVA ($P = 0.001$) predicted better final BCVA. Complications were retinal detachment (11.4%), macular atrophy (7%), subretinal hemorrhage (4.5%), epiretinal membrane (4.5%), recurrent choroidal neovascular membrane (4.5%), macular hole (3.4%), and cystoid edema (3%).

Conclusion: Autologous RPE–choroid patch achieved long-lasting BCVA improvement and central fixation, in eyes with choroidal neovascular membrane and intact external limiting membrane. Atrophic maculopathies only obtained temporary visual benefit.

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Various types of exudative or atrophic maculopathies have in common primary damage to the retinal pigment epithelium (RPE) and secondary damage to the retina. Autologous transplantation of RPE and choroid (RPE–choroid patch) is a surgical technique that has been investigated for the treatment of age-related macular degeneration (AMD) with geographic atrophy and advanced exudative AMD with RPE tear or massive subretinal hemorrhage. The rationale is to transport healthy RPE, from a more peripheral location to the submacular space, where the original RPE and choroidal tissue are damaged by disease progression.

This concept was first described by Peyman et al[1] in 1991 and further modified by Stanga et al[2] using an RPE–choroid patch from an area directly adjacent to the excised choroidal neovascular membrane (CNV) and van Meurs and Van Den Biesen,[3] harvesting the patch from the mid periphery.[3,4]

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In previous years, suspension's transplantation of autologous RPE cells, harvested from the periphery during submacular surgery for removal of hemorrhage/CNV, had been proposed, but this surgery has shown limited benefits[5]; cell suspensions have difficulties attaching to the diseased Bruch membrane and often do not form the monolayer required for optimal RPE function and may undergo apoptosis. An RPE–choroid patch may thus provide a better cell source with more chance of regaining normal retinal structures and thus visual acuity.[6,7]

Furthermore, Van Zeeburg et al[6] suggested that RPE–choroid patch could be a possible alternative treatment for patients affected by AMD refractory to standard treatment with anti–vascular endothelial growth factor (anti-VEGF) therapy or in whom anti-VEGF treatment is contraindicated.[7,8] The same authors also showed that a higher gain in vision might be achieved with an RPE–choroid patch surgery as compared to anti-VEGF treatment, in absence of complications.[7,8] In 2011, we reported about the feasibility and safety of the RPE–choroid patch in eyes with advanced subfoveal CNV using a peripheral retinotomy, to lower the risk of surgical complications.[9] Autologous RPE–choroid patch was reported in geographic atrophy cases as well.[10] The authors reported maintenance of visual function at 3 years in 2 of 10 operated eyes. However, long-term results of RPE–choroid patch in dry AMD, hereditary maculopathies, and exudative maculopathies other than AMD have not yet been reported.

In this study, we sought to report a detailed description of the surgical technique of our RPE–choroid patch technique and to evaluate the long-term functional and anatomical results, the prognostic factors, and the complication rate of this surgery, in a large cohort of eyes with exudative and atrophic maculopathies due to AMD, retinal dystrophies, and other causes.

Material and Methods

Study Eyes and Data Categorization

We retrospectively reviewed the medical charts of all consecutive eyes that underwent RPE–choroid patch surgery for exudative and atrophic macular degeneration by the same surgeon (B.P.). The analysis of data was performed in 2017 and included eyes operated between 2007 and 2015. Collected data included demographics, best-corrected visual acuity (BCVA), reading ability, retinal biomicroscopy, fixation stability based on microperimetry, as well as qualitative data from spectral domain optical coherence tomography (OCT), color fundus photography, fundus autofluorescence (FAF), fluorescein angiography (FA), and indocyanine green (ICG) angiography, in addition to surgical time, intraoperative and postoperative surgical complications.

Surgery indication from 2007 to March 2011 was limited to exudative AMD with RPE tear and/or massive subretinal hemorrhage and eyes with chronic active AMD refractory to frequent (6 or more) injections of anti-VEGF therapeutics, associated with progressive visual loss. From April 2011, the inclusion criteria were limited to eyes with significant visual loss for no longer than 1 year but extended to include eyes with neovascular maculopathies other than AMD and atrophic maculopathies, associated with retinal dystrophies. For this study, we excluded eyes with lack of sufficient postoperative BCVA data and those who had a follow-up of less than 2 years, regardless of the degree of visual improvement or the presence of postoperative complications. These eyes mainly belonged to patients who were referred from distant locations, followed at our institution for a limited time after the operation, and subsequently returned to their local ophthalmologists. The study was conducted in accordance with the Declaration of Helsinki.

Functional Parameters

Distance vision and reading ability. Best-corrected visual acuity was measured in Snellen notation and converted to logarithm minimum angle of resolution (logMAR) for analyses. If more than one assessment of BCVA was made during that period, the BCVA demonstrating the greatest improvement from baseline was chosen. For the purpose of analysis, we classified two subgroups based on preoperative vision: the low preoperative BCVA group that included eyes with preoperative BCVA $<$20/200 ($>$1 logMAR) and the high preoperative BCVA group that included eyes with preoperative vision $\geq$20/200 ($\leq$1 logMAR). Reading ability was defined as ability to read, at 30 cm, at least point 12 of print letters.

Microperimetry. Macular Integrity Assessment Device system was used to test the retinal sensitivity and the preferred retinal locus of fixation. In this study, we used a low vision test, consisting of 68 test loci covering the central 20°, with a 4 to 2 projection strategy, that changes the light intensity in 4 dB steps, until there is a change from "not seen" to "seen" or from "seen" to "not seen." Then, the intensity changes in 2 dB steps until the stimulus is not seen again. Classification of fixation stability is represented in the Macular Integrity Assessment Device by the indices P1 and P2, which are defined as the percentage of fixation points falling inside a circle of 11 and 21 radii,

respectively, centered on the preferred retinal locus. Eyes with P1 greater than 75% are classified as stable. If P1 is less than 75% and P2 is more than 75%, fixation is classified as relatively unstable. If both P1 and P2 are less than 75%, the pattern is described as representing unstable fixation as defined.[11]

Morphological Parameters

Optical Coherence Tomography. Optical Coherence Tomography images were acquired on the Heidelberg (Heidelberg Engineering, GmbH, Dossenheim, Germany), Triton-Topcon (Topcon Corp, Tokyo, Japan), and Cirrus HD-OCT (Carl Zeiss Meditec, Oberkochen, Germany). Preoperatively, we evaluated the integrity of the external limiting membrane (ELM) to correlate the integrity of external retinal layers with the postoperative functional outcomes. Postoperatively, the OCT was used to study the RPE–choroid patch morphology and the occurrence of postoperative macular complications through qualitative analysis of the retinal and choroidal tissues morphology. We also evaluated the presence of subretinal fluid and cystoid macular edema (CME) before and after surgery.

Fundus autofluorescence. Fluorescein angiography (Heidelberg Engineering, GmbH) was used to detect location and extension of RPE atrophy in preoperative time and to monitor RPE alterations during postoperative time.

Color fundus photography. Color Fundus photography (Daytona; Optos, Marlborough, MA) was used to document postoperative appearance of the fundus and, in particular, the changes of patch morphology, the amount of bleeding in the macular region, and in patch harvesting site.

