

Subretinal Fluid Levels of Signal-Transduction Proteins and Apoptosis Molecules in Macula-Off Retinal Detachment Undergoing Scleral Buckle Surgery

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PURPOSE. To evaluate signal transduction and early apoptosis protein levels in subretinal fluid collected during scleral buckling surgery for macula-off rhegmatogenous retinal detachment (RRD). Our aim was to assess both their relation with RRD features and their influence on the posttreatment outcome.

METHODS. Thirty-three eyes of 33 RRD patients scheduled for scleral buckle surgery were enrolled in the study. Undiluted subretinal fluid samples were collected during surgery and analyzed via magnetic bead-based immunoassay. All patients underwent a complete ophthalmologic evaluation at baseline and at each follow-up visit (months 1, 3, and 6). Moreover, both at baseline and at the postsurgery month 6 visit, the patients were tested by means of spectral-domain optical coherence tomography (SD-OCT) in order to evaluate the average ganglion cell-inner plexiform complex thickness, as well as the photoreceptor inner segment/outer segment junction status.

RESULTS. Patients' clinical features (retinal detachment size, detachment duration, and occurrence of proliferative vitreoretinopathy) were associated with several early apoptotic factors (caspase-8, caspase-9, and B-cell lymphoma 2 [Bcl-2]-associated death promoter [BAD]). Furthermore, both early apoptosis factors (caspase-8, Bcl-2, and p53) and signal-transduction proteins (ERK 1/2) were found to influence the postsurgery month 3 OCT characteristics.

CONCLUSIONS. Signal-transduction proteins and early apoptosis proteins are associated with different clinical features and postsurgery outcomes.

Keywords: retinal detachment, subretinal fluid, signal-transduction proteins, apoptosis

Rhegmatogenous retinal detachment (RRD) is one of the most common sight-threatening eye diseases.¹⁻⁴

The visual prognosis following RRD is primarily dependent on the macula status on presentation³; this aspect implicates the macula-off RRD being considered a relevant illness worldwide. Understanding the mechanisms that underlie the retinal damage is crucial to the development of new strategies that could improve the visual outcome.

Abundant evidence shows that signal-transduction proteins and apoptosis molecules play an important role in the outcome after retinal detachment repair. Nevertheless, this evidence is from either basic science or animal studies.⁵⁻¹² The cellular response to stress, such as that experienced by the retinal cell during RRD, is a complex mechanism, depending on both molecular pathways stimulating the cell survival and molecular pathways leading to apoptosis. Several signaling pathways contribute to cell survival and are activated by cellular response to stimuli, such as stress, cytokines, and free radicals.¹³ Apoptosis is a complex process regulated by different cell signals leading to DNA fragmentation and cell death. Two different signaling cascades have been identified: extrinsic

(initiated when the death receptors on cell surfaces are bound by the death ligands, causing the initiator caspase activation) and intrinsic (triggered by stress stimuli, such as hypoxia, genotoxic stress, UV irradiation, hormones, and cytokine deprivation and leading to mitochondrial permeabilization and, eventually, the consequent release of cytochrome c into the cytosol).¹⁴⁻¹⁶ A final common pathway for both the intrinsic and the extrinsic cascades involves the activation of the effector caspases.¹⁶

In the present prospective study, we evaluated signal-transduction and early apoptosis protein levels in subretinal fluid collected during scleral buckling surgery for macula-off RRD, and we assessed their relations with clinical features.

METHODS

Study Participants

A total of 33 eyes of 33 patients (18 males and 15 females; mean age 59.5 ± 13.4 years; range, 27-88 years) were enrolled in the study.



All patients had a clinical and imaging diagnosis of macula-off RRD scheduled for scleral buckle surgery. Patients consecutively presented at the University Gabriele D'Annunzio Department of Ophthalmology between January 2015 and December 2015. This study was approved by the institutional ethics committee, and patients provided signed informed consent for the use of their data. The study adhered to the tenets of the Declaration of Helsinki.

