Vitreomacular adhesion: its treatment and effect on neovascular age-related macular degeneration therapeutics

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VITREOMACULAR ADHESION: ITS TREATMENT AND EFFECT ON NEOVASCULAR AGE-RELATED MACULAR DEGENERATION THERAPEUTICS

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Declaration

I, James E. Neffendorf, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed: 

Submitted December 2021

Abstract

Background

Vitreomacular interface conditions can substantially affect vision. In addition, vitreomacular status may influence the clinical course of other diseases such as neovascular age-related macular degeneration (nAMD).

Aims

To investigate the efficacy and safety of intravitreal ocriplasmin or gas for symptomatic vitreomacular adhesion (sVMA).

To assess the effect of vitreomacular interface status (attached or detached vitreous) on anti-vascular endothelial growth factor (anti-VEGF) drug clearance in nAMD.

To determine the effect of the vitreomacular interface status on the pharmacodynamics and systemic safety of anti-VEGF drugs.

Methods

- A meta-analysis of intravitreal ocriplasmin versus sham or placebo injection for sVMA.
- A pilot study investigating the effect of intravitreal ocriplasmin on hue discrimination measured by serial Farnsworth-Munsell 100 (FM-100) testing.
- A literature synthesis of intravitreal gas for sVMA.
- A clinical trial (VITCLEAR) using serial blood sampling to investigate the systemic pharmacokinetic and pharmacodynamic properties of two different anti-VEGF drugs (ranibizumab and aflibercept) for nAMD. Serum concentrations of the anti-VEGF drug, a panel of cytokines (including VEGF), high sensitivity C-reactive protein (hs-

CRP), and systemic renal function markers were measured at baseline and 11 time points over a time period of one month after intravitreal anti-VEGF injection.

Results

- The meta-analysis of intravitreal ocriplasmin included 932 participants from four randomised controlled trials. Ocriplasmin, when compared to control, was more likely to result in VMA release within 28 days (risk ratio 3.46, 95% confidence interval 2.00 to 6.00). Those receiving ocriplasmin were more likely to have an adverse event than control participants (risk ratio 1.22, 95% confidence interval 1.09 to 1.37).
- Thirteen patients were included in the hue discrimination pilot study. The FM-100 mean total error score worsened from 331.4 to 371.6 at one week ($p = 0.29$), and reduced further to 397.1 at one month ($p = 0.40$), before recovering to 349.1 at one year ($p = 0.19$).
- The literature synthesis of gas for sVMA included 91 eyes and found anatomic success in 48% at one month, and 57% at final review. Safety was acceptable, although there were retinal detachments in two highly myopic eyes.
- The VITCLEAR study included 53 participants. The systemic half-life of aflibercept was decreased when a posterior vitreous detachment was present compared to those with an attached posterior hyaloid, but not significantly so (20.6 days versus 23.3 days; $p = 0.66$). Compared to baseline, those who received aflibercept had a significant reduction in their serum VEGF concentration at one week and one month (104.5 vs 19.5 vs 39.6 ng/L, respectively; $p < 0.01$ at both time points compared to baseline), whereas those receiving ranibizumab had no change (139.3 vs 125.9 vs 133.4 ng/L, respectively; $p = 0.10$ and $p = 0.61$). Serum concentrations of renal

function markers were unchanged from baseline to one month in both anti-VEGF drug groups.

Conclusion

- For sVMA, there is evidence that ocriplasmin is an effective first line treatment, with an acceptable safety profile.
- Further studies are required to determine the effect of ocriplasmin on hue discrimination.
- Gas appears to be an effective treatment for sVMA, but retinal detachment can occur and larger studies are needed to determine the relative risk versus benefit, particularly in myopic eyes.
- The vitreomacular interface may influence the systemic anti-VEGF drug pharmacokinetics of aflibercept, but larger studies are needed to verify this non-significant difference. Intravitreal aflibercept results in a significant reduction of systemic VEGF concentration, whereas ranibizumab does not. Further work is needed to determine whether those with reduced serum VEGF levels have an altered systemic safety profile.

Contents

List of figures and tables

List of acronyms

Abbreviation	Meaning
AD	autosomal dominant
AE	adverse event
AIN	allergic interstitial nephritis
AMD	age-related macular degeneration
ANCHOR	anti-vascular endothelial growth factor antibody for the treatment of predominantly classic choroidal neovascularization in age-related macular degeneration
Ang	angiogenin
Ang-2	angiopoietin 2
ANGPTL4	angiopoietin like 4
ANOVA	analysis of variance
AR	autosomal recessive
ATE	arterial thromboembolic event
AUC	area under curve
anti-VEGF	anti-vascular endothelial growth factor
BCVA	best corrected visual acuity
BEAVRS	British and Eire association of vitreoretinal surgeons
bFGF	basic fibroblast growth factor
BL	baseline
BRB	blood-retinal barrier
B-Scan US	b-scan ultrasound
C2F6	hexafluoroethane
C3F8	octafluoropropane
CATT	comparison of age-related macular degeneration treatments trial
CENTRAL	Central register of controlled trials
CI	confidence interval
CKD-EPI	chronic kidney disease epidemiology collaboration
Cmax	maximum observed concentration
CRF	Clinical research facility
CRP	c-reactive protein
CSR	central serous retinopathy
CTIMP	clinical trial of an investigational medicinal product
CXCL-1	chemokine ligand 1
CXCL-9	chemokine ligand 9
CXCL-10	chemokine ligand 10
CXCL-12	chemokine ligand 12
CXCL-13	chemokine ligand 13
DMO	diabetic macular oedema
EDTA	ethylenediaminetetraacetic acid
EGF	epidermal growth factor
eGFR	estimated glomerular filtration rate
ELISA	enzyme-linked immunosorbent assay
ERM	epiretinal membrane
ETDRS	early treatment diabetic retinopathy study
Fc	fragment crystallizable
FDA	Food and drug administration
FM-100	Farnsworth munsell-100
FTMH	full thickness macular hole
GS	glomerulosclerosis

HGF	hepatocyte growth factor
hsCRP	high sensitivity c-reactive protein
HTN	hypertension
IC_{50}	inhibitory concentration
ICAM-1	intercellular adhesion molecule 1
ICG	indocyanine green
ICTRP	International clinical trials registry platform
IFN-γ	interferon gamma
IGF-1	insulin-like growth factor 1
IL-1α	interleukin 1 alpha
IL-1β	interleukin 1 beta
IL-2	interleukin 2
IL-3	interleukin 3
IL-4	interleukin 4
IL-6	interleukin 6
IL-8	interleukin 8
IL-10	interleukin 10
IL-12	interleukin 12
IL-13	interleukin 13
ILM	internal limiting membrane
INJECT	investigation of jetrea in patients with confirmed vitreomacular traction
IOP	intraocular pressure
IRC	intraretinal cysts
ISE	ion selective electrodes
IVAN	inhibition of vascular endothelial growth factor in age-related choroidal neovascularisation
LOCF	last observation carried forward
LOD	limit of detection
logMAR	logarithm of the minimum angle of resolution
M1	month 1
MARINA	minimally classic/occult trial of the anti-vascular endothelial growth factor antibody ranibizumab in the treatment of age-related macular degeneration
MedDRA	Medical dictionary for regulatory activities
MCP-1	monocyte chemoattractant protein
MIG	monokine induced by interferon-gamma
MGN	membranous glomerulonephritis
MH	macular hole
MRI	magnetic resonance imaging
nAMD	neovascular age-related macular degeneration
NCA	non-compartmental analysis
NEI-VFQ	National eye institute visual function questionnaire
NICE	National institute for health and care excellence
NO	nitric oxide
NOS	nitric oxide synthase
nPVD	no posterior vitreous detachment
NRES	National research ethics service
OCT	optical coherence tomography
ONL	outer nuclear layer
OPL	outer plexiform layer
PCR	posterior capsular rupture
PDGF-AA	platelet derived growth factor aa
PEDF	pigment epithelium derived factor

PET-CT	positron emission tomography/computed tomography
PGI-2	prostaglandin i2
PlGF	placental growth factor
PPV	pars plana vitrectomy
PRISMA	preferred reporting items for systematic reviews and meta-analysis
PRN	pro re nata
PrONTO	prospective optical coherence tomography imaging of patients with neovascular age-related macular degeneration treated with intra-ocular ranibizumab
PVD	posterior vitreous detachment
RCT	randomised controlled trial
READ-3	ranibizumab for edema of the macular in diabetes
RIVAL	randomised clinical trial comparing ranibizumab and aflibercept
RPE	retinal pigment epithelium
RR	risk ratio
RRD	rhegmatogenous retinal detachment
RVO	retinal vein occlusion
SAE	serious adverse event
SANA	systemic avastin for neovascular amd
SEM	standard error of the mean
SF6	sulfur hexafluoride
SRF	subretinal fluid
SST	serum separator tube
SUSAR	suspected unexpected serious adverse reaction
sVMA	symptomatic vitreomacular adhesion
$t_{1/2}$	half life
T&E	treat and extend
TES	total error score
TGF-β	transforming growth factor beta
TNF-α	tumour necrosis factor alpha
TMA	thrombotic microangiopathy
T_{max}	time to maximum concentration
VA	visual acuity
VCAM-1	vascular cell adhesion protein 1
VEGF	vascular endothelial growth factor
VIEW	vegf: trap-eye investigation of efficacy and safety in wet amd
VFQ	visual function questionnaire
VFQ-25	visual function questionnaire 25
VMA	vitreomacular adhesion
VMT	vitreomacular traction
W1	week one
WHO	World health organisation
XLR	x-linked recessive
Y1	year 1

Publications and manuscripts in preparation

The following papers were published as a result of work described in this thesis.

Neffendorf JE, Kirthi V, Pringle E, Jackson TL. Ocriplasmin for symptomatic vitreomacular adhesion. Cochrane Database Syst Rev. 2017 Oct 17;10(10):CD011874

Neffendorf JE, Kirthi V, Soare C, Jackson TL. The effect of intravitreal ocriplasmin on hue discrimination. Optom Vis Sci. 2021 Dec 1;98(12):1394-99.

Neffendorf JE, Simpson ARH, Steel DHW, Desai R, McHugh DA, Pringle E, Jackson TL. Intravitreal gas for symptomatic vitreomacular adhesion: a synthesis of the literature. Acta Ophthalmol. 2018 Nov;96(7):685-91.

Acknowledgements

I started retinal research 8 years ago, and this has dovetailed with a journey in clinical ophthalmology. I am now a consultant ophthalmologist with a specialist interest in vitreoretinal surgery. My career thus far has taught me extensively about my strengths and weaknesses, and has enabled me to understand how to interpret, appreciate and contribute to science.

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To the patients involved in my projects. It was a pleasure to look after all of you, and I will never forget the generosity you each gave with your time and effort to be involved with this work.

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1 Introduction

Due to the aging population, diseases that involve the vitreomacular interface are an increasing problem. They can affect central vision and therefore have the potential to cause significant loss of vision.[1] Disease and the anatomical status of the vitreomacular interface are also known to impact macular disease.[2] It is therefore important that research into the vitreomacular interface is prioritised. Furthermore, investigation into how the vitreomacular interface influences the elimination of intravitreal therapy is warranted. It is also important to further understand the systemic safety of intravitreal therapy after it leaves the eye.

1.1 **The vitreomacular interface**

The term vitreomacular interface refers to the anatomical location where the vitreous gel is in contact with the macula portion of the neuroretina. The macula is responsible for highly detailed vision and the appreciation of colour.

1.1.1 Anatomy of the vitreous

The vitreous is a gel-like structure which occupies the vitreous cavity of the eye, extending anteriorly from the lens at Weigert's ligament to the retina posteriorly. It is transparent and has a refractive index of 1.33.[3] The main constituent of vitreous is water, accounting for 98.5 – 99.7% of the total volume, with the remainder made up of hyaluronic acid, collagen, fibronectin, fibrillin and opticin.[4] The collagen fibres, 8 – 12 nm in diameter, fill the vitreous body and are interspersed with hyaluronic acid. Aside from a small number of hyalocytes, the vitreous is acellular.[3]

The vitreous has a central zone which is surrounded by a cortical zone. The cortical vitreous is most strongly attached to the vitreous base at the *ora serrata*, and has other strong attachment points at the optic disc, fovea and retinal vessels due to the higher concentrations of collagen in these locations.[5, 6]

Its anterior attachment to the lens occurs at Weigert's ligament, which circumferentially surrounds Berger's space (Figure 1.1). This potential space is continuous with Cloquet's canal, the remnant of the primary embryological vitreous, which stretches posteriorly to form a ring around the optic nerve head. The posterior border of the vitreous is the posterior

hyaloid membrane, which is a condensation in contact with the internal limiting membrane of the retina (ILM).

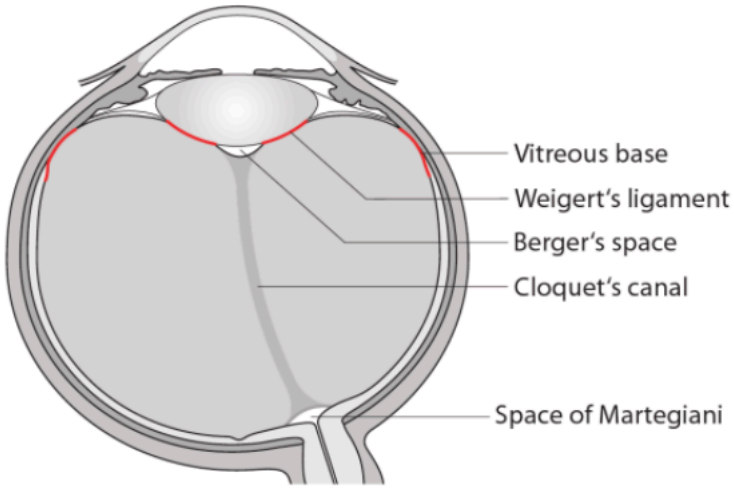

Figure 1.1: A cross-sectional view through the eye demonstrating the anatomical attachments of the vitreous gel.[7]

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1.1.2 Anatomy of the macula

The macula is the central part of the retina which is responsible for detailed vision and the appreciation of colour. It measures approximately 5.5 mm in diameter, sitting between the superotemporal and inferotemporal vascular arcades of the retina. At the centre of the macula is the fovea, a ≈1.5 mm diameter zone which contains the highest concentration of cone photoreceptor cells; those responsible for colour vision and fine visual discrimination. The central ≈500 µm of the fovea is free of capillaries, which enables a dense network of photoreceptors to provide high-quality vision. In contrast to other parts of the retina,

histologically the fovea constitutes only four layers; the ILM, outer plexiform layer (OPL), outer nuclear layer (ONL) and photoreceptor layer (Figure 1.2).

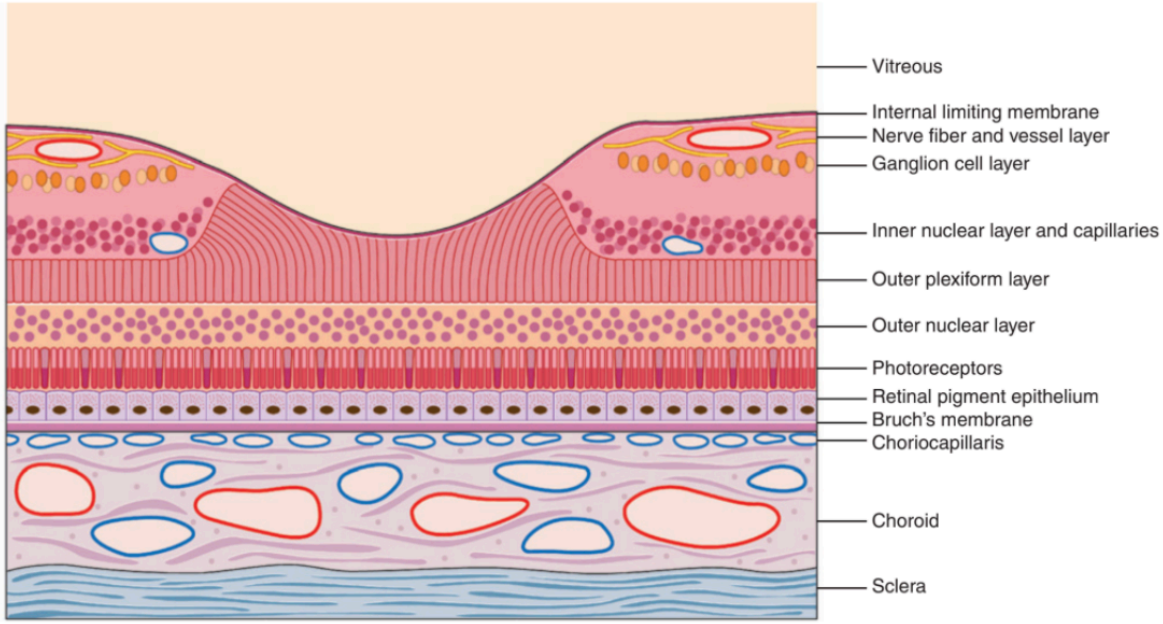


Figure 1.2: Schematic drawing of a microscopic section of the macula.[8]

This article was published in Ryan's Retina Sixth Edition by C.P. Wilkinson, D.R. Hinton, S.R. Sadda and P. Wiedermann, Chapter 1 – Fluorescein Angiography: Basic Principles and Interpretation, Page 10, Copyright Elsevier (2018).

1.1.3 Anatomy of the vitreomacular interface

The area at which the macula and posterior vitreous face meet is termed the vitreomacular interface. The point of connection is the vitreous cortex, which may be in direct continuity with the vitreous gel proper, or separated by a disc-shaped posterior premacular vitreous pocket (Figure 1.3). This pocket of fluid is also referred to as the premacular bursa, and usually forms in early childhood.[9]

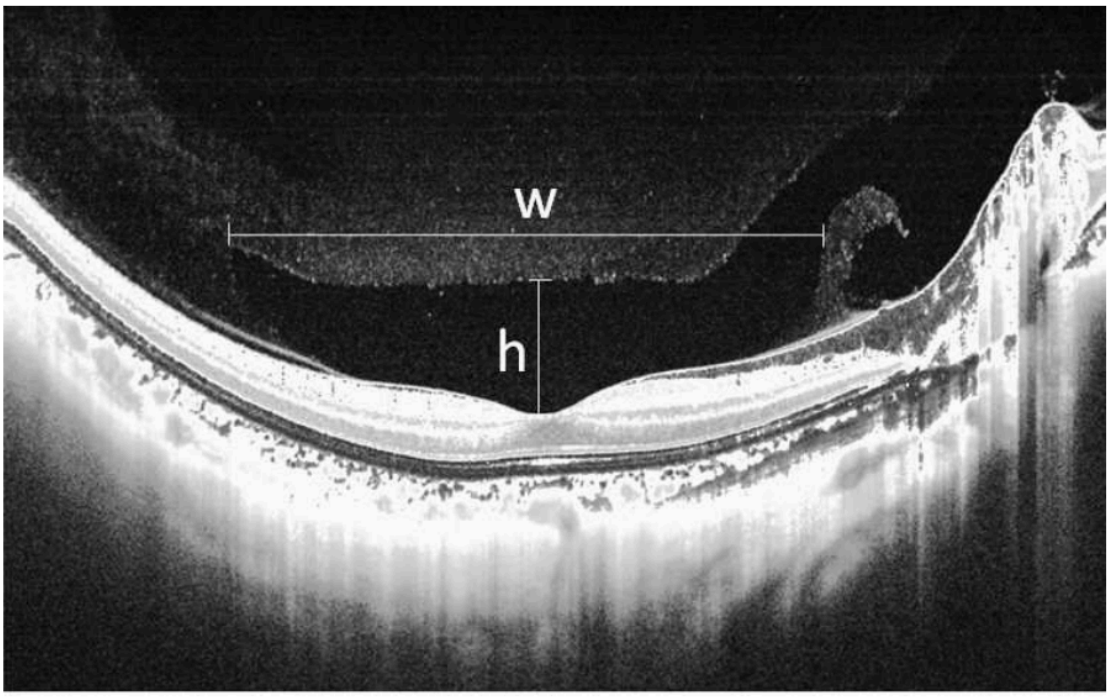


Figure 1.3: A swept-source optical coherence tomography image of the premacular bursa.[10] The height is defined as the distance between the fovea and the anterior hyaloid border (h) and the width (w) is the maximum diameter in the scan through the fovea and the disc.[10] Reproduced by permission from The Association for Research in Vision and Ophthalmology: Observation of posterior precortical vitreous pocket using swept-source optical coherence tomography by Itakura et al. Copyright The Association for Research in Vision and Ophthalmology 2013.

1.2 **Aging change at the vitreomacular interface: posterior vitreous detachment**

As with many structures in the eye, biochemical and anatomical changes occur with aging. In the vitreous, the main aging event is known as posterior vitreous detachment (PVD). Posterior vitreous detachment generally occurs with increasing age, but can be seen in younger patients in the context of myopia, trauma and diabetes (Figure 1.4).[11-14] It can also be

induced by cataract surgery.[15] Histological studies have shown PVD to be present in less than 10% of patients under 50, compared to 63% of patients over the age of 70.[16, 17]

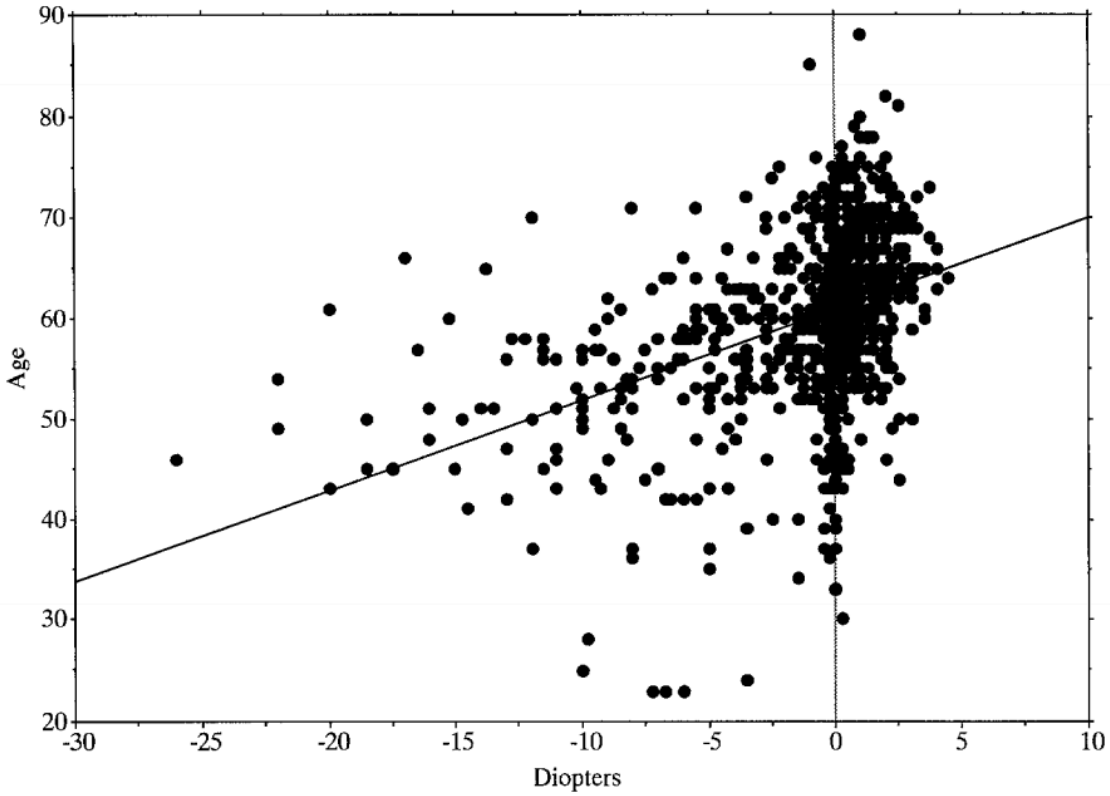


Figure 1.4: Scatter diagram and regression line of posterior vitreous detachment onset age and refractive error.[18]

Reprinted by permission from Springer Nature: Springer Nature Graefe's Archive for Clinical and Experimental Ophthalmology The age of onset of posterior vitreous detachment by J Yonemoto et al. Copyright 1993.

