PROLIFERATIVE VITREORETINOPATHY (PVR) - THE USE OF ADJUVANT THERAPY IN THE PREVENTATIVE TREATMENT OF PVR AND THE STUDY OF CLINICAL AND BIOLOGICAL RISK FACTORS

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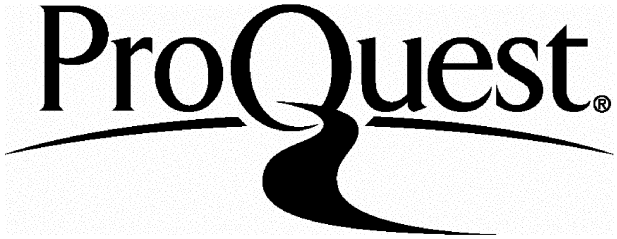

Abstract

Proliferative vitreoretinopathy (PVR) is a major cause of failure of retinal detachment surgery and is thought to complicate 5 to 10% of all detachments. This thesis has investigated the use of adjuvant therapy in the preventative treatment of PVR and the identification of clinical and biological risk factors involved in PVR.

A prospective randomised control study was conducted comparing intravitreal infusion of either 5 Fluorouracil (5-FU) and heparin or placebo in high-risk patients undergoing primary vitrectomy for rhegmatogenous retinal detachment surgery. There were 87 patients in each group. The incidence of postoperative PVR was significantly lower (P= 0.019) in the treatment group. Of the placebo group 26.4% (23/87) and 12.6% (11/87) of the 5-FU/heparin group developed postoperative PVR. In the 5-FU/heparin group the number of patients undergoing more than one operation was 19.5% (17/87) and the number of reoperations due to PVR was 52.9% (9/17). In the placebo group the number of patients undergoing more than one operation was 25.3% (22/87) and the number of reoperations due to PVR was 72.7% (16/22). Patients in the placebo treated group had a significantly worse visual acuity outcome ($p=<0.05$).

This study also investigated the accuracy of a "predictive risk formula" for the development of PVR and to assess its use in a clinical setting. Complete data were available on 214 out of 220 patients. Nine point two percent of the low risk (12/130) and 27.4% (23/84) of the high risk patients developed postoperative PVR ($p<0.0001$).

Further risk factor analysis was also performed on these patients. Multiple regression analysis revealed only the existence of preoperative PVR, higher levels of bFGF and protein to be significant independent risk factors ($p<0.05$) for the development of PVR. Combined multiple logistic regression on clinical and biological risk factors revealed only preoperative PVR to be a significantly independent risk factor ($p=0.01$) for the development of postoperative PVR.

Table of Contents

Title page 1

Abstract 2

Table of Contents 4

List of Figures 13

List of Tables 15

List of Abbreviations 17

Acknowledgements 18

List of Presentations and Publications 19

Chapter 1: Introduction 20

History of retinal detachment 20

Definition 20

Overview 20

Methods of Examination 20

Early period (1704 - 1850) 21

Modern period (1851 -1970) 22

Concepts of Pathogenesis 25

Early period (1853 - 1919) 26

Modern period (1923 - 1950) 28

Treatment 29

Early period (1805 - 1922) 29

Modern period (1923 – 1950) 31

Closure of retinal breaks by extra ocular surgery 32

Closure of retinal breaks by intra ocular surgery 37

Early vitrectomy 37

Modern vitrectomy 38

Closed vitrectomy 38

Open-sky vitrectomy 39

What does history tell us? 39

Proliferative vitreoretinopathy (PVR) 41

Definition 41

Terminology 41

Pathophysiology and clinico-pathological correlation of PVR 45

Cellular constituents of PVR membranes 45

RPE cell component 46

Macrophage and inflammatory cell component 48

Glial cell component 52

Fibroblast component 54

Extracellular matrix 54

Proteins 55

Structural proteins 55

Cell adhesion proteins 56

Proteins with counter-adhesive properties (Matricellular proteins): 58

Thrombospondin 1, Osteonectin and Tenascin

Membrane contraction 59

Immune system involvement in PVR membranes 61

The role of growth factors and cytokines in PVR 61

Transforming growth factor-beta (TGF-ß) 62

Platelet derived growth factor (PDGF) 62

Interleukin-1-beta (IL-1ß) 64

Interlekin-6 (IL-6) 65

Fibroblast growth factors (FGF) 66

Tumour necrosis factor α (TNFα) 67

Interferon gamma (IFN Y) 67

Epidermal growth factor (EGF) 67

Risk Factors for developing PVR 68

Treatment of PVR 71

Closure of all breaks 71

Counteract traction 74

Retinotomy 74

Intraocular tamponade 75

Long acting gases 75

Silicone oils 76

Silicone or gas? 85

Adjuvant therapy for the treatment of PVR 88

Stages of the PVR process 88

Cellular activation 88

Proliferation 89

Extracellular matrix elaboration and remodelling 89

Contraction 90

Classes of adjuvant agents 90

Anti-inflammatory agents 90

Agents that inhibit cellular proliferation 91

Fluoropyrimidines 91

Daunorubicin (daunomycin) 95

Retinoids 98

Immunotoxins 100

Taxol 101

Colchicine 101

X-ray irradiation 102

Other agents 102

Drugs that act on the ECM and cell surface 102

Drugs that interfere with collagen biosynthesis 102

Antimetabolites 103

cis-Hydroxyproline 103

Penicillamine 103

Drugs that effect cell adhesion 103

RGDS 104

Heparin 104

Summary 106

Chapter 2: Justification and Aims 108

Chapter 3: Patients, Materials and Methods 110

Clinical Study- 110

The use of adjuvant 5-FU and heparin in the treatment of patients at high-risk of developing PVR

Preoperative assessment 110

Allocation to clinical risk category 111

Inclusion and Exclusion Criteria 114

Randomisation 114

Sample calculation size 115

Surgical Technique 115

Intraoperative and follow up assessments 115

Outcome Measures 116

Prospective analysis of risk factor formula and assessment of risk factors 116

Preoperative assessment 117

Intraoperative and follow-assessments 117

Collection and storage of vitreous 118

Cytokine and growth factor analysis 118

Enzyme-Linked Immunosorbent Assays (ELISA) 118

Cytokine assays 124

IL-ß 124

IL-6 124

Basic-FGF 124

TGF-ß2 124

Protein assay 125

Data Handling and Statistical Methods 126

Chapter 4: Results 128

Clinical Study –The use of adjuvant 5-FU and heparin in the treatment of patients at high risk of PVR 128

Patient profile 128

Clinical outcome 130

Postoperative PVR 130

Relationship between preoperative PVR and postoperative PVR 130

Relationship between position of break and postoperative PVR 132

Reoperations 132

Relationship between preoperative PVR and final visual acuity 137

Relationship between postoperative PVR and final visual acuity 138

Complications 138

Summary of clinical trial 139

Assessment of clinical risk factor formula 140

Patient profiles 140

Development of postoperative PVR 142

Relationship between preoperative PVR and surgical outcome 142

Relationship between preoperative PVR and postoperative PVR 144

Relationship between postoperative PVR 146

and further reattachment surgery

Relationship between preoperative PVR and final visual acuity 148

Relationship between postoperative PVR and final visual acuity 150

Relationship between reoperations and final visual acuity 151

Summary of results 151

Vitreal cytokine and protein levels 153

Relationship between cytokine levels and preoperative PVR 153

Relationship between cytokine levels and risk group 155

Relationship between cytokine levels and postoperative PVR 157

Relationship between cytokine levels and postoperative PVR

compared by treatment group 159

and postoperative PVR compared by treatment group.

Summary of results 159

Risk factor analysis 160

Clinical risk factors 160

Biological risk factors 162

Combined clinical and biological risk factors 164

"Fitting" the discriminant rule into the data set 165

Using the discriminant rule for clinical risk factors 165

Using the discriminant rule for protein 167

Using the discriminant rule for clinical risk factors and protein combined 169

Chapter 5: Discussion 171

PVR is a wound healing response 171

Using clinical risk factors to define high-risk patients 172

The use of 5-FU and heparin in the preventative treatment of patients at risk of developing PVR 174

Choice of Agents 176

Low Molecular weight heparin 176

5-Fluorouracil (5-FU) 177

Daunomycin 180

Adjuvant therapy with 5-FU and LMWH 181

Validity of clinical risk formula 185

Clinical risk factors for the development of PVR 186

Univariate analysis 187

Multiple logistic regression analysis 192

Role of Cytokines and Growth factors in PVR 194

Cytokine and protein levels as risk factors for developing PVR 198

Cytokine and protein levels as risk factors for developing PVR 199

Chapter 6: Conclusion 201

References 203

Appendix 227

List of Figures

Figure 1.1	Use of The Retina Society Classification System.	43
Figure 1.2	Blood born macrophages leaving the circulation and entering PVR tissue.	50
Figure 1.3	T cell reaction in PVR tissue.	51
Figure 1.4	Intense glial proliferation in PVR tissue.	53
Figure 1.5	Complications caused by silicone oil tamponade	80
Figure 1.6	The inflammatory and destructive damage caused by silicone oil tamponade.	81
Figure 2.1	Risk equation used and clinical examples.	112
Figure 2.2	A diagrammatic representation of enzyme - linked immunosorbent assay for cytokines.	120
Figure 2.3	Coloured end product of the enzyme – linked immunosorbent assays.	121
Figure 2.4	Amplification reaction in the high sensitivity (Quantikine ™ HS) ELISA kits.	123
Figure 3.1	Pre and postoperative visual acuity in the treated group.	135
Figure 3.2	Pre and postoperative visual acuity in the placebo treated group.	136
Figure 3.3	Relationship between preoperative PVR and surgical outcome.	143
Figure 3.4	Relationship between preoperative PVR and postoperative PVR.	145
Figure 3.5	Relationship between postoperative PVR and surgical outcome.	147
Figure 3.6	Pre- and postoperative visual acuity.	148

Figure 3.7 Levels of TGF-β2, b-FGF, IL-1β, IL-6 and protein in the vitreous of patients who did and did not have preoperative PVR. 154

Figure 3.8 Levels of TGF-β2, b-FGF, IL-1β, IL-6 and protein in the vitreous of patients compared by treatment group. 156

Figure 3.9 Cytokine levels and postoperative PVR. 158

Figure 4.1 Graphical representation of the wound healing response and treatment strategy. 175

List of Tables

Table 1.1	Classification of proliferative vitreoretinopathy by grade (Machemer et al., 1991).	44
Table 1.2	Grade C PVR (Machemer *et al.*, 1991).	44
Table 2.1	Clinical risk factors studied.	113
Table 3.1	Preoperative patient details.	129
Table 3.2	Relationship between pre and postoperative PVR in treatment group.	131
Table 3.3	Relationship between pre and postoperative PVR in placebo group.	131
Table 3.4	Relationship between position of break and postoperative PVR.	132
Table 3.5	Vitreoretinal reoperations.	133
Table 3.6	Final change in visual acuity between the two treatment groups.	134
Table 3.7	Preoperative PVR and final visual acuity in the treatment group.	137
Table 3.8	Preoperative PVR and final visual acuity in the placebo group.	137
Table 3.9	Postoperative PVR and final visual acuity.	138
Table 3.10	Preoperative patient details.	141
Table 3.11	Relationship between preoperative PVR and surgical outcome.	142
Table 3.12	Relationship between postoperative PVR and preoperative PVR.	144
Table 3.13	Relationship between reoperation and postoperative PVR.	146
Table 3.14	Relationship between final visual acuity and preoperative PVR.	149
Table 3.15	Relationship between final visual acuity and postoperative PVR.	150

Table 3.16	Relationship between reoperations and final visual acuity.	151
Table 3.17	Cytokine levels and preoperative PVR.	153
Table 3.18	Cytokine levels and treatment group.	155
Table 3.19	Cytokine levels and postoperative PVR.	157
Table 3.20	Cytokine levels in patients with postoperative PVR and treatment group.	159
Table 3.21	Estimated coefficients and odds ratios for the risk factors.	161
Table 3.22	Estimated coefficients and odds ratios for risk factors.	164
Table 3.23	Predictive power using the training set.	165
Table 3.24	Predictive power using the validation set.	166
Table 3.25	Predictive power using the training set.	167
Table 3.26	Predictive power using the validation set.	168
Table 3.27	Predictive power using the training set.	169
Table 3.28	Predictive power using the validation set.	169

List of Abbreviations

a-FGF	acidic fibroblast growth factor
ANOVA	analysis of variance
b-FGF	basic fibroblast growth factor
cDNA	complementary DNA
CF	counting fingers (visual acuity)
CI	confidence interval
Da	Dalton
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
FDUR	fluorodeoxyuridine
FGF	fibroblast growth factor
5-FU	5-fluorouracil
FUR	fluorouridine
HM	hand movement (visual acuity)
IL	interleukin
kDa	kilo-Dalton
mRNA	messenger ribonucleic acid
MMP	matrix metalloproteinase
NPL	no perception of light (visual acuity)
PDGF	platelet-derived growth factor
PL	perception of light (visual acuity)
PVR	proliferative vitreoretinopathy
RNA	ribonucleic acid
SE	standard error
TGF	transforming growth factor
TIMP	tissue inhibitor of matrix metalloproteinases
vol	volume
wt	weight

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Chapter 1

Introduction

History of retinal detachment

Definition

Rhegmatogenous retinal detachment is a condition where a full thickness break occurs in the neurosensory retina resulting in its separation from the retinal pigment epithelium.

Overview

Great inventions are seldom the result of a totally new concept. They are nearly always based on previous ideas that did not mature to their full potential. This was true for ophthalmoscopy and for the discovery of the treatment of retinal detachment. The early period was characterised by "fishing expeditions" in various directions without real knowledge of the treatment methods used. Once the fundamental principals were established progress was finally made. It is interesting to note that the early treatments already contained the embryonic ideas that were developed in the modern period and that were not initially perused to logical conclusions. To portray this evolution of treatment this chapter has been divided into methods of fundal examination and understanding the pathogenesis of retinal detachment.

Methods of Examination

The evolution of methods of examination can be divided into an early (1704 – 1850) and a modern (1851 – 1970) period.

Early period (1704 – 1850)

Detailed examination of the fundus was not possible until the invention of the ophthalmoloscope. Despite this disadvantage a number of pathological observations were still made about retinal detachments during this period. In 1691, the first anatomic description of a retinal detachment was recorded by Maitre-Jan. It was not until 1707 that his work was first published in the first modern textbook on ophthalmology. His work originated from observing a dislocated lens, total retinal detachment, and retraction of the vitreous body in the eye of a dead cow. He followed these observations by studying the incidence of retinal detachment following contusion or perforation of the globe in patients examined at autopsy (Schepens, 1983).

In 1722, De Saint Yves described clinical symptoms of retinal detachment that he called a "separation of the retina from the choroid." He reported "kind of shadows" in the visual field that corresponded to the detached area. However, he did not make the distinction between the visual effects of retinal detachment and those of dense vitreous opacities (de St Yves, 1741) . In 1765, Morgagni described a retinal detachment in a patient with an intraocular tumour (Schepens, 1983) . Many other authors went on to describe their observations in eyes with retinal detachment. Wardrop in his book, *Essays on the Morbid Anatomy of the eye*, published pathologic observations of retinal detachment in human eyes (Schepens, 1983) . At the time all the descriptions made on detachments were based on observing the pupillary reflex and sometimes the white glossy detachment that could be seen through the dilated pupil (Duke-Elder and Dobree, 1967).

Until the development of the ophthalmoscope very little progress was made. Méry in 1704 accidentally saw details of a cat fundus that he was drowning to study it's pupillary reaction. He failed to provide the correct explanation for what he observed. De la Hire correctly explained Méry's observation five years later. He explained that when the cat was placed under water the refractive power of the cornea was neutralised. In addition, he drew a diagram that showed the optical effect of using what in fact amounted to a contact lens with a flat anterior surface (Schepens, 1983) . Unfortunately advantage was not taken of these early observations.

Modern period (1851 –1970)

The modern period is defined by the development of the ophthalmoscope. Charles Babbage, the British mathematician, actually first invented the ophthalmoscope in 1847, but the surgeon friend he gave it to failed to use it, and it was not reported until 1854. Hermann Helmholtz therefore has been credited with its development. He described the principals of ophthalmoscopy in his famous 40-page monograph published in 1851. Helmholtz's discovery was not greeted with enthusiasm. One famous ophthalmologist warned against the dangers of using his instrument "as it shone a naked light into the eye" and another "felt that the mirror was useful for ailing ophthalmologists with reduced sight only and as he possessed good eyesight he was having nothing to do with it" (Michels *et al.*, 1990) . Helmholtz's instrument was notoriously difficult to use. In 1852 Reute introduced the first indirect ophthalmoscope. As Reute's instrument was easy to use and allowed improved visualisation it was the first clinically useful ophthalmoscope. Coccius and Loring further refined these early monocular direct and indirect ophthalmoscopes. In 1861Giraud-Teulon devised the first direct ophthalmoscope and later incorporated an

electric light source in the instrument. This ophthalmoscope was a hand held device and required the examiner to hold a convex lens in the other hand. This was a time of great invention; between 1851to1880 no less than 78 different ophthalmoscopes were described. In 1883, Adams described a monocular indirect ophthalmoscope attached to a headband with illumination using a separate light source (Michels *et al.*, 1990). In 1911, Gullstrand after carefully studying the parameters affecting ophthalmoscopy and basing his work on the mathematics of the optics of the eye, developed reflex-free ophthalmoscopy. This instrument could be used monocularly or binocularly. It provided a clear image of the fundus with excellent magnification and illumination (Schepens, 1983). The principals of this instrument are used in modern fundus cameras.

In 1945 Schepens described the first effective binocular indirect headband ophthalmoscope and was specifically designed to aid examination of retinal detachments. This instrument had two components: a headband to hold the stereoscopic viewing system and a powerful light source fixed on a flexible arm. In addition, the observer held a condensing lens in front of the patient's eye to obtain an aerial image of the fundus. This left the other hand free to perform additional procedures. In 1951, Schepens modified his instrument by incorporating the viewing system and the light source into the headband. He also described scleral indentation using a scleral depressor mounted on a thimble. Using this instrument it was much easier to examine the retinal periphery. Combined with the binocular indirect ophthalmoscope, Schepens technique of scleral depression became the method of choice for examination and treatment of vitreoretinal pathology. Using his ophthalmoscope he reported a series of 400 consecutive cases of

idiopathic retinal detachment and found one or more breaks in 99 percent of the cases (Schepens, 1947).

Pomerantzeff in 1968 optimised the Schepens ophthalmoscope by applying Gullstrand's principal. The Schepens-Pomerantzeff ophthalmoscope is most useful for seeing through a small pupil or through media opacities and for optimising the view and stereopsis when examining the periphery of the fundus (Amoils and Kaufmann, 1972).

Both direct and indirect ophthalmoscopes have had their proponents through the years. Gonin, the father of modern retinal detachment surgery, used monocular indirect ophthalmoscopy. Although electric hand-held ophthalmoscopes were popular in the 1930s and are still widely used at present, binocular indirect ophthalmoscopy has proved to be the instrument of choice. It is a credit to Schepens that none of the current models of binocular indirect ophthalmoscopes are fundamentally different from the original Schepens and the Schepens-Pomerantzeff models.

Slit-lamp microscopy evolved gradually and with the use of various contact and non contact lenses has increased the ability to study vitreous and retinal pathology. Early models of the modern slit-lamp were based on instruments devised by Czapski (binocular corneal microscope, 1899) and Gullstrand (slit-beam illumination system, 1911). It is worth noting that Gullstrand, in the same year, won a Nobel Prize for his work related to the optics of the eye.

Koeppe building on Gullstrand's work described the use of a flat contact lens with a dioptric power of minus 69.4 diopter and a diameter of 17 mm to examine the posterior sections of the eye. Although using this lens was not easy accurate detailed examination of the posterior pole was possible. In 1946, Hruby described his concave, minus 55

diopter, non-contact lens for use with the slit lamp. Goldmann in 1948 introduced the three-mirror contact lens that provides an excellent detailed view of the periphery and macula. El Bayadi in 1953 introduced the planoconvex lens of plus 60 D with the slit-lamp microscope. All three types of lenses are in use today (Michels *et al.*, 1990).

The landmark achievements in the development of methods of examination in the modern period can be summarised as follows:

1851 – Helmholtz; Monocular ophthalmoscope

1861- Giraud Teulon; Binocular ophthalmoscope

1900 – Trantas; Scleral depression

1911 - Gullstrand; Reflex free ophthalmoscopy

1912 - Gullstrand; Development of the slit lamp

1918 – Koeppe; Slit lamp microscopy of the fundus with a flat contact lens

1942 – Hruby; Slit lamp microscopy of the fundus with a concave precorneal lens

Concepts of Pathogenesis

Before the advent of ophthalmoscopy progress could not be achieved on the pathogenesis of retinal detachment. As the most defining moment in the pathogenesis of retinal detachment is Gonin's discovery of the importance of the retinal break in 1919. We can divide the concepts of pathogenesis into an early (1853 – 1919) and a modern (1920 – 1950) post Gonin period.

Early period (1853 – 1919)

Following the description of ophthalmoscopy numerous theories began to emerge to account for the occurrence of retinal detachment. This early period that lasted 66 years ended in 1919 with Gonin recognising the all-important role of the retinal break. Coccius in 1853 was the first to describe retinal breaks but the significance of this finding was not realised.

Many theories were put forward during this time. Although there was no agreement on which theory was correct, most ophthalmologists at the time classified retinal detachment into idiopathic (spontaneous or serous) and secondary types (neoplasm or inflammatory) (Duke-Elder and Dobree, 1967) . Arlt proposed that the main cause was choroidal exudation. Von Graefe felt that retinal detachment occurred secondary to either choroidal effusions or haemorrhage. Later, he observed the higher incidence of detachment in myopes and suggested that the distension of the globe stretched the globe to the point that it separated from the choroid. He felt that retinal breaks were part of the healing process and not a cause of retinal detachment.

The possible role played by the vitreous was also recognised. Stellwag introduced the theory of " hypotony " and suggested that the vitreous normally held the retina in place and that a lowering of the pressure in the vitreous cavity caused the retina to fall off the pigment epithelium. Muller observed traction bands in the vitreous cavity that may pull the retina. Leber also postulated that the vitreous exerted tractional forces on the retina. Ivanoff in 1869 was the first observer to notice that a vitreous detachment preceded retinal detachment and was probably a precipitating factor.

During this period it was accepted that retinal breaks were an outcome and not the cause of retinal detachment. Despite this belief De Wecker and de Jaeger in 1870 thought that retinal breaks were important in causing retinal detachment. They postulated that the cause of detachment in eyes with a posterior staphyloma was a hypersecretion of fluid between the vitreous gel and retina. They also postulated that vitreous traction was responsible for detachments in eyes following trauma. However, their theories did not extend to spontaneous detachments. Leber in 1882 rejected the theories de Wecker and de Jaeger. He felt that "alterations of the vitreous that were so tenuous as to be clinically undetectable at the time" were of fundamental importance. Also, if retinal breaks occurred they caused an increase in the extent of detachment but were not the cause of retinal detachment. De Wecker in defence, insisted on the frequent nature of retinal breaks in retinal detachments even when the observer was unable to detect them clinically.

Leber's original hypothesis was heavily criticised due to a lack of ophthalmoscopic evidence. In 1908 after studying microscopic sections of eyes with long-standing retinal detachments he postulated that retinal breaks were caused by extensive preretinal organisation. Interestingly, this was an early description of proliferative vitreoretinopathy. Dufour and Gonin supported Leber's theory, suggesting that retinal tears were due to localised vitreoretinal adhesions. Interestingly in 1916 Leber reported that 75% of retinal detachments had a retinal break. In 1924 Lister pointed out that if there was a retinal break in a detachment spontaneous resolution did not occur (Pomerantzeff, 1968) . Unfortunately, the importance of retinal breaks was still not appreciated.

Modern period (1923 - 1950)

It was Gonin in 1920 that stressed the invariable occurrence of retinal breaks and the pathogenesis of retinal detachment. Gonin's work resulted from pathological studies as oppose to clinical observations. Gonin's work was not recognised at the time. Despite Gonin presenting successful results of retinal reattachment to the French Ophthalmological Society in 1920 progress on treatment was hampered due to very few ophthalmologists accepting his theory. This was particularly true in English speaking countries where the direct ophthalmoscope was still used exclusively for fundus examination (Duke-Elder and Dobree, 1967) .

As methods of retinal examination improved both Vogt in 1936 and Hruby in 1950 gave further support to Gonin's theories. Also previous hypothesis such as vitreous traction, retinal distension and preretinal organisation were seen in a more informative light. This gradual understanding of the pathogenesis of retinal detachment resulted in dramatic changes in the treatment of retinal detachments.

The landmark achievements of the developments in concepts of pathogenesis may be summarised as follows:

1853 - Coccius; discovery of retinal breaks

1870 - de Wecker; suspicion that retinal breaks exacerbate retinal detachments

1882 - Leber; retinal breaks caused isolated invisible areas of vitreoretinal traction

1908 - Leber; role of preretinal organisation

1920 - Gonin; first clear account of role played by the vitreous and retinal break in detachments

Treatment

As our understanding of the theories on the pathogenesis of retinal detachment developed so did the success of treatment. As is evident in the review of treatments in the early period the success of surgery was exceptionally low. It was not until Gonin's theories were accepted that the outcome of surgery improved.

Early period (1805 - 1922)

In 1805 Ware described the first recorded treatment of retinal detachment when he perforated the sclera to allow the "effusion of fluid between the choroid coat and retina". This resulted in the immediate escape of a "yellow coloured fluid." He was describing drainage of subretinal fluid and subsequent treatments that followed also focused on drainage of fluid. As previously mentioned von Graefe thought that retinal detachments were caused by choroidal exudation pushing the retina forward and compressing the vitreous gel into a retracted mass. Based on this theory he punctured the sclera and retina, effectively creating a retinal break, in order to equalise the pressure between the subretinal and vitreous space (Duke-Elder and Dobree, 1967) . Other authors described using gold wire to create permanent drainage. Grizou described threading a gold thread through the sclera and choroid bringing the needle out a short distance away thereby creating a permanent tract for drainage of the fluid (Michels *et al.*, 1990) . Galezowski transfixed the conjunctiva, sclera and choroid with a piece of gold wire. However, due to the high infection rates this procedure was abandoned.

Unsurprisingly, these methods were spectacularly unsuccessful and so the ophthalmologists of the day resorted to more conservative treatments of prolonged bed rest. Stellwag and later Donders, who felt that eyes with detachments should be kept in

state of immobility, originally proposed this treatment. They achieved this by sandwiching the patient's head between sandbags. They positioned the patient so that the subretinal fluid would be in the most dependent position. This was one of the first descriptions of postoperative positioning. Later, bandaging and compressing the eye combined with many weeks of bed rest became popular. Sometimes a salt free diet was included as part of the therapy to help the resorption of fluid. To improve the success of surgery topical mercury ointment and pilocarpine injections were tried. Others, who felt that there was a relationship between retinal detachment and glaucoma, recommended iridectomy as either a treatment or even a prophylactic measure in cases of retinal detachment (Duke-Elder and Dobree, 1967).

In order to explain the poor outcome of surgery De Wecker gave renewed support to Leber's theory, i.e. that detachments were caused by adhesions of the retracting vitreous to the retina. Based on these assumption treatments were than directed to cause an adhesive chorioretinitis or push the retina against the choroid. Various methods were tried to achieve chorioretinal adhesion such as galvanocautery, electrolysis, sutures in the retina, and injection of tincture of iodine into the vitreous and into the subretinal space. Unfortunately, the adhesive chorioretinitis was not aimed at closing the retinal breaks.

Treatments based on pathogenic theories of globe distension and vitreous traction were developed. Aliamo in 1893 was the first to describe excision of a full thickness strip of sclera followed by Muller in 1903. Blaskowics in 1911 described lamellar resection. These resection operations were recommended exclusively for the treatment of detachments in myopes (Pomerantzeff, 1968).

However, as no effort was made to close the retinal break the success of reattachment surgery was very poor. In 1911, results from a large survey of American ophthalmologists carried out by Vail in Cincinnati reported the success rate to be less than one in one thousand. Of 281 replies, 90% of ophthalmologists admitted that they had never cured a case of retinal detachment. The final recommendation was that "vigorous treatment should not be attempted until a more favourable outcome could be achieved". An extensive review conducted by the Ophthalmological Society of the United Kingdom in 1916 revealed only 85 cases of successful long- term reattachment lasting longer than 6 months. This led a prominent American ophthalmologist at the time to quote, when asked how he managed retinal detachments, to "send them to the most disagreeable of my colleagues". Improvements in outcome were unfortunately slow. Sir William Lister in an address to the British Medical Association in 1927 concluded,

" treatment should not be urged except as a last clutch at a straw of hope " (Lister, 1927) .

The notable developments during this early period were as follows:

1805 - Ware; release of subretinal fluid

1882 -Weber; injection into the vitreous

1893 - de Wecker; chorioretinal adhesions by galvanocautery

1895 - Deutschmann; injection of animal vitreous and sectioning of vitreous bands

1911 - Blaskowics; lamellar scleral resection

Modern period (1923 – 1950)

After Gonin in 1920 emphasised the relationship between vitreous detachment and traction resulting in retinal tears treatment was aimed at closing the retinal break. It is interesting to note that Gonin's summary of the aim of retinal reattachment surgery

stands true even today: " Retinal reattachment to be durable requires that the traction exerted on the retina by the vitreous be eliminated or be counterbalanced by an appropriate chorioretinal adhesion. The possibility of such a reattachment is conceivable only after closure of the retinal break(s) " (Gonin, 1930).

Based on his theory two types of operation were devised; an extra- ocular approach to close the retinal break and an intra-ocular approach to eliminate or neutralise vitreous traction on the retina.

Closure of retinal breaks by extra ocular surgery

Inspired by Leber, Gonin proposed transscleral thermocauterisation to close retinal breaks referred to as *Gonin's ignipuncture technique.* His treatment protocol started pre operatively. He advocated careful preoperative examination using monocular indirect ophthalmoscopy to identify all breaks. Intraoperatively he used scleral indentation to confirm break location. Once all the breaks had been identified a radial sclerostomy about 2 to 3mm long was made at the site of the break. The subretinal space was entered and subretinal fluid drained. A thermocautery was inserted into the sclerostomy and left for 2 to 3 seconds. The aim was to transfix the retinal break after removal of the subretinal fluid. The patient was than rested for a number of days. This technique dramatically improved the success of retinal reattachment surgery from less than 6% to more than 50%. It should be noted that in using cautery he deliberately perforated the sclera and as a result drained sub retinal fluid. In addition, the documentation and recording of retinal breaks used by Gonin is still in use today in preoperative fundus charts (Amsler and Dubois initially devised this chart in 1928).

Various modifications on his technique emerged but the basic principals remained the same. In 1930 Heim and Weve independently introduced diathermy, which was less aggressive and produced more chorioretinal reaction than cautery. The diathermy was applied either on the scleral surface or through holes trephined in the sclera (Michels *et al.*, 1990). In 1931 Guist trephined the sclera around the break and cauterised the choroid with a caustic potash stick, while Arruga used sodium chloride as the chemical agent. Lindner injected potassium hydroxide into the supra-choroidal space after undermining the sclera with a spatula.

As the success rate improved, techniques that had been used in the "*Early Period*" were now revived and used to treat localised breaks and areas of chorioretinal degeneration. In 1936 Vogt modified electrolysis and called it catholysis. Gonin had observed that individual treatment of multiple breaks and areas of degeneration was difficult and could be ineffective. With this observation, both Gonin and Lindner separately described building a barrage of chorioretinal adhesion between affected and non-effected retina (Schepens, 1983). The bases of prophylactic laser used today.

Diathermy was most widely used method of retinopexy both in Europe and America until the mid-1950s. However, diathermy produced unacceptable scleral necrosis, and if the thermocoagulation was too intense, led to a severe choroidal reaction and retinal shrinkage. Scleral dissection techniques to perform safer diathermy were difficult and time consuming. Therefore, cryotherapy and light photocoagulation were developed.

In 1934 Biette introduced cryotherapy using carbon dioxide snow, but this was not popular and quickly forgotten. Cooper revived this technique by introducing liquid nitrogen and Lincoff than reported its use clinically in the treatment of retinal detachment

(Lincoff *et al.*, 1964) . To increase surgical control a silver envelop was used to contain the freezing agent. The next significant breakthrough was in 1968 when Amoils (Amoils, 1968) developed a carbon dioxide cryoprobe in which the temperature could be varied between -40^0 to -70^0. Cryotherapy is still in use today. Although, uncertainty still persists over whether cryotherapy destroys more retinal pigment epithelium than either diathermy or photocoagulation and leads to more cases of proliferative vitreoretinopathy (Campochiaro *et al.*, 1985) .

Maggiore first suggested the use of light energy for retinopexy (Michels *et al.*, 1990) . This was followed by Meyer-Schwickerath in 1949 who initially using solar energy, but later developed the carbon arc photocoagulator. He went on to develop the xenon arc in 1966, that was used until the late 1970s when it was replaced by laser (Michels *et al.*, 1990) . Cambell in 1963 introduced the ruby laser but it was found to be clinically unreliable because the exposure time was short and fixed (Campbell *et al.*, 1963) . L'Esperance two to three years later developed the argon laser, which was initially proposed by Gordon (L'Esperance, 1968) . L'Esperance also helped develop other wavelength lasers, including krypton and dye lasers (Michels *et al.*, 1990) . Therefore, a whole range of lasers with a variety of wavelengths and delivery systems became available.

Despite closing retinal breaks with chorioretinal adhesion and releasing subretinal fluid there still remained a high failure rate. Therefore, it was felt that other pathological mechanisms must be present to account for this. To reduce the effect of vitreous traction techniques to reduce the size of the globe were revisited. Lindner and Hildesheimer used full thickness scleral resection in addition to treating retinal breaks. Pischel and Miller in

1939 described a similar technique in the United States (Duke-Elder and Dobree, 1967). In 1951 Sharpland and two other surgeons reintroduced Blaskowics' lamellar resection (Sharpland, 1951). The first technique to treat PVR was suggested by Weve in 1949 in which he described a full thickness scleral infolding technique to treat star-shaped retinal folds. Everett wrote descriptions of a scleral out folding technique. Lemoine and co-workers described partial thickness scleral imbrication. The aim of these techniques was to shorten the sclera thereby resulting in an inward buckle. These techniques had little effect on reducing vitreoretinal traction (Duke-Elder and Dobree, 1967; Michels *et al.*, 1990).

Other methods involved injecting substances into the subchoroidal space to temporally reduce ocular volume. Strampelli injected subchoroidal plasma in1933 and Smith injected air under the choroid in 1952. This was the first description of using air as an agent. Unfortunately, as procedures to reduce ocular volume multiplied less importance was spent in closing the retinal breaks and success of surgery declined (Schepens, 1983). Two factors increased the success of surgery in the 1950s: a revival of binocular ophthalmoscopy with scleral depression and surgical procedures that emphasised the importance of closing retinal breaks.

The use of scleral buckling in the area of the retinal break was probably first described by Jess in 1937. He temporarily placed a gauze pad on the sclera overlying the retinal break. It is Custodis who has the acclaim of describing the external buckling technique in 1949 for the all-important reason for supporting the retinal break (Lincoff *et al.*, 1965). Custodis used polyviol, a mixture of polyvinyl alcohol and Congo red, as the buckling material. He treated breaks with diathermy but advised against drainage as this may lead

to radial folds interfering with reattachment. Custodis'method was a major step forward in treating retinal detachments as the buckle closed the break and also reduced vitreoretinal traction.

Schepens and colleagues coined the term *scleral buckling* and used polyethylene implants either as segmental (Schepens, 1956) or encircling buckles (Schepens *et al.*, 1957) . Arruga used polyamide thread to encircle the globe at the equator but this occasionally cut through the sclera. McDonald suggested the use of silicone rubber. Silicone rubber had several advantages over polyethylene (Michels *et al.*, 1990) . It was a softer material and could be molded into a variety of shapes and sizes. It was also less irritant to the surrounding tissue and resulted in a lower infection rate (Schepens *et al.*, 1960) . Schepens later abandoned polyethylene buckles in favor of silicone rubber for buckling after lamellar dissection. Lincoff and co-workers introduced the silicone sponge as an episcleral implant in 1960 (Lincoff *et al.*, 1965) . They also designed an improved scleral needle, used cryotherapy rather than diathermy and recommended a non-drainage procedure. These modifications contributed greatly to the advancement of retinal detachment surgery and the elements are still in use today.

Dynamic implants whose buckling characteristics that could be manipulated, intra and post operatively, were tried including an inflatable silicone rubber implant that was sutured to the sclera. A similar device was a silicone balloon that was inserted in the episclera and removed as soon as chorioretinal adhesion could be achieved. Finally, Gelatin was used as an absorbable implant, inserted under scleral flaps, and was absorbed within 6 months (Schepens, 1983) . The use of scleral buckling resulted in two major

improvements; the success of surgery in unfavorable cases was improved and the need for immobilisation was eliminated.

Closure of retinal breaks by intra ocular surgery

After Gonin, it has been accepted that two main causes of retinal reattachment failure are traction on the retina by a degenerate or partly organised vitreous body and the proliferation of preretinal and subretinal membranes. As surgical techniques advanced the outcome of difficult cases improved.

Early vitrectomy

Vitreous surgery is not new, in 1863 Von Graefe attempted to cut a dense posterior vitreous membrane by inserting a cutting needle into the vitreous cavity through the pars plana ciliaris (Michels *et al.*, 1990) . However, systematic and safe vitreous surgery had to wait until the introduction of indirect ophthalmoscopy and better instrumentation.

The first of these instruments were forceps or scissors that fitted in to a needle 1.4 mm in diameter. Visualisation was either by indirect ophthalmoscopy or through a microscope with axial illumination and a flat contact lens.

At this time removal of the vitreous was considered to be dangerous and it was not until cataract surgeons routinely excised prolapsed vitreous that this view changed. Kasner in 1962 treated a ruptured globe with vitreous prolapse by drawing the vitreous out with a sponge and cutting it with scissors. A technique still practiced by cataract surgeons today. He followed this by the planned removal of vitreous in a patient with amyloidosis in 1966. He performed this technique by enlarging the corneal section, removing the crystalline lens, grasping the vitreous with a pair of forceps and cutting it with a pair of

scissors. Other surgeons also demonstrated that removal of vitreous is well tolerated by the eye by repeating this technique (Machemer *et al.*, 1972).

Modern vitrectomy

The above findings and the improvements in surgical instrumentation led to the development of modern vitrectomy. Two basic types of modern vitrectomy were developed: closed technique and open sky vitrectomy.

Closed vitrectomy

Machemer is without doubt the pioneer of modern vitreous surgery. Machemer described a technique involving removal of the vitreous through a posterior approach, leaving the anterior segment intact and thereby avoiding the complications of a large corneal wound (Machemer, 1972). Machemer and associates also devised a vitreous infusion suction cutter (VISC) and were the first to describe its use in humans. The instrument was introduced through the pars plana and surgery was performed using a binocular-operating microscope. Endoillumination was provided axially by the incorporation of a fibre optic sleeve around the VISC. Machemer using his new machine reported successful results on 150 cases that at the time were deemed inoperable.

Improvements soon began to appear to Machemer's system. Machemer had demonstrated that lateral illumination with fiberoptics was more useful than axial light (Machemer, 1974). O'Malley and Heintz (O'Malley and Heintz, 1972) used a third port to provide infusion of fluid thereby maintaining a constant intra ocular pressure. The instruments were of the same diameter allowing them to be freely interchangeable. The three- port set-up greatly improved the surgeon's ability to perform complicated vitreous surgery in a controlled environment and has become the standard technique used today.