Exclusion criteria were (1) macular detachment (MD) duration ≥ 5 weeks; (2) proliferative vitreoretinopathy (PVR) at baseline of a grade greater than B according to Machemer's classification¹⁷; (3) any previous vitreoretinal surgery; (4) any complicated cataract surgery (posterior capsular rupture or postsurgery neodymium-doped yttrium aluminum garnet (Nd:YAG) laser capsulotomy); (5) ocular media opacity (e.g., cataract greater than Lens Opacities Classification System (LOCS) grade 3 or vitreous hemorrhage); (6) any maculopathy affecting the patient before the RRD, including age-related macular degeneration; (7) any history of vascular diseases, including diabetic retinopathy or previous retinal vein occlusion in the study eye, because their influence on ganglion cell-inner plexiform layer complex (GC-IPL) thickness is known¹⁸; (8) any optic neuropathy, including glaucoma, or any condition increasing the risk of secondary glaucoma (e.g., pigment dispersion syndrome or pseudoexfoliation syndrome); and (9) intraocular pressure (IOP) > 21 mm Hg.

Study Protocol

At baseline, all patients underwent a complete ophthalmic evaluation, including assessment of best-corrected visual acuity (BCVA) using Early Treatment Diabetic Retinopathy Study (ETDRS) charts, tonometry, slit-lamp biomicroscopy, indirect fundus ophthalmoscopy, and spectral-domain optical coherence tomography (SD-OCT). Furthermore, all patients were tested by means of B-scan ultrasonography in order to confirm the diagnosis and the extension of the RRD. We defined the moment of retinal detachment as the subjective reduction of VA, as considered in several studies,^{19,20} taking into account that not all patients experiencing RRD are able to identify the beginning of the peripheral scotoma and that symptoms, such as floaters and phosphenes, are not specific for RRD.

Subjects were evaluated at baseline and were followed up at the postsurgical visits for months 1, 3, and 6. At each follow-up visit, patients were evaluated with a complete ophthalmologic evaluation. Moreover, both at baseline and at the postsurgical month 6 visit, patients were tested by means of SD-OCT in order to evaluate the average GC-IPL thickness, as well as the photoreceptor inner segment/outer segment (IS/OS) junction status (normal; disrupted/absent).

Surgical Procedure

The same expert surgeon performed all the scleral buckle surgeries within 3 days after the baseline evaluation. During the surgery, undiluted subretinal fluid (SRF) samples were collected. In brief, after the 360° conjunctival peritomy was completed, indirect ophthalmoscopy was performed in order to determine the localization of the tears and the most appropriate SRF drainage site. The drainage was performed at the buckling in relation to the highest retina elevation, and far enough from the retinal tears and the choroidal site of the vortex veins. In order to realize the drainage puncture, a scleral pocket was performed. Subsequently, a small incision through the residual sclera and the choroid was carried out after a careful cauterization of scleral and choroidal vessels. In order to facilitate SRF collection, drainage by means of a cannula (Terumo SurFlash Polyurethane I.V. Catheter 20G; Tokyo,

Japan) was immediately performed under direct observation, so that the fluid was collected immediately into a 3-mL syringe. Then, a nonabsorbable polyester suture was placed through the two edges of the scleral incision to close the margin of the pocket at the end of the drainage.

