


Perspective

Novel insights into the pathophysiology of proliferative vitreoretinopathy: The role of vitreoschisis-induced vitreous cortex remnants

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ABSTRACT.

Purpose: We previously hypothesized a causal relationship between vitreoschisis-induced vitreous cortex remnants (VCR) and the development of proliferative vitreoretinopathy (PVR). This study aims to substantiate this association through histopathological analysis of surgical specimens in support of strategies to improve therapeutic outcomes.

Methods: A descriptive, prospective, non-consecutive case series. Histopathological and immunohistochemical analyses were performed on membranes removed from the peripheral retinal surface during initial vitrectomy for primary rhegmatogenous retinal detachment (RRD) ($n = 11$) or recurrent retinal detachment ($n = 12$). The clinical aspect of the membranes ranged from loose-meshed membranes visualized with triamcinolone to more fibrotic membranes stained with trypan blue.

Results: Consistent with the clinical presentation, histopathological analysis revealed membranes with different area characteristics. Paucicellular lamellar collagen-rich areas, suggestive of VCR, appeared to transition to areas of increased cellularity and eventually more fibrotic areas of low cellularity. Five different area characteristics could be identified that seemed to correspond to five histopathological stages in PVR formation, with lamellar VCR collagen acting as an essential precondition: 1. Lamellar collagen, low cellularity (hyalocytes). 2. Lamellar collagen, increased cellularity (hyalocytes, glial cells). 3. Lamellar collagen, high cellularity (macrophages, glial cells, RPE-cells). 4. Early fibrosis, decreased cellularity (myofibroblasts). 5. Fibrosis, low cellularity (myofibroblasts).

Conclusion: These findings confirm the role of VCR in preretinal PVR formation posterior to the vitreous base. We propose that the presence of VCR over the retinal surface should be qualified as a risk factor for PVR formation. Detection and adequate removal of VCR may improve the success rate of vitreoretinal surgeries.

Key words: histopathology – immunohistochemistry – primary rhegmatogenous retinal detachment – proliferative vitreoretinopathy – recurrent retinal detachment – vitreoschisis-induced vitreous cortex remnants

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Introduction

In most cases, surgery for primary RRD is successful, while in about 10%–15%, additional surgery is required for a redetachment (Enders *et al.* 2017). The primary cause of surgical failure is the development of preretinal proliferative vitreoretinopathy (PVR), a clinical syndrome characterized by the formation of contractile fibrocellular membranes, which can lead to retinal break formation/reopening, macular pucker, and macular hole (Leaver 1995; Pastor 1998; Sebag & Green 2013; Coffee *et al.* 2014). Reattachment rates for RRD associated with PVR are much lower, leading to more surgical procedures, extended follow-up, and disappointing functional outcomes.

Despite significant advances in surgical instrumentation and techniques, the incidence of retinal redetachment due to PVR has largely remained unchanged persisting at approximately 10%–15% (Joeres *et al.* 2006; Sundar *et al.* 2018). Moreover, extensive research into the pathophysiology of PVR, leading to the use of adjuvants to counteract the disease process, has not led to improvements in the prevention and management of PVR (Charteris 2020). The prevailing hypothesis on the pathogenesis of preretinal PVR focuses on the breakdown of the

blood–retina barrier and the consequences of intravitreal dispersion of retinal pigment epithelial (RPE) cells, but may have underestimated the role of vitreous and, in particular, vitreoschisis-induced vitreous cortex remnants (VCR).

Conventional teaching on spontaneous or surgically induced posterior vitreous detachment (PVD) reports that the vitreous cortex separates completely, leaving the inner retinal surface clean from collagen remnants up to the area described as the vitreous base (where the vitreous collagen inserts into the inner retina making a clean separation impossible). However, others have suggested that incomplete separation of the vitreous cortex may occur (Sebag 2008). In case of vitreoschisis, the outermost lamellae of the vitreous cortex remain attached to the retinal surface as VCR. The presence of VCR over the macula (mVCR) has been associated with the development of idiopathic macular pathologies such as a macular hole and macular pucker as well as macular PVR (Yamashita *et al.* 2008; Gupta *et al.* 2011; Sebag & Green 2013; Kato *et al.* 2021).

In previous studies, we found that VCR can be much more widespread across the retina and is a far more common finding than previously thought (Van Overdam *et al.* 2019, Van Overdam 2020). The presence of VCR over the peripheral retina (pVCR) has been reported in 35% of patients undergoing vitrectomy for RRD (Van Overdam 2020; Assi & Khoueir 2021). Although the use of triamcinolone acetonide (TA) to visualize vitreous and facilitate vitreous base shaving has been extensively reported and recommended (Peyman *et al.* 2000; Sakamoto & Ishibashi 2009; Veckeneer & Wong 2009), we suggested that more specifically the presence of pVCR is often missed, necessitating repeated TA applications targeted to the peripheral retinal surface for adequate pVCR detection.

Furthermore, we found that detection and removal of pVCR during primary RRD surgery can improve surgical outcomes and reduce retinal redetachment rates to less than 5% (Van Overdam 2020), also recently confirmed by other researchers (Sartini *et al.* 2022; Rizzo *et al.* 2021). In all patients who developed a redetachment

due to PVR (in highly myopic as well as in emmetropic eyes), pVCR were detected but were not or not completely removed during the first surgery (Van Overdam 2020). Therefore, we suggest VCR to be a causal link in the development of preretinal PVR membranes over the macula and peripheral retina posterior to the vitreous base where VCR can act as a scaffold for fibrocellular proliferation, while hyalocytes (resident vitreous macrophages) present in VCR also play a role in PVR membrane formation (Van Overdam 2020).

This theory offers potential improvements in therapeutic intervention by removing VCR and embedded hyalocytes from the retinal surface to reduce the risk of postoperative PVR formation and retinal redetachment. This study aims to further substantiate the relationship between the presence of VCR over the retinal surface and the formation of PVR by histopathology.

Methods

A descriptive, prospective, non-consecutive case series was performed. The study was granted Institutional Review Board approval and complied with the tenets of the Declaration of Helsinki. Twenty-three membranes were removed from the peripheral retinal surface posterior to the vitreous base, during 23-gauge pars plana vitrectomy in 23 eyes of 23 patients with RRD ($n = 11$) or redetachment ($n = 12$) by the first author at the Rotterdam Eye Hospital and sent for histopathological and immunohistochemical analysis at the Erasmus University Medical Center, Rotterdam. The clinical aspect of the membranes varied from loose-meshed membranes suggestive of VCR to more fibrotic and adherent membranes resembling PVR.