Fluorescein angiography and indocyanine green angiography. Preoperatively FA and ICG angiography (HRAII Heidelberg Engineering, GmbH) were used for the diagnosis. Postoperatively, we used these test modalities to evaluate the revascularization of the RPE–choroid patch and to follow its evolution. Based on OCT, FAF, FA, ICG angiography, we further categorized the study eyes into four maculopathy pattern subgroups: CNV-AMD subgroup; CNV-AMD-Hem subgroup for cases with subretinal hemorrhage; atrophy subgroup for atrophic maculopathies; and other CNV subgroup for CNV due to an etiology other than AMD.

Surgical Technique in Details

All patients had consented to the surgery after discussion of the benefits, and the potential risks and discomfort of the RPE–choroid patch procedure. A standard 3-port pars plana vitrectomy was performed with 20-gauge (G) instrumentation. In phakic patients, pars plana vitrectomy was routinely combined with phacoemulsification, and a 3-piece intraocular lens was implanted in the capsular bag. After sclerotomy creation, posterior vitreous detachment was induced when not already present, and vitrectomy was completed in the usual manner with removal of the vitreous till the anterior vitreous base, assisted by scleral indentation and triamcinolone staining. The inferotemporal sclerotomy was cut perpendicularly and occupied by an illuminated infusion cannula, sutured to the sclera. The superior sclerotomies were cut tangentially to the scleral plane. Residues of vitreous, which remained incarcerated into the sclerotomies, were aspirated and removed with the vitrectomy probe. The internal limiting membrane (ILM) was peeled routinely since 2011. A retinal detachment (RD) was then induced by injecting balanced salt solution into the subretinal space using a 41-G needle, connected to the active pump of the viscous fluid injection apparatus of vitrectomy machine, set at 40 mmHg, to obtain a steady stream during the injection in the subretinal space. The RD was then extended with a fluid–air exchange. Under balanced salt solution, an approximately 200° peripheral retinotomy was performed with scissors near to the ora serrata in the superior and temporal quadrant. The temporal retina was folded on the nasal retina, to expose the temporal superior and inferior subretinal space. Choroidal neovascular membrane, when present, was removed using retinal forceps, and endodiathermy was applied to the feeder vessels after CNV removal. Endodiathermy was also applied in multiple spots at the macular area, in all operated eyes including those with atrophic maculopathy in absence of a CNV, with the aim of creating microtrauma of the choriocapillaris to enhance the revascularization of the RPE–choroid patch. The location of the harvesting site of the patch was chosen by visually selecting an area of normally appearing RPE in temporal, superior, or inferior quadrants, avoiding the vortex veins. An intense diathermy was first applied around the margins of the intended patch (measuring approximately three optic disk diameters). Using a 20-G vertical-cutting intraocular scissor, the combined RPE and choroidal tissue were then cut down, till the clear white sclera was visible, within the margins of the diathermy burns, starting at the posterior aspect of the intended patch graft. Any encountered choroidal bleeding was mainly controlled with additional diathermy and raising the infusion pressure for several minutes. When raising the infusion pressure, the sclerotomy was

kept closed by instruments to avoid retina incarceration into the sclerotomy. After dissecting the posterior part of the patch, perfluorocarbon liquid (PFCL) was injected into the subretinal space and over the isolated edges of the patch, to avoid the rolling or elevation of dissected edges of the RPE–choroid patch. Subsequently, the anterior half of the patch was cut, and the RPE–choroid patch was transplanted using nontoothed retinal forceps, onto the submacular area under PFCL. Care was taken to flatten the border of the RPE–choroid patch with a spatula. At the harvesting site, remaining residues of choroid were removed from the sclera with aspiration and/or forceps, and the borders of incised RPE–choroid were heavily treated with endodiathermy. Perfluorocarbon liquid was progressively and slowly aspirated and subsequently reinjected in the epiretinal space to flatten retina on the top of the patch and to center fovea on visually healthy RPE. If the patch was not centered, then PFCL was moved again subretinally, the patch position was recentered, and then the maneuver of moving PFCL from the subretinal to the epiretinal space was repeated. Retina was then completely reattached with PFCL, and retinotomy was sealed with multiple rows of laser. Peripheral laser was applied to the retinotomy edge and extended to 360°. The peripheral retina at the ora serrata anterior to the retinotomy was carefully removed with vitrectomy probe. Perfluorocarbon liquid was finally exchanged directly with 1,000 cSt silicone oil. Silicone oil was removed after at least 3 months. Patients received further surgery in case of postoperative RD or epiretinal membrane (ERM) formation.

Statistical Analysis

We used SPSS Software Version 22.0 (IBM Corporation, New York, NY) for the statistical analysis. Because of the fact that the distribution of our sample was not a normal distribution, we used nonparametric tests for analysis. Spearman's rho correlation analysis was used to evaluate correlation between the preoperative quantitative and qualitative variables and postoperative visual outcome. The relationship between preoperative (age, preoperative BCVA, perfusion of the RPE–choroid patch, and ELM integrity) and postoperative BCVA was evaluated with linear regression analysis. To compare preoperative and postoperative BCVA for the whole group of patients and for each subgroup of maculopathy patterns, Mann–Whitney *U* test was performed. For all tests, *P* value <0.05 was considered statistically significant.

Results

From the original cohort of 120 eyes operated since 2007, 88 eyes (36 right and 52 left) of 84 patients (41 women and 43 men) were eligible for analysis. The remaining 32 eyes were excluded because of a follow-up time shorter than 24 months or for lack of sufficient visual acuity data. For the 88 eyes included in the analysis, the mean age was 71.9 ± 9.7 years (range: 42–88, median 74). Seventy-seven eyes of 75 patients were affected by neovascular maculopathy, 11 eyes of 9 patients by atrophic maculopathy (Table 1). All 88 study eyes had a follow-up time of at least 24 months. Forty-nine (56%) eyes had a follow-up of 3 years to 5 years, 15 (17%) eyes had a follow-up of 6 years to 8 years, and 4 eyes (4.5%) had a follow-up of 8 years to 9 years.

Anatomical Results

Biomicroscopy and color fundus photograph. For 59 eyes (67%) that did not have hazy media early postoperatively, the RPE–choroid patch was well visible, between 1 day and 1 week, in the subfoveal area as a pigmented thick oval to round tissue, with an average size of 3-disk diameters (Figure 1A). In temporal quadrants, a whitish area of bare sclera was visible, identifying the harvesting site of the patch. Subretinal blood remnants at the borders of the harvesting site were a common finding and lasted for the first 4 weeks to 6 weeks (Figure 1, A and B). Three months after surgery (Figure 1B), the RPE–choroid patch had more defined and flat thin edges. A progressive reabsorption of blood was observed at the harvesting site.

Commonly, the patch had a darker pigmentation with respect to the surrounding tissue (Figure 1, A and B). Silicone oil was removed in 78 (89%) eyes at mean time of 5.5 months after surgery (range: 3–8). After silicone oil removal, the appearance of the patch and the harvesting site did not change through follow-up. In a subgroup of 39 eyes (44%), we observed a fibrotic thin scar such as a whitish prophyle surrounding the patch. This scar extended to the harvesting site, in eyes in which the shortest distance between the edge of the patch and the harvesting site was inferior to the diameter of the harvesting site (Figure 2).