Collection and Multiplex Analysis of Samples

Approximately 250 μ L fluid was collected during the surgery and immediately chilled in ice. Within 1 hour, the samples were centrifuged at 17,949g for 15 minutes at 4°C and stored at -70°C until assayed. The total protein concentration for each sample was evaluated by bicinchoninic acid protein assay (Pierce Biotechnology, Rockford, IL, USA). Undiluted retinal fluid samples (25 μ L neat per well) were used to detect changes in apoptosis or signaling pathways, using the MILLIplex MAP 5-Plex Early Apoptosis Magnetic Bead Kit to test B-cell lymphoma 2 [Bcl-2]-associated death promoter (BAD), Bcl-2, caspase-9, p53, and caspase-8 (Cat. no. 48-669MAG; Merck-Millipore, Vimodrone, Italy) and the MILLIplex MAP 7-Plex Multi-Pathway Magnetic Bead Kit to test response element binding (CREB), nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), p38, extracellular signal-regulated kinases 1 and 2 (ERK 1/2), p70S6K, and signal transducer and activator of transcription 3 and 5 protein (STAT3, STAT5) (Cat. no. 48-680MAG, Merck-Millipore), respectively, according to the manufacturer's protocol. Briefly, 25 μ L neat samples was added to 25 μ L 1 \times magnetic beads and incubated overnight at 4°C with shaking. At the end of the incubation, the plate was washed twice in an assay buffer and then incubated for 1 hour with 25 μ L 1 \times detection antibody at room temperature (RT). Then, always at RT, the plate was incubated for 15 minutes with 1 \times streptavidin-phycoerythrin, and for another 15 minutes with amplification buffer. Therefore, the plate was washed twice and incubated with 150 μ L assay buffer for 5 minutes. The plate was run immediately on a Luminex 100200 platform (Luminex Corporation, Austin, TX, USA) with xPONENT 3.1 software. The assay was performed in a 96-well plate, using all the assay components provided in the kit. All incubation steps were performed in the dark to protect the beads from light. Positive and negative controls were supplied with the kit. The results were expressed as mean fluorescence intensity (MFI).

Imaging

Patients were tested using a Cirrus SD-OCT (Carl Zeiss Meditec, Inc., Dublin, CA, USA), a commercially available device with a scan speed of 27,000 axial scans per second and an axial resolution of 5 μ m. All scans were acquired by the same operator after pupil dilation using eye drops containing 0.5% tropicamide and 10.0% phenylephrine hydrochloride. Cirrus SD-OCT was used to acquire two macular scans using the macular cube 512 \times 128 scan protocol. The GCA algorithm, incorporated into Cirrus SD-OCT software version 6.5, was used to process and measure the thickness of the macular GC-IPL within a 14.13-mm² elliptical annulus area centered on the fovea. The GCA algorithm automatically segmented the GC-IPL based on the three-dimensional data generated from the macular cube 512 \times 128 scan protocol. The average, minimum, and six sectoral GC-IPL thickness values (superotemporal [ST], superior [S], superonasal [SN], inferonasal [IN], inferior [I], and inferotemporal [IT]) were measured from the elliptical annulus centered on the fovea. A detailed description of the algorithm has been presented previously.²¹

During the scanning, the subject's pupil was first centered and focused on the iris viewport. The line-scanning ophthalmoscope with auto focus mode was then used to optimize the

TABLE 1. Clinical Characteristics of the Enrolled Patients

RRD Patients, <i>n</i> = 33	
Age, y	59.5 ± 13.4
Sex, <i>n</i>	
Male	18
Female	15
Retinal detachment size, number of quadrants, <i>n</i> (%)	
≤2	21 (63.6)
≥3	12 (36.4)
Detachment duration, weeks, <i>n</i> (%)	
≤2	23 (69.7)
≥3	10 (30.3)
BCVA, logMAR, at baseline, median (IQR)	
Baseline	2.00 (0.66–2.00)

n, number of patients; BCVA, best-corrected visual acuity (logMAR [logarithm of the minimum angle of resolution]); IS/OS junction, inner segment/outer segment photoreceptor junction.

view of the retina. The center and enhance modes were used to optimize the Z-offset and the scan polarization, respectively, in order to maximize the signal. After each capture, the motion artifact was checked with the line-scanning ophthalmoscope image with an en face OCT overlay. Rescanning was performed if a motion artifact, indicated by blood vessel discontinuity, was detected.

Only good-quality scans, defined as scans with signal strength ≥ 6 , were used for the analysis.