Surgery

For the detection of VCR over the retinal surface, TA (20 mg/ml; Kenacort-A, Bristol-Myers Squibb, New York, NY, USA) was gently injected towards the macula, retinal mid-periphery and far-periphery in all four quadrants in the absence of heavy liquid (Van Overdam *et al.* 2019, Van Overdam 2020). After the removal of free-floating TA from the vitreous

cavity, this procedure was repeated at least once. Removal of VCR from the retinal surface was performed according to the previously described Vitreous Wiping technique (Van Overdam *et al.* 2019) in combination with the vitrectome. More fibrotic and adherent membranes (PVR) were peeled using end-gripping forceps after staining with Membrane Blue-Dual (MBD; 0.025% brilliant blue and 0.15% trypan blue; DORC International, Zuidland, The Netherlands), which was applied under air. Peeling of preretinal membranes (VCR/PVR) in combination with the underlying internal limiting membrane (ILM) was enhanced using intracryanine green (IfCG; 2.5 mg/ml; SERB Laboratories, Paris, France) staining.

Removed VCR attached to the Vitreous Wipe (a piece of polyvinyl alcohol (PVA), Eyetec™ Instrument Wipe, Network Medical Products, North Yorkshire, UK) and peeled membranes placed on a piece of PVA, were fixated in neutral-buffered formalin 10% and sent to the pathology department at the Erasmus Medical Center, Rotterdam, The Netherlands, for further processing and analysis.

Pathology

Small, fragile tissue samples were described macroscopically, stained using crystal violet (Merck Millipore, #115940) for optimal visualization during processing, and transferred to be processed by the Hologic Cellient (Hologic, Inc., Marlborough, MA 01752 USA) (Van Ginderdeuren *et al.* 2014). After processing, the FFPE (formalin-fixed paraffin-embedded) blocks were cut at 4 μm to a maximum of 6–8 serial sections and mounted on slides for histochemical and immunohistochemical staining.

Histochemical staining

Routine staining protocols were used for standard haematoxylin and eosin (HE) staining and Sirius Red (SR) staining to detect fibrosis using an automated staining system (HE600, Ventana Medical Systems, Tucson, AZ, USA). For SR staining, in brief, following deparaffinization, slides were rehydrated by passage through decreasing ethanol series, 5 min predifferentiation step using 0.2% phosphomolybdic acid followed by 45 min incubation with 0.1% SR solution.

Immunohistochemical staining

Immunohistochemistry was performed with an automated, validated, and accredited staining system (Ventana Benchmark ULTRA, Ventana Medical Systems, Tucson, AZ, USA) using ultraview or optiview universal DAB detection kit. In brief, following deparaffinization and heat-induced antigen retrieval, the tissue samples were incubated according to their optimized time with the antibody of interest (Table 1). According to the manufacturer's instructions, the incubation was followed by a haematoxylin II counterstain for 8 min, and a blue colouring reagent for 8 min (Ventana). Tonsil tissue was used as a positive control for all antibodies, except for glial fibrillary acidic protein (GFAP) where normal brain tissue was used.

Results

Characteristics of the 23 patients and 23 harvested membranes are shown in Table 2. Twelve patients were male (52%), mean age was 63.5 years (range 51–84), mean axial length (AL) was 24.7 mm (range 22.84–29.48), and mean spherical equivalent (SE) before cataract surgery was -1.5 dioptre (D; range -9.0 to $+3.25$). No analysis could be performed on the VCR membranes of three patients (patients 1, 2 and 3) as these were lost during histopathological processing. All three patients had a recent onset macula-off RRD, where VCR were detected over the entire retinal surface, removed as thin loose-meshed sheets from attached and detached retina, and sent attached to the Vitreous Wipe for further processing and analysis (Fig. 1).

Consistent with the clinical presentation, histopathological analysis revealed membranes with different area characteristics. Paucicellular lamellar collagen-rich areas, suggestive of VCR (with or without ILM), appeared to transition to areas of increased cellularity and eventually more fibrotic

areas of low cellularity. We could identify five different area characteristics that seemed to correspond to five histopathological stages in PVR formation, with VCR acting as an essential precondition (Table 3, Fig. 2). In all membranes, later-stage areas were accompanied by adjacent earlier-stage areas, indicating the transition from one stage to another.

Histopathological VCR-PVR stages

In the *first stage*, hypocellular VCR are detected as thin lamellar crumpled collagen membranes highlighted by SR staining. Immunohistochemistry does not show reactive glial cells, but only sparse hyalocytes/macrophages.

The *second stage* shows VCR with increased cellularity associated with gliosis as identified by GFAP-positive glial cell staining. CD3 and CD163 are largely negative.

The *third stage* demonstrates high cellularity with the proliferation of RPE cells and an influx of a few CD3-positive lymphocytes and many CD163-positive macrophages. The cells have an activated phenotype and show strong positivity for HLA-DR.

The *fourth stage* is characterized by a decrease in cellularity and early signs of fibrosis with CD163-positive macrophages and RPE cells that transdifferentiate into smooth muscle actin (SMA)-positive myofibroblasts.

The *fifth stage* shows increased fibrosis with thick layers of SR staining collagen and a further decrease of cellularity consisting mainly of SMA-positive myofibroblasts.

Hereunder, the five stages are illustrated by case examples for each different stage.

Case examples

Case 1, patient 8, VCR-PVR stage 1

Clinical details. A 51-year-old, phakic woman with in her left eye (SE -5.25

D, AL 25.03 mm) a primary macula-off RRD from 1 to 8 o'clock. Vitreous cortex remnants (VCR) were detected over the detached macula and detached temporal quadrants and removed by ILM peeling over the macula towards the temporal mid-periphery, and by vitreous wiping over the mid-periphery and far-periphery, more in small pieces rather than in a sheet.

Histopathological findings. Only VCR removed with ILM could be used for analysis. Vitreous cortex remnants attached to the vitreous wipe were lost during processing. The thin membrane contained thin layers of collagen which was demonstrated in the SR staining. With HE staining we observed a thin one-to-multi-layered curled membrane containing approximately 5 detectable nuclei. Glial fibrillary acidic protein staining revealed that there was no glial component in this membrane, nor were CD3 T-cells present. There were few CD163-positive M2-profibrotic hyalocytes/macrophages which showed an activated phenotype as they stained positive for HLA-DR. The presence of collagen with few macrophages (hyalocytes) in the absence of gliosis corresponds to VCR-PVR stage 1 (Fig. 3).