1.2.1 Process of posterior vitreous detachment

Over time, the internal structure of the vitreous undergoes synchysis, a liquefaction process, resulting in a reduced adhesive force between the cortical vitreous and the ILM of the retina. By the age of 70, it is estimated that approximately 50% of the vitreous has liquified.[17]

Vitreous degeneration also causes lacuna formation and collapse of the vitreous gel. These changes frequently result in the vitreous detaching from its points of adhesion, except the vitreous base anteriorly, and is known as PVD.[17]

Posterior vitreous detachment usually starts with focal superior perifoveal detachment and this gradually extends to a complete release of vitreopapillary traction.[19-21] Sometimes the PVD is incomplete, leaving the vitreous in contact with the macula, optic disc or both. Some studies have suggested PVD can occur asymptomatically below the age of 50, and then a further 'acute' PVD, sometimes accompanied by characteristic visual symptoms, may occur many years later.[21]

Acute symptomatic PVD can be a rapid process where patients sometimes describe floaters, or a change in their existing floaters, and photopsia (flashing lights), often in the temporal periphery of the visual field.[22, 23] Females and myopes are more likely to present with visual symptoms during the PVD process.[22] The floaters can be vitreous fibre opacities, haemorrhage, retinal pigment epithelium (RPE) pigment, or a ring of tissue resultant from vitreous detaching from the optic nerve (Weiss ring).

Once a PVD has developed, 90% of patients will develop PVD in the fellow eye within 3 years (Figure 1.5).[24] The timing of fellow eye PVD development appears unrelated to age, but may occur sooner in the context of high myopia.[24]

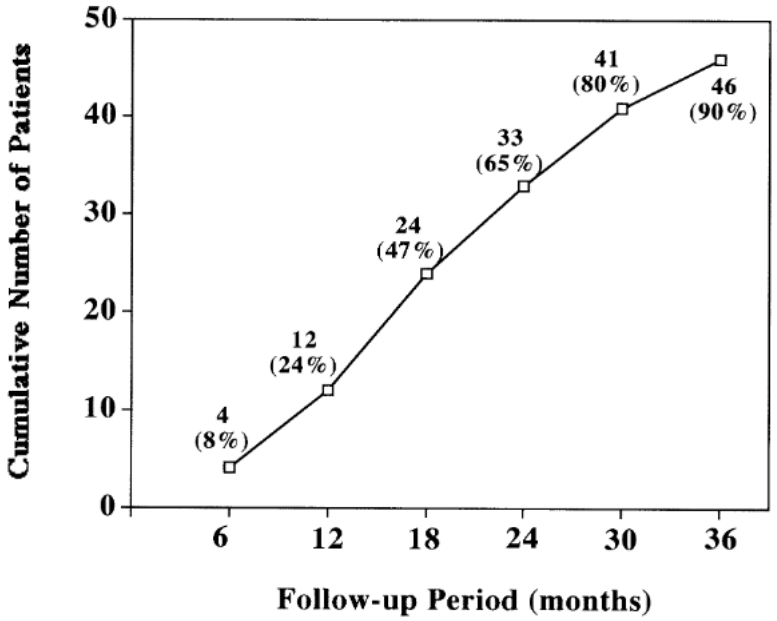


Figure 1.5: Time of onset of posterior vitreous detachment in the fellow eyes after it developed in the first eye.[24]

Squares represent the cumulative number of patients.

Reprinted from Ophthalmology, Volume 111, Issue 9, T Hikichi et al, Time course of development of posterior vitreous detachment in the fellow eye after development in the first eye. Copyright (2004) with permission from Elsevier.

1.2.2 Clinical signs of posterior vitreous detachment

In order to assess whether a PVD is present, a number of different examination techniques can be used. Firstly, slit-lamp examination with a hand-held fundus lens may reveal signs such as a Weiss ring, a so-called 'crinkly membrane' formed by the posterior hyaloid membrane, or an optically clear space between the posterior hyaloid membrane and the retina. However, it may be difficult to accurately determine whether the vitreous is attached with clinical examination alone. Investigations such as optical coherence tomography (OCT) and B-scan ultrasonography (B-scan US) can be useful to confirm the vitreous status.

In 2013, the international vitreomacular traction study group demonstrated that OCT can be used to accurately document vitreous status when it classified alongside vitreomacular adhesion (VMA) and macular hole (MH).[25] In certain cases it can be obvious whether the posterior hyaloid is attached, but occasionally, particularly if the posterior hyaloid has migrated significantly in the anterior direction, it can be harder to determine on OCT (Figure 1.6). Furthermore, the presence of the premacular bursa can affect diagnostic accuracy. It is therefore important that the OCT findings are carefully correlated with clinical examination.

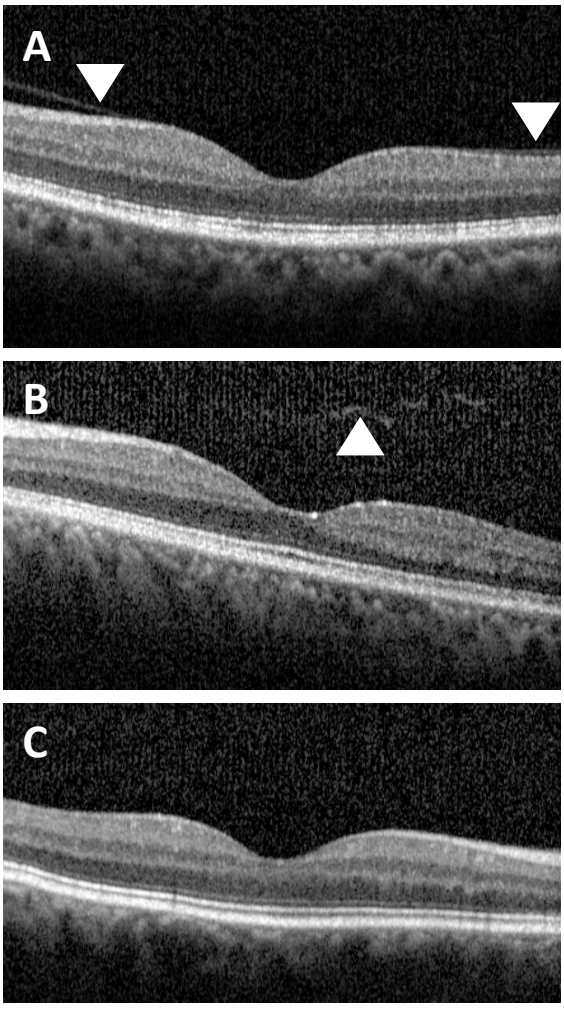


Figure 1.6: Optical coherence tomography imaging showing (A) vitreomacular adhesion, (B) posterior vitreous detachment, and (C) uncertain vitreous status.

The white triangles in A and B identify the signal from the vitreous face.

Another investigation that can be used to assess PVD status is B-scan US. This can be time-consuming and may not always be available or indicated in routine clinical practice, but it is informative in equivocal cases. In some cases, for example if the fundal view is poor in the context of vitreous haemorrhage or dense cataract, B-scan US is important to delineate anatomical information about the posterior segment. Asking the patient to move their eye during the assessment gives dynamic information about the vitreous, and can be used to determine whether or not a PVD is present (Figure 1.7).

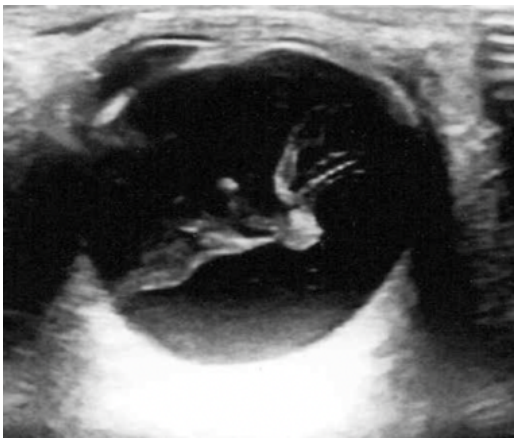


Figure 1.7: B-Scan ultrasound of a posterior vitreous detachment. The posterior hyaloid face is seen in the mid-vitreous cavity.

1.2.3 Complications of posterior vitreous detachment

The clinical evolution of PVD takes time, and is associated with a number of vitreoretinal diseases due to the tractional effect of the vitreous on the retina. Retinal tears, usually at the anterior vitreous near the ora serrata, can occur in 10-16% of patients with a symptomatic PVD, due to gel movement causing traction on the retina (Figure 1.8).[26, 27]

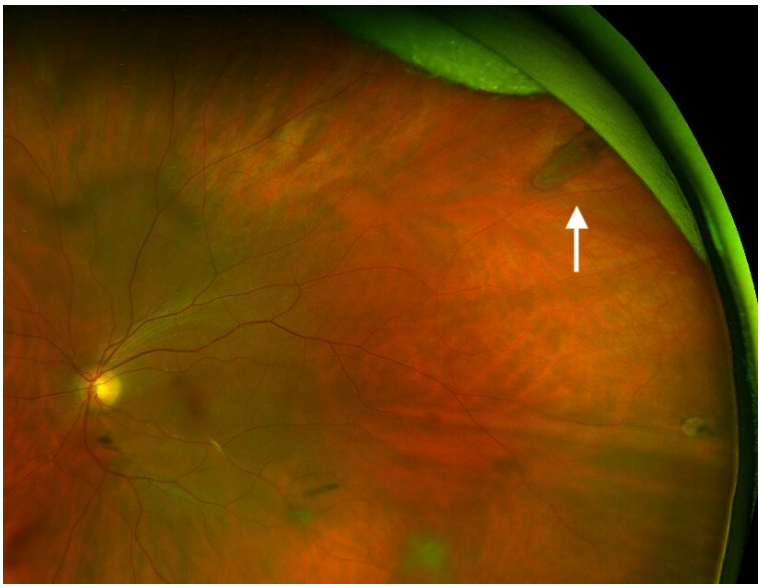


Figure 1.8: Fundus photograph of a retinal tear. A white arrow shows the position of the tear in the superotemporal quadrant of the left eye.

The highly-predictive Shafer's sign (RPE pigment in the anterior vitreous) is a key to the diagnosis of a retinal tear.[28, 29] If a retinal tear occurs in association with PVD, it will usually occur early and be present at the time of initial examination. However, it has been shown that 1.8 - 3.4% of tears can develop late, which has implications for follow-up planning in PVD patients.[27, 30, 31] The identification of retinal tears during examination of PVDs is important, due to the high risk of subsequent retinal detachment if they are missed and left untreated. Retinal tears can be treated with laser or cryo-retinopexy, with a high rate of success in providing chorioretinal adhesion and preventing retinal detachment.[32, 33]

In addition to retinal traction, PVD can result in a pulling force on the surface blood vessels of the retina or optic nerve head, which can lead to a vitreous haemorrhage either in addition to, or independent of, a retinal break.[34, 35] Red blood cells can be seen in the anterior vitreous, and are differentiated from RPE pigment by their smaller size. The degree of haemorrhage

can vary from a mild vitreous haze to a complete loss of the fundal view. Many clinicians consider a loss of fundal view due to unexplained vitreous haemorrhage as an indication for urgent vitrectomy, to be treated as for a macula-on rhegmatogenous retinal detachment (RRD), due to the risk of associated retinal breaks.[25]

1.3 **Tractional diseases of the vitreomacular interface**

Whilst, anatomically, VMA may refer to a normal asymptomatic state, clinically, the term is used when VMA occurs in the context of an incomplete PVD.

A spectrum of VMA exists associated with incomplete PVD, ranging from asymptomatic, non-tractional VMA to extensive distortion of the retinal structure due to vitreomacular traction (VMT) and MH with loss of visual function (Table 1.1). It is difficult to predict why some patients will develop an incomplete PVD with persisting adhesion to the macula. Histological studies have shown astrocytes, fibrocytes and collagen are present in the attached complex.[36] The characterisation of disease tends to be based on OCT findings, sometimes in reference to defined standards (Table 1.1 and Figure 1.9).[25, 37, 38] However, it is important to note that the OCT changes, which may include retinal thickening and intraretinal oedema, do not always correlate with visual function and symptoms.[39, 40]

Anatomic State	IVTS Classification System for Vitreomacular Adhesion, Traction, and Macular Hole
VMA	Definition
	Evidence of perifoveal vitreous cortex detachment from the retinal surface
	Macular attachment of the vitreous cortex within a 3-mm radius of the fovea
	No detectable change in foveal contour or underlying retinal tissues
	Classification
	By size of attachment area
	Focal (≤1500 μm)
	Broad (>1500 μm, parallel to RPE and may include areas of dehiscence)
	By presence of concurrent retinal conditions
	Isolated
	Concurrent
VMT	Definition
	Evidence of perifoveal vitreous cortex detachment from the retinal surface
	Macular attachment of the vitreous cortex within a 3-mm radius of the fovea
	Association of attachment with distortion of the foveal surface, intraretinal structural changes, and/or elevation of the fovea above the RPE, but no full-thickness interruption of all retinal layers
	Classification
	By size of attachment area
	Focal (≤1500 μm)
	Broad (>1500 μm, parallel to RPE and may include areas of dehiscence)
	By presence of concurrent retinal conditions
	Isolated
	Concurrent
FTMH	Definition
	Full-thickness foveal lesion that interrupts all macular layers from the ILM to the RPE
	Classification
	By size (horizontally measured linear width across hole at narrowest point, not ILM)
	Small (≤250 μm)
	Medium (>250 μm and ≤400 μm)
	Large (>400 μm)
	By presence or absence of VMT
	By cause
	Primary (initiated by VMT)
	Secondary (directly due to associated disease or trauma known to cause macular hole in the absence of prior VMT)
LMH	Definition
	Irregular foveal contour
	Defect in the inner fovea (may not have actual loss of tissue)
	Intraretinal splitting (schisis), typically between the outer plexiform and outer nuclear layers
	Maintenance of an intact photoreceptor layer
Macular Pseudohole	Definition
	Invaginated or heaped foveal edges
	Concomitant ERM with central opening
	Steep macular contour to the central fovea with near-normal central foveal thickness
	No loss of retinal tissue

Abbreviations: ERM = epiretinal membrane; FTMH = full-thickness macular hole; ILM = internal limiting membrane; IVTS = International Vitreomacular Traction Study; LMH = lamellar macular hole; RPE = retinal pigment epithelium; VMA = vitreomacular adhesion; VMT = vitreomacular traction.

Table 1.1: The International Vitreomacular Traction Study classification system for vitreomacular adhesion, traction and macular hole.[25]

ERM, epiretinal membrane; FTMH, full-thickness macular hole; ILM, internal limiting membrane; IVTS, International Vitreomacular Traction Society; LMH, lamellar macular hole; RPE, retinal pigment epithelium; VMA, vitreomacular adhesion; VMT, vitreomacular traction.

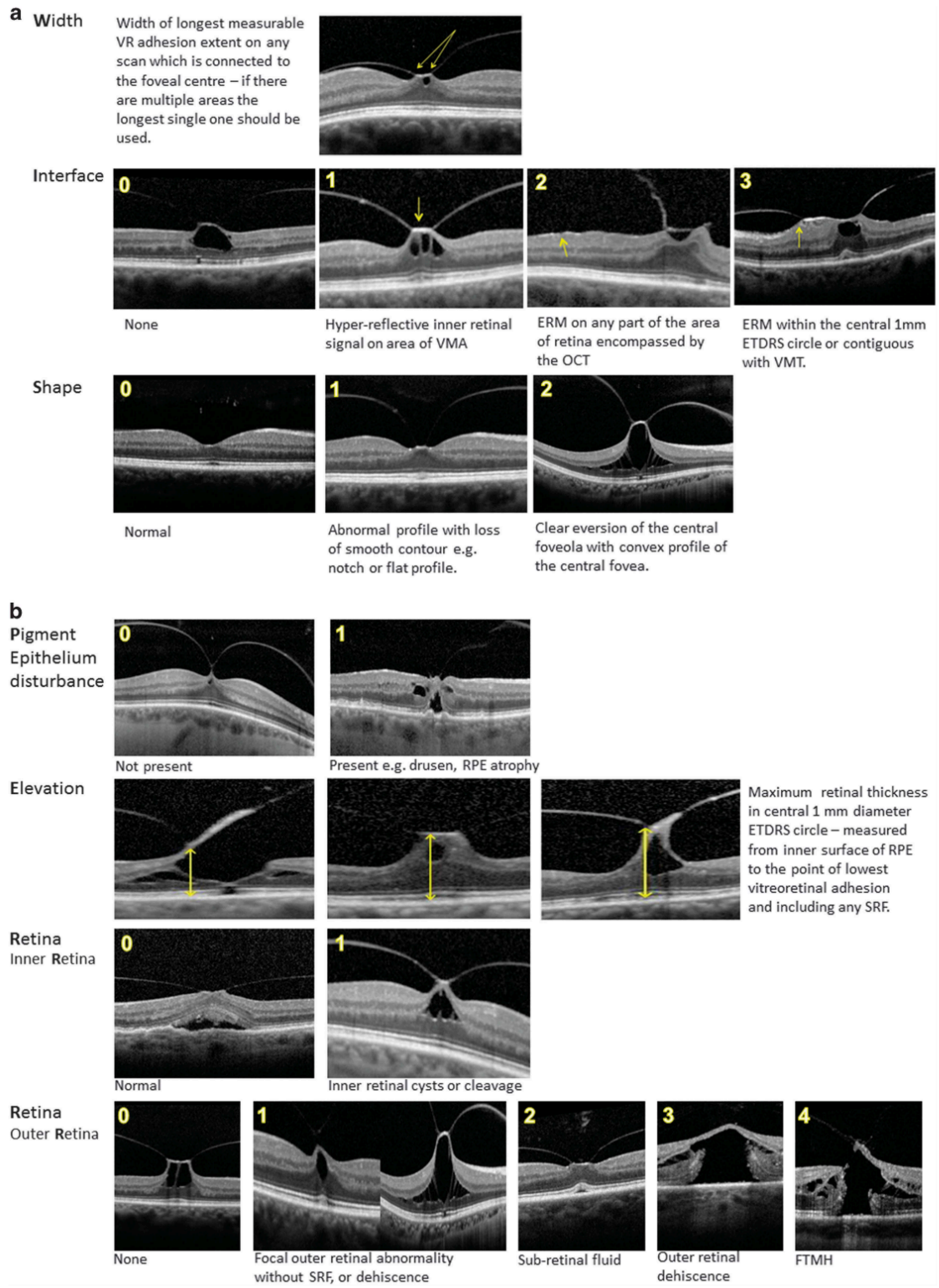


Figure 1.9: The focal vitreomacular traction classification tool: WISPERR.[38] A, width of vitreous attachment (W), interface features (I), and foveal shape (S). B, retinal pigment epithelial changes (P), elevation of vitreous attachment (E), and inner and outer retinal changes (R).

ERM, epiretinal membrane; ETDRS, early treatment of diabetic retinopathy study; FTMH, full thickness macular hole; OCT, optical coherence tomography; RPE; retinal pigment epithelium; SRF, subretinal fluid; VMA, vitreomacular adhesion; VMT, vitreomacular traction; VR, vitreoretinal.

Reproduced by permission from Springer Nature: Springer Nature Eye The design and validation of an optical coherence tomography-based classification system for focal vitreomacular traction by DHW Steel et al. Copyright 2016.

1.3.1 Symptomatic vitreomacular adhesion

Symptomatic vitreomacular adhesion (sVMA) is defined as visual loss secondary to foveal damage caused by abnormal VMT. Symptomatic VMA includes isolated VMT, impending MH and MH with persisting vitreous attachment.[41]

There is a degree of disease overlap – for example, impending MH is often grouped with VMT and epiretinal membrane (ERM) often coexists with sVMA. For this reason, it is difficult to define the prevalence and visual impact of sVMA. One study reported that VMA may occur in isolation or in association with other eye disease in approximately 1.5% of the population.[41] However, the majority of these cases occurred alongside ERM, and thus the VMA may not be responsible for visual loss. Excluding cases associated with ERM reduced the prevalence to 0.35% in the same population-based study; however, this figure also included cases with other diseases, such as neovascular age-related macular degeneration (nAMD) and diabetic macular oedema (DMO).[41] If only cases of isolated VMA/VMT with or without MH were considered, then the prevalence of sVMA was 171.5 per 100,000 population.[41]

The natural history of sVMA varies. Symptomatic VMA may spontaneously resolve, with detachment of the posterior vitreous face from the ILM.[1] One pre-OCT era study of 53 eyes showed a complete PVD occurred in only 11% of eyes over 60 months' follow-up.[42] A more recent review by Steel *et al* reported spontaneous resolution occurs in 10-11 %.[1] It has been shown that many, if not most, full-thickness macular holes (FTMH) result from persistent VMT which either fully detaches from the retina causing a FTMH, or remains attached at the edge of the hole.[43-46]

1.3.2 Treatment options for symptomatic vitreomacular adhesion

As would be expected with its wide range of disease severity, treatment strategies for sVMA vary. Asymptomatic VMT or minimally symptomatic sVMA can be observed, since separation of the posterior vitreous face may occur spontaneously and without visual compromise. However, a longer duration of VMT may lead to loss of vision and possibly lower efficacy of any subsequent intervention, and therefore treatment is often considered if symptoms are significant or visual acuity (VA) is reduced.[42, 47, 48] If VMT progresses to FTMH then intervention is usually advisable, and an evolving VMT/impending MH may similarly necessitate intervention.