Furthermore, two cross-sectional SD-OCT scans through the fovea (one horizontal and one vertical) were obtained for each eye. The integrity of the IS/OS junction (normal; disrupted/absent) was evaluated by an experienced retinal physician. The IS/OS junction was assumed to be “normal” when at least 50% of its length in the central 1500- μ m zone from the fovea was continuous and clearly visible; otherwise it was defined as “disrupted/absent.”

Statistical Analysis

Statistical calculations were performed using the Statistical Package for Social Sciences (version 20.0; SPSS, Inc., Chicago, IL, USA). To detect departures from normal distribution, the Shapiro-Wilk test was performed for all variables. The variables were presented as follows: qualitative variables as frequency and percentage; quantitative variables as median and interquartile range (IQR).

In order to test the influence on clinical patients' features of signal-transduction and apoptosis proteins, patients were divided based on their clinical features. Then, the Mann-Whitney *U* test was performed in order to obtain *P* values. Moreover, the linear univariate regression model was applied to test the relation between BCVA at the postsurgical month 6 visit and the analyzed molecules.

The chosen level of statistical significance was $P < 0.05$.

RESULTS

Patients' clinical characteristics are reported in Table 1.

Table 2 shows the percentage of samples in which the studied molecules resulted in upregulation, after the comparison with the entire group of analyzed patients.

At baseline, the BCVA was 2.00 (0.66–2.00) logMAR. During the follow-up, the BCVA was 0.50 (0.37–0.75) logMAR at the postsurgical month 1 visit, 0.40 (0.15–0.70) logMAR at the

TABLE 2. Percentage of Patients Who Resulted in Upregulated Molecules

Tested Molecules	Values	Percentage of Upregulated
Early apoptosis factors		
Caspase-8	87 (73–116)	42.4
Caspase-9	62 (49–157)	21.2
BAD	300 (232–2335)	30.3
Bcl2	62 (47–83)	45.5
p53	50 (36–265)	-
Signal-transduction proteins		
NF- κ B	49 (36–71)	45.5
p38	130 (76.5–795)	-
ERK1/2	296 (123–906)	9.1
STAT3	85 (65–515)	36.4
STAT5	107 (74.5–795)	39.4
CREB	60 (46.5–320)	33.3
p70	60 (44–355)	51.5

Mean fluorescence intensity is the unit of measure. Data are presented as median (interquartile range).

postsurgical month 3 visit, and 0.40 (0.10–0.70) logMAR at the postsurgical month 6 visit.

The presurgical foveal detachment was 437 (200–867) μ m, as evaluated at the baseline OCT.

During the follow-up period, 13 patients (39.4%) experienced PVR. Moreover, three patients (9.1%) were retreated for RRD recurrence. Of the latter patients, two out of the three had a recurrence secondary to PVR; one out of the three had a new retinal tear complicated by RRD recurrence.

Finally, at the month 6 OCT examination, 17 out of 33 patients (51.5%) showed a disrupted/absent IS/OS junction, and 14 patients (42.4%) had a reduced average GC-IPL thickness.

Early Apoptosis Factors

Caspase-9 protein was greater in quantity in the three- to four-quadrant RD group than in the one- to two-quadrant group (median: 65 MFI and IQR: 48–82 MFI versus median: 62 MFI and IQR: 49–465 MFI; $P = 0.048$). Moreover, the caspase-9 quantity was higher in those patients affected by 1- to 2-week RDs (median: 66 MFI and IQR: 51–455 MFI) than in patients with 3- to 4-week RDs (median: 52 MFI and IQR: 32–74 MFI, $P = 0.048$). Furthermore, the caspase-9 quantity was significantly less in patients experiencing PVR during the follow-up period (median: 54 MFI and IQR: 38–82 MFI in PVR patients; median: 64 MFI and IQR: 51–437 MFI in no-PVR patients; $P = 0.025$) (Table 3).

The Bcl-2-associated death promoter protein was 854 (241–2795) MFI in the PVR group and 289 (223–358) MFI in the no-PVR group ($P = 0.049$) (Table 3).