Fundus autofluorescence. For all eyes, the FAF of the patch was comparable with the FAF of the peripheral choroid. Multiple areas of hypoautofluorescence were noticed at the borders of the patch, where the necessary surgical manipulation was applied (Figure 3B). Fifty-nine eyes (67%) presented scattered areas of hypoautofluorescence also over the patch and

Table 1. Demographic, Morphological, and Functional Data

Demographic and Functional Data					
No. of Patients, No. of Eyes	Age, Years, Mean SD (Range)	Gender, Female/ Male, Number (%)	Eye, Right/Left, Number (%)	Preop BCVA-Treated Eye, Snellen, Mean SD (Range) logMAR	Preop BCVA Fellow Eye, Snellen, Mean SD (Range) logMAR
84, 88	71.9 ± 9.06 (50–88)	41 (48.8)/43 (51.2)	36 (40.9)/52 (59.1)	20/252, 1.1 ± 0.2 (0.4–1.5)	20/74, 0.57 ± 0.5 (0–1.5)

Maculopathy Pattern Subgroups					
Exudative Maculopathies			Atrophic Maculopathies		
Subtype	Number	%	Subtype	Number	%
AMD-CNV	48	62.3	Dry AMD	1	9.1
Hemorrhagic CNV-AMD	23	29.8			
Myopic CNV	2	2.6	Heredodystrophic/atrophic maculopathies	7	63.6
CNV secondary to angioid streak	1	1.3			
Idiopathic CNV	2	2.6	Pattern dystrophies	3	27.3
CNV secondary to laser for CSCR	1	1.3			
Total	77	100	Total	11	100

CSCR, central serous chorioretinopathy.

in the posterior pole, around the patch, in longer follow-up (Figure 3C).

Optical Coherence Tomography. The patch appeared as a subfoveal layer of RPE and choroid, with open large hyporeflective spaces corresponding to perfused choroidal vessels (Figure 4). We observed the disappearance of subretinal fluid and CME in the immediate postoperative follow-up, in all cases that presented subretinal fluid (40%) and CME preoperatively (25%). The visibility of external layers of retina changed in time, being rarely visible for at least 1 month after surgery and becoming more defined in the long follow-up (Figure 4).

Fluorescein and indocyanine angiography. One month after surgery, FA and indocyanine green confirmed the vascularization of the patch in all but seven eyes (7.9%). The choroidal vessels into the patch exhibited different direction as compared to the vessels of surrounding choroid (Figure 5, B and C). Vascularization of the patch was homogenous in 77 (87%) eyes, whereas in 4 (4.5%) eyes, the patch showed a nonhomogeneous vascularization pattern, meaning that some areas of the patch were less vascularized than others and presented sectorial hypoperfusion. Figure 5 summarizes the evolution of one case followed from baseline and for more than 5 years with FA, ICG, OCT, FAF, and microperimetry.

Functional Results

Visual outcomes. Mean baseline BCVA was 20/320 (logMAR 1.2 ± 0.2) and 20/200 (logMAR 0.94 ± 0.36 range: 0.4–1.5, median 1.1), respectively. By the end of follow-up time, mean BCVA improved in the whole cohort of eyes to 20/200 (logMAR 0.94 ± 0.36 range:

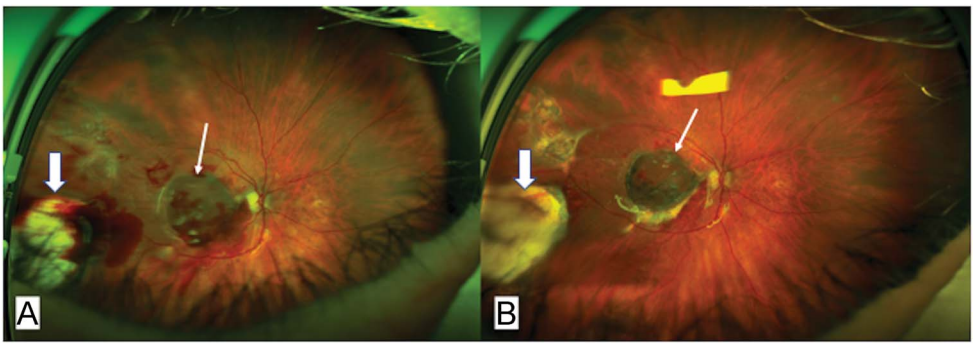

Fig. 1. A. Fundus photograph of an eye, 1 week after RPE–choroid patch surgery. The patch is well visible, in the subfoveal area (white thin arrows), as a pigmented thick oval—round tissue, with an average size of three disk diameters. In the inferotemporal quadrant, a whitish area of bare sclera (white thick arrow) identifies the harvesting site of the patch. Subretinal blood remnants at the borders of the harvesting site and at the borders of the patch are visible. The vitreous cavity is silicone-filled. **B.** Fundus photography of the same eye, 3 months after surgery. The patch has more defined and flat thin edges (white thin arrows), and reabsorption of blood is observed at the harvesting site (white thick arrow) and around the patch. The patch has a darker pigmentation with respect to the surrounding tissue. The whitish area of bare sclera in the inferotemporal quadrant (white thick arrow) corresponds to the harvesting site.

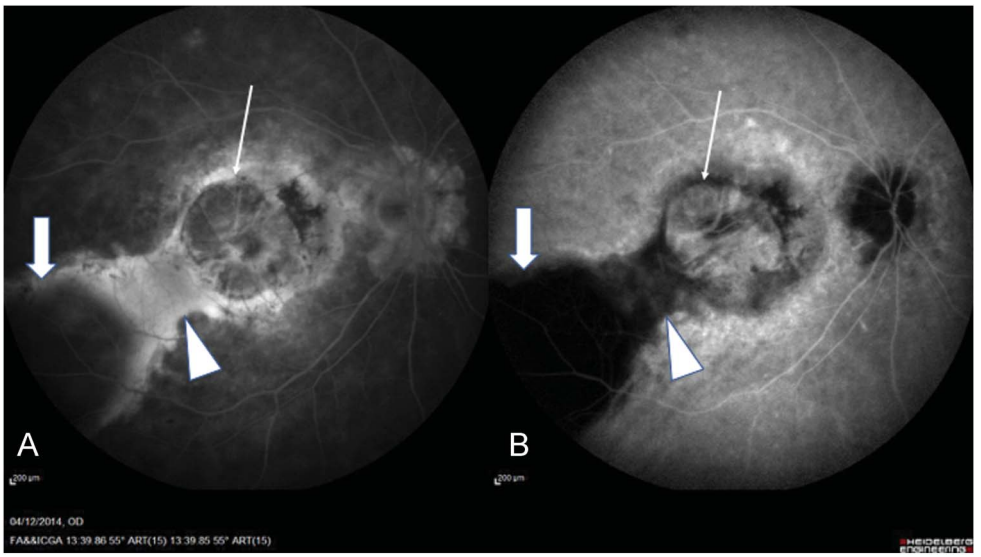

Fig. 2. A. Fluorescein angiography (FA) and (**B**) indocyanine green angiograph images of an eye, 2 years after patch surgery. The patch is vascularized. A fibrotic thin scar surrounds the patch, as a whitish line on FA and a black in ICG (thin white arrow). The fibrotic scar (arrowhead) extends to the dark harvesting site (thick white arrow). Note that the shortest distance between the edge of the patch and the harvesting site is inferior to the diameter of the harvesting site.