Case 2, patient 11, VCR-PVR stage 2

Clinical details. A 68-year-old, phakic man with in his left eye (SE -0.25 D, AL 23.91 mm) a primary macula-off RRD from 2 to 9 o'clock with a giant retinal tear from 2 to 5 o'clock with curled edge. Vitreous cortex remnants were detected over the detached macula and detached inferior quadrants with two mid-peripheral retinal starfolds at 6 and 7 o'clock. Peeling of the ILM together with the overlying VCR was performed over the macula towards the inferior mid-periphery. The more fibrotic and adherent membranes in the area of the starfolds blocked further ILM peeling. These immature PVR membranes (stained with MBD) were peeled

Table 1. Immunohistochemistry information.

Tissue/cells	Antibody	Type	Concentration ($\mu\text{g/ml}$)	Company	Clone	Procedure	Incubation (min)
T-cells	CD3	AR	0.4	Ventana	2GV6	OV 32'	32
Hyalocytes/Macrophages	CD163	AM	0.23	Cell Marque	MRQ26	OV 32'	20
Myofibroblasts	SMA	AM	1.2	Biosite	BS66	OV 32'	12
Glial cells	GFAP	AR	4.69	Cell Marque	EP672Y	UV 8'	8
Activation marker	HLA-DR	AM	1.2	DAKO	CR3/43	OV 8'	32

AR = Anti-rabbit, AM = Anti-mouse, OV = Optiview CC1, UV = Ultraview CC1.

Table 2. Patient characteristics, surgical outcome and histopathological findings.

Patient	Age	Gender	Axial Length	Spherical Equivalent	Previous Treatments	Diagnosis	Macula	Additional Findings	PVR*	Surgical Treatment	Anatomical Outcome	BCVA Preop	BCVA 12 m	Clinical aspect Membrane	VCR-PVR stages Membrane
1	62	M	25.92	-4.25	-	RRD	OFF	-	-	PPV + C ₃ F ₈	RA	2.48	0.18	VCR	ML
2	52	F	24.64	-0.25	-	RRD	OFF	-	-	PPV + SF ₆	RA	0.48	0.20	VCR	ML
3	63	M	25.60	-3.50	-	RRD	OFF	-	-	PPV + SF ₆	RA	1.70	0.20	VCR	ML
4	67	M	24.50	+0.75	Laser	RRD	OFF	MP	B	PPV + SO	RA	1.30	0.48	ILM, VCR	1, 2
5	47	F	29.48	-9.00	Laser	RRD	ON	LRT	-	PPV + SF ₆	RA	0.00	0.10	VCR	1, 2
6	62	M	23.69	-1.00	-	RRD	OFF	HT	C3	PPV + SO	RA*	1.50	0.30	VCR, PVR	2, 3
7	50	M	25.48	-4.25	-	RRD	OFF	LRT	D1	PPV + SO	RA*	2.48	1.00	VCR, PVR	2, 3, 4
8	51	F	25.03	-5.25	-	RRD	OFF	-	-	PPV + SO	RA	1.30	0.18	ILM, VCR	1
9	63	F	23.46	-1.00	Laser	RRD	OFF	-	-	PPV + C ₃ F ₈	RA	0.18	0.10	ILM, VCR	1, 2
10	71	F	22.84	+1.00	Laser	RRD	OFF	-	C3	PPV + SO	RA*	2.48	0.80	VCR, PVR	2, 3, 4
11	68	M	23.91	-0.25	-	RRD	OFF	GRT	C1	PPV + SO	RA*	2.00	0.40	ILM, VCR†	1, 2
12	73	M	25.55	-1.00	PPV + C ₃ F ₈	re-RD	ON	MP	B	PPV + SO	RA	0.10	0.18	VCR, PVR	2, 3, 4
13	74	F	23.11	-1.50	PPV + SF ₆	re-RD	ON	MP	B	PPV + SO	RA*	0.40	0.20	VCR, PVR	4, 5
14	73	M	24.10	+0.25	PPV + SO; SOR; PPV + C ₃ F ₈	re-RD	OFF	-	C1	PPV + SO	RA	2.48	0.80	ILM, VCR, PVR	1, 2, 3
15	68	M	25.31	+0.50	SB, PPV + SF ₆	re-RD	OFF	HT, VH	C2	PPV + SO	RD	1.70	0.70	VCR, PVR	2, 3
16	60	M	25.40	-3.25	PPV + SF ₆	re-RD	ON	MH	B	PPV + SO	RA	0.80	0.80	VCR	1, 2
17	66	F	23.03	+1.00	PPV + C ₃ F ₈	re-RD	OFF	MH, UV	C1	PPV + SO	RA*	2.00	1.70	VCR, PVR	2, 3, 4
18	70	M	24.11	-0.50	PPV + SF ₆ (2x); Laser (2x)	re-RD	ON	MP	C2	PPV + C ₃ F ₈	RA	0.30	0.10	ILM, VCR, PVR	1, 2, 3, 4, 5
19	84	F	23.65	-0.75	PPV + SO	re-RD	OFF	HT, VH	B	PPV + SO	RA†	2.48	2.48	VCR, PVR	2, 3, 4
20	64	M	24.82	+3.25	PPV + SO	re-RD	OFF	UV	B	PPV + C ₃ F ₈	RA	1.70	1.00	VCR, PVR	2, 3
21	56	F	24.06	-1.00	PPV + C ₃ F ₈	re-RD	OFF	-	C2	PPV + SO	RA	0.80	0.48	ILM, VCR, PVR	2, 3, 4
22	57	F	24.55	-3.00	PPV + SF ₆	re-RD	OFF	MH, MP	C1	PPV + SO	RA*	0.48	0.40	ILM, VCR, PVR	2, 3
23	59	F	25.76	-2.25	PPV + C ₃ F ₈	re-RD	OFF	-	C2	PPV + SO	RA*	2.48	0.30	VCR, PVR	3, 4, 5

M = male, F = female, Laser = laser treatment retinal tears, PPV = vitrectomy, SF₆ = sulfurhexafluoride gas, C₃F₈ = perfluoropropane gas, SO = silicone oil (5000cs), SOR = silicone oil removal, SB = scleral buckle surgery, RRD = primary rhegmatogenous retinal detachment, re-RD = recurrent retinal detachment, re-RD = recurrent retinal detachment, re-RD = recurrent retinal detachment (2 clock hours), GRT = giant retinal tear (3 clock hours), HT = hypotony, MP = macular pucker, MH = macular hole, VH = vitreous haemorrhage, UV = uveitis, BCVA: best corrected visual acuity (decimal converted to LogMAR), Preop = preoperative, 12 m = 12 months postoperative, VCR = Vitreochisis-induced Vitreous Cortex Remnants, PVR = Proliferative Vitreoretinopathy, ILM = Internal Limiting Membrane, ML = membrane lost during histopathological processing, RA = retina attached after first surgery and after silicone removal (if applicable), RD = retina detached after first surgery due to PVR in areas where VCR/PVR could not be completely removed during first surgery, but attached after one additional vitrectomy with membrane peeling and silicone tamponade and after silicone oil removal.

