Outcome of Transplantation of Autologous Retinal Pigment Epithelium in Age-Related Macular Degeneration: A Prospective Trial

Susanne Binder,1,2 Ilse Krebs,1,2 Ralf-Dieter Hilgers,5 Ali Abri,1,2 Ulrike Stolba,1,2 Adele Assadouлина,2 Lukas Kellner,1,2 Boris V. Stanzel,1,2 Christian Jahn,1,2 and Hans Feichtinger4

PURPOSE. To present the outcome of a consecutive series of patients who had foveal choroidal neovascularization (fCNV) in age-related macular degeneration (AMD) and were treated with subretinal surgery combined with simultaneous transplantation of autologous retinal pigment epithelial (RPE) cells.

METHODS. Patients with fCNV who were not eligible for laser or photodynamic therapy were included in the study. They underwent subretinal membrane excision with simultaneous transplantation of autologous RPE cells. Eyes with membrane excision alone served as the control. Tests included best corrected visual acuity for far and near with Early Treatment Diabetic Retinopathy Study (ETDRS) and Jaeger charts, multifocal (mf)ERG, central visual field analysis, optical coherence tomography (OCT), and angiography, before surgery, and 1 month and 3 months after treatment, and at 3-month intervals thereafter.

RESULTS. The results of final examinations of 53 eyes are presented. In 39 eyes, RPE transplantation was performed (group 1); 14 eyes had membrane excision alone (group 2). In group 1, visual acuity improved significantly, two or more lines in 21 patients (53.8%); patients; remained stable in 12 patients (30.8%); and decreased two or more lines in 6 patients (15.4%); P = 0.0062). In group 2, the corresponding values were 21.1%, 57.8%, and 21.1% (P = 0.5377 NS). Statistical analysis of results in the two groups showed a trend in favor of group 1 (P = 0.9714). The difference in reading acuity was significant between the two groups (mean change in group 1: 1.85 ± 0.42 vs. 0.43 ± 0.47 in group 2; P = 0.0001). mfERG response density changes were significantly different between groups 1 and 2 (P = 0.0094). No significant decreases in central visual field defects were detected. OCT showed the postoperative median retinal thickness in the lesion area in group 1 to be higher (242.31 ± 12.30 µm) than in group 2 (202.07 ± 10.68 µm), showing a trend (P = 0.0682).

CONCLUSIONS. Patients undergoing fCNV removal with autologous transplantation of RPE reached significantly better reading acuity and higher mfERG-response density than control subjects. The results provide evidence that autologous transplantation of RPE is a beneficial supplement to membrane excision alone in patients with fCNV in AMD and may be regarded as a reasonable treatment option.

Retinal pigment epithelial (RPE) cells play a central role in maintaining visual function of the retina. 1 Dysfunction of the RPE may alter the extracellular environment of the photoreceptors (PRs) and thereby contribute to the pathogenesis of a variety of sight-threatening diseases, including age-related macular degeneration (AMD), which is now the major cause of permanent visual loss after age 50 in Western countries.

Conventional treatment of the neovascular form of AMD, such as laser photocoagulation, has limited success, and photodynamic treatment remains palliative. Moreover, only 20% of patients with neovascular AMD are eligible for the latter therapies. Membrane excision alone has shown disappointing visual results in AMD because of the mechanical removal of RPE and around the membrane during surgery and the primary dysfunction of the RPE.

In addition, progression of RPE and choriocapillaris atrophy after membrane excision leads to further loss of vision in these patients.

One potentially curative treatment is subretinal transplantation of healthy RPE, which has some advantages over neovascular transplantation, as it concerns only one cell type that is not involved in neural networking. Because of delayed immune reaction, the transplantation of homologous RPE has shown no visual benefit in patients with AMD. Thus, interest has been focused on autologous cells to overcome the problem of immune tolerance.

Although transplantation of full sheets containing an RPE-choriocapillaris-choroidal complex have resulted in sequestration and no visual benefits thus far, and some remaining functions have been demonstrated only with scanning laser ophthalmoscopy (SLO), the transplantation of autologous RPE suspensions and iris pigment epithelium (IPE) suspensions in eyes with foveal choroidal neovascularization (fCNV) have shown the first beneficial results, as reported by our group and others. Because these have been interventional case series without control subjects, we conducted a clinical trial to evaluate the effect of simultaneous transplantation of autologous, freshly harvested RPE at the time of membrane excision in patients with neovascular AMD and compared these results with those in a control group in which only membrane excision was performed. A prospective, nonrandomized, controlled clinical observational trial to compare the results in a

_________________________

From the 1Department of Ophthalmology, 2The Ludwig Boltzmann Institute for Retinology and Biomicroscopic Laser Surgery, and the 3Department of Pathology and Bacteriology, Rudolf Foundation Clinic, Vienna, Austria; and the 4Institute for Medical Statistics, RWTH, Aachen, Germany.

Presented at the annual meeting of the Association for Research in Vision and Ophthalmology, Fort Lauderdale, Florida, May 2002.

Supported by Vienna Mayor’s Funds (SB, BVS) and an unrestricted research grant from the L. Boltzmann Institute.

Submitted for publication February 6, 2004; revised July 6 and July 26, 2004; accepted August 2, 2004.

Disclosure: S. Binder, None; I. Krebs, None; R.-D. Hilgers, None; A. Abri, None; U. Stolba, None; A. Assadouлина, None; L. Kellner, None; B.V. Stanzel, None; C. Jahn, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked ‘advertisement’ in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Susanne Binder, L. Boltzmann Institute for Retinology and Biomicroscopic Lasersurgery, Department of Ophthalmology, Rudolph Foundation Clinic, Juchgasse 25, 1030 Vienna, Austria; susanne.binder@wienkav.at.


Copyright © Association for Research in Vision and Ophthalmology 4151

4151

Downloaded from iovs.arvojournals.org on 03/18/2019
consecutive series of transplant recipients with those of control subjects was performed.