0.2–1.3, median 1.1). The difference was statistically significant (P = 0.009). Specifically, the statistical analysis demonstrated significant improvement of BCVA in the CNV-AMD (P = 0.0063), CNV-AMD-Hem (P = 0.0074), and other CNV subgroups (P = 0.0030). Visual acuity improvement was not statistically significant in the atrophy subgroup (Table 2). A gain in vision of at least 15 letters was obtained,

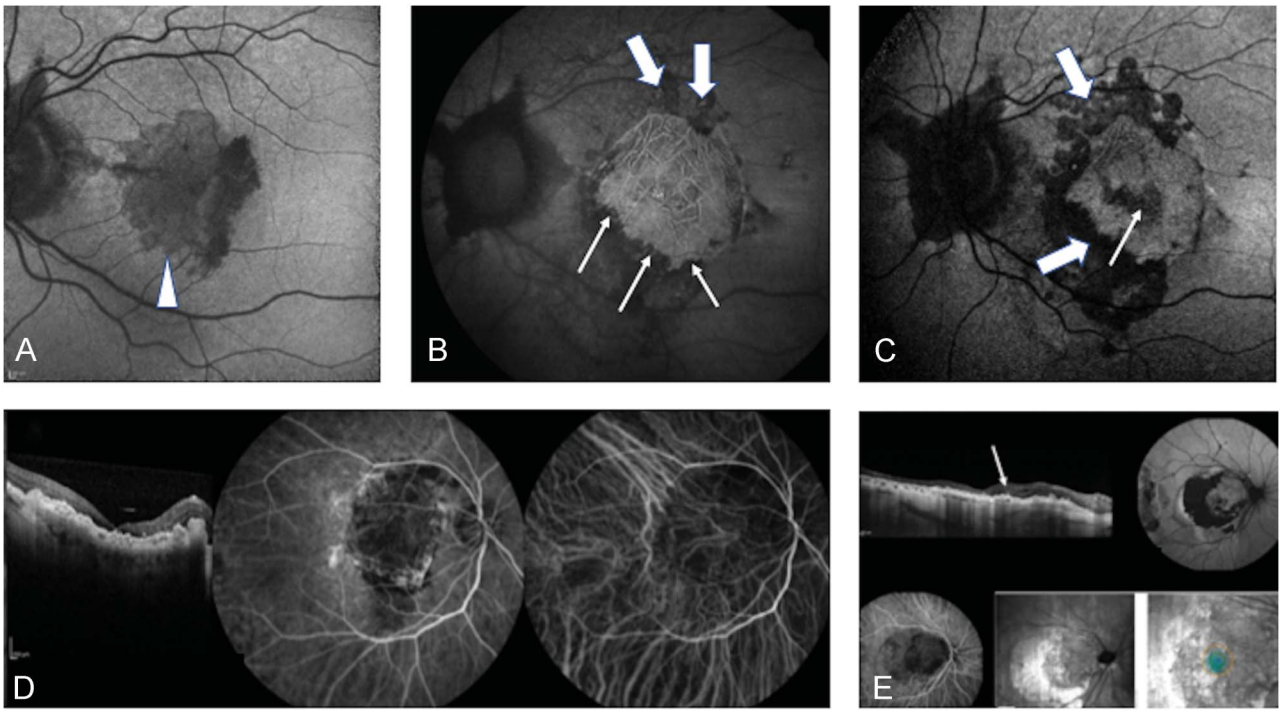

Fig. 3. A. Fundus autofluorescence before surgery in an eye with CNV and angioid streak. The hypofluorescent area corresponds to the CNV (arrowhead). **B.** Fundus autofluorescence of the same eye, 6 months after surgery. Over the area of the patch, FAF appeared comparable with the FAF of the surrounding choroid, although the presence of wrinkles in the patch is visible as lines of hypofluorescence and hyperfluorescence. Multiple areas of hypoautofluorescence are noticed at the borders of the patch, where the necessary surgical manipulation was applied (thin white arrows). However, new areas of hypoautofluorescence are present outside the borders of the patch (thick white arrows). **C.** Fundus autofluorescence of the same eye, 4 years after surgery. In this eye, scattered areas of hypoautofluorescence developed over the patch during the follow-up, as a sign of progressive atrophy (thin white arrow). The hypoautofluorescence surrounding the patch increased significantly over the follow-up (thick white arrows). **D.** Optical coherence tomography showing external retinal atrophy over a patch, which is vascularized, as documented by FA and ICG. **E.** Optical coherence tomography of a patch exhibiting revascularization only in the nasal half. Note the thicker retinal thickness (thin white arrow) over the vascularized half of the patch.

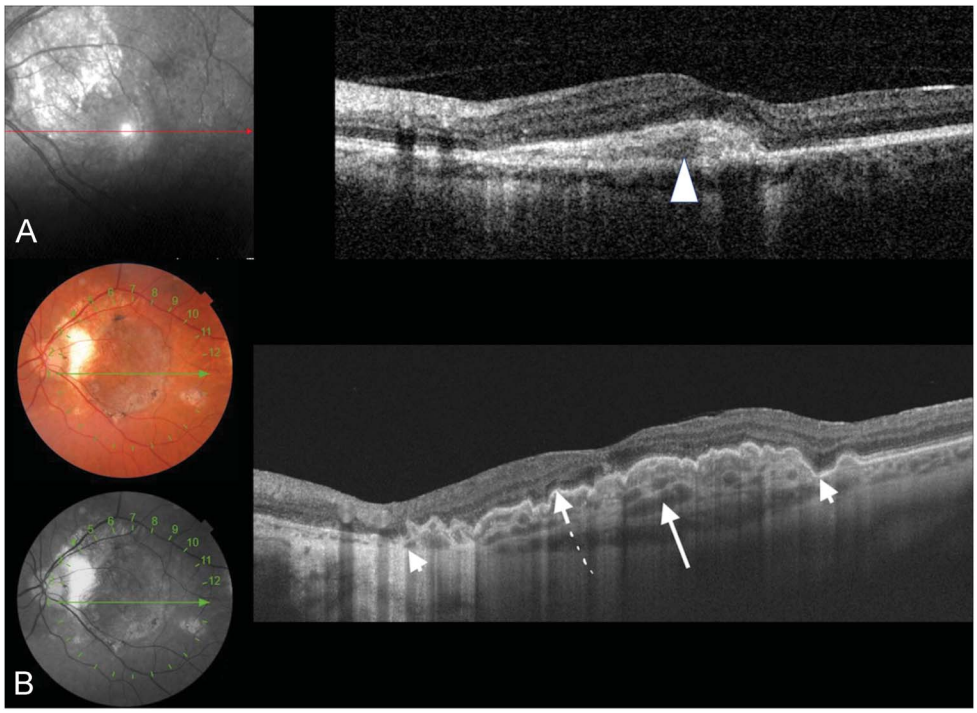

Fig. 4. A. Infrared and OCT images of an eye with subfoveal choroidal neovascularization, in fibrotic stage (arrowhead). **B.** Color, infrared, and OCT images of the same eye, 5 years after RPE–choroid patch surgery. The patch appears as a subfoveal layer of RPE and choroid with open large hyporeflective spaces corresponding to perfused choroidal vessels (white thin arrow). The external layers of the retina are visible (dashed white arrow). The edges of the patch are marked by white arrowheads.