Further progress in the management of severe or previously untreatable retinal detachment were made with improvements in instrumentation such as the operating microscope, various types of forceps and scissors, endodiathermy and cauterisation, endolaser and contact lenses.

Open-sky vitrectomy

In this technique a large corneal incision is made and the crystalline lens removed to gain access to the posterior chamber. This method was first described in 1960 (Schepens, 1981) . Kasner et al was the first to publish a successful series using this method. Schepen's and co-workers made further improvements (Schepens, 1981) . Two methods are described both of which use an operating microscope with long focal length. In the first technique a 300-degree corneal section is made and the cornea bathed in hyaluronic acid and tissue culture solution. The second technique uses a 170-degree pars plana incision that affords better protection to the cornea. Two iridotomies are fashioned and an intracapsular cataract extraction performed. The vitreous gel is removed with a vitrectomy instrument. Any scar tissue and pre retinal membranes are dissected with intraocular scissors, forceps or picks.

The main indications for open-sky vitrectomy are for cases with predominantly anterior pathology, for the removal of foreign bodies or in cases of retinopathy of prematurity. Open sky vitrectomies are very rarely performed in this country.

What does history tell us?

From the above section it is evident that treatment of retinal detachment was only possible after the invention of the ophthalmoscope in 1851. Ophthalmoscopy was probably discovered in 1847 but clinicians at the time felt that it would not assist them to

improve the efficacy of their treatment. Similarly, binocular ophthalmoscopy was described a century before it was practised and scleral indentation fifty years after it was first used.

Our understanding of pathogenesis was also hampered by scepticism. In 1882, Leber could not convince his colleagues that retinal breaks and vitreoretinal traction were the key factors involved and it was 40 years later that Gonin offered indisputable evidence to support this. Despite this, it still took another 30 yeas for the majority of ophthalmologists to accept that closure of retinal breaks was the key to successful retinal reattachment surgery. This illustrates the slowness of clinicians in realising the importance of new findings from researchers.

Proliferative vitreoretinopathy (PVR)

Definition

Proliferative vitreoretinopathy (PVR) is defined as the growth and contraction of membranes within the vitreous cavity and both surfaces of the retina following rhegmatogenous retinal detachment. These membranes can exert traction and reopen previously closed retinal breaks, create new breaks, and distort or obscure the macula. PVR is the primary cause of final failure of retinal reattachment surgery (The Retina Society Terminology Committee, 1983).

Terminology

The definitions used to describe the development of membranes on both surfaces of the retina have changed over time depending on the observed underlying pathology.

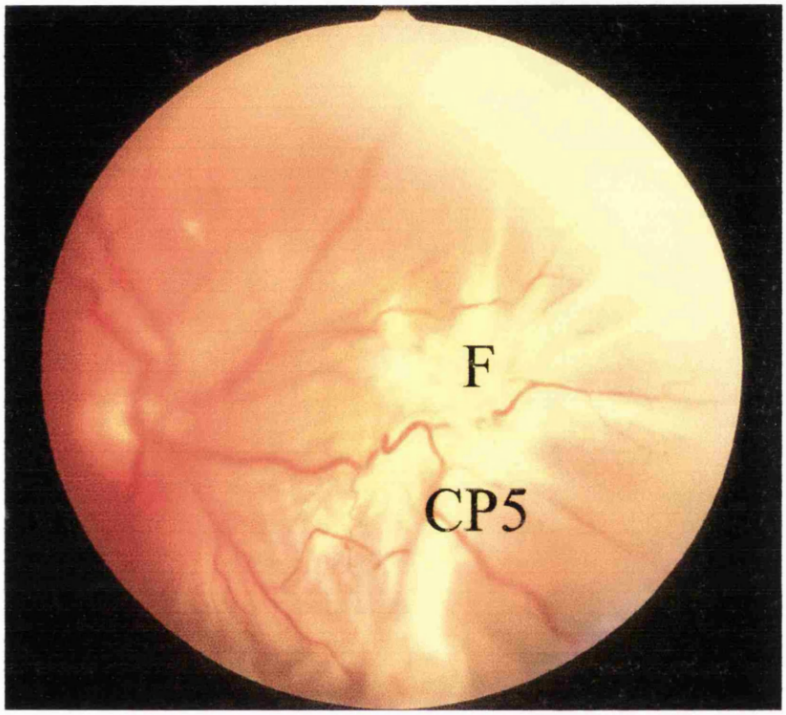
Muller in 1858 first hypothesised that the vitreous exerted tractional forces on the retina and was central to the PVR process. Leber in 1881 supported the role of traction. However, in his model the vitreous played a passive role and that it was inflammatory proliferation of new tissue from the ciliary epithelium forming a contractile membrane that pulled the retina off the choroid. He termed this complication as "pre-retinitis or hyalitis" and the disease was thought incurable (Duke-Elder and Dobree, 1967).

Tolentino et al (Tolentino *et al.*, 1967) introduced the term "massive vitreous retraction" and Cibis (Charteris, 1995) defined the process as "massive preretinal retraction" to emphasise cellular activity in, and retraction of, the vitreous together with the appearance of retinal folds. This was changed to "massive periretinal proliferation" by Machemer and Laqua (Machemer and Laqua, 1978) to acknowledge the massive proliferation of cells on the surface of the retina and vitreous resulting in periretinal membrane formation.

The Retina Society Terminology Committee in 1983 (The Retina Society Terminology Committee, 1983) proposed the term "proliferative vitreoretinopathy" to take into account the location of the pathology and the proliferation of cellular tissue. The classification divided the disease process in to four stages of PVR (A-D) of increasing order of severity. Grade A refers to vitreous haze and pigment. Grade B describes retinal stiffness and wrinkling, rolled edges of retinal breaks and vascular tortuosity. Grade C refers to fixed retinal folds and is divided into stages depending on the number of quadrants involved. For example involvement of 1,2 and 3 quadrants are staged C1, 2 and 3 respectively. Grade D referred to fixed retinal folds in 4 quadrants either in an open or closed funnel configuration. This system has proved to be very useful in classifying PVR and is still used in some clinical centres. A major fault in the system was that it did not classify pathology to the location of PVR and excluded anterior PVR. To account for these factors a revised classification was published by the Retina Society in 1991 (Machemer *et al.*, 1991).

In the new classification, Grade A and B remained unchanged. Grade C was expanded into anterior and posterior locations, the dividing line being the equator. The extent of proliferation in each area is expressed by the number of clock hours of retinal involvement (1-12). Grade C was also subdivided into the type of contraction involved. This classification is summarised in tables 1.1 and 1.2. Figure 1.1 shows how this classification system works in practice.

Figure 1.1 Use of The Retina Society Classification System.



In this case there is one star fold posterior to the vitreous base involving 5 clock hours of the retina.

Table 1.1 Classification of proliferative vitreoretinopathy by grade (Machemer et al., 1991).

Grade	Features
A	Vitreous haze; pigment clumps; pigment clusters on inferior retina
B	Wrinkling of inner retinal surface; retinal stiffness; vessel tortuosity; rolled and irregular edge of break; decreased mobility of vitreous
C P 1-12	Posterior to equator: focal, diffuse, or circumferential full-thickness retinal folds*; subretinal strands*
C A 1-12	Anterior to equator: focal diffuse, or circumferential full-thickness retinal folds*; subretinal strands*; anterior displacement*; condensed vitreous strands

*Expressed in the number of clock hours involved

Table 1.2 Grade C PVR (Machemer *et al.*, 1991).

Type	Location (in relation to equator)	Features
1. Focal	Posterior	Starfold posterior to vitreous base
2. Diffuse	Posterior	Confluent starfold posterior to vitreous base. Optic disc may not be visible
3. Subretinal	Posterior / anterior	Proliferation under the retina: annular strand near disc; linear strands; moth-eaten-appearing sheets
4. Circumferential	Anterior	Contraction along posterior edge of vitreous base with central displacement of the retina; peripheral retina stretched; posterior retina in radial folds
5. Anterior displacement	Anterior	Vitreous base pulled anteriorly by proliferative tissue; peripheral retina trough; ciliary processes may be stretched, may be covered by membrane; iris may be retracted

This classification system has helped to standardise the terminology and grading used to describe the severity of PVR and has allowed comparisons to be made between different studies. A problem of this system is that it does not take into account risk factors involved in the PVR process such as the number, size and type of retinal breaks.

Pathophysiology and clinico-pathological correlation of PVR

PVR is considered to be an abnormal wound healing response in a specialised tissue. This results in the formation of cellular membranes on both surfaces of the retina and vitreous. Organisation of the membranes can lead to tractional retinal detachments.

This is a very dynamic process involving several steps (Glaser *et al.*, 1993) . An initial insult e.g., cryotherapy, leads to inflammation and breakdown of the blood retinal barrier with release of cytokines and growth factors. This is followed by a cellular response resulting in the release and migration of various cell types including macrophages and retinal pigment epithelial cells (RPE) cells into the vitreous and onto the surface of the retina. Organisation of the inflammatory tissue occurs and a contractile membrane can form that may lead to traction on the retina.

Cellular constituents of PVR membranes

The cellular composition of PVR membranes has been extensively studied and four main categories of cells have been found. These are RPE (Clarkson *et al.*, 1977; Hiscott *et al.*, 1984a; Kampik *et al.*, 1981) , glial (Clarkson *et al.*, 1977; Morino *et al.*, 1990; Newsome *et al.*, 1981) , inflammatory (macrophages and lymphocytes) (Charteris *et al.*, 1992; Charteris *et al.*, 1993; Hiscott *et al.*, 1985; Jerdan *et al.*, 1989; Kampik *et al.*, 1981) and fibroblasts (Clarkson *et al.*, 1977; Hiscott *et al.*, 1985; Walshe *et al.*, 1992) .

RPE cell component

RPE cells have been found in intravitreal and periretinal PVR membranes (Clarkson *et al.*, 1977; Hiscott *et al.*, 1984a; Kampik *et al.*, 1981) . As the RPE cells are in an ectopic position they have migrated from their normal location. Two mechanisms are thought to play a role in RPE cell dispersion:

1. Machemer and Laqua (Machemer and Laqua, 1975) have shown that RPE cells are avulsed and shed into the vitreous at the time of retinal tear formation. Alternatively, the RPE cells remain attached to the flap of retina and latter become seeded in to the vitreous cavity. Following separation as the RPE cells have lost there normal cellular contacts and feedback mechanisms they undergo morphological changes and are eventually "pinched off" Bruch's membrane and form motile RPE macrophages (Johnson and Foulds, 1977) . An observation to support this mechanism is the increased incidence of PVR with larger retinal breaks (Yoshida *et al.*, 1984) .
2. In the second theory RPE cells are dispersed intraoperatively during cryotherapy and scleral depression (Singh *et al.*, 1986) . Many observers have noticed a cloud of pigment at time of cryotherapy and it is thought that this represents the release of RPE cells (Hilton, 1974) . Glaser et al (Glaser *et al.*, 1993) have shown that vitreous samples from eyes treated with cryotherapy contained significantly more viable RPE cells than control vitreous. This was supported by Campochiaro et al (Campochiaro *et al.*, 1985) in a bovine model showing increased release of colony forming RPE cells following cryotherapy compared with diathermy or a sham procedure. It is interesting to note that the location of PVR is largely inferior regardless of the location of the retinal break (Michels, 1984) . In fact, even in cases where the retinal

breaks are exclusively superior the disease is most extensive inferiorly. It is felt that once the free cells are released gravity causes them to settle inferiorly. Of all the cell types found in PVR membranes it is only RPE cells that behave in this manner. Therefore, it is possible that RPE cells are the most important cell type in the initiation of the disease process. RPE cells have also been shown to be capable of producing factors that can stimulate both fibroblasts and astrocytes (Bryan and Campochiaro, 1986) . Therefore, cryotherapy can cause the release of RPE cells that can stimulate the recruitment of fibroblasts and astrocytes, the three main cells found in PVR contractile membranes.

The role of RPE cells in PVR is complex. It has been shown histologically that RPE cells can give rise to three different cell types (Machemer and Laqua, 1975) :

1. Pigment epithelial macrophages that form "pigment clumps" rather than membranes (Gilbert *et al.*, 1988) ,
2. RPE cells that retain epithelial characteristics,
3. RPE cells that have undergone mesenchymal differentiation into fibroblast like cells.

Mandelcorn (Mandelcorn *et al.*, 1975) using an in vivo model of the owl monkey has shown that RPE cells can differentiate into both macrophages and fibroblasts. Mueller-Jensen (Muller-Jensen, 1974) observed that RPE cells in vitro transformed into macrophages with microvilli and were filled with phagocytosed material but retained some pigment epithelial characteristics. Vidaurri-Leal et al found that when RPE cells when exposed to vitreous collagen or fibrin transformed into fibroblast like cells (Vidaurri-Leal *et al.*, 1984; Vidaurri-Leal and Glaser, 1984) .

RPE cells are capable of secreting factors that are chemotactic for other cells, including macrophages and lymphocytes. They can also synthesise collagen, a major component of PVR membranes (Morino *et al.*, 1990) . This has been shown both in animal models (Mueller-Jensen *et al.*, 1975; Newsome and Kenyon, 1973) and in vitro by human RPE cells (Campochiaro *et al.*, 1986a) .

Macrophage and inflammatory cell component

Inflammatory cells have consistently been identified in PVR membranes.

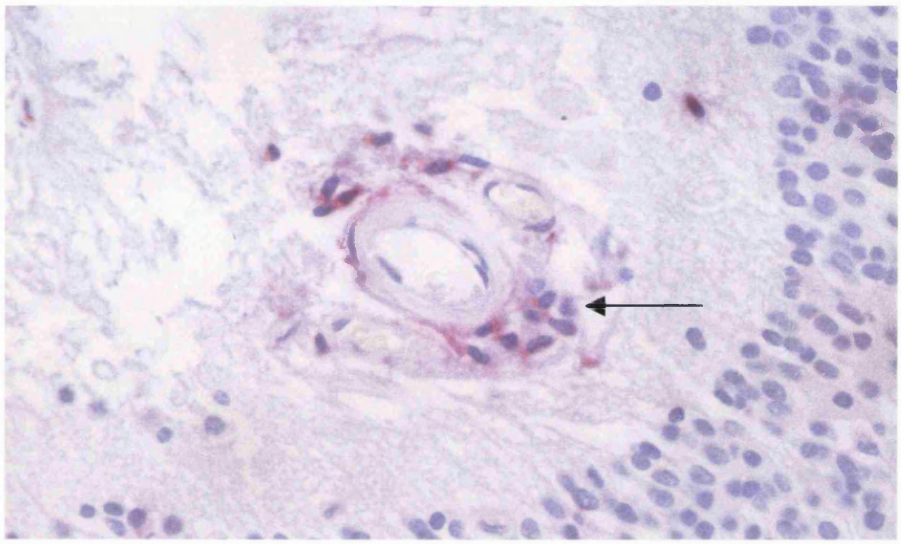
Macrophages were first thought to derive from circulating blood monocytes and are described by there morphological characteristics (Hiscott *et al.*, 1985; Kampik *et al.*, 1981; Newsome *et al.*, 1981) . Immunohistochemical studies of PVR membranes have confirmed the acute inflammatory cell type origin of the macrophages found in severe intraocular proliferation (Esser *et al.*, 1993) . Other studies have shown that macrophages are derived from RPE cells (Baudouin *et al.*, 1990; Jerdan *et al.*, 1989; Vinores *et al.*, 1990) . The origin of the macrophages still remains unresolved; it is likely that early membranes contain predominantly blood born macrophages and that established membranes contain a mixed population. Figure 1.2 shows macrophages leaving the circulation in an eye with PVR.

Macrophages play two significant roles in the disease. Firstly, they act as scavenger cells phagocytosing inflammatory debri and secondly, they secrete growth factors and cytokines that recruit and stimulate other cells involved in the disease process. It is not surprising that RPE cells also have similar functions.

Lymphocytes have also been identified in PVR membranes. Charteris et al (Charteris *et al.*, 1992; Charteris *et al.*, 1993) has consistently shown T lymphocytes in both epiretinal

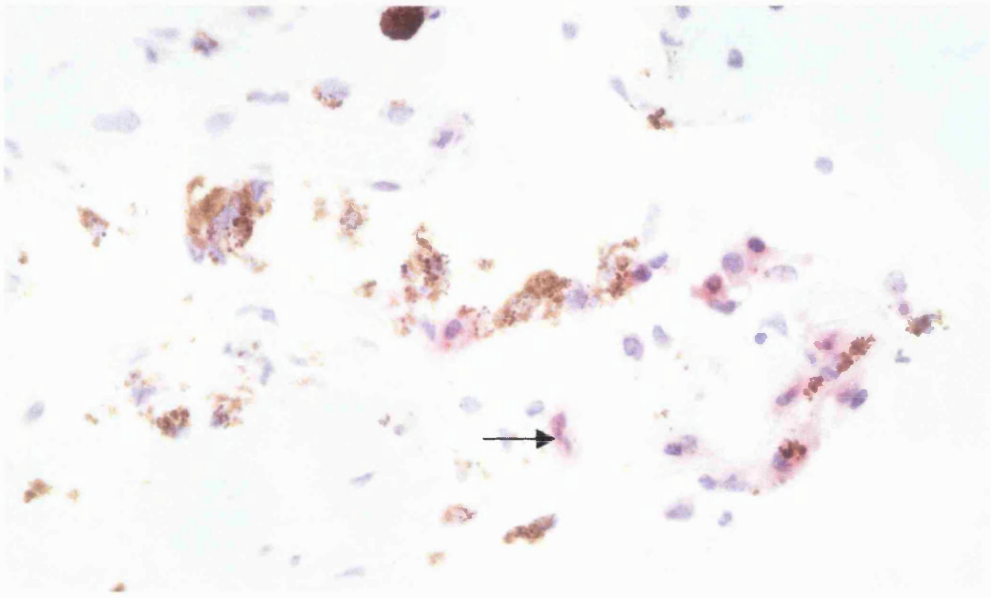
and subretinal membranes. Figure 1.3 shows T cells in a PVR membrane. Immunohistochemical typing has shown these lymphocytes to be of both CD4+ and CD8+ subsets. Expression of the MHC class II antigen (HLA-DR) and T cells expressing the interleukin 2 receptor has also been found. HLA-DR antigens are usually expressed by immunocompetent cells and are a marker of cell activation. MHC class II antigen enable mononuclear phagocytes to present antigens to T-lymphocytes resulting in the release of cytokines and subsequent recruitment of inflammatory cells. Limb et al (Limb *et al.*, 1993a) has shown the presence of cell adhesion molecules (CD11a, CD11c, CD18 and ICAM-1) in PVR membranes. T-lymphocytes and macrophages play a fundamental role in wound healing and have been shown to have reciprocal interactions in the wound healing process. More significantly macrophages have been shown to produce a soluble factor which can prolong T cell survival and hence potentiate the role of T cells at foci of fibrogenesis (Kovacs and Kelley, 1985) . The presence of T lymphocytes and macrophages does not imply an autoimmune process in the development of PVR. None the less, they do play a significant role in the development of PVR affecting the process through the secretion of growth factors and subsequent regulation of cellular chemotaxis, proliferation and the secretion of extracellular matrix components.

Figure 1.2 Blood born macrophages leaving the circulation and entering PVR tissue



A photomicrograph showing macrophages (CD68 immunostain x 160). Arrow shows macrophages near a capillary.

Figure 1.3 T cell reaction in PVR tissue.



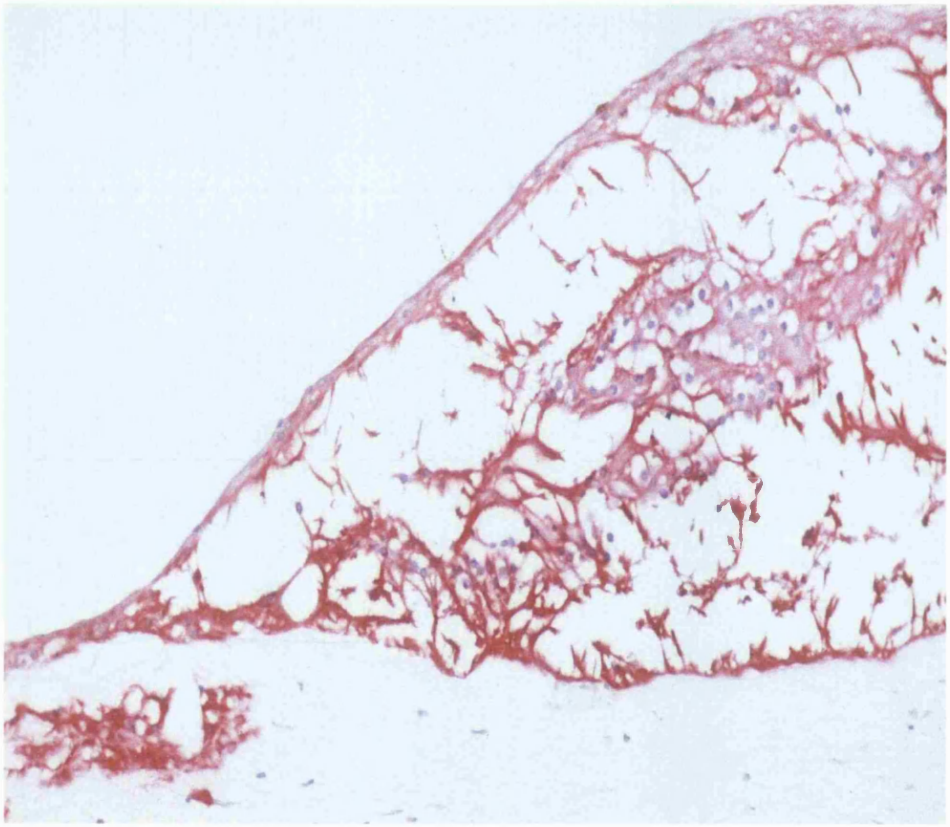
A photomicrograph showing T cells (UCHL-1 immunostain x 160). It is interesting to note the close proximity of the T cells to macrophages. (pigmented cells).

Glial cell component

Glial cells are frequently found in PVR membranes (Hiscott *et al.*, 1989; Morino *et al.*, 1990; Van Horn *et al.*, 1977) within a mixed population of cells. Morino et al (Morino *et al.*, 1990) found that as the membrane aged the glial cell ratio increased. Interestingly, "simple" epiretinal membranes (ERM) are composed solely of glial cells (Kampik *et al.*, 1980) and therefore other cellular components are required to produce the contractile and tractional properties of PVR membranes. Also "simple" ERM can form at sites of retinal breaks and holes (Clarkson *et al.*, 1977; Foos, 1974) . The cellular origins of glial cells remain uncertain. Müller cells, astrocytes, microglia, and perivascular glia all have the potential to proliferate and contribute to periretinal membrane formation (Foos, 1974; Hiscott *et al.*, 1984b) .

The cellular composition of subretinal bands is similar. These bands can form as diffuse sheets or as taut bands (Hiscott and Grierson, 1991; Machemer, 1980; Sternberg, Jr. and Machemer, 1984) and may prevent the surgical reattachment of the retina. Immunohistological and ultrastructural studies have also demonstrated the benign nature of purely glial membranes; diffuse membranes are purely glial (Schwartz *et al.*, 1988; Trese *et al.*, 1985; Wilkes *et al.*, 1987) whereas subretinal bands are formed from a mixed population of cells including RPE, fibroblast and glial cells. The main cellular component of taut subretinal bands are RPE cells (Hiscott *et al.*, 1989) with a minimal glial cell component. Figure 1.4 shows an intense glial cell reaction in PVR tissue.

Figure 1.4 Intense glial proliferation in PVR tissue



A photomicrograph staining for glial tissue (GFAP immunostain x 100). The strong red staining shows the intense glial proliferation that has occurred.

Fibroblast component

Immunohistological studies have identified fibroblasts in PVR membranes (Clarkson *et al.*, 1977; Hiscott *et al.*, 1984a; Kampik *et al.*, 1981) . Studies have shown that the fibroblasts represent transformed RPE cells (Machemer and Laqua, 1975; Mandelcorn *et al.*, 1975) (Mueller-Jensen *et al.*, 1975) . Hiscott et al (Hiscott *et al.*, 1984b) found no evidence of RPE cell morphology of fibroblasts in their specimens. Other theories suggest that fibroblasts originate from vascular epithelial cells, glia or hyalocytes (Hiscott *et al.*, 1984b; Kampik *et al.*, 1981; Rosenthal *et al.*, 1997) . Fibroblasts have been found to contain myofibrils (Hiscott *et al.*, 1985; Kampik *et al.*, 1981; Walshe *et al.*, 1992) and therefore can cause contraction of membranes.

As the cellular components in PVR membranes can change both their morphology and immunology the exact derivation and contribution of different cell types is difficult to define.

Extracellular matrix

The ECM provides the scaffold for membrane formation. Many classes of molecules, including large families of closely related proteins have been identified in the extracellular matrix (ECM) (Jerdan *et al.*, 1989; Morino *et al.*, 1990; Scheiffarth *et al.*, 1988) . These molecules range from glycosaminoglycans such as heparin sulphate proteoglycans (Jerdan *et al.*, 1989; Rentsch, 1977; Rodrigues *et al.*, 1981) to components of the clotting and fibrinolytic system including plasminogen, plasmin and fibrinogen (Esser *et al.*, 1997; Immonen *et al.*, 1988; Peczon *et al.*, 1983; Weller *et al.*, 1988; Weller *et al.*, 1989) . Peczon et al (Peczon *et al.*, 1983) described two types of membranes that are found during vitrectomy. The first is an established membrane that is

composed predominantly of collagen and a second newly formed membrane consisting predominantly of fibrin. He suggested that this newly formed membrane might provide a scaffold upon which further membrane can form. This assumption is shared by others (Charteris, 1995) . This newly formed membrane may also be amenable to pharmacological treatment.

Proteins

Most investigations have concentrated on three classes of proteins found in the ECM (Hiscott *et al.*, 1999) : structural proteins (the collagen and elastic families), cell adhesion proteins (fibronectin, laminin and vitronectin) and proteins with counter-adhesive properties (thrombospondin, tenascin, and osteonectin or SPARC which are a group of glycoproteins sometimes known as matricellular proteins).

Structural proteins

1. Collagens

Abundant collagen has been found in PVR membranes. Collagen types 1 to V have all been identified (Scheiffarth *et al.*, 1986) . Immunohistochemical analysis of membranes has consistently shown the presence of types I, III and IV and only variable amounts of type II collagens (Morino *et al.*, 1990) . It is not clear whether type II collagen represents incarcerated vitreous (collagen type II is present in normal vitreous) or whether it is newly produced.

As new collagen is present this suggests that it locally produced by the cells in the membrane (Jerdan *et al.*, 1989; Morino *et al.*, 1990) . RPE cells are the probable source of the collagen production. RPE cells in vitro can produce various subtypes of collagen (Campochiaro *et al.*, 1986a; Newsome and Kenyon, 1973) and following experimental

retinal detachment collagen has been found in the subretinal space (normally devoid of collagen) (Hiscott *et al.*, 1999). The presence of several subtypes of collagen (Hiscott *et al.*, 1985) and the co-localisation of collagen and RPE cells (Morino *et al.*, 1990) in PVR membranes is further evidence that RPE cells may be responsible for the collagen found in membranes.

As the membrane matures and the collagen content increases there is a corresponding decrease in the RPE and cellular content. RPE cells that remain undergo metaplasia and take on a fibroblast like morphology and a decrease in proliferative ability (Hiscott *et al.*, 1985; Morino *et al.*, 1990).

2. Elastic precursors

The elastic fibre family comprise of either fibrillin related microfibrills (known as oxytalan), intermediate fibres (eluanin) which consist of microfibrills set in elastin and mature elastic fibres which consist of a central core of elastin surrounded by microfibrills (Hiscott *et al.*, 1999).

Although, mature elastic fibres have not been found in PVR membranes oxytalan fibres have been found (Alexander *et al.*, 1992). The role of oxytalan fibres is uncertain. Oxytalan fibers have been found in various tissues undergoing mechanical deformation and may play a role in strengthening the structural properties of PVR membranes (Alexander *et al.*, 1992).

Cell adhesion proteins

The cell adhesion molecules comprise of three main classes laminins, fibronectins and vitronectins.

1. Laminin

Laminin is a member of a family of extracellular matrix proteins and that has been shown to be co-distributed with glial cells, RPE cells and with fragments of inner limiting lamina of the retina (Jerdan *et al.*, 1989; Rodrigues *et al.*, 1981) . Again there is circumstantial evidence that laminin is produced by RPE cells by the co-localisation RPE cells and laminin in PVR membranes (Machemer *et al.*, 1978) and the ability of RPE cells in-vitro to produce laminin (Campochiaro *et al.*, 1986a) .

The functional significance of laminin is unclear. Laminins can effect cell differentiation, expression of phenotypes and are involved in anchoring epithelial cells to their substrates (Hiscott *et al.*, 1999) .

2. Fibronectin

As mentioned, in the early stages of wound healing, RPE cells are thought to settle on the surface of the retina. There is evidence that the fibronectin at the vitreoretinal juncture in early PVR originates from both local production by RPE and glial cells and from plasma following breakdown of the blood-retinal barrier (Hiscott *et al.*, 1992) . There is evidence that fibronectin and fibrinogen may provide a temporary scaffold or substrate upon which early PVR membrane formation can occur (Hiscott *et al.*, 1999) . Abundant fibronectin has been found in PVR membranes (Hiscott *et al.*, 1985) and its presence in early PVR membranes lends support to this theory (Hiscott *et al.*, 1992) . Further evidence that fibronectin plays a role in early membrane formation is supported by the co-distribution of fibronectin and thrombospondin (Hiscott *et al.*, 1992) . Thrombospondin is thought to bind to fibronectin fibrils thereby contributing to the provisional matrix. Such a matrix may play a role not only in cell adhesion but also in recruiting and activating RPE cells to the provisional matrix. RPE cells in vitro have been found to have an increased affinity to

fibronectin compared to a range of other substrates (Wagner *et al.*, 1995) . Fibronectin has been shown to be chemoattractant for RPE cells (Campochiaro *et al.*, 1984; Campochiaro *et al.*, 1986a) .

3. Vitronectin

Another family of molecules thought to have a predominant role in cell adhesion are vitronectins (Casaroli Marano *et al.*, 1995) . Vitronectins are involved in the coagulation and complement cascades as well as mediating cell adhesion and motility. There are several studies that have identified vitronectin in PVR membranes (Casaroli Marano *et al.*, 1995; Casaroli Marano and Vilaro, 1994; Esser *et al.*, 1994; Heidenkummer *et al.*, 1996) . The distribution of vitronectin is usually pericellular. (Hiscott *et al.*, 1999) . Controversy exists to whether vitronectin is increased or decreased in PVR vitreous compared to normal vitreous (Hiscott *et al.*, 1999) .

Proteins with counter-adhesive properties (Matricellular proteins):

Thrombospondin 1, Osteonectin and Tenascin.

These molecules are termed "matricellular" as they interact with many molecules in the extracellular matrix as well as cell receptors (Hiscott *et al.*, 1999) . These proteins are involved in many cellular processes including phagocytosis, adhesion, proliferation and migration (Akimoto *et al.*, 1998) . For wound healing to occur partial cell detachment is required and is thought to be mediated through three matricellular proteins which can exhibit anti-adhesive properties; thrombospondin 1, osteonectin and tenascin (Hiscott *et al.*, 1999) .

The presence of thrombospondin (Esser *et al.*, 1991) and the co-localisation of thrombospondin 1 and fibrinonectin (Nunes *et al.*, 1998) in PVR membranes suggests

that these two molecules play an essential role in the formation of early proliferative disease. However, with the discoveries of tenascin and osteonectin in PVR membranes it seems that a "cocktail" of adhesion modulating proteins are involved (a situation similar to that found in dermal wound repair) (Hiscott *et al.*, 1999).

Matrix remodelling

The ECM is in continual flux through out the wound healing process and a family of enzymes called matrix metalloproteinases (MMPs), that degrade the extracellular matrix, are central to this activity (Matrisian, 1990).

MMPs are zinc dependent proteases that have degradative effect on extracellular matrix components. They are secreted in the latent form and need activation for their proteolytic activity (Woessner, 1991). There are three broad categories of MMPs based on their substrate specificity. The first class are the collagenases (MMP 1 and 8) which degrade collagen types I-III (Ennis and Matrisian, 1993). The second are the stromelysins (MMP3) which degrade proteoglycans, fibronectin, laminin and collagen types III-V (Ennis and Matrisian, 1993; Matrisian, 1990). The third class are the gelatinases (MMP2 and 9). These gelatinases degrade denatured collagens type I-III and intact collagen type IV, V and VII. The role of MMPs 2 and 9 has been extensively studied recently (Matsubara *et al.*, 1991) and their role in the PVR process is being unravelled.

MMPs 2 and 9 have recently been shown to be present in subretinal fluid (Immonen *et al.*, 1996) and in the vitreous (Kon *et al.*, 1999) of patients with PVR. MMPs can be produced by a variety of cells including neutrophils, macrophages, fibroblasts, and vascular smooth cells (Birkedal-Hansen *et al.*, 1993) and by ectopic RPE cells (Alexander *et al.*, 1990; Alexander *et al.*, 1991). Their production is regulated by a

number of growth factors and cytokines (Bauer *et al.*, 1985; Hunt *et al.*, 1993) and their tissue activity is inhibited by tissue inhibitors of matrix matelloproteinases (TIMPs). Therefore, the overall matrix digestion is dependent on the balance of MMPs and TIMPs (Matrisian, 1990; Matrisian, 1992).

Membrane contraction

It is the contraction of the complex periretinal and vitreous membranes that are responsible for the clinical picture seen in PVR. The exact mechanism of membrane contraction is uncertain. However, most authors would agree that RPE cells are central to the process.

Gabbiani (Majno *et al.*, 1971), Cleary et al (Cleary and Ryan, 1981) and Ryan (Ryan, 1985) have all suggested that membranes contract in a similar fashion to muscle contraction. They described cells with features of both fibroblasts and smooth muscle cells and called them "myofibroblasts".

Others have suggested that RPE cells with fibroblast create contractile forces as they migrate through the membrane. RPE cells have been shown to move by extending lamellipodia covered coated pits which than adhere to collagen fibres and then retract. RPE cells can "reel" in collagen at a rate of 5mm of fibre in a 24-hour period (Ehrlich and Rajaratnam, 1990; Grierson *et al.*, 1996; Harris *et al.*, 1981). In vitro collagen gel studies have shown that "seeded" RPE cells quickly dedifferentiate into fibroblastic cells and begin to migrate (Mazure and Grierson, 1992).

Most authors would agree that the RPE cells are capable of producing wound contraction and that glial cells produce little or no contraction (Hiscott *et al.*, 1984a; Hiscott *et al.*, 1984b; Raymond and Thompson, 1990). However, glial cells can provide a scaffold

upon which other cells cause contraction. De Juan et al (de Juan, Jr. *et al.*, 1989) and Guidry (Guidry *et al.*, 1992) disagree and suggest a more dynamic role for glial cells.

Immune system involvement in PVR membranes

A role for the immune system has been suggested in PVR. The presence of MHC class II histocompatability antigen HLA-DR on both RPE and inflammatory cells suggests an involvement of the immune system in PVR membranes (Baudouin *et al.*, 1990; Jerdan *et al.*, 1989; Limb *et al.*, 1993a) . Macrophages have also been found in PVR membranes (Baudouin *et al.*, 1990; Esser *et al.*, 1993; Jerdan *et al.*, 1989) . Diffuse deposits of IgG, IgA, IgE, components of the complement system (Baudouin *et al.*, 1990) , B lymphocytes (Baudouin *et al.*, 1990) and T lymphocytes (Charteris *et al.*, 1993) have all been found in PVR membranes. The cell adhesion molecules CD11a, CD11c, CD18, ICAM-1, and LFA-1 have bee identified in PVR membranes and vitreous (Heidenkummer and Kampik, 1992; Limb *et al.*, 1993a) .

A cellular and humoral response (Brinkman *et al.*, 1979; Brinkman and Broekhuyse, 1978) to retinal antigens following retinal detachments and to anti-S antigen antibodies in the sera of patients with PVR (Grisanti *et al.*, 1994) have been described. An autoimmune response has also been demonstrated in a rabbit model of PVR to IRBP, S antigen and opsin (Broekhuyse *et al.*, 1990) . A true autoimmune response appears unlikely, although a role for T lymphocytes and macrophages is better established.

The role of growth factors and cytokines in PVR

A lot of work has been done on the role of growth factors and cytokines on the pathogenesis of PVR. Cytokines, by binding to cell receptors, act as cellular mediators of chemotaxis, proliferation, contraction and extracellular matrix elaboration by the cells

involved in the PVR process. Their exact role is complex and has not been fully explained, although they appear central to the wound healing response (Wiedemann, 1992).

RPE cells react to vitreous fluid, following break down of the blood retinal barrier, by mitogenesis and chemotaxis (Campochiaro *et al.*, 1986b). Serum is also chemotactic for RPE cells (Campochiaro and Glaser, 1985) suggesting that chemical mediators are involved. The response of RPE and glial cells to individual growth factors has also been investigated (Campochiaro and Glaser, 1985; Leschey *et al.*, 1990) . Although these experiments are informative direct assumptions can not be made from in vitro studies as cell responses can be effected by the culture medium, cell density, presence/and or absence of other growth factors and interactions with extracellular matrix. The following is an account of some commonly found growth factors and cytokines in PVR.

Transforming growth factor-beta (TGF-ß)

TGF-ß molecules are a family of dimeric polypeptides with a molecular weight of 25 kilodaltons (kDa). There are 3 main forms of TGF-ß (type 1-3) and the degree of homology between them ranges from 64% to 82% (Massague *et al.*, 1992) . TGF-ß is secreted by a variety of cells including lymphocytes, fibroblasts and macrophages (Connor, Jr. *et al.*, 1989) . TGF-ß2 is the main isoform in the retina and vitreous (Wakefield *et al.*, 1988) . TGF-ß2 is usually secreted in a latent form and has similar activities to TGF-ß1 (Smiddy *et al.*, 1989). TGF-ß has complex biological activities and is important in wound repair processes such as PVR (Connor, Jr. *et al.*, 1989).

TGF-ß upregulates the synthesis of fibronectin (Wiedemann, 1992) and the deposition of fibronectin and collagen in the ECM (Ignotz and Massague, 1986; Varga *et al.*, 1987)

. TGF-ß is chemotactic for monocytes (Wahl *et al.*, 1987) and fibroblasts (Postlethwaite *et al.*, 1987) . TGF-ß has been found to have a negative effect on RPE cell proliferation and to inhibit the stimulatory effect of basic fibroblast growth factor (b-FGF), insulin like growth factor (IGF-1) and platelet derived growth factor (PDGF) (Leschey *et al.*, 1990) . In contrast Wiedemann (Wiedemann, 1992) found that TGF-ß upregulates the genes that code for b-FGF, PDGF and interleukin-1 (IL-1). Cellular aspirates from PVR vitreous and subretinal fluid have demonstrated immunoreactivity to TGF-ß (Baudouin *et al.*, 1993) . TGF-ß has also been found elevated in PVR vitreous (Connor, Jr. *et al.*, 1989) . Limb et al (Limb *et al.*, 1991) found no difference in patients with and without PVR. Kon et al (Kon *et al.*, 1999) found the mean vitreous level of TGF-$ß_2$ to be 1.5 times higher in PVR compared to normal vitreous.

Platelet derived growth factor (PDGF)

PDGF was first purified from human platelets (Antoniades, 1981) . It has a molecular weight ranging from 28 to 35kDa and exists as a polymer of two polypeptide chains (A and B) linked by a disulphide bond. Therefore, 3 potential dimer combinations can be formed: AA, AB and BB (Robbins *et al.*, 1994) . It is released from platelets during thrombus formation but is also produced by monocytes, endothelial cells and fibroblasts (Ross *et al.*, 1986) . PDGF is chemotactic for neutrophils, monocytes, fibroblasts (Rutherford and Ross, 1976) , RPE (Campochiaro and Glaser, 1985) and glial cells (Harvey *et al.*, 1987) .