**Materials and Methods**

**Patients**

In this study, patients with AMD and fCNV due to AMD were included. Eligibility criteria were fCNV not suitable for laser or photodynamic therapy, patients aged more than 50 years, and progression of the disease combined with visual loss over the past 3 to 6 months.

Exclusion criteria were presence of other retinopathies, uncontrollable glaucoma and optic nerve atrophy, the presence of severe general disease necessitating chemotherapy, and inability to cope with the requirements of follow-up.

No limit was put on visual acuity, which was in most subjects ≤20/200. Membrane size was not a limiting factor, unless it exceeded the large superior and inferior vascular arcades, indicating long duration of the disease with considerable fibrotic areas along with advanced damage of the PRs, which would have substantially increased the surgical trauma and size of the retinotomy.

The patient was informed about alternative therapeutic modalities, including other surgical techniques, – including the benefits of advanced damage of the PRs, which would have substantially increased the surgical trauma and size of the retinotomy.

The patient was informed about alternative therapeutic modalities, including other surgical techniques, and the benefits and complications of each treatment. After this extensive description, an informed consent was signed including an explanation that subretinal membrane excision would be supplemented by the transplantation of autologous RPE isolated from the nasal retinal area of the same eye, only if sufficient RPE cells could be safely transferred. Otherwise, membrane excision alone would be performed. Patients were randomized according to the quality of the cell suspension.

During the surgery, the RPE cells were harvested subretinally from the nasal side of the optic disc and given to a sterile pathologist who was in the operating room. The pathologist, who had been uninvolved in the case selection and postoperative follow-up, centrifuged the freshly harvested RPE cells and counted in a hemocytometer and evaluated them under a microscope. If there were at least 1500 RPE cells, then they were diluted in 0.1 to 0.2 mL physiologic saline (BSS+; Alcon Surgical, Fort Worth, TX) and returned to the surgeon for transplantation.

If there were fewer than 1500 RPE cells and the aspirate was mostly hemorrhagic, the cells were not prepared for transplantation by the pathologist. Simultaneous with the examination and preparation of the RPE cells by the pathologist, the surgeon removed the subretinal membrane, whether the patient was to receive the cell suspension or not. With the exception that one group had subretinal delivery of a cell suspension and the other group had none, every step was performed similarly in both groups.

Patients 70 years of age or those with mild signs of cataract—less than NO3, NC3, or C3P3 on the Lens Opacification Classification System (LOCS) Charts devised by Chylak et al. were advised to undergo simultaneous cataract removal, together with an implantation of a posterior chamber lens. Those with a substantial cataract—NO3, NC3, C3P3, or more—underwent cataract surgery as a first procedure. The decision for further treatment options for fCNV and included in the study was made on adequate judgment on the fluorescein angiogram of the CNV 3 to 4 weeks after cataract surgery.

The study conformed to the Declaration of Helsinki and was approved by the Ethics Committee of the Rudolf Foundation Clinic, government of the City of Vienna. A data safety and monitoring board was impaneled.

**Surgery**

The principles and details of the surgical technique have been described in the pilot study. In this series, two aspects were modified: First, the sequence of surgical steps was changed so that after vitrectomy and posterior hyaloid removal, the nasal retinotomy and bleb detachment were created first with a Ca2+- and Mg2+-free solution (BSS+; Alcon Surgical) to facilitate the separation of the retina from the pigment epithelium. While guidance of the bent cannula (20 gauge, angled vitrectorinal cannula; Visitec, Sarasota FL) subretinal and gentle mobilization the RPE was performed by the surgeon, the aspiration via a tube connected to a microsyringe was performed by the assistant. The RPE suspension was handed to the pathologist and prepared by centrifugation of the aspirate at 100g for 5 minutes. Cells were counted in a hemocytometer, and viability tests (Live/Dead Viability and Cytotoxicity assay; Molecular Probes, Eugene, OR) were performed in selected cases. If there were at least 1500 RPE cells, they were diluted in 0.1 to 0.2 mL physiologic saline (BSS+, Alcon Surgical) in a 1-mL microsyringe, now directly connected to the subretinal cannula, and handed back to the surgeon for transplantation. If there were fewer than 1500 RPE cells and the aspirate was mostly hemorrhagic, the cells were not prepared for transplantation by the pathologist.

Simultaneous with the examination and preparation of the RPE cells by the pathologist, the surgeon continued the surgery and removed the subretinal membrane. During this procedure, the second retinotomy was performed superiorly and temporally or nasally of the fCNV. The membrane was gently mobilized with hydrosuction and scissors if necessary and slowly removed with forceps, while the intraocular pressure was increased.

Second, a small perfluorocarbon bubble (PFCL, Octo-line-Perfluorocarbon; Bausch & Lomb Inc., Waterford, Ireland) covering the posterior pole and the two retinotomies was used to prevent bleeding after membrane removal, to allow laser treatment of the retinotomies, and to guarantee safe delivery of the transplant into the subfoveal area. If a suitable transplant was prepared by the pathologist, it was now provided to the surgeon and delivered gently subretinally. The grayish cell cloud was usually visible in the subretinal space under the PFCL bubble. A few minutes were given to allow the cells to settle, and the anterior vitreous was cleaned of circulating debris a second time. Then, the PFCL was removed and a fluid-air exchange with a nonexpandable mixture of sulfo-hexa-fluoridine (SF6, Minican 3.0; Linde AG, Unterschleissheim, Germany) performed. If no transplant could be prepared by the pathologist, the surgeon proceeded in the same way with PFCL removal and fluid-gas exchange. These cases formed the control group. With the exception that RPE cell suspensions containing too few cells were not transplanted, every step was performed similarly in both groups. Thus, the decision to perform transplantation neither depended on the circumstances of the case nor on the surgeon.