The caspase-8 quantity was 82 (67–113) MFI in the SRF of patients affected by a retinal detachment size of one to two quadrants and 95 (87–580) MFI in patients experiencing an RRD of three to four quadrants ($P = 0.048$). The caspase-8 amount was significantly higher in patients with an MD duration of 3 to 4 weeks than in patients who experienced symptoms for 1 to 2 weeks before the surgery (median: 101 MFI and IQR: 83–270 MFI, median: 82 MFI and IQR: 67–353 MFI, respectively; $P = 0.035$). Finally, caspase-8 was 98 (82–735) MFI in those patients with an OCT disrupted/absent IS/OS junction at the postsurgical 6 month follow-up and 82 (67–88) MFI in the OCT normal IS/OS junction group ($P = 0.042$) (Tables 3, 4).

TABLE 3. Early Apoptosis Factors and Clinical Characteristics of Enrolled Patients

Early Apoptosis Factors	Retinal Detachment Size, Number of Quadrants			Detachment Duration, Weeks			PVR Development During Follow-Up		
	1–2, <i>n</i> = 21	3–4, <i>n</i> = 12	<i>P</i> Value	1–2, <i>n</i> = 23	3–4, <i>n</i> = 10	<i>P</i> Value	Yes, <i>n</i> = 13	No, <i>n</i> = 20	<i>P</i> Value
Caspase-9	62 (49–465)	65 (48–82)	0.048	66 (51–455)	52 (32–74)	0.048	54 (38–82)	64 (51–437)	0.025
BAD	292 (232–2335)	361.0 (232–2309)	0.562	300 (241–1594)	328 (215–2600)	0.884	854 (241–2795)	289 (223–358)	0.049
Caspase-8	82 (67–113)	95 (87–580)	0.048	82 (67–353)	101 (83–270)	0.035	98 (82–735)	83 (67–101)	0.113
Bcl2	56 (42–74)	63 (58–83)	0.367	60 (44–73)	68 (54–84)	0.441	67 (47–87)	57 (45–73)	0.298
p53	41 (34–265)	53 (40–260)	0.459	52 (36–350)	40.0 (26–57)	0.201	52 (28–66)	48.0 (36–295)	0.761

n, number of patients; IS/OS junction, inner segment/outer segment photoreceptor junction; mean fluorescence intensity is the unit of measure. Data are presented as median (interquartile range). The Mann-Whitney *U* test was performed in order to obtain *P* values.

The Bcl-2 protein was increased in the SRF of those patients showing a disrupted/absent IS/OS junction on OCT images performed at the postsurgical month 6 visit (median: 73 MFI and IQR: 61–287 MFI in the altered IS/OS junction group; median: 53 MFI and IQR: 45–62 MFI in the preserved IS/OS junction group; *P* = 0.003) (Table 4).

The amount of p53 was greater in those patients showing a normal IS/OS junction (median: 43 MFI and IQR: 36–58 MFI in the disrupted/absent IS/OS junction group; median: 325 MFI and IQR: 41–435 MFI in the normal IS/OS junction group; *P* = 0.029) (Table 4).

Signal-Transduction Proteins

Extracellular signal-regulated kinases 1 and 2 were increased in those patients with detachment durations of 1 to 2 weeks, compared with the 3- to 4-week-duration MD group (median: 432 MFI and IQR: 172–1129 MFI; median: 119 MFI and IQR: 65–371 MFI; *P* = 0.025) (Table 5). Furthermore, ERK 1/2 was higher in quantity in those patients without a reduced average GC-IPL thickness at the month 6 visit (median: 321 MFI and IQR: 160–2270 MFI in the reduced GC-IPL group; median: 367 MFI and IQR: 169–1028 MFI in the normal GC-IPL group; *P* = 0.028) (Table 6).