The role of PDGF in PVR has been extensively studied. Campochiaro and Glaser (Campochiaro and Glaser, 1985) demonstrated that a heat stable component was responsible for RPE cell chemotaxis. In their experiment they heated bovine serum for 5

minutes to denature the heat labile fibronectin. They found that after boiling the serum still retained 66% of its ability to stimulate RPE migration and concluded that PDGF is the probable serum component responsible. They have also shown that PDGF is chemotactic for RPE cells (Campochiaro and Glaser, 1985) and that RPE cells in culture produce PDGF –like proteins that are capable of stimulating fibroblast migration and proliferation (Campochiaro *et al.*, 1989). A role for PDGF in PVR is implicated by the presence of PDGF and PDGF receptors in PVR tissue (Robbins *et al.*, 1994).

Interleukin-1-beta (IL-1ß)

Interleukin 1 (IL-1) includes two distinct proteins, IL-1α and IL-1ß, that play a role in acute and chronic inflammation (Dinarello and Mier, 1986). They are produced as procytokines that are than cleaved to produce active compounds of approximately 17kDa. IL-1ß is produced primarily by monocytes and macrophages. Other cells capable of producing IL-1ß include fibroblasts, T and B lymphocytes, astrocytes and microglial cells (Callard and Gearing, 1994a). Triggers for IL-1ß include bacterial endotoxin and a variety of non- microbial inflammatory substances. The most extensively studied function of IL-1ß is initiation of inflammation. IL-1ß induces vascular endothelial cells to secrete chemokines (e.g., MCP-1) and to express vascular adhesion molecules (e.g., ICAM or VCAM) (Callard and Gearing, 1994a). IL-1 is chemotactic for monocytes and neutrophils (Kampschmidt, 1983) and is a stimulant for other cytokines such as IFN-γ and IL-6 (Van Snick, 1990). IL-1 also induces expression of MMPs leading to ECM degradation, monocyte migration and to MMP catalysed degradation of IL-1ß in a negative feedback loop (Ito *et al.*, 1996). IL-1 is chemotactic for RPE and glial cells (Harvey *et al.*, 1987; Kirchhof and Sorgente, 1989). IL-1ß and IL-1ß mRNA expression

has been shown in PVR membranes (Limb *et al.*, 1991; Limb *et al.*, 1994). IL-1 has also been found in PVR vitreous (Limb *et al.*, 1991) . Kon et al found that IL-1ß was significantly raised in patients with preoperative PVR compared to patients with control patients (Schonfeld, 1998).

Interleukin-6 (IL-6)

IL-6 is a pleotropic cytokine that plays a central role in the network of cytokines that maintain homeostasis. It has a molecular weight is 26kDa and is secreted by many types of cells including macrophages, monocytes, T and B lymphocytes, fibroblasts and endothelial cells (De Vos *et al.*, 1992) . IL-6 is a major mediator of the acute phase reaction in the inflammation and immune response. Its functions include differentiation and antibody production by B cells. It potentiates phytohemagglutinin activation of T cell production of IL-2 and IL-2 receptor and enhances the IL-2 and IFN-γ differentiation of cytotoxic T cells. One of the fundamental events in acute phase inflammation is the production of IL-1. IL-1 stimulates the production of IL-6 and these two cytokines contribute synergistically to acute pathological changes found in inflammation, including cellular infiltration, growth and differentiation of T and B lymphocytes (Dinarello and Mier, 1986; Mizel, 1989). Cells with mRNA expression to IL-6 have been found in PVR membranes (Limb *et al.*, 1993b) . Limb et al (Limb *et al.*, 1991) have shown the presence of IL-6 in PVR vitreous. Kauffmann et al (Kauffmann *et al.*, 1994) found higher levels of IL-6 in the vitreous of patients with PVR than a miscellaneous group of patients with conditions ranging from ocular trauma, macular pucker, vitreous haemorrhage secondary to vein occlusion and macular degeneration.

Kon et al (Kon *et al.*, 1999) found IL-6 to be independent predictive risk factors for the development of PVR.

Fibroblast growth factors (FGF)

FGF exist as a family of seven closely related factors showing an overall sequence homology of 30-50%. Basic-FGF (b-FGF) is the most extensively studied member of the family and when isolated has a molecular weight of 18kDa (Gospodarowicz *et al.*, 1986) . Acidic and basic FGF have very similar biological activities and interact with the same receptor (Gospodarowicz *et al.*, 1986) .

B-FGF has been shown to stimulate the proliferation of cells of mesodermal and neuroectodermal origin, including fibroblasts, endothelial cells, astrocytes, oligodendrocytes, neuroblasts, bovine lens epithelial cells and melanocytes.

Acidic and basic FGF have been shown to stimulate DNA synthesis and cellular proliferation of RPE cells (Fredj-Reygrobellet *et al.*, 1991) and to have an effect on extracellular matrix synthesis (Fredj-Reygrobellet *et al.*, 1991) .

Acidic FGF has been found in cellular aspirates of PVR vitreous (Baudouin *et al.*, 1993) and in PVR membranes (Fredj-Reygrobellet *et al.*, 1991) (Malecaze *et al.*, 1991) . Kon et al (Kon *et al.*, 1999) found elevated levels of bFGF in the vitreous of patients with preoperative PVR. These observations, as well as the observations that inappropriate expression of bFGF and other members of the FGF family can result in tumour production (Frenzel *et al.*, 1998; Rogelj *et al.*, 1989a; Rogelj *et al.*, 1989b) , suggest that FGF may play a prominent role in proliferative conditions and uncontrolled angiogenesis.

Tumour necrosis factor α (TNF α)

Tumour necrosis factor α (TNF α) is a potent paracrine and endocrine mediator of inflammatory and immune functions. It is also known to regulate growth and differentiation of a wide variety of cell types. TNF α is selectively cytotoxic for many transformed cells, especially in combination with IFN γ. TNF α is secreted by activated monocytes, macrophages, fibroblasts, T and B cells (de Boer *et al.*, 1998). TNF α has been found in vitreous (Limb *et al.*, 1991) and in PVR membranes (Limb *et al.*, 1994).

Interferon gamma (IFN γ)

IFN γ is a pleiotropic cytokine involved in the regulation of nearly all phases of the immune and inflammatory responses, including the activation, growth and differentiation of T cells, B cells, macrophages and fibroblasts (Callard and Gearing, 1994b). IFN γ has also been found in vitreous (Limb *et al.*, 1991) and in PVR membranes (Limb *et al.*, 1994).

Epidermal growth factor (EGF)

EGF is a 53 amino acid cytokine that is proteolitically cleaved from a large integral membrane protein precursor. EGF acts to stimulate growth of epithelial cells and is involved in wound healing (Callard and Gearing, 1994c).

EGF in vitro has been shown to stimulate DNA synthesis and cellular proliferation of RPE cells (Leschey *et al.*, 1990). EGF has been found in the vitreous, subretinal fluid (Baudouin *et al.*, 1993) and in epiretinal membranes (Fredj-Reygrobellet *et al.*, 1991) of patients with PVR.

Risk Factors for developing PVR

Many studies have been done to investigate the risk factors for developing PVR. The majorities of these studies have been retrospective and have looked at individual risk factors only. In fact most ophthalmologists rely on anecdotal evidence only at present to assess patient risk. As mentioned a number of studies have been published on risk factors but due to differences in methodology, definitions and statistical analysis they cannot be directly compared. Until recently the risk of developing PVR had not been investigated in a prospective study.

Tolentino et al in 1967 (Tolentino *et al.*, 1967) suggested that vitreous haemorrhage and vitreous syneresis were risk factors. However, his observations were anecdotal and did not involve any statistical analysis.

Chignell et al (Chignell *et al.*, 1973) found aphakia to be a significant risk factor. They postulated that aphakia made the localisation of small holes difficult and therefore lead to treatment failure. With the use of vitrectomy and internal search this may no longer be an independent risk factor. It was the theoretical failure to close all retinal breaks that led to PVR in their thinking.

Rachal and Burton (Rachal and Burton, 1979) found repeat surgery to be associated with the development of PVR.

Bonnet et al (Bonnet, 1984) in a retrospective study found the following risk factors to be associated with post operative PVR: preoperative grade C plus PVR, repeat retinal reattachment surgery and large retinal breaks exposing more than 3 disc diameters of pigment. In a later prospective study Bonnet (Bonnet, 1988) found preoperative Grade B and vitreous haemorrhage to be associated with the development of PVR.

Yoshida et al (Yoshida *et al.*, 1984) in a large retrospective study of patients with PVR treated with conventional scleral buckling procedures found size of retinal breaks, severity of preoperative PVR, vitreous haemorrhage and postoperative choroidal haemorrhage to be risk factors.

Lambrou et al (Lambrou *et al.*, 1987) reported that the use of silicone oil increases the risk of PVR. They looked at the effect of silicone oil, perfluoropropane gas or fluid in the vitreous cavity of rabbits. They reported that a higher proportion of silicone-filled eyes (83%) had severe proliferative vitreoretinopathy than either the perfluoropropane- filled (30%) or fluid-filled (10%) eyes. An in vitro proliferation assay using the vitreous samples showed that the silicone-filled vitreous had increased mitogenic activity for retinal pigment epithelial cells compared with the gas-filled or fluid-filled vitreous. They felt that silicone oil appears to increase proliferation by stimulating the release of more or different mitogenic factors as well concentrating active factors into a smaller volume near the retina. Lewis et al (Lewis *et al.*, 1988) suggested that silicone oil causes perisilicone proliferation in eyes treated for advanced PVR. They found that in 19 (61%) out of 31 eyes developed perisilicone proliferation and that this lead to redetachment in 15 (49%) eyes. Zilis et al (Zilis *et al.*, 1989) also found a high incidence of perisilicone proliferation. In their series 21/55 (38%) developed perisilicone proliferation. The result of these studies must be viewed with caution as they were descriptive and not randomised or case controlled. Also other risk factors were not analysed. In addition the silicone oil studies showed that both C_3F_8 and silicone oil were equally effective in the treatment of severe PVR (Abrams *et al.*, 1997).

Cowley et al (Cowley *et al.*, 1989) in 1989 retrospectively analysed 607 eyes undergoing retinal reattachment surgery. Using stepwise discriminant analysis they found the use of vitrectomy to be a strong risk factor. Other risk factors in order of importance were the presence of preoperative PVR, preoperative choroidal detachment, and the amount of cryopexy applied.

Malbran et al (Malbran *et al.*, 1990) in a retrospective study of 1180 patients found vitreous traction on horseshoe or crescent- shaped tears tear to be the determining factor for the development of proliferative vitreoretinopathy (PVR) and that small or round holes were not complicated by PVR.

Fleury at al (Fleury and Bonnet, 1992) prospectively analysed 60 eyes complicated by recurrent retinal detachment and PVR. They report a strong association with new breaks and postoperative PVR with a worse prognosis for new posterior tears. They concluded that new posterior breaks related to severe tangential traction and therefore represented a worse prognosis.

Nagasaki et al (Nagasaki *et al.*, 1991; Nagasaki *et al.*, 1993) looked at risk factors in aphakic eyes using regression analysis. They identified the following risk factors for developing PVR in order of significance: choroidal detachment, duration of retinal detachment longer than one month, occurrence of retinal detachment within one year following cataract surgery, and history of vitreous loss in cataract surgery.

Girard et al in 1994 conducted a large retrospective study of 1020 retinal detachments with none or mild preoperative PVR. Using multiple regression analysis they identified ten significant predictive variables for PVR. They were in increasing order of importance: minor intra- or postoperative haemorrhage, grade A preoperative PVR,

preoperative choroidal detachment, giant tears, air tamponade, detachment involving more than 2 quadrants, cumulative break area larger than 3 optic disks, postoperative choroidal detachment, signs of uveitis at initial examination, and grade B preoperative PVR. More recent work by Kon et al (Kon *et al.*, 1998a; Kon *et al.*, 1999) has looked at biological risk factors. They have shown that vitreous protein and cytokines to be predictive of PVR. Using multiple logistic regression analysis they found vitreous protein, matrix metalloproteinases 2 and 9 and IL-6 to be independent risk factors. They also looked at clinical risk factors and found the presence of preoperative PVR and aphakia to be independent risk factors (Kon *et al.*, 2000) . Limb et al also looked at biological risk factors and found that IL-6, IL-1 and IFN to be associated with PVR (Franks *et al.*, 1992) . They also found the cell adhesion molecule ICAM to be a risk factor for PVR (Limb *et al.*, 1997; Limb *et al.*, 1999) .

Treatment of PVR

The basic principles of treating retinal detachment complicated by PVR are similar to those of detachments generally. These involve the identification and closure of retinal breaks and the complete release of retinal traction. To these can be added the prevention of reoccurrence of the proliferative process and its resulting traction.

Closure of all breaks

Closure of all retinal breaks is a prerequisite to achieving retinal reattachment. A retinal break causes a short-circuiting of the fluid dynamics so that the retinal pigment epithelial pump cannot cope with the volume of vitreous fluid flowing into the subretinal space. If the break is closed the forces tending to reattach the retina can overcome the traction

tending to reopen the breaks. These forces can often overcome what appears to be clinically quite severe PVR.

Closure of the retinal break is achieved by creating a chorioretinal scar along the edges of the retinal break. This adhesion is usually achieved using either cryotherapy or laser.

External scleral buckling procedures are usually used for treating mild PVR. In general, laser photocoagulation is preferred as cryotherapy has been shown to increase the dispersion of RPE cells (Glaser *et al.*, 1993) and theoretically the development of PVR.

Conventional scleral buckling procedures have been reported to achieve anatomical success in up to 74.7% cases (Grizzard and Hilton, 1982) . However, if you look at the success rate in severe PVR this drops to 19% in the same series. Others have also reported low success rates following conventional scleral buckling procedures for severe PVR; Yoshida et al (Yoshida *et al.*, 1984) and Sternberg and Machemer (Sternberg, Jr. and Machemer, 1985) reported success rates of 46.9% and 33% respectively.

In bullous detachments locating the retinal break and applying cryotherapy can be difficult leading to either under or over treatment of the break. To overcome these difficulties Stanford and Chignell (Stanford and Chignell, 1985) and Gilbert and Macleod (Gilbert and McLeod, 1996) simultaneously described a technique to treat bullous detachments. The technique was called D-ACE, Drainage (D) through a sclerostomy first, followed by injection of air (A), cryotherapy (C) and placement of scleral explant (E). Using this technique Wong et al (Wong *et al.*, 1992) achieved a primary success rate of 85.5% with an overall success rate of 97% in 97 cases. It is important to follow the D-ACE procedure as described.

Vitrectomy techniques are the treatment of choice for moderate to advanced PVR. Machemer, who popularised the treatment, achieved a success rate of 34% (17 eyes out of 47) using vitrectomy, peeling of membranes, scleral buckling and fluid/gas exchange in cases that were considered to have a very poor prognosis or "hopeless".

The instrument system used by most surgeons is the "three port pars plana vitrectomy" set up. The fibreoptic light pipe and vitrectomy probe enter through two of the ports and can be interchanged; the infusion cannula is introduced through the third port.

The crystalline lens is removed if necessary if visualisation is poor. Some surgeons advocate removing the crystalline lens routinely (Leaver *et al.*, 1984; Michels, 1984). The intraocular lens in pseudophakic eyes is usually left in-situ.

The vitrectomy probe is used to excise the central vitreous core; attention is then given to the anterior vitreous. After removal of as much as possible of the anteroperipheral fibrous tissue attention is paid to the posterior segment. After intravitreal and periretinal membranes are excised retinal breaks are marked using diathermy and a fluid gas exchange is performed. A retinectomy is sometimes needed to relieve traction and a high encircling scleral buckle is used to offset residual traction at the vitreous base. A primary success rate of 81% was reported by Lewis et al (Lewis and Aaberg, 1991) with a final reattachment rate of 90% in patients with PVR. The main cause of failure was the presence of new and recurrent anterior PVR.

Counteract traction

Several methods exist to counteract traction on the retina. The ability to achieve this is especially important in areas of retina that contain the retinal break. The methods available include external scleral buckling, peeling of the vitreous base, internal tamponade, relaxing retinectomies and retinotomies.

Retinotomy

To mobilise a stiff retina a relaxing retinotomy is used. Machemer first described this technique (Doft, 1986) to relieve traction on the retina. The retinotomy is usually positioned nasally and superiorly to the disc. A retinectomy is performed only after other procedures to relieve traction have been unsuccessful.

Morse et al (Morse *et al.*, 1990) reviewed 100 consecutive eyes undergoing relaxing retinotomies. With a minimum follow-up of 6 months, 58 eyes were completely attached, 8 were partially attached (macula on), and 34 were detached. Han et al (Han *et al.*, 1990) looked at 54 consecutive eyes undergoing relaxing retinotomy for proliferative vitreoretinopathy (42 eyes) and trauma (12 eyes) to determine whether perioperative factors, including size and location of the retinotomy influenced visual or anatomic outcome. After 6 months' minimum follow-up, anatomic success (retina attached posterior to buckle and an intraocular pressure of 3 mm Hg or more) was achieved in 35 eyes (64%). Functional success (visual acuity of 5/200 or better) was achieved in 14 eyes (26%). Preoperative visual acuity of hand motions or better and location of the retinotomy in the superior four clock hours of the fundus were two factors that were predictors of functional success. One of the main causes of failure was recurrent PVR.

In a more recent publication the fifth Silicone Study Group compared relaxing retinotomy in 117 of 404 eyes with severe PVR (Grade C3 or worse) undergoing vitrectomy and treatment with long- acting gas or silicone oil. The patients were divided into two groups. Group 1 had not undergone previous vitrectomy and group 2 had. Group 1 eyes not undergoing retinotomy had better anatomic and visual outcomes and less hypotony than eyes that did regardless of tamponade. Silicone oil decreased the likelihood of hypotony in eyes undergoing retinotomy. These differences were not found in group 2 eyes. The report concluded that eyes undergoing a vitreous operation for the first time for the treatment of PVR in most instances could be successfully treated by conventional techniques without the need for relaxing retinotomy. Retinotomy may be required more often in patients undergoing repeat vitreous surgery for PVR, in which case both silicone oil and long-acting perfluoropropane gas appear to be equally effective

Intraocular tamponade

Following a vitrectomy intraocular tamponade is needed to appose the break and to flatten the retina while chorioretinal adhesion develops. A bubble larger than the break will not enter the subretinal space due to its surface tension and when positioned over the break and thereby prevents the passage of fluid into the break. The time for chorioretinal adhesion to develop is dependent on the type of retinopexy employed. Air is unsuitable as it is absorbed quickly. Long acting gases and silicone oil are more suitable.

Long acting gases

As early as 1911 Ohm (Michels, 1984) reported the use of intraocular gas for the treatment of retinal detachment. Interest in the clinical use of sulphur hexafluoride (SF6)

and the perfluorocarbon gases has grown because of the expansile qualities and longer duration of mechanical tamponade that these agents provide.

SF6 was introduced by Norton in 1973 (Norton and Fuller, 1976) as a vitreous substitute. SF6 is a non-toxic and inert gas. It is expansile because of its relative insolubility compared to gases in the blood. Pure SF6 will expand to twice its volume and last for about 10-14 days in the vitrectomised eye (Chang, 1994).

Vygantas et al (Vygantas *et al.*, 1973) in the same year introduced octofluorocyclobutane (C_4F_8) a perfluorocarbon gas that lasts even longer than SF6.

Lincoff et al studied the longevity of three gases, perfluoromethane, perfluoroethane, and perfluoropropane (CF_4, C_2F_6, and C_3F_8, respectively) for their possible value as intravitreal tamponades in retinal surgery. They found that C_3F_8 remained 28 days, nine times as long as air and four times as long as SF_6. They also commented on the cataractogenic properties of these gases. However, most of the effect was temporary and resolved when the gas was removed.

Silicone oils

Silicone oils are linear synthetic organic-inorganic polymers and consist of a common chain made of repeating units of siloxane [-Si-O-] units. The chains length determines its molecular weight. The major differences among the silicone oils are in the chemical structure of radical side groups, radical termination of the chain and the chain's length. The commonest silicone oil used in ophthalmology is polydimethylsiloxane (PDMS). The PDMS chains are synthesised by polymerisation of linear and low molecular weight siloxanes. Various amounts of low molecular weight components (LMWC) have been detected in different silicone oils (Gabel *et al.*, 1995). It is felt that these impurities lead

to emulsification of silicone oils and that LMWC may be responsible for the chronic toxicity of the oils leading to their infiltration into the surrounding ocular tissues (Gabel *et al.*, 1995; Nakamura *et al.*, 1991) . This has caused great concern and has led to improved standards and classification of silicone oils paying particular attention to the percentage of LMWC and the amount of reactive hydroxyl end groups.

Polydimethyl siloxanes are assumed to be stable in living systems because of their intrinsic properties such as thermal stability and non-adhesiveness to tissues. This has not been the case for PDMS used in breast augmentation. There have been many reports that the oils are not stable and undergo chemical modifications to a variety of hydrolysed species and the presence of free silicone oil being localised in surrounding tissues and regional lymph nodes.

Many studies have shown that when highly purified silicone oils of various viscosities are compared with regard to their in-vitro stability a positive correlation between the degree of viscosity and an increase in stability is found. With higher viscosity oils being more stable and theoretically safer (Heidenkummer and Kampik, 1991; Hoing *et al.*, 1991; Kampik *et al.*, 1992; Lakits *et al.*, 1999) .

Studies by several workers in 1958 and 1968 indicated that the PDMS were none toxic to ocular tissues. Armaly, Cibis et al and Levine and Ellis (Leaver, 1994) found no evidence of inflammatory or hypersensitivity reactions when silicone oils were left in the vitreous cavity of rabbits, cats and monkeys; although when left in the anterior chamber of rabbits they caused corneal decompensation after only a few months.

Cibis (Cibis *et al.*, 1962) went on to treat a large series of complicated retinal detachments using intraocular silicone oil. He reported remarkable success in cases at the

time thought to be "inoperable" if treated with conventional scleral buckling. Cibis was aware of the complications; particularly cataract and keratopathy associated with silicone oil but in view of the "hopelessness" of the cases he was operating on he felt that it was an acceptable risk to take.

However, a major flaw lay in these early trials in that the duration of study was short and the inherent differences that exist between the vitreous of intact animal eyes and of human eyes complicated by retinal detachment.

Cibis died in 1965 before he could report the long-term safety of silicone oil. Okun and Okun and Arribas (Okun, 1968) were able to follow their cases and those of Cibis. Watzke, Cockerham and Schepens and others in the United States described their own series (Leaver, 1994) . Unfortunately, these reports were not favourable. Okun (Cockerham *et al.*, 1970) reported a 58% incidence of cataract at 3 years, Watzke (Cockerham *et al.*, 1970) reported only 2 out of 33 patients maintained their 6 month visual acuity at 3 years. More significantly, Mukai et al wrote of retinal toxicity (Mukai *et al.*, 1972; Mukai *et al.*, 1975) and his reports lead to dampened enthusiasm for the use of silicone oil especially in the USA.

Meanwhile Scott (Scott, 1972; Scott, 1975) in Cambridge began to popularise the use of silicone oil in the treatment of complicated retinal detachments. Others in Europe supported his work (Leaver, 1994) and they found silicone oil an indispensable tool for dealing with otherwise inoperable cases of retinal detachment. Furthermore, later publications on retinal toxicity failed to confirm the occurrence of retinal toxicity (Meredith *et al.*, 1985; Momirov *et al.*, 1983) . Subsequent improvements in microsurgical techniques and the manufacture of silicone oils have reduced the incidence

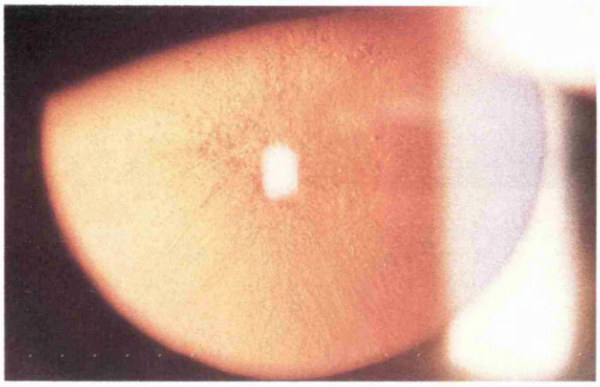
of complications. The main complications that occur now are cataract, glaucoma, keratopathy and possibly retinopathy in the long term.

Most workers would agree that the complications of silicone oil arise as a result mechanical effects on ocular tissues in contact with the oil as appose to direct toxicity.

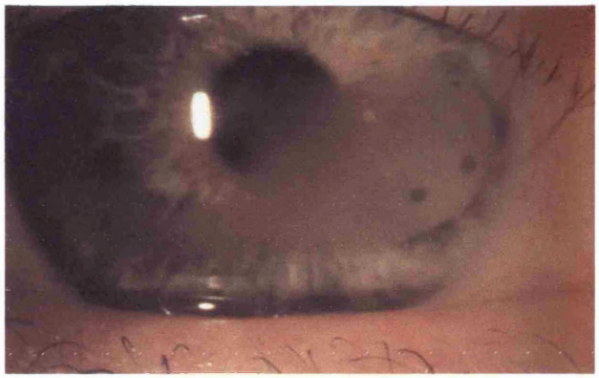
Figure 1.5 and 1.6 show the various complications found with using silicone oil.

The presence of silicone oil in the anterior chamber in contact with the corneal endothelium can lead to corneal decompensation (Chan and Okun, 1986; Cibis *et al.*, 1962) . Silicon oil can migrate into the anterior chamber around the intact lens resulting in complications both in aphakic and phakic patients. By careful searching silicone oil on gonioscopy can be found in up to 40% of phakic cases (Leaver *et al.*, 1979) . Light and electron microscopy have revealed the presence of vacuoles in the endothelial cytoplasm (Leaver *et al.*, 1979) . Band keratopathy can occur as a result of contact between silicone oil and the endothelium (Cibis *et al.*, 1962; Leaver *et al.*, 1979) . Prolonged silicone oil contact leads to stromal vascularisation, photophobia, pain, lacrimation and eventually perforation (Leaver *et al.*, 1984; Martola and Dohlman, 1963) .

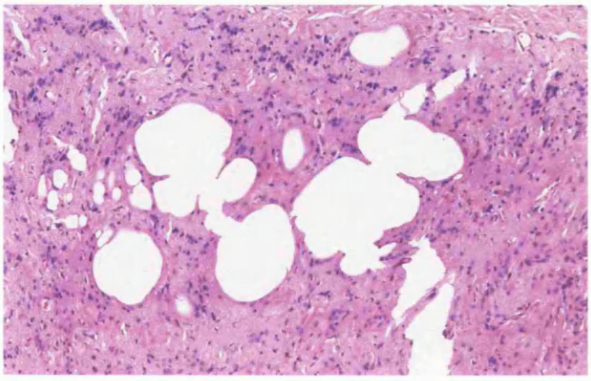
Figure 1.5 Shows the complications caused by silicone oil tamponade



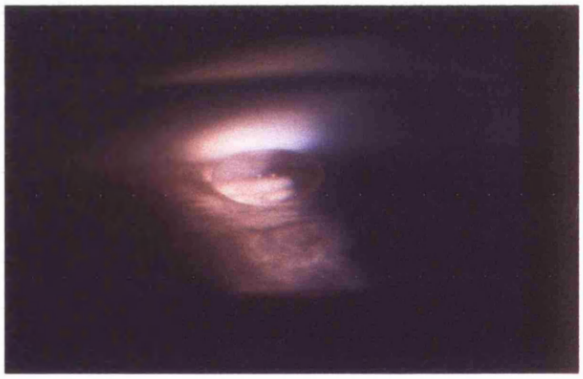
A silicone oil cataract



Band keratopthy in a silicone oil filled eye

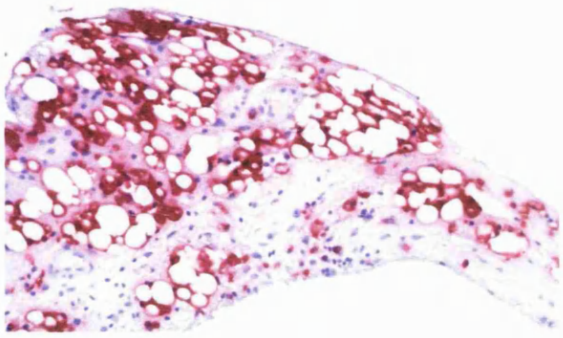


Silicone oil infiltration of the optic nerve. The empty spaces are presumed to have contained silicone oil. (H &E x 80)

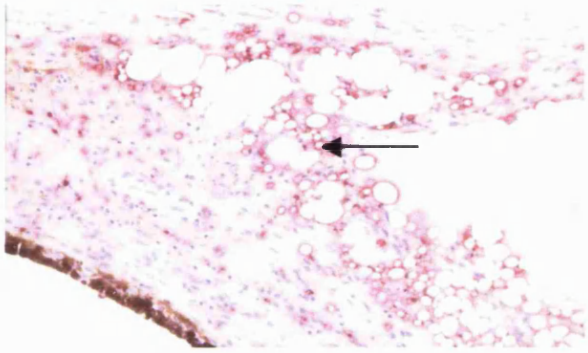


A gonioscopic view showing silicone oil in the angle.

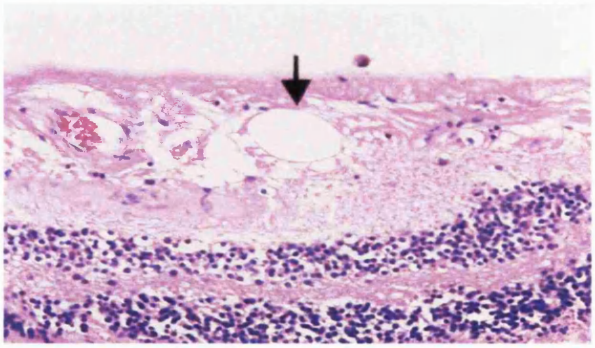
Figure 1.6 Shows the inflammatory and destructive damage caused by silicone oil tamponade



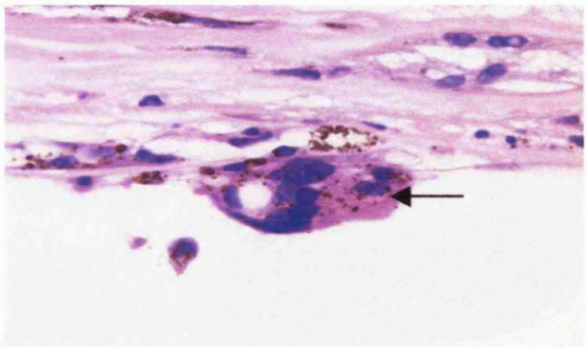
Intense macrophage (red staining) reaction to silicone oil in a PVR membrane (PGM1 x 120)



Destruction of the angle by silicone oil. The angle (arrow) architecture has been destroyed by the oil. (PGM1 x 100)



Intra retinal silicone oil (arrow). The empty round space is presumed to be silicone oil. (H & E x 80)



A photomicrograph showing a giant cell reaction to silicone oil (arrow). (H & E x 300)

Transient or sustained raised intraocular pressure (IOP) after silicone oil injection is common (Grey and Leaver, 1977; Leaver *et al.*, 1979; McCuen *et al.*, 1985a; McCuen *et al.*, 1985b) . An immediate postoperative rise in IOP is seen in 7-48% of eyes (Burk *et al.*, 1988; Lucke *et al.*, 1987; Nguyen *et al.*, 1992) . The pathogenesis of this IOP rise is uncertain but is probably related to anterior chamber activity, obstruction to aqueous out flow by choroidal effusions, the buckle or a combination of these elements. In most cases it is short lived and settles with medical treatment alone. Another cause of an immediate rise in IOP is pupillary block. Acute pupillary block glaucoma is uncommon if an Ando's inferior iridectomy is performed but can occur if the iridectomy is too small or blocked (Leaver, 1994) .

Chronic open angle glaucoma can also occur following silicone oil tamponade. Infiltration of the angle with silicone oil is considered to be the most important cause of intermediate and late onset glaucoma (Ni *et al.*, 1983) . Emulsification of silicone oil has been reported in up to 56% of oil filled eyes (Valone and McCarthy, 1994) . Other mechanisms leading to chronic glaucoma include neovascular glaucoma, peripheral anterior synechiae, steroid response and pre-existing glaucoma and angle problems (Leaver, 1994) . Leaver et al (Leaver *et al.*, 1979) reported 15% of 93 eyes had persistently raised intraocular pressure of more than 22 mmHg in the long term. Chun and Okun (Chan and Okun, 1986) reported an incidence of glaucoma in 16.8% of all eyes, 36% of which were phakic. Honavar et al (Honavar *et al.*, 1999) looked at 150 eyes and found an incidence of secondary glaucoma in 40% of eyes. Using multivariate analysis independent risk factors for developing glaucoma were in relative importance; rubeosis iridis, aphakia, diabetes, silicone oil in the anterior chamber and anatomic

failure. They also showed that poor prognostic factors for the control of glaucoma were silicone oil emulsification and diabetes.

Cataract is the most common complication following the use of silicone oil. Grey and Leaver (Grey and Leaver, 1977) reported that some lens changes were present in 57% of eyes at 1 year and this rose to 85% at 2 years. Chan and Okun (Chan and Okun, 1986) reported that 62.1% developed cataract after 1 month follow up. Some investigators have shown that cataract occurs earlier in eyes that the retina remains detached after surgery (Scott, 1972; Watzke, 1967).

Early removal of silicone oil has no significant effect on the incidence of cataract (Billington and Leaver, 1986; Casswell and Gregor, 1987; Franks and Leaver, 1991; Gonvers, 1985).

The question of retinal toxicity still remains a controversial issue. Lee et al (Lee *et al.*, 1969) looked at the effects of silicone oil in rabbit and owl monkey eyes. He reported swelling and vacuolisation in the retinal ganglion cells and the inner nerve fibre layer of the retina. This lead to partial loss of the ganglion cells within the compressed nerve fibre layer of retina. He also noted suppression of the ERG. Mukai et al (Mukai *et al.*, 1975) supported these findings and also described similar changes in owl monkeys. They found empty spaces in the inner segment of the retina adjacent to the vitreoretinal interface corresponding to the intercellular spaces between Muller's fibers. These spaces contained numerous spherical bodies encircled by a homogeneous electron-opaque material suggesting a silicone-phospholipid complex. Marked degeneration of small ganglion cells was seen. Muller fibers appeared to be shrivelled. Such changes were not found in control eyes suggesting a direct effect due to silicone oil. These reports discouraged further work

on using silicone oil in complicated retinal detachments until work published by Momirov et al (Momirov *et al.*, 1983) and Meredith et al (Meredith *et al.*, 1985) that changed opinion. Momirov found the ERG to be lower in silicone filled eyes that remained detached compared to eyes that remained attached suggesting pre existing retinal changes and the effects of surgery already performed as the cause of the ERG changes. Meredith looking at rabbit eyes found no difference in ERG responses in silicone filled eyes compared to control eyes.

Inferences drawn from these studies are difficult as silicone is used in the treatment of complicated retinal detachments and it is difficult to exclude the effects of the retinal pathology from the toxic effects of treatment. Also the changes occurring in an experimental animal model are inherently different by nature to those in a clinical setting. Interestingly, Kirchhof et al (Kirchhof *et al.*, 1986) suggested that silicone oil penetrates the retina only in areas of detached retina and not where the retina remains attached. However, the possibility of some kind of silicone oil-related retinopathy cannot be discounted and further research needs to be done.

Early removal of silicone oil, when clinically safe, is usually advocated and is practiced whenever possible. Gonvers (Gonvers, 1985) and Gonvers et al (Gonvers and Thresher, 1983) reported that the incidence of late complications of silicone oil were reduced if silicone oil was removed routinely at 6 weeks and reported a 62% reattachment rate at six months. The risk of retinal redetachment and hypotony remain a real problem, but in the majority of cases the retina can be reattached with re- injection of intraocular gas or silicone oil.

Silicone oil or gas?

Whether to use silicone oil or gas has been addressed by a large multicenter trial set up in 1985. The Silicone Study Group was set up to evaluate the benefits and risks of using long-acting intraocular gas or silicone oil tamponade following vitrectomy in eyes with severe PVR.

The Silicone Study report 1 (The Silicone Study Group, 1992a) investigated vitrectomy with silicone oil or SF6. 101 eyes with rhegmatogenous retinal detachment and severe (with a classification of at least C-3) PVR but without prior vitrectomy were treated with vitrectomy and randomised to either a mixture of 20% SF6 gas and air or to 1000 centistokes of silicone oil. Significantly more eyes that received silicone oil had visual acuity better than or equal to 5/200 compared to those eyes that received SF6 gas. The macula attachment was significant higher in eyes that received silicone oil compared to those that received SF6 gas. Hypotony, keratopathy and blind eyes were considerably more frequent in eyes randomised to SF6. This report implies that longer intraocular tamponade improves the outcome of surgery in severe PVR.

The second report (Anonymous, 1992) compared silicone oil and C_3F_8 gas in 265 eyes with severe PVR. 131 eyes had not undergone prior vitrectomy (group 1) while 134 eyes had undergone vitrectomy with intraocular gas tamponade (group 2). At the last examination, there were no differences between gas and silicone oil in achieving a visual acuity of greater than or equal to 5/200 and complete posterior retinal reattachment with either treatment modality. For group 1 eyes followed up for at least 18 months, there was an advantage favouring gas in achieving complete posterior retinal reattachment. The rates of reoperation and keratopathy were similar, while hypotony in group 2 eyes was

more prevalent in eyes randomised to gas. The rate of keratopathy was similar for both treatment modalities and either tamponade produced better results than those seen with SF_6 gas.

The third report (McCuen *et al.*, 1993) compared the outcome in eyes, using silicone oil and C_3F_8 gas, that underwent primary vitrectomy (group 1) with eyes that had undergone previous vitrectomy with intraocular gas tamponade (group 2). No differences were found between eyes in the two groups in achieving visual acuity of 5/200 or better, macular reattachment, or complete retinal reattachment. In eyes undergoing more than one operation to reattach the retina the final visual acuity was worse.

The fourth report (Barr *et al.*, 1993) looked at IOP as an outcome. Low IOP, 5 mmHg or less, was defined as hypotony, or elevated IOP, more than 25 mmHg. They found that 5% eyes had chronically elevated IOP and 24% had chronic hypotony. Chronically elevated IOP was significantly more prevalent in eyes randomised to silicone oil than in those randomised to C3F8 gas. Chronic hypotony was significantly more prevalent in eyes randomised to C3F8 gas than in those randomised to silicone oil, in eyes with anatomic failure and correlated with a significantly poorer postoperative vision. Factors prognostic of chronic hypotony included preoperative hypotony, diffuse contraction of the retina anterior to the equator, rubeosis, and large retinal breaks. In a multivariate analysis, diffuse contraction of the retina anterior to the equator remained an independent factor prognostic of chronic hypotony regardless of whether the retina was attached postoperatively.

The sixth report (Hutton *et al.*, 1994) evaluated the effects of removal of silicone oil. Silicone oil was removed in 45% eyes (oil- removed eyes). Compared with the eyes that

did not undergo silicone oil removal (oil-retained eyes), oil-removed eyes were more likely to be attached ($P < .0001$), have a visual acuity of 5/200 or greater ($P < .0001$), and not be hypotonous ($P < .001$). There was no association between the length of oil retention and incidence of recurrent retinal detachment after oil removal. Eyes with attached retinas at the time of oil removal generally improved in visual acuity at the last follow-up examination ($P < .0001$), that was not evident in eyes with detached retinas at the time of oil removal. There was an increased risk for recurrent retinal detachment at the last follow-up examination in the oil-removed eyes.