The patient was asked to lie on his or her back for 1 to 2 hours and then turned to a prone position for the remainder of the day and overnight. A head-down position was sustained as much as possible for the first 3 to 4 days.

**Postoperative Course**

Postoperative treatments consisted of topical corticosteroids with antibiotics (Tobradex; Alcon Ophthalmica GmbH, Vienna, Austria), non-steroidal anti-inflammatory eye drops (Voltaren-Ophtha; Novartis Pharma GmbH, Vienna, Austria) four times per day for 4 weeks, and mydriatic drops (Mydriaticum; Agepha, Vienna, Austria) when needed. Eyes with a postoperative pressure increase received topical antiglaucoma therapy and sometimes acetazolamide (Diamox; Goldschield, Pharmaceuticals Ltd., Croydon, UK) 500 mg/day orally for a short period. Biomicroscopy of the anterior and posterior segment was also performed daily during the first postoperative week, together with visual acuity tests.

**Far and Near Visual Acuity**

Distance acuity was tested with Early Treatment Diabetic Retinopathy Study (ETDRS) charts at a distance of 2 m. The log minimum angle of resolution (logMAR) was scored by the smallest line in which at least three letters were identified correctly. Missed letters in this line were added to the score, letters read in smaller lines were subtracted from the score (0.01 letter). When a patient was able to read the smallest line, the test was repeated at a distance of 4 m (letters that were read correctly at a 4-m distance were multiplied by 0.02). The largest line at
the 4-m distance corresponded to 1.0 logMAR or 0.1 in decimal visual acuity; 0.0 logMAR equals 1.0 in decimal visual acuity. A 0.1 logMAR interval counted as 1 line. Worsening of 3 lines meant doubling of the MAR.

Reading acuity was examined with Jaeger charts (Jg) at a distance of 30 ± 5 cm/s. Jg 1 is the smallest and Jg 16 the largest type. No reading acuity was registered as Jg 17. Three to 4 spherical diopeters were added to the best corrected distance acuity for reading. This test regimen was not changed in the follow-up examinations. Reading acuity was calculated in the same way as far visual acuity. A change of two or more Jg type sizes was counted as improvement in or loss of acuity.

Multifocal Electroretinography

Multifocal electoretinograms (mERGs) were recorded on computer (Retiscan, ver. 5.1; Roland-Consult, Wiesbaden, Germany). The recordings present the summation potentials of cones and cone bipolar cells and can be presented in hexagon-related single plots or three dimensionally. The stimulus array consisted of 105 hexagonal elements that were presented on a high-resolution cathode ray tube monitor (60 Hz). The eye monitor distance was 35 cm. The central 30° of the retina was simulated by flickering hexagons independently between black and white according to a pseudorandomized binary m-sequence (mean luminance, 180 cd/m²). Hexagon size was scaled with eccentricity to evoke focal responses of approximately the same amplitude in the response arrays. Each record was collected in five to six segments. Pupils were fully dilated with 1% cyclopentolate (cyclopentolate 1%, Thilo; Alcon Ophthalmika GmbH, Vienna, Austria). A lens of +3.0 D was added to the lens holder in front of the eye, which was refracted for best visual acuity for far distance. A diagonal cross was the fixation target, and the fellow eye was occluded. DTL conjunctival electrodes were used for the recording. The reference electrode was attached to the lateral margin of the same eye, and the ground electrode was placed on the forehead after cleaning and application of a conductive paste.

The recording signals were amplified (50,000×) and band-pass filtered (10–300 Hz). The quality of the records was controlled by real-time monitoring. The presence of a “blind spot” was an indication of a good-quality recording. The noisy records were discarded and re-recorded. In this study, b-wave amplitudes were used for evaluation.

Central Visual Field

Ten-degree static threshold perimetry was performed (Octopus 101 Perimeter; Interzeag, Switzerland). The background was 4-asb. The stimulus size was Goldmann III, and the stimulus exposure time was 100 ms. The program started by testing 45 locations in the central 4° area, which resulted in a foveal resolution of 0.7°. The examination was completed with an additional 36 points between 4° and 9.5° eccentricity. The patients were alerted by an acoustic signal before the stimulus was presented. In addition, the instrument produced acoustic signals without actually presenting a light. Positive responses to these audible signals were counted as false-positive answers. Negative responses to strong stimuli presented in an area where the patient demonstrated higher sensitivity in prior testing were called false-negative responses. Examinations with >15% false-positive answers were repeated, whereas a larger number of false-negative catch trials were accepted in cases with poor visual acuity and strongly depressed visual fields. Gray scales based on the actual measured values and defect curves were examined. The mean defect of the baseline perimetry (i.e., the average defect of the differences between the age-corrected normal data) and the actual measured results were compared with the values at the follow-up examinations.

Optical Coherence Tomography

With the patient’s pupils dilated, optical coherence tomography (OCT) was performed with an OCT scanner (Carl Zeiss Meditec, Dublin, Ireland). The color-coded reflectivity of multiple horizontal retinal diameters was registered in three different areas of the lesion: central, superior, and inferior. Unrelated to the size of the lesion, scans with a size of 2.83 mm were performed, measured, and calculated. The median values were registered and the postoperative values compared with those in the normal retina. In smaller lesions, scans at the edges were performed to compare the scans of healthy areas of the retina with the area of the lesions.

Fluorescein and Indocyanine Green Angiography

Fluorescein (FA) and indocyanine green angiography (ICG-A) was performed with a retina angiograph (Heidelberg Engineering GmbH, Heidelberg, Germany), for judgment of membrane characteristics and to measure the largest diameter of the lesion before surgery and during follow-up. Both methods were used to detect recurrences. Distances in the angiograms were measured in millimeters with the angiograph software. For angiography, six to eight images were taken in the early phase after injection, then every 15 seconds for 1 minute, after 2 minutes, and finally after 5 and 10 minutes.