If the decision is made to treat sVMA, various strategies can be considered. Traditionally, pars-plana vitrectomy (PPV) has been the standard approach for VMT or FTMH.[1] Pars plana vitrectomy for this indication carries risks including iatrogenic retinal breaks (11-16%), retinal detachment (2-6%), vitreous haemorrhage (5%), hypotony (3-16%) and endophthalmitis (0.02-0.13%).[49-56] Furthermore, cataract formation is common in phakic eyes following vitrectomy, and this usually commits the patient to further surgery that can carry a

higher risk than a routine cataract procedure due to posterior capsular defects and changes in the iris-diaphragm complex.[55]

Alternatively, small uncontrolled studies have shown that an intravitreally injected expansile gas bubble can pneumatically release VMT (including if associated with MH), without the need for PPV, with success rates varying from 71% to 95%.[57-59]

A newer option for treating VMT and MH with persisting VMA is pharmacological vitreolysis with ocriplasmin.[60-63] Ocriplasmin is administered via intravitreal injection at a dose of 125 μg in 0.1 mL. It has marketing authorisation in Europe, the US and elsewhere and is supported by the UK's National Institute for Health and Care Excellence (NICE) for the treatment of VMT, including when associated with MH of diameter of 400 μm or less, in the absence of ERM.[64, 65]

1.4 The impact of vitreomacular status on visual function

Disease of the vitreomacular interface can have a major effect on visual function. Visual acuity is a measure of the spatial resolution of the visual processing system. Common visual acuity measures utilise the Snellen or Early Treatment Diabetic Retinopathy Study (ETDRS) vision charts, which produce a value (e.g. 6/6 or 85 letters, respectively) that is often used to define the level of visual impairment. These values are widely used for driving standards, registration of visual impairment, and suitability for treatment in conditions such as nAMD and cataract.[66-69]

Symptomatic VMA is often first noticed when a patient complains of deteriorating vision. Visual acuity can often underestimate the visual deterioration experienced by the patient, because symptoms such as a metamorphopsia can have a major effect on the quality of vision, for example with facial recognition, yet not impact VA to the same degree.[70, 71] In addition, contrast sensitivity and quality of colour vision theoretically could also be affected by sVMA. For these reasons, patient reported outcome measures including visual function questionnaires (e.g. the US National Eye Institute's Visual Function Questionnaire 25, (VFQ-25)) have been designed to estimate the impact of eye disease on visual function, as well as determining response to treatment.[72, 73]

1.5 Association of macular disease and vitreomacular status

In addition to causing visual disturbance, VMA may influence the clinical course of, or may be associated with, macular diseases such as diabetic maculopathy, retinal vein occlusion (RVO) and nAMD, although the data are sometimes conflicting.[2, 37, 41, 74-77] Whilst there may be an association between sVMA and these diseases, it is not certain that this is causal.[37] Furthermore, it is possible that VMA may act as a biomarker when predicting response to treatment. Previous PPV may also influence how macular disease responds to treatment. Overall, it seems that vitreous attachment alters the prognosis of macular disease, with the strongest evidence present for nAMD.[2]

1.5.1 Neovascular age-related macular degeneration

A major review found an increased prevalence of VMA in patients with nAMD.[37] In addition, a meta-analysis of 2,156 patients found that those with VMA had an inferior

response to anti-vascular endothelial growth factor (anti-VEGF) therapy when compared to those with PVD.[78]

There are numerous factors which may explain the apparent association between nAMD disease activity and vitreomacular status. Firstly, direct tractional force may induce local inflammatory changes within the macula and stress of the RPE cells, which in turn may promote nAMD development and disease activity.[79] Traction can also reduce retinal interstitial tissue pressure, resulting in an influx of fluid according to Starling's law.[8] Another theory relates to vitreous oxygenation and vascular endothelial growth factor (VEGF) expression, specifically that a detached vitreous may increase retinal oxygenation and therefore be protective.[37] A magnetic resonance imaging (MRI) study confirmed PPV significantly increases vitreous oxygenation.[80]

When a PVD occurs, the viscosity of the medium (aqueous vs vitreous) in contact with the macula reduces. This may enable growth factors and disease modulators to diffuse away from the macula more quickly and thus result in reduced disease activity.[81] Conversely, anti-VEGF drugs may diffuse away from their target site faster when a PVD is present or the eye is vitrectomised. A study of 204 eyes found eyes with VMA needed more anti-VEGF injections over the course of 1 year in a treat-and-extend (T&E) protocol compared to those without VMA (8.4 vs 7.4, respectively), although VA was not significantly different between the two groups.[82]

1.5.2 Diabetic maculopathy and retinal vein occlusion

Other macular diseases such as diabetic maculopathy and RVO can also be influenced by vitreomacular status.

Numerous studies have found that the status of the posterior hyaloid (attached vs detached) has an effect on diabetic maculopathy. The prevalence of PVD is lower in cases with coexisting DMO, and PVD is associated with a better central macular thickness response to triamcinolone treatment.[83, 84] A study of 105 eyes found anti-VEGF therapy to be less effective in treating DMO in the presence of VMA or VMT, compared to eyes with no vitreoretinal interface abnormality.[85] In addition, retrospective analysis of data from the Ranibizumab for Edema of the Macular in Diabetes (READ-3) study found those with VMA at baseline who developed PVD during the study had a greater potential for visual improvement with anti-VEGF treatment than those with PVD at baseline.[86] Another group also found DMO response to ranibizumab improved if VMA spontaneously resolved during a course of treatment.[87]

Prior PPV can also impact peripheral diabetic retinopathy. Laidlaw reviewed the subject and found PPV resulted in better vision and reduced macular thickness, but noted that most evidence was uncontrolled and retrospective.[88] He concluded that vitrectomy for DMO should be restricted to those with signs of traction, which was supported by a subsequent systematic review and meta-analysis.[89] The previously described oxygenation theory may explain the clinical improvement after vitrectomy. Interestingly, PPV has been shown to reduce retinal neovascularisation, yet increase iris neovascularisation in patients with diabetic tractional retinal detachment, particularly if lensectomy is performed as a combined

procedure.[90, 91] This may be due to easier diffusion of VEGF anteriorly through the vitreous cavity towards the iris.

Posterior vitreous detachment is also thought to have an effect on the prognosis of central and branch RVO. The incidence of secondary macular oedema is lower if PVD is present.[92] Kumagai, *et al* demonstrated that visual improvement after PPV continues to improve one year after surgery, suggesting an ongoing benefit after release of VMA.[93, 94] Other studies have shown that PPV's effect on macular oedema and VA is temporary and does not affect the long-term outcome.[95]

1.6 Age-related macular degeneration

Age-related macular degeneration (AMD) is the leading cause of irreversible and progressive loss of vision in individuals over 50 years of age in developed nations.[96-98] It can have a substantial impact on quality of life, and affects more than 500,000 people in the UK alone.[99] It affects approximately 0.5% of people over the age of 60, rising to 20% of those over the age of 90.[99] Early AMD is characterised by RPE pigmentary changes and the presence of extracellular drusen deposits between RPE cells and Bruch's membrane.[100] Late AMD is defined by the presence of atrophic or neovascular disease.[101] Approximately 10% of those with late AMD have the neovascular form of the disease (characterised by the presence of choroidal neovascularisation), which is clinically more destructive and aggressive, but also more amenable to treatment.

There are many risk factors for AMD, the most significant being increasing age.[101] Those of European ethnicity have a higher risk of developing AMD than Asian, African and Hispanic ethnicities.[102] Environmental factors such as smoking and poor diet also increase the risk.[103]

There is also a substantial genetic component. Coding variants of the complement factor H and factor I genes as well as the age-related maculopathy susceptibility 2 locus have been strongly associated.[104]

Aside from genetic polymorphisms, there are other indicators that link inflammation to AMD. Retinal pigment epithelium cell disruption promotes chronic inflammatory change in the retina and choroid.[105] Furthermore, numerous markers of inflammation such as cytokines are known to be altered both locally and systemically in AMD patients.[106] Research is ongoing to identify inflammatory biomarkers which will help delineate the mechanisms of AMD as well as aiding the development of new therapeutics.

Currently, there is no treatment available for atrophic AMD. Current research is focused on complement inhibition, but as yet, there is no approved treatment.[107] The primary form of treatment for nAMD is intravitreal anti-VEGF therapy with regular clinical review from the point of diagnosis.[108] A substantial amount of research is ongoing regarding future nAMD treatments. These range from port delivery systems, which involve an implanted refillable reservoir of anti-VEGF, to adjunctive stereotactic radiotherapy.[109] Other possible options include gene therapy, complement inhibitors, longer acting anti-VEGF drugs, and cell-based therapies.[110, 111]

1.6.1 Anti-vascular endothelial growth factor treatment of neovascular age-related macular degeneration

Neovascular AMD involves choroidal neovascularisation, wherein new immature blood vessels invade the sub-retinal space. It is a complex process of endothelial cell proliferation

and migration, resulting in vascular leakage. One of the major driving forces is VEGF-A, and therefore this is the main pharmacological target. Vascular endothelial growth factor-A is part of a large family of growth factors (VEGF A-E and placental growth factor (PlGF)) which exert their influence via numerous receptors (VEGFR-1-3, neuropilins and cell surface heparin proteoglycans).[112] Therapeutic agents may be molecules targeting VEGF isoforms, inhibitors of VEGF receptors, or targets for downstream processes.[113] Vascular endothelial growth factor is also known to act as a survival factor in both *in vivo* and *in vitro* models, which is why some authors postulate that geographic atrophy can occur in patients receiving anti-VEGF treatment.[114-116]

Currently, there are two established licensed anti-VEGF drugs for nAMD treatment in the UK: ranibizumab (Lucentis®; Novartis Pharma AG, Basel, Switzerland) and aflibercept (Eylea®; Bayer Pharma AG, Berlin, Germany). They have transformed nAMD management as well as other conditions such as diabetic maculopathy and retinal vein occlusion.[117, 118] Another drug, brolucizumab (Beovu®; Novartis Pharma AG, Basel, Switzerland) is approved by the US Food and Drug Administration for nAMD and has recently gained marketing authorisation in the EU.[119]

Standard initial treatment of nAMD is three injections of either ranibizumab, aflibercept or brolucizumab at one month intervals. Following this loading dose, there is considerable choice in planning treatment and follow-up intervals.[120]

Landmark studies showed monthly ranibizumab 0.5 mg prevented sight loss in over 90% of patients with nAMD, with an average increase in best-corrected visual acuity (BCVA) of between 1 and 2 lines (Figure 1.10).[121, 122] The VEGF:Trap-Eye: Investigation of Efficacy

and Safety in Wet AMD (VIEW)-1 and VIEW-2 studies, comparing bimonthly aflibercept 2 mg with monthly ranibizumab 0.5 mg, showed similar efficacy and safety between the two drugs on a fixed re-treatment schedule (Figure 1.11).[123, 124] A meta-analysis confirmed bimonthly aflibercept treatment produces similar outcomes to monthly ranibizumab.[125]

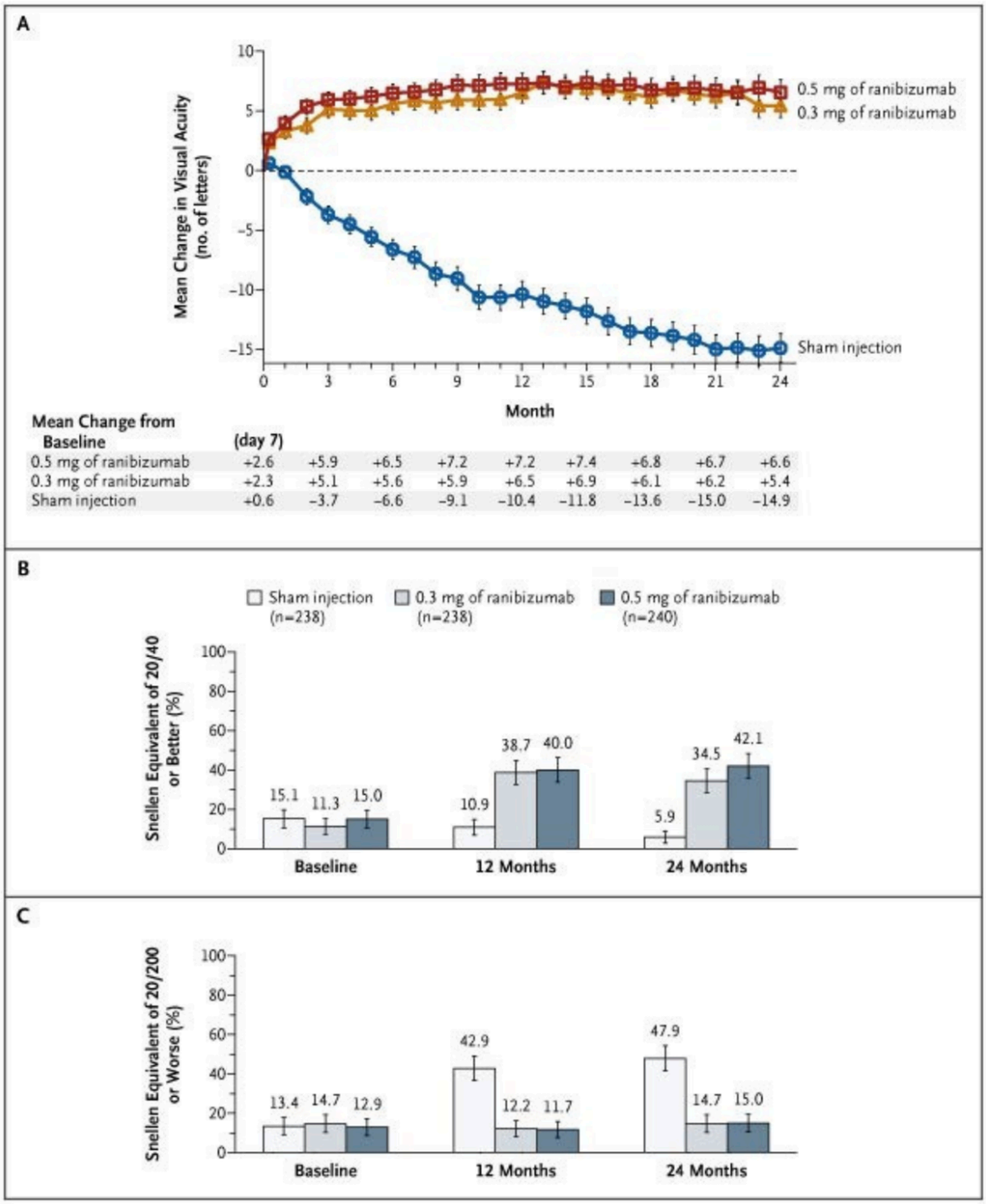
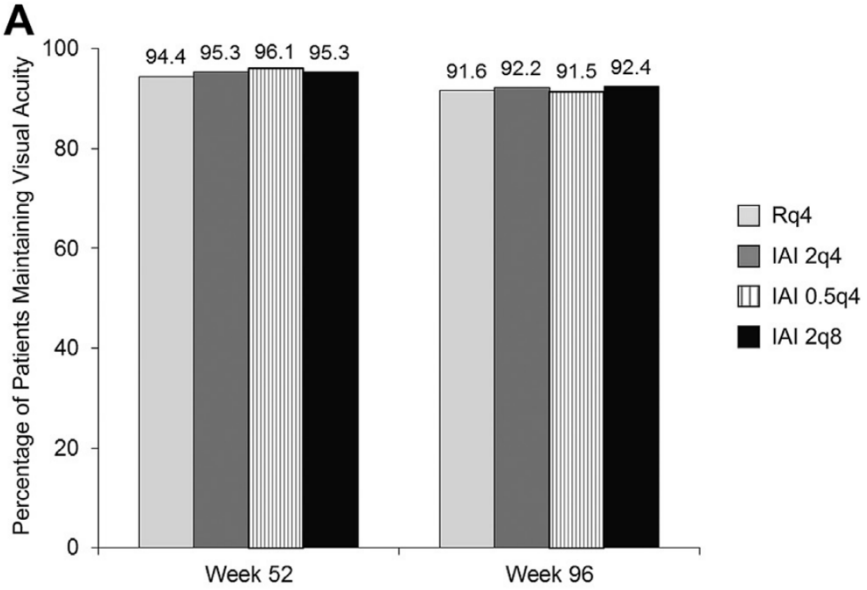
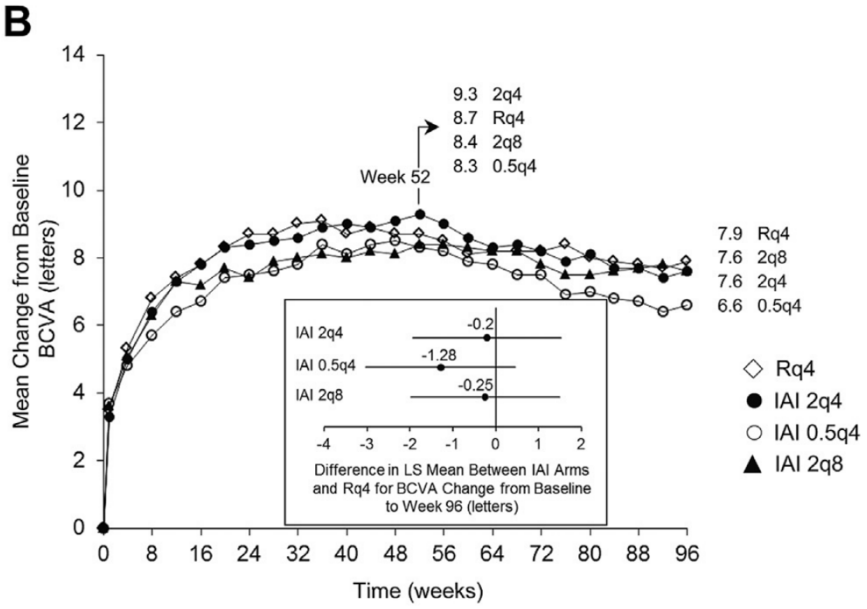


Figure 1.10: Mean changes from baseline in visual acuity and snellen equivalents at 12 and 24 months in the minimally classic/occult trial of the anti-vascular endothelial growth factor antibody ranibizumab in the treatment of neovascular age-related macular degeneration study.[122] Panel A shows the mean change from baseline in visual acuity during a 24-month period. At each monthly assessment, the comparison between each ranibizumab group and the sham-injection group significantly favoured ranibizumab ($P<0.001$). Panels B and C show the change from baseline in the percentage of patients with a Snellen equivalent of 20/40 or better and the percentage of patients with 20/200 or worse, respectively, at 12 and 24 months ($P<0.001$ for the comparison between each ranibizumab group and the sham-injection group at 12 and 24 months).


A
Percentage of Patients Maintaining Visual Acuity
100
80
60
40
20
0
94.4
95.3
96.1
95.3
91.6
92.2
91.5
92.4
Week 52
Week 96
Rq4
IAI 2q4
IAI 0.5q4
IAI 2q8


B
Mean Change from Baseline BCVA (letters)
14
12
10
8
6
4
2
0
9.3 2q4
8.7 Rq4
8.4 2q8
8.3 0.5q4
Week 52
7.9 Rq4
7.6 2q8
7.6 2q4
6.6 0.5q4
IAI 2q4
IAI 0.5q4
IAI 2q8
-0.2
-1.28
-0.25
-4 -3 -2 -1 0 1 2
Difference in LS Mean Between IAI Arms and Rq4 for BCVA Change from Baseline to Week 96 (letters)
0 8 16 24 32 40 48 56 64 72 80 88 96
Time (weeks)
Rq4
IAI 2q4
IAI 0.5q4
IAI 2q8

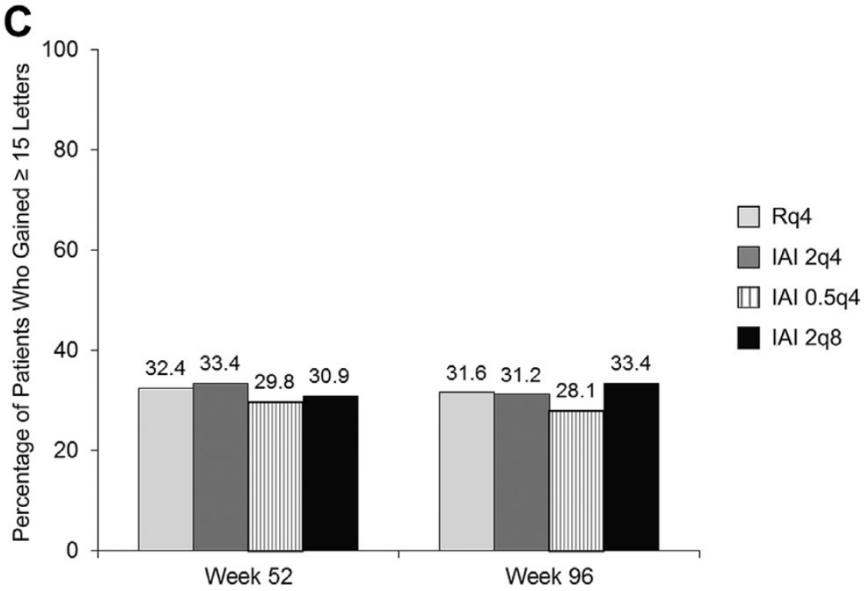

C
Percentage of Patients Who Gained ≥ 15 Letters
100
80
60
40
20
0
32.4
33.4
29.8
30.9
31.6
31.2
28.1
33.4
Week 52
Week 96
Rq4
IAI 2q4
IAI 0.5q4
IAI 2q8

Figure 1.11: Graphs showing visual acuity outcomes in the VIEW-1 and VIEW-2 studies.[124] A, Proportion of patients maintaining visual acuity (losing <15 Early Treatment Diabetic Retinopathy Study letters). B, Mean change from baseline best-corrected visual acuity. The inset shows the difference in least square mean (with 95% confidence interval) between intravitreal aflibercept arms and ranibizumab (aflibercept minus ranibizumab) for best-corrected visual acuity change from baseline to week 96, in the full analysis set. C, Proportion of patients who gained 15 letters of more, full analysis set. The outcomes for the aflibercept and ranibizumab groups were similar in (A), (B), and (C) at both weeks 52 and 96. IAI = intravitreal aflibercept injection; Rq4 = 0.5 mg intravitreal ranibizumab every 4 weeks; 2q4 = 2 mg every 4 weeks; 0.5q4 = 0.5 mg every 4 weeks; 2q8 = 2 mg every 8 weeks after 3 initial monthly injections.