The seventh report (Abrams *et al.*, 1995) looked at the incidence of corneal abnormalities. This was the first study to find that the incidence of corneal abnormalities were equal with oil and gas. Using multivariate analysis independent prognostic factors for corneal abnormalities at 24 months were iris neovascularisation, aphakia or psuedophakia, postoperative aqueous flare and reoperations. Corneal abnormalities were correlated with poor visual acuity and hypotony.

The eight report (Cox *et al.*, 1995) analysed the incidence of macular pucker. They found a prevalence of 15% and an incidence of 4.7% of postoperative macular pucker. There was no difference between prevalence of postoperative macular pucker between group 1 and group 2 eyes or between eyes randomised to gas versus silicone oil. This would go against the theory of perisilicone proliferation.

The tenth report (Diddie *et al.*, 1996) found that in eyes with anterior PVR predictors of poor visual outcome were severe preoperative PVR (grade D-1 or worse) and the use of C_3F_8 gas as the intraocular tamponade. The choice of tamponade did not affect the anatomical outcome.

The eleventh report (Abrams *et al.*, 1997) looked at long term follow up. They compared 36 months follow up with 72 months follow up. They found that silicone oil and perfluoropropane gas were equal in most respects for the management of retinal detachments with PVR. Primary success is paramount for obtaining better visual results. Overall, surgery for PVR had a high likelihood of retinal reattachment, and if anatomically and visually successful at 3 years, there is an excellent chance that the results will be maintained.

Adjuvant therapy for the treatment of PVR

In spite of the improvements in vitreoretinal surgery there still remains a proportion of cases that fail reattachment surgery. The main cause of this failure is PVR. As a result, the importance of pharmacological and other forms of treatment to prevent membrane formation is becoming more important. The ideal agent should be effective, highly specific, non-toxic, and have a favourable pharmacokinetic profile.

The development of these contractile vitreoretinal forces are akin to those occurring generally in a wound healing response and involve both proliferative and contractile elements. Therefore it is important to understand the pathogenesis of the proliferative response. The different classes of drugs developed so far act at different stages of the processes. A brief reminder of the processes is worthwhile.

Stages of the PVR process

Cellular activation

It is thought that RPE cells, glial cells and fibroblasts are activated and displaced in response to various stimuli leading to membrane formation and traction. Inflammation and the breakdown of the blood retinal barrier play an important part in the early stage of

the PVR process. Drugs that exert their action before any amplification occurs, such as corticosteroids and non-steroidal anti-inflammatory agents, can be particularly effective as they exert their affect early in the process.

Proliferation

Under normal circumstances RPE and glial cells are in the resting phase of the cell cycle and become activated in response to injury. The precise pathways are poorly understood. Once a resting cell actively enters the growth cycle, an orderly sequence of steps, including nucleic acid and protein synthesis (S phase) occurs, resulting in mitosis (M phase) and the generation of two daughter cells. During the S phase no microscopic changes are visible in the cell, although nucleoprotein synthesis is occurring in preparation for cell division. During the replicating phase cells are more susceptible to pharmacological intervention, lasting typically 24 hours for RPE cells and slightly less for fibroblasts. Drugs that act to inhibit cellular proliferation when cells are in the growth phase are referred to as cell specific. Drugs that act on defined phases are referred to as phase specific (Calabresi and Parks, 1985).

Extracellular matrix elaboration and remodelling

There is a large amount of ECM in PVR membranes (Machemer, 1977), consisting mainly of collagen. Much of the collagen is newly secreted collagen and not derived from native vitreous collagen (collagen type 2). Collagen is secreted as procollagen, a precursor of collagen, and undergoes hydroxylation of certain proline and lysine residues, attachment of various carbohydrate residues, chain association, triple helix formation, fiber formation and cross-linkage (Eyre, 1980). Different classes of drugs act on different stages of the process. Antimetabolites, such as 5-fluorouracil, inhibit ribosome

synthesis of procollagen. Antimetabolites, such as vincristine and colchicine, inhibit secretion along the microtubular system. Most drugs affect more than one stage of the process (Verdoorn *et al.*, 1986).

Contraction

Drugs such as taxol, colchicine and 5-FU prevent cellular contraction of collagen lattices. These drugs affect the cell cytoskeleton effecting both migration and contraction. As cellular attachment to collagen is a prerequisite for gel contraction, a process greatly facilitated by fibronectin, this represents another point of attack. Drugs such as the synthetic tetrapeptide RGDS block attachment of cells to fibronectin and thereby collagen (Avery and Glaser, 1986; Klein *et al.*, 1991). Having identified the sequential steps in the vitreoretinal scarring process we can discuss specific agents that have shown promise.

Classes of adjuvant agents

Anti-inflammatory agents

Steroids were the first class of agents shown to inhibit the vitreoretinal scarring process (Tano *et al.*, 1980a). Steroids exert their antiproliferative effect by inhibiting DNA, RNA and protein synthesis as well as altering cell membrane permeability (Chen *et al.*, 1992). Although they were originally chosen for their ability to inhibit fibroblast proliferation it is now accepted that their primary effect is to dampen the inflammatory response in the eye, especially moderating the break down of the blood retinal barrier (Blumenkranz and Hartzer, 1994) . Steroids have been shown to have a bimodal effect on cellular proliferation with stimulation of cellular proliferation at relatively low doses and inhibition at supra physiological doses (Blumenkranz *et al.*, 1987).

Tano et al (Tano *et al.*, 1980a) was the first to use dexamethasone intravitreally in the rabbit eye. In their PVR model a single intravitreal injection of 1 mg of dexamethasone alcohol significantly reduced number of retinal detachments from 57% in control to 24% in treated eyes. Retinal neovascularisation was also inhibited in treated eyes (4% versus 19%) compared to control eyes. He also looked at the affect of using triamcinolone (Tano *et al.*, 1980b) . A single intravitreal injection of 1 mg of triamcinolone inhibited fibroblast growth and significantly reduced the number of retinal detachments (84% to 34%). Retinal neovascularisation was also reduced (72% to18%). Chandler et al (Chandler *et al.*, 1992) found that triamcinolone had no effect on irradiated pre-retinal membranes suggesting that steroids probably have a minimal effect in established membranes. Also, they also proposed that a combination of surgical and pharmacological treatment would be required to treat PVR. In a prospective trial comparing dexamethasone to 5-FU both treatments were found to be equally effective (Garcia-Layana *et al.*, 1995) .

The non-steroidal anti-inflammatory agents indomethacin and meclofenemate also reduce cellular proliferation in vitro (Blumenkranz and Hartzer, 1994) . When indomethacin was used in combination with 5-FU in an experimental model of PVR there was a slight additive effect (Blumenkranz *et al.*, 1982) .

Agents that inhibit cellular proliferation

Fluoropyrimidines

The fluoropyrimidines were the first family of antimetabolites to be used in PVR. They have a high potency in reducing proliferation associated with low toxicity at even high doses (Blumenkranz *et al.*, 1987) . Fluoropyrimidines exert their effects on rapidly

growing cells (Blumenkranz *et al.*, 1987) . 5-FU was the first member to be tested. Fluorouracil is a pyrimidine analogue. For it to have antiproliferative or anticontractile properties it must first be enzymatically converted into either a ribose or deoxyribose nucleotide. Active ribonucleotide forms are incorporated into RNA where they affect protein synthesis. 5-FU is enzymatically converted by thymidine phosphorylase and thymidine kinase to form 5-fluorodeoxyuridine monophosphate (5-FUDRP) that irreversibly inhibits thymidylate synthetase. This enzyme is the rate-limiting step in the generation of deoxythymidylate, a necessary building block for DNA synthesis (Blumenkranz *et al.*, 1987) . An alternative pathway is the conversion of 5-FU by uridine phosphorylase to 5-fluorouradine and uridine-cytidine kinase to 5-fluorouradine triphosphate (5-FURTP). The ribonucleotides of 5-fluorouradine are incorporated into messenger and ribosomal RNA producing coding errors and faster translation times than unmodified RNA (Blumenkranz and Hartzer, 1994) . The ribonucleosides (5-FUR) and nucleotides (5-FURP) but not 5-FU have been shown to have anticontractile properties on human fibroblasts and pigment epithelial cells (Hartzer *et al.*, 1989) .

5-FU has a dose dependent inhibition of rabbit dermal and conjunctival fibroblast proliferation (Blumenkranz *et al.*, 1984a) when used in vitro. The ID_{50} of 5-FU for a variety of ocular tissues varies between 0.35 and 0.71 ug/ml (Blumenkranz *et al.*, 1984b; Blumenkranz *et al.*, 1982; Blumenkranz *et al.*, 1984a) .

Blumenkranz et al (Blumenkranz *et al.*, 1982) injected fluorouracil intravitreally in an experimental model of PVR. They found that a single injection was effective and well tolerated. When supplemented by repeated 10-mg subconjunctival injections of fluorouracil or in combination with intravitreally administered indomethacin the effect

was additive. Stern at al (Stern *et al.*, 1983a) injected 0.5 mg fluorouracil intravitreally every 24 hours for seven days in an animal model of tractional retinal detachment. Four weeks after injection the rate of tractional retinal detachment was significantly less than control eyes. The height of the traction retinal detachment at four weeks was reduced by 50% in treated eyes compared to control eyes. Histological studies using thymidine labelling suggested that 5-FU significantly reduced the proliferation of RPE cells. However, proliferation and ERM formation still occurred even at high doses but was delayed for up to 3 weeks. This delay may be beneficial to allow surgical healing to take place.

Ward et al (Ward *et al.*, 1993) investigated the relative effects of 5-FUR versus 5-FU in an animal model of PVR. They found that a single 100ug injection of 5-FUR prevented proliferation in 70% of eyes compared with 41% in eyes that received a 1mg injection of 5-FU. In the same PVR model 1.0mg 5-fluorouridine 5'-monophosphate (FUMP) encapsulated in multivesicular liposomes decreased the frequency of tractional retinal detachment by 92% compared to controls. The half- life of the drug in the vitreous was 124 hours compared to only 4.5 hours of the non-encapsulated drug. In a rabbit model intravitreal injection of 0.1mg of 5-FUR resulted in retinal toxicity with a reduction in retinal protein synthesis (Leon *et al.*, 1990) suggesting that with an increase in potency there is a parallel increase in toxicity.

When 5-FU was injected into the vitreous cavity of rabbits it produced no morphological or electrophysiological damage to the retina in doses up to 1mg in the non-vitrectomised eye (Blumenkranz *et al.*, 1984b; Blumenkranz *et al.*, 1982).

Blumenkranz et al (Blumenkranz *et al.*, 1984b) in a pilot study injected 5-FU subconjunctivally or intravitreally in 22 patients with severe PVR. Retinal reattachment was achieved in 60% of patients at 6 months postoperatively. No serious systemic or ocular complications were observed with using 5-FU, although delayed healing of corneal epithelial defects occurred in 18% of cases and subtle subepithelial scarring in 31.8%.

Stern et al investigated the toxicity of 5-FU in the rabbit eye after lensectomy and vitrectomy. In eyes receiving repeated intravitreal injections of 1.25 mg of 5-FU all eyes had opaque corneas by three days that had not cleared by four weeks. Histological studies showed loss of photoreceptor outer segments and loss of ribosomes in all the retinal cells examined. The ERG b-wave decreased to 0% of the baseline value (no b-wave) but recovered after three weeks. In eyes receiving 1.25 mg of 5-FU every 24 hours for seven days corneal opacification increased to a maximum after two weeks and gradually decreased by four weeks. The ERG b-wave diminished to 9.6% of the baseline value at two weeks but later recovered to 62.5% of the baseline value at three weeks. Histological studies showed loss of photoreceptor outer segments and ribosomes at nine days that returned to near normal after five weeks. In eyes receiving 0.5 mg of 5-FU every 24 hours for seven days clinical, electrophysiological, and histological studies showed no toxicity. This reduced dosage of fluorouracil still exerted a significant antiproliferative effect on injected retinal pigment epithelial cells.

Nao-I et al (Nao-i and Honda, 1983) injected 5-FU in albino rabbit eyes. Concentrations of up to 10ug/ml had no effect on the ERG. Higher concentrations reduced the b wave response; 0.1mg/ml decreased the response to 62% and 1mg/ml to 41% of normal. The

ERG responses returned to baseline values within 60 minutes. The B wave response disappeared at a dose of 5mg/ml after 3 minutes although the amplitude recovered to 89% of baseline if the infusion was replaced with a 5-FU free medium after 5 minutes.

A limiting factor in the use of fluoropyrimidines for the treatment of PVR has been the rapid clearance of the drug from the eye. Jarus et al (Jarus *et al.*, 1985) showed that the clearance of intravitreal 5-FU in a vitrectomised aphakic rabbit eye was twice as fast as that in a phakic non vitrectomised eye. Using multivesicular liposomes or slow release biodegradable implants it is possible to prevent very high drug concentrations thereby avoiding toxicity while maintaining an effective therapeutic drug concentration (Garcia-Arumi *et al.*, 1997; Giordano *et al.*, 1993) . Rubsamen et al (Rubsamen *et al.*, 1994) developed a cylindrical solid implant moulded from copolymers of lactide and glycolide impregnated with 1 mg of 5-FU. In a rabbit model of PVR 89% of eyes that received the implant were attached compared to only 11% in eyes that received the control polymer without the drug. They did not find any ERG or histological evidence of toxicity.

Daunorubicin (daunomycin)

Daunomycin is an anthracyline antibiotic that has complex biological effects on DNA binding, free radical formation, membrane binding and metal ion chelation. The anthracyline ring portion inserts itself between base pairs of the double helix of the DNA molecule. Its amino acid group is also thought to interact with the phosphate backbone and copper chelation (Khawly *et al.*, 1991) .

Daunomycin is a very effective antiproliferative agent and its action is independent of the cell cycle (Wiedemann *et al.*, 1987) . Daunomycin arrests cell proliferation and migration but not contraction in vitro (Verdoorn *et al.*, 1986) . Exposure of RPE cells

and fibroblasts to daunomycin (7.5 mg/ml) for ten minutes resulted in complete growth arrest suggesting that a single intraoperative dose may be effective in clinical practice (Wiedemann *et al.*, 1985).

In an animal model daunomycin has been shown to have an antiproliferative action. Khawly et al injected homologous fibroblasts to induce PVR in gas compressed rabbit eyes. When daunomycin was injected at the same time as the fibroblasts 91% of retinas remained attached compared to none in the control eyes. This rate decreased to 27% if the drug was administered 3 days after fibroblast injection. Using 9 nmol of daunomycin in another study (Santana *et al.*, 1984) the incidence of retinal detachment was reduced by 50% in the rabbit model. Doses higher than 30 nmol per eye are toxic to the retina. Chen et al investigated the affect of combining daunorubicin (15 nmol) and triamcinolone acetonide (2 mg) with that of daunorubicin alone in a refined experimental model of PVR. Both treatments were effective in preventing retinal detachment. However, there was no significant difference in the rate of retinal detachment between the two treatment groups. Wiedemann went on to study the pharmacokinetic properties of intraocular daunomycin. The half-life of daunomycin in the vitreous is 131 min, indicating that a critical concentration is maintained in the eye for longer than 4 hr after a single injection. Using labelled daunomycin the authors found that the drug is eliminated across the retina and no significant binding of the drug to vitreous occurs.

Studies have shown that doses of up to 15 nmol of daunomycin are safe and associated with no histological or ERG changes (Bresgen *et al.*, 1994; Santana *et al.*, 1984; Wiedemann *et al.*, 1987; Wiedemann, 1989; Wiedemann and Heimann, 1990) .

Wiedemann (Wiedemann and Heimann, 1990) also defined a therapeutic window for the use of daunomycin in vitrectomised patients.

There are only a few reports on the use daunomycin in human eyes. In a series of 15 eyes (Wiedemann *et al.*, 1987) with PVR following trauma infusion of 7.5ug/ml for 10 minutes of daunomycin after pars plana vitrectomy resulted in anatomic success in 93% of patients. Visual acuity improved in all patients and there were no clinical signs of toxicity to the cornea, lens, retina or optic nerve.

Wiedemann (Wiedemann *et al.*, 1991) went on to treat a further 69 patients with advanced PVR with vitrectomy and silicone oil tamponade and adjuvant daunomycin therapy. After long term follow up 73% were attached and 89% had a vision of 20/800 or better. They found no specific toxicity due to daunomycin.

The authors also found that the reoperation rate to be less with treatment.

Both these studies gave the impetus to test the use of daunomycin in a randomised control trial in patients with PVR. Two hundred eighty-six patients with stage C2 (Retina Society Classification, 1983) or more advanced PVR in which surgery with silicone oil were randomised. Standardized surgery plus adjunctive daunorubicin perfusion (7.5 ug/ml for 10 minutes) was compared with surgery alone. Six months after standardized surgery, complete retinal reattachment without additional vitreoretinal surgery was achieved in 62.7% of eyes in the daunorubicin group versus 54.1% in the control group (P = .07, one-sided). However, in the daunorubicin group, significantly fewer vitreoretinal reoperations were performed within 1 year postoperatively (P = .005, one-sided) to achieve the same overall 1-year retinal reattachment rate. The rate of primary success rate was 65.5% in the daunorubicin group versus 53.9% in the control group.

There was no difference in the best-corrected visual acuity between the two groups. No severe adverse effects related to daunorubicin were found. To date this is the largest randomised trial for adjuvant therapy in PVR. The authors did not recommend routine use of this treatment but did conclude that pharmacological intervention is possible.

Retinoids

Retinoids play an important role in the differentiation and proliferation of a number of cell types. Vitamin A is esterified by RPE cells and plays a major role in visual pigment regeneration. The mechanism of action is uncertain but there is evidences that retinoic acid modifies gene expression through the cyclic AMP-dependent protein kinase (Campochiaro *et al.*, 1991) . This can have an affect on the cytoskeleton and ECM and therefore cell phenotype and growth. Cells grown in the presence of 1 uM retinoic acid do not exhibit cellular overgrowth and maintain characteristics associated with the morphologic appearance of mature RPE cells in vivo. Growth curves and 3H-thymidine incorporation studies suggest that retinoic acid inhibits RPE cell growth primarily after the cells have reached confluence. Thereby promoting density-dependent growth arrest.

Kim and Stern (Kim and Stern, 1990) looked at the effects of Vitamin A and retinoic acid on cultured retinal fibroblasts, they found that dermal fibroblast proliferation was stimulated by retinoic acid and not affected by vitamin A. In contrast, both agents stimulated scleral fibroblasts proliferation and inhibited fibroblast contraction of collagen gels.

Verstraeten et al (Verstraeten *et al.*, 1992) looked at the effects of Vitamin A on RPE cells. Using doses of 17.5-70um RPE cell proliferation and migration was significantly inhibited. Cellular morphology also changed from a regular polygonal shape to an

elongated stellate shape. A decrease in the amount of ECM fibronectin and an alteration of actin fibers was also found. Cell mediated contraction of collagen lattices was also reduced. All these effects were reversed on removal of vitamin A from the culture medium.

In a rabbit model of PVR intravitreal injection of Vitamin A in silicone oil (Araiz *et al.*, 1993) significantly reduced the rate of tractional retinal detachment and no adverse toxicity effects were found associated with treatment.

Giordano et al (Giordano *et al.*, 1993) investigated a sustained delivery system of retinoic acid in a rabbit model of PVR. They used microspheres of biodegradable polymers (poly [D, L-lactic-co-glycolic] acid 50-50). A significant reduction in tractional retinal detachment (36% in treated eyes versus 100% in control eyes) was found. Histological studies showed only a mild inflammatory response consisting of macrophages and giant cells surrounding the microspheres in the preretinal vitreous.

In a pilot study of 20 patients (Fekrat *et al.*, 1995) with recurrent retinal detachment due to PVR patients were randomised to either 40 mg oral 13-cis-retinoic acid twice daily for 4 weeks postoperatively or control. All eyes underwent surgical repair using similar techniques. Nine of ten eyes in the study group remained attached during a mean follow-up of 8.3 months, whereas four of ten eyes in the control group remained attached ($P = 0.061$) during a mean follow-up of 9.6 months. The rate of macular pucker was similar between the groups. Of the six eyes in the control group that detached four had 6 or more clock hours of PVR. The final visual acuity was better than 20/400 in six study eyes and four control eyes. The authors concluded that a prospective, randomised, controlled clinical trial is now needed to access the use of retinoic acid as adjuvant therapy.

Immunotoxins

The use of immunotoxins is a new therapy available for the treatment of a variety of diseases. They were initially developed for the treatment of malignancies. The antibody portion of the molecule binds to a specific antigen on the target cell, the molecule then sets off a sequence of events leading to death of the target cell. Using a murine conjugate antibody against human transferrin receptors and the A chain of ricin, a plant derived toxin that inhibits protein synthesis by ribosomal inactivation, Davis et al (Davis *et al.*, 1990) showed that protein synthesis of non confluent RPE cells was significantly reduced. In a similar study Jaffe et al (Jaffe *et al.*, 1990) found the proliferation of RPE cells was significantly reduced when exposed to the immunotoxin. There was a marked decrease in DNA that accompanied the immunotoxin-mediated decrease in cell number. Viable cells remaining after exposure to the immunotoxin were morphologically abnormal; typically the cells had elongated spindle-shaped processes and had lost their normal cuboidal appearance. In contrast, cell number was not decreased in confluent human retinal pigment epithelial cells after treatment with maximal doses of immunotoxin. Morphologic changes similar to those seen in proliferating cells were observed in confluent cells exposed to high dosages of (more than 100 ng/mL) immunotoxin. Similar changes have been found with human fibroblasts grown on a collagen gel (Hermsen *et al.*, 1990).

Taxol

Taxol is extracted from the plant toxus brevifolia and has anti-tumour activity (Wani *et al.*, 1971) . It acts as a promoter of microtubule (Schiff *et al.*, 1979) assembly and completely inhibits proliferation of exponentially growing cells at low concentration. In an in vitro model (van Bockxmeer *et al.*, 1985) using rabbit choroidal fibroblasts taxol reduced gel mediated contraction and proliferation by 50% at concentrations of 2×10^{-8}M and 3×10^{-9}M respectively. In a rabbit model of PVR concentrations of as little as 0.5 ug virtually abolished tractional retinal detachment and was associated with no significant histological or electrophysiological toxicity. At a higher dose optic neuropathy was noted.

Colchicine

Colchicine is an alkaloid extracted from the autumn crocus plant. It has been used for the treatment of articular pain from the sixth century A.D and more recently for the treatment of gout. Colchicine interferes with the sol-gel transformation in mitotic spindles thereby arresting cell division in metaphase. Colchicine also inhibits cell motility (Davidson *et al.*, 1983) . Colchicine at doses well below toxicity was found to be a potent inhibitor of astrocyte, fibroblast, and RPE cell proliferation and migration. A colchicine concentration of 1.3×10^{-8} mol/L and 1.7×10^{-8} mol/L showed a 50% inhibition of proliferation for RPE cells, astrocytes, and fibroblasts, respectively. At concentrations of 10^{-7} mol/L colchicine inhibited 44%, 46%, and 93% of RPE cells, astrocyte and fibroblast migration respectively. Colchicine has been shown to have toxic effects on the retina at concentration levels of 2.5×10^{-6} mol/L. In the monkey eye optic atrophy (Davidson *et al.*, 1983) was ophthalmoscopically evident within 4 weeks after a single dose of 10 μg

and retinal toxicity was observed after as little as 1 μg of colchicine. To overcome the toxicity of colchicine Lemor et al (Lemor *et al.*, 1986) administered 3.5 mg of colchicine orally for 5 weeks to rabbits in an experimental model of traumatic retinal detachment. Colchicine treated eyes showed a detachment rate of 29.6% compared with 74% in the untreated group. Histopathological studies showed no retinal toxicity.

X-ray irradiation

In an animal model of PVR (Meredith *et al.*, 1988) low dose radiation therapy (6Gy) reduced the rate of tractional detachment. In an in vitro study (Ohuchi *et al.*, 1991) using irradiation doses of 1000-3000 cGy cell proliferation and DNA synthesis was reduced.

In a prospective randomised study of patients with severe PVR irradiation doses of 3000 cGy (Binder *et al.*, 1994) did not reduce the incidence of postoperative detachment although the onset of the PVR process was delayed.

Other agents

Additional agents have been tested for their potential to inhibit PVR scarring such as thiotepa, VP16, vincristine, bleomycin, methotrexate and mitomycin-C (Barrada *et al.*, 1984; Blumenkranz *et al.*, 1984a; Hoffman *et al.*, 1998) . None of these treatments has resulted in clinical application.

Drugs that act on the ECM and cell surface

Drugs that interfere with collagen biosynthesis

As mentioned collagen is the major non- cellular component of PVR membranes and therefore drugs that effect collagen biosynthetic pathway may have effect on the treatment of PVR scarring (Hiscott *et al.*, 1989; Morino *et al.*, 1990) .

Antimetabolites

A number of antimetabolites exhibit a non-specific inhibition of collagen synthesis. Vinblastin, colchicine, 5-FU and 5-FUR all reduce the ECM secretion of collagen by fibroblasts and RPE cells (Hartzer *et al.*, 1989). In addition antimetabolites, including colchicine and vinblastine, limit the secretion of synthesised procollagen by their effects on intracellular microtubular transport (Takahashi *et al.*, 1997).

***cis*-Hydroxyproline**

Agents such as *cis*-hydroxyproline destabilise the collagen triple helix by interfering with hydroxyproline synthesis resulting in functionally unstable collagen fibers (Yamada, 1983).

Penicillamine

Penicillamine is a degradation product of penicillin and is used to treat rheumatoid arthritis. It chelates copper, mercury, zinc and lead. By chelating copper, a cofactor for lysyl oxidase, it prevents the cross linkage and polymerisation of procollagen molecules into collagen in the ECM. In a rabbit trauma model of PVR (Weiss and Belkin, 1981) proliferation was reduced in treated eyes.

Drugs that effect cell adhesion

Many of the interactions of cells and their ECM occur at the level of the basement membrane. These interactions are mediated through non-collagenous proteins and glycoproteins such as laminin, heparin-sulphate proteoglycan and fibronectin (Yamada, 1983). Fibronectin is a major secretory product of cultured fibroblasts. It facililatates cell binding to collagen. Fibronectin also has binding sites for heparin, laminin and integrins.

Agents that interfere binding of fibronectin to cells can produce changes in cell morphology, migration and contraction of collagen lattices.

RGDS

RGDS is synthetic tetrapeptide, arg-gly-asp-ser (RGDS) derived from the cell- binding domain of fibronectin. It has been shown to interfere with cell adhesion. An in vitro study (Avery *et* al., 1986) has shown that RGDS inhibit RPE cell attachment to fibronectin in a dose-dependent manner with 70% inhibition at 1 mg/ml.

Heparin

Heparin is formed from long chain mucopolysaccharides of varying molecular lengths from 5 to 60kDa. Heparin is ubiquitous in the body and binds to several ECM proteins, including fibronectin, laminin and vitronectin (Yamada, 1983) . Heparin also interferes with cell adhesion. Heparin has significant anticoagulant properties. By binding to antithrombin III the resulting antithrombin-thrombin complex inactivates thrombin (Walter and Israel, 1987) . Heparin also binds to activated factor X that converts prothrombin to thrombin (Blumenkranz *et al.*, 1992) . Heparin also has other biochemical properties (Yamada, 1983) . It binds to a number of growth factors, including PDGF, EDGF and FGF. Soluble heparin prevents fibroblast adhesion to fibronectin- coated substrates and produces changes in the cytoskeleton of smooth muscle cells and pericytes after short exposures (Hoover *et al.*, 1980) . Heparin also inhibits the polymerisation of Type I collagen (Blumenkranz and Hartzer, 1994) .

In vivo studies of heparin on three dimensional collagen lattices have shown a significant reduction in fibroblast- mediated contraction (Blumenkranz *et al.*, 1992) . Heparin inhibits proliferation of human scleral fibroblasts (Del Vecchio *et al.*, 1988) . Heparin

reduces RPE cell proliferation in a dose dependent manner with concentrations of 200 units/ml for 6 days reducing proliferation by 30% (Blumenkranz and Hartzer, 1994).

The breakdown of the blood retinal barrier and fibrin formation are important steps in the PVR process. The use of heparin has been investigated in the rabbit eye after vitrectomy and cyclocryotherapy (Johnson *et al.*, 1987). Several different routes were used to administer heparin: single anterior chamber injection of heparin (15 IU), heparin infusion (2 IU/100ml or 4 IU/100ml) or a single intravenous injection. All treatments resulted in a statistically significant reduction of postoperative intraocular fibrin. No ocular bleeding complications developed postoperatively and a constant heparin intraocular infusion of 10 IU/cc did not change the bleeding time after sectioning of a retinal vessel.

A prospective trial investigating the use of systemic heparin or heparin infusion during vitrectomy to prevent fibrin formation has been done (Johnson and Blankenship, 1988). A total of 73 eyes were randomised; 26 served as the control group, 23 received 10,000 IU of intravenous bolus of heparin and 12 eyes each underwent vitrectomy with an infusion solution containing 10 or 5 IU/100 ml of heparin. The single intravenous bolus produced a minimal inhibition of fibrin formation. A 10-IU/100 ml heparin infusion resulted in a statistically significant reduction in postoperative fibrin formation ($P = 0.04$) but increased intraoperative bleeding ($P = 0.02$). A trend toward reduced postoperative fibrin formation was noted in the intravenous heparin and 5 IU/cc infusion groups.

Heparin has also been used successfully to reduce postoperative fibrin formation after vitrectomy in infants undergoing vitrectomy for retinopathy of prematurity (MacDonald *et al.*, 1985). Using heparin coated collagen shields anterior chamber fibrin formation was significantly reduced (Murray *et al.*, 1990).

A problem with using standard heparin is the risk of bleeding. To avoid the undue risks of bleeding investigators have produced low molecular weight heparin (LMWH) fragments. LMWH are produced by chemical or enzymatic depolymerisation of unfractionated heparin. The LMWH used clinically have mean molecular weights in the range of 4kDa to 6kDa. LMWH are weaker inhibitors of thrombin but inhibit the coagulation enzyme Xa to a similar degree (Bratt *et al.*, 1985) as unfractionated heparin. They also inhibit platelets to a lesser degree (Salzman *et al.*, 1980).

In a rabbit model (Iverson *et al.*, 1991a) of lensectomy, vitrectomy and retinotomy intraocular fibrin formation following infusion of 5 IU/ml of LMWH was significantly reduced. There was no significant difference in the degree of vitreous haemorrhage between the groups. Corneal clarity was improved in the heparin-treated group.

In an animal model of PVR injection of 100 IU of LMWH reduced the rate of tractional retinal detachment from 45% to 27% at 4 weeks (Blumenkranz *et al.*, 1992). In a more suitable model of lensectomy and partial vitrectomy 5IU LMWH/ml in the infusion for approximately 1 hour reduced the rate of tractional retinal detachment from 77% to 28% at 3 month (Iverson *et al.*, 1991b).

Summary

After Machemer introduced modern vitrectomy techniques the success of retinal reattachment surgery has dramatically improved with anatomical success being achieved in up to 90% of cases. This success has been achieved with a better understanding of the surgical principals involved in treating PVR. The arena is now set to try and improve this already very good success rate with the use of pharmacological adjuvant therapy. By

reducing the rate of PVR both the success of surgery and the visual outcome can be hopefully improved.

Chapter 2

Justification and Aims

The major cause of failure of retinal reattachment surgery for rhegmatogenous retinal detachment is proliferative vitreoretinopathy (PVR). As our understanding of the pathophysiology of PVR has improved so has the outcome of surgical treatment. It is unlikely that further improvements in surgical technique will significantly improve the success rate of surgery. Any further improvements will involve the use of adjuvant pharmacological treatment. Until now the majority of the research into pharmacological treatment has concentrated on in vitro and in vivo studies in animal models and only one prospective randomised clinical trial has been completed. The results of these studies have been very encouraging and have led us to undertake this study.

5-fluorouracil (5-FU) has been shown to be effective in reducing the rate of PVR in animal models. Toxicity studies using either single or multiple intravitreal injections of 5-FU produced no morphological or electrophysiological changes in the rabbit retina at low dosages. A prospective study in human eyes showed that a single injection of 10 mg of 5-FU was well tolerated. Recent laboratory work from our center has shown that short exposures to 5FU result in prolonged cellular growth arrest of Tenon's capsule fibroblasts. Subsequent experiments have shown that thirty- minute exposure *in vivo* can cause cellular growth arrest of RPE cells.

Low molecular weight heparin (LMWH) is a multipotential drug useful in the treatment of PVR. LMWH has been shown to reduce postoperative fibrin following vitrectomy and to have less haematological complications compared with non- fractionated heparin.

Heparin binds to fibronectin and to a wide range of growth factors including acidic and basic fibroblast growth factors and platelet derived growth factors.

Animal work has shown that LMWH is effective in reducing the rate of tractional retinal detachment and produced no toxic effects in the rabbit eye when infused using a dose of 5IU/ml.

As 5-FU and LMWH are effective in different aspects in the PVR process it was felt that a syngergistic approach to the prevention of PVR would be advantageous. Furthermore, recent studies in our center have identified patients at highest risk of PVR.

The aims of this study were

1. To test the use of adjuvant 5-fluorouracil and low molecular weight heparin as preventative therapy in the surgical treatment of patients at high risk of PVR
2. To test the clinical validity of a regression formula to detect patients at high risk of PVR in a clinical setting
3. To further investigate the clinical and biological risk factors in the development of PVR

Chapter 3

Patients, Materials and Methods

Clinical Study - The use of adjuvant 5-FU and heparin in the treatment of patients at high-risk of PVR.

A prospective, randomised, double masked, controlled trial was conducted using 5-FU and LMWH in the preventative therapy of patients at risk of developing PVR. All patients presenting with a diagnoses of rhegmatogenous retinal detachment undergoing primary vitrectomy were screened. All patients considered at high risk of developing PVR were randomised to receive either 5-FU and heparin or placebo in the infusion during vitrectomy. During surgery a vitreous sample was obtained to study the role of proteins and cytokines in the proliferative response.

174 high- risk patients were recruited to take part in the trial from June 1997 to January 1999. Patients received either 5-FU and LMWH or placebo in the infusion during routine pars plana vitrectomy.

Preoperative assessment

A complete medical and ophthalmic history was taken and a full examination performed on all patients. Patients were examined both at the slit lamp and with indirect ophthalmoscopy. Specific attention was paid to the risk factors under investigation (**Table 2.1**) which included age, duration of symptoms, refractive status, preoperative use of cryotherapy/laser, presence of preoperative PVR, presence of preoperative uveitis, presence of preoperative vitreous haemorrhage, size of detachment, preoperative macula status (detached or not) and preoperative lens status ("phakic" or "aphakic":

pseudophakic eyes with intact posterior capsules were classified as "phakic"; eyes, including those that were pseudophakic, that did not have intact posterior capsule were classified as "aphakic"). Preoperative PVR was considered to be present if there was greater than 1-clock hour of Grade C PVR according to the updated version of the Retina Society classification (Machemer *et al.*, 1991). All clinical details were entered directly into a database **(Appendix I)**. If patients were at high risk they were counseled about the study and were invited to participate. A printed explanation form was given **(Appendix II)** and the patients were allowed to think about the study before informed consent was obtained **(Appendix III)**.

Allocation to clinical risk category

In a previous study using a similar data set, we devised a discriminant function (formula) to predict patients at risk of developing PVR **(see appendix IV)**. We applied this formula on our patients to identify those patients most at risk.

Figure 2.1 shows the formula and clinical examples showing how the formula is used. In summary, multiple logistic regression analysis was performed on a number of clinical risk factors and an odds ratio of PVR obtained (e^b). The following risk factors were chosen: anterior uveitis, aphakia, preoperative vitreoretinopathy, and preoperative cryotherapy, size of detachment, vitreous haemorrhage. These risk factors were chosen as they have consistently been reported in the literature to be predictive of developing PVR. The mean risk value of developing PVR was then calculated. Following examination of patients the risk data was added to the formula and a risk score was obtained. If the risk score is above the mean than the patient was assigned as "high risk". The mean was used as an arbitrary level.

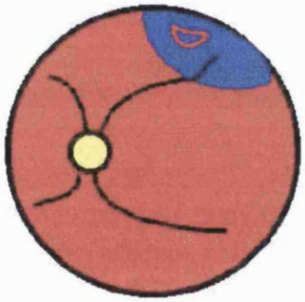
Figure 2.1 Risk equation used and clinical examples

The following equation was used:

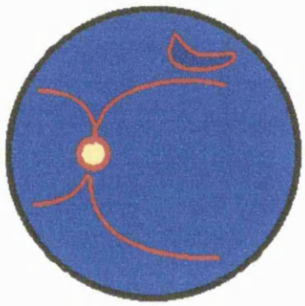
Risk of developing PVR = 2.88 x (Grade C PVR) + 1.85 x (Grade B PVR) + 2.92 x (Aphakia) + 1.77 x (Anterior Uveitis) + x 1.23 (Quadrants of Detachment) + 0.83 x (vitreous haemorrhage) + 1.23 x (Previous Cryotherapy)

If a risk factor is present than 1 is added to the equation, if the risk factor is absent than 0 is added. For quadrants detached a figure of 1-4 should be added. If the risk factor score is greater than 6.33 than the patient is at high risk.

Example 1



Example 2



Risk Factor	*Example 1*	*Example 2*
PVR	0	1.85
Aphakia	0	2.92
Uveitis	1.77	1.77
Cryotherapy	0	1.23
Vitreous Haemorrhage	0	0
Quadrants of Detachment	1.23	4.92
Total	3	12.69

Table 2.1 Clinical risk factors studied.

Factors	Description	Classification
1. Age (years)	continuous data	
2. Duration of symptoms (days)	continuous data	
3. Degree of myopia	not myopic	no
	refraction 0.00 to -5.00	low
	refraction > -5.00	high
4. Preoperative lens status	phakia	yes
		no
	psuedophakia	yes
		no
	aphakia	yes
5. Preoperative cryotherapy/laser	none or >3 months ago	no
	< 3 months ago	yes
6. Preoperative PVR	no PVR	no
	> 1 clock hours grade C	yes
7. Uveitis	presence of cells in A/C	yes
	absence of cells	no
8. Preoperative vitreous haemorrhage	presence	yes
	absence	no
9. Size of detachment (quadrants)	quadrants	1
		2
		3
		4
10. Macula status	macula on	on
	macula off	off
11. Intraoperative cryotherapy	used	yes
	not used	no
12. Intraoperative tamponade	SF_6	yes
		no
	C_3F_8	yes
		no
	silicone oil	yes
		no

Inclusion and Exclusion Criteria

Inclusion criteria were all patients undergoing primary vitrectomy for rhegmatogenous retinal detachment found clinically to be at high risk of developing PVR.

The following exclusion criteria were used:

1. Giant retinal tears
2. Posterior penetrating trauma
3. Proliferative diabetic retinopathy
4. Primary open angle glaucoma
5. Corneal opacity sufficient to impair surgical view
6. NPL vision
7. Previous vitrectomy
8. Inability to give informed consent
9. Inability to complete follow up program
10. Unwillingness to accept randomisation
11. Pre-menopausal women
12. Children under the age of 16 years

Randomisation

Randomisation was carried out after the patient had been listed for surgery and recruited. Randomisation was done using the help of the medical statistics support office and a randomisation schedule was sent to the pharmacy department who dispensed coded vials of treatment drugs or placebo. Block randomisation was used in order to keep the numbers of subjects in the two different groups as closely balanced as possible. The examiner did not know how the sequence was constructed.