Best-Corrected Visual Acuity at the Postsurgery Month 6 Visit

Linear regression analysis was performed to evaluate the relationship between BCVA at the postsurgical month 6 visit and the tested molecules. Both ERK 1/2 and CREB were related to the month 6 BCVA (Table 7).

DISCUSSION

In this prospective study, we investigated the levels of both signal-transduction proteins and early apoptosis proteins in the SRF collected during scleral buckling surgery for macula-off

RRD. Overall, we found that these proteins are associated with different clinical features and postsurgical outcomes.

Several studies have demonstrated that cytokines are correlated with the RRD repair outcome.^{22,23} In fact, abundant laboratory-based evidence is available showing that inflammatory cytokines and inflammatory cells could underlie the pathologic changes leading to the development of PVR.^{24–26} Laboratory and animal studies also showed that signal-transduction proteins and early apoptosis molecules influence the outcome after surgery for RRD repair.^{5–12} However, to the best of our knowledge, no clinical study has evaluated the association between the levels of these molecules in the SRF and the clinical features, as well as the postsurgical results, in patients with macula-off RRD undergoing scleral buckle surgery.

In our patients, caspase-9 concentration was found to be higher both in RRD with a greater size and in RRD characterized by less time elapsed after the onset of symptoms. These results are not surprising for the following reasons: (1) With a greater level of detachment, more outer retinal cells start to deteriorate from the reduction of nutrients and oxygen supply, leading to the activation of the apoptotic pathway; and (2) it is known that apoptosis occurs quickly after RRD onset.⁵ Interestingly, the extrinsic pathway was also found to be involved in RRD, and caspase-8 level was increased in patients affected by a bigger RD.

Ricker et al.²⁵ enrolled 75 patients affected by RRD and scheduled them for scleral buckle surgery. In their study, the authors collected the SRF during the surgery and demonstrated that the level of several cytokines was higher in patients in whom PVR developed. In the present study, we showed that early apoptosis molecules are associated with PVR development. Indeed, we found that a lower concentration of caspase-9 characterized the SRF of patients in whom PVR developed within 6 months after the surgery. The intrinsic pathway, which has been well established as involving caspase-9, is triggered by several stress factors.^{14–16} Retinal detachment reduces nutrients and oxygen supply for the outer retina. In

TABLE 4. Early Apoptosis Factors and Postsurgical Month 6 Optical Coherence Tomography Features of Enrolled Patients

Early Apoptosis Factors	Reduced Average GC-IPL Thickness			IS/OS Junction		
	Yes, <i>n</i> = 14	No, <i>n</i> = 19	<i>P</i> Value	Disrupted/Absent, <i>n</i> = 17	Normal, <i>n</i> = 16	<i>P</i> Value
Caspase-9	56 (40–350)	82 (54–286)	0.422	82 (50–455)	55 (49–66)	0.111
BAD	326 (234–1962)	344 (284–848)	0.470	359 (220–1674)	298 (241–2775)	0.926
Caspase-8	100 (68–847)	92 (79–735)	0.406	98 (82–735)	82 (67–88)	0.042
Bcl2	66 (53–73)	62 (47–121)	0.434	73 (61–287)	53 (45–62)	0.003
p53	35 (28–45)	62 (40–360)	0.335	43 (36–58)	325 (41–435)	0.029

n, number of patients; IS/OS junction: inner segment/outer segment photoreceptor junction. Data are presented as median (interquartile range). The Mann-Whitney *U* test was performed in order to obtain *P* values.