Fixed monthly or bimonthly injections of ranibizumab or aflibercept, respectively, have significant service implications due to the chronicity of disease and increasing disease prevalence. Attempts to reduce the treatment burden by changing the regimen after the monthly loading dose have been investigated. These include quarterly, *pro re nata* (*PRN*), and T&E methods.[123, 126-135] Quarterly dosing has been shown to result in significantly worse visual outcomes than monthly dosing.[133, 136]

In a *PRN* approach, patients receive an injection if disease activity or visual loss secondary to nAMD is detected at fixed follow-up intervals.[120] The prospective optical coherence tomography imaging of patients with neovascular age-related macular degeneration treated

with intra-ocular ranibizumab (PrONTO) study found comparable visual outcomes using *PRN* versus fixed monthly dosing with ranibizumab.[132] Conversely, the comparison of age-related macular degeneration treatments trials (CATT) study reported better visual outcomes with monthly anti-VEGF (ranibizumab or bevacizumab) than *PRN* at two years.[127] The inhibition of vascular endothelial growth factor in age-related choroidal neovascularisation (IVAN) study did not find a significant difference in visual outcome between a fixed monthly (ranibizumab or bevacizumab) or *PRN* regimen at two years.[137] A meta-analysis concluded that monthly dosing did not result in a clinically relevant benefit compared to PRN dosing. Furthermore, it reported that monthly dosing probably resulted in a higher risk of endophthalmitis, and was less cost-effective.[120]

Treat-and-extend involves fixed treatment intervals until clinical remission, followed by increasing the treatment interval up to a maximum of 12-14 weeks.[138-140] The principle behind T&E is to optimise visual outcomes by maintaining disease control, whilst also minimising unnecessary visits by titrating injections to match the maximum effective dosing interval. A T&E approach has been shown to produce better visual outcomes than *PRN* regimens in a large observational study.[141] Various T&E studies have tried to establish the optimal methodology for treating nAMD. This has involved different numbers of loading injections, maximum interval, and criteria for interval reduction.[126, 139, 142-150] The recent randomised clinical trial comparing ranibizumab and aflibercept (RIVAL) study showed no statistically significant difference in VA outcomes or the number of injections required between ranibizumab and aflibercept T&E therapy at two years.[151]

Studies have compared the cost-effectiveness and effect on quality of life of anti-VEGF drugs. A review of previously published data in Japan found better effectiveness with

aflibercept T&E compared to *PRN* or monthly ranibizumab.[152] However, a UK study of meta-analysis data showed ranibizumab T&E may afford better use of resources that aflibercept T&E.[153] There is also evidence that switching between drugs can be of benefit to those with treatment-resistant disease, perhaps due to tachyphylaxis.[154] There is no strong evidence to suggest that either ranibizumab or aflibercept is superior to the other drug.

Brolucizumab is given monthly for a 3 month loading dose, followed by dosing every 12 weeks.[119] The dose can be reduced to 8-weekly if disease activity is present. It was found to be non-inferior to aflibercept at the week 48 end point, with a similar safety profile.[119] Recently, however, post-marketing surveillance has suggested it may carry an increased risk of intraocular inflammation and its adoption has been slow in many nations.[155]

Another anti-VEGF drug, bevacizumab (Avastin®; Genentech, South San Francisco, CA, USA) is also used to treat nAMD. Bevacizumab was developed as a systemic anti-VEGF treatment for colorectal cancer.[156] There was initially interest in whether it could be beneficial as a systemic treatment for nAMD. The Systemic Avastin for Neovascular AMD (SANA) study in 2006 found 2-3 doses of systemic bevacizumab (5 mg/kg) resulted in a 14 letter mean VA gain and reduction of 112 μm in OCT central retinal thickness at 24 weeks.[157] However, a large clinical trial did not occur due to the potential risks associated with off-label systemic anti-VEGF, and the perceived better safety profile of intravitreal treatments.[157]

Intravitreal bevacizumab, given off-label, was first used in 2005 mainly due to its cheap cost ($ 7 USD per dose).[158] However, it required compounding the drug into syringes and therefore raised concerns about maintaining sterility. The results of a single intravitreal injection of bevacizumab were shown to be similar to intravenous bevacizumab or

intravitreal ranibizumab.[158] However, its manufacturer did not pursue clinical trials and licensing steps for bevacizumab to be used for nAMD.

Two non-commercial major studies subsequently compared ranibizumab with bevacizumab for nAMD; CATT and IVAN.[127, 130, 159, 160] The CATT study found equivalent visual acuity results at two years between the two drugs when administered by the same schedule.[127] The IVAN study also found similar visual outcomes at two years, with a reduction in the frequency of retreatment resulting in a small loss of efficacy irrespective of the drug.[160] Based on these results and cost implications, some clinicians routinely use bevacizumab for nAMD.[158]

1.7 Intravitreal drug distribution and elimination

Intravitreal injection is the main route of administration for posterior segment diseases such as nAMD. This route delivers better therapeutic concentrations to the retina than other ocular methods (e.g. topical and peri-ocular).[161] Oral medications have poor bioavailability due to the blood-ocular-barrier.[162] A 30-gauge needle is inserted 3.5 – 4 mm posterior to the corneal limbus through the pars plana, followed by injection of the drug.

Drug distribution in the vitreous cavity is dependent on diffusion, convection, and vitreous interactions. Diffusion is determined by concentration gradient, molecular weight, viscosity, and net charge. Higher molecular weight molecules are slowed down by the vitreous mesh.[163] Drugs with a positive charge are attracted to the negatively charged hyaluronic acid in the vitreous, slowing their passage.[164] Convection refers to the movement of aqueous humor towards the retina, due to pressure and temperature gradients, and whilst it may have subtle

effects on drug distribution, it is not thought to be a major factor.[161] Protein in the vitreous humor can also theoretically affect drug distribution and elimination by binding. These interactions may reduce pharmacological effect and slow drug diffusion, but they are poorly understood.[165]

Another important factor in drug distribution is vitreous humor liquefaction. With age, the vitreous liquifies and a PVD often occurs. This could potentially speed up drug diffusion and elimination which might influence the efficacy of intravitreal therapy. Goldenberg *et al* used a rabbit model to show bevacizumab absorbs quicker through the retina in an eye with a PVD versus a non-PVD eye.[166] It is also highly plausible that drug diffusion is significantly altered following PPV because vitreous is approximately 300 to 2000 times more viscous than aqueous, and the speed of molecular diffusion is inversely related to viscosity.[81] Animal models have shown this to be the case with various antibiotics and anti-VEGF drugs.[167-173] There is also anecdotal evidence that patients with nAMD who have previously undergone a vitrectomy may have disease reactivation sooner after their anti-VEGF treatment than those who have not. Vitrectomy could therefore potentially reduce the efficacy of intravitreal injections, and influence a decision on dose interval or drug choice.

After passage through the vitreous cavity, intravitreal drugs targeting the retina and choroid need to cross the retina. The first barrier is the ILM, which has a thickness of approximately 4 μm.[174] The ability of a substance to cross the ILM is both size and charge dependent.[164] Negatively charged macromolecules pass easily, whereas strongly positively charged substances can be blocked. A study showed a 2000 kDa negatively charged dextran penetrates bovine retina more efficiently than a 20 kDa positively charged dextran.[175] Once

through the ILM, drug passage through the retina can be limited by the tightly packed structure, particularly for larger molecules.[164]

Molecular size is also thought to influence diffusion across the retina.[176] This may be particularly relevant to biological drugs, due to their large size. Although bevacizumab (149 kDa) is larger than its parent molecule (ranibizumab, 48 kDa), aflibercept (115 kDa) and brolucizumab (26 kDa), studies indicate it can nonetheless cross the entire retina.[177]

Drugs undergo elimination from the eye either posteriorly through the blood-retinal-barrier (BRB), or anteriorly via the trabecular meshwork and uveoscleral pathway (Figure 1.12). The BRB consists of retinal capillary endothelial cells (inner BRB) and the RPE cells (outer BRB). The inner BRB has tight junctions which allow paracellular transport of molecules below 2 nm, as well as active transport of some larger molecules, although this route is prone to saturation.[165, 178] The outer BRB has tight, gap and adherent junctions that can be crossed by passive and active diffusion, dependent on molecular size, charge and lipophilicity.[165] Lipophilic molecules cross the RPE transcellularly whereas small hydrophilic substances can pass through tight junctions.[165] Once a drug reaches the choroid, it rapidly enters the systemic circulation.[161] Drugs leaving via the trabecular meshwork and uveoscleral outflow circulate through the vitreous cavity before passing through the zonules into the anterior chamber. This route is more useful for larger molecules and hydrophilic substances which cannot cross the BRB.[165]

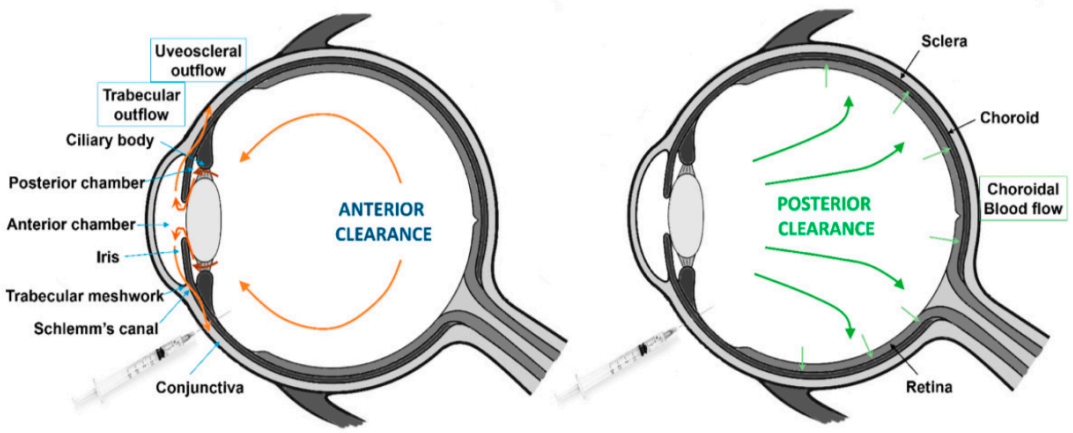


Figure 1.12: Schematic representation of the anterior and posterior clearance from the vitreous humor.[165]

1.8 Pharmacokinetics of intravitreal anti-vascular endothelial growth factor drugs

The above principles of drug distribution and elimination apply to anti-VEGF drugs. They are unlikely to be substantially restricted by the vitreous meshwork.[179, 180] Protein binding of anti-VEGF drugs to vitreous constituents is poorly understood, but potentially could influence speed of diffusion. Evidence is conflicting on whether previous vitrectomy affects their half-life and efficacy.[171, 181] Some studies suggest anti-VEGF drugs are more likely to leave the eye via the anterior route, whereas others suggest the posterior route has more importance.[182-187]

Most anti-VEGF half-life research has been performed on animal models due to the invasive requirement of sampling at multiple time points. However, animal eyes differ structurally from human eyes. For example, the rabbit eye is smaller than the human eye (vitreous volume 1.5 ml vs 4.5 ml), with a proportionately larger lens, and therefore pharmacokinetic

data should be interpreted with caution.[188] Species with bigger eyes have longer diffusion paths, and therefore have slower vitreous clearance.[189] Human experiments involving multiple vitreous samples over time are impractical for determining pharmacokinetic properties.

1.8.1 Ranibizumab

Ranibizumab is a monoclonal antibody fragment measuring 48 kDa which binds to the receptor binding site of VEGF-A (Figure 1.13).[190] An early study investigating its pharmacokinetic properties was performed on monkeys.[189] After intravitreal injection, ranibizumab distributed rapidly to the retina (within 24 hours), and the ocular half-life measured 3.0 days, with a terminal systemic half-life of 0.5 days. Rabbit model assays have calculated ocular half-life between 2.7 – 3.2 days (Table 1.2).[181, 191-193]

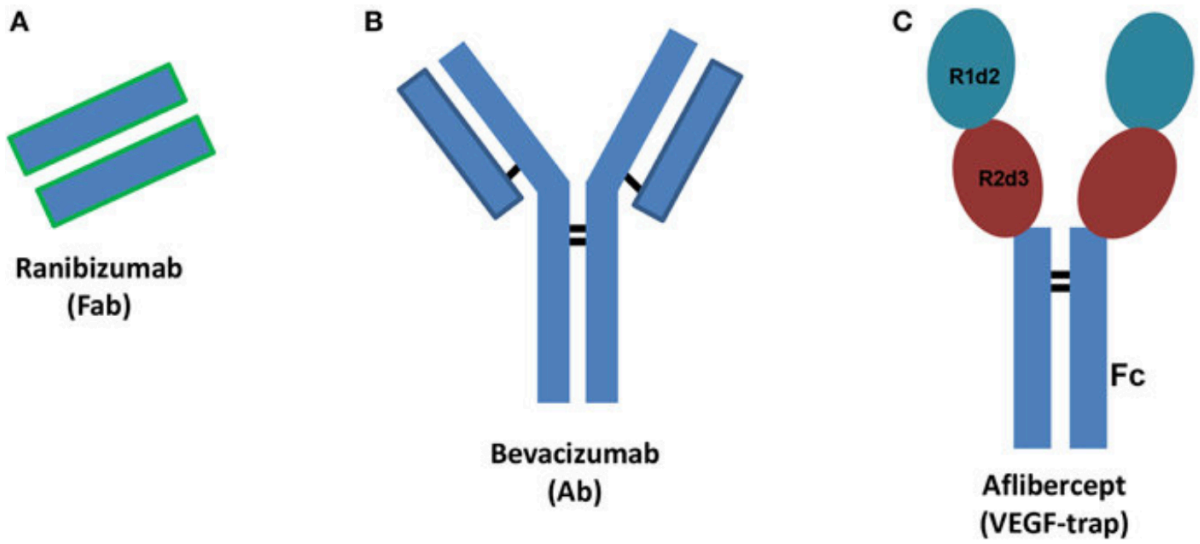


Figure 1.13: Schematic structure of ranibizumab (A), bevacizumab (B) and aflibercept (C).[194] *Ab, antibody; Fab, fragment antigen binding; Fc, fragment crystallizable region.*

Author	Model	Sample	Vitreous status	Ocular half-life (days)
Ahn *et al*[181]	New Zealand white rabbit	VH	Non-vitrectomised Vitrectomised	2.8 2.5
Gaudreault *et al*[191]	New Zealand white rabbit	AH	Non-vitrectomised	3.0
Shatz *et al*[193]	New Zealand white rabbit	VH	Non-vitrectomised	3.2
Bakri *et al*[192]	Dutch belted rabbit	AH	Non-vitrectomised	2.9
Christoforidis *et al*[195]	Dutch belted rabbit	VH	Non-vitrectomised Vitrectomised	2.8 2.1
Niwa *et al*[173]	Cynomolgus monkey	AH	Non-vitrectomised Vitrectomised	2.3 1.4
Gaudreault *et al*[189]	Cynomolgus monkey	AH	Non-vitrectomised	3.0
Roche (unpublished)[196]	Cynomolgus monkey	AH	Non-vitrectomised	2.6
Christoforidis *et al*[197]	Owl monkey	VH	Non-vitrectomised	2.7
Krohne *et al*[187]	Human (n=18)	AH	Non-vitrectomised	7.2
Avery *et al*[198]	Human (n=43)	Serum	Non-vitrectomised	5.8
Xu *et al*[199]	Human (n=229)*	Serum	Non-vitrectomised	8.6
Zhang *et al*[200]	Human (n=876)*	Serum	Non-vitrectomised	6.5 – 7.2

Table 1.2: Studies measuring ocular half-life of ranibizumab

*AH, aqueous humor; VH, vitreous humor.*population approach non-linear mixed-effect model.*

A monkey model found the dose to the retina was approximately one third that in the vitreous, indicating that the drug was partially cleared through the anterior chamber.[189] The data indicated that there was minimal intraocular metabolism of ranibizumab.[189] Furthermore, the serum measured half-life after intravitreal administration was similar to the ocular half-life (3.0 vs 3.5 days, respectively).[189]

Another less invasive method of pharmacokinetic analysis is radiolabelling with positron emission tomography/computed tomography (PET/CT) scanning. The intravitreal ranibizumab ocular half-life in rabbits was 2.8 days, which is consistent with the assay method.[195] The same technique in a monkey model calculated an ocular half-life of 2.7 days.[197] Another technique using fluorescence imaging found a longer ocular intravitreal ranibizumab half-life (3.3 days) in monkeys.[201]

The radiolabelling technique has also demonstrated that ranibizumab ocular half-life in rabbits is significantly reduced after either vitrectomy or lensectomy, compared to controls (2.1 days vs 1.8 days vs 2.8 days, respectively).[171] A shorter ranibizumab ocular half-life has also been reported in vitrectomised compared to non-vitrectomised monkey eyes (1.4 days vs 2.3 days).[173] Another study did not find a significant difference in vitrectomised rabbit eyes compared to non-vitrectomised eyes (2.5 days vs 2.8 days, respectively).[181]

There has been one human study calculating ocular half-life of intravitreal ranibizumab using ocular samples.[187] Eighteen patients had an aqueous humor sample taken between 1 and 37 days post-injection at the time of cataract surgery. The ocular half-life measured 7.2 days, which, as expected due to the larger human eye size, was longer than any of the animal models. Studies using human serum have found ocular half-lives in the range of 5.8 – 8.6 days.[198-200] Ranibizumab is rapidly excreted by the kidneys with an estimated systemic half-life of 2 hours.[199]

1.8.2 Bevacizumab

Bevacizumab has a whole antibody structure with a molecular weight of 149 kDa (Figure 1.13). Rabbit models have estimated the ocular half-life to be 4.2 – 7.1 days, which is longer than ranibizumab (Table 1.3).[183, 184, 195, 202, 203] The ocular half-life in monkey models has been estimated as 2.8 – 3.9 days.[196, 197, 204] Human studies have calculated the ocular half-life of bevacizumab at 9.8 – 11.7 days using an aqueous sample taken at the time of cataract surgery.[185, 186] Previous PPV has been shown to reduce the ocular half-life of bevacizumab in rabbits (4.2 vs 2.3 days).[195] Bevacizumab is cleared by the kidneys with a systemic half-life of 21 days; far slower than ranibizumab due to its increased size.[205]

Author	Animal	Sample	Vitreous status	Ocular half-life (days)
Simapis et al[202]	New Zealand white rabbit	VH	Non-vitrectomised	6.6
Ahn et al[203]	New Zealand white rabbit	VH	Non-vitrectomised Vitrectomised	7.1 7.0
Bakri et al[184]	Dutch belted rabbit	VH	Non-vitrectomised	4.3
Nomoto et al[183]	Dutch belted rabbit	VH	Non-vitrectomised	6.0
Christoforidis et al[195]	Dutch belted rabbit	VH	Non-vitrectomised Vitrectomised	4.2 2.3
Miyake et al[204]	Cynomolgus monkey	AH	Non-vitrectomised	2.8
Roche (unpublished)[196]	Cynomolgus monkey	AH	Non-vitrectomised	3.3 – 3.9
Christoforidis et al[197]	Owl monkey	VH	Non-vitrectomised	3.6
Krohne et al[186]	Human (n=30)	AH	Non-vitrectomised	9.8
Meyer et al[185]	Human (n=16)	AH	Non-vitrectomised	11.7
Avery at al.[198]	Human (n=7)	Serum	Non-vitrectomised	18.7

Table 1.3: Studies measuring ocular half-life of bevacizumab

AH, aqueous humor; VH, vitreous humor.

1.8.3 *Aflibercept*

Aflibercept is a recombinant fragment crystallizable (Fc) fusion protein measuring 115 kDa (Figure 1.13). It is a trap molecule, comprising fused components from different endogenous receptors.[206] In contrast to ranibizumab, it also binds to VEGF-B and PlGF.[207] It has a larger molecular weight than ranibizumab and therefore would be expected to have a longer ocular half-life.

Rabbit models have calculated an ocular half-life of 3.9 – 4.6 days (Table 1.4).[208-210] The ocular half-life in monkey models was 2.2 – 2.4 days.[173, 197] Stewart mathematically estimated the human ocular half-life of aflibercept to be 7.1 days based on known rabbit and monkey values.[211] Further small human studies have found an ocular half-life of 9 days.[196] This value sits between the half-lives for ranibizumab and bevacizumab. The systemic half-life has been calculated at 1.5 days following intravenous administration, which is

considerably shorter than bevacizumab (20 days), but longer than ranibizumab (2 hours).[199, 212]

Author	Animal	Sample	Vitreous status	Ocular half-life (days)
Furfine et al.[208]	New Zealand rabbit	VH	Non-vitrectomised	4.5
Park et al.[210]	New Zealand rabbit	VH	Non-vitrectomised	3.9
Christoforidis et al.[209]	Dutch belted rabbit	VH	Non-vitrectomised	4.6
Niwa et al.[173]	Cynomolgus monkey	AH	Non-vitrectomised Vitrectomised	2.2 1.5
Christoforidis et al.[197]	Owl Monkey	VH	Non-vitrectomised	2.4
Caruso et al.[196]	Human (mathematical model)	AH	Non-vitrectomised	9.5
Avery at al.[198]	Human (n=39)	Serum	Non-vitrectomised	11.4

Table 1.4: Studies measuring ocular half-life of aflibercept

AH, aqueous humor; VH, vitreous humor.

There has been minimal work on the ocular half-life of anti-VEGF drugs for retinal disease comparing eyes with and without a PVD. In addition, the literature on half-life in vitrectomised eyes is limited.

1.9 Safety of anti-vascular endothelial growth factor treatment

Another important aspect of anti-VEGF drugs is their safety profile. This can be divided into ocular and systemic adverse events.

1.9.1 Ocular adverse events

The most serious ocular adverse event of intravitreal anti-VEGF is infective endophthalmitis, which has been calculated at 0.056% for each injection in a large meta-analysis of over 350,000 patients combining real world and clinical trial data.[213] The cumulative risk to a

patient over a two year course of injections is likely to be higher and has been estimated at 1%.[214] Non-infectious endophthalmitis is also rare (<0.1%), with no known difference in the event rate difference between ranibizumab and aflibercept.[215]

Anti-vascular endothelial growth factor intravitreal injections also increase the risk of complications during subsequent cataract surgery. The most widely acknowledged marker of cataract surgery safety is the posterior capsular rupture (PCR) rate, which a large UK database study estimated as 1.92%.[216] The PCR risk in an eye which has previously received an intravitreal anti-VEGF injection is higher, with an odds ratio of 1.66 versus eyes that have not received anti-VEGF injections.[217] The higher risk is most likely due to lens capsule or zonular trauma during the injection procedure.[218]

As would be expected, a volume-driven acute intraocular pressure (IOP) rise occurs immediately after an anti-VEGF intravitreal injection.[219] This rise in IOP is well tolerated and tends to be transient.[220] In the rare event of sustained high IOP and central retinal artery occlusion, an anterior chamber paracentesis can be performed. Chronic sustained ocular hypertension can occur with time, particularly in the context of pre-existing glaucoma and multiple injections.[221] For this reason, clinicians are advised to monitor IOP at injection visits and investigate accordingly if elevated levels are detected.[222]

Non-severe side effects such as sub-conjunctival haemorrhage from local needle trauma, are common, but of no significant impact.[223] Corneal abrasions can occur from the eyelid speculum, but usually heal within a few days at most.