Sample calculation size

A power of 80% was used to calculate the sample size. The sample size was calculated using a statistical software package with the help of the statistics department. The sample size was calculated prior to obtaining ethical committee approval. The calculation was performed as for categorical data as the primary outcome measure is a binary outcome measure. In order to detect a halving of the risk of PVR from 40% to 20%, 91 patients in each arm were required for 95% precision and 80% confidence.

Surgical Technique

Before starting surgery the coded vials were added to the infusion bag. Strict precautions were taken in accordance with theatre policy in the use of cytotoxic drugs. A stop clock was started at the beginning of the infusion and stopped when air exchange was done. If the infusion continued longer than 1 hour than the trial infusion was replaced with a new infusion bag. The volume of infusion was measured by weighing the infusion bag at the end of surgery. The concentration of 5-FU and LMWH used was 200 ug/ml and 5 IU/ml respectively. Normal saline was used for the placebo. Surgery was standard 3 port pars plana vitrectomy, retinopexy using endolaser where possible, and indirect laser for anterior breaks inaccessible with laser. Cryotherapy was allowed when neither form of laser delivery was possible.

Intraoperative and follow up assessments

Intraoperative details were entered directly into a database. Details included for each patient included operative findings, type of retinopexy used, type of intraocular tamponade used, any intraoperative complications, length of surgery, length of infusion and volume of infusion used.

Outcome Measures

The patients were followed for a minimum of 3 months and assessed for

1. Development of PVR
2. Status of retinal attachment
3. The development of any complications including keratopathy, glaucoma, hypotony, hyphaema, iatrogenic breaks, vitreous haemorrhage and retinal appearance.
4. Details of any operations if any.

Patient details were added directly to a database. Primary success was defined as a completely attached retina 3 months without any further vitreoretinal procedures. Any vitreoretinal procedure necessary to achieve retinal reattachment was entered as a reoperation. The following procedures were considered as non- vitreoretinal: cataract surgery, photocoagulation in attached retina and removal of gas due to raised intraocular pressure. Anatomical success was defined as completely attached retina (posterior to the scleral buckle if used) at the 6-month follow up.

Prospective analysis of risk factor formula and assessment of risk factors

Non clinical trial patients

After clinical examination of patients with rhegmatogenous retinal detachment undergoing vitrectomy who were not at high risk of developing PVR were included in this analysis. 130 patients were followed prospectively and were compared to 82 high-risk patients from the placebo treated group.

The exclusion criteria were similar:

1. Penetrating eye injury
2. History of blunt trauma to eye less than 6 months
3. Concurrent eye conditions, e.g., infection
4. History of intraocular eye surgery of less than 6 months
5. Current steroid treatment, topically or systemically

Preoperative assessment

A complete medical and ophthalmic history was taken and a full examination performed on all patients. Patients were examined both at the slit lamp and with indirect ophthalmoscopy. Specific attention was paid to the risk factors under investigation that included age, duration of symptoms, refractive status, preoperative use of cryotherapy/laser, presence of preoperative PVR, presence of preoperative uveitis, presence of preoperative vitreous haemorrhage, size of detachment, preoperative macula status (detached or not) and preoperative lens status ("phakic" or "aphakic"). All clinical details were entered directly into a database.

Intraoperative and follow-assessments

Operative details were recorded for each patient, including the use of cryotherapy and type of tamponade. The patients were followed for a minimum of six months and were assessed for the:

1. Retinal status
2. Development of postoperative PVR
3. Development of postoperative complications including cataract, glaucoma, infection and iatrogenic breaks

4. Details of any further surgery

Collection and storage of vitreous

At the beginning of the three port pars plana vitrectomy a neat vitreous biopsy was collected, before the infusion was started. The sample was added to a siliconised Eppendorf tube and stored at –70°C. Silicone Eppendorfs were used to prevent growth factors in the vitreous from adhering to the inner surface of the tubes. Prior to analysing the vitreous samples they were divided, labelled and alloquoted and re-frozen until used.

Cytokine and growth factor analysis

The following factors were studied in the vitreous samples collected: TGF-ß, b-FGF, IL-6 and IL-1 ß. Commercially available kits were used. These factors were chosen because they are involved in the wound healing response and also because Kon et al in risk factor study had chosen these factors (Kon *et al.*, 1999) . By repeating a similar analysis we were able to verify their results and also to correlate our clinical data with biological data.

Enzyme-Linked Immunosorbent Assays (ELISA)

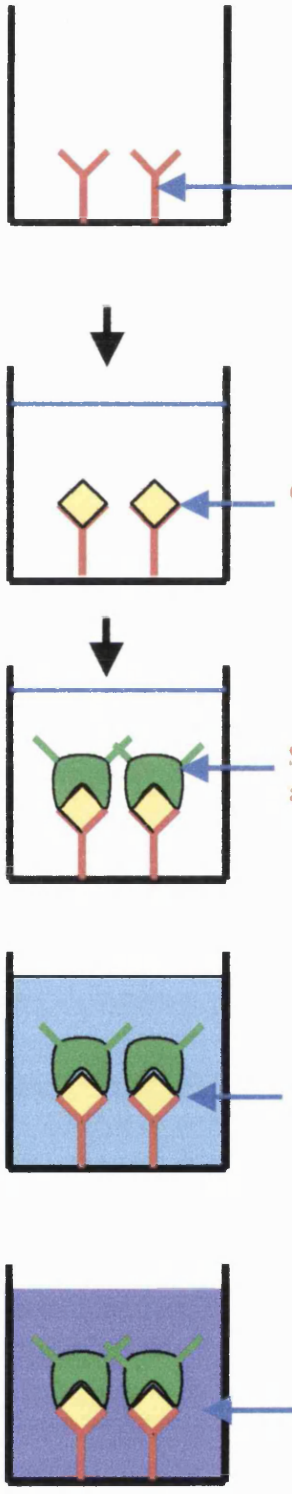
Cytokines and growth factors can be analysed in a number of methods. We chose ELISA in this study as there are readily available commercial kits and the results are reproducible. High sensitivity kits were used for b-FGF, IL-6 and IL-1 ß (HS, Quantikine™). Normal sensitivity kit was used for TGF-ß (Quantikine™). High-sensitivity kits for this factor were not available at the time of the study.

All vitreous samples were centrifuged for 10 minutes at 200g and the supernatants collected for assay. A pilot study was done to determine the correct dilution factor used in the assays.

All the assays used employed a similar technique **(Figure 2.2)**. Briefly the assays employed a quantitative- sandwich enzyme immunoassay. A monoclonal antibody specific to the appropriate cytokine had been coated onto a micro plate. Cytokine standards and samples were pipetted into the wells in duplicate. The immobilised antibody binds any cytokines present. After the appropriate incubation period the plates are washed in buffer to remove any unbound complexes. An enzyme linked polyclonal antibody is added that is specific to the cytokines. The plate is again incubated at room temperature. After washing, to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. After an incubation period, an amplifier solution is added to the wells and a colour reaction occurs in proportion to the amount of cytokine initially present **(Figure 2.3)**.

The optical density in each well is measured using a Titertek Plus MS2 micro plate reader and a standard curve (concentration against optical density) for the cytokine is plotted. The standard curve is obtained using the soft ware supplied by the plate reader. Any readings outside the standard curve were repeated after adjusting the dilution appropriately.

Figure 2.2 A diagrammatic representation of enzyme - linked immunosorbent assay for cytokines



A specific monoclonal antibody has been pre-coated onto a micro plate (Step 1)

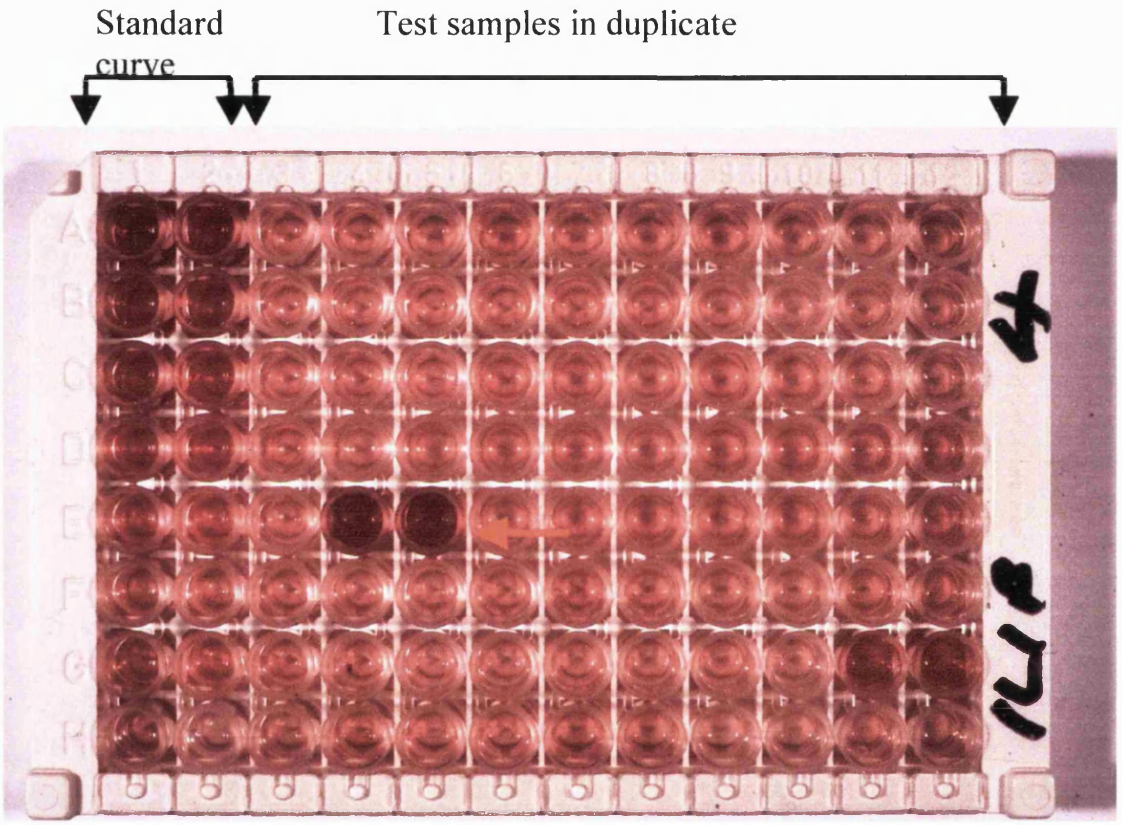
Cytokine in vitreous or standard binds to monoclonal antibody (Step 2)

An enzyme linked specific polyclonal antibody conjugate binds to the cytokine complex (Step 3)

Following a wash to remove any bound antibody-enzyme reagent, a substrate solution is added (Step 4)

After an incubation period, an amplifier solution is added to the wells and a colour reaction develops in proportion to the cytokine levels in step 1 (Step 5)

Figure 2.3 Coloured end product of the enzyme – linked immunosorbent assays

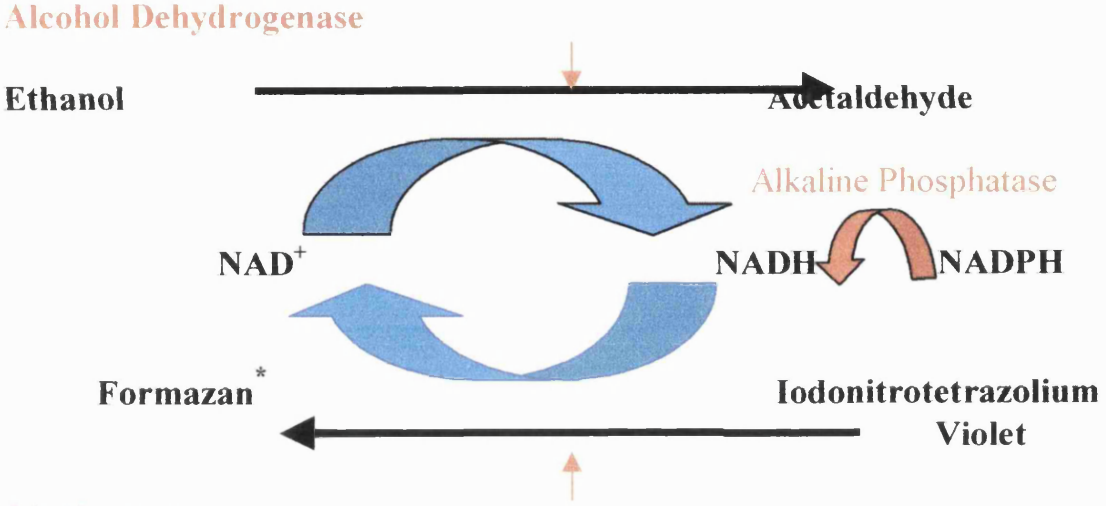


A typical plate showing illustrating the colour reaction product of the assay. The greater the intensity of the colour the higher the cytokine level. In readings where the intensity level is very high the assay for that sample is repeated with an appropriate dilution (arrow).

(NADPH) to reduced nicotinamide adenine dinucleotide (NADH). The NADH serves as a cofactor that activates the redox cycle driven by the secondary enzyme system consisting of alcohol dehydrogenase and diaphorase. In the reaction catalysed by diaphorase, NADH reduces tetrazolium salt (INT-violet or iodonitrotetrazolium violet) to produce an intensely coloured formazen dye and NAD^+. NAD^+ is reduced by ethanol, in an alcohol dehydrogenase-catalysed reaction, to regenerate NADH that can than re-enter the redox cycle. The rate of reduction of the tetrazolium salt and thus the amount of coloured product formed are directly proportional to the amount of cytokine bound in the first stage of the reaction cycle **(figure 2.4)**.

Figure 2.4 Amplification reaction in the high sensitivity (Quantikine ™ HS)

ELISA kits



* Coloured end product

Cytokine assays

IL-ß

IL-ß was measured using the high sensitivity assay system (Quantikine™ HS, R & D Systems). 150µl of standards or sample (1:3 dilution [Serum Calibrator Diluent HD6]) were added to the wells. The concentrations used to plot the standard curve were 8, 4, 2, 1, 0.5, 0.25, 0.125 and 0 pg/ml. The optical density was made at 490 nm.

IL-6

IL-6 was measured using the high sensitivity assay system. 200 µl standards or sample (1:75 dilution [Urine Calibrator Diluent HD5B]) were added to the wells. The concentrations used to plot the standard curve were 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156 and 0 pg/ml. The optical density was made at 490 nm.

Basic-FGF

Basic FGF was measured using the high sensitivity assay system. 150 µl standards or sample (1:15 dilution [Urine Calibrator Diluent HD5B]) were added to the wells. The concentrations used to plot the standard curve were 16, 8, 4, 2, 1, 0.5, 0.25 and 0 pg/ml. The optical density was made at 490 nm.

TGF-ß2

TGF-ß2 assay measures the activated form of TGF- ß2 and therefore any latent TGF- ß2 needs to be activated first. This was achieved by the adding 25µl of 1N HCL 125µl to vitreous, incubating for 10 minutes and than adding 25µl of 1N NaOH/0.5M HEPES. 100 µl standards or activated sample (1:24 dilution [Urine Calibrator Diluent RD51]) were added to the wells. The concentrations used to plot the standard curve were 2000, 1000, 500, 250, 125, 62.5, 31.2 and 0 pg/ml. The optical density was made at 450 nm.

Protein assay

The total protein concentration in the vitreous was measured using the Bio-Rad Protein Microassay (Bio-Rad). This is a colorimetric assay that allows the rapid measurement of multiple samples. It uses a solution of cupric ions that form a copper/protein complex (coloured component). The vitreous samples were first centrifuged at 1000g for 10 minutes and the supernatant used. A standard curve was prepared by diluting bovine serum albumin (BSA; Sigma) in PBS; the concentrations used were 4000, 2000, 1000, 500, 250, 125, 125, 62.5 μg/ml. 5 μl of samples or standards were added in triplicate to a sterile 96 well microtiter plate. 25μl of reagent A (alkaline copper tartrate solution) followed by 200μl of reagent B were added to each well. The plate was than gently agitated to mix the reagents and incubated for 15 minutes at room temperature. The optical density of the coloured product was measured at 650 nm using a microplate reader (Titertek Plus MS2 reader; ICN Flow). A standard curve was constructed using the software supplied by the plate reader. The level of protein was calculated and expressed in μg/ml. If the reading of the vitreous protein was above 4000μg/ml than the sample was diluted appropriately and a further analysis preformed.

Data Handling and Statistical Methods

All patient details were entered directly into a database using Microsoft Access 97 (Microsoft Cooperation). **(Appendix I)**. All data was treated as according to the provisions of the Data Protection Act (1984). The data was backed up daily onto a CD Rom.

All statistical analysis was done with the help of the Statistics Department. *Multiple regression analysis* was used to analyse the risk factors (both clinical and biological). Multiple regression analysis yields a *regression model* in which the dependent variable (e.g., development of PVR) is expressed as a combination of the explanatory variables (e.g., preoperative PVR, size of detachment).

Multiple regression analysis was used as it allows us to:

1. Remove any possible "nuisance" from a study of the relationship of two variables of interest. E.g., if we look at preoperative PVR we would want to remove the effect of silicone oil, as silicone oil may be used in cases of preoperative PVR.
2. It allows us to explore variables that we would not expect to have prognostic significance. E.g., the effect of racial origin.
3. It allows us to develop a prognostic index from several variables for predicting the outcome of interest. E.g., to develop a multivariate rule.

As in many cases of multivariate analysis it is usual to first analyse the simple relation between each potential variable and the outcome variable of interest whilst ignoring all other variables. In other words *linear regression* was first performed on the data set to define the *independent risk factors*. These were than analysed in the regression model.

The *chi-squared test* was used for any linear associations between the development of PVR and treatment given (including other variables) in the trial patients. The chi-squared was also used to analyse the association of vitreous cytokine levels and PVR. The *two-tailed* independent sample T-test was used to analyse the results for individual protein and cytokine levels in the vitreous.

Chapter 4

Results

Clinical Study - The use of adjuvant 5-FU and heparin in the treatment of patients at high risk of PVR

Patient profile

A total of 174 patients were recruited to take part in the study. At the 6- month follow up visit complete data entry was available for 167 patients. All patients attended the 2 week and 8 week follow up. There was missing data for 5.7% (5/87) in the placebo group for both the 3 month and 6 month follow up and 2.3 % (2/87) for the combined treatment group.

One patient (placebo group) died during the follow up. Three patients had silicone oil tamponade (clinically indicated intra-operatively) two in the adjuvant and one in the placebo group. **Table 3.1** shows the preoperative characteristics of the study group population.

Table 3.1 Preoperative patient details.

Characteristic	Placebo group	Treatment group
Age (years)	64.3 (range 18– 93)	62 (range 27– 90)
Myopia greater than –5.00 diopter	13 (14.9%)	16 (18.4%)
Lens status: Aphakia	35 (40.2%)	38 (43.7%)
Phakia	52 (59.8%)	49 (56.3%)
Uveitis	68 (78.2%)	64 (73.6%)
Cryotherapy/ laser within 3 months	18 (20.7%)	13 (14.9%)
Previous scleral buckle	13 (14.9%)	10 (11.5%)
Preoperative PVR: None	36 (41.4%)	32 (36.8%)
Grade B	36 (41.4%)	43 (49.4%)
Grade CP1	15 (17.2%)	12 (13.8%)
Vitreous haemorrhage	20 (23.0%)	29 (33.3%)
Size of detachment: 1 quadrant	2 (2.3%)	3 (3.5%)
2 quadrants	19 (21.8%)	27 (31.0%)
3 quadrants	37 (42.5%)	32 (36.8%)
4 quadrants	29 (33.3%)	25 (28.7%)
Macula status: On	11 (12.6%)	12 (13.8%)
Off	76 (87.4%)	75 (86.2%)

Clinical outcome

The primary outcome measure was postoperative PVR. PVR was considered present if there was greater than 1 clock hour of grade C PVR as defined by the updated version of the Retina Society classification (Machemer *et al.*, 1991). Secondary outcome measures included primary success rate of surgery, no of reoperations, final visual acuity and any intraoperative or postoperative complications.

Postoperative PVR

The primary outcome measure of postoperative PVR could be evaluated in 167 patients (82 in the placebo and 85 in the combined group). This data was missing in 7 patients (5 in the placebo and 2 in the combined group). The rate of postoperative PVR in the placebo group was 26.4% (23/87) and 12.6% (11/87) in the combined treatment group. The Mantel-Haenszel chi square test yielded a P value of 0.02.

Relationship between preoperative PVR and postoperative PVR

The rate of postoperative PVR in the placebo group was 26.4% (23/87) and 12.6% (11/87) in the combined treatment group. **Table 3.2 and 3.3** show the relationship between postoperative PVR and the presence of preoperative PVR for both treatment groups.

Table 3.2 Relationship between pre and postoperative PVR in treatment group.

Postoperative PVR	Preoperative PVR		
	Grade A	Grade B	Grade CP1
No	26 (81.2%)	41 (95.3%)	9 (75%)
Yes	6 (18.8%)	2 (4.9%)	3 (25%)
Total	32 (100%)	43 (100%)	12 (100%)

$X^2_{(2)} = 5.226$, p=0.078

There was no statistical significant difference between the incidence of postoperative PVR compared with preoperative PVR

Table 3.3 Relationship between pre and postoperative PVR in placebo group.

Postoperative PVR	Preoperative PVR		
	Grade A	Grade B	Grade CP1
No	28 (77.8%)	29 (80.5%)	7 (46.7%)
Yes	8 (22.2%)	7 (19.5%)	8 (53.3%)
Total	36 (100%)	36 (100%)	15 (100%)

$X^2_{(2)} = 6.81$, p=0.03

A significantly higher (p=0.03) proportion of patients with preoperative PVR developed postoperative PVR.

Relationship between position of break and postoperative PVR

Table 3.4 shows the relationship between position of break and postoperative PVR for both treatment groups.

Table 3.4 Relationship between position of break and postoperative PVR.

	Placebo group			**Treatment group**		
	Position of break			**Position of break**		
	Superior	**Inferior**	**Combined**	**Superior**	**Inferior**	**Combined**
No PVR	46 (76.67%)	10 (66.67%)	8 (66.67%)	58 (89.23%)	8 (88.89%)	10 (76.92%)
PVR	14 (23.33%)	5 (33.33%)	4 (33.33%)	7 (10.77%)	1 (11.11%)	3 (23.08%)
	P= 0.414 (Fisher's exact)			P=0.563 (Fisher's exact)		

There was no statistical difference between position of break and postoperative PVR for either treatment group.

Reoperations

The primary success rate of retinal reattachment without the need for further surgery was 58/87 (66.7%) and 66/87 (75.9%) in the placebo and treatment group respectively. Seven (8.3 %) patients in the placebo group and 2 (2.3%) patients in the combined group developed postoperative PVR and either declined surgery or intervention was not indicated.

The number of one or more reoperations in the placebo group was 22 (3 patients had more than one). The number of reoperations due to PVR was 16. The total number of one or more reoperations in the combined treatment group was 19 (3 patients had more than

one). The number of reoperations due to PVR was 9. **Table 3.5** shows the results of the secondary outcome of vitreoretinal reoperations.

Table 3.5 Vitreoretinal reoperations.

	Primary success (single vitreoretinal procedure)	**One or more reoperations**	**Final reattachment rate**
Treatment group	66/87 (75.9%)	19 (21.8%)	79/87 (90.1%)
Placebo group	58/87 (66.7%)	22 (25.3%)	75/87 (86.2%)

Visual acuity outcome

Presenting visual acuity and visual acuity at last follow up are shown **Figure 3.1 and 3.2** for both treated and placebo groups respectively. At the last follow up in the treated group 12/87 (13.79%) had no change in visual acuity, 53/87 (60.92%)had improved visual acuity and 22/87 (25.29%)had worse visual acuity. In the placebo group 11/87 (12.64%)had no change in visual acuity, 40/87 (45.98%) had improved visual acuity and 36/87 (41.38%)had worse visual acuity. **Table 3.6** shows the final change in visual acuity between the two treatment groups.

Table 3.6 shows the final change in visual acuity between the two treatment groups.

Treatment group	No change	Better	Worse
Placebo	11/87 (12.64%)	40/87 (45.98%)	36/87 (41.38%)
Treatment	12/87 (13.79%)	53/87 (60.92%)	22/87 (25.29%)

$X^2_{(2)} = 3.9$, p=0.048

Patients in the placebo group had a significantly worse ($X^2_{(2)} = 3.9$, p=0.048) final visual acuity (patients who were worse and no change were combined and compared to those who were better). Naturally macular status preoperatively was highly linked to postoperative visual acuity.

one). The number of reoperations due to PVR was 9. **Table 3.5** shows the results of the secondary outcome of vitreoretinal reoperations.

Table 3.5 Vitreoretinal reoperations.

	Primary success (single vitreoretinal procedure)	**One or more reoperations**	**Final reattachment rate**
Treatment group	66/87 (75.9%)	19 (21.8%)	79/87 (90.1%)
Placebo group	58/87 (66.7%)	22 (25.3%)	75/87 (86.2%)

Visual acuity outcome

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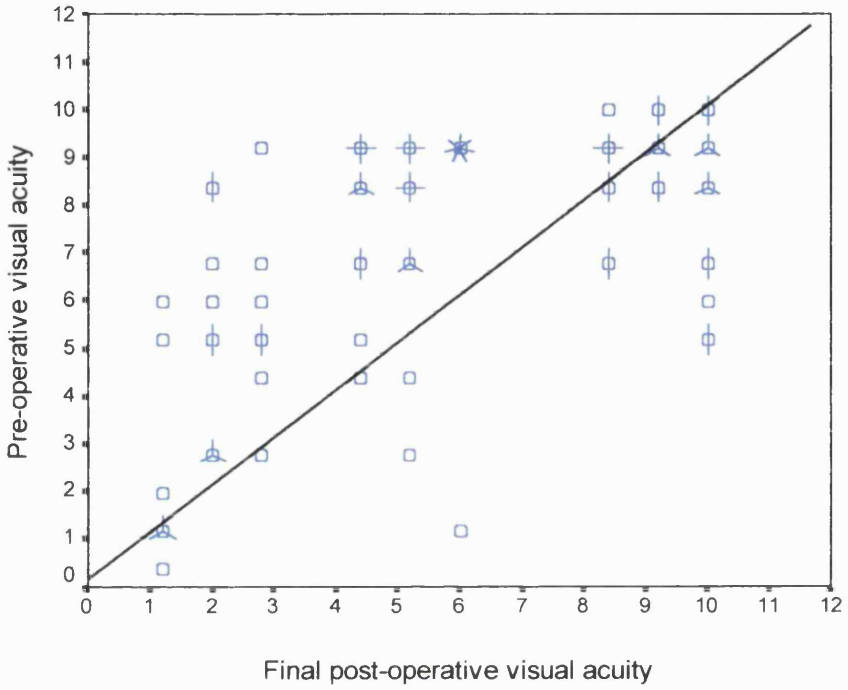
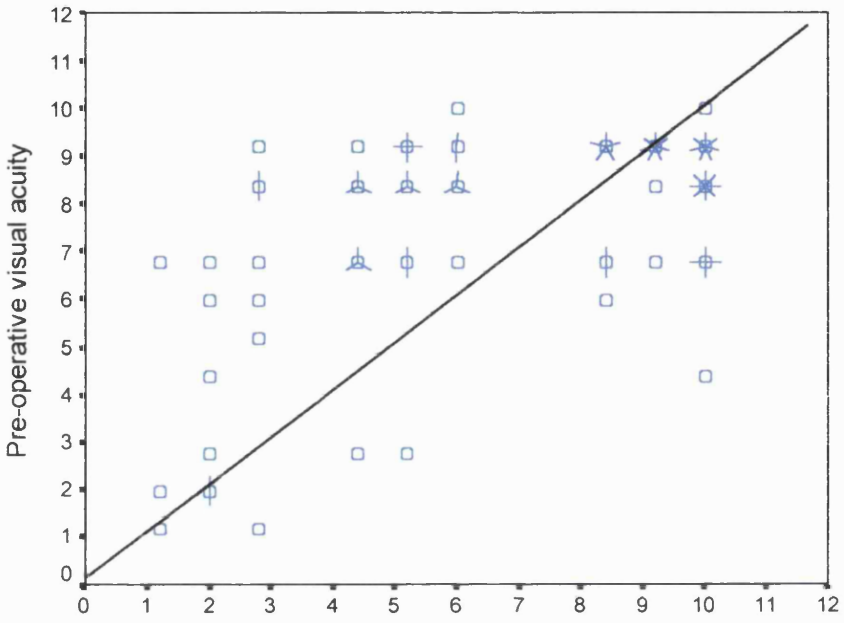
Figure 3.1 Pre and postoperative visual acuity in the 5-FU/ heparin treated group



Key:
1= 6/5
2= 6/6
3= 6/9
4= 6/12
5= 6/18
6= 6/24
7= 6/36
8= 6/60
9= CF
10= HM
11= PL
12= NPL

A scatter plot (sunflower) of visual acuity, each line of the sunflower patel represents a patient, and so the figure below represents three cases.

Figure 3.2 Pre and postoperative visual acuity in the placebo treated group.



Key:
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3= 6/9
4= 6/12
5= 6/18
6= 6/24
7= 6/36
8= 6/60
9= CF
10= HM
11= PL
12= NPL

A scatter plot (sunflower) of visual acuity, each sunflower petal represents a case.

Relationship between preoperative PVR and final visual acuity

Table 3.7 and 3.8 show the final visual acuity of the two treatment groups classified by preoperative PVR.

Table 3.7 Preoperative PVR and final visual acuity in the treatment group.

Final Visual acuity	Preoperative PVR		
	Grade A	Grade B	Grade CP1
6/60 or better	19 (59.38%)	32 (74.42%)	4 (33.33%)
Worse than 6/60	13 (40.62%)	11 (25.58%)	8 (66.67%)

P=0.164 (Fisher's exact)

There was no statistically significant difference between preoperative PVR and final visual acuity of worse than 6/60.

Table 3.8 Preoperative PVR and final visual acuity in the placebo group.

Final Visual acuity	Preoperative PVR		
	Grade A	Grade B	Grade CP1
6/60 or better	17 (47.22%)	20 (55.56%)	4 (26.67%)
Worse than 6/60	19 (52.78%)	16 (44.44%)	11 (73.33%)

P=0.031 (Fisher's exact)

A significantly higher proportion (p=0.031) of patients achieved a visual acuity of 6/60 or worse if they had preoperative grade CP1 PVR.

Relationship between postoperative PVR and final visual acuity

The final visual acuity classified into those that developed postoperative PVR and those that did not is shown in **Table 3.9.**

Table 3.9 Postoperative PVR and final visual acuity.

Final visual acuity	**Postoperative PVR**	
	No	**Yes**
6/60 or better	94 (70.15%)	2 (6.06%)
Worse than 6/60	40 (29.85%)	31 (93.94%)
Total	134 (100%)	33 (100%)

P<0.0001 (Fisher's exact)

Patients who developed postoperative PVR had a significantly worse final visual acuity ($p<0.0001$).

Complications

There were very few complications in either treatment group. Two significant intraoperative complication were recorded in the 5-FU/heparin group, in one there was retinal incarceration and in the other a choroidal haemorrhage. In one patient of the placebo group the infusion cannula entered the subretinal space.

The only other complications recorded were mild corneal oedema in two cases (5-FU/ heparin group) that did not significantly impair visualisation of the surgical field. There were 10 postoperative hyphaemas divided equally between the two groups. All the hyphaemas were mild and settled with conservative treatment alone.

Summary of clinical trial

The incidence of postoperative PVR was significantly lower (P= 0.019) in the 5-FU/heparin therapy compared to the placebo group. Of the placebo group 26.4% (23/87) and 12.6% (11/87) of the 5-FU/heparin group developed postoperative PVR.

In the placebo group the number of patients undergoing more than one operation was 25.3% (22/87) and the number of reoperations due to PVR was 16/87 (18.4%). In the 5-FU/heparin group the number of patients undergoing more than one operation was 21.8% (19/87) and the number of reoperations due to PVR was 10.3% (9/87). Patients in the placebo group had a significantly worse (p=0.048) final visual acuity compared to the 5-FU/heparin group. There were no differences in complication rates between the two groups.

Assessment of clinical risk factor formula

Patient profiles

Complete data were collected on 212 patients out of 220. Out of these patients complete data was available on 97.7% (130/132) of the low risk patients and 94.3% (82/87) of the high-risk patients. The mean age of the low risk group was group 56.4 years (range 15-88) and in the high risk 64.3 years (range 15-93). The male to female ratio in the low risk group was 72:58 and 52:35 in the high-risk group. **Table 3.10** shows the preoperative characteristics.

Table 3.10 Preoperative patient details

Characteristic	High risk group	Low risk group
Age (years)	64.8 (range 15– 93)	56.5 (range 15– 88
Myopia greater than –5.00 diopter	13 (15.8%)	36 (27.6%)
Lens status: Aphakia	35 (42.7%)	13 (10.0%)
Phakia	52 (57.3%)	119 (90.0%)
Uveitis	64 (78.0%)	40 (30.8%)
Cryotherapy/ laser within 3 months	18 (22.0%)	14 (10.8%)
Preoperative PVR: None	34 (41.5%)	117 (90.0%)
Grade B	34 (41.5%)	11 (8.5%)
Grade CP1	14 (17.1%)	2 (1.5%)
Vitreous haemorrhage	20 (24.4%)	13 (10.0%)
Size of detachment: 1 quadrant	2 (2.4%)	33 (25.4%)
2 quadrants	19 (23.1%)	70 (53.8%)
3 quadrants	34 (41.5%)	20 (15.4%)
4 quadrants	27 (32.9%)	5 (3.8%)
Macula status: On	11 (13.4%)	46 (35.4%)
Off	71 (86.6%)	84 (64.6%)

Development of postoperative PVR

In the low risk group 9.1% (12/132) developed postoperative PVR compared to 28.0% in the high risk group (23/84). The incidence of postoperative PVR was significantly higher in the high-risk group (p=0.0001). It was possible to predict postoperative PVR using clinical risk factors only.

Relationship between preoperative PVR and surgical outcome

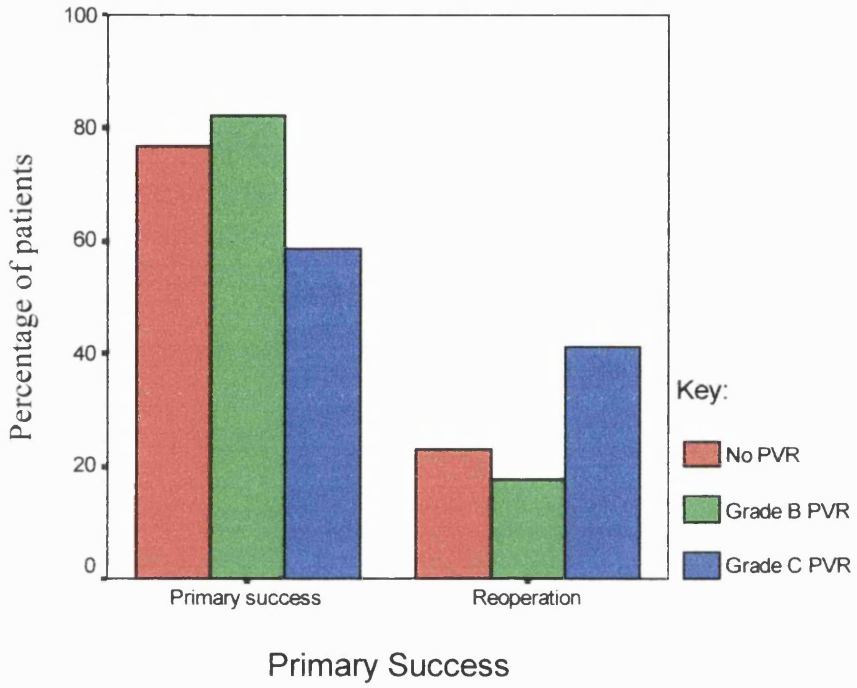
The overall primary success rate for vitrectomy was 75.9%. For the high-risk group the primary success rate was 71.2% and for the low risk group 78.8%. **Table 3.11 and Figure 3.3** shows the relationship between preoperative PVR and surgical outcome.

Table 3.11 Relationship between preoperative PVR and surgical outcome.

Outcome	**Preoperative PVR**		
	No	**Grade B**	**Grade C**
Primary success	112 (76.19%)	38 (84.44%)	7 (46.67%)
Redetachment	35 (23.81%)	7 (15.56%)	8 (53.33%)
Total	147 (100%)	45 (100%)	15 (100%)

There is a statistically significant difference between the rate of primary success between preoperative PVR Grade C plus ($X^2_{(2)} = 8.797$, p=0.012) compared to either Grade B or no PVR.

Figure 3.3 Relationship between preoperative PVR and surgical outcome.



Primary success rate of patients with no preoperative PVR, grade B and grade C PVR. The primary success rate (only a single vitreoretinal procedure was required to achieve a complete anatomical reattachment) was significantly greater in those patients with either no or Grade B PVR compared to grade C PVR.

Relationship between preoperative PVR and postoperative PVR

In the low risk group 9.1% (12/132) developed postoperative PVR compared to 28.0% in the high risk group (23/84). **Table 3.12 and Figure 3.4** shows the association between preoperative PVR and postoperative PVR

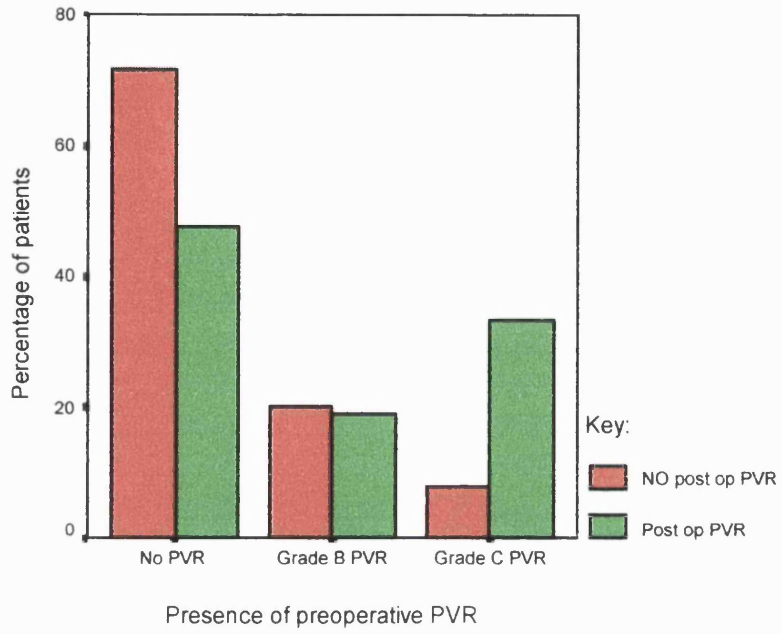
Table 3.12 Relationship between postoperative PVR and preoperative PVR.

Postoperative PVR	**Preoperative PVR**		
	No	**Grade B**	**Grade C**
Yes	19 (12.58%)	7 (15.56%)	9 (56.25%)
No	132 (87.42%)	38 (84.44%)	7 (43.75%)
Total	151 (100%)	45 (100%)	16 (100%)

P=<0.0001 (Fisher's exact)

A significantly greater number (p=<0.0001) of patients with preoperative PVR (Grade CP1 plus) developed postoperative PVR.

Figure 3.4 Relationship between preoperative PVR and postoperative PVR



Development of postoperative PVR compared with the presence of preoperative PVR. The rate of postoperative PVR was significantly higher ($p<0.05$) in those patients who presented with preoperative PVR.