respectively, in 24/58 (41%) of the low preoperative BCVA subgroup and in 11/30 (37%) of the high preoperative BCVA subgroup. Forty-four (50%) eyes had BCVA of ≥20/200 after 4 years. Six (7%) eyes had BCVA of ≥20/40 after 4 years. Twenty-five eyes (28%) showed a BCVA gain between 25 letters and 50 letters at final measurement, after 4 years. Among the eyes with final improvement in BCVA, we observed different pattern of visual change. A progressive and fast improvement in BCVA, within 6 months after surgery, was observed in 17 eyes (20%) of patients with mean age of 64 years (range 42–64); a progressive but slow improvement in BCVA, in the first postoperative year, was observed in 14 eyes (15%) of patients with mean age of 75 years (range 65–81); a late and sudden improvement of BCVA occurred more than 1 year after surgery in 3 eyes (3.4%) of patients, who were, respectively, 66, 75, and 76 years old (mean age of 72 years). Reading ability was recovered in 43% of cases. Regarding the effect of the pattern of maculopathy on the postoperative visual acuity outcome, we found improvement of mean BCVA in the CNV-AMD (7 letters), CNV-AMD-Hem (11.5 letters), and other CNV subgroups (26.7), whereas BCVA improvement was not statistically significant in the atrophy subgroup (2 letters) as compared to baseline. Table 3 shows the percentage of eyes gaining and losing letters of vision for each functional and maculopathy pattern subgroup. The mean time to recover the final vision was 12 months. Based on the Spearman's rho correlation analysis, only 2 predictive factors were found to be associated significantly with final BCVA. The first one was the integrity of ELM (P = 0.009, R = 0.567): cases with ELM that seemed tomographically intact were associated with a greater final BCVA than those with nonintact ELM. The second predictive factor was the preoperative BCVA (P = 0.001, rho R = 0.814). To further confirm this finding, linear regression analysis demonstrates a positive correlation between preoperative BCVA and final BCVA (P = 0.001). Although age did not significantly correlate with final BCVA, linear regression analysis between age and gain in vision shows a tendency to a lower gain in vision with increasing age (Figure 6). In fact, in few patients (4 eyes) between 78 years and 85 years of age, we observed a significant improvement in vision between 10 letters and 40 letters.

Microperimetry. Twenty eyes, with final reading ability of at least 12 pt, underwent microperimetry, which became available to us only recently. All eyes showed a central foveal fixation with average sensitivity of 10.5 dB (range 5.1–14.8 dB) and average fixation stability of P1 = 37.8% (range 10%–87%) and P2 = 73.5%. Fixation was located over the patch (Figures 3E, 5F, and 7A). The microperimetry grid was superimposed on the OCT B scan by matching the color fundus photographs. This allowed us to provide good correlation between the functional result in terms of retinal sensitivity and the anatomical results, point by point, on the OCT scan (Figure 7B).

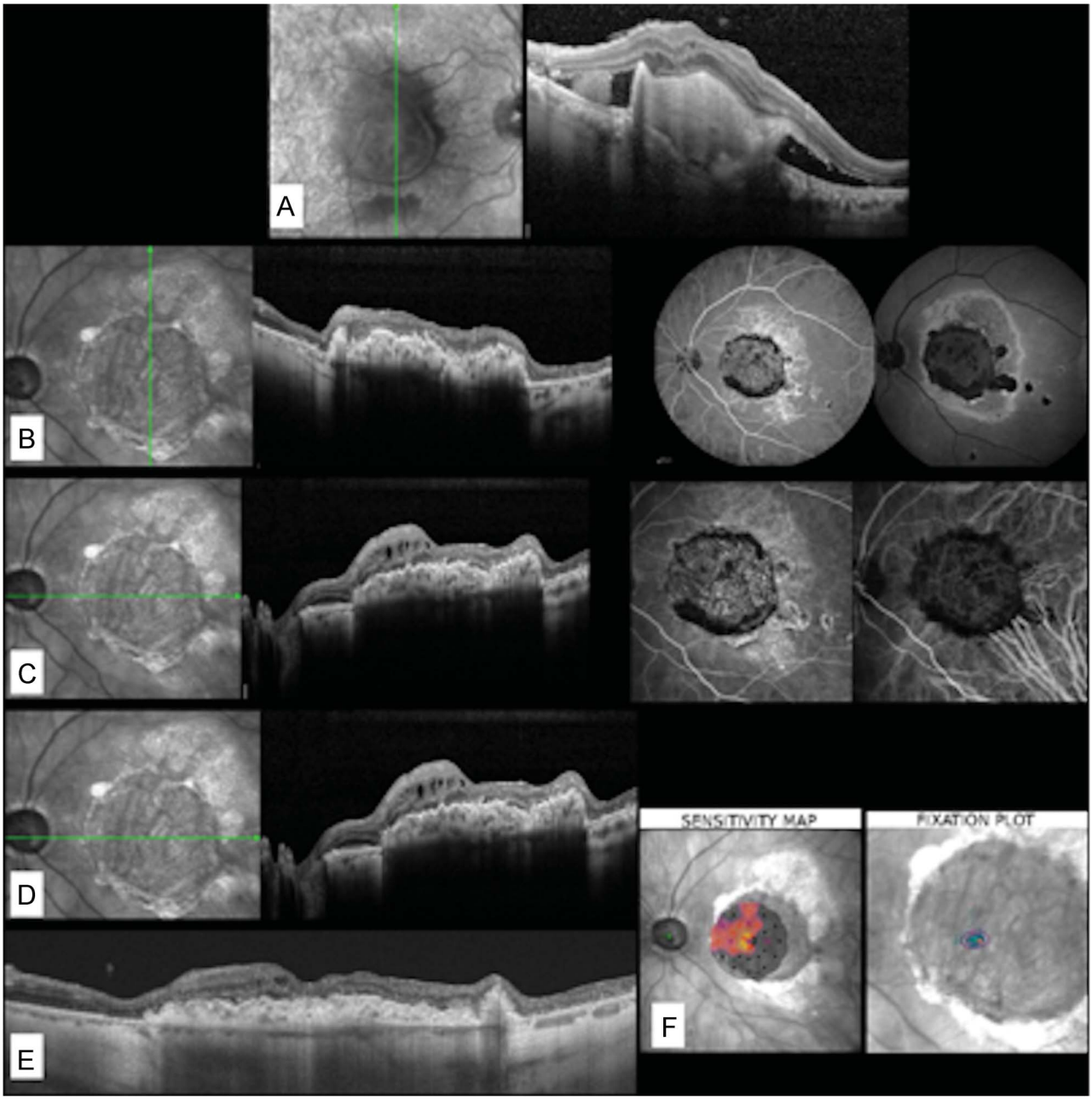

Fig. 5. Evolution of one eye with subfoveal CNV from baseline to 5-year follow-up. **A.** Preoperative OCT shows the retina with visible external layers and ELM, the subfoveal CNV, and subretinal fluid. **B.** Infrared, OCT, FA, and ICG of an RPE–choroid patch, 3 months after surgery. The patch is well vascularized, and BCVA is 20/40. **C.** Infrared, OCT, FA, ICG, 2 years after surgery, with partial CME over the nasal half of the patch, BCVA 20/60. **D.** Infrared OCT, 2.5 years after surgery, with edema still present; BCVA 20/60. **E.** Optical coherence tomography and microperimetry, 3 years after surgery, with disappearance of cystoid edema (**E**), and restoration of vision (BCVA 20/40) and central fixation (**F**).