1.9.2 Systemic vascular endothelial growth factor suppression

The VEGF signalling pathway has a major modulatory effect on vasoconstriction, atherosclerosis, platelet activation and thrombosis.[224] Vascular endothelial growth factor also has a role in other processes such as tissue repair, inflammation, haematopoiesis and lymphogenesis.[130, 225-228] Suppression of VEGF could therefore potentially alter normal physiology.

Systemic plasma levels of ranibizumab and aflibercept, when given intravitreally, exceed the inhibitory concentration (IC_{50}) of VEGF.[206] It has also been shown that the VEGF concentration falls after intravitreal anti-VEGF drugs.[229 230] Furthermore, those with bilateral retinal disease have been shown to have a treatment effect in the fellow eye with single eye injection, supporting the hypothesis that systemic VEGF suppression occurs.[231]

Drugs with an Fc domain (aflibercept and bevacizumab) undergo slower systemic elimination than those without (ranibizumab), due to their binding affinity for endothelial cells and therefore may be more likely to cause systemic VEGF supression.[232] The recent RIVAL study supported this assumption. Mean plasma VEGF concentration reduced significantly after aflibercept, but not ranibizumab, most likely due to delayed elimination (Figure 1.14).[151]

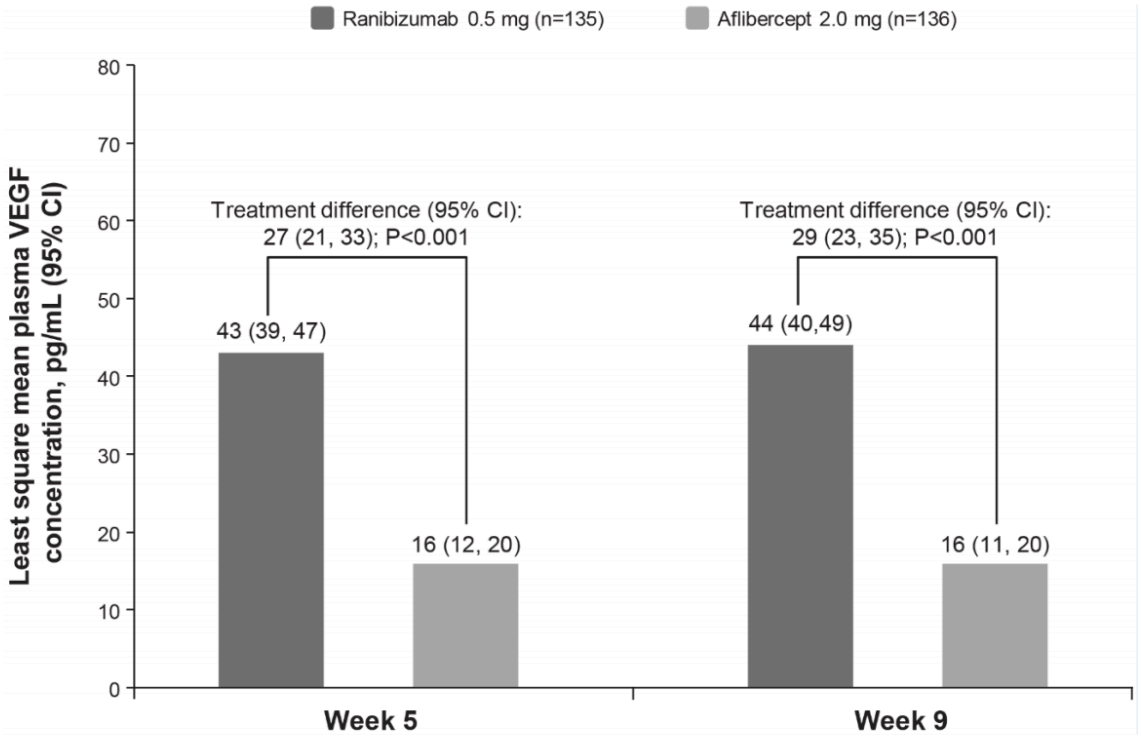


Figure 1.14: Plasma vascular endothelial growth factor concentration change for ranibizumab and aflibercept, compared to baseline, at one week after the second and third injections. The baseline plasma vascular endothelial growth factor concentration was 44pg/ml and 42 pg/ml in the ranibizumab and aflibercept groups, respectively. This figure demonstrates that aflibercept reduced plasma vascular endothelial growth factor concentration significantly more than ranibizumab, at one week post-second and third injections.[151]

The proposed mechanisms that link VEGF suppression and vascular dysregulation are complex (Figure 1.15). An animal model showed VEGF administration results in hypotension, which was reversible by inhibiting nitric oxide synthase (NOS).[233] Conversely, inhibiting the VEGF pathway causes hypertension, again reversible by NOS inhibition.[234] A reduction in nitric oxide (NO) production reduces the ability for the vascular endothelium to vasodilate in response to acetylcholine, and therefore results in higher blood pressure .[224]

Nitrite and nitrate are also involved in this pathway, because they generate NO when NOS activity is suppressed.[235]

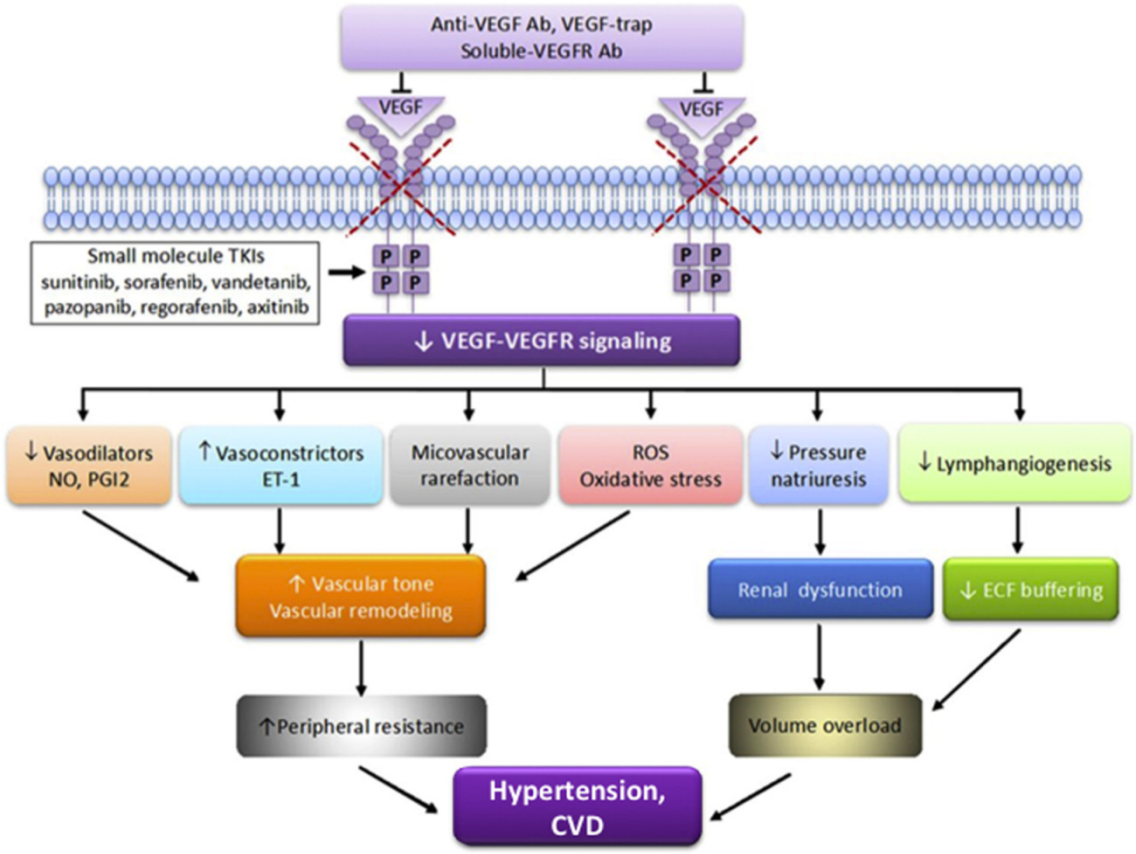


Figure 1.15: Physiological processes linking anti-vascular endothelial growth factor drugs to hypertension.[224]

Ab, antibody; CVD, cardiovascular disease; ECF, extracellular fluid; ET-1, endothelin 1; NO, nitric oxide; P, phosphorylation site of tyrosine kinase; PGI2, prostacyclin; ROS, reactive oxygen species; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor

Another vasodilator, prostacyclin (also known as prostaglandin I2 (PGI-2)) is also downregulated by VEGF.[236] Intravenous bevacizumab given to healthy patients reduced endothelial vasodilation within 15 minutes, supporting the link between anti-VEGF drugs and endothelial dysfunction.[237] There are also other potential mechanisms of VEGF suppression

resulting in hypertension and cardiovascular disease. The common final pathway outcomes are either increased peripheral resistance or volume overload.[224]

Another side effect of anti-VEGF treatment is arterial thromboembolic events (ATE). These are mainly due to endothelial cell mechanisms. Endothelial dysfunction can result in coronary artery vasospasm, myocardial infarction and cerebrovascular ischaemia.[238] In addition, endothelial dysfunction predisposes to atherosclerosis, vascular thrombosis and altered haemostasis, all of which increase the risk of ATE.[239] Microvascular changes, such as depletion of pericytes, can also occur with anti-VEGF drugs, further adding to the risk of ATE.[224]

The evidence suggests that systemic VEGF suppression carries the potential of serious vascular side effects, but the risk is likely to vary with the drug, dose, and route of delivery.

1.9.3 Risk of arterial thromboembolism

A meta-analysis of oncology patients showed the incidence of ATEs was higher in intravenous bevacizumab groups compared to controls (3.8 % vs 1.7 %).[240] Patients with a history of ATE were at highest risk. Despite this, they continue to be used for their powerful tumour anti-angiogenic effects.

The landmark minimally classic/occult trial of the anti-vascular endothelial growth factor antibody ranibizumab in the treatment of neovascular age-related macular degeneration (MARINA) study showed a higher, but non-significant, rate of ischaemic cerebrovascular events with intravitreal ranibizumab, but this was not replicated in the anti-vascular

endothelial growth factor antibody for the treatment of predominantly classic choroidal neovascularization in age-related macular degeneration (ANCHOR) study.[121, 122] A large pooled analysis did not find an increased risk of ATEs with ranibizumab versus sham for nAMD.[241] A comprehensive review of aflibercept use in large trials did not find a statistically different risk of ATEs compared to controls, which included those receiving ranibizumab therapy.[242] Recently, a population-based study of 504 AMD patients undergoing anti-VEGF treatment did not find an increased risk of stroke, myocardial infarction or death compared to control patients either with or without AMD.[243]

Most intravitreal anti-VEGF trials were not powered to detect significant differences in ATEs event rates, and often excluded those with significant cardiovascular morbidities. These are the patients who may be at the highest risk of ATEs, and therefore the results may not be generalisable to routine clinical practice.[122, 123] In addition, patients with nAMD are thought to have an increased risk of stroke that is independent of anti-VEGF treatment, confounding an analysis of ATE risk.[244] Therefore the risk of embolic cardiovascular and cerebrovascular events has not been fully defined.

1.9.4 Risk of hypertension

Most oncology clinical trials have shown that intravenous anti-VEGF drugs, including bevacizumab, increase blood pressure.[245] This is most likely a local effect on the endothelium, and is more common in patients over the age of 60 with pre-existing hypertension.[246] Hypertension stimulates a hypertrophy in the myocardium, which is usually matched by an angiogenic compensatory response. Such a counteraction may not be possible in patients on anti-VEGF therapy.[224] Hypertension is also well known to cause

atherosclerosis and atrial fibrillation, and is a greater risk to patients with additional cardiovascular risk factors.[224]

Clinical trials are thought to under recruit from ischaemic heart disease groups and therefore these patients may be at risk of hypertension with intravitreal anti-VEGF. A study of 82 patients specifically assessing blood pressure changes with intravitreal bevacizumab found dysregulation in the anti-VEGF treated group.[247] Despite similar findings with intravenous aflibercept, no increased risk of hypertension has been identified in the VIEW studies of intravitreal aflibercept use.[124]

1.9.5 Risk of renal disease

Oncology trials showed renal disorders can occur with systemic anti-VEGF drugs, ranging from benign proteinuria to severe renal failure.[248, 249] This was thought to be due to renal podocytes in the glomerular basement membrane requiring VEGF signalling to maintain normal function.[250] Similar to cardiovascular complications, those most at risk have pre-existing disease.[251] This is of particular relevance to diabetic patients who often have nephropathy.

An animal model study showed that intravitreal aflibercept significantly inactivates VEGF in glomerular podocytes and disrupts the basement membrane.[252] The same study did not find a similar effect with ranibizumab.

There have been various reports of worsening renal function attributed to intravitreal anti-VEGF therapy.[253-261] A case series found 3 patients receiving intravitreal bevacizumab and

aflibercept suffered a substantial decline in renal function (worsening proteinuria and creatinine concentration).[262] One reported case had proteinuria and increased creatinine after intravitreal bevacizumab which stabilised after a treatment switch to ranibizumab.[263] These cases raised concern that drugs with a longer half-life (aflibercept and bevacizumab) may carry a higher risk. However, renal dysfunction has also been reported after ranibizumab.[253, 255, 258] Therefore, the effect of intravitreal anti-VEGF on clinical renal function has not been fully delineated.

1.10 Problems and Aims

1.10.1 Summary of introduction and thesis scope of work

The preceding introduction detailed vitreomacular anatomy, the process of PVD and the visual problems that can occur at the vitreomacular interface, particularly in the context of incomplete PVD. The treatment of symptomatic vitreomacular adhesion was outlined. In addition, the impact of the vitreomacular interface and vitreous status on macular disorders was reviewed. This interaction relates to factors local to the vitreomacular interface, which can physically modulate underlying macular disease, and the state of the vitreous, which alters how physiological molecules and drugs move to and from their target tissue. This was followed by an overview of the efficacy and safety of anti-VEGF drugs in nAMD.

This thesis focusses on the clinical impact of changes to the vitreous and vitreomacular interface. The scope of work encompasses their local role as a primary disease, to their impact on the systemic levels of drugs injected into the vitreous cavity, and from this a wider consideration of the safety of anti-VEGF drugs once they have left the vitreous cavity.

1.10.2 Thesis questions and aims

Question 1: What is the best method to treat symptomatic vitreomacular adhesion?

Pars plana vitrectomy is the standard treatment for sVMA, but other treatment options have emerged. Randomized controlled trials support ocriplasmin treatment for sVMA.[62, 63, 264, 265] Despite this, ocriplasmin has not been widely adopted, with debate persisting about its safety and efficacy.[266, 267] An intravitreal expansile gas bubble has been investigated in numerous small case series as a means of releasing VMA, but there is no pooled analysis of its safety and efficacy.[57-59] Whether either of these treatment options is superior to PPV remains unknown.

Aim 1: To assess the likelihood of successful vitreous separation with intravitreal ocriplasmin

A meta-analysis addressing the efficacy of intravitreal ocriplasmin treatment for sVMA is warranted to provide high level evidence regarding the management of this visually significant condition. This will enable clinicians to better understand the benefits of ocriplasmin.

Aim 2: To perform a pooled analysis of trial data on the safety of ocriplasmin

Ocriplasmin's mechanism of action is based on anti-laminin and anti-fibronectin activity. These substances are widely present in the eye, and therefore it is possible that ocriplasmin may exert unwanted effects away from its vitreomacular interface target. A pooled analysis

of adverse events following ocriplasmin intravitreal injection will better inform ophthalmologists about the safety of this treatment.

Aim 3: To determine the risk of dyschromatopsia after ocriplasmin

There have been reports of subjective dyschromatopsia following ocriplasmin.[268] Symptoms have been reported as early as 4 hours after ocriplasmin treatment, and can persist for months.[268, 269] Dyschromatopsia is highly subjective, and there is a lack of objective colour vision measurement in the literature. A study will be performed to measure Farnsworth Munsell-100 colour hue discrimination before and after ocriplasmin treatment. Measurements will be performed up to 1 year following treatment to assess whether there is any objective colour vision defect and if it is transient or longstanding.

Aim 4: To assess the safety and efficacy of an intravitreal expansile gas bubble for symptomatic vitreomacular adhesion

A pooled analysis of data in the literature using gas for sVMA would be beneficial to determine the effectiveness of this treatment.

Question 2: Does the status of the vitreomacular interface influence anti-VEGF drug clearance from the eye to the systemic circulation?

The vitreous status can influence the clinical course of nAMD.[2] Given the changes in intra-ocular fluid dynamics depending on vitreous status, it is possible that anti-VEGF drugs leave the eye faster when a PVD is present or if the eye is vitrectomised. This pharmacokinetic

change may influence systemic drug concentrations and interactions with homeostatic mechanisms.

Ever since their introduction, there has been debate about whether intravitreal anti-VEGF drugs have systemic safety risks. Most major studies and pooled analyses have found no significant risk, but these studies often exclude patients with systemic comorbidities. These are the patients who may be at the highest risk. A recent review concluded that current data are insufficient to confirm the safety of intravitreal anti-VEGF drugs and that pharmacovigilance needs to be improved to determine the true risk of ATEs.[270] In addition, a number of case reports have identified deteriorating renal function following anti-VEGF drugs, and have suggested that pre-existing renal disease may confer an additional risk.[257-259]

Aim 5: To assess whether the status of the vitreomacular interface affects anti-VEGF drug pharmacokinetics

A study will be performed to measure systemic ranibizumab and aflibercept concentrations at various time points after intravitreal injection for nAMD. Pharmacokinetics will be compared in eyes with a PVD, no PVD, and previous PPV.

Aim 6: To investigate whether intravitreal anti-VEGF drugs affect renal function

Case series have found disruption of renal function with intravitreal anti-VEGF. In these cases, creatinine concentration has risen, often irreversibly.[262, 263] There are limited data in the literature regarding creatinine concentration after intravitreal anti-VEGF. A study will be

performed to measure systemic creatinine, urea, estimated glomerular filtration rate and electrolyte concentrations before and after intravitreal ranibizumab and aflibercept.

Aim 7: To assess whether systemically absorbed anti-VEGF drugs affect the systemic inflammatory environment

Studies have found significant differences in systemic cytokine concentrations between nAMD patients and healthy controls.[271] In addition, the use of anti-VEGF drugs has been shown to affect systemic concentration of VEGF, particularly with aflibercept.[272] The thesis will investigate the effect of anti-VEGF drugs on systemic cytokine and inflammatory marker concentrations, including analysis on the effect conferred by differences in the vitreous status.

2 Ocriplasmin for symptomatic vitreomacular adhesion

This chapter is the published meta-analysis of ocriplasmin for symptomatic vitreomacular adhesion. The same text appears in the final published pdf version (Appendix 1).

Citation

Neffendorf JE, Kirthi V, Pringle E, Jackson TL. Ocriplasmin for symptomatic vitreomacular adhesion. Cochrane Database Syst Rev. 2017 Oct 17;10(10):CD011874

DOI:10.1002/14651858.CD011874.pub2

2.1 Abstract

2.1.1 Background

Symptomatic vitreomacular adhesion (sVMA) is a recognised cause of visual loss and by tradition has been managed by pars plana vitrectomy (PPV). A less invasive alternative to surgery in some people is enzymatic vitreolysis, using an intravitreal injection of ocriplasmin.

2.1.2 Objectives

To assess the efficacy and safety of ocriplasmin compared to no treatment, sham or placebo for the treatment of sVMA.

2.1.3 Search methods

We searched the Cochrane Central Register of Controlled Trials (CENTRAL) (which contains the Cochrane Eyes and Vision Trials Register) (2017, Issue 1), MEDLINE Ovid (1946 to 24 February 2017), Embase Ovid (1947 to 24 February 2017), PubMed (1946 to 24 February 2017), the ISRCTN registry (www.isrctn.com/editAdvancedSearch); searched 24 February 2017, ClinicalTrials.gov (www.clinicaltrials.gov); searched 24 February 2017 and the World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) (www.who.int/ictrp/search/en); searched 24 February 2017. We did not use any date or language restrictions in the electronic searches for trials.

2.1.4 Selection criteria

We included randomised controlled trials (RCTs) of people with sVMA. The intervention was intravitreal ocriplasmin 125 μg injection, and this was compared to placebo or sham injection (control). Placebo was defined as a single intravitreal injection of 0.10 mL placebo with identical drug vehicle diluted with saline. A sham injection was defined as the syringe hub or blunt needle touching the conjunctiva to simulate an injection.

2.1.5 Data collection and analysis

Two authors independently selected relevant trials, assessed methodological quality and extracted data. We graded the certainty of the evidence using the GRADE approach.

2.1.6 Main results

This review included four RCTs conducted in Europe and the USA with a total of 932 eyes of 932 participants. Participants were 18 to 97 years of age, with evidence of focal vitreomacular adhesion (VMA) on optical coherence tomography (OCT) imaging, with a best corrected visual acuity (BCVA) of 20/25 or worse in the study eye and 20/400 or better in the fellow eye. The interventions compared were intravitreal ocriplasmin versus sham (two RCTs) or placebo (two RCTs) injection. Both sham and placebo injection were classified as the control group. The main outcome measures were assessed at 28 days and six months. Overall, we judged the studies to have a low or unclear risk of bias. All four RCTs were sponsored by the manufacturers of ocriplasmin.

Compared with control, ocriplasmin treatment was more likely to result in VMA release within 28 days (risk ratio (RR) 3.46, 95% confidence interval (CI) 2.00 to 6.00; 859 eyes, 4 RCTs, high-certainty evidence). Approximately 97/1000 eyes will have VMA release within 28 days without treatment. An additional 237 eyes will have VMA release within 28 days for every 1000 eyes treated with ocriplasmin (95% CI 96 more to 482 more).

Treatment with ocriplasmin was also more likely to result in macular hole (MH) closure (RR 2.87, 95% CI 1.50 to 5.51; 229 eyes, 3 RCTs, high-certainty evidence). Approximately 123/1000 eyes with MH will have closure with no treatment. An additional 231 eyes will have MH closure for every 1000 eyes treated with ocriplasmin (95% CI 62 more to 556 more).

Eyes receiving ocriplasmin were also more likely to have complete posterior vitreous detachment (PVD) within 28 days (RR 2.94, 95% CI 1.39 to 6.24; 689 eyes, 3 RCTs, high-certainty evidence). Approximately 40/1000 eyes will have complete PVD within 28 days without treatment. An additional 78 eyes will have complete PVD within 28 days for every 1000 eyes treated with ocriplasmin (95% CI 16 more to 210 more).