Patient Follow-Up

All patients were examined before and during surgery and were scheduled to return after 1 month and 3 months and then at 3-month intervals after surgery (within 2 weeks before and after that date). At each regularly scheduled follow-up visit, a protocol refraction, best corrected visual acuity measurement, ophthalmoscopic examination, color fundus photography, and fluorescein angiography were performed in both eyes.

Each examination was performed by two independent ophthalmologists uninvolved in the selection of patients and surgery and without knowledge of the group to which the patient belonged. They were specialists, either in retinal medicine or electroretinography. Patients were scheduled at the same time of day for each follow-up examination and had breaks to relax between the different examinations.

Statistical Methods

The parameters of interest for statistical evaluation—namely, visual acuity (logMAR), reading acuity, mERG, OCT, and central visual field defect—were analyzed based on the respective measurement at the last examination.

Analyses were adjusted to the baseline visual acuities. Because of the various baseline values and follow-up periods, these two factors were considered to be covariates in the statistical analysis. Thus, a three-factor analysis of covariance model (between the factors: treatment groups, covariable baseline measurement, and follow-up period) was fitted to the data of the six parameters just listed. P < 0.05, as a result of the statistical twosided tests, was considered significant. Furthermore, the data were described by appropriate statistical measures. Computations were conducted on computer (SAS, ver. 8 [proc means, mixed, glm] for Windows XP; SAS, Cary, NC).

Results

Patients

Fifty-seven eyes of 56 patients were entered into the study. One patient, with two affected eyes was included in the analysis with the first treated eye. During follow-up three eyes had to be excluded because only visual acuity and clinical data were available, but additional mfERG, OCT, and central visual field values were lacking. One of these three patients showed visual improvement, and two had unchanged visual acuities after surgery. No complications occurred. Thus, the final analysis is based on 53 eyes of 53 patients: 39 with RPE transplantation (group 1) and 14 with membrane excision alone (group 2). The median age of the 53 patients was 77.4 years, ranging between 62 and 85 years; 40 (75.4%) were women.
Visual Acuity for Far Viewing over the Follow-Up Period Stratified by Therapy Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable</th>
<th>n</th>
<th>Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Follow-up period</td>
<td>14</td>
<td>8.79</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>Before surgery</td>
<td>14</td>
<td>1.37</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>1 Month</td>
<td>14</td>
<td>1.29</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>3 Months</td>
<td>14</td>
<td>1.24</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>6 Months</td>
<td>12</td>
<td>1.11</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>9 Months</td>
<td>8</td>
<td>1.15</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>12 Months</td>
<td>7</td>
<td>1.18</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>Final examination</td>
<td>14</td>
<td>1.26</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Difference (final vs. preop)</td>
<td>14</td>
<td>−0.11</td>
<td>0.18</td>
</tr>
<tr>
<td>1</td>
<td>Follow-up period</td>
<td>39</td>
<td>12.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Before surgery</td>
<td>39</td>
<td>1.32</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>1 Month</td>
<td>39</td>
<td>1.20</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>3 Months</td>
<td>39</td>
<td>1.18</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>6 Months</td>
<td>39</td>
<td>1.13</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>9 Months</td>
<td>39</td>
<td>1.12</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>12 Months</td>
<td>39</td>
<td>1.11</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Final examination</td>
<td>39</td>
<td>1.11</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Difference (final vs. preop)</td>
<td>39</td>
<td>−0.21</td>
<td>0.07</td>
</tr>
</tbody>
</table>

**Surgery**

The surgery was performed in patients under general anesthesia by one surgeon (SB) and was uneventful in all cases. Membrane excision, together with RPE transplantation, was performed in 39 (75.8%) eyes, 14 (24.2%) eyes were solely treated by membrane removal. All except five eyes had simultaneous cataract surgery. Three eyes had undergone cataract extraction earlier.

**Postoperative Course**

During the early postoperative time between 1 and 21 days, inflammation was similar to vitreous surgery with and without cataract surgery. A transient pressure increase was observed in eight (15%) eyes and was always treatable with anti-glaucoma therapy. In 12 (22.6%) eyes, a slight hemorrhage in the vitreous and around the surgical areas was observed. In one (1.8%) eye a choroidal and vitreous hemorrhage developed 48 hours after surgery and a second vitrectomy with silicone oil tamponade was performed immediately. The blood was removed and the retina attached, but a considerable per- and subretinal fibrosis developed later in this patient.

In the late postoperative course (3 months), OCT detected a macular hole in one eye. A retinal detachment developed in five (9.4%) eyes: four (7.5%) in the transplantation group and one (7%) in the control group. They occurred between 3 and 9 months after surgery. Each case started with the formation of a preretinal membrane followed by reopening of the central retinotomy and consecutive progression to retinal detachment. A second vitrectomy, gas (SF6) or silicone oil tamponade (Acri. Siloil 5000 ct/10 ml; Askin & Co. GesmbH, Vienna, Austria) with (n = 4) and without (n = 1) an encirclement (FCI, silicone cord 2-mm diameter; Askin & Co. GesmbH) was performed in these eyes. All retinas were reattached, and three eyes have already had the silicone oil removed.

**Visual Acuity for Far and Near**

In group 1, far visual acuity improved by two or more lines in 53.8% (21 patients), remained the same within 1 line in 30.8% (12 patients), and decreased two or more lines in 15.4% (6 patients). In 15 (38.4%) cases, improvement was 3 to 8 lines. Statistical evaluation (pair-wise t-test) showed significant improvement after 3, 6, 9, and 12 months, but was the most significant after 6 months (P = 0.0014) and the final examination (P = 0.0062), respectively. In group 2, visual acuity improved in 21.1% (three patients), remained the same in 57.8% (eight patients), and deteriorated in 21.1% (three patients; P = 0.5377, NS).

The mean follow-up period in group 2 (8.79 ± 0.97 months) was shorter than in group 1 (12 ± 0.00 months). Compared with the preoperative value, mean visual acuity deteriorated over time (Table 1). The statistical evaluation shows no significant variation over the time points (P = 0.2871), but the final results correlate positively with preoperative visual acuity (P ≤ 0.00005). In neither group did we observe differences (P = 0.9714) or time dependency of visual acuity at the final examination.