* PVR classification from the Retina Society Terminology Committee (Hilton *et al.* 1983).

† Immature PVR part of the epiretinal membrane could not be removed as a sheet, and therefore, not sent for histopathological analysis.

* Additional limited membrane peeling from attached retina during oil removal surgery in areas where VCR/PVR could not be completely removed during first surgery.

‡ Retina was attached but patient did not want additional surgery for silicone oil removal.

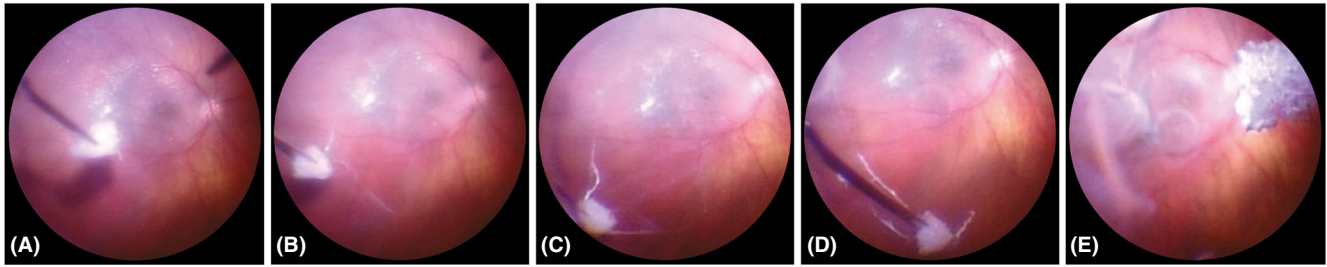


Fig. 1. In patient 1, vitreous wiping was performed after vitrectomy, internal limiting membrane (ILM) peeling, and staining with triamcinolone acetate (TA). Peripheral vitreoschisis-induced vitreous cortex remnants (pVCR) covering the mid-peripheral and far-peripheral retinal surface were stained by TA, and most visible at the edge of the area of ILM peeling (A). Macular VCR (mVCR) were already removed by ILM peeling. The Vitreous Wipe was gently passed over the retinal surface, removing the layer of VCR towards the vitreous base (B–D), resulting in a more mobile retina without retinal damage (E). Perfluorocarbon liquid (PFCL) was used to provide counter-traction away from the direction of the wiping movement. A small residual PFCL bubble can be seen on the retinal surface (D, E). The Vitreous Wipe with attached VCR (E) was sent for histopathological analysis.

Table 3. Histopathological stages in VCR-PVR development.

Stage	Main characteristics	Main cell types	Clinical appearance
1	Lamellar collagen, low cellularity	Hyalocytes	VCR
2	Lamellar collagen, increased cellularity	Hyalocytes, Glial cells	↓
3	Lamellar collagen, high cellularity	Macrophages, Glial cells, RPE cells	Premature PVR
4	Early fibrosis, decreased cellularity	Myofibroblasts	↓
5	Fibrosis, low cellularity	Myofibroblasts	Mature PVR

VCR = vitreoschisis-induced vitreous cortex remnants; PVR = proliferative vitreoretinopathy

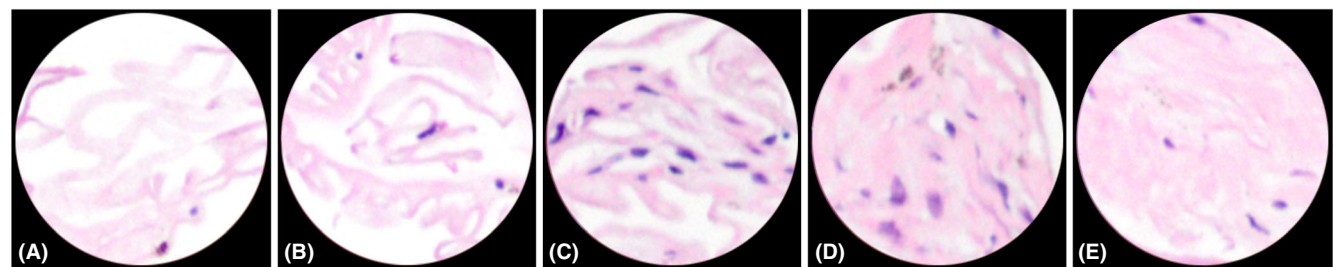


Fig. 2. Five different membrane areas corresponding to five histopathological VCR-PVR stages as identified in a HE-stained membrane (patient 18): 1. Lamellar collagen with low cellularity (A); 2. Lamellar collagen with increased cellularity (B); 3. Lamellar collagen with high cellularity (C); 4. Early fibrosis with decreased cellularity (D); 5. Fibrosis with low cellularity (E).

in small pieces which could not be used for histopathologic analysis.

Histopathological findings. On HE staining, we observed a thin multilayered curled membrane containing more than 5 detectable nuclei. The thin membrane contained thin layers of collagen demonstrated in the SR staining. Glial fibrillary acidic protein staining revealed a minimal glial component in this membrane, CD3 T-cells were sparse. There was an increased number of CD163-positive M2-pro-fibrotic macrophages, part of which had an activated phenotype as HLA-DR stained positive. The gliosis and slightly increased cellularity with activated macrophages indicate VCR-PVR

stage 2, accompanied by adjacent stage 1 and early stage 3 areas (Fig. 3).

Case 3, patient 14, VCR-PVR stage 3

Clinical details. A 73-year-old pseudophakic man with in his left eye (SE +0.25 D, AL 24.10 mm) a total redetachment with a temporal retinal tear at the edge of previous laser scars. Three surgeries had previously been performed by the referring surgeon: vitrectomy with silicone oil for a long-standing primary RDD with PVR, vitrectomy with oil removal, and vitrectomy with C₃F₈ gas for a redetachment. During the fourth surgery, the whole retina had a stiffer appearance. An epiretinal membrane was detected

over a very wide surface area and was removed as completely as possible with, in some areas, peeling of the underlying ILM.