Eyes receiving ocriplasmin were more likely to achieve 3-line or greater improvement in BCVA at six months (RR 1.95, 95% CI 1.07 to 3.53; 674 eyes, 3 RCTs, moderate-certainty evidence). Approximately 61/1000 eyes will have a 3-line or greater improvement in BCVA at six months without treatment. An additional 58 eyes will have 3-line or greater improvement in BCVA at six months for every 1000 eyes treated with ocriplasmin (95% CI 9 more to 154 more).

Receiving ocriplasmin also reduced the requirement for vitrectomy at six months (RR 0.67, 95% CI 0.50 to 0.91; 689 eyes, 3 RCTs, moderate-certainty evidence). Approximately 265/1000 eyes will require vitrectomy at six months without treatment and 87 fewer eyes will require vitrectomy for every 1000 eyes treated with ocriplasmin (95% CI 24 fewer to 132 fewer).

Treatment with ocriplasmin resulted in a greater improvement in validated visual function questionnaire form score at six months (mean improvement difference 2.7 points, 95% CI 0.8 to 4.6; 652 eyes, 2 RCTs, moderate-certainty evidence).

Eyes receiving ocriplasmin were more likely to have an adverse event (RR 1.22, 95% CI 1.09 to 1.37, 909 eyes, 4 RCTs, moderate-certainty evidence). Approximately 571/1000 eyes will have an adverse event with sham or placebo injection and 106 more eyes will have an adverse event for every 1000 eyes treated with ocriplasmin (95% CI 52 more to 212 more).

2.1.7 Authors' conclusions

Evidence from a limited number of RCTs suggests that ocriplasmin is useful in the treatment of sVMA. However, up to 20% of eyes treated with ocriplasmin will still require additional treatment with PPV within six months. There were more ocular adverse events in eyes treated with ocriplasmin than control (sham or placebo injection) treatment. Many of these adverse events, particularly vitreous floaters and photopsia, are known to be associated with PVD. At present however, there is minimal published long-term safety data on eyes treated with ocriplasmin. Further large RCTs comparing ocriplasmin with other management options for sVMA would be beneficial.

2.2 Plain language summary

2.2.1 Ocriplasmin for symptomatic vitreomacular adhesion

What is the aim of this review

The aim of this Cochrane Review was to find out how well ocriplasmin works in the treatment of sVMA. Cochrane Review authors collected and analysed all relevant studies to answer this question and found four studies.

Key messages

People with sVMA treated with ocriplasmin have an increased chance of release of sVMA and improved vision compared with people who are not treated with ocriplasmin (high-certainty evidence). They are also probably less likely to require surgery, but one in five people with sVMA treated with ocriplasmin will probably still require surgery at a later date to treat sVMA (moderate-certainty evidence).

What was studied in the review?

With age, the gel-like substance (vitreous) that fills the eye begins to pull away from the back of the eye (retina). Sometimes the vitreous remains attached to the retina and causes damage to the retina as it pulls away, leading to visual loss. This is known as sVMA. Symptomatic VMA includes two related conditions, vitreomacular traction (VMT) and MH.

The standard treatment for sVMA is surgery. Ocriplasmin is an alternative, less invasive, treatment. This is an enzyme that can be injected directly into the eye to release the vitreous from the retina.

What are the main results of the review?

Cochrane Review authors found four studies that compared ocriplasmin with control (sham or placebo treatment) for the treatment of sVMA. All four studies were sponsored by the manufacturers of ocriplasmin.

The review showed that:

- ocriplasmin increases the chance of sVMA resolution compared with no treatment (high-certainty evidence).
- people with sVMA treated with ocriplasmin have improved vision compared with people who are not treated with ocriplasmin (high-certainty evidence).
- treatment with ocriplasmin probably reduces the requirement for surgery, but approximately one in five people treated with ocriplasmin may require further surgery at a later date (moderate-certainty evidence).
- there were more ocular adverse events in eyes treated with ocriplasmin than control (sham or placebo injection) treatment.

How up-to-date is this review?

Cochrane Review authors searched for studies that had been published up to 24 February 2017.

2.3 Summary of findings

Patient or population: people with symptomatic vitreomacular adhesion

Settings: eye hospital

Intervention: ocriplasmin injection

Comparison: sham or placebo injection

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of eyes (studies)	Certainty of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Sham or placebo injection	Ocriplasmin injection				
Complete release of vitreous adhesion Follow-up: 28 days	**97 per 1000**	**334 per 1000** (193 to 579)	**RR 3.46** (2.00 to 6.00)	859 (4 studies)	⊕⊕⊕⊕ **High**	-
Closure of macular hole Follow-up: 28 days to 24 months	**123 per 1000**	**354 per 1000** (185 to 679)	**RR 2.87** (1.50 to 5.51)	229 (3 studies)	⊕⊕⊕⊕ **High**	-
Complete posterior vitreous detachment Follow-up: 28 days	**40 per 1000**	**118 per 1000** (56 to 250)	**RR 2.94** (1.39 to 6.24)	689 (3 studies)	⊕⊕⊕⊕ **High**	-
3-line or greater improvement in best-corrected visual acuity Follow-up: 6 months	**61 per 1000**	**119 per 1000** (70 to 215)	**RR 1.95** (1.07 to 3.53)	674 (3 studies)	⊕⊕⊕⊖ **Moderate**[a]	-
Requirement for vitrectomy Follow-up: 6 months	**265 per 1000**	**178 per 1000** (133 to 241)	**RR 0.67** (0.50 to 0.91)	689 (3 studies)	⊕⊕⊕⊖ **Moderate**[a]	-
Mean change in validated visual function questionnaire score from baseline Score ranges from 0 to 100, higher scores are better visual function Follow-up: 6 months	**Mean change in NEI-VFQ score was 0.7**	**NEI-VFQ score was 2.7 higher** (0.8 higher to 4.6 higher)	-	652 (2 studies)	⊕⊕⊕⊖ **Moderate**[a]	-
Any ocular adverse event Follow-up: 6 months	**571 per 1000**	**697 per 1000** (623 to 783)	**RR 1.22** (1.09 to 1.37)	909 (4 studies)	⊕⊕⊕⊖ **Moderate**[a]	-

Table 2.1: Summary of findings for the main comparison. Ocriplasmin injection compared with control for symptomatic vitreomacular adhesion.

*The basis for the assumed risk (e.g. the median control group risk across studies) is provided in footnotes. The corresponding risk (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).

CI, confidence interval; NEI-VFQ, National Eye Institute Visual Function Questionnaire; RCT, randomised controlled trial; RR, risk ratio.

GRADE Working Group grades of evidence

High-certainty: Further research is very unlikely to change our confidence in the estimate of effect.

Moderate-certainty: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low-certainty: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low-certainty: We are very uncertain about the estimate.

[a]*Downgraded one level for imprecision (-1).*

2.4 **Background**

2.4.1 Description of the condition

In healthy eyes, the posterior vitreous face lies in contact with the internal limiting membrane (ILM) of the retina with various points of stronger adhesion such as the macula, vasculature and optic disc. Over time, the structure of the vitreous liquefies in a process known as synchysis, with reduction in the adhesive forces between vitreous and ILM. This often results in the vitreous gel detaching from all parts of the retina, except at the vitreous base anteriorly, in a normal process known as PVD.[17] The process usually starts with focal detachment in the perifovea of the superior quadrant and then extends slowly for years until eventually resulting in a complete PVD with release of vitreopapillary adhesion.[19, 20, 273] However, in certain cases, incomplete PVD may occur, leaving the vitreous in contact with the macula or optic disc, or both.

Although, anatomically, VMA may refer to a normal asymptomatic state, clinically, the term is used when VMA occurs in the context of an incomplete PVD. There is a spectrum of VMA associated with incomplete PVD, which ranges from asymptomatic, non-tractional VMA to extensive distortion of the retinal structure due to VMT which may result in loss of visual function. These distinctions tend to be based on OCT, sometimes in reference to defined photographic standards.[37] However, it is important to note that the OCT changes, which may include retinal thickening and intraretinal oedema, do not always correlate with visual function and symptoms.

Symptomatic vitreomacular adhesion is defined as visual loss secondary to foveal damage caused by abnormal VMT. Symptomatic vitreomacular adhesion includes isolated VMT, impending MH and MH with persisting vitreous attachment.[41] Impending MH is often grouped with VMT. Epiretinal membrane (ERM) often coexists with sVMA. It is possible that VMA influences the clinical course of, or may be associated with, other diseases such as diabetic macular oedema (DMO), retinal vein occlusion (RVO) or neovascular age-related macular degeneration (nAMD), although the data are sometimes conflicting.[2, 37, 41, 74-77] Whilst there may be an association between sVMA and these other diseases, it is not certain that this is causal.[37] Consequently, it is difficult to define the prevalence of sVMA. One study reported that VMA may occur in isolation or in association with other eye disease in approximately 1.5% of the population.[41] However, the majority of these cases occurred alongside ERM, and thus the VMA may not be responsible for visual loss. Excluding cases associated with ERM reduced the prevalence to 0.35% in the same population-based study; however, this figure also included cases with other diseases, such as nAMD and DMO.[41] If only cases of isolated VMA/VMT with or without MH were considered, then the prevalence of sVMA was 171.5 per 100,000 population.[41]

The natural history of sVMA varies. Symptomatic vitreomacular adhesion may spontaneously resolve, with detachment of the posterior vitreous face from the ILM.[1] One study of 53 eyes showed a complete PVD occurred in 11% of eyes over 60 months' follow-up.[42] Weinand and colleagues reported that approximately 10% of cases of VMT syndrome resolve spontaneously.[274] Other studies have found spontaneous resolution in 17% to 35% of cases with VMT.[275-277] Eyes with VMT and isolated inner retinal distortion, as well as those receiving vitreous injections, have an increased likelihood of VMT release.[275] Poor prognostic indicators for spontaneous release include the presence of ERM and large

horizontal adhesion diameter.[276-279] It has been shown that many, if not most, MHs result from persistent VMT which either fully detaches from the retina causing an MH, or remains attached at the edge of the hole.[43-46, 280]

2.4.2 Description of the intervention

Treatment strategies for VMA vary depending on disease severity. Asymptomatic VMT can be observed, since separation of the posterior vitreous face may occur spontaneously and without sequelae. However, a longer duration of VMT may lead to loss of vision and possibly lower efficacy of any subsequent intervention, and therefore treatment is often considered if symptoms are significant or visual acuity is reduced.[42, 47, 48] If VMT progresses to MH then intervention is usually advised, and an evolving VMT/impending MH may likewise necessitate intervention.

If intervention is considered for sVMA, various strategies may be considered. Traditionally, PPV is the standard approach for VMT or MH.[1] Small uncontrolled studies reported that an intravitreal gas bubble can pneumatically release VMT, without the need for PPV, with success rates varying from 71% to 95%.[57-59]

Pharmacological vitreolysis has been investigated as an alternative treatment for VMT, and for MH with persisting VMA.[60-63] Autologous plasmin, an enzyme that breaks down the laminin and fibronectin bonds maintaining vitreous adhesion, has been used perioperatively to induce a PVD during vitrectomy.[281-283] However, autologous plasmin is not suited to the treatment of VMT due to its autolytic instability.[284] Based on autologous plasmin, a recombinant DNA molecule, initially referred to as microplasmin, and more recently

ocriplasmin (Jetrea; ThromboGenics, Leuven, Belgium), was developed to provide the same catalytic properties but with greater stability.

Ocriplasmin is administered as a single intravitreal injection of 125 μg in 0.1 mL. It has marketing authorisation for the treatment of VMT, including when associated with MH of diameter of 400 μm or less.[65] In the UK, the National Institute for Health and Care Excellence (NICE) supports the use of ocriplasmin for adults with VMT causing severe sight problems or a MH up to 400 μm, in the absence of ERM.[65]

2.4.3 How the intervention might work

Ocriplasmin is a proteolytic enzyme which targets laminin and fibronectin, both of which are important structural components of the interface between the vitreous and the retina. It is a truncated form of the human serine protease plasmin which functions in a two-stage mechanism; liquefaction of the vitreous and vitreoretinal separation.[285]

2.4.4 Why it is important to do this review

Ocriplasmin has marketing authorisation in Europe and the USA and is the only licensed, non-surgical treatment for sVMA. Macular hole is the second most common indication for PPV, and both MH and VMT can cause substantial visual problems.[286] This review is important as it assessed the efficacy and safety of ocriplasmin treatment.

2.4.5 Objectives

To assess the efficacy and safety of ocriplasmin compared to no treatment, sham or placebo for the treatment of sVMA.

2.5 **Methods**

2.5.1 Criteria for considering studies for this review

Types of studies

We included RCTs only.

Types of participants

We included participants with a diagnosis of sVMA, including VMT and MH of 400 μm or less with persisting VMA. There were no restrictions with regards to gender, age or ethnicity.

Types of interventions

We included any RCT in which intravitreal ocriplasmin was compared to no treatment, sham injection or placebo.

Types of outcome measures

Primary outcome measures

- Proportion of eyes with complete release of vitreous adhesion as determined by analysis of OCT images captured 28 days after ocriplasmin, sham or placebo treatment

Secondary outcome measures

- Proportion of eyes with closure of MH as determined by analysis of OCT images captured 28 days after ocriplasmin, sham or placebo treatment.
- Proportion of eyes with complete PVD as measured by clinical examination or B-scan ultrasonography 28 days after ocriplasmin, sham or placebo treatment.

- Proportion of eyes with 3-line or greater improvement in BCVA from baseline, measured using Early Treatment Diabetic Retinopathy Study (ETDRS) at 4 m or Snellen chart, at six months after ocriplasmin, sham or placebo treatment.
- Proportion of eyes requiring PPV within six months of ocriplasmin, sham or placebo treatment (as recommended by the investigator if the underlying condition deteriorated, BCVA worsened by more than 2 lines on ETDRS or Snellen chart, or if the underlying condition had not improved within 28 days after treatment).
- Mean change in validated VFQ score from baseline, measured at six months after ocriplasmin, sham or placebo treatment.

Safety outcome measures

- Description of ocular adverse events and serious adverse events, and any non-ocular serious events attributed to ocriplasmin or no treatment/sham/placebo.

2.5.2 Search methods for identification of studies

Electronic Searches

The Cochrane Eyes and Vision Information Specialist conducted systematic searches in the following databases for RCT and controlled clinical trials. There were no language or publication year restrictions. The date of the search was 24 February 2017.

- Cochrane Central Register of Controlled Trials (CENTRAL; 2017, Issue 1) (which contains the Cochrane Eyes and Vision Trials Register) in the Cochrane Library (searched 24 February 2017) (Appendix 2)
- MEDLINE Ovid (1946 to 24 February 2017) (Appendix 3)
- Embase Ovid (1980 to 24 February 2017) (Appendix 4)

- PubMed (1946 to 24 February 2017) (Appendix 5)
- ISRCTN registry (www.isrctn.com/editAdvancedSearch; searched 24 February 2017) (Appendix 6)
- US National Institutes of Health Ongoing Trials Register ClinicalTrials.gov (www.clinicaltrials.gov; searched 24 February 2017) (Appendix 7)
- World Health Organization ICTRP (www.who.int/ictrp; searched 24 February 2017) (Appendix 8)

Searching other resources

We searched the reference lists of included studies for other possible studies. We did not search proceedings from conferences specifically, because such RCTs presented at these meetings were searched by Cochrane Eyes and Vision and included in CENTRAL.

2.5.3 Data collection and analysis

Selection of studies

Three authors (JN, VK and TJ) independently assessed the results identified by the searches and classified each record as either possibly relevant or definitely not relevant. We then obtained full-text copies of all possibly relevant records, and three authors (JN, VK and TJ) classified them as definitely include, unsure or definitely exclude based on the criteria for inclusion. In the event of any difficulty in classification due to lack of clarity or data, we contacted study investigators for further information. All contacted authors responded to our requests. We resolved discrepancies by consensus following discussion between authors (JN, VK and TJ) and documented this in the review. All excluded records were documented.

Data extraction and management

Two authors (JN and VK) independently extracted trial data for the primary and secondary outcomes onto paper data extraction forms developed by Cochrane Eyes and Vision. Subsequently, data were transcribed into Review Manager 5 by one author (JN) and verified by a second author (VK).[287] Any discrepancies were resolved by consensus between authors (JN, VK and TJ) and documented in the review.

We collected the following information on study characteristics (Appendix 9):

- study design: parallel group RCT/within-person RCT/one or both eyes reported
- participants: country, total number of participants, age, sex, inclusion and exclusion criteria
- intervention and comparator details: including number of people (eyes) randomised to each group
- primary and secondary outcomes as measured and reported in the trials, adverse events
- length of follow-up
- date study conducted;
- funding and conflicts of interest.

We extracted the following data from each included study for intervention and comparator groups separately:

- number of events and number of participants for outcome data collected for dichotomous variables (release of vitreous adhesion at 28 days, closure of MH at 28 days and complete PVD at 28 days)

- mean, standard deviation and number of participants for outcome data measured for continuous variables (change in BCVA at six months and change in validated VFQ at six months). To compare visual acuity across studies, the mean BCVA was converted to logarithm of the minimum angle of resolution units (logMAR). Counting fingers vision was assigned a logMAR acuity of 1.6, hand movements 1.9, light perception 2.2 and no light perception 2.5.[288] The default VFQ assessed was the National Eye Institute Visual Functioning Questionnaire - 25 (NEI-VFQ25)

We collected evidence of harm from RCTs only.

Assessment of risk of bias in included studies

Two authors (JN and VK) independently assessed the included trials for bias using the methods and grades described in Chapter 8 of the Cochrane Handbook for Systematic Reviews of Interventions.[289] We assessed the following: methods of sequence generation used for randomisation; allocation concealment; masking (blinding) of outcome assessors; masking of participants and personnel; incomplete outcome data; selective outcome reporting; other bias. We considered the use, or not, of independent masked OCT image analysis assessors in the assessment of bias. We then classified each item as 'low,' 'high' or 'unclear' risk of bias.

Measures of treatment effect

We presented dichotomous data as RR with 95% CI;

- Primary outcome:
    - Resolution of VMA
- Secondary outcomes
    - closure of MH

    - complete PVD
    - proportion of eyes with 3-line or greater gain in BCVA
    - requirement for PPV
- We presented continuous data as mean differences with 95% CIs:
    - change in validated VFQ measure

Unit of analysis issues

Trials randomised one or both eyes to the intervention or comparator. If people were randomly allocated to treatment but only one eye per person was included in the trial then there was no unit of analysis issue. In these cases, we documented how the eye was selected and if this was done before randomisation. If people were randomly allocated to treatment but both eyes were included and reported, we planned to analyse as 'clustered data,' that is, adjust for within-person correlation. If the study was a within-person study, that is, one eye was randomly allocated to intervention and the other eye received the comparator, then we planned to analyse as paired data. We planned to contact the trial investigators for further information to do this if necessary.

Dealing with missing data

In the event of missing trial outcome data, we contacted the authors of the trial to understand why the data were missing. If no response was received within four weeks, we used the information provided in the published articles. Missing data were handled in accordance with the guidelines given in Chapter 16 of the Cochrane Handbook for Systematic Reviews of Interventions.[290] We planned to perform sensitivity analyses on the impact of missing data and comment on the findings in the discussion of the review.

Assessment of heterogeneity

We assessed heterogeneity and inconsistency among trials statistically using an I^2 value (> 50%) to assess if variability in effect was due to sampling error. We also planned to assess diversity among studies by reviewing participant characteristics and trial methodology.

Assessment of reporting biases

We assessed selective outcome reporting by comparing intended outcomes in published protocols, published methods papers and clinical trial registries to reported outcomes in the results sections of trial reports. If there were 10 or more eligible RCTs, we planned to use a funnel plot to assess for study-reporting bias.

Data synthesis

If there were three or fewer eligible RCTs then we planned to use a fixed-effect model for the meta-analyses. If there were more than three included trials, we planned to use a random-effects model instead. If we had evidence of high heterogeneity (e.g. $I^2 > 50\%$), it would not be sensible to pool the data from different trials; in which case, we planned to do a narrative summary of the results.

Subgroup analysis and investigation of heterogeneity

If trials demonstrated clinical heterogeneity and sufficient data were available, including age (< 65 years, 65 years and over), presence of ERM, size of adhesion (less than 1500 μm, 1500 μm or greater) and sVMA subtype (isolated VMT, and MH with persisting vitreous attachment), we planned to perform subgroup analyses for the primary outcome.

Sensitivity analysis

We planned to conduct one sensitivity analysis, excluding studies that were at high risk of bias in one or more domains.

'*Summary of findings' table*

We prepared a 'Summary of findings' table for the following outcomes:

- resolution of VMA at 28 days
- complete PVD at 28 days
- closure of MH at 28 days
- proportion gaining 3-line or greater improvement in BCVA at six months
- requirement of PPV at six months
- change in validated VFQ measure at six months
- adverse and serious adverse events.

Two authors (JN and VK) independently graded the overall certainty of the evidence for each outcome using the GRADE Working Group classification.[291]

2.6 **Results**

2.6.1 Description of Studies

Results of the search

The electronic searches yielded 418 records (Figure 2.1). The Cochrane Information Specialist scanned the search results, removed 136 duplicates and then removed 123 references which were irrelevant to the scope of the review. We screened the remaining 159 reports and obtained 14 full-text reports for further assessment. We included five reports of four RCTs, three reports (Haller et al; Stalmans et al; Varma et al.) analysed separate outcomes from the same two RCTs (TG-MV-006 and TG-MV-007).[63, 264, 278] We excluded nine reports of nine studies. We did not identify any ongoing studies from our searches of clinical trials registries.

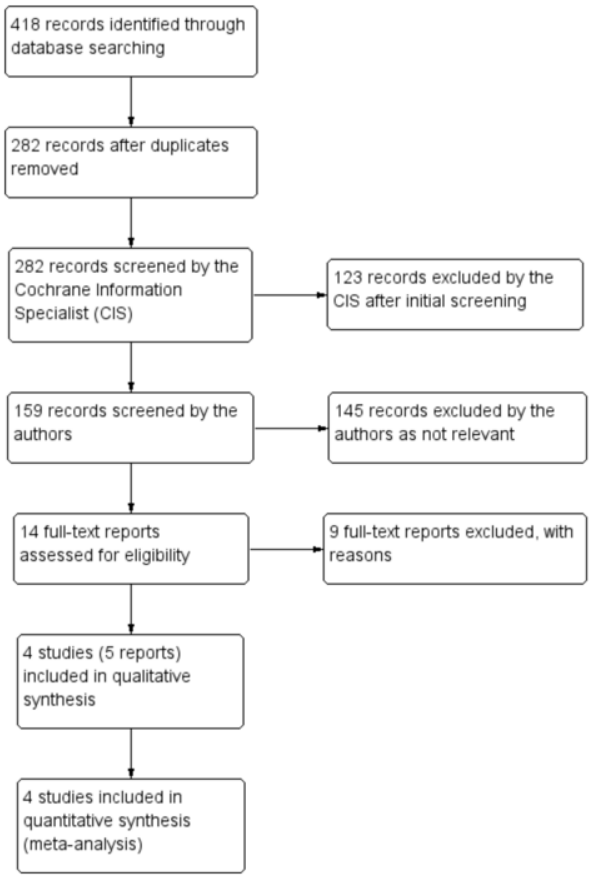


Figure 2.1: Study flow diagram

2.6.2 Included studies

The following is a summary of the characteristics of the four RCTs that met the review inclusion criteria (MIVI-IIT; OASIS; TG-MV-006; TG-MV-007).[62, 63, 264, 265, 278] All data were initially obtained from published literature, then verified for discrepancies using the clinical trials registries described in the methods section (Appendices 10 - 13).