In group 1 near-reading acuity, assessed with Jg charts, improved two or more Jg type sizes in 53.8% (21 patients), remained unchanged plus/minus one size in 43.8% (17 patients), and deteriorated more than two sizes in 2.56% (1 patient) of the cases. Reading acuity between Jg 1 and 10 was achieved in 10.2% (4 patients) of the cases. In group 2, reading acuity improved by two or more Jg type sizes in 28.5% (four patients), was stable in 57.0% (eight patients), and worsened in 14.5% (two patients). All eyes had reading acuities lower than Jg 12.

The mean follow-up period in group 2 (8.79 ± 0.97 months) was shorter than in group 1 (14.38 ± 0.42 months). The mean reading acuity was nearly unchanged over the observation period in groups 1 and 2 (Table 2). However, the final reading acuity was clearly dependent on the follow-up period (P < 0.00005) and, adjusted for time, showed significant group differences (P < 0.00005). Thus, higher mean deteriorations were found in group 2 (−0.43 ± 0.47) than in group 1 (1.85 ± 0.42). The mean final reading acuity was nearly identical in both groups (group 1: 14.38 ± 0.42; group 2: 14.79 ± 0.42).

**Multifocal Electroretinography**

The mean follow-up period in group 2 was again shorter than in group 1 (median, 7.93 ± 1.16 vs. 11.92 ± 0.8 months). The comparison of the maximum mfERG response density for the b-wave showed higher mean values after 12 months in the transplantation group (Figs. 1, 2). The respective preoperative values in groups 1 and 2 were 93.32 ± 4.81 and 95.51 ± 5.29 nV/deg², respectively; the levels after treatment were 90.69 ± 3.53 nV/deg² after 12 months in the transplantation and 69.56 ± 5.39 nV/deg² in the control group (P = 0.0093; Table 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable</th>
<th>n</th>
<th>Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Follow-up period</td>
<td>14</td>
<td>8.79</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>Before surgery</td>
<td>14</td>
<td>15.21</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>1 Month</td>
<td>10</td>
<td>15.60</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>3 Months</td>
<td>11</td>
<td>15.36</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>6 Months</td>
<td>9</td>
<td>15.00</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>9 Months</td>
<td>5</td>
<td>14.60</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>12 Months</td>
<td>7</td>
<td>14.00</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>Final examination</td>
<td>14</td>
<td>14.79</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Difference (final vs. preop)</td>
<td>14</td>
<td>−0.43</td>
<td>0.47</td>
</tr>
<tr>
<td>1</td>
<td>Follow-up period</td>
<td>39</td>
<td>14.38</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Before surgery</td>
<td>39</td>
<td>16.23</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>1 Month</td>
<td>39</td>
<td>15.10</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>3 Months</td>
<td>39</td>
<td>14.53</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>6 Months</td>
<td>39</td>
<td>14.59</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>9 Months</td>
<td>39</td>
<td>14.36</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>12 Months</td>
<td>39</td>
<td>14.38</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Final examination</td>
<td>39</td>
<td>14.38</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Difference (final vs. preop)</td>
<td>39</td>
<td>−1.85</td>
<td>0.42</td>
</tr>
</tbody>
</table>
Follow up b-wave Tx vs. control (nV/deg²)

3). Whereas in group 1 the highest mean response density occurred after 1 and 3 months, in group 2, the mean density decreased over time. The final mfERG values were independent of the follow-up period ($P = 0.6285$) and of the initial values ($P = 0.5478$).

Central Visual Field
Before surgery, patients in group 1 showed a mean defect of $22.14 \pm 0.81$ with 0.7 false-positive and 6.9 false-negative answers. The respective results in group 2 were $19.93 \pm 1.68$, 0.8 false-positive and 6.6 false-negative answers. At 12 months, the mean defect was $19.50 \pm 1.10$ in group 1 and $17.79 \pm 2.82$ in group 2. Although the median mean defect in the control group was lower than in the treatment group, a reduction of the scotoma of 12% was observed in both groups. There was no trend for a significant shift in the mean defect in the central visual field over time (Table 4), the statistical evaluation showed no significant variation over the time points ($P = $)

Follow up a and b wave
Amplitude Tx versus control (nV/deg²)
The mean follow-up period in group 1 was shorter than in group 2 (11.92 ± 0.08 months versus 12.00 ± 0.00 months). At the last visit, the mean OCT values in group 1 were higher (202.07 ± 10.68 μm) than in the control group (202.31 ± 12.30 μm), showing a trend but no significant difference between the two groups (P = 0.0682).

Fluorescein and Indocyanine Green Angiography

Most membranes were occult membranes with minimum classic areas (50/53; 94.3%), four (7.5%) eyes presented with a large pigment epithelium detachment (PED), and three (5.3%) eyes had a chorioretinal anastomosis. Four (7.5%) eyes had one or more PDT treatments before surgery, and one (1.8%) eye had a pigment epithelium tear after laser treatment.

After surgery, angiography revealed recurrence of extraretinal CNV in two (5.1%) eyes of the transplantation group after 3 and 12 months and in one (7.8%) eye of the control series after 12 months. Laser treatment was applied to two of these membranes, and, in the third case, the neovascular membrane became inactive during follow-up without further treatment. Before surgery, the medium size of the lesions was comparable in both groups (median, 5.9 mm; range, 3.6–10.2 mm in group 1 versus median, 6.0 mm; range, 3.1–10.1 mm in group 2).

Discussion

To improve or maintain vision in patients with fCNV related to AMD, the transplantation of RPE seems a logical approach and has shown promising results in a small consecutive case series.24 Herein, we present the outcome of a prospective trial in which subretinal surgery with membrane excision alone was compared with simultaneous transplantation of autologous RPE cells together with membrane excision (Fig. 3).