TABLE 5. Signal-Transduction Proteins and Clinical Characteristics of Enrolled Patients

Signal-Transduction Proteins	Retinal Detachment Size, Number of Quadrants			Detachment Duration, Weeks			PVR Development During the Follow-Up		
	1-2, n = 21	3-4, n = 12	P Value	1-2, n = 23	3-4, n = 10	P Value	Yes, n = 13	No, n = 20	P Value
NF-κB	48 (36-64)	59.0 (40.25-156.75)	0.228	51 (36-66)	48 (38-262)	0.658	58 (39-70)	48 (36-72)	0.955
p38	142 (91-1005)	102.5 (65.5-700)	0.274	142 (96-905)	87 (65-154)	0.089	99 (65-835)	136 (93-882)	0.166
ERK1/2	302 (136-1062)	211.0 (72.75-757.5)	0.242	432 (172-1129)	119 (65-371)	0.025	157 (93-675)	428 (143-1200)	0.123
STAT3	85.0 (62.5-440)	87.5 (70.35-652.5)	0.326	85 (65-495)	75 (57-547)	0.524	71 (57-585)	85 (65-505)	0.510
STAT5	111.0 (82-775)	84.0 (70.75-890)	0.839	111 (77-825)	96.5 (69-785)	0.743	81 (73-755)	115 (79-827)	0.355
CREB	60.0 (46.5-255)	68.5 (46.75-447.5)	0.471	62 (45-275)	57.5 (47-365)	0.985	58 (37-475)	61 (48-267)	0.925
p70	89.0 (45-400)	51.0 (36.25-169.5)	0.193	60 (37-375)	60 (45-251)	0.893	53 (32-193)	64 (44-387)	0.396

n, number of patients; mean fluorescence intensity is the unit of measure. Data are presented as median (interquartile range). The Mann-Whitney *U* test was performed in order to obtain *P* values.

fact, several studies have demonstrated that histopathologic changes in the outer retinal cells, following retinal detachment, are related to the activation of the apoptotic cascade,^{6,7} and apoptosis is considered as the major cause of the outer retinal cell death after RD.^{27,28} Our results might suggest a protective role of the apoptotic activation against the PVR development. The fact that a decrease in apoptotic activation leads to a higher risk of PVR after RRD has already been shown in several studies evaluating the influence of gene polymorphism.^{29,30} Pastor-Idoate et al.²⁹ speculated that reduced levels of apoptotic proteins in retinal cells might activate other cell death pathways, which would increase the intraocular inflammation after RRD. The increased inflammation could generate a cascade of tissue responses that generate and amplify the hostile microenvironment, where activated RPE can migrate and transdifferentiate, with eventual PVR development.

Despite significant progress in the surgical management of RD, patients still often have significant visual loss, largely secondary to photoreceptor death. There are several studies reporting the use of OCT to study the photoreceptor layer after macula-off RD repair.³¹⁻³³ All these studies demonstrated that OCT can detect photoreceptor damage following RRD, and OCT showed that photoreceptor impairment after the surgery was between 50% and 60%.³¹⁻³³ As specified above, apoptosis is considered the leading cause of photoreceptor death during the RRD.^{27,28} Our results showed that subretinal apoptotic protein levels were correlated with the integrity of the IS/OS junction on OCT images at the postsurgical month 6 visit. These results should be considered relevant because we demonstrated, for the first time by an imaging approach, the importance of the apoptotic pathway for the post-RRD photoreceptor status. Furthermore, it is known that inhibitors of apoptosis can only partly rescue photoreceptors, implying

the existence of other pathways inducing the RD-associated photoreceptor death.²⁸ Nevertheless, we did not find a difference in the level of signal-transduction proteins between patients with and without IS/OS junction alteration. Interestingly, we also found that p53 protein concentration is increased in the SRF of patients without OCT photoreceptor damage. Once activated, following cellular stress, p53 induces a cell cycle arrest to allow either repair and survival or apoptosis.³⁴ This indicates that the stress following the RD could induce an increase in p53 concentration that seems to be protective to the photoreceptors, probably because the repair and survival process prevails until the detachment repair is performed.