Types of participants

The four RCTs included enrolled 932 participants (932 eyes). All participants received individually randomised, parallel group treatment to a single eye. The age range of all included participants was 18 to 97 years. All included participants had evidence of focal

VMA on OCT, BCVA of 20/25 or worse in the study eye and 20/400 or better in the fellow eye (ETDRS acuity chart). Exclusion criteria were: active proliferative diabetic retinopathy, high myopia (axial length greater than 26 mm or more than -8 dioptres), previous vitrectomy or uncontrolled glaucoma, previous intravitreal injections within the past three months in the study eye, intraocular surgery or laser photocoagulation within the past three months in the study eye or rhegmatogenous retinal detachment in either eye. Additional exclusion criteria in TG-MV-2012 were: nAMD, RVO, aphakia, MH greater than 400 μm in diameter, vitreous opacification or lenticular or zonular instability.[63, 264, 278] In OASIS, eyes with an ERM were also excluded from enrolment.[265]

Types of interventions

MIVI-IIT compared a single injection of ocriplasmin 75 μg, ocriplasmin 125 μg or ocriplasmin 175 μg with sham injection (conjunctiva touched with a blunt needle to simulate an injection) to establish the optimal dose.[62] A fourth cohort of participants underwent an initial injection of ocriplasmin 125 μg, but also a repeat injection at four and eight weeks if VMA was still present on OCT. Therefore, only data from participants receiving ocriplasmin 125 μg in this study were extracted and pooled for analysis. TG-MV-006 and TG-MV-007 both compared a single injection of ocriplasmin 125 μg with placebo injection (of the same vehicle used in the ocriplasmin injection).[63, 264, 278] OASIS compared a single injection of ocriplasmin 125 μg with sham injection (syringe hub pressed into conjunctiva to simulate an injection).[265]

Types of outcome measures

All four studies reported data for some of our primary and secondary outcome measures. No trial reported data for every outcome measure. Two trial reports (OASIS; Varma et al.) provided data on participant-reported outcome measures using the NEI-VFQ25.[264, 265]

Data synthesis, subgroup and sensitivity analyses

As the search identified four trials, we used a random-effects model. As there was no evidence of significant heterogeneity for the primary outcomes ($I^2 < 50\%$), we pooled data and performed no subgroup analyses of the primary outcome. Since no studies had a high risk of bias in any domain, we did not conduct a sensitivity analysis.

2.6.3 Excluded studies

We excluded nine articles after reviewing full-text copies (Table 2.2).[39, 60, 61, 292-297]

Study	**Reason for exclusion**
Benz 2010[60]	Indication for ocriplasmin was not symptomatic vitreomacular adhesion. It was investigating whether 125 μg microplasmin would induce vitreous release in people scheduled for PPV
De Smet 2009[61]	Investigated safety and efficacy of 4 different doses of intravitreal microplasmin prior to preplanned PPV. Subsequent PPV occurred either 1-2 hours, 24 hours or 7 days following ocriplasmin, meaning the participant population and outcome measures were not eligible for inclusion in our review
Dugel 2015[292]	Post hoc analysis of data from studies we already extracted data from (TG-MV-006 and TG-MV-007)
Elbendary 2011[293]	Autologous plasmin injected into participants with diabetic macular oedema associated with vitreomacular traction
Jackson 2017[39]	Incorrect study design; post hoc analysis
Lanzetta 2014[294]	Postmarket surveillance study, not an RCT, therefore not eligible for inclusion
Lanzetta 2014[295]	Post-hoc analysis of data, not an RCT, therefore excluded
Lescrauwaet 2016[296]	Not an RCT
Novack 2015[297]	Eligible participants for this study required exudative age-related macular degeneration, which did not meet inclusion criteria for our review

Table 2.2: Characteristics of excluded studies

PPV, pars plana vitrectomy; RCT, randomised controlled trial.

2.6.4 Risk of bias in included studies

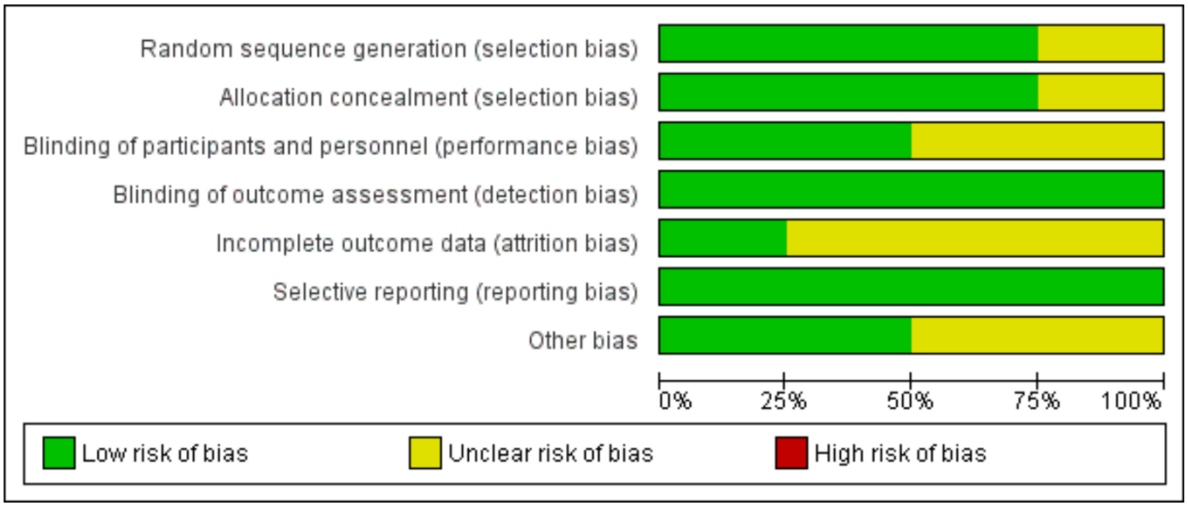


Figure 2.2: Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

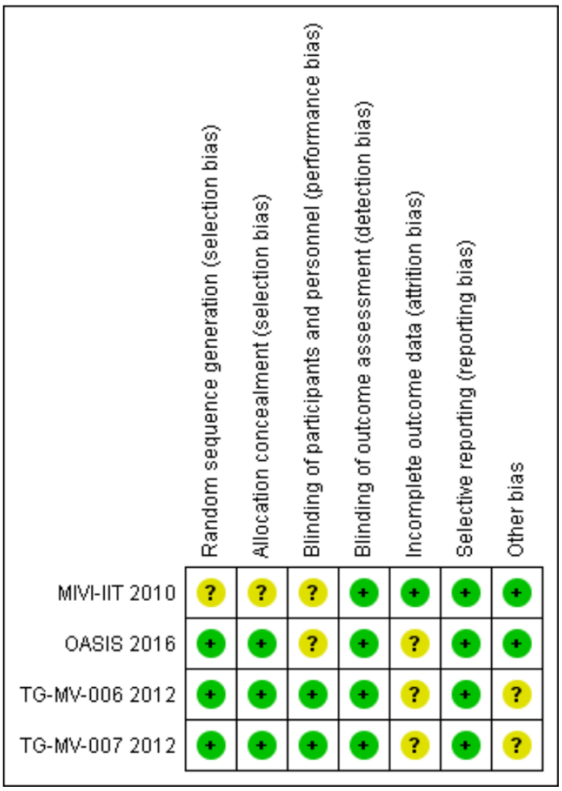


Figure 2.3: Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

Allocation

MIVI-IIT did not describe the method of sequence generation, and provided insufficient information to also assess allocation concealment.[62] TGI-MV-006 and TG-MV-007 clearly described randomisation and allocation concealment, which as a centralised telephone-based system with blocks of treatment assigned to sites.[63, 264, 278] OASIS clearly described the method of randomisation, which used a centralised interactive voice response system.[265]

Blinding

Two trials adequately masked participants and investigators (TG-MV-006; TG-MV-007).[63, 264, 278] However, two trials (MIVI-IIT; OASIS) did not mask investigators to sham injections, which may have induced a different sensation to a true injection.[62, 265] The risk of performance bias was graded as unclear for both studies.

Incomplete outcome data

We graded risk of bias as low in one study (MIVI-IIT), and unclear in the other three studies (OASIS; TG-MV-006; TG-MV-007).[62, 63, 264, 265, 278] Unclear risk was due to losses to follow-up not being reported and being unequal in different study groups. In addition, OASIS randomised 200 participants, but 50 participants were later found to be incorrectly enrolled by the central reading centre for a variety of reasons including MH greater than 400 μm, presence of ERM or no VMA at baseline.[265] A subgroup analysis of this smaller cohort of participants, who met the inclusion and exclusion criteria, was performed, but only on outcome data for VMA release.

One trial (MIVI-IIT) reported a dilution error, which resulted in an extra participant treated in the ocriplasmin 125 μg cohort and one less participant in the ocriplasmin 175 μg cohort.[62]

Selective reporting

All studies reported on all prespecified primary and secondary outcomes (MIVI-IIT; OASIS; TG-MV-006; TG-MV-007).[62, 63, 264, 265, 278]

Other potential sources of bias

Two studies (TG-MV-006; TG-MV-007) reported a baseline imbalance between study groups as pseudophakia was more common in the ocriplasmin group than in the placebo group and there were more women in the ocriplasmin group than in the placebo group.[63, 264, 278] Therefore, this was at unclear risk of bias.

2.6.5 Effects of interventions

See summary of findings for the main comparison (Table 2.1).

Proportion of eyes with complete release of vitreous adhesion

All four RCTs provided data for proportion of eyes with complete release of vitreous adhesion as determined by analysis of OCT images captured 28 days after ocriplasmin, sham or placebo treatment (MIVI-IIT; OASIS; TG-MV-006; TG-MV-007).[62, 63, 264, 265, 278] After excluding participants with protocol violations from OASIS, analysis of the pooled data showed higher complete release of vitreous adhesion in the ocriplasmin group compared with control (placebo or sham) treatment (RR 3.46, 95% CI 2.00 to 6.00; 859 eyes; 4 studies; high-certainty evidence; Figure 2.4). A total of 97/1000 eyes had VMA release within 28 days without treatment. An additional 237 eyes had VMA release within 28 days for every 1000 eyes treated with ocriplasmin (95% CI 96 more to 482 more).

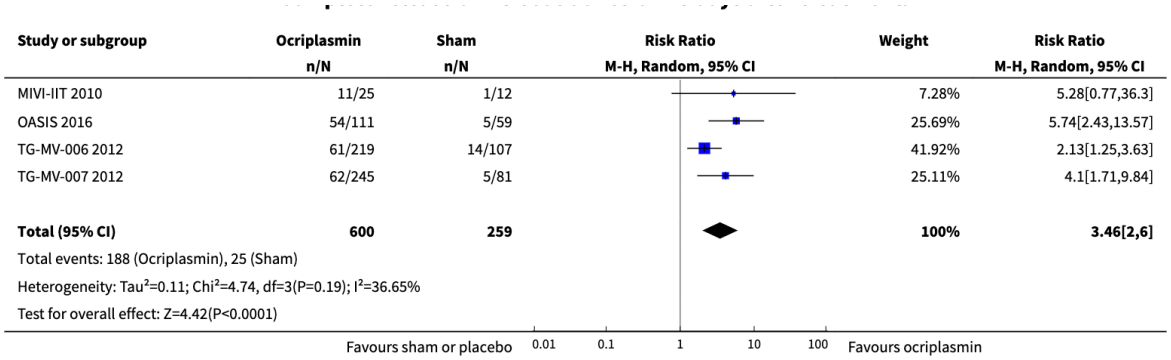


Figure 2.4: Ocriplasmin versus sham injection, Outcome 1 complete release of vitreous adhesion 28 days after treatment.

CI, confidence interval.

Proportion of eyes with closure of macular hole

Three studies (OASIS; TG-MV-006; TG-MV-007) provided data for proportion of eyes with closure of MH as determined by analysis of OCT images captured 28 days after ocriplasmin, sham or placebo treatment; data from MIVI-IIT could not be included in this analysis as the original paper did not provide a breakdown of the ocriplasmin doses used to treat MH.[62, 63, 264, 265, 278] OASIS measured MH closure at three months and the closure rate remained the same to the end of the study at 24 months. After excluding 14 participants incorrectly enrolled in OASIS due to MH being greater than 400 μm, analysis of the pooled data showed higher closure of MH in the ocriplasmin group compared with control (placebo or sham) treatment (RR 2.87, 95% CI 1.50 to 5.51; 229 eyes; 3 studies; high-certainty evidence; Figure 2.5). A total of 123/1000 eyes with MHs had closure with no treatment. An additional 231 eyes had MH closure for every 1000 eyes treated with ocriplasmin (95% CI 62 more to 556 more).

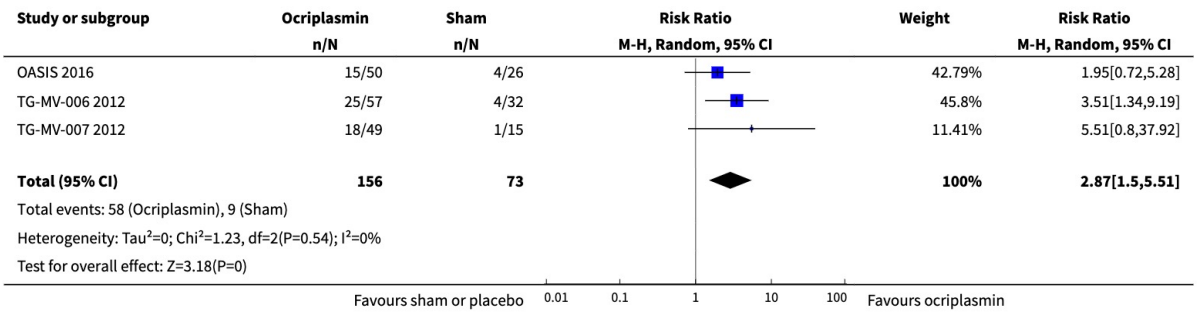


Figure 2.5: Ocriplasmin versus sham injection, Outcome 2 closure of macular hole 28 days after treatment.

CI, confidence interval.

Proportion of eyes with complete posterior vitreous detachment

Three studies (MIVI-IIT; TG-MV-006; TG-MV-007) provided data for proportion of eyes with complete PVD as measured by clinical examination or B-scan ultrasonography 28 days after ocriplasmin, sham or placebo treatment.[62, 63, 264, 278] Analysis revealed a higher incidence of complete PVD at 28 days in eyes treated with ocriplasmin compared with control (placebo or sham) treatment (RR 2.94, 95% CI 1.39 to 6.24; 689 eyes; 3 studies; high-certainty evidence; Figure 2.6). A total of 40/1000 eyes had complete PVD within 28 days without treatment. An additional 78 eyes had complete PVD within 28 days for every 1000 eyes treated with ocriplasmin (95% CI 16 more to 210 more).

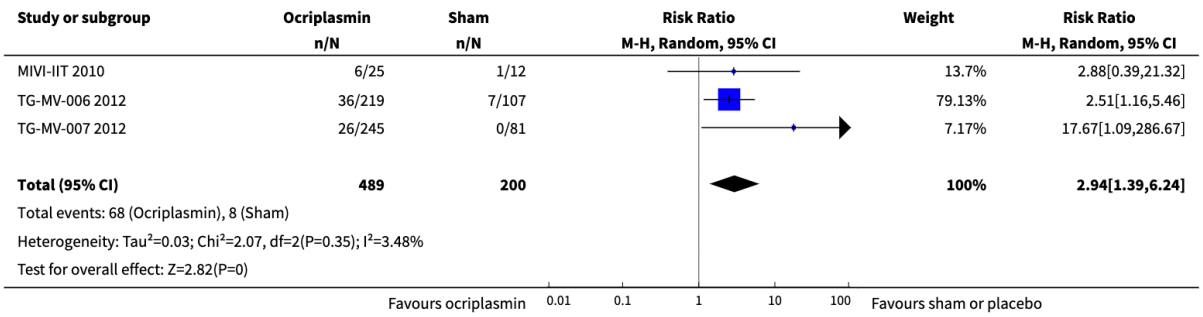


Figure 2.6: Ocriplasmin versus sham injection, Outcome 3 complete posterior vitreous detachment 28 days after treatment.

CI, confidence interval.

Proportion of eyes with 3-line or greater improvement in best corrected visual acuity

Three studies (MIVI-IIT; TG-MV-006; TG-MV-007) provided data for proportion of eyes with 3-line or greater improvement in BCVA measured using the ETDRS scale, at six months after ocriplasmin, sham or placebo treatment.[62, 63, 264, 278] Due to separate outcomes reported for eyes with and without full-thickness MH, and large numbers of participants not meeting eligibility criteria, data were not included from OASIS. Eyes that had undergone PPV in MIVI-IIT during this six-month period were also excluded.[62] Analysis of the pooled data revealed that eyes treated with ocriplasmin without PPV were more likely to achieve 3-line or greater improvement in BCVA than control (sham or placebo) eyes (RR 1.95, 95% CI 1.07 to 3.53; 674 eyes; 3 studies; moderate-certainty evidence; Figure 2.7). A total of 61/1000 eyes had 3-line or greater improvement in BCVA at six months without treatment. An additional 58 eyes had 3-line or greater improvement in BCVA at six months for every 1000 eyes treated with ocriplasmin (95% CI 9 more to 154 more).

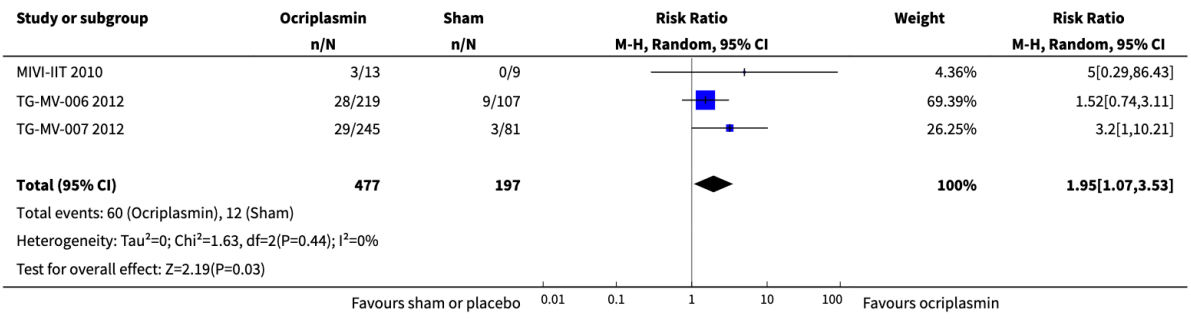


Figure 2.7: Ocriplasmin versus sham injection, Outcome 4 greater than 3-line improvement in best corrected visual acuity 6 months after treatment.

CI, confidence interval.

Proportion of eyes requiring vitrectomy within six months of ocriplasmin, sham or placebo treatment

Three studies provided data for proportion of eyes requiring vitrectomy (MIVI-IIT; TG-MV-006; TG-MV-007).[62, 63, 264, 265, 278] All three RCTs defined the requirement for vitrectomy as "recommended by the investigator if the underlying condition deteriorated, BCVA worsened by more than two lines on ETDRS or Snellen chart, or if the underlying condition had not improved within 28 days after treatment." Due to separate outcomes reported for eyes with and without full-thickness MH, and large numbers of participants not meeting eligibility criteria, data were not included from OASIS. Analysis revealed a lower requirement for vitrectomy in eyes treated with ocriplasmin compared with control (placebo or sham) treatment (RR 0.67, 95% CI 0.50 to 0.91; 689 eyes; 3 studies; moderate-certainty evidence; Figure 2.8). A total of 265/1000 eyes required vitrectomy at six months without treatment and 87 fewer eyes required vitrectomy for every 1000 eyes treated with ocriplasmin (95% CI 24 fewer to 132 fewer).

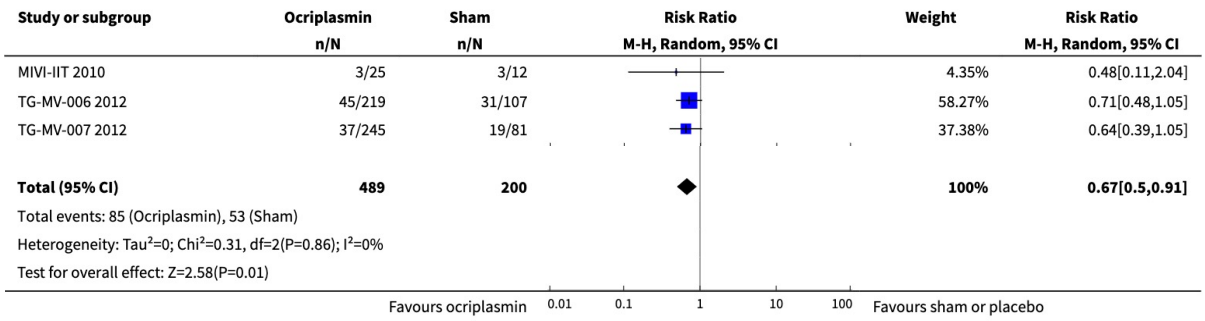


Figure 2.8: Ocriplasmin versus sham injection, Outcome 5 requirement for pars plana vitrectomy at month 6.

CI, confidence interval.

Mean change in validated Visual Function Questionnaire score from baseline measured at six months after ocriplasmin, sham or placebo treatment

One trial (Varma et al.) reported data for mean change in validated VFQ score from baseline, which analysed pooled participant-reported visual function outcomes for TG-MV-006 and TG-MV-007.[63, 264, 278] In all eyes across both studies, mean increases in the composite NEI-VFQ25 score at six months from baseline were greater in eyes treated with ocriplasmin (464 eyes) than placebo (188 eyes) (mean change: 3.4 with ocriplasmin versus 0.7 with placebo; P = 0.005). We calculated the mean difference as 2.7 (95% CI 0.8 to 4.6). Visual function data was also reported in OASIS, but this was not reported for the subgroup who met the inclusion and exclusion criteria following central reading centre analysis.