In the largest surgical series of membrane excision alone in patients with AMD, Thomas et al.11 reported a 14% visual improvement, which was in contrast to much better results in younger patients with ocular histoplasmosis.35,36 In a meta-analysis of subretinal membrane excision over a 5-year period in 677 cases, we found improvement in 33% overall and deterioration in 25%; however, small studies with shorter observation times and classic as well as occult membranes were included.37 When membrane excision was compared with laser treatment in a randomized prospective multicenter study comparable results were found after 2 years.38 Nevertheless, laser and PDT treatment have limited indications related to the membrane characteristics on fluorescein angiography, exact boundaries, and lesion size and are applicable in only 20% of cases of neovascular AMD.7–10 In contrast, subretinal membraneotomy has the clear advantage that it is not limited by these factors and does not require multiple treatments for an indefinite period.9,10 Whereas the outcome of our control group is in accordance with that obtained in the studies just mentioned, better functional results were observed after simultaneous transplantation of autologous RPE.

In our study, recruitment of patients began on April 25, 2000, when only predominately classic membranes were an indication for treatment with PDT. Verteporfin (Visudyne; Novartis) for occult fCNV was approved in Austria in August 2002. The last patient underwent surgery on February 13, 2003, and only six patients from our study underwent surgery between September 2002 and February 2003. In none of these cases was an occult-only fCNV that might have been an indication for this treatment present. All had rather large, mixed, minimal classic membranes with very low visual acuities. Therefore, at that time none of our patients would have been a candidate for PDT.

To collect as much information as possible, we performed a wide range of clinical tests in this trial. Far visual acuity measured with ETDRS charts and converted to logMAR showed a significant pre- to postoperative improvement in the transplantation group, whereas this was not the case in the control group. However, the difference in postoperative values between the two groups reached no significance but showed a trend in favor of the transplantation group.

Reading acuity has received little attention in studies of treatment options for AMD, although it is one of the most important factors for the patient. This deficiency was partially due to the lack of standardized reading tests. They exist to-day,59 but were not available in German when this study was started. Jaeger charts, which are generally used and accepted in European countries, were used in our study. Reading tests

Data are expressed in microvolts per square degree.

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable</th>
<th>n</th>
<th>Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Follow-up period</td>
<td>14</td>
<td>7.93</td>
<td>1.16</td>
</tr>
<tr>
<td></td>
<td>Before surgery</td>
<td>14</td>
<td>93.51</td>
<td>5.29</td>
</tr>
<tr>
<td></td>
<td>1 Month</td>
<td>10</td>
<td>89.91</td>
<td>7.58</td>
</tr>
<tr>
<td></td>
<td>3 Months</td>
<td>10</td>
<td>74.63</td>
<td>5.34</td>
</tr>
<tr>
<td></td>
<td>6 Months</td>
<td>5</td>
<td>75.40</td>
<td>5.45</td>
</tr>
<tr>
<td></td>
<td>9 Months</td>
<td>3</td>
<td>80.30</td>
<td>8.11</td>
</tr>
<tr>
<td></td>
<td>12 Months</td>
<td>7</td>
<td>69.56</td>
<td>5.39</td>
</tr>
<tr>
<td></td>
<td>Final examination</td>
<td>14</td>
<td>71.32</td>
<td>4.11</td>
</tr>
<tr>
<td></td>
<td>Difference (final vs. preop)</td>
<td>14</td>
<td>-22.19</td>
<td>5.78</td>
</tr>
</tbody>
</table>

TABLE 4. Central Visual Field Mean Defect over the Follow-Up Period Stratified by Therapy Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable</th>
<th>n</th>
<th>Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Follow-Up Period</td>
<td>12</td>
<td>10.08</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td>Before surgery</td>
<td>12</td>
<td>19.95</td>
<td>1.68</td>
</tr>
<tr>
<td></td>
<td>1 Month</td>
<td>4</td>
<td>18.80</td>
<td>4.35</td>
</tr>
<tr>
<td></td>
<td>3 Months</td>
<td>5</td>
<td>21.27</td>
<td>3.87</td>
</tr>
<tr>
<td></td>
<td>6 Months</td>
<td>4</td>
<td>18.95</td>
<td>3.19</td>
</tr>
<tr>
<td></td>
<td>9 Months</td>
<td>2</td>
<td>22.75</td>
<td>5.25</td>
</tr>
<tr>
<td></td>
<td>12 Months</td>
<td>7</td>
<td>17.79</td>
<td>2.82</td>
</tr>
<tr>
<td></td>
<td>Final examination</td>
<td>12</td>
<td>16.28</td>
<td>1.79</td>
</tr>
<tr>
<td></td>
<td>Difference (final vs. preop)</td>
<td>12</td>
<td>-3.64</td>
<td>1.87</td>
</tr>
</tbody>
</table>

1 Follow-Up Period | 37 | 12.00 | 0.00 |
| Before surgery    | 37 | 22.14 | 0.81 |
| 1 Month           | 36 | 20.36 | 1.04 |
| 3 Months          | 37 | 19.93 | 1.08 |
| 6 Months          | 37 | 20.13 | 1.09 |
| 9 Months          | 37 | 20.21 | 1.08 |
| 12 Months         | 37 | 19.50 | 1.10 |
| Final examination | 37 | 19.50 | 1.10 |
| Difference (final vs. preop) | 37 | -2.64 | 0.75 |
were performed in an identical manner at all control examinations, allowing no magnifying glasses. In reading acuity, the difference between the transplantation group and control groups turned out to be significant (P < 0.0001). Useful reading vision was achieved in approximately 10% of the transplantation group, whereas none of the control group reached a level higher than Jg 12. This result may be influenced by the fact that the medium size of the membranes in this series was rather large (mean, 5.9 in group 1 vs. mean, 6.0 in group 2) and consisted of patients who had predominately occult membranes with minimal classic CNV. Therefore, very poor or even a complete lack of reading ability was present before surgery in these series. Similar findings were present in our pilot study where 21% (3/14) gained useful reading vision (between Jg 1 and 4). In both studies we found that patients with smaller membranes had a greater chance to regain useful reading acuity with the suspension technique of RPE transplantation.