Complications

Postoperative complications were observed between 3 days and 3 months after surgery, except RPE–choroidal atrophy, which appeared later and progressively. In 10 eyes (11.4%), RD occurred and promptly underwent surgery. The detachment occurred early, within the 1st week (3 eyes), or 3 weeks to 6 weeks after the primary surgery with proliferative vitreoretinopathy (5 eyes), or after silicone oil removal surgery (2 eyes). Epiretinal membrane developed in 4 eyes (4.5%). These eyes subsequently received further surgery with ERM and ILM peeling. Because internal limiting removal was performed during the primary surgical procedure, we no longer observed ERM development in the absence of RD. Four eyes (4.5%) presented postoperative subretinal

Table 2. Analysis of BCVA in Whole Group and in Maculopathy Pattern Subgroups

Maculopathy Pattern Subgroups	No. of Eyes (%)	Preoperative BCVA, Snellen, Mean SD (Range) logMAR	Final BCVA, Snellen, Mean SD (Range) logMAR	*P*
CNV-AMD	48 (54.54)	20/252, 1.1 ± 0.23 (0.6–1.5)	20/187, 0.97 ± 0.39 (0.2–1.3)	*0.0063
CNV-AMD-Hem	23 (26.14)	20/317, 1.2 ± 0.17 (0.7–1.5)	20/200, 1 ± 0.35 (0.3–1.3)	*0.0074
Atrophy	11 (12.5)	20/200, 1.0 ± 0.15 (1.0–1.3)	20/182, 0.96 ± 0.336 (0.4–1.3)	0.2991
Other CNV	6 (6.82)	20/317, 1.2 ± 0.27 (0.7–1.0)	20/100, 0.7 ± 0.34 (0.4–1.3)	*0.0030
Whole group	88 (100)	20/252, 1.1 ± 0.2 (0.4–1.5)	20/182, 0.96 ± 0.37 (0.3–1.3)	*0.0090

*P-value < 0.05.

hemorrhage, occurring 1 day after surgery. These eyes were in the CNV-AMD-Hem group and were undertaking anticoagulant medications, which had been stopped 15 days before surgery but were restarted 2 days to 3 days after surgery. These eyes lost significant vision due to the complications with an average final vision of 20/500. Progressive atrophy of the RPE–choroid patch occurred in six (7%) eyes, five eyes affected by exudative maculopathy and one eye affected by atrophic maculopathy. One eye (1.1%) had no vascularization in the patch, and another eye showed revascularization only in one half of the patch with progressive retinal atrophy occurring only over the nonvascularized half of the patch. Gain in vision in this eye was slow, and only after 3 years, the patient gained BCVA of 0.2 Snellen and central fixation (Figure 3E). However, atrophy could be observed even in eyes with vascularized patch (Figure 3D). Four eyes (4.5%) operated for CNV-related maculopathy developed recurrent CNV in an extrafoveal location and were treated with anti-VEGF injections and/or focal laser. Three eyes (3%) developed CME and were treated with topical steroid obtaining only partial benefit (Figure 5). Two eyes (2.3%) showed patch decentration that was evident a month after surgery. Overall, 10 eyes (11%) had final postoperative BCVA lower than preoperative BCVA due to complications.

Surgical time. The mean surgical time was 2 hours and 50 minutes (range 2 hours 30 minutes−4 hours 10 minutes) for the first 40 eyes and decreased to 1 hour 40 minutes (range 1 hour 20 minutes−2 hours 55 minutes) for the subsequent cases.

Discussion

This study found out that the transplantation of a full-thickness patch of RPE and choroid under the fovea may improve the vision, in a selected group of patients affected by various types of maculopathies. Overall, the mean final vision significantly improved, when compared with the mean preoperative vision. Moreover, 35 eyes (40%) showed an improvement in vision of at least 15 letters, 25 eyes (28%) had an improvement of 25 to 50 letters, and 36 eyes (41%) remained stable. Hence, 81% of the study eyes did not experience visual loss over a long follow-up, with no further treatment, except for silicone oil removal. Furthermore, eyes that experienced visual improvement, not only gained far sight,

Table 3. Gain in Letter Score in Maculopathy Pattern Subgroups and Functional Subgroups

	n	Gain of at Least 15 Letters, Number (%)	Loss of at Least 15 Letters, Number (%)	Stable (±10 Letters), Number (%)	Reading Ability, Number (%)
Maculopathy pattern subgroups					
CNV-AMD	48	15 (31)	6 (12)	27 (56)	18 (37)
CNV-AMD-Hem	23	12 (52)	1 (4)	11 (48)	10 (43)
Atrophy	11	2 (18)	2 (18)	7 (63)	6 (54)
Other CNV	6	5 (83)	0	1 (17)	4 (66)
Functional subgroups					
Low preop BCVA subgroup	58	22 (38)	1 (2)	35 (60)	19 (33)
High preop BCVA subgroup	30	11 (37)	8 (27)	11 (37)	19 (63)
Whole group	88	33 (38)	9 (10)	46 (52)	38 (43)

Low preop BCVA subgroup <1 logMAR or ≤30 letters.
High preop BCVA subgroup >1 logMAR or >30 letters.