Adverse effects

Due to inconsistencies between the studies and differences in control groups (placebo injection versus sham injection), we did not perform a pooled analysis of adverse events. Instead, a descriptive account of the types of ocular adverse event is provided below, based on data from three studies (OASIS; TG-MV-006; TG-MV-007).[63, 264, 265, 278] Although a large number of participants were incorrectly enrolled in OASIS, safety data are presented for all participants who underwent intervention with ocriplasmin or control treatment.

Any ocular adverse events

These were defined as any ocular adverse event that did not meet the criteria for a serious ocular adverse event. All four RCTs provided data for any ocular adverse event (MIVI-IIT; OASIS; TG-MV-006; TG-MV-007).[62, 63, 264, 265, 278] Analysis revealed more ocular adverse events in eyes treated with ocriplasmin compared with placebo or sham-treated eyes (RR 1.22, 95% CI 1.09 to 1.37; 909 eyes; 4 studies; moderate-certainty evidence; Figure 2.9).

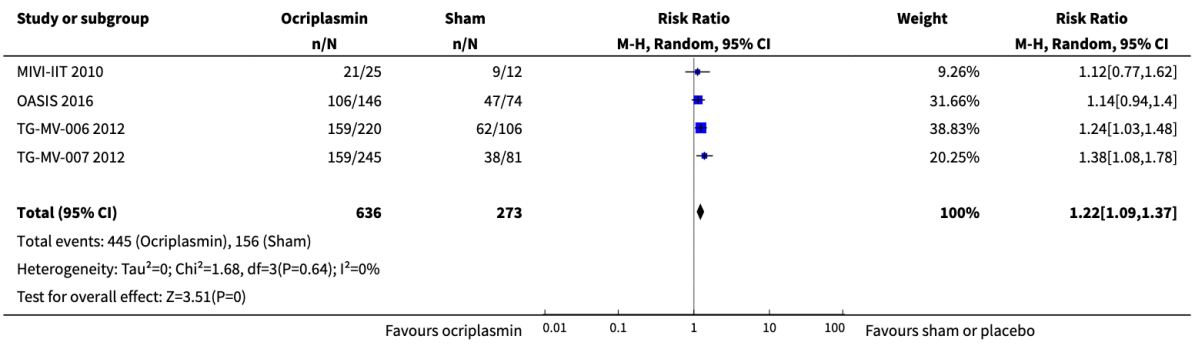


Figure 2.9: Ocriplasmin versus sham injection, Outcome 6 any ocular adverse event

CI, confidence interval.

A breakdown of the most frequently reported ocular adverse events is listed in the table below (n = number of eyes affected, not total number of events). The first five ocular adverse events were participant-reported. The most commonly reported ocular adverse events following ocriplasmin treatment were vitreous floaters (affecting 133/611 eyes or 21.8%), photopsia (affecting 98/611 eyes or 16.0%) and injection-related eye pain (affecting 83/611 eyes or 13.6%). The incidence of vitreous floaters, photopsia, injection-related eye pain, blurred vision and visual impairment was significantly greater in eyes treated with ocriplasmin than those treated with sham or placebo injection.

Study	TG MV-006 2012		MIVI-IIT 2010		TG-MV-007 2012		OASIS 2016	
	Ocriplasmin (n = 25)	Control (n = 12)	Ocriplasmin (n = 220)	Control (n = 106)	Ocriplasmin (n = 245)	Control (n = 81)	Ocriplasmin (n = 246)	Control (n = 74)
Any ocular adverse event	21	9	159	62	159	38	106	47
Vitreous floaters[a]	-	-	42	9	36	5	55	6
Photopsia[a]	-	-	36	4	19	1	43	5
Injection-related eye pain[a]	-	-	33	6	30	5	20	6
Blurred vision[a]	-	-	24	4	16	2	27	4
Visual impairment[a]	-	-	21	3	4	0	21	4
Conjunctival haemorrhage	8	3	34	14	34	10	14	1
Increased intraocular pressure[a]	-	-	9	10	9	0	10	10
Retinal tear[a]	-	-	5	2	1	3	2	5
Cataract[a]	-	-	14	12	12	5	19	10
Anterior chamber cells[b]	1	0	-	-	-	-	-	-
Iridicyclitis[b]	1	0	-	-	-	-	-	-
Vitritis[b]	3	0	-	-	-	-	-	-

Table 2.3: Ocular adverse events

[a] *Ocular adverse events not reported in MIVI-IIT*

[b] *Ocular adverse events not reported in OASIS 2016, TG-MV-006 2012 or TG-MV-007 2012.*

Note: the control group in MIVI-IIT 2010 and OASIS 2016 was sham injection. The control group in TG-MV-007 2012 and TG-MV-006 2012 was placebo injection.

Any serious ocular adverse events

Two studies (TG-MV-006; TG-MV-007) defined serious ocular adverse event as: an event resulting in persistent or clinically significant disability, incapacity or both; an event requiring inpatient hospitalisation or prolongation of an existing hospital stay; or an event that was considered to be medically important.[63, 264, 278] One study (OASIS) did not provide a

definition of a serious ocular adverse event.[265] MIVI-IIT reported no instances of serious ocular adverse events.[62]

A breakdown of the most frequently reported serious ocular adverse events is listed in the table below (n = number of eyes affected, not total number of events). The total incidence of serious ocular adverse events was 66/611 (10.8%) in eyes treated with ocriplasmin compared with 35/261 (13.4%) treated with sham or placebo injection. Most frequently reported was an increased or new macular hole, which occurred in 47/611 (7.7%) of eyes treated with ocriplasmin compared with 26/261 (9.9%) of eyes treated with sham or placebo injection. None of the included studies reported any cases of endophthalmitis.

Study	**TG MV-006 2012**		**MIVI-IIT 2010**		**TG-MV-007 2012**		**OASIS 2016**	
	Ocriplasmin (n = 25)	Control (n = 12)	Ocriplasmin (n = 220)	Control (n = 106)	Ocriplasmin (n = 245)	Control (n = 81)	Ocriplasmin (n = 246)	Control (n = 74)
Any serious ocular adverse event	0	0	21	11	15	9	30	15
Macular hole (increased or new)	-	-	15	11	9	5	23	10
Retinal Detachment	-	-	2	2	0	1	1	1
Reduced visual acuity	-	-	1	0	2	1	18	18
Endophthalmitis	0	0	0	0	0	0	0	0

Table 2.4: Serious ocular adverse events

Note: the control group in MIVI-IIT 2010 and OASIS 2016 was sham injection. The control group in TG-MV-007 2012 and TG-MV-006 2012 was placebo injection.

2.7 **Discussion**

Summary of main results

We identified four RCTs, with 932 eyes, comparing ocriplasmin with control (placebo or sham injection) treatment. On full-text analysis, we excluded 50 participants due to breaches of our inclusion criteria, and 23 participants because they received a different dose of ocriplasmin, giving 859 eyes for outcome analysis. The studies were conducted in Europe and the USA. We found that treatment with ocriplasmin increased the likelihood of complete release of vitreous traction compared to control (sham or placebo injection) treatment. Ocriplasmin was also associated with a 3-line or greater improvement in BCVA and improvement in participant-reported visual function.

There were however, more ocular adverse events in eyes treated with ocriplasmin than control (placebo or sham injection) treatment. Many of these adverse events, particularly vitreous floaters and photopsia, are known to be associated with PVD. Of the serious ocular adverse events, increased or new MH was the most frequently reported. Given the high incidence in all eyes regardless of treatment, this most likely represents the natural history of VMT in a significant proportion of patients.

Overall completeness and applicability of evidence

Three of the included studies (OASIS; TG-MV-006; TG-MV-007) were large and contributed the majority of included participants (834) for our analysis.[63, 264, 265, 278] The other study (MIVI-IIT), designed to determine the appropriate dose, contributed a relatively small number (25) of participants.[62] The control groups in the trials also varied, with participants in TG-MV-006 and TG-MV-007 receiving a placebo injection, and participants in MIVI-IIT

and OASIS receiving a sham injection. Due to the mechanical nature of the primary outcome, the variation in control group intervention could impact on the validity of the results, particularly adverse events. All four trials reported the same primary outcome and follow-up periods were identical. One trial (OASIS) reported additional secondary outcome data at 24-months.

It is important to note that OASIS initially randomised and treated 220 participants, but subsequent central reading centre analysis revealed 50 participants were ineligible due to lack of sVMA, presence of ERM or presence of MH greater than 400 µm. To comply with the inclusion and exclusion criteria of this review, we used only data from this smaller, central reading centre verified cohort of participants. Despite this attrition bias, sufficient pooled data were available, hence the impact of this bias was deemed small.

Quality of the evidence

Generally, we graded the risk of bias as low. However, two studies (TG-MV-006; TG-MV-007) reported cases that did not complete the study on the ClinicalTrials.gov database (see Characteristics of included studies table) but the publications did not describe these losses to follow-up. The authors confirmed using the last-observation-carried-forward (LOCF) method for their missing outcome data, assuming the outcome was unlikely to change after discontinuation of treatment and likely to improve spontaneously over time. As these losses to follow-up were not described in the original papers, we judged the risk of bias for incomplete outcome data as unclear.

Potential biases in the review process

We followed a standard Cochrane protocol, to minimise potential methodological biases in the review process.

Agreements and disagreements with other studies or reviews

In the UK, NICE recommends the use of ocriplasmin for adults with VMT causing severe sight problems or a MH up to 400 μm, in the absence of ERM. Our findings support this.

Subsequent publications and postmarket surveillance studies have addressed the safety of ocriplasmin. One large postmarket surveillance study found lower rates of adverse events than were reported in the registration studies, but noted that under-reporting is common in post-market surveillance studies.[298] Members of the British and Eire Association of VitreoRetinal Surgeons (BEAVRS) have reported their experience with ocriplasmin in comparison to the MIVI-TRUST trial data.[299] They found a lower rate of MH closure and increased incidence of adverse events with ocriplasmin compared to the registration studies, but there is an uncertain risk of reporting bias.

Our review found a higher rate of vitreous floaters and photopsia with ocriplasmin, but no increased risk of loss in visual acuity and retinal detachment. There have been reports of acute reduction in visual acuity, electroretinography changes, dyschromatopsia, phacodonesis and OCT ellipsoid zone alteration, but the majority have been transient.[267, 300]

Various studies and reviews have suggested certain subgroups of sVMA participants may be more likely to respond successfully to ocriplasmin treatment based on baseline characteristics such as adhesion diameter, lack of coexisting ERM, and the angle between the posterior

vitreous cortex and the ILM.[278, 279, 301] However, such analyses are exploratory, and without confirmatory prospective RCTs they are beyond the scope of this review.

There are different approaches to potentially manage sVMA including PPV, intravitreal gas injection, ocriplasmin and observation. Further research, ideally in a head-to-head trial, would be beneficial.

2.8 Authors' conclusions

Implications for practice

We found evidence to support the use of ocriplasmin for the treatment of sVMA, although the number of studies was low. There are reported concerns about the safety of ocriplasmin treatment and there is debate within the vitreoretinal community regarding the advantages and disadvantages of ocriplasmin.

Implications for research

Further large RCTs would augment our current understanding of the safety and efficacy of ocriplasmin. Ideally these would compare ocriplasmin with other commonly used management options, in particular observation or PPV. Randomised controlled trials recruiting participants with baseline characteristics thought to improve the efficacy of ocriplasmin are warranted.

Acknowledgements

We acknowledge Cochrane Eyes and Vision for creating and executing the electronic search strategies. We thank David Steel and Jennifer Evans for their comments on the protocol and review, Catey Bunce for her comments on the review and Ana Quartilho for her comments throughout the editorial process.

3 Effect of ocriplasmin on colour hue vision

In this chapter, patients receiving an intravitreal injection of ocriplasmin were assessed to determine whether there was any change in their colour vision. Work from this chapter was used for the following publication, although due to the publisher's copyright the pdf cannot be reproduced in this thesis.

Citation

Neffendorf JE, Kirthi V, Soare C, Jackson TL. The effect of intravitreal ocriplasmin on hue discrimination. Optom Vis Sci. 2021 Dec 1;98(12):1394-99. DOI: 10.1097/OPX.0000000000001811

3.1 Introduction

The previous chapter was a meta-analysis of ocriplasmin, combining evidence from four randomised controlled trials.[302] This showed an improved likelihood of vitreomacular adhesion (VMA) release within 28 days with ocriplasmin, compared to sham or placebo treatment (risk ratio [RR] 3.46, 95% confidence interval (CI) 2.00 to 6.00). Furthermore, treatment with ocriplasmin was found to be more likely to result in macular hole (MH) closure (RR 2.87, 95% CI 1.50 to 5.51), and more likely to result in complete posterior vitreous detachment (PVD) (RR 2.94, 95% CI 1.39 to 6.24). As well as visual improvement, ocriplasmin treatment was shown to result in better visual function, when measured with validated questionnaires.[39, 302, 303]

There have been multiple reports of safety issues with ocriplasmin, often attributed to its non-selective targeting of fibronectin and laminin.[299] Reassuringly, most adverse events have tended to be transient. In terms of visual function, there have been reports of reduced contrast sensitivity, dyschromatopsia and sudden severe sight loss which have usually been reversible.[300, 304] Acute neuroretinopathy has been reported, as well as electroretinogram abnormalities and ellipsoid zone changes on optical coherence tomography (OCT).[305] Most of these occured in patients in whom ocriplasmin had successfully released VMA.[306-308] There have also been reports of zonular instability during subsequent cataract surgery.[299]

Patients who have described dyschromatopsia following ocriplasmin tend to find it is transient and self-limiting.[267, 309] Data from the MIVI-TRUST trials and post-marketing surveillance studies estimated the risk of dyschromatopsia at 0.5-9.1%, although these are potentially at risk of recall bias and underreporting.[63, 267, 298, 310] In general, the vision was

subjectively described as being 'yellowish'.[298] A retrospective case series of 19 patients described subjective complaints of colour abnormalities or brightness reduction in 36.8%, all of whom had increased sub-retinal (SRF) fluid and ellipsoid zone attenuation on OCT, which tended to settle at 3 months post-injection.[268] The dyschromatopsia has been reported to occur rapidly after ocriplasmin, within 4 hours.[269]

The Investigation of JETREA in Patients with Confirmed Vitreomacular Traction (INJECT) study reported a worsening of subjective colour vision abnormalities from 6.6% at baseline to 11.9% at one month, before improving to 5% at one year.[305] Members of the Macula Society were surveyed in 2017 on their experiences with ocriplasmin, and reported a 10% rate of subjectively decreased colour vision.[311]

Dyschromatopsia is a highly subjective symptom, and objective measurement of colour vision is largely lacking from the literature. Given the concerns about colour vision abnormalities we incorporated colour hue discrimination testing as part of the routine care pathway for patients receiving intravitreal ocriplasmin at King's College Hospital, London.

This chapter explores the effect of ocriplasmin on colour vision.

3.1.1 Colour vision

Colour vision is a highly complex neurological process that relies on various mechanisms such as healthy photoreceptor function, retinal transmission and cortical processing. Those with a congenital defect of colour vision describe a static isolated defect which is usually bilateral and often a result of X-chromosome sequence errors.[312] Patients with congenital

defects are often asymptomatic. Acquired colour vision deficiencies are secondary to ocular or visual pathway disease (which may themselves be hereditary). In contrast to a congenital defect, acquired colour vision deficiency is often symptomatic, may often deteriorate or improve with time, and be asymmetric or unilateral.[313]

Cone and rod photoreceptors are responsible for mediating vision over a range of illumination levels. The following terms are used to describe different illumination levels: scotopic, where only rods are sensitive; mesopic, mixed rod and cone sensitivity; photoptic, only cones are sensitive. Cones are mediators of vision in daylight light levels, and provide colour perception and good visual acuity. They are most highly concentrated at the fovea, meaning tests of their function are conducted with foveal targets. Rods are responsible for mediating vision at low illumination levels, and therefore are important for night vision. The retinal midperiphery is the area where the greatest sensitivity to light occurs, and is the target for rod function testing.

When the eye is in a dark-adapted state, the peak luminosity occurs at 500 nm, which is in the blue-green range (Figure 3.1). A rightward shift of the spectral sensitivity curve, termed the Purkinje shift, occurs in photopic conditions with a peak luminosity of 555 nm. The difference between the two curves, akin to the difference in brightness of a light being perceived as a colour rather than a light, is known as the photochromatic interval.

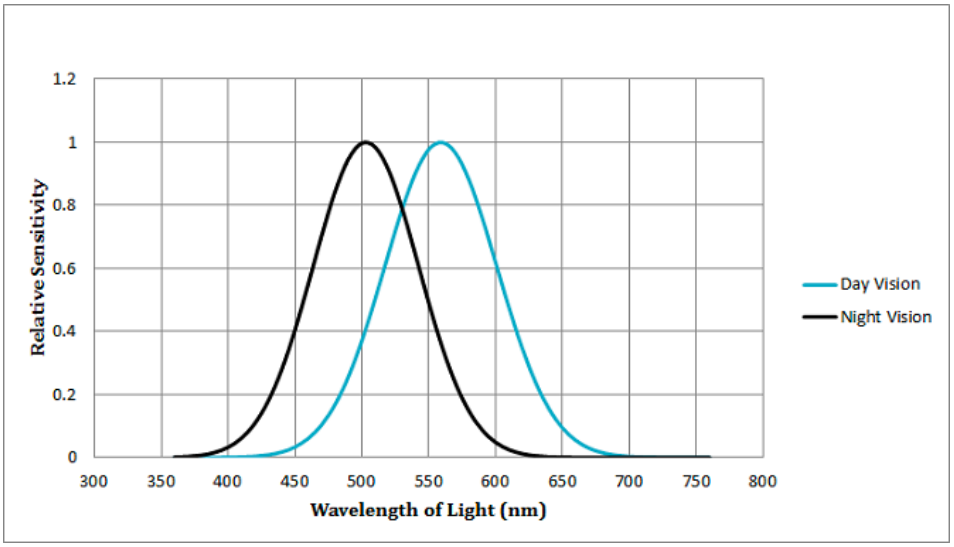


Figure 3.1: Spectral sensitivity curves for day and night vision.[314]

There are three types of cone photoreceptors; short (S-), medium (M-) and long (L-) wavelength-sensitive which have maximal sensitivities of 419 nm, 531 nm and 558 nm, respectively. Short-wavelength sensitive cones make up 7-10% of the normal cone population, whereas the M- and L- cones have a highly variable ratio, ranging from 1.1:1 to 16.5:1 respectively.[315] The three cone types have overlapping spectral wavelength sensitivities which allow their combined contribution to perceive approximately 8,000 colours and hues, in a process called trichromacy (Figure 3.2). This allows humans to distinguish approximately 8,000,000 shades and tints. Table 3.1 summarises the affected cones and potential aetiology of congenital and acquired colour vision deficiencies.

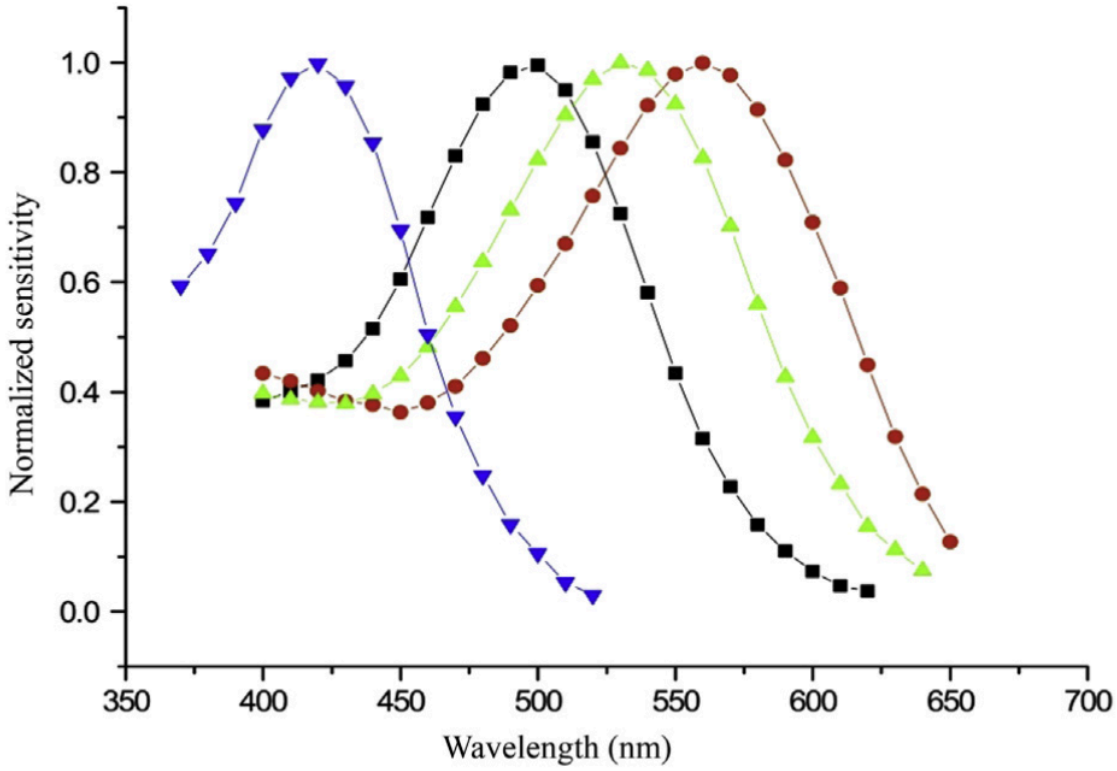


Figure 3.2: The spectal sensitivities of the 3 classes of cone photoreceptors (S-cones, blue inverted triangles; M-cones, green triangles; L-cones, red circles) and of the rods (black squares) plotted against wavelength in nm.[313]

	Deficiency and severity	Cone affected or FM-100 hue axis	Aetiology and example conditions
Congenital	Anomalous Trichromacy		
	Protanomaly	L – cones	XLR
	Deuteranomaly	M -cones	XLR
	Tritanomaly	S - cones	AD
	Dichromacy		
	Protanopia	L – cones	XLR
	Deuteranopia	M – cones	XLR
	Tritanopia	S - cones	AD
	Monochromacy		
	M – cone	L – and S – cones	Combined XLR and AD
	L – cone	M – and S – cones	Combined XLR and AD
	S – cone	M – and L – cones	XLR
	Rod monochromacy	S - , M - , and L - cones	AR
Acquired	No defined axis		
	Trichromatic	Mild red-green and tritan	Macular cysts, toxic amblyopia
	Monochromatic	No colour discrimination	End-stage of type I - III acquired colour vision defects
	Type I red-green		
	Trichromatic	Mostly between protan/deutan	Choroidal atrophy
	Dichromatic	As above, then between deutan and tritan	