Menke et al.³⁵ recently showed postsurgical GC-IPL layer thinning in patients undergoing RRD surgery. We demonstrated that patients not showing a thinning of the GC-IPL layer at the postsurgical month 6 visit had a greater concentration of subretinal ERK1/2 protein. Extracellular signal-regulated kinases 1 and 2 are thought to be critical for neuronal differentiation and to have an important prosurvival activity in neurons,³⁵ as well as in retinal ganglion cells.³⁶ Thus, their overexpression could promote ganglion cell survival and, finally, GC-IPL thickness preservation. Finally, ERK1/2 were shown to be related to the 6-month BCVA, which also indicates the functional importance of this protein.

Our study has some limitations. The series presented here is relatively small. However, one should assess the current series in light of the low RRD incidence and the strict inclusion criteria, which increase the rarity of the patients suitable for recruitment. Furthermore, this study lacked a control group, and we were unable to evaluate the expression of signal-transduction proteins, as well as the level of early apoptosis proteins, in healthy subjects and to compare them with patients with RRD. Although it is not well known, SRF

TABLE 6. Signal-Transduction Proteins and Postsurgical Month 6 Optical Coherence Tomography Features of the Enrolled Patients

Signal-Transduction Proteins	Reduced Average GC-IPL Thickness			IS/OS Junction		
	Yes, n = 14	No, n = 19	P Value	Disrupted/Absent, n = 17	Normal, n = 16	P Value
NF-κB	62 (49-515)	44 (34-58)	0.221	58 (39-390)	49 (35-59)	0.133
p38	145 (117-1445)	151 (80-852)	0.473	130 (66-1085)	168 (119-795)	0.385
ERK1/2	321 (160-2270)	367 (169-1028)	0.028	425 (152-1062)	296 (111-1163)	0.837
STAT3	290 (69-615)	225 (70-577)	0.341	85 (67-385)	67 (54-665)	0.592
STAT5	109 (65-842)	88 (76-787)	0.360	111 (71-820)	545 (86-830)	0.536
CREB	59 (39-64)	157 (47-392)	0.557	58 (46-365)	62 (46-345)	0.742
p70	96 (44-432)	64 (35-345)	0.323	89 (51-380)	46 (34-335)	0.113

n, number of patients; IS/OS junction, inner segment/outer segment photoreceptor junction; data are presented as median (interquartile range). Mean fluorescence intensity is the unit of measure. The Mann-Whitney *U* test was performed in order to obtain *P* values.

TABLE 7. Relationship Between Best-Corrected Visual Acuity at the Postsurgical Month 6 Visit and the Tested Molecules

RRD Patients, <i>n</i> = 33		
Tested Molecules	Regression Coefficient ± SE	<i>P</i> Value
p38	−0.542 ± 2.131	0.050
ERK1/2	−0.517 ± 2.636	0.022
CREB	−0.851 ± 3.645	0.003

n, number of patients; SE, standard error; linear regression analysis was used to evaluate the relationship between best-corrected visual acuity at the postsurgery month 6 visit and the tested molecules. Only the statistically significant results are shown.

accumulation in RD is thought to be due to different mechanisms, involving the vitreous, serum, and retina.^{37,38} Therefore, SRF may be better considered as a separate pathologic entity. Hence, even though the concentration of the tested molecules was estimated in the vitreous of healthy subjects,³⁹ we believe that these values should not be compared to those of our study patients, since we tested the same molecules directly in the SRF instead of the vitreous. Another major limitation is that we followed the patients only up to 6 months after the scleral buckle surgery.

In conclusion, we report the first study testing the level of pro-survival and apoptotic proteins in the SRF of eyes experiencing macula-off RRD and operated by scleral buckle surgery. The apoptotic pathway is crucial in the pathologic events following RRD: Its activation is early, in the first 2 weeks, and both leads to the photoreceptor damage and has a protective role against PVR. Data from this study might help to understand pathologic processes in retinal detachment. In particular, the early activation of the apoptotic pathway and the subsequent photoreceptor damage could explain why after 10 days of RRD occurrence, the final visual outcome is clinically comparable and independent of further delay of surgery for up to 30 days.²⁰ Furthermore, we envisage that these proteins may become a therapeutic target once their role is further clarified.

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