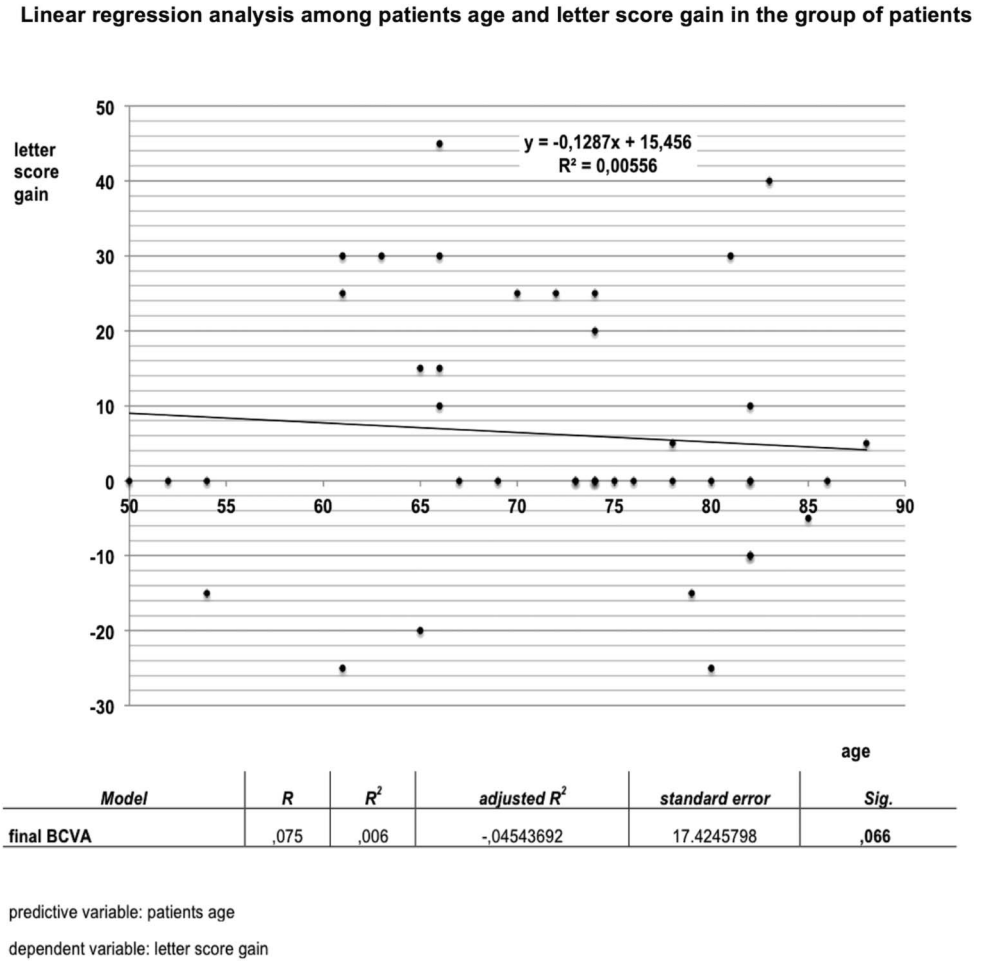

Fig. 6. Linear regression analysis of the relationship between patients' age and letter score gain for the whole cohort of eyes operated by RPE–choroid patch graft surgery.

but also achieved reading ability and central fixation. In fact, 43% of eyes reached the ability to read again using the area of retina over the patch, as demonstrated by microperimetry. The course of visual acuity was analyzed in different subgroups, based on preoperative BCVA, the diagnosis, and type of maculopathy. The gain in vision resulted statistically significant for eyes operated with low preoperative BCVA. However, the

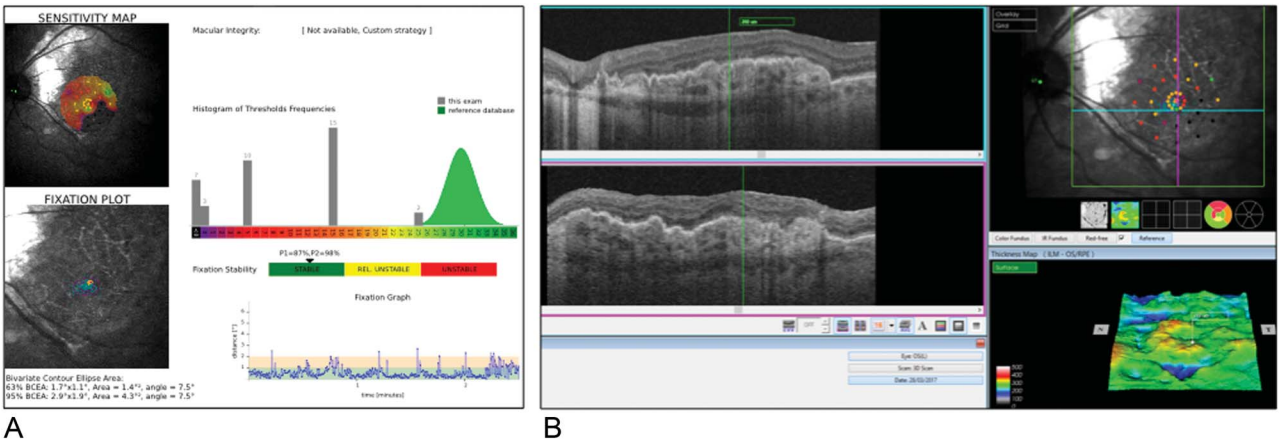

Fig. 7. Microperimetry analysis. **A.** Microperimetry plot of a patient, 3 years after patch surgery. The plot shows central fixation on the patch with high fixation stability: P1 = 87%, P2 = 98%. **B.** The microperimetry grid of the same eye superimposed on the OCT B scan, by matching the color fundus photograms.

mean final vision was 20/200 in the low preoperative BCVA subgroup and 20/125 in high preoperative BCVA subgroup. Hence, if the eye is operated when the preoperative BCVA is higher, the amount of improvement might be lower, but the absolute value of final vision will be higher. In our group of patients, the lowest preoperative BCVA was 20/600 in 5 eyes (6%). Long-term visual gain was significant also for eyes with hemorrhagic CNV secondary to AMD and for CNV of other etiologies (myopia, iatrogenic, and idiopathic), but not for atrophic maculopathies. It has to be noted that the eyes that gained vision, maintained it in the long term, with no further treatment.

The ANCHOR study[12] reported that eyes with exudative AMD, receiving monthly injection of 0.5-mg ranibizumab, gained 15 letters or more in 40.3% after 1 year. The MARINA STUDY[13] reported a mean gain of 7.2 letters after 2 years of monthly ranibizumab injections. The VIEW study[14] reported a gain of 17.2 letters at 12 months for patients with baseline BCVA <20/200 and treated with aflibercept. However, the functional benefit obtained with monthly doses of anti-VEGF was lost over long-term follow-up, as reported in studies describing real-life outcomes.[15,16] In our study, a gain in vision of at least 15 letters was obtained in 41% of the low preoperative BCVA subgroup with vision and in 37% of the high preoperative BCVA subgroup with preop vision. Ross et al[17] reported that eyes treated with ranibizumab for CNV-AMD showed a change of BCVA from baseline to 1 year of +6.5, +7.5, +1.7, and −1.5 letters, respectively, for baseline visual acuity categories of 23 to 35, 36 to 55, 56 to 70, and >70 letters. Furthermore, a large study conducted in the United Kingdom, published in 2014,[18] reported the results of using ranibizumab for CNV-AMD in 12,951 eyes. Mean visual acuity (letters) for eyes followed up for at least 3 years from a baseline of 55 letters was 57 (+2) letters at 1 year, 56 (+1) letters at 2 years, and 53 (−2) letters at 3 years. Overall, our results compare favorably with those obtained from anti-VEGF injection in eyes with neovascular AMD, although direct comparison may not be possible due to differences in study designs, inclusion criteria, and preoperative vision.

The visual course was different in exudative and atrophic maculopathies. Eyes affected by dry AMD showed an initial improvement in vision, which remained stable for approximately 2 years. The comparison between BCVA at the end of the follow-up with the preoperative BCVA did not result statistically significant, because of the reoccurrence of choroidal atrophy 2 years after surgery. In patients with atrophic heredodystrophies as well, we have seen that the disease might relapse after a few years, compromising the initial significant benefit of surgery. Nevertheless, we think that even prolonging the time of useful vision, for these patients with no other options, might be helpful, while waiting for more appropriate approaches such as genetic therapy.

The follow-up in this study was at least two years. However, 19 eyes had a follow-up between 6 years and 10 years. The long follow-up has allowed us to understand that eyes showing an improvement in vision may present different pattern of speed of visual gain, as previously reported by Van Zeeburg et al[6] ranging from fast improvement within the first 6 months or slow and progressive visual improvement in the 1st year, and finally but unexpectedly, presenting sudden visual improvement more than 1 year after surgery.