Relationship between postoperative PVR and further reattachment surgery

The association between reoperation and postoperative PVR is shown in **Table 3.13** and **Figure 3.5.**

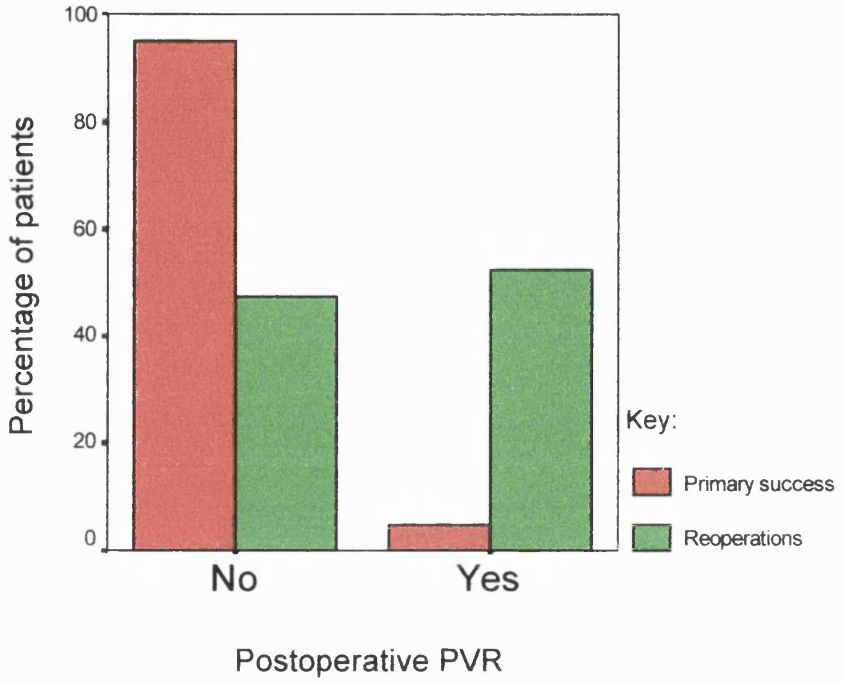
Table 3.13 Relationship between reoperation and postoperative PVR.

Outcome	Postoperative PVR	
	No	Grade C
Reoperation	24 (13.79%)	26 (78.79%)
Primary success	150 (86.21%)	7 (21.21%)
Total	174 (100%)	33 (100%)

$X^2_{(2)} = 63.962$, p=<0.0001

A significantly greater proportion of patients (p=<0.0001) achieved retinal reattachment with a single operation if they did not develop postoperative PVR.

Figure 3.5 Relationship between postoperative PVR and surgical outcome.

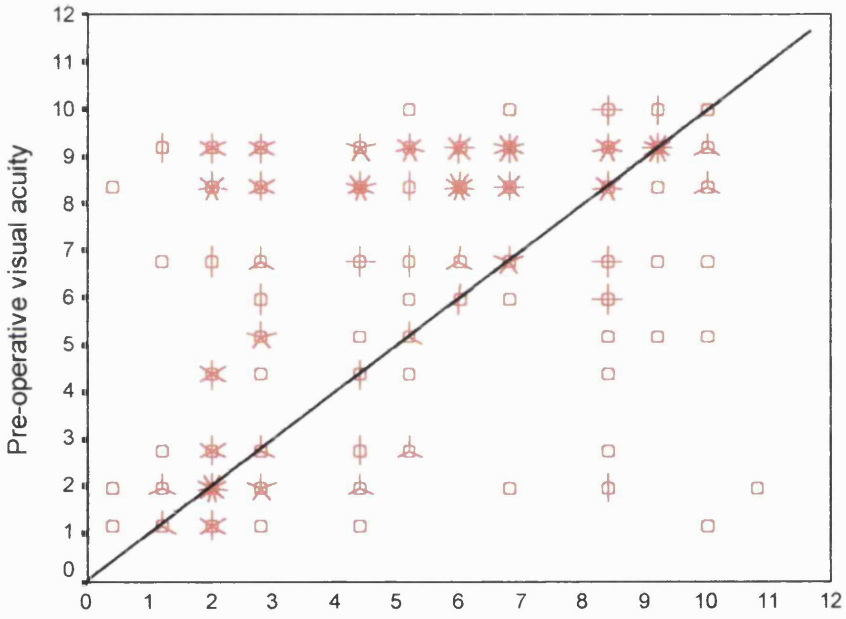


The primary success rate in patients that did or did not develop postoperative PVR. The primary success rate (complete anatomical retinal reattachment) was significantly higher ($p<0.05$) in those patients that did not develop postoperative PVR.

Relationship between preoperative PVR and final visual acuity

The change in visual acuity at presentation compared to the last follow up is shown in **Figure 3.6.** At the last follow up visual acuity had improved in 51.83%, had decreased in 31.65% and remained the same in 16.51% patients.

Figure 3.6 Pre- and postoperative visual acuity.



Key:
1= 6/5
2= 6/6
3= 6/9
4= 6/12
5= 6/18
6= 6/24
7= 6/36
8= 6/60
9= CF
10= HM
11= PL
12= NPL

A scatter plot (sunflower) of visual acuity, each sunflower petal represents a case.

Table 3.14 shows the final visual acuity classified by preoperative PVR. A significantly higher proportion (p=<0.0001) of patients achieved a visual acuity of worse than 6/60 in the preoperative PVR Grade C group.

Table 3.14 Relationship between final visual acuity and preoperative PVR.

Final visual acuity	**Preoperative PVR**		
	No	**Grade B**	**Grade C**
6/60 or better	132 (87.42%)	38 (84.44%)	7 (43.75%)
Worse than 6/60	19 (12.58%)	7 (15.56%)	9 (56.25%)
Total	151 (100%)	45 (100%)	16 (100%)

P=<0.0001 (Fisher's exact)

Relationship between postoperative PVR and final visual acuity

Table 3.15 shows the final visual acuity of the classified by postoperative PVR.

Table 3.15 Relationship between final visual acuity and postoperative PVR.

Final visual acuity	**Postoperative PVR**	
	No	**Grade C**
6/60 or better	40	1
Worse than 6/60	20	21
Total	60	22

P=<0.0001 (Fisher's exact test)

A significantly higher proportion of patients achieved a visual acuity of 6/60 or better in the non- postoperative PVR group.

Relationship between reoperations and final visual acuity

Table 3.16 shows the relationship between final visual acuity and reoperations. A reoperation was any vitreoretinal procedure performed to reattach the retina or remove PVR membranes. Operations such as cataract extraction and removal of silicone oil were excluded.

Table 3.16 Relationship between reoperations and final visual acuity.

Final visual acuity	**One or more reoperations**	
	No	**Yes**
6/60 or better	90 (89.11%)	18 69.23%)
Worse than 6/60	11 (10.89%)	8 (30.77%)
Total	101 (100%)	26 (100%)

P=0.026 (Fisher's exact)

The results show that eyes that had undergone only one operation achieved a final visual acuity of 6/60 or better in a significantly higher number of cases (p=0.026) compared to those that had multiple operations.

Summary of clinical results

Complete data were available on 212 out of 220 patients. Out of these patients complete data was available on 97.7% (130/132) of the low risk patients and 94.3% (82/87) of the high-risk patients. The primary success rate of retinal reattachment surgery was 75.9%. For the high-risk group the primary success rate was 71.2% and for the low risk group 78.8%.

A significantly greater proportion of patients in the high- risk group developed postoperative PVR ($p=<0.0001$) compared to the low risk group.

A significantly greater proportion ($p=<0.05$) of eyes that had developed postoperative PVR had an unsuccessful primary reattachment rate and worse final visual acuity compared to those that did not develop postoperative PVR.

In cases that had undergone more than one operation the final visual acuity was significantly worse ($p=<0.05$) compared to those that had undergone only one operation.

Vitreal cytokine and protein levels

Relationship between cytokine levels and preoperative PVR

Table 3.17 and figure 3.7 show the median cytokine and protein levels of patients with and without preoperative PVR.

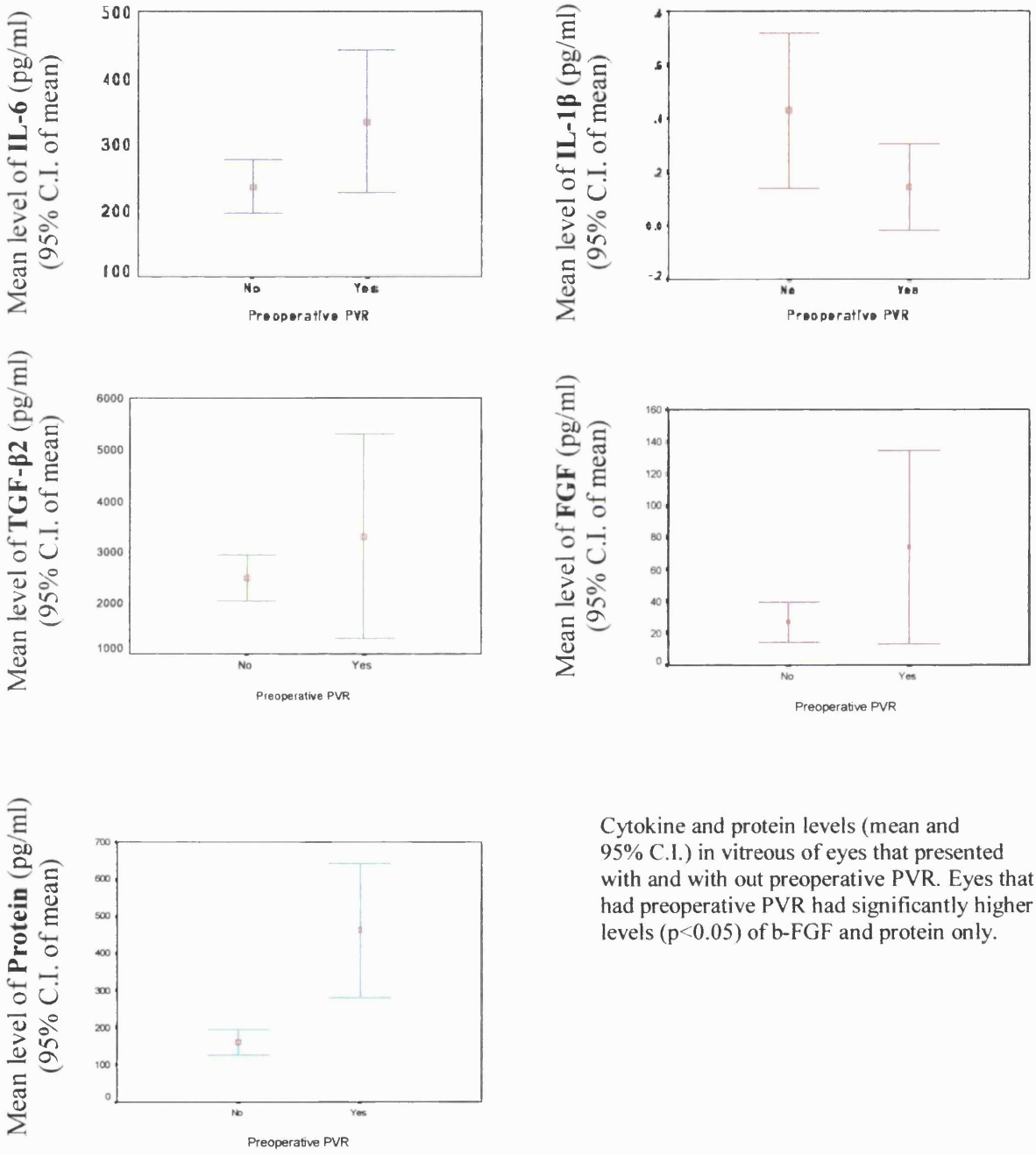
Table 3.17 Cytokine levels and preoperative PVR.

Factor	Preoperative PVR		
	No Median (inter quartile range)	**Yes Median (inter quartile range)**	**P value**
TGF-β2 (pg/ml)	911.04 (29.55 to 167.3)	1239.84 (24.75 to 153.28)	0.173
b-FGF (pg/ml)	2.31 (0.77 to 11.15)	12.92 (6.59 to 19.61)	<0.001
IL-1β (pg/ml)	0.03 (0.001 to 0.238)	0.03 (0.001 to 0.123)	0.890
IL-6 (pg/ml)	106.88 (32.33 to 369.68)	321.83 (63 to 628.58)	0.60
Protein (μg/ml)	75.5 (30.92 to 152.5)	173 (112 to 625.5)	<0.001

Two-sample Wilcoxon rank sum test

The median levels of b-FGF (p=<0.001) and protein (p=<0.001) were significantly higher in patients with preoperative PVR compared to those without preoperative PVR. There was no statistically significant difference in the mean levels of IL-6, IL-1β and TGF-β2.

Figure 3.7 Levels of TGF-β2, b-FGF, IL-1β, IL-6 and protein in the vitreous of patients who did and did not have preoperative PVR



Cytokine and protein levels (mean and 95% C.I.) in vitreous of eyes that presented with and with out preoperative PVR. Eyes that had preoperative PVR had significantly higher levels ($p<0.05$) of b-FGF and protein only.

Relationship between cytokine levels and risk group

Table 3.18 and figure 3.8 show the relationship between cytokine levels classified by treatment group

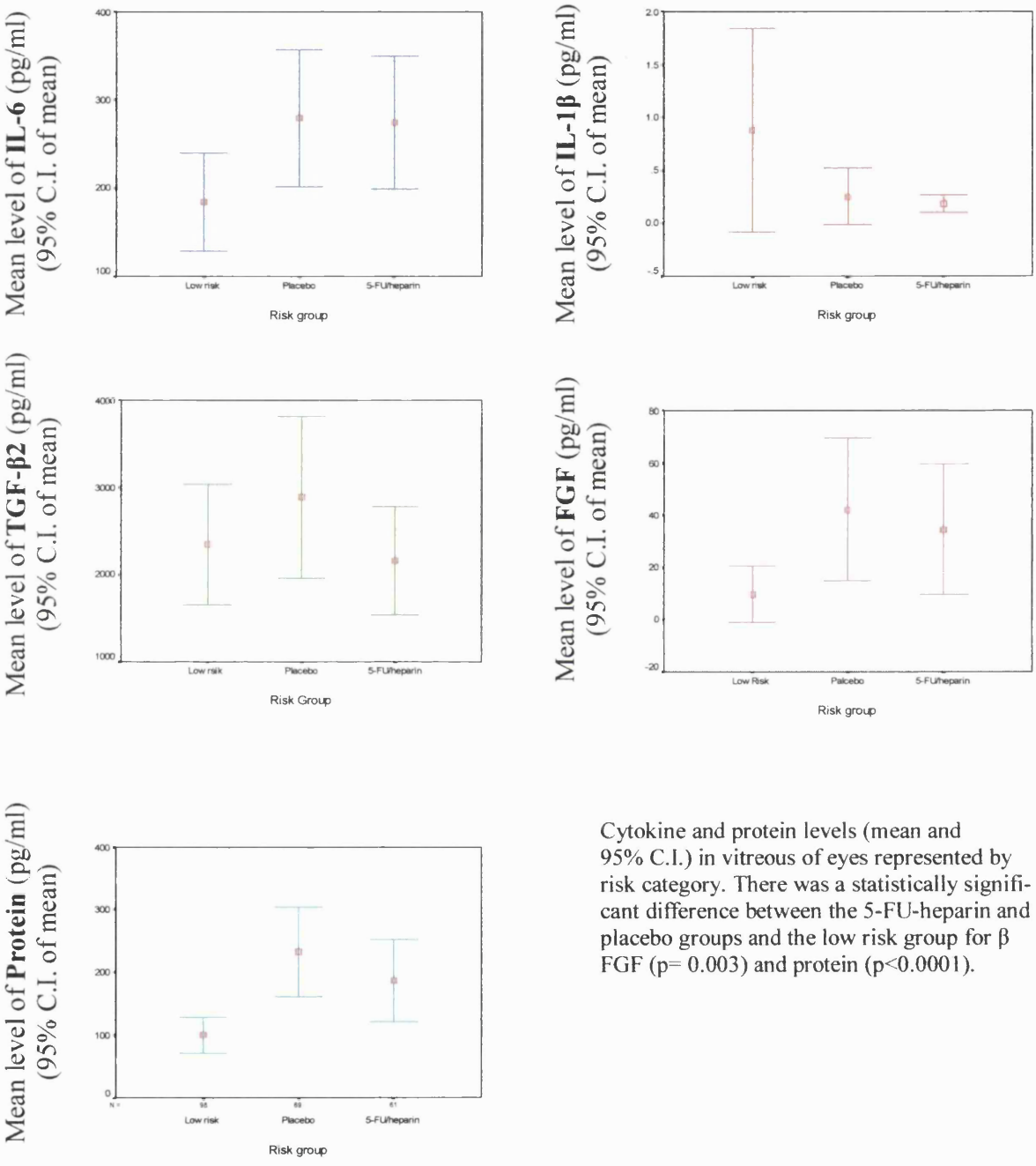
Table 3.18 Cytokine levels and treatment group.

Factor	Treatment group				
	5FU-Heparin treated Median (Inter-quartile range)	**Placebo treated** Median (Inter- quartile range)	**Low risk group** Median (Inter- quartile range)	**P_1**	**P_2**
TGF-β2 (pg/ml)	1279.44 (703.2 to 3596.4)	1459.92 (703.2 to 3596.4)	1630.56 (934.8 to 3143.04)	0.450	0.402
b-FGF (pg/ml)	4.5 (0.81 to 19.61)	6.05 (1.28 to 15.71)	1.7 (0.54 to 5.19)	0.866	0.003
IL-1β (pg/ml)	0.056 (0.001 to 0.207)	0.03 (0.001 to 0.181)	0.006 (0.001 to 0.291)	0.531	0.742
IL-6 (pg/ml)	190.24 (48.15 to 422.67)	144.27 (31.73 to 560.18)	84.79 (28.28 to 287.75)	0.758	0.07
Protein (μg/ml)	97 (41 to 181)	112 (53.19 to 249.44)	53.5 (22 to 117)	0.159	<0.0001

P_1 (5-FU/heparin versus placebo)
P_2 (5-FU/heparin – placebo versus low risk group)

There was no statistical significance of any of the factors investigated between the 5-FU-heparin and placebo groups. There was a statistically significant difference between the 5-FU-heparin and placebo groups and the low risk group for β FGF ($p= 0.003$) and protein ($p<0.0001$). The p values for Il-6 and IL-1β are 0.007 and 0.742.

Figure 3.7 Levels of TGF-β2, b-FGF, IL-1β, IL-6 and protein in the vitreous of patients who did and did not have preoperative PVR



Cytokine and protein levels (mean and 95% C.I.) in vitreous of eyes represented by risk category. There was a statistically significant difference between the 5-FU-heparin and placebo groups and the low risk group for β FGF ($p= 0.003$) and protein ($p<0.0001$).

Relationship between cytokine levels and postoperative PVR

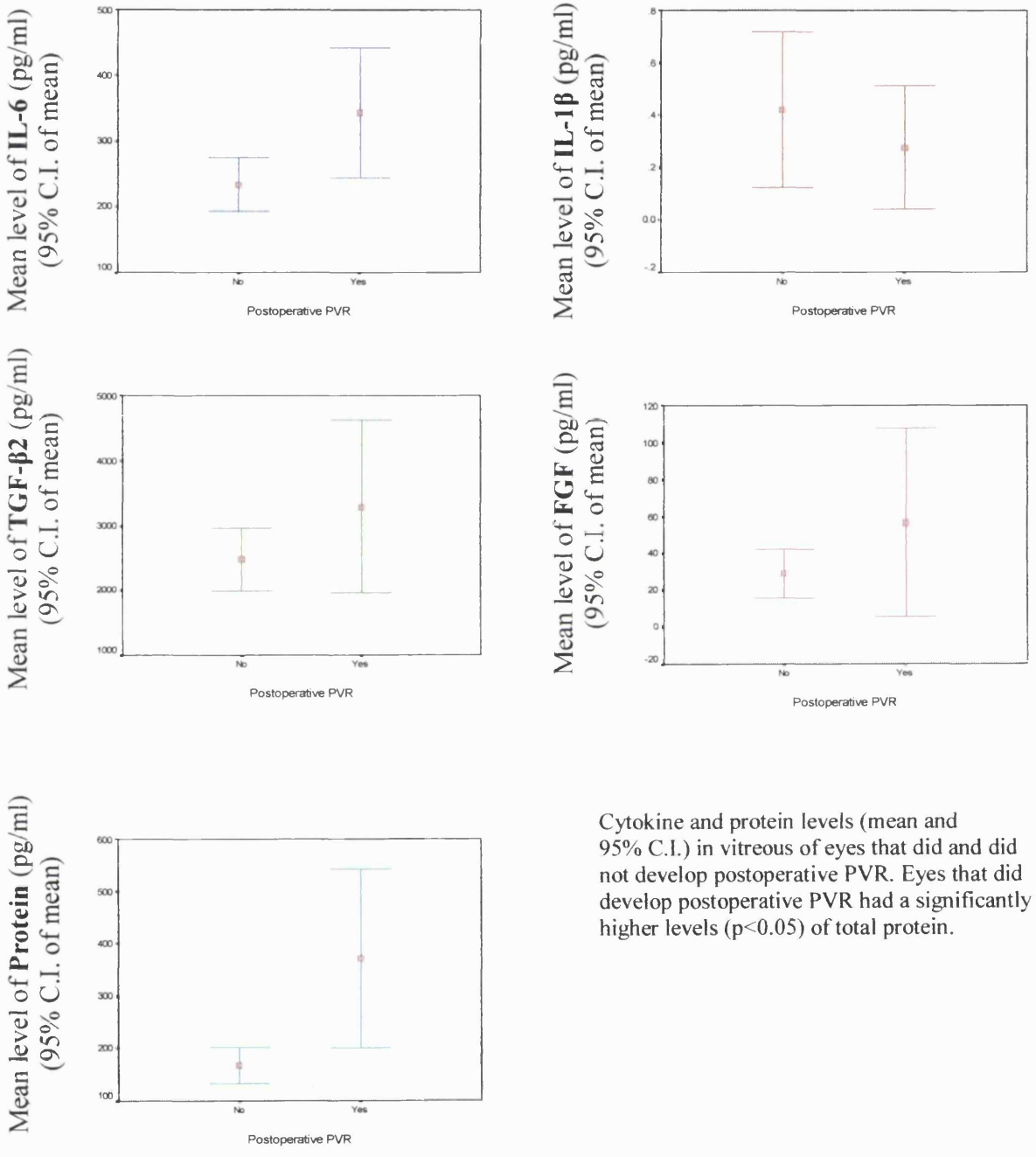
The levels of cytokines and protein in the vitreous of patients (placebo and low risk groups) who did and did not develop postoperative PVR and the difference between the two groups are shown in **Table 3.19 and figure 3.9.**

Table 3.19 Cytokine levels and postoperative PVR.

Factor	**Postoperative PVR**		
	No (inter-quartile range)	**Yes** (inter-quartile range)	**P value**
TGF-β2 (pg/ml)	1558.08 (703.2 to 3143.04)	1825.32 (1113.12 to 4747.8)	0.129
b-FGF (pg/ml)	2.24 (0.77 to 8.52)	6.05 (1.56 to 14.15)	0.083
IL-1β (pg/ml)	0.015 (0.001 to 0.255)	0.003 (0.001 to 0.273)	0.686
IL-6 (pg/ml)	89.25 (42.56 to 347.85)	252.68 (25.28 to 609.75)	0.236
Protein (μg/ml)	67 (27 to 149)	115 (82 to 272)	0.002

The median level of protein in the vitreous was significantly higher (p=0.002) in patients who developed postoperative PVR compared to those that did not. There was no statistically significant difference in the median levels of the cytokines between the two groups.

Figure 3.7 Levels of TGF-β2, b-FGF, IL-1β, IL-6 and protein in the vitreous of patients who did and did not have preoperative PVR



Cytokine and protein levels (mean and 95% C.I.) in vitreous of eyes that did and did not develop postoperative PVR. Eyes that did develop postoperative PVR had a significantly higher levels ($p<0.05$) of total protein.

Relationship between cytokine levels and postoperative PVR compared by treatment group.

The cytokines and protein levels in the vitreous of patients who developed postoperative PVR compared by treatment group are shown in **Table 3.20**

Table 3.20 Cytokine levels in patients with postoperative PVR and treatment group.

Factor	5FU-Heparin treated (A) Median (inter quartile range)	Placebo treated (B) Median (inter quartile range)	% Difference (A/B)	P-value
TGF-β2 (pg/ml)	1332.72 (1173.6 to 2086.08)	1946.40 (1259.04 to 5319.12)	- 68.47	0.290
b-FGF (pg/ml)	36.40 (18.07 to 103.59)	8.54 (2.39 to 25.58)	426.23	0.450
IL-1β (pg/ml)	0.20 (0.09 to 0.29)	0.03 (0.01 to 0.225)	666.67	0.472
IL-6 (pg/ml)	421.2 (321.83 to 594.53)	252.68 (29.18 to 628.58)	166.69	0.784
Protein µg/ml	286 (156.37 to 663.18)	135.96 (71.12 to 403)	210.36	0.456

Two-sample Wilcoxon rank-sum test

There was no statistically significant difference in vitreous levels between those patients who developed postoperative PVR in the 5-FU/heparin treated and placebo groups. However, the percentage differences between the 5-FU/heparin versus the placebo treated groups are large.

Summary of cytokine results

Significantly higher levels of b-FGF ($p=<0.001$) and protein ($p=<0.001$) were found in patients with preoperative PVR (Grade CP1 plus) compared to those without preoperative PVR.

Significantly higher levels of protein ($p=0.002$) were found in patients who developed postoperative PVR compared to those that did not.

There was no statistically significant difference in vitreous levels between those patients who developed postoperative PVR in the 5-FU/heparin treated and placebo groups. However, the percentage difference between the two groups was very large for b-FGF, IL-1β, IL-6 and protein. This difference suggests that those patients treated with 5-FU/heparin that developed postoperative PVR had higher preoperative levels of these growth factors and protein.

Risk factor analysis

Clinical risk factors

The following clinical risk factors were analysed; degree of myopia, preoperative lens status (phakia, aphakia or psuedophakia), preoperative use of cryotherapy or laser, preoperative PVR, uveitis, vitreous haemorrhage, size of detachment, macula status, intraoperative use of cryotherapy and type of internal tamponade (gas or silicone oil).

Univariate analysis of individual risk factors revealed preoperative PVR (>1 clock hours, Grade C PVR) and size of detachment to be significant ($p<0.05$) clinical risk factors for the development of PVR.

Multivariate regression analysis of all the clinical risk factors revealed preoperative PVR (>1 clock hours, Grade C PVR) to be the only independent predictive risk factor for the development of PVR. **Table 3.21** shows the estimated coefficients and odds ratios for the risk factors.

Table 3.21 Estimated coefficients and odds ratios for the risk factors.

Variable	Odds ratio	P-value	95% C.I
Preoperative PVR Grade C	8.93	<. 0001	2.98 to 26.80
Preoperative PVR Grade B	1.28	0.607	0.50 to 3.27
"Aphakia"	0.90	0.821	0.36 to 2.26
Duration of symptoms	1.006	0.196	1.00 to 1.02
Myopia > 5.00	0.379	0.074	0.13 to 1.10
Macula attached	0.302	0.031	0.10 to 0.90
Preoperative cryotherapy	1.86	0.199	0.72 to 4.78
Intraoperative cryotherapy	0.66	0.274	0.31 to 1.39
Preoperative uveitis	1.380	0.495	0.55 to 3.48
Size of detachment (Quadrants) **2** **3** **4**	 1.662 2.424 4.848	 0.454 0.205 0.027	 0.439 to 6.29 0.62 to 9.52 1.20 to 19.65
C_3F_8 tamponade	3.53	0.002	1.61 to 7.75
Silicone oil tamponade	7.31	0.006	1.76 to 30.44
Retinotomy	1.69	0.169	0.80 to 3.59
Fibrin	2.57	0.118	0.79 to 8.38

Using recognised risk factors the following multivariate rule (equation) was obtained:

log (odds risk of PVR) = -3.41 – 0.02(aphakia) + 1.46 (preop PVR grade CP1 plus) + 2.85 (total detachment) + 1.81 (preop cryo) – 0.65 (uveitis) + 0.342 (vitreous haemorrhage)

Key: Preop =preoperative

In this equation a value of 1 is added if the risk factor is present and 0 if the risk factor is absent.

Using this equation an arbitrary cut off value can be set that can predict the risk of developing PVR to take into account both a high sensitivity and specificity.

Biological risk factors

Multivariate logistic regression analysis of the cytokines and protein revealed protein to be the only significant ($p=<0.03$) independent risk factor for the development of PVR.

Table 3.21 shows the estimated coefficients and the odds ratios for the cytokines and protein as risk factors.

Table 3.21 Estimated coefficients and the odds ratios for the cytokines and protein

Variable	Odds ratio	P Value	95% C.I
TGF-β2	1.01*	0.188	1.00 to 1.02
b-FGF	1.03**	0.312	0.98 to 1.08
IL-1β	0.478**	0.482	0.061 to 3.74
IL-6	1.001*	0.119	0.97 to 1.32
Protein	1.182***	0.029	1.02 to 1.37

* Odds ratios were calculated per change of 100pg/ml for TGF-β_2 and IL-6
** Odds ratios were calculated per change of 10pg/ml for b-FGF and IL-1β
*** Odds ratios were calculated per change of 100μg/ml for protein

By using protein concentration analysis the following discriminant rule (formula) is obtained: **log (odds risk of PVR) = -1.98 + 0.166 x protein (ug/100).**

Using this equation an arbitrary cut off value can be set that can predict the risk of developing PVR to take into account both a high sensitivity and specificity.

Combined clinical and biological risk factors

Multivariate logistic regression on both biological and clinical risk factors revealed preoperative PVR (> 1 clock hours of Grade C) and high protein levels to be the only independent predictive risk factors for PVR. **Table 3.22** shows the estimated coefficients and the odds ratios for the combined risk factors

Table 3.22 Estimated coefficients and odds ratios for risk factors.

Variable	Odds ratio	P Value	95% C.I
Preoperative PVR Grade C	13.651	0.001	2.80 to 66.47
Preoperative PVR Grade B	1.00	1.000	0.28 to 3.56
"Aphakia"	0.946	0.938	0.23 to 3.81
Preoperative cryotherapy	1.982	0.308	0.53 to 7.38
Preoperative uveitis	0.523	0.277	0.16 to 1.68
Size of detachment (Quadrants)			
2	2.681	0.248	0.50 to 14.27
3	0.749	0.777	0.10 to 5.57
4	3.057	0.251	0.45 to 20.62
Vitreous haemorrhage	2.306	0.238	0.58 to 9.22
Protein	1.15*	0.155	0.95 to 1.38

* Odds ratios were calculated per change of 100μg/ml for protein

By using protein concentration and clinical risk factors analysis the following discriminant rule (formula) is obtained: **log (odds risk of PVR) = -2.81 - 0.05 x (aphakia) + 2.6 x (preop PVR grade CP1 plus) + 1.1 x (total detachment) + 0.68 x (preop cryo) – 0.65 x (uveitis) + 0.84 x (vitreous haemorrhage) + 0.14 x protein (ug/100).**

Using this equation an arbitrary cut off value can be set that can predict the risk of developing PVR to take into account both a high sensitivity and specificity.

"Fitting" the discriminant rule into the data set

To access the validity of the discriminant ("formula") rule the data set was randomly divided into a "training set" and a "validation" set. The proportion of PVR cases was similar in each group. The clinically relevant risk factors were then fitted into a multiple regression model, using the training data set, to form the allocation rule. Error rates were then calculated by classifying both the training data set and the validation data set using the derived rule.

Using the discriminant rule for clinical risk factors

A probability cut off of 0.1 was chosen for the development of PVR. A patient is predicted to develop PVR if the calculated probability is above 0.1 and predicted not to develop PVR if lower than 0.1. **Table 3.23** shows the predictive power of the formula on the training data and **Table 3.24** using the validation data.

Table 3.23 Predictive power using the training set.

	Value
Sensitivity	92.5%
Specificity	30.77%
Positive predictive value	21.74%
Negative predictive value	95.24%
Correctly classified	41.40%

Table 3.24 Predictive power using the validation set.

	Value
Sensitivity	75.00%
Specificity	27.27%
Positive predictive value	15.79%
Negative predictive value	85.71%
Correctly classified	34.62%

Using the discriminant rule on the training and validation data 41.40% and 34.62% of cases were correctly classified.

Using the discriminant rule for protein

A probability cut off of 0.15 was chosen for the development of PVR. A patient is predicted to develop PVR if the calculated probability is above 0.15 and predicted not to develop PVR if lower than 0.15. **Table 3.25** shows the predictive power of the formula on the training data and **Table 3.26** using the validation data.

Table 3.25 Predictive power using the training set.

	Value
Sensitivity	73.68%
Specificity	46.81%
Positive predictive value	21.88%
Negative predictive value	89.80%
Correctly classified	51.33%

Table 3.26 Predictive power using the validation set.

	Value
Sensitivity	100%
Specificity	53.85%
Positive predictive value	25.00%
Negative predictive value	100.00%
Correctly classified	60.00%

Using the discriminant rule on the training and validation data 51.33% and 60.00% of cases were correctly classified.

Using the discriminant rule for clinical risk factors and protein combined

A probability cut off of 0.1 was chosen for the development of PVR. **Table 3.27** shows the predictive power of the formula on the training data and **Table 3.28** using the validation data.

Table 3.27 Predictive power using the training set.

	Value
Sensitivity	100%
Specificity	53.85%
Positive predictive value	31.15%
Negative predictive value	100%
Correctly classified	61.82%

Table 3.28 Predictive power using the validation set.

	Value
Sensitivity	50%
Specificity	46.15%
Positive predictive value	12.50%
Negative predictive value	85.71%
Correctly classified	46.67%

Using the discriminant rule on the training and validation data 61.82% and 46.67% of cases were correctly classified. By adding protein to the discriminant rule the predictive outcome increased by 20.42% on the training data and 12.06% on the validation data.

Chapter 5

Discussion

The success rate of retinal reattachment has improved dramatically over the past two decades with rates of over 90% being achieved. It is unlikely that further advances in surgical instrumentation or technique will improve the overall success rate. The major cause of failure of final reattachment is postoperative PVR. With a better understanding of the pathophysiology of PVR and identification of those at risk it is hoped that adjuvant pharmacological treatments may improve the success of surgery.

This project set out to determine whether it is possible to identify those at risk of developing PVR, to prevent postoperative PVR using adjuvant 5-FU and LMWH and to further our understanding of the role of clinical and biological risk factors in the PVR process.

PVR is a wound healing response

PVR is a wound healing response in a specialised tissue with the formation of membranes on both surfaces of the retina and vitreous base. There are three main phases of a wound healing response: exudation/inflammation, proliferation of cellular elements and wound regeneration.

An initial injury, e.g., surgery or cryotherapy, leads to a breakdown in the blood retinal barrier with the release of growth factors and cytokines that are thought to be central in the PVR process (Campochiaro *et al.*, 1986b).

The second phase, proliferation and migration, is centred on the RPE cell. RPE cells are able to secrete factors that are capable of stimulating both fibroblasts and astrocytes

(Bryan and Campochiaro, 1986) . RPE cells have been shown histologically to give rise to three different cell types in PVR membranes (Machemer and Laqua, 1975) : pigment epithelial macrophages (Gilbert *et al.*, 1988) , RPE cells with retained epithelial characteristics and RPE cells that have undergone mesenchymal differentiation into fibroblast like cells (Vidaurri-Leal *et al.*, 1984; Vidaurri-Leal and Glaser, 1984) .

RPE cells can also synthesise collagen, a major component of PVR membranes (Morino *et al.*, 1990) .

The third phase involves the laying down of the extracellular matrix, remodelling and finally production of mature scar tissue (Hiscott *et al.*, 1985; Jerdan *et al.*, 1989; Scheiffarth *et al.*, 1986; Scheiffarth *et al.*, 1988) .

The role of cytokines should also be emphasised as there are also central to the PVR process. Cytokines act as chemical mediators between cells, are chemoattractant and regulators of cellular proliferation and secretion of extracellular matrix. They also regulate their own secretion and are involved in contraction of extracellular matrix (Ignotz and Massague, 1986; Wahl *et al.*, 1987; Wiedemann, 1992) .

Using this knowledge it may be possible to modulate the early phases of the wound healing response and prevent postoperative PVR. This study has attempted to use our understanding of the PVR process to successfully treat patients who were at high risk of developing PVR.

Using clinical risk factors to define high-risk patients

As previously discussed we used a discriminant rule (formula) to define patients at high risk of developing PVR. If we are able to identify patients at highest risk of developing PVR we would than be able to better target these patients. This is particularly important

when the treatment used has potentially significant side effects. The formula used was based on a prospective trial on all patients undergoing primary vitrectomy for rhegmatogenous retinal detachment (Kon *et al* ., 2000). This study was used as it analysed a number of risk factors associated with the development of PVR and it was at the time the largest prospective study of PVR (n=140). On the basis of this study we chose the following preoperative risk factors: PVR (Grade C or Grade B), uveitis, cryotherapy, vitreous haemorrhage, size of detachment and aphakia. Using logistic regression analysis we were able to generate a regression formula to estimate the probability of a patient to develop postoperative PVR.

When we used this formula prospectively we were able to significantly (p=0.0001) predict the development of postoperative PVR. In those patients defined as low risk 9.1% (12/132) developed postoperative PVR compared to 28.0% in those defined as high risk (23/84). The formula is easy and quick to use and has enabled us to target patients at risk of developing PVR. However, this formula was tested in a trial setting and therefore the validity of the formula may be compromised in a busy clinical environment. It may be more difficult to apply this in a clinical setting with varying degrees of clinical expertise and observer variability. In this study there was only one clinical observer but in clinical practice observer variation would become an inherent problem in using a clinical risk factor formula. However as the formula is simple to use and does use clinically distinct observations it should prove to be useful.

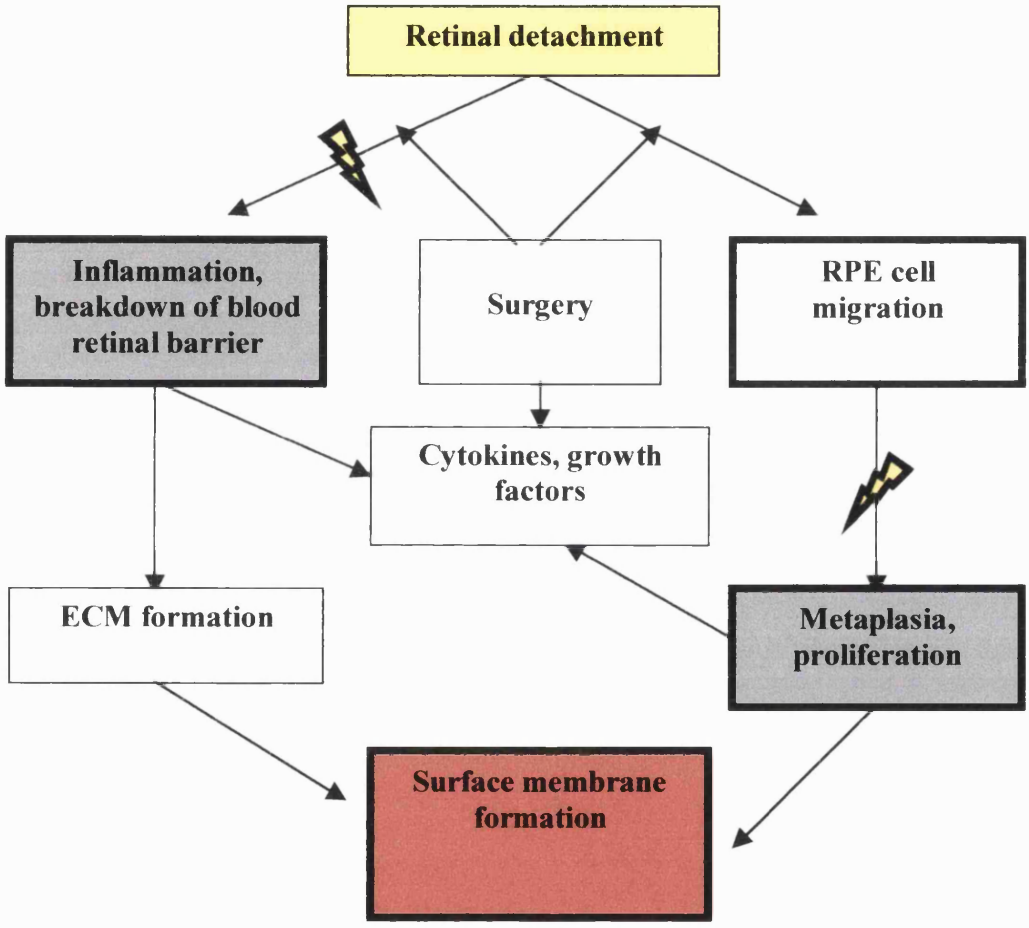
Alternatively vitreous protein levels can be used to prospectively predict PVR. The use of biological risk factors may allow for a more objective assessment of risk. Unfortunately,

at the present time accurate and immediate measurement of these biological factors perioperatively is not possible.