In parallel with improvement of visual acuity, mfERG response density turned out to be significantly better in the transplantation group than in the control. mfERG measures the potentials of the cone and cone bipolar cells of the retina and thus represents a more sensitive and objective test for central retinal functions than does visual acuity. Whereas constant

FIGURE 3. Red-free images and mid-phase angiograms in three patients (1, 2, and 3; patients 32, 39, and 5, respectively) before surgery (A, A’, and 3 (B, B’) and 12 (C, C) months after surgery. (1, 2) Fundus photographs of two transplant-recipient eyes. (3) Eye of a control patient. (1) In patient 32, red-free images (line 1) show the largest diameter (arrows) of the lesion to be unchanged over the 12-month period (5.4 mm before surgery to 5.2 mm after 3 months and 12 months). Note the disappearance of hard exudates (bright areas) on the temporal side over time. On fluorescein angiograms (line 2), an area of blocked fluorescein surrounded by a rim of mottled hyperfluorescence was present consistent with an occult neovascular lesion before surgery (1A’). After surgery, the largest diameter of the atrophic area was unchanged. Islands of hyper- and hypofluorescence were visible within the surgical area. (2) In patient 39, indocyanine green angiograms (line 3) and fluorescein angiograms (line 4) are shown. Before surgery, a bright hyperfluorescence with leakage surrounded by hypofluorescence (retinal edema and exudates) was present on the fluorescein angiogram (2A, line 4), consistent with retinal angiomatous proliferation (RAP), which is shown clearer on the indocyanine green angiogram (2A, line 3). After surgery at 3 and 12 months, only a small area of atrophy was present on both angiograms (2B, 2C) and (2B’, 2C’, respectively). The largest diameter of the lesion decreased from 6.5 mm before to 3.3 mm after surgery (arrows). (3) In patient 5, red-free images (line 5) and fluorescein angiograms (line 6) of the control eye are shown. The largest diameter (arrows) of the lesion increased substantially after surgery, from 5.0 mm (3A) to 7.2 mm (3C). On the angiogram, bright hyperfluorescence indicated a fibrotic part of the lesion, and mottled hyperfluorescence was present temporally and superiorly (occult membrane) before surgery (3A’). Three months after surgery (3B) the RPE had atrophied (hyperfluorescence), with atrophy of the superior and inferior choriocapillaris (lack of fluorescence). At 12 months, additional areas of choriocapillaris atrophy were present (3C’).
mild deterioration of the postoperative response densities was observed in the control group, increased levels were found in the transplantation group after 1 and 3 months, but they returned to levels comparable to the preoperative values after 6, 9, and 12 months, respectively. In a smaller series of patients comparable results were obtained. We recognized a large variation in the response density and implicit time. This can be explained by the stage of disease, which is reflected in the degree of damage to the integrity of the microstructure in the central retina generally present in macular diseases. In this study, correlations were determined that showed the maximum amplitude of the b-wave potentials to be the most important indicator, but not their latencies. The reason for improved amplitudes after 1 and 5 months could be the reduction of retinal edema as well as support provided by the transplanted RPE. In the later course, these amplitudes reached preoperative levels in group 1, whereas a constant decrease was measured in group 2.

Central visual field defects did not show significant differences between group 1 patients and group 2 control subjects, but, before surgery, smaller scotomas were recorded in the latter.

OCT was performed to evaluate either the stability of the retinal thickness or progression to retinal atrophy. Stable thickness was found in the transplantation group and a trend toward maintenance when retinal thickness in healthy areas and over the lesion was compared with control cases. This will be observed further in studies with larger samples and with ultra-high-resolution OCT, with which the condition of single cell layers can be examined with greater precision.

One might suggest that the cataract surgery in eyes with substantial cataracts and neovascular AMD may have some influence in the early postoperative weeks after surgery, but, in the later course, the macular situation is the dominant influence for further visual development.

Because only eyes with no or very mild cataracts were included in this study, we do not believe that the cataract surgery had an influence on vision. Combined cataract surgery with vitrectomy was performed in all control subjects and in 90% of the transplant-recipient eyes. This rules out the possibility that cataract surgery may have biased the final visual outcome between the two groups.

Although observational studies are likely to be affected by inhomogeneity of the comparison groups, in our study we compared two groups that showed good homogeneity, according to the main demographic patients' characteristics as well as to the baseline findings. Note that because of the presence of the disease, it can be assumed that the condition of the control eyes that had the shorter follow-up period (mean, nearly 9 months) will be worse after 1 year. Consequently, the estimate of the group difference is conservative.

The effect of size, comparing two groups of 14 and 39 patients, which can be detected with a statistical test (t-test), at the 5% significance level with a power of 80%, is 0.886. One can argue that the power is highly affected by the high imbalance of the sample size. However, using a balanced design (27 to 27) the effect size increases only slightly (to 0.777). Thus, the empiric power of the test may be acceptable for establishment of large differences. Nevertheless, this implies that there is a great chance to overlook possible clinically relevant effects.

The weakness of this study is that it was not a randomized trial. Patients were randomly selected according to the quality and amount of the cell suspension we were able to harvest. Therefore, cases are not distributed evenly and some control subject data are incomplete. Thus, the statistical methods used to compare the two groups should be viewed with caution. Confounding and bias may play a role in this study.

The problem that we faced in the study design was that the autologous RPE cells we harvested were the possession of the patient. If the number seems to be sufficient and the quality good, it seems ethically correct to give the patient back his own RPE cells. Therefore, it was decided that the selection of the cases should be based on the number and quality of the RPE cells. We believe that we came as close as possible to randomization in this study, in which the decision for group distribution was performed by a person uninvolved in case selection and follow-up.