Although there was a trend for a lower gain in vision with increasing age, the age of the patient was not a limiting factor to reach a positive result. However, we think that there might be a relation between age and speed of visual gain after surgery; the younger patients presented a possible recovery in vision within the first 6 months, whereas the older patients showed a slow and progressive or a late but sudden improvement. The threshold age seemed to be approximately 70 years.

Indications for surgery were initially limited to CNV secondary to AMD in patients with RPE tear, subretinal hemorrhages, or patients losing vision after anti-VEGF injections. Because of the encouraging results, indications were extended after March 2011 to eyes with exudative or atrophic maculopathies affecting primarily the RPE, with a preoperative BCVA ≥20/200 and visibility of the outer retina on OCT. Having improved the selection criteria, the efficacy of surgery improved significantly. In previous publication on patch surgery for AMD,[19,20] patients were selected based on relatively large subfoveal lesions or poorly demarcated CNVs not treatable with laser or photodynamic therapy. Leakage on fluorescein angiography plus history of recent loss of reading vision (less than 3 months) was a main perquisite for surgery. Eligible eyes had BCVA corresponding to Snellen fractions of 20/40 to 20/800. Based on the results of this study, we think that eligible eyes should have preserved outer retinal layers, a visual acuity ranging between 20/200 and 20/80 or recent higher loss of vision due to subretinal hemorrhage or RPE tear. Our study is in accordance with a more recent study published by Van Romunde et al[21] that highlighted the importance of the state of the outer retina layers at baseline, to promote a functional improvement. Among the prognostic factors that we analyzed, only the visibility of the ELM on preoperative OCT

and preoperative BCVA were statistically significant. We recommend using these parameters as a good indicator for a successful result of surgery. The less damaged is the retina preoperatively, the better will be the final functional and anatomical outcome.

Patch revascularization was observed in 92% of the eyes. This high rate of revascularization could be due to the heavy diathermy applied to the macular choroidal surface in multiple areas before transplanting the patch of choroid, even in absence of CNV removal, as in the atrophic cases. We think that this maneuver might create a microtrauma to the choriocapillaris that might favor the revascularization.

We observed a complication rate that was comparable with what reported in literature for the same subject.[6,21,22] Eyes with RD were reoperated and could preserve visual. During primary surgery, an extensive and meticulous vitrectomy is performed, and peripheral retina is carefully reviewed. Retinotomy is performed near the ora serrata, involving the superior temporal and inferior temporal quadrant. All RD cases originated from retinotomy, either early and due possibly to incomplete reattachment at the time of final tamponade exchange, or late with the occurrence of proliferative vitreoretinopathy at the edge of retinotomy.

Choroidal neovascular membrane occurred at the edge of the patch and was probably due to iatrogenic intraoperative trauma to the choroid. After the occurrence of 4 cases of postoperative ERM, the ILM peeling was added to the surgical technique, and since then, the occurrence of ERM was no longer observed. As well, we observed that ILM peeling during primary surgery preserved the macular area from proliferative vitreoretinopathy. Two macular holes occurred postoperatively and two intraoperatively during the detachment of the retina with subretinal injection. It might be possible that eyes with preoperative subnormal retinal thickness area at higher risk although we were not able to statistically associate the occurrence of iatrogenic macular hole with preoperative retinal thickness. To avoid this complication, we recommend detaching retina first in the periphery and not in the submacular area. Also, when peeling the ILM to avoid the postoperative occurrence of ERM on the macula, we advocate to perform a foveal sparing ILM peeling technique, to lower the risk of developing intraoperative iatrogenic macular hole (personal observation of the surgeon). The cases that showed macular hole as postoperative complications were operated before 2015. The patients refused further surgery. In case of occurrence of macular hole at the present time, in absence of ILM (already peeled during primary surgery), we would suggest additional procedures as recently described.[23,24]

Subretinal hemorrhages occurred in patients who were undertaking anticoagulant therapy. For all of them, anticoagulant therapy had been suspended 15 days before surgery. Since then, anticoagulant therapy was suspended at least 20 days before surgery and substituted with heparin. Patients that presented severe contraindication to anticoagulant suspension were excluded from surgery. By doing so, severe subretinal hemorrhage was no longer observed.

Patch decentration may jeopardize the result of surgery. Intraoperatively, care was taken to center the patch to the center of the fovea. However, a small patch might partially migrate in the weeks after surgery for a process of fibrosis of the edges. This is one of the complications observed in the 1st years of surgery. Since then, we started to isolate larger patch, at least 3 disk diameters large. The large size allowed for easier intraoperative manipulation of the patch and compensated for the postoperative contraction and fibrosis of the edges.

The most sight-threatening complication was postoperative progressive macular atrophy. The explanation for progressive macular atrophy is not completely understood. Some of these cases were associated with lack of patch revascularization. In other eyes, atrophy could be explained only with persistence of original pathology despite the RPE–choroid patch surgery. A recent article[25] hypothesizes that contiguity between native and transplanted RPE could be protective factor against the progression of patch atrophy.

Silicone oil was not removed in all patients. Cases that underwent subsequent RD surgery and had low functional expectations or patients that were not willing to undergo further surgery were left under silicone oil–filled, as long as the intraocular pressure was under control and the eye had no signs of inflammation.

This study has limitations. First, our results are subjects to bias inherent to the retrospective design and different follow-up times. Second, the surgical technique for RPE–choroid patch graft has a steep learning curve. In fact, the operating time decreased to almost half in the last 49 eyes, as well as the occurrence of complications. We believe that a significant part of the success of the surgery relies on the intraoperative course, both in terms of efficacy and safety.

Two operations are required with the present technique because the tamponade in the first surgery is always silicone oil 1,000 cSt. Although our preference is still to use 20 g instrumentation for the surgery, the procedure could also be performed with smaller gauge instrumentation based on the surgeon preference.

To our knowledge, RPE–choroid patch surgery has not been reported in eyes with CNV secondary to high

myopia, idiopathic CNV, pattern dystrophies, or heredodystrophy. We previously reported the outcome of this surgery for CNV secondary to angioid streaks.[26] We think that this surgical technique could be indicated to nearly all the eyes with diseases affecting primarily the RPE and the choroid. By inserting healthy RPE and choroid under relatively preserved photoreceptors, surgery may prolong the macular function and prevent retinal atrophy. However, because the transplant is autologous, it is possible to obtain good functional results with a less complex surgery technique as shown in the recent literature.[27]

Conclusion

Autologous RPE–choroid transplantation has shown to improve visual function in eyes with maculopathies of different origins, both in terms of distance vision, reading vision, and central fixation. Based on our results, we recommend autologous RPE–choroid transplantation surgery only in patients with exudative maculopathies of various origins, with visible ELM and/or photoreceptors at the preoperative OCT, even when a recent subretinal hemorrhage is associated. A preoperative BCVA equal or greater than 20/200 might lead to better final functional results. Considering the possible postoperative complications with the present surgical technique, we recommend to indicate surgery only to patients with vision equal or lower than 20/100. In atrophic maculopathies, surgery might be considered only as a last resource for patients with poor vision in the other eye, and a guarded outcome should be highlighted.

Key words: AMD, atrophic maculopathy, choroid, choroidal neovascular membrane, exudative maculopathy, retinal pigment epithelium, macula, macular degeneration, patch, RPE–choroid transplantation.

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