Histopathological findings. On HE staining, we observed a layered curled membrane of varying thickness containing many nucleated cells. The membrane contained thin layers of collagen as demonstrated in the SR staining

indicating VCR. Glial fibrillary acidic protein staining revealed an extensive GFAP-positive glial component in this membrane, CD3 T-cells were sparsely present. There were many CD163-positive M2-pro-fibrotic macrophages which showed an activated phenotype (HLA-DR-positive staining). The highly increased cellularity with a high number of activated macrophages was classified as VCR-PVR stage 3. Smaller adjacent stage 1 and 2 areas could also be distinguished (Fig. 3).

Case 4, patient 23, VCR-PVR stage 4

Clinical details. A 59-year-old phakic female with in her left eye (SE -2.25, AL 25.76 mm) a total redetachment

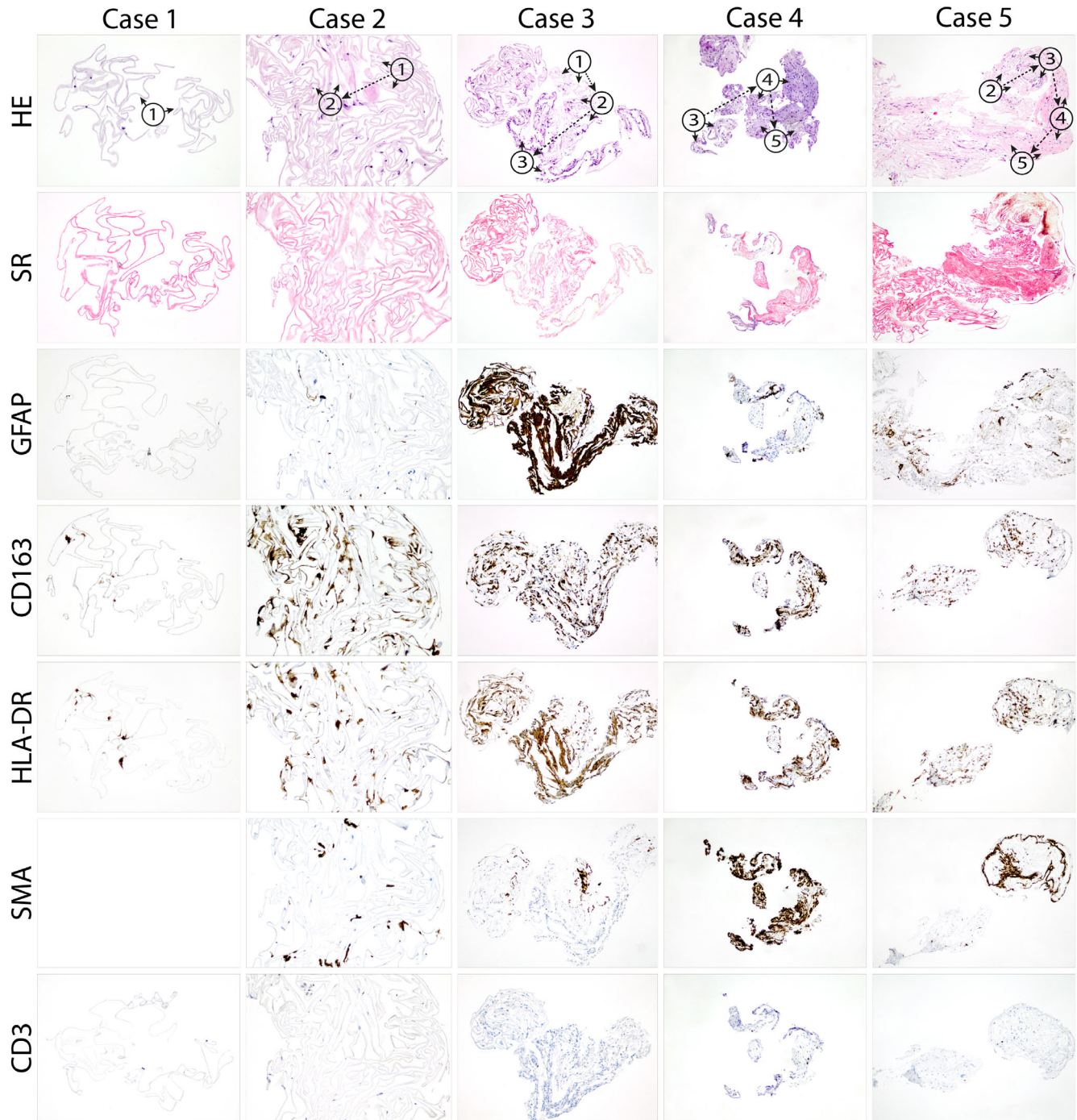


Fig. 3. Histochemical and immunohistochemical staining of five exemplary cases illustrating the five histopathological stages in maturation from VCR to PVR (patients 8, 11, 14, 23, and 18, respectively), depicted in columns (cases 1–5): HE staining (top row); SR staining (second row); GFAP staining (third row); CD163 staining (fourth row); HLA-DR staining (fifth row); SMA staining (sixth row); CD3 staining (bottom row). For case 1, insufficient material was harvested to perform SMA staining. The original magnification is $\times 200$ (cases 1 and 2) or $\times 100$ (cases 3–5). The encircled numbers indicate different VCR-PVR stage areas for each case connected by arrows indicating the transition to adjacent later-stage areas.

with a reopened retinal tear at 6 o'clock. One surgery had previously been performed by the referring surgeon: vitrectomy with C_3F_8 gas for a primary macula-off RRD in the temporal quadrants with peeling of a macular pucker and removal of VCR from the detached and stiffer-

appearing temporal quadrants. As described in the surgery report, VCR were also detected over the attached nasal quadrants, but the surgeon decided to limit VCR removal to the detached retina as this was already time-consuming and it was believed that VCR over detached retina may

pose a higher risk of PVR formation than VCR over attached retina. During the second surgery, VCR and PVR were detected over the stiffer-appearing detached nasal quadrants with three mid-peripheral starfolds. Vitreous cortex remnants (VCR) and Proliferative vitreoretinopathy (PVR) were removed

as completely as possible. No VCR and PVR were detected in the more mobile temporal quadrants.