The use of 5-FU and heparin in the preventative treatment of patients at risk of developing PVR

As previously discussed PVR is a wound healing response consisting of three different phases. By using adjuvant therapy we had hoped to modulate both the inflammatory and proliferative responses. We used combination therapy on the presumption that an additive effect would be gained. The use of combination therapy in treatment protocols is well practiced in other disciplines of medicine, e.g., cancer medicine. **Figure 4.1** represents graphically the treatment strategy we have used.

Figure 4.1. Graphical representation of the wound healing response and treatment strategy.



Notes: Heparin has a primary effect on inflammation and breakdown of the blood retinal barrier and 5-FU has a primary effect on RPE cell proliferation.

Therefore the use of both 5-FU and heparin was used to have a synergistic effect in the prevention of surface membrane formation.

Choice of Agents

Low Molecular weight heparin

Heparin consists of a chain of mucopolysaccharides of varying molecular lengths ranging from 5 to 60kDa. Heparin's primary action is on the coagulation pathway. Heparin has significant anticoagulant properties leading to inactivation of thrombin (Walter and Israel, 1987) . Heparin also interferes with cell adhesion by binding to several ECM proteins, including fibronectin, laminin and vitronectin (Yamada, 1983). Heparin is truly a multipotential drug (Yamada, 1983) ; it binds to a number of growth factors, including PDGF, EDGF and FGF, prevents fibroblast adhesion to fibronectin- coated substrates and produces changes in the cytoskeleton of smooth muscle cells and pericytes after short exposures (Hoover *et al.*, 1980) .Heparin also inhibits the polymerisation of Type I collagen (Blumenkranz and Hartzer, 1994) . In vitro studies of heparin on three dimensional collagen lattices have shown a significant reduction in fibroblast- mediated contraction (Blumenkranz *et al.*, 1992) . Heparin has been shown to reduce RPE cell proliferation in a dose dependent manner with concentrations of 200 units/ml for 6 days reducing proliferation by 30% (Blumenkranz and Hartzer, 1994) .

In vivo studies examining the use of heparin after vitrectomy in rabbit eyes (Johnson *et al.*, 1987) have shown a significant reduction of postoperative intraocular fibrin. A prospective trial investigating the use of systemic heparin or heparin infusion during vitrectomy to prevent fibrin formation has been done (Johnson and Blankenship, 1988). A 10-IU/100 ml heparin infusion resulted in a statistically significant reduction in postoperative fibrin formation ($P = 0.04$) but increased intraoperative bleeding ($P = 0.02$). A trend toward reduced postoperative fibrin formation was noted using an infusion

concentration of 5 IU/100 ml. Another study has also shown heparin to reduce postoperative fibrin formation after vitrectomy in infants undergoing vitrectomy for retinopathy of prematurity (MacDonald *et al.*, 1985).

A significant complication of using standard heparin is the risk of bleeding. Fractionated heparin (LMWH) has a similar effect on coagulation but inhibits thrombin and platelets to a lesser degree (Bratt *et al.*, 1985) (Salzman *et al.*, 1980). Thereby, resulting in the same anticoagulation properties but with less bleeding tendencies.

In a rabbit model (Iverson *et al.*, 1991b) of lensectomy, vitrectomy and retinotomy intraocular fibrin formation following infusion of 5 IU/ml of LMWH was significantly reduced. In an animal model of PVR, involving lensectomy and partial vitrectomy, 5IU /ml of LMWH in the infusion for approximately 1 hour significantly reduced the rate of tractional retinal detachment from (Iverson *et al.*, 1991a). To date there have been no prospective clinical trials using LMWH as adjuvant therapy in human subjects.

5-Fluorouracil (5-FU)

It was Blumenkranz et al who first introduced the use of 5-FU in ophthalmic surgery (Blumenkranz *et al.*, 1982) and 5-FU still remains the most extensively studied antiproliferative agent in humans. 5-FU interferes with cell replication by inhibiting DNA and RNA synthesis (Blumenkranz *et al.*, 1987). Blumenkranz et al (Blumenkranz *et al.*, 1984b) in a pilot study injected 5-FU subconjunctivally or intravitreally in 22 patients with severe PVR. Retinal reattachment was achieved in 60% of patients at 6 months postoperatively. No serious systemic or ocular complications were observed with using 5-FU, although delayed healing of corneal epithelial defects occurred in 18% of cases and subtle subepithelial scarring in 31.8%. Garcia-Layana et al in a randomised clinical trial

compared subconjunctivally injected dexamethasone and 5-FU following vitrectomy in patients with rhegmatogenous retinal detachment at high risk of developing PVR (Pastor, 1998) . They found that dexamethasone (8 mg daily for 7 days) and 5-FU (10 mg daily for 7 days) significantly reduced the incidence of postoperative PVR. However, there were no differences between the two drugs. Both these studies were encouraging but did not lead to further trials until this study.

The safety of 5-FU has been studied extensively studied. A single intravitreal injection of 5-FU into the vitreous cavity of rabbits produced no morphological or electrophysiological damage to the retina in doses up to 1mg (Blumenkranz *et al.*, 1984b; Blumenkranz *et al.*, 1982) . Sequential repeated 0.5mg (0.5 mg of 5-FU daily for 7 days) injections in vitrectomised eyes of rabbits also produced no evidence of toxicity (Stern *et al.*, 1983b) . At higher doses of 1.25 mg daily for 7 days corneal opacification resulted that gradually decreased by four weeks. The b–wave of the ERG diminished to 9.6% its baseline value but recovered to 62.5% at three weeks. Histological studies showed loss of photoreceptor outer segments and ribosomes at nine days; both returned to near normal after five weeks.

Nao-I et al (Nao-i and Honda, 1983) injected 5-FU in albino rabbit eyes. Concentrations of up to 10ug/ml had no effect on the ERG. Higher concentrations reduced the b wave response; 0.1mg/ml decreased the response to 62% and 1mg/ml to 41% of normal. Both the ERG responses returned to baseline values within 60 minutes. The B wave response disappeared at a dose of 5mg/ml after 3 minutes although the amplitude recovered to 89% of baseline if the infusion was replaced with 5-FU free medium after 5 minutes.

We found no evidence of clinical toxicity relating to the use of 5-FU and LMWH. Intraoperative complications were minimal. One patient developed corneal oedema that made visualisation more difficult, one patient developed retinal incarceration and one patient had a choroidal haemorrhage. There were 10 postoperative hyphaemas divided equally between the two groups. All the hyphaemas were mild and settled with conservative treatment alone.

The use of short exposure treatments of 5-FU to prevent postoperative scarring is well established in glaucoma surgery (Khaw *et* al.,1992; Khaw *et al.*, 1993a; Khaw *et* al., 1993b). Single intravitreal injections of high concentrations of 5-FU can be toxic to the retina and result in prolonged exposure to the drug. Occleston et al studied the effects of single 5 minute exposures of 5-FU and mitomycin–c on ocular fibroblast mediated collagen lattice contraction (Occleston *et al.*, 1994) . Both drugs were effective in inhibiting lattice contraction although mitomycin-c also had a significant effect on cell viability. Kon at al (Kon *et al.*, 1998b) investigated the effects of single, short-term (5 or 30 minutes) exposures to thiotepa or 5-FU on collagen lattice contraction and RPE cell proliferation. Both thiotepa and 5-FU at concentrations above 0.06 mg/ml and 0.25 mg/ml respectively (for both 5 and 30 minute treatments) significantly inhibited ($p < 0.05$) contraction of collagen lattices and proliferation of RPE cells compared with controls. Cell death did not occur except for exposure of the RPE cells in collagen lattices to the highest concentration of thiotepa (4 mg/ml). 5-FU despite having an effect on replicating cells was still effective in inhibiting long term cell proliferation. More importantly, there was a similar degree of inhibition of proliferation and lattice contraction with 5 and 30-minute exposures. This would imply that intracellular

accumulation of the drug is rapid and reaches a saturation point after short exposures. On the basis of this study and the animal work discussed above we used an infusion of 200 μg/ml of 5-FU and 5 IU/ml heparin during vitrectomy as a safe treatment regimen.

Daunomycin

Daunomycin is the only antiproliferative agent that has been used in a prospective randomised intention to treat trial until this study. This trial was based on two previous clinical studies. In the first study (Wiedemann *et al.*, 1987) 7.5 μg/ml of daunomycin was infused over a 10 minute period before either intraocular gas or silicone oil tamponade in 15 patients with post traumatic detachments. Postoperatively, 14/15 patients were anatomically attached and only 1 patient had recurrent tractional retinal detachment. Visual acuity improved in all patients postoperatively. In the second study the authors (Wiedemann *et al.*, 1991) went on to treat a further 69 patients with advanced PVR with vitrectomy, silicone oil tamponade and adjuvant daunomycin therapy. After long term follow up 73% were attached and 89% had a vision of 20/800 or better. The authors felt that the success rate of surgery was favourable compared to similar series and the reoperation rate was less with adjuvant treatment.

These results led to a prospective clinical trial using daunomycin. The results of the randomised control trial (Wiedemann *et al.*, 1998) that followed were favourable although they were reported as being non significant. In the trial 286 patients with stage C2 (Retina Society Classification, 1983) or more advanced PVR were randomised to standardised surgery plus adjuvant daunomycin perfusion (7.5 ug/ml for 10 minutes) and surgery alone. Six months after standardized surgery, complete retinal reattachment without additional vitreoretinal surgery was achieved in 62.7% of eyes in the

daunorubicin group versus 54.1% in the control group. The Mantel-Haenszel chi-squared test gave a one sided P value of 0.07. The overall final anatomic success rates with or without reoperations were 77.2% after 6 months and 80.9% after 1 year. When these results were compared to treatment effect 76.5% and 80.2% in the daunomycin group versus 78.0% and 81.8% in the control group were finally reattached. These rates did not defer between treatment groups. When the vitreoretinal reoperation rate was compared at 1 year, significantly fewer vitreoretinal reoperations were performed within 1 year postoperatively (P = .005, one-sided) in the daunomycin group. The rate of patients with no vitreoretinal reoperations was 65.5% in the daunorubicin group versus 53.9% in the control group. When the time to reoperation was examined 25% of the control patients had a reoperation within 94 days compared to 183 days in the daunomycin group. There was no difference in the best-corrected visual acuity. No severe adverse effect related to daunorubicin was identified. In conclusion, 20% of patients in both groups had redetached after 1 year despite modern vitreoretinal surgery. The development of new PVR was not an outcome measure, the number of patients who had oil removed was not analysed and the cause of failure, especially late redetachment, was not analysed. Despite this data a definite trend to primary success was seen in the daunomycin group. The authors do not recommend the routine use of adjuvant therapy with daunomycin in patients with severe PVR.

Adjuvant therapy with 5-FU and LMWH

Our study was designed as a preventative treatment against the development of PVR. The patients were chosen to be at risk of developing PVR using clinical risk factors based on previous work in our center (Kon *et al.*, 2000) . We have found the chosen criteria to be

significantly predictive of developing PVR. Most other treatment trials have treated patients either with existing PVR or a mixed cohort of cases. This is certainly true of the daunomycin trials described above in which only patients with established PVR were enrolled. Other agents have been tried with variable success. Colchicine (Berman and Gombos, 1989) has been used in humans on a mixed group of patients with PVR, including diabetic, sickle cell and trauma cases. At the doses used there was no significant difference in the control and treated groups. Subconjunctivally injected dexamethasone (Garcia-Layana *et al.*, 1995) as adjuvant therapy in patients at risk of developing PVR significantly reduced the incidence of postoperative PVR from 80% to 36% in controls and treated patients respectively. In another pilot study of 20 patients with recurrent retinal detachment due to PVR the use of retinoic acid was investigated. Patients were randomised to either 40 mg oral 13-cis-retinoic acid twice daily or control for 4 weeks postoperatively (Fekrat *et al.*, 1995). All 20 eyes underwent surgical repair using similar techniques. Nine of ten eyes in the study group remained attached during a mean follow-up of 8.3 months, whereas four of ten eyes in the control group remained attached ($P = 0.061$) during a mean follow-up of 9.6 months. The rate of macular pucker was similar between the groups. Of the six eyes in the control group that detached, 4 had 6 or more clock hours of PVR. The p value was again nearing significance and the authors felt that a randomised prospective trial is now needed. Binder et al in a prospective study (Binder *et al.*, 1994) studied the effect of postoperative radiation therapy for the prevention of reproliferation of membranes and recurrent PVR (PVR grade D1 to D3). Half the eyes (30 eyes) received a total dose of 3000 cGy after surgery the other half remained untreated. After 6 months in the control group 57% (17/30)

remained attached and 43% (13/30) had detached again. In the irradiated group 63% (19/30) were attached and 37% (11/30) had detached. However, there was no statistically significant difference between the two groups (P = 0.479, Fisher's Exact Test). After 14 months the number of cured and uncured eyes remained the same in the control group, while in four of the eyes in the irradiated group a later onset of reproliferation and detachment occurred. This late onset of reproliferation and detachment was also found in the daunomycin trial. A final cure rate of 57% (17/30) was achieved in the control group and a 50% (15/30) cure rate in the irradiated group. Thus the failure rate was 43% (13/30) in the control group and 50% (15/30) in the irradiated group (P = 0.473, Fisher's Exact Test). This study suggests that radiation therapy has an antiproliferative effect and temporarily inhibits the proliferative process.

A prospective trial investigating the use of systemic heparin or heparin infusion during vitrectomy to prevent fibrin formation has been done (Johnson and Blankenship, 1988). The patients were a mixed group consisting of diabetic retinopathy, severe PVR, trauma and endophthalmitis. Patients with minimal PVR (Grade B or less) were excluded. This is in direct contrast to our study that enrolled patients with Grade A to Grade CP1 PVR. A total of 73 eyes were randomised; 26 served as the control group, 23 received 10,000 IU of intravenous bolus of heparin, and 12 eyes each underwent vitrectomy with an infusion solution containing 10 or 5 IU/100 ml of heparin. The single intravenous bolus produced a minimal inhibition of fibrin formation. A 10-IU/100 ml heparin infusion resulted in a statistically significant reduction in postoperative fibrin formation (P = 0.04) but increased intraoperative bleeding (P = 0.02). A trend toward reduced postoperative fibrin formation was noted in the intravenous heparin and 5 IU/cc infusion groups. As discussed

above the only study in humans using intraocular 5-FU involved patients with severe PVR in a non- randomised study.

In our trial we have chosen a very well defined group of patients that would gain the most clinical benefit from preventative treatment. Patients with severe PVR (Grade CP1 plus) were excluded from the study. A preventative treatment is always more effective than treating an established disease.

At the start of surgery there was no difference in baseline difference between in the 5-FU/ LMWH and the control group. At the 6 month follow up there was missing data for 5.7% (5/87) in the placebo group and 2.3% (2/87) for the 5-FU/heparin group.

The rate of postoperative PVR was significantly higher ($p=0.02$) in the placebo group compared to the 5-FU/heparin group. The primary success rate of retinal reattachment was 75.9% in the 5-FU/heparin group compared to 66.7% in the placebo treated group. The final reattachment rate was 90.1% in the 5-FU/heparin group compared to 86.2% in the placebo treated group. Both these results show a trend to a better success rate for the 5-FU/heparin group treated group.

Visual acuity outcome is a very significant denominator of success as oppose to anatomical success for patients. As our patient group included patients with a favourable visual prognosis (macular attached retinal detachments) visual outcome is an important secondary outcome. The final visual acuity was significantly better ($p=<0.05$) in the 5-FU/heparin treated group versus the placebo group. The significantly worse visual acuity outcome ($p=<0.0001$) found in patients that developed postoperative PVR supports the improved visual acuity outcome in the 5-FU/heparin group.

One of the difficulties of adjuvant therapy is to achieve therapeutic drug levels in the vitreous in a modality that is practical and safe. We have shown that an intraoperative infusion of 5-FU/heparin is a safe and effective method of drug administration. The treatment also does not incur any further inconvenience to the patient.

In conclusion we have shown that by using adjuvant treatment with 5-FU and LMWH it is possible to significantly reduce the rate of postoperative PVR. In view of the efficacy in preventing PVR, the lack of side effects and the relatively low cost (the cost of combined treatment is $6.00), we advocate the use of this treatment in the future management of all patients with an increased risk of developing postoperative PVR.

The effect of treatment on all patients with a retinal detachment undergoing vitrectomy is unclear and a further study to address this is required and will soon be undertaken. A large multicenter trial based in our center using the same treatment regimen on patients with severe PVR is also nearing completion.

Validity of clinical risk formula

As we now have a prophylactic treatment available for the prevention of PVR it is important to be able to define those patients at highest risk so that treatment can be targeted especially if the treatment may be potentially toxic. One of the most important areas of epidemiological research is the identification of risk factors for specific disorders. Such research is usually aimed at unravelling pathogenesis, but risk factors can also be used as screening tests.

As mentioned previously, we have reported (Kon *et al.*, 2000) , using univariate and multiple regression analysis, clinical risk factors for PVR. Using a discriminant rule

based on these risk factors, a formula was developed to further identify prospectively those patients most likely to develop PVR.

Using this formula we were able to predict correctly 28% of cases of PVR in a group defined as high- risk. This compared well to a PVR rate of 9.1% in a group defined as low risk. This predictive power was statistically significant (p=0.0001).

A risk factor has to be strongly associated with a disease within a population before it can be considered to be potentially useful as a screening test. It is not unusual for a strong risk factor of aetiological importance to be proposed as a screening test for the disorder. All the risk factors we used had a relative odds ratio of less than 10. Only a few risk factors in epidemiology are associated with a higher relative odds ratio. A risk factor with a relative odds ratio of less than 10 would perform poorly as a screening test. This would explain why we were able to correctly predict only 28% of cases of PVR in our high-risk group. The fact that strong risk factors can be poor screening tests can be explained by recognising that the relative odds ratios (or relative risks) are usually assessed by comparing the risk of disease at each end of the distribution of the risk factors. In this way the effect of being highly "exposed" to the factors is compared to being slightly "exposed". The groups being compared are mutually exclusive and most people in the middle of the distribution are ignored.

Clinical risk factors for the development of PVR

The study of risk factors can help us to understand better the pathophysiology of PVR. As mentioned in a separate study (Kon *et al.*, 2000) in our department we found aphakia, preoperative PVR grade CP1 plus, size of detachment and the use of silicone oil to be significant risk factors for the development of PVR. Using logistic regression only

aphakia and preoperative PVR were found to be independent predictive risk factors for PVR. This study has enabled us to consolidate further our knowledge of the risk factors involved in the PVR process.

A large number of studies have been done investigating risk factors. Most have been retrospective and have used univariate analysis. Univariate analysis investigates the relationship of any two variables without taking into effect the possible influence of other variables. A practical example of this is the effect of silicone oil. It is usually found that silicone oil is used in cases that have a poor prognosis and consequently a high proportion of severe preoperative PVR. Therefore to look at silicone oil as an individual risk factor may be misleading. In contrast multiple logistic regression analysis helps us to investigate the effect of multiple clinical risk factors (variables) on the development of PVR.

Another problem encountered in comparing different studies investigating risk factors has been the different classifications used to describe PVR and therefore a direct comparison between studies has not been possible.

Our study population was particularly important as there is now an effective prophylactic treatment available for patients at risk of developing PVR and this study group has helped us to define those at greatest risk.

Univariate analysis

Univariate analysis revealed preoperative PVR, macular attached, total retinal detachment, C_3F_8 and silicone oil to significant risk factors for the development of postoperative PVR. In contrast to our first study we did not find aphakia to be a

significant risk factor and found both macular attached retinal detachments and C_3F_8 to be risk factors.

The existence of preoperative PVR would suggest that the cellular and extra cellular events that are responsible for the PVR process have already began and it is not unreasonable to expect the presence of preoperative PVR to lead to further postoperative PVR. We did not find Grade B PVR to be a risk factor for the development of PVR as oppose to other investigators who have felt that Grade B PVR represents an immature form of PVR with a high potential for progression. Girard et al (Girard *et al.*, 1994) in a retrospective study of 1020 patients found both Grade A and Grade B PVR to be significant risk factors. They did not find Grade C1 to be a significant risk factor. They felt that Grade A and B PVR represented immature forms of PVR with a definite potential for progression, whereas grade C1 may represent a spontaneously arrested, non-evolutive form of the disease. Our findings contradict those of Girard, although the studies are not directly comparable as our studies were prospective and included only those patients undergoing vitrectomy. In Girard's series only 176 patients (17.2%) underwent vitrectomy. Bonnet (Bonnet, 1988) also found Grade B PVR to be a significant risk factor in a prospective study comparing Grade O –A to B PVR preoperatively. None of the patients underwent vitrectomy. In a previous retrospective study Bonnet (Bonnet, 1984) had found preoperative fixed retinal folds (Grade C PVR) to be a risk factor. Cowley et al (Cowley *et al.*, 1989) using stepwise discriminant analysis also found preoperative Grade C to be a risk factor. Both these studies support our findings.

Macular attached at time of presentation was found to be a risk factor in our study. The nature of the association is uncertain. It may be that macular on detachments present later than macular off detachments and that duration of detachment is more important. We did not find a statistical difference between duration of symptoms and macular attachment status and more importantly duration of symptoms was not found to be a risk factor. It must be noted that duration of symptoms is a poor surrogate maker for length of detachment but is easily ascertained. Yoshino et al (Yoshino *et al.*, 1989) in a retrospective study found that duration of symptoms greater than 1 month was a risk factor suggesting that chronicity aggravated the PVR process .

It is possible to explain why size of detachment may be a risk factor. In the normally attached retina the RPE cells lie in their normal position between the sensory retina and choroid. In this environment there are under physiological control processes and perform their normal physiological functions. In retinal detachment, they are exposed and can settle in ectopic sites where they are not under normal physiological control. It is than possible that they can proliferate and undergo metaplasia with the resultant development of PVR. Therefore, the larger the size of detachment the greater the area of exposed RPE and the increased chance of developing PVR. Girard et al (Girard *et al.*, 1994) found that detachment greater than 2 quadrants is a significant risk factor. The size of retinal breaks have also been found to be a risk factor (Girard *et al.*, 1994; Yoshino *et al.*, 1989) . Again the area of exposed RPE may be important especially after cryotherapy is applied (Bishara and Buzney, 1991; Glaser *et al.*, 1993) .

The choice of intraocular tamponade and in particular the use of silicone oil has been extensively studied. Lambrou et al in 1987 (Lambrou *et al.*, 1987) reported that the use

of silicone oil increases the risk of PVR. They looked at the effect of silicone oil, perfluoropropane gas or fluid in the vitreous cavity of rabbits. They reported that a higher proportion of silicone-filled eyes had severe proliferative vitreoretinopathy than either the perfluoropropane- filled or fluid-filled eyes. An in vitro proliferation assay using the vitreous samples showed that the silicone-filled vitreous had increased mitogenic activity for retinal pigment epithelial cells compared with the gas-filled or fluid-filled vitreous. They felt that silicone oil appears to increase proliferation by stimulating the increased release of mitogenic or different factors as well possibly by concentrating active factors into a smaller volume near the retina. Lewis et al (Lewis *et al.*, 1988) in a non-randomised study suggested that the use of silicone oil resulted in a high incidence of perisilicone proliferation.

Whether to use silicone oil or gas has been addressed by a large multicenter trial set up in 1985. The Silicone Study Group was set up to evaluate the benefits and risks of using long-acting intraocular gas or silicone oil tamponade following vitrectomy in eyes with severe PVR (The Silicone Study Group, 1992a; The Silicone Study Group, 1992c; The Silicone Study Group, 1992b) . In essence the take home message was that C_3F_8 and silicone oil were equally good and better than SF_6 in the management of severe PVR. Also there was a slight benefit in using silicone oil in the presence of severe anterior PVR. Silicone oil also has clinical advantages, it is technically easier to use and controllable, is optically clear and does not obscure the operating view. Post- operative vision is restored earlier and patients can also travel by air. Another valuable property is that it is not absorbed and therefore its tamponading effect is maintained for longer.

We again confirmed that the use of silicone oil was a risk factor and we also found C_3F_8 to be a risk factor. In contrast Girard et al (Girard *et al.*, 1994) found using logistic regression air tamponade as a risk factor.

Bonnet et al looked at the effect of cryotherapy. They reported that the use of cryotherapy in the treatment of retinal detachments with horse shoe tears with curled posterior edges or giant retinal tears greater than 180 degrees was a risk factor (Bonnet *et al.*, 1996) . Cowley et al also found cryotherapy to be a risk factor (Cowley *et al.*, 1989) . The only study of the effect of cryotherapy in human vitrectomised eyes (Glaser *et al.*, 1993) showed increased intravitreal dispersion of RPE cells compared to "sham" cryotherapy. Both cryotherapy and laser photocoagulation (Jaccoma *et al.*, 1985) result in break down of the blood retinal barrier although with cryotherapy this is more severe. We did not find cryotherapy to be a risk factor.

In contrast to our first study aphakia was not found to be an independent risk factor. We classified psuedophakia without an intact posterior capsule as "aphakia" because we believe that the posterior capsule plays an important role in the blood retinal barrier breakdown irrespective of the presence of an intraocular lens. This is supported by studies that have shown that vitreous loss in aphakic patients is a significant risk factor for the development of PVR (Greven *et al.*, 1992; Nagasaki *et al.*, 1991; Nagasaki *et al.*, 1993) .

Aphakia has been found to be a risk factor for PVR in other studies. Chignell et al (Chignell *et al.*, 1973) suggested that aphakia was a significant risk factor for the failure of retinal reattachment surgery. They postulated that small round holes in the periphery were difficult to find and remained untreated. The rate of PVR was also significantly

higher in their aphakic patients. In our series the difficulty in identifying breaks was not a major contributing factor as we were using an internal approach. Yoshino et al (Yoshino *et al.*, 1989) also found aphakia to be a risk factor. Other authors agree with this study and have not found an association with aphakia and PVR (Bonnet, 1984; Girard *et al.*, 1994) . Greven et al (Greven *et al.*, 1992) found no difference in either PVR or reattachment rate between aphakic and pseudophakic patients. However, they found aphakic patients to have a significantly worse visual outcome.

Intraocular inflammation (Campochiaro *et al.*, 1986b; Jaccoma *et al.*, 1985) and break down of the blood retinal barrier are thought to be important in the pathogenesis of PVR . Girard et al (Girard *et al.*, 1994) found anterior uveitis to be a risk factor. We did not find anterior uveitis to be a significant risk factor.

Vitreous haemorrhage is considered to be one of the most important risk factors for PVR (Bonnet, 1994; Cowley *et al.*, 1989; Yoshino *et al.*, 1989) . Blood acts as a potent inflammatory stimulus and as a major source of growth factors (Wiedemann, 1992) . We did not find preoperative vitreous haemorrhage to be a significant risk factor. Duquesne et al in a prospective study looking at the effect of vitreous haemorrhage confirmed our findings (Duquesne *et al.*, 1996) .

Multiple logistic regression analysis

Multiple logistic regression analysis helps us to investigate the effect of multiple clinical risk factors (variables) on the development of PVR. Univariate analysis investigates the relationship of any two variables only without taking into effect the possible influence of other variables.

An advantage of using multiple regression analysis is that a regression equation can be generated and used to estimate the probability of developing a disease. Odds ratios can also be obtained for individual risk factors and can be used in screening tests. As mentioned for a risk factor to be useful as a screening test it must be strongly associated with the disease. The strength of the association between a risk factor and a disorder can be quantified by its relative risk or odds ratio.

Using logistic regression only preoperative PVR Grade CP1 plus was found to be an independent predictive risk factor for the development of postoperative PVR with an odds ratio of 5.98. The size of detachment, macula status and type of tamponade used were not found to be independent risk factors.

Using clinical risk factors and fitting them in a regression model, patients can be classified as either "high risk" or "low risk". It is not possible to use an individual risk factor alone in this study as an odds ratio of 5 between the highest and lowest fifths of the distribution of a risk factor is equivalent to only a 14% detection rate for a 5% false positive rate (if the standard deviations of the risk factor in people with and without the disease are the same). Therefore, if we use preoperative PVR Grade CP1 plus only as a screening test than our detection rate would only be approximately 14%.

With any regression model it is difficult to determine the efficiency of the model. Ideally a further prospective study would be required to test the validity of the model.

We tested the model by dividing our patient data into a training set and a validation set. Using this model we were able to correctly predict postoperative PVR in 34.6% of patients. This is very similar to our current predictive rate of 27.2% using the clinical risk formula used in defining our high- risk patients.

Role of Cytokines and Growth factors in PVR

PVR is a wound healing response in a specialised tissue. It is highly probable that cytokines and growth factors play a central role in inflammation, cell proliferation and migration, secretion and remodelling of extracellular matrix. Many studies have investigated the role of cytokines in PVR (Baudouin *et al.*, 1993; Connor, Jr. *et al.*, 1989; Limb *et al.*, 1991; Limb *et al.*, 1993a; Malecaze *et al.*, 1991).

In a previous study looking at the effect of cytokine and protein expression in the vitreous of eyes with retinal detachment and PVR (Kon *et al.*, 1999). At the time it was the only prospective study in which the relationship of these chemical mediators to postoperative PVR had been studied. Four cytokines (TGF-β_2, bFGF, IL-1-β and IL-6) and protein were analysed. Of these, significantly higher levels ($p=<0.05$) of TGF-β2, bFGF, IL-1-β and protein were found to be associated with the presence of preoperative PVR.

The role of TGF-β has been extensively studied. TGF-β has been isolated in human choroid, retina and vitreous and has been implicated as a regulatory cytokine in many biologic events including wound healing, chemotaxis, angiogenesis and cellular proliferation (Ignotz and Massague, 1986; Mustoe *et al.*, 1987; Postlethwaite *et al.*, 1987; Varga *et al.*, 1987; Wahl *et al.*, 1987). TGF-β has been shown to enhance RPE induced collagen gel contraction (Raymond and Thompson, 1990). Connor et al (Connor, Jr. *et al.*, 1989) found a raised level of TGF-β2 in the vitreous of eyes with preoperative PVR (Grade C or worse). Limb et al (Limb *et al.*, 1991) found no difference in TGF-β2 levels between PVR, none PVR and control vitreous (from cadaveric eyes). However, Limb et al included Grade B PVR in their series. In our present study we did not find a difference between PVR and none PVR vitreous. This is

in contrast to Kon et al's findings (Kon *et al.*, 1999) . These conflicting results may be due to a difference in population group as Kon et al's study had a preoperative PVR rate of 39.0% in comparison to 7.55% in our series. The median level of TGF-β2 in PVR vitreous in our study was similar to the mean levels found by Connor et al, but much less than in Kon et al's series (1.24ng/ml Vs 1.2ng/ml Vs 3.81ng/ml).

The role of FGF has also been studied in the past. FGF has been shown to be present in epiretinal membranes (Fredj-Reygrobellet *et al.*, 1991) and in PVR vitreous (Baudouin *et al.*, 1993) . Kon et al (Kon *et al.*, 1999) have shown that the mean levels of bFGF in patients with preoperative PVR was significantly raised (84.78 Vs 31.52). Our study agrees with that of Kon et al and supports the growing evidence that FGF may be involved in the pathogenesis of PVR. However, we found the median level of b-FGF to be 5.6 times higher in the PVR group (median 12.92ng/ml) compared to Kon et al's (Kon *et al.*, 1999) report of greater than 2.2 times the difference.

IL-6 is a 26-kDa molecule that is secreted by may types of cells, including macrophages, monocytes, fibroblasts, epithelial cells, endothelial cells, smooth muscle cells and lymphocytes (De Vos *et al.*, 1992) . IL-6 and IL-1 are related cytokines that have broad spectrum of activity in inflammation and immune reactions (Mizel, 1989) . IL-1 stimulates the production of IL-6 and these two cytokines contribute synergistically to the acute phase of the inflammatory reaction. Investigators have tried to implicate these two cytokines in the PVR process. Limb et al (Limb *et al.*, 1991) concluded that IL-1 and IL-6 were the predominant cytokines found in the vitreous of PVR and retinal detachment vitreous. They also showed that levels of IL-6 were 20 times greater in PVR vitreous compared to uncomplicated retinal detachments. Kauffmann et al (Kauffmann *et al.*,

1994) also found higher levels of IL-6 in PVR patients compared to a "mix bag" of patients that included PDR, macular pucker, giant retinal tears and vitreous haemorrhage. They could not detect IL-1 in most of their samples. We also found very low levels of IL-1 in our samples. Kon et al (Kon *et al.*, 1999) reported mean levels of 5.04 pg/ml and 217.43 pg/ml of IL-1β and IL-6 respectively in PVR vitreous. The corresponding levels in non-PVR vitreous were 3.62 pg/ml and 203.75 pg/ml respectively. In our study we found median levels of 321.83 pg/ml of IL-6 in PVR vitreous and 106.88 in non- PVR vitreous. The studies by Kauffmann et al and Limb et al were different in many respects, including differences in definition, laboratory and statistical methodology. Limb et al converted their results into categorical data using 1 pg/ml for IL-1 and 20 pg/ml for IL-6 to define high and low levels. A possible explanation for this could be that their detection test could not analyse low levels of cytokines and therefore could not use interval data. Another possible problem is their sample size of 15 patients in each group. Both the sample size in Kon et al and our study were much larger, n=136 and n=192 respectively, and the ELISA test used had a higher sensitivity. We were able to measure IL-6 on all the samples tested with levels comparable to those of Kon et al. Kon et al found much higher levels of IL-1 in their samples compared to our study. As mentioned their preoperative PVR rate was much higher. Our samples also underwent one freeze thaw cycle extra than Kon et al (personnel communication) and this extra freeze thaw cycle may have had an affect on the composition and activity of the cytokines. Kauffmann et al found undetectable levels of IL-1 in 15/16 samples. This may indicate that the levels of cytokines may fluctuate, confirming the dynamic nature of the wound healing response, and as we are sampling only one point in evolution of the process the levels of cytokines

may be dependent on sampling time. However, our results and those of Kon et al showed that there was no correlation between duration of treatment and cytokine or protein levels.

The role of IL-1 in PVR is still unclear. IL-1 plays a role in the wound healing response as described. Hint et al found TGF- β plus IL-1β greatly increased collagen gel contraction (Hunt *et al.*, 1993) . They went on to show that TGF- β and IL-1β (Hunt *et al.*, 1994) cause increased presentation of cell surface integrins that bind directly to extracellular matrix molecules to cause contraction. However, despite the non-statistically significant levels in this study, the role of these two cytokines is probably central to the PVR process.

Due to the complex interactions of cytokines, it may not be possible to link one cytokine as a predominant factor in the PVR process and measurement of total protein may better represent the PVR potential of vitreous. The total protein represents a "soup" of all the detectable proteinaceous components in the vitreous and is a measure of inflammation, break down of the blood retinal barrier and wound healing. Kon et al (Kon *et al.*, 1999) found a significantly higher protein levels in PVR vitreous compared with non-PVR vitreous (mean 5.72 mg/ml Vs 2.89 mg/ml). We also found a significantly higher ($p=<0.001$) of protein (median 0.17 mg/ml Vs 0.08mg/ml) in PVR vitreous compared to non-PVR vitreous. The differences between the two levels were similar in both studies (2.1 Vs 2.0). Both Connor et al (Connor, Jr. *et al.*, 1989) and Kauffmann et al (Kauffmann *et al.*, 1994) also found significant differences with a fivefold and three fold difference respectively.

Kon et al (Kon *et al.*, 1999) went on to investigate the relationship of cytokines and protein levels and postoperative PVR.

Cytokine and protein levels as risk factors for developing PVR

Many investigators have looked at the relationship of preoperative PVR and cytokines. Kon et al (Kon *et al.*, 1999) were the first group to look at cytokine levels and postoperative PVR. Using univariate analysis they found that TGF-β_2, bFGF, IL-6 and protein to be significant risk factors ($p=0.05$). In our study we only found protein to be a risk factor ($p=0.02$). Using multiple logistic regression analysis Kon et al (Kon *et al.*, 1999) found Il-6 and protein to be independent predictive risk factors. As expected from our univariate analysis only protein was an independent risk factor.

Our results suggest that cytokines are involved in the PVR process, although their exact role is unclear. It is very unlikely that a single or just a few growth factors are only responsible. In contrast it is more likely that the PVR process involves an intricate pathway of agonist and antagonist interactions between many of the growth factors.

It is also not surprising that our results are different from those of Kon et al. In our study the peroperative PVR rate was 7.5% versus 35.0% in Kon et al study. Also our postoperative PVR rate was much smaller, 16.2% versus 29.4% respectively. They explained that the high rate of postoperative PVR was caused by their patients representing a high- risk group as they all required vitrectomy. As a result, their results can only be applied to a similar high risk- group. Our study had a postoperative PVR rate that is similar to other published trials (Bonnet, 1988; Duquesne *et al.*, 1996; Rachal and Burton, 1979). Therefore, our patient group may better represent the majority of patients

requiring vitrectomy for rhegmatogenous retinal detachment. It is also surprising that IL-6 remained an independent risk factor after accounting for protein.

When we investigated the cytokine and protein profile of patients who developed postoperative PVR in the trial patients we found that the levels of bFGF, IL-1β, Il-6 and protein were much higher in the 5-FU/heparin versus the placebo treated group. The levels did not reach statistical significance due to the low incidence of postoperative PVR in the 5-FU/heparin treated group. This suggests that very high levels of cytokines and protein were present and that despite adjuvant treatment these patients developed PVR.

In summary, it is likely that the wound healing response is dependent on complex interactions between numerous cytokines and that single cytokine measurement are poor makers for the overall picture. Further work in animal models and human vitreous samples needs to be done to further understand the complex interactions of cytokines and the role they play in PVR.

Cytokine and protein levels as risk factors for developing PVR

We have already discussed the clinical risk factor model to predict PVR. We can also construct a model using protein. Using this model with a probability cut off value of 0.15 we were able to correctly predict postoperative PVR in 60.0% of patients.

By combining cytokine, protein and clinical risk factors and using multiple logistic regression on the individual factors only preoperative PVR grade CP1 plus remained an independent risk factor (p=0.001). Using logistic regression Kon et al (Kon *et al.*, 1999) found IL-6, protein and aphakia to be independent risk factors.

Using a regression equation with clinical risk factors and protein increased the predictive power of the model by 12.06% compared to the clinical model. However, this represents

a drop of 13.27% in prediction compared to just using protein alone. This discrepancy may represent the difficulty in formulating an efficient statistical model to test the regression equation. Ideally, a further prospective study would be required to test the model.