In contrast, an interesting aspect of the study is that at 12 months, 15% or 56% of patients had a 3- to 8-line gain in vision. This rarely occurs in the natural course of the disease.

So far, several attempts have been made to transplant cells in sheets, to preserve a well-organized, polarized cell layer with a basal lamina. Homologous transplants with human adult RPE sheets showed immune reactions. Even immunosuppression and transplantation to the correct place with proper orientation resulted in disorganization of the sheet, as reported by del Priore et al. in a clinicopathologic study. In a small series, amblyopia full-thickness transplants from the edges of the original CNV were translocated subfoveally by Awylard et al. They observed encapsulation of these sheets and a lack of visual improvement. Technically, transplantation of an RPE suspension was improved by placing the cells under a PFCL bubble. This approach avoided reflux of the cells into the vitreous, guaranteed safe delivery into a small subretinal space, and therefore enhanced contact between the RPE cells and thus the probability of RPE survival. A refinement of the instrumentaton for safer RPE harvesting and transplantation, the use of a Ca²⁺- and Mg²⁺-free solution to facilitate RPE removal at the nasal retinal area and to prevent possible damage to the RPE cells were additional measures in the surgical strategy. The condition of RPE and cell amounts were analyzed by light microscopy in every case, and cell-viability tests showed approximately 80% viable cells when performed. In a parallel experimental study, the minimal number of aged, human RPE needed for monolayer regrowth in vitro were examined (Stanzel BV, et al. IOVS 2002;43:ARVO E-Abstract 3437). We found that at least 1500 to 2300 cells were necessary for confluence, with a chance of 40% to 100% that they would reach confluence. Therefore, aspirates with very low cell amounts and visibly hemorrhagic were not used for transplantation. The ability of RPE obtained in pars plana vitrectomy to reach confluence was shown experimentally by another group (Ivert LI, et al. IOVS 2002;43:ARVO E-Abstract 3449).

Areas of atrophy of the RPE and choriocapillaris can increase over time. That this effect can be related to the course of the disease was shown clearly after laser treatment in juxtafoveal membranes, but was also reported after membrane excision and attributed to the removal of adjacent RPE. In AMD, changes of Bruch's membrane may be severe, and membrane excision could result in additional traumatic defects. In histopathologic studies of excised membranes, the presence of Bruch’s membrane was found in approximately 30%; however, this varies considerably between cases, leaving the question of the condition of the section bed and the amount of the residual Bruch’s membrane at the time of membrane removal unclear. Tezel et al. have shown that the fate of retinal pigment epithelium seeded on Bruch’s membrane is partially dependent on the extracellular matrix component exposed. Thus, Tezel et al. found much better cell survival on basal lamina or outer collagenous layers than on deeper elastin layers. This includes the caveat that (1) the microenvironment of RPE cells seeded onto Bruch’s membrane in tissue culture...
may be different from that in the subretinal space, because the neurosensory retina, choriocapillaris, and choroid were not present in vitro, and (2) that accumulations of age-related debris in different layers of Bruch’s membrane also influence RPE cell growth.50

RPE wound-healing experiments performed by Wang et al.51 on Bruch’s membrane explants demonstrated that aged RPE basement membrane supports aged RPE resurfacing of localized RPE defects; however, the deeper portions of inner collagenous layers (ICLs) of aged submacular human Bruch’s membrane were limited in their ability to support RPE resurfacing when compared with RPE basal lamina.52 In an experimental study performed on rabbit eyes by Phillips et al.,52 in which autologous RPE transplantation was performed on mechanically denuded Bruch’s membrane, better PR and choriocapillaris survival was provided in the transplant areas than in the denuded areas.52 Clearly, the behavior of the transplanted RPE depends on the environment in which the cells are seeded. Therefore patients with advanced, but not end-stage, disease were selected for our surgery.

Because of the lack of in vivo cell markers that can be safely used in a human trial, we currently do not know the fate of the transplanted RPE cells nor their precise function. Whether they act as bystanders in the digestion of cellular debris, secrete growth factors to facilitate repopulation from the edges of the damaged areas, or adhere as small islands and/or are capable of restoring the RPE monolayer is still unclear. Under ideal conditions one would like to achieve both. However, one might speculate that improvement of reading acuity as well as better/higher mfERG response densities, both reflecting a function of the foveolar retinal area, would indicate a profit from the presence of the transplanted RPE cells and support of photoreceptor survival.

Overall, surgery combined with transplantation turned out as a relatively safe procedure in all eyes, and postoperative complications were not higher than in vitrectomy and subretinal surgery alone, for which, in general, retinal detachments are reported with a frequency between 7% and 15%.45,53,54

Clearly, emmetropic patients with AMD rarely have a retinal detachment develop in the natural course of the disease; however, patients in this study had progressive visual loss due to mostly occult ICNV, in which laser or PDT treatment was not a treatment option.

Those patients in our series with retinal detachment characterizedly presented with a preretinal membrane followed by reopening of the centrally located retinotomy. In no case did the retinal detachment develop from the denuded area around the nasally created retinotomy.

Other surgical techniques, such as like 360° rotation, currently used in eyes with ICNV with and without hemorrhages in single cases are reported to have a rather high percentage of postoperative complication, including proliferative vitreoretinopathy at a rate of between 20% and 40%.50–54 Limited rotation techniques are prone to a lesser complication rate than full-rotation techniques and do not involve multiple surgical procedures, including additional muscle surgery and silicone removal, but are indicated in small membranes that are mostly treated with PDT today.

In summary, we provide evidence that membrane removal supplemented by simultaneous RPE transplantation is a feasible and safe technique, when compared with other surgical procedures, resulting in clinically relevant improvement. This combined procedure seems superior to membrane excision alone in patients with advanced AMD, justifying further clinical evaluation and research.

References