Histopathological findings. On HE staining, fibrosis was observed with relatively high cellularity which was mainly represented by spindle cell activated myofibroblasts that stain positive for CD163, HLA-DR, and SMA. Gliosis could still be observed through GFAP-positive glial cells. The fibrosis was composed of thick layers of SR-staining collagen. The early relatively cellular fibrosis was classified as VCR-PVR stage 4. Small adjacent stages 2 and 3 areas and early stage 5 areas could also be identified (Fig. 3).

Case 5, patient 18, VCR-PVR stage 5

Clinical details. A 70-year-old pseudophakic man with in his right eye (SE -0.50, AL 24.11 mm) an extensive tractional PVR membrane over the macula extending to the mid-periphery in the temporal quadrants, and also nasally around the optic disc. Two surgeries and two additional laser treatments had previously been performed by the referring surgeon: vitrectomy with SF₆ gas for a primary RDD, laser treatment for new retinal holes inferiorly, vitrectomy with SF₆ for a re-RD, and laser treatment for new retinal holes superiorly. During the third surgery, a large continuous membrane was peeled from the superior arcade towards the inferior temporal mid-periphery.

Histopathological findings. On HE staining, we observed a multilayered curled membrane with a notable fibrotic area containing more than five detectable nuclei. The membrane contained thick layers of collagen (fibrosis) which were shown by SR staining. Glial fibrillary acidic protein staining revealed a glial component in this membrane. Few CD3 T-cells were present. There were CD163-positive M2-profibrotic macrophages which showed an activated phenotype (HLA-DR-positive staining). The extensive fibrosis indicates VCR-PVR stage 5, although the cellularity is still rather high in other fibrotic parts of the sample which fits VCR-PVR stage 4. Adjacent stage 1–3 areas were also present in this membrane (Fig. 3).

Discussion

The histopathological analysis in combination with the clinical findings of

this descriptive study supports the hypothesis that VCR can serve as a scaffold for fibrocellular proliferation, and that VCR containing hyalocytes may actually be the ‘missing link’ in our understanding of how PVR membranes may grow over the retinal surface posterior to the vitreous base in the presence of a clinical PVD (van Overdam 2020). On the basis of various membrane area characteristics, we were able to distinguish five histopathological stages of proliferation from VCR to PVR, with the presence of VCR in the initial phase being an important risk factor to allow further preretinal proliferation in later stages.

These histopathological stages correspond well with the clinical aspect of the membranes (Table 3), suggesting a changing membrane composition over time, previously described as membrane maturation (Hiscott *et al.* 1985). In thin, loose-meshed VCR membranes, mainly collagen with hyalocytes and gliosis areas with low cellularity were identified (stages 1 and 2). Thicker, more adherent premature PVR membranes also contained areas of high cellularity and early fibrosis (stages 3 and 4). In firmer, more fibrotic, mature PVR membranes, areas with fibrosis and low cellularity predominated (stages 4 and 5). In concordance with other studies, this resembles the different stages of a wound healing process: an initial inflammatory phase leading to a proliferative response, followed by remodelling which causes contractile characteristics (Grinnell 1994; Minchiotti *et al.* 2008).

The variability in membrane formation across the retinal surface posterior to the vitreous base is likely to be determined by differences in the presence and interaction of well-known PVR-determinants, such as hyalocytes/macrophages, glial cells, RPE cells, cytokines, and growth factors (Sakamoto & Ishibashi 2011; Pennock *et al.* 2014; Guenther *et al.*; 2019; Song *et al.* 2021). The cell type variation found between the different membrane areas supports our suggestion that these areas represent different stages in PVR formation: in early-stage paucicellular, collagen-rich areas, hyalocytes/macrophages and GFAP-positive glial cells were detected, while in areas with high cellularity activated CD163

and HLA-DR-positive macrophages, and keratin-positive RPE cells were found, which have previously been shown to play an essential role in the development of PVR as they can transdifferentiate into SMA-positive myofibroblasts, which were seen in the more fibrotic areas (Grinnell 1994; Minchiotti *et al.* 2008; Sakamoto & Ishibashi 2011; Pennock *et al.* 2014; Guenther *et al.* 2019; Song *et al.* 2021).

Macrophages can be phenotypically characterized as M1 and M2 subtypes, with M1 type macrophages being proinflammatory, and M2 type macrophages playing a significant role in wound healing and tissue remodelling by inhibiting inflammation and stimulating new extracellular matrix (ECM) formation (Song *et al.* 2021). Using CD163, a highly specific marker for M2 type macrophages in combination with HLA-DR, we provide evidence of an activated profibrotic state. Our observations confirm earlier observations of CD163 and HLA class II expression by M2 type hyalocytes in patients with macular hole or macular pucker (Boneva 2020). M2 type macrophages are known to secrete cytokines such as IL-10, transforming growth factor beta (TGF-beta), and platelet-derived growth factor (PDGF), inducing the synthesis of ECM components such as collagens and fibronectin (Sakamoto & Ishibashi 2011; Song *et al.* 2021). Factors like fibronectin and PDGF attract GFAP-positive glial cells and RPE cells. Under influence of the M2 type hyalocytes/macrophages, these cells can transform into SMA-expressing myofibroblasts increasing collagen production and contraction of the membrane (Song *et al.* 2021). The role of CD3-positive T-lymphocytes in PVR appears to be limited since these cells were not observed in high numbers in any stage. This is in concordance with the anti-inflammatory role associated with the M2 polarized hyalocytes and macrophages (Sakamoto & Ishibashi 2011).

Our current findings cannot exclude that the collagen seen in the early-stage membrane areas may be newly formed collagen rather than native vitreous/VCR collagen, and that later-stage areas could also have arisen *de novo*, in the absence of VCR collagen. However, one would expect higher cellularity in the early-stage areas if the collagen was newly formed.

Furthermore, previously published clinical findings suggest that anomalous PVD with vitreoschisis may leave a layer of VCR collagen over the macular and peripheral retinal surface, and that removal of VCR may reduce the risk of macular and peripheral PVR formation, indicating that it is most likely native VCR collagen seen in the early-stage areas (Van Overdam *et al.* 2019; Van Overdam 2020; Kato *et al.* 2021; Sartini *et al.* 2022; Rizzo *et al.* 2021). In addition, a recent ultrastructural evaluation has revealed the presence of native vitreous collagen (regularly arranged fibrils <16 nm) adjacent to newly formed collagen (irregularly arranged fibrils >16 nm)

in macular PVR (mPVR) and idiopathic macular puckers (iMP). In PVR, a predominance of myofibroblasts and RPE cells surrounded by native vitreous collagen was found, while myofibroblasts, fibroblasts, and hyalocytes represented the main cell types in iMP. Native vitreous collagen was embedded as a continuous layer between the premacular cells and ILM (Guenther *et al.* 2019). We have updated the schematic diagram of anomalous PVD (Van Overdam 2020) to better indicate this association between mVCR and mPVR (Fig. 4).