At present there are no practical ways to measure biological risk factors perioperatively and this limits the usefulness of a model based on biological risk factors. The information gained from this study does however contribute further to developing a clinical model to predict postoperative PVR in patients undergoing primary vitrectomy for rhegmatogenous retinal detachment.

Chapter 6

Conclusions

We have seen an improvement in surgical outcome from no success to over 90% anatomical success since Gonin's era. However, there are still a proportion of patients who progress to develop postoperative PVR. This study has shown that it is possible to reduce the risk of postoperative PVR in high-risk patients by using adjuvant treatment with 5-FU and LMWH. This is the first study to show a clear treatment effect and in view of the lack of side effects and the relatively low cost (the cost of combined treatment is $6.00), we advocate the use of this treatment in the future management of all patients with an increased risk of developing postoperative. A further prospective study will be required to access its efficacy in treating all patients undergoing primary vitrectomy for rhegmatogenous retinal detachment.

This study has again shown that final visual acuity is significantly worse in patients that develop postoperative PVR. If it is possible to prevent PVR than the visual outcome will be improved. Therefore, we would expect the final visual acuity outcome of patients treated with adjuvant therapy to be improved. This was shown.

This study highlights the difficulty in comparing studies in which; definitions, outcome criteria, methodology and statistical tests differ. Even in studies designed to be similar in methodology comparison is difficult.

This study has shown that it is possible to define high- risk patients using a simple formula based on easily identifiable risk factors.

The study has also given us a further opportunity to advance our knowledge of the pathogenesis of PVR and in particular the risk factors involved. We have confirmed that preoperative PVR grade CP1 is a significant independent risk factor for developing PVR and have refuted some of the previously accepted risk factors.

This study has also highlighted the complex interactions involved in the PVR process and the need for further research especially as there is now an effective treatment available to prevent PVR.

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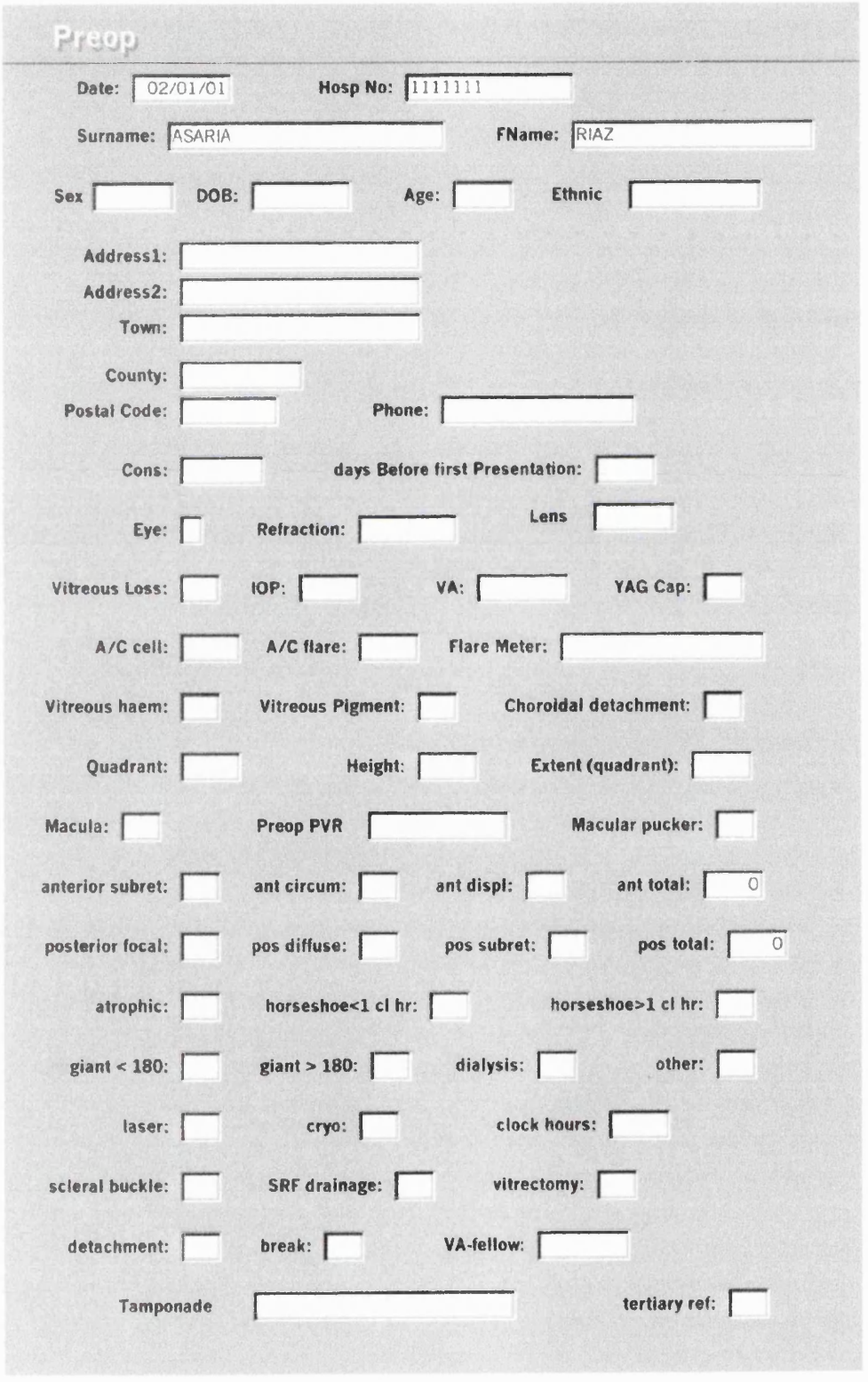
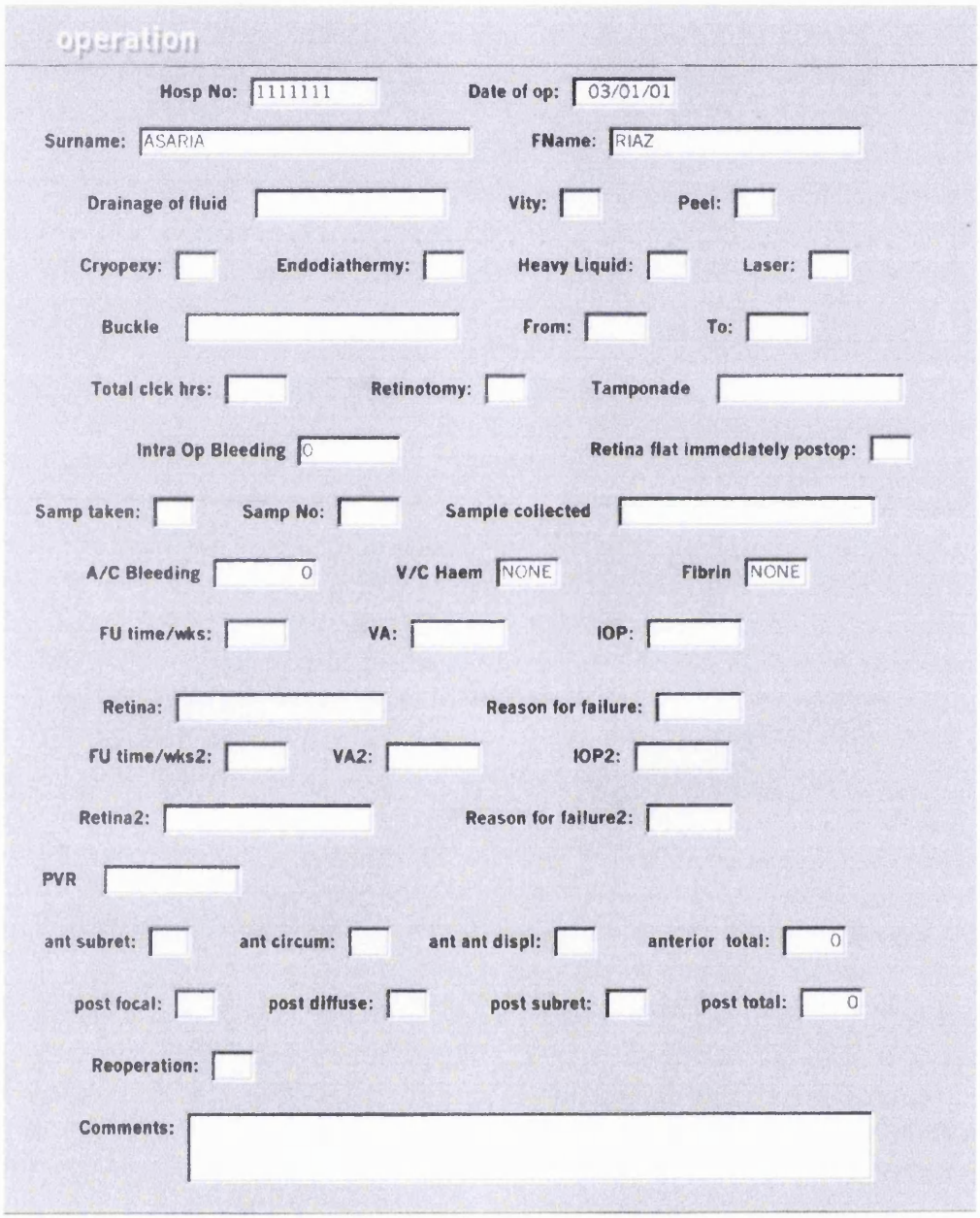
Appendix I
Data Base Entry Forms




operation
Hosp No: 1111111
Date of op: 03/01/01
Surname: ASARIA
FName: RIAZ
Drainage of fluid
Vity:
Peel:
Cryopexy:
Endodiathermy:
Heavy Liquid:
Laser:
Buckle
From:
To:
Total clck hrs:
Retinotomy:
Tamponade
Intra Op Bleeding 0
Retina flat immediately postop:
Samp taken:
Samp No:
Sample collected
A/C Bleeding 0
V/C Haem NONE
Fibrin NONE
FU time/wks:
VA:
IOP:
Retina:
Reason for failure:
FU time/wks2:
VA2:
IOP2:
Retina2:
Reason for failure2:
PVR
ant subret:
ant circum:
ant ant displ:
anterior total: 0
post focal:
post diffuse:
post subret:
post total: 0
Reoperation:
Comments:

Appendix II
Patient Explanation Form

Title

Combination adjuvant therapy using to surgery using low molecular weight heparin & 5 fluorouracil for proliferative vitreoretinopathy.

Background

The retina is a thin layer which lines the inside of the eye. It is sensitive to light (like the film in a camera) and is necessary for vision. Your retina is detached, because one or more holes have developed in it, allowing fluid to pass underneath it. Treatment is aimed at closing the holes but this is often complicated by the development of scar tissue on the surface of the retina. This scar tissue pulls the retina into a knot, preventing the holes from being closed. This is known as proliferative vitreoretinopathy (PVR). If PVR develops than the retina may re- detach, and further treatment is necessary.

There is good laboratory evidence that the development of scar tissue might be prevented by using a combination of medicines given at the time of surgery. We do not yet know whether such additional treatment is helpful or not in real patients.

Purpose of the study

This study is being conducted in order to find out whether the use of a combination of such medicines can reduce the risk of PVR. The two medicines to be used are ; low-molecular weight heparin and 5-fluorouracil.

The medicines being used are not new, and have been extensively used for other medical conditions. Heparin is an anticoagulant used to treat blood clots and 5-Fluorouracil is an

antiproliferative agent used in cancer treatment. Both these agents have previously been used in the eye and have been found to be safe. Therefore we do not anticipate any unknown side effects.

Study design

If you agree to enter the study, you will be randomly allocated to one of two groups. If you are in the treatment group, you will be given the two drugs which will be added to the fluid which is infused into your eye while the operation is being carried out. The operation and follow up will be the same in each group. Neither, you nor your doctor will know which group you are in until the study is completed.

Procedures involved

The operation will be the standard procedure for your particular detachment. The jelly in the middle of your eye is removed, along with any cataract you may have. Any scar tissue present may be removed, if the retina is still not flat a cut may be made to the retina to relieve the tension and aid flattening.

Gas is then put in the eye to replace the jelly. The gas floats in the eye and prevents fluid from getting through any holes. A laser is then used to "spot weld" the retina so that it remains permanently attached. A plastic strip may be placed on the eye to form an indent (a "buckle") which helps to keep the holes closed.

After the operation, you will be asked to "posture", that is to sit or lie in a particular position for about 50 minutes in each hour for 5 to 10 days. This is so the gas can push up against the retina and close the holes.

Consequence of procedures

The eye is often uncomfortable for several weeks after surgery. No extra discomfort is expected as a result of the medications that you may receive if you are in the treatment group.

Benefits

The results of this study will tell us whether the extra medication reduces the risk of postoperative PVR. This will be of particular importance should a detachment occur in the other eye.

Termination

If the results of the study show a significant difference between the two groups before the study has finished, it will be stopped and all patients will be offered the better treatment. Should any significant side effects occur, the study will be terminated

Confidentiality

Information derived from the study will be treated as completely confidential. Information will be stored in electronic and paper form and kept in a secure location. All electronic data will comply with the requirements if the data protection act.

Voluntary participation

If you do not wish to participate in this study you will not be disadvantaged in any way and will receive the normal clinical management. If you do wish

to participate your General Practitioner will be informed. You will be free to withdraw from the study at any stage and will not be disadvantaged.

No one who is pregnant or could become pregnant should participate in the study

Contact name and telephone number

Mr Riaz Asaria FRCOphth - 0171-253 3411, bleep 172.

Appendix III

Consent record

I agree to/prefer not to (delete as appropriate) participate in the clinical study entitled: Combination adjuvant therapy using low molecular weight heparin and 5 fluorouracil in those patients at risk of developing Proliferative Vitreoretinopathy.

I agree to participate in the study. The nature of the study has been fully explained to me and understood by me. The written explanation given to me is attached and I have been given a copy of this consent form to keep. I am aware that there could also be unforeseen risks and that I must assume that if I suffer injury I will only receive compensation that I am entitled to receive under the law. In other words I shall be in the same position I would be in if I were receiving normal clinical management.

The nature of this study and treatment has been fully explained to and understood by me. I understand I am required t inform you of any involvement I may have in other studies. I agree that my GP be informed of my involvement in this study.

Print name..... Signature.....

Date.....

The explanation of this trial has been given to me by:

Print name..... Signature.....

Date.....

The explanation of this trial has been given to me in the presence of:

Print name..... Signature.....

Date.....

Appendix IV

Risk factors for proliferative vitreoretinopathy – a prospective study

Key Words : proliferative vitreoretinopathy, vitrectomy, risk

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ABSTRACT

Aim: To assess clinical variables and vitreous protein as risk factors for the development of postoperative proliferative vitreoretinopathy (PVR).

Methods: A prospective study was conducted on 140 patients with a rhegmatogenous retinal detachment in whom a primary vitrectomy was performed. 12 clinical variables were recorded and vitreous samples obtained for measurement of protein concentration. Univariate and multivariate logistic regression analysis were used to determine the risk factors for PVR.

Results: Complete data were available for 136 out of 140 patients. 40 of the 136 patients (29.4%) developed postoperative PVR. Univariate regression revealed that significant ($p<0.05$) risk factors included aphakia, presence of preoperative PVR, size of detachment, the use of silicone oil and high vitreous protein level. Multivariate regression analysis revealed only aphakia (odds ratio 2.72), the presence of preoperative PVR (odds ratio 3.01) and high vitreous protein concentration (odds ratio 1.11) to be significant ($p<0.05$) independent, predictive risk factors for the development of PVR.

Conclusions: Our study has shown that the significant risk factors for PVR are preoperative PVR, aphakia and high vitreous protein levels. Two models (clinical factors only and clinical factors and vitreous protein) were constructed to predict the probability of developing postoperative PVR and may be used to identify those at risk for possible intravitreal pharmacological treatment.

INTRODUCTION

The success rate of retinal detachment surgery has now reached over 90%. The major cause of failure is attributable to the development of proliferative vitreoretinopathy (PVR).[1;2] It is a complex process comprised of events that are similar to those of the wound healing response with inflammation, migration and proliferation of a variety of cells.[3-5] To improve the prognosis of retinal detachment surgery, recent research has focused on the use of intravitreal pharmacological agents to prevent the occurrence of PVR [6-12], although none of these agents are used routinely in clinical practice due to concerns of retinal toxicity. However, if risk factors for the development of PVR could be identified, these potentially toxic intravitreal treatments could be targeted at those patients at greatest risk. A similar principle is used to maximise efficacy and minimise side-effects of anti-scarring therapy in glaucoma filtration surgery. [13]

Many studies have investigated and identified a number of clinical risks factors for PVR. These include preoperative vitreous haemorrhage,[14-16] PVR at presentation,[15; 17; 18] aphakia,[16; 19] large retinal break,[16; 20] use of cryopexy,[18] duration of detachment,[16] the presence of choroidal detachment preoperatively,[17; 18] horse-shoe tear,[16; 20] and the use of silicone oil.[21; 22] However, results from these studies are often contradictory and inconclusive. Girard et al[17] cited that the reason is partly due to "the absence of a clear distinction between preoperative and postoperative PVR and to the use of quantitative methods of statistical analysis". To this, we believe, may be added the unavoidable bias of retrospective studies.

We have conducted a prospective study to identify the risk factors for the development of PVR in patients undergoing a primary vitrectomy for a rhegmatogenous retinal

detachment. This study population was chosen because intraocular access is part of the operation, and the benefit:risk of instillation of intravitreal treatments is most favourable in these patients. We studied both clinical variables and protein levels in the vitreous. The risk factors were analysed using univariate and multivariate logistic regression.

MATERIALS AND METHODS

140 consecutive patients with rhegmatogenous retinal detachment in whom vitrectomy was considered necessary for a number of reasons including giant retinal tear, posterior retinal break, the presence of preoperative PVR and media opacities were enrolled into this prospective study between January 1995 and February 1996. Eyes with the following conditions were excluded: penetrating eye injury, history of blunt trauma to eye of less than 6 months, concurrent eye conditions e.g. infection, history of intraocular eye surgery of less than 6 months and patients on steroid treatment, topically or systemically. Informed consent was obtained from all participating patients.

Preoperative Assessment

A medical and ophthalmic history were taken and examination performed on all patients. Specific attention was paid to the risk factors under investigation, which included age, duration of symptoms, degree of myopia, preoperative lens status ("phakia" or "aphakia": eyes, including pseudophakic eyes, with intact posterior capsules were classified as "phakia"; eyes, including those which were pseudophakic, that did not have intact posterior capsule were classified as "aphakia"), preoperative use of cryotherapy/laser, presence of preoperative PVR, presence of preoperative uveitis, presence of preoperative vitreous haemorrhage, size of detachment and preoperative macula status (detached or

not) **(Table 1)**. Preoperative PVR was considered present if there was greater than 1 clock hours, Grade C PVR in the updated version of the Retina Society classification.[23]

Intraoperative and Follow-up Assessments

Details of the procedures during the operation were recorded for each patient, including the use of cryotherapy and the type of tamponade employed. The patients were followed up for a minimum of 3 months and assessed for:

1. the status of the retina and the development and grade of PVR, if any,
2. the development of complications including cataract, glaucoma, iatrogenic breaks, infection, vitreous haemorrhage and hyphaema,
3. details of further operations, if any.

Postoperative PVR was defined as either the presence of new PVR of greater than 1 clock hours of Grade C PVR in a detached retina after the vitrectomy or, in a successfully attached retina, new clinically visible periretinal membranes or bands of greater than 1 clock hours. A successful outcome was defined as a completely attached retina without an internal tamponade at the last follow-up.

Collection , Storage and Assay of Vitreous Protein

At the beginning of the routine 3-port pars plana vitrectomy, a vitreous sample was collected using the vitreous cutter without an infusion to prevent dilution of the sample. The samples were placed into siliconised Eppendorfs (Sigma, Poole, U.K.), immediately frozen and kept at -70°C for protein level analysis.

The total protein concentration of the vitreous samples was measured using the Bio-Rad Protein Microassay (Bio-Rad, Herts., U.K.). This colorimetric assay utilises a solution of cupric ions which forms a copper/protein complex (coloured compound) with the protein.

This method was chosen as it allows the rapid screening of multiple small volume vitreous samples and results are obtained within 45 minutes.

Data Handling and Statistical Methods

All statistical analyses were carried out using the computer software program SPSS for Windows Release 6.0 (SPSS Inc., Chicago, U.S.A.). The two-tailed independent sample T-test was used to analyse the results of protein level in the vitreous. The forward stepwise logistic multiple regression analysis was used to analyse the protein and clinical risk factors together to predict the risk of developing of postoperative PVR. This method of analysis involves developing a mathematical model that uses a combination of the values of a group of explanatory variables (protein levelsand clinical risk factors) to predict the value of a dependent variable (postoperative PVR). Initially the risk factors were analysed in a univariate analysis then the multivariate logistic regression analysis was used to reveal independent risk factors. Multivariate analysis was first applied to the clinical data alone and then to clinical data and protein level combined.

Results

Patient Profile

Complete data were available for 136 out of 140 patients. 4 patients were lost to follow-up due to transfer of care to other hospitals or non-attendance at follow-up clinics. Of the 136 patients, 94 were male and 42 were female. The mean age was 59.0 years (range 16 to 86 years). The patients were followed up for at least 3 months with a mean follow-up time of 8.3 months. **Table 1** shows the clinical risk factors and the frequency of occurrence.

Clinical Results

40 of the 136 patients (29.4%) developed postoperative PVR. **Table 2** shows the association between the presence of preoperative PVR and the development of postoperative PVR. A significantly higher ($p<0.05$) proportion of patients with preoperative PVR developed postoperative PVR.

The rate of successful outcome (complete anatomical retinal reattachment) was 78.7%. **Table 3** shows the outcome in relation to the presence of preoperative PVR. The rate of successful outcome was significantly higher ($p<0.05$) in patients with no preoperative PVR compared to those with preoperative PVR. The relationship between postoperative PVR and outcome is shown in **Table 4**. A successful outcome was achieved in a significantly higher proportion ($p<0.05$) of eyes that did not develop postoperative PVR compared to those that did.

Presenting visual acuity and visual acuity at last follow-up are shown in **Figure 1**. At the last follow-up, 83 patients (61.0 %) had improved visual acuity whilst 24 patients (17.7%) had worse visual acuity than preoperatively. 29 (21.3%) had no change in visual acuity. **Table 5** shows the final visual acuity of the successful cases (n=107) classified into those who had preoperative PVR and those who did not. A significantly higher proportion ($p<0.05$) of patients achieved a visual acuity of 6/60 or better in the non-preoperative PVR group. The final visual acuity of successful cases (n=107), classified into those who developed postoperative PVR and those who did not is shown in **Table 6**. A significantly higher proportion ($p<0.05$) of patients achieved a visual acuity of 6/60 or better in the non-postoperative PVR group.

Vitreous Protein

The mean protein concentration (with 95% confidence interval) was significantly higher ($p<0.05$) in patients who had preoperative PVR (5.72 mg/ml (3.68 - 7.76)) compared to those who did not (2.89 mg/ml (1.96 - 3.82)). The mean protein concentration (with 95% confidence interval) was also significantly higher ($p<0.05$) in patients who developed postoperative PVR (6.83 mg/ml (4.57-9.16)) compared to those who did not (2.81 mg/ml (1.87-3.75)). The percentage of patients developing PVR and its relationship to vitreous protein is shown in **Figure 2**.

Risk Factor Analysis

Univariate analysis. Analysis of individual factors in a univariate regression revealed that significant ($p<0.05$) risk factors included "aphakia" (all eyes in this group did not have an intact posterior lens capsule including pseudophakic eyes), presence of preoperative PVR (>1 clock hours, Grade C PVR), size of detachment, the use of silicone oil and high vitreous protein level.

Multivariate analysis of clinical risk factors. Multivariate logistic regression analysis on the clinical data revealed only "aphakia" and the presence of preoperative PVR to be significant ($p<0.05$) independent, predictive risk factors for the development of PVR. **Table 7** shows the estimated coefficients and the odds ratios for the significant clinical risk factors. Based on the above estimated coefficients, the logistic regression equation of the probability of developing PVR was worked out which would allow quantification of changes in risk in relation to clinical status.

Estimated probability of developing PVR =

$$\frac{1}{1+e^{1.7802 - 1.1408(\text{aphakia}) - 1.2302(\text{preop PVR})}}$$

In the equation, a value of 1 is entered for aphakia and 0 is entered for phakia. If preoperative PVR is present, a value of 1 is entered with 0 entered for no preoperative PVR. It is estimated that when the lens status (preoperatively) changes from phakia to aphakia, the odds of developing PVR are increased by 3.13 times. The presence of preoperative PVR increased the odds by 3.42 times.

Multivariate analysis of clinical risk factors and vitreous protein. Multivariate logistic regression analysis of the clinical data and protein level revealed that "aphakia", the presence preoperative PVR and high vitreous protein level were significant ($p<0.05$) independent, predictive risk factors for the development of PVR. **Table 8** shows the estimated coefficients and the odds ratios for the significant risk factors.

Estimated probability of developing PVR =

$$\frac{1}{1+e^{2.0918 - 0.9993(\text{preop PVR}) - 1.1029(\text{aphakia}) - 0.1029(\text{protein})}}$$

In the equation, if preoperative PVR is present, a value of 1 is entered with 0 entered for no preoperative PVR. A value of 1 is entered for aphakia and 0 is entered for phakia. Protein is entered in mg/ml. It is estimated that in the presence of preoperative PVR, the odds of developing postoperative PVR are increased by 2.72 times. When the lens status (preoperatively) changes from phakia to aphakia, the odds of developing PVR are increased by 3.01 times. For each mg increase in the protein level the odds are increased by 1.10 times.

DISCUSSION

This prospective study has shown that PVR (presence preoperatively and development postoperatively) has an adverse effect not only on the surgical outcome but also on the final visual acuity achieved in successful cases. Using multifactorial analysis, the study has also shown that significant risk factors for the development of postoperative PVR are preoperative PVR, aphakia and high vitreous protein levels.

The existence of preoperative PVR suggests that the cellular, extracellular and chemical elements required for wound healing are present. It is therefore not unreasonable to expect preoperative PVR to be a risk factor for the development of postoperative PVR. Girard *et al*'s[17] retrospective study of preoperative PVR Grades B and C1 found only Grade B but not Grade C1 preoperative PVR to be a significant risk factor. They hypothesised that Grade B PVR may represent an immature form of PVR with a definite potential for progression, whereas Grade C1 PVR may represent a spontaneously arrested, non-evolutive form of the disease. Our study did not evaluate Grade B PVR as a risk factor as most patients with Grade B PVR did not require vitrectomy and therefore the number of patients enrolled was small (n=9). However, we found Grade C PVR involving more than 1 clock hours to be a significant risk factor. Although this contrasts with Girard *et al's* findings, the two studies are not directly comparable as our study was prospective, used different PVR gradings and included only patients undergoing vitrectomy.

Chignell *et al*[19] described aphakia as one of the significant preoperative factors contributing to failure of retinal detachment surgery. They cited that the majority of

failures were due to inaccurate preoperative assessment (e.g. failure to observe holes) and that this was particularly true for aphakic eyes where small holes in the periphery were difficult to identify and thus remained untreated. They revealed that reattachment surgery failed in 53 (11.7%) eyes despite repeated operations. 37 (69.8%) of these failures had developed postoperative PVR and in this group, 12 (32.4%) eyes were found to be aphakic. Yoshino *et al*[16] also found that amongst others, aphakia was a risk factor for the development of postoperative PVR. Other studies[17; 24] however, did not find aphakia to be a risk factor. The pathological mechanism by which aphakia could be related to the development of PVR is unclear. However, the breakdown of blood-ocular barrier may be significant[16]. Miyake[25; 26] found that there was more disruption to the blood-retinal barrier after intracapsular compared to extracapsular cataract extraction. Miyake *et al*[27] also found that the outward active transport of fluorescein from the vitreous was reduced in aphakic compared to phakic eyes. They suggested that the posterior lens capsule may protect the anterior uvea (site of active transport) from mechanical and physical irritation by the vitreous gel. The disruption of blood-retinal barrier would, in theory, allow serum factors e.g. fibronectin to enter and remain in the vitreous and may enhance the development of PVR. We have classified pseudophakia without an intact posterior capsule (e.g. capsular dehiscence during cataract surgery) as "aphakia" because we believe that the posterior capsule plays an important role in the blood-retina barrier irrespective of the presence of an intraocular lens.

The total protein level represents the sum of all the detectable proteinaceous components in the vitreous and therefore does not provide specific information regarding individual enzymatic or cytokine activity. Nevertheless, the total protein level can provide

information on the state of inflammation, breakdown of blood-retinal barrier and the severity of wound healing. In our study, significantly higher ($p<0.05$) protein levels were found in the vitreous of eyes with preoperative PVR compared to those without (mean of 5.72 mg/ml vs 2.89 mg/ml). This finding is in agreement with previous studies[3; 28] although the difference in protein level between the PVR and non-PVR groups in our study is smaller. Connor *et al*[28] found a five-fold, Kauffmann *et al*[3] found a three-fold, while our study only found a two fold increase. As far as we are aware, there is no report in the literature relating vitreous protein concentration to the development of postoperative PVR. In our series the mean concentration of vitreous protein was significantly higher in those patients who developed postoperative PVR compared to those who did not (mean of 6.83 mg/ml vs 2.81 mg/ml) and for each mg increase in the protein level the odds of developing PVR is increased by 1.10 times.

2 statistical models were constructed to predict the probability of an individual patient developing PVR. The first model used the clinical risk factors alone whilst the second included both the clinical risk factors and the vitreous protein level. As with any statistical model, it is often difficult to determine the efficiency of the model in predicting the risk. Ideally, a further prospective study applying the findings discussed above would be required to test the efficiency of the model. However, a less ideal method of assessing the "goodness-of-fit" of the model was used in this study. This method compared our predictions to the observed outcome from the original data. A probability cut-off value of 0.5 was chosen for the development of PVR. A patient is predicted to develop postoperative PVR if the calculated probability is above 0.5 and predicted not to develop PVR if it is lower than 0.5. Using this method, our first model (clinical risk factors alone)

correctly predicted the outcome in 72.8% of patients. The second model (combined clinical risk factors and protein level) correctly predicted the outcome in 76.5 % of the patients. Therefore, although the clinical risk factor model can be helpful in identifying those at risk, the combined model has a greater predictive value.

The identification of these risk factors in our group of patients (primary vitrectomy for retinal detachment) is of particular practical importance. As intraocular access forms part of the operation, those at risk can receive intravitreal instillation of pharmacological treatment without the need for further procedures.

At present, the measurement of protein concentration in the vitreous in our laboratory can be achieved within 45 minutes. If this duration could be shortened, this would allow measurement of vitreous protein concentration during the operation. The result, together with clinical information, may be used in our combined postoperative PVR model to identify patients at risk of developing PVR. The identification of these high risk patients is of vital importance if they are to be targeted for aggressive treatment and if improvements in success rate of retinal detachment surgery are to be achieved.

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Legends

Table 1 Frequency data on clinical risk factors

Table 2 Relationship between pre- and postoperative PVR

Table 3 Relationship between preoperative PVR and surgical outcome

Table 4 Relationship between postoperative PVR and surgical outcome

Table 5 Preoperative PVR and final visual acuity in successful cases (n=107)

Table 6 Postoperative PVR and final visual acuity in successful cases (n=107)

Table 7 Estimated coefficients and odds ratios for the significant clinical risk factors

Table 8 Estimated coefficients and odds ratios for the significant clinical risk factors and vitreous protein

Figure 1 Scatter plot (sunflower) of visual acuity

Key:

CF – count fingers
HM – hand movement
PL – perception of light
NPL – no perception of light

Each sunflower petal represents 1 case

Figure 2 Percentage of patients who developed postoperative PVR and its relationship to vitreous protein.

Table 1 Frequency data on clinical risk factors

Factors			Frequency	Percentage
1. Age (years)	range	16 - 86 years		
	mean (S.D.)	59.0 years (16.37)		
2. Duration of symptoms	range	1- 540 days		
	mean (S.D.)	50.9 days (92.96)		
3. Degree of myopia		not myopic	78	57.4
		refraction 0.00 to -5.00	26	19.1
		refraction > -5.00	32	23.5
4. Preoperative lens status		"phakia"	96	70.6
		"aphakia"	40	29.4
5. Preoperative cryotherapy/laser		none or >3 months ago	90	66.2
		< 3 months ago	46	33.8
6. Preoperative PVR		no PVR	83	61.0
		> 1 clock hours grade C	53	39.0
7. Uveitis		presence of cells in A/C	24	17.6
		absence of cells	112	82.4
8. Preoperative vitreous haemorrhage		presence	19	14.0
		absence	117	86.0
9. Size of detachment (quadrants)		1 quadrant	14	10.3
		2 quadrants	42	30.9
		3 quadrants	32	23.5
		4 quadrants	48	35.3
10. Macula status		macula on	38	27.9
		macula off	98	72.1
11. Intraoperative cryotherapy		used	74	54.4
		not used	62	45.6
12. Intraoperative tamponade		SF_6	71	52.2
		C_3F_8	25	18.4
		silicone oil	40	29.4

Table 2 Relationship between pre- and postoperative PVR

		Preoperative PVR	
		No	**Yes**
Postoperative PVR	**No**	65 (78.3 %)	31 (58.5 %)
	Yes	18 (21.7 %)	22 (41.5 %)
Total		83 (100.0 %)	53 (100.0 %)

$\chi^2 = 6.122$; $p<0.05$

Table 3 Relationship between preoperative PVR and surgical outcome

		Preoperative PVR	
		No	**Yes**
Outcome	**success**	70 (84.3 %)	37 (69.8 %)
	failure	13 (15.7 %)	16 (30.2 %)
Total		83 (100.0 %)	53 (100.0 %)

$\chi^2 = 4.068$; $p<0.05$

Table 4 Relationship between postoperative PVR and surgical outcome

		Postoperative PVR	
		No	**Yes**
Outcome	**success**	83 (86.5 %)	24 (60.0 %)
	failure	13 (13.5 %)	16 (40.0 %)
Total		96 (100.0 %)	40 (100.0 %)

$\chi^2 = 11.782$; $p<0.05$

Table 5 Preoperative PVR and final visual acuity in successful cases (n=107)

		Preoperative PVR	
		No	**Yes**
Final Visual Acuity	**6/60 or better**	72.9 %	40.5 %
	worse than 6/60	27.1 %	59.5 %
Total		100.0 %	100.0 %

$\chi^2 = 10.696$; $p<0.05$

Table 6 Postoperative PVR and final visual acuity in successful cases (n=107)

		Postoperative PVR	
		No	**Yes**
Final Visual Acuity	**6/60 or better**	68.7 %	37.5 %
	Worse than 6/60	31.3 %	62.5 %
Total		100.0 %	100.0 %

$\chi^2 = 7.655$; $p<0.05$

Table 7 Estimated coefficients and odds ratios for the significant clinical risk factors

Variable	Coefficient (B)	p-value	odds ratio (e^B)	95% C.I. of e^B
"Aphakia"	1.14	0.008	3.13	1.35 to 7.26
Preoperative PVR	1.23	0.003	3.42	1.54 to 7.62

Table 8 Estimated coefficients and odds ratios for the significant clinical risk factors and vitreous protein

Variable	Coefficient (B)	p value	odds ratio (e^B)	95% C.I. of e^B
Preoperative PVR	0.10	0.019	2.72	1.18 to 6.27
"Aphakia"	1.10	0.013	3.01	1.26 to 7.22
Protein	0.10	0.016	1.11	1.02 to 1.21

Preoperative
visual acuity

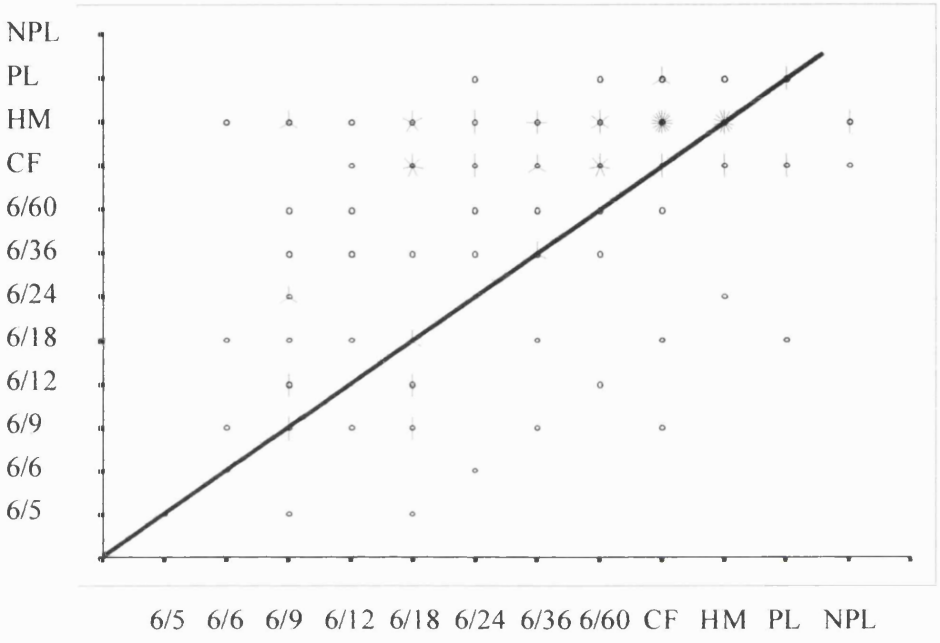
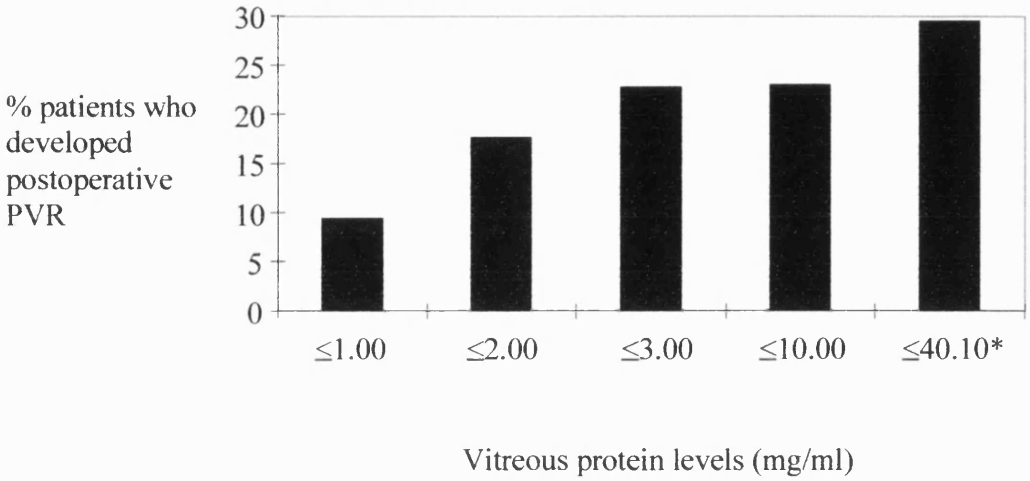


Figure 1 Scatter plot (sunflower) of visual acuity

Key:

CF – count fingers
HM – hand movement
PL – perception of light
NPL – no perception of light

Each sunflower petal represents 1 case



*highest level of vitreous protein detected

Figure 2 Percentage of patients who developed postoperative PVR and the relationship to vitreous protein.

Appendix V

Notes from oral examination

1. Following completion of the thesis the primary outcome measure was available for all the patients in the active group and not available for two of the patients in the placebo group. This has strengthened the statistical outcome.
2. Three patients in the trial received silicone oil, two in the active treated and one in the placebo treated group.
3. Missed breaks and posterior vitreous seperation were not analysed as risk factors