Previous studies have already emphasized the essential role of vitreous in the development of PVR.

Proliferative vitreoretinopathy determinants present in the vitreous body provide a PVR-promoting environment; the lamellar structure of the vitreous cortex provides a scaffold for fibrocellular proliferation, and hyalocytes in the cortical vitreous play a significant role in ECM synthesis and modulation of immune reaction and inflammation (Elnor *et al.* 1988; Sakamoto & Ishibashi 2011; Wiedemann *et al.* 2013; Boneva *et al.* 2020). Therefore, complete removal of the vitreous body and cortex with shaving at the vitreous base has become a widely accepted surgical goal to prevent post-operative PVR development (Kuhn & Aylward 2014).

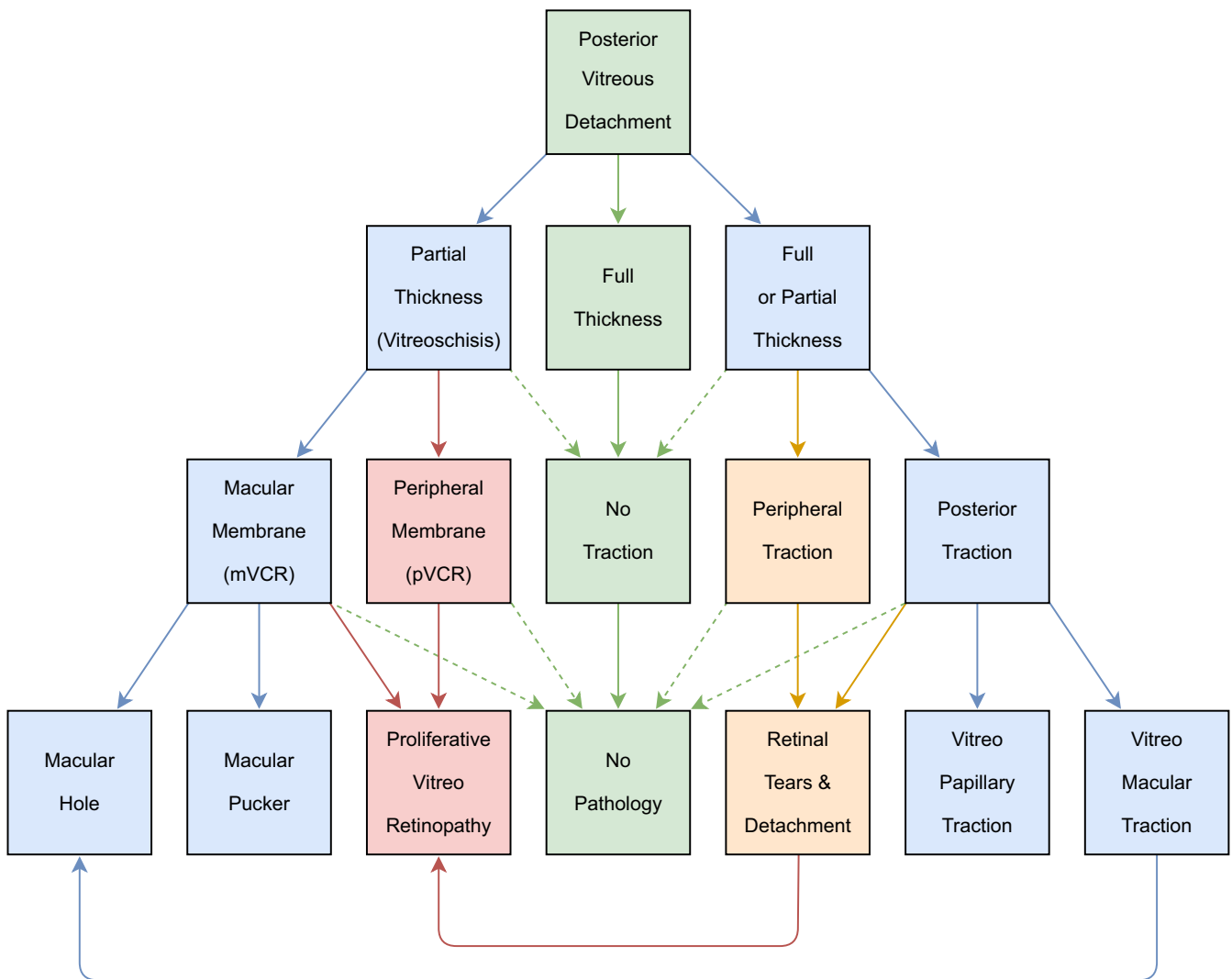


Fig. 4. Updated version of the schematic diagram of anomalous PVD (Van Overdam 2020) with VCR as the ‘missing link’ in the pathophysiology of PVD and PVR indicated in red. In this version, the link between a posterior membrane (macular VCR) and PVR is more clearly indicated. A PVD may be full thickness (without vitreoschisis) or partial thickness (with vitreoschisis). Full or partial thickness PVD may lead to posterior and/or peripheral traction, which may result in vitreopapillary traction, vitreomacular traction, macular hole, retinal tears, and retinal detachment. Partial thickness PVD may lead to macular membranes (macular VCR; mVCR) and/or peripheral membranes (peripheral VCR; pVCR), which may result in macular hole, macular pucker, macular PVR (mPVR) and/or peripheral PVR (pPVR).

The current study adds further support to the usefulness of detection and removal of VCR across the retinal surface as a novel and critical part of a complete vitrectomy to prevent pre-retinal PVR formation posterior to the vitreous base. However, not all surgeons routinely use TA (or at least not as described in this article), and VCR detection and removal can be challenging and time consuming depending on membrane composition, thickness, and adherence to the retina. This may hinder broader acceptance of this hypothesis and technique. In addition, we have noticed that it is still not clear to all surgeons as to what is meant by VCR, which may be mistaken for vitreous remnants at the vitreous base after vitrectomy with incomplete shaving. To avoid this misunderstanding, Fig. 5 shows more clearly where VCR can be located.

Factors that can affect VCR detection include TA concentration,

application method, and number of applications. In our experience, repeated targeted staining with TA is necessary. The contrast between the white TA particles and the underlying retinal and subretinal layers can also be of influence, making VCR more visible over detached retina and VCR visualization in highly myopic eyes much more challenging. In addition, the fibrocellular proliferation stage of the membrane also plays a role. The irregular surface of early-stage VCR, to which the TA crystals adhere, appears to decrease as fibrocellular proliferation increases. Application of trypan blue, preferably under air, could provide better visualization of the membrane in these cases. More effective and easier ways for VCR detection and removal would be helpful, and more research is needed to determine which patients would benefit most from removing VCR, and to what extent VCR should be removed. This

probably depends on the presence of other PVR risk factors.

Most known PVR risk factors are associated with intravitreal dispersion of RPE cells, disruption of the blood-retina barrier, and retinal hypoxia. These factors include a longer duration and larger extent of the retinal detachment, larger size and a greater number of retinal tears, and the presence of vitreous haemorrhage or inflammation (Pastor *et al.* 2002; Wickham *et al.* 2011). The presence of VCR over the retinal surface would be an additional and potentially even more important, modifiable risk factor. Removing VCR from the retinal surface may eliminate profibrotic M2-activated hyalocytes, as well as the scaffold for fibrocellular proliferation, thereby reducing the risk of preretinal PVR formation.

Our additional theory for PVR formation is not only of interest for PVR prevention but also for PVR

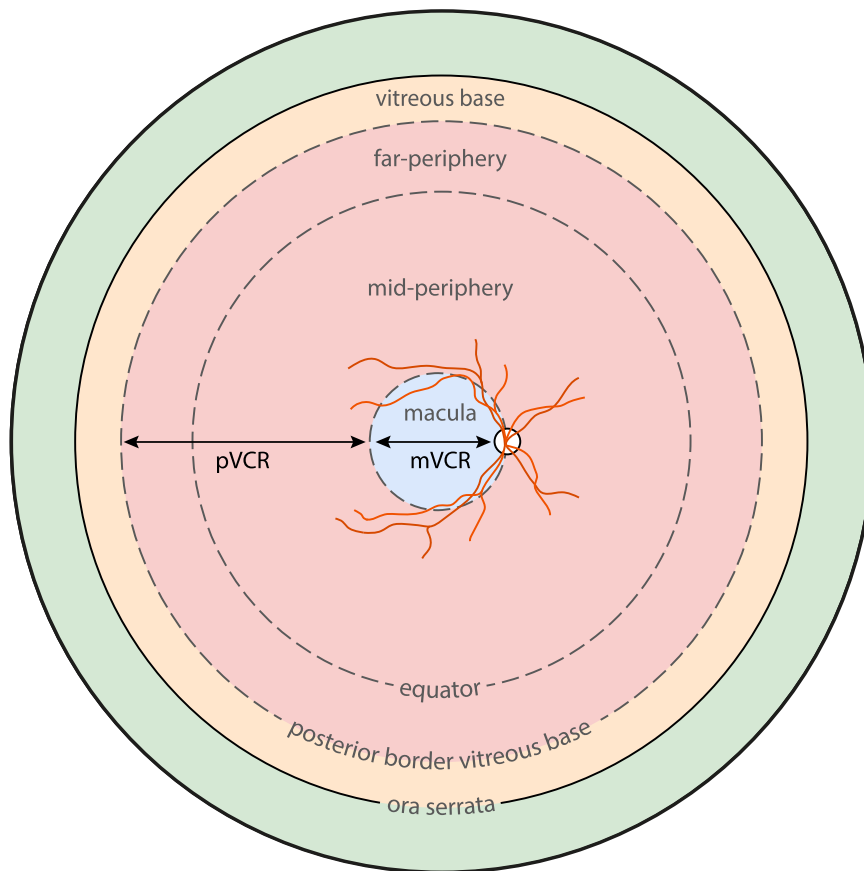


Fig. 5. Schematic representation of the different retinal areas: the macula and the peripheral retina, which can be divided into the mid-periphery (from the anterior border of the macula to the equator), the far-periphery (from the equator to the posterior border of the vitreous base), and the retinal part of the vitreous base. Vitreoschisis-induced vitreous cortex remnants (VCR) can be present over the macula (mVCR) and the peripheral retina posterior to the vitreous base (pVCR), which should not be mistaken for vitreous remnants at the vitreous base after vitrectomy with incomplete shaving.

management. It is important to consider the extent of VCR and the different histopathologic VCR-PVR stages. The presence of VCR may be limited to the macula or focal areas of the peripheral retina. More often, however, VCR covers the entire retinal surface and may be responsible for diffuse retinal stiffness in the absence of focal fibrotic membranes, a clinical picture commonly referred to as PVR B. In long-standing retinal detachments, intraretinal changes with gliosis due to hypoxia may be responsible for retinal stiffness and retinal shortening (Charteris 2020), but in less long-standing detachments, meticulous VCR removal may result in a more mobile retina. Conversely, performing laser photocoagulation or a retinectomy without removing VCR may activate M2 profibrotic hyalocytes and promote fibrocellular proliferation, resulting in more traction. In addition, in the case of a focal fibrotic membrane with a retinal star fold (clinical aspect of PVR C), solely removing the fibrotic part from the centre of the fold, or performing a retinectomy, may flatten the retina, but the remaining surrounding VCR poses a risk for further PVR formation and surgical failure.

We acknowledge that the current dataset is limited by the number of patients included, and the difficulties in obtaining representative samples for histopathology. The possible histopathological absence of stage 1–2 areas in two membranes with stage 3–5 areas may be related to the fact that these fragile parts of the membrane were lost during processing, or perhaps only the fibrous part could be grasped and collected during surgery. This may have resulted in an incomplete overview of the processes underlying PVR formation in these patients. However, the current data do indeed support our theory that originated from clinical observations. It would be difficult to think of a prospective *in vivo* approach that would further investigate this theory other than a change to clinical practice in a large, randomized trial. Such an approach, however, could cause an ethical dilemma for the surgeon responsible for the individual patient. The authors would like to suggest that with the current study, sufficient theoretical support is

provided for the removal of VCR to prevent PVR in surgical practice.

In conclusion, this study and the presented histopathological evidence, support the hypothesis that VCR and hyalocytes play an important role in PVR development. Detection and adequate removal of VCR may improve the success rate of surgery for primary as well as failed retinal detachment. Further research is needed to improve the knowledge of VCR and its role in PVR formation and surgical failure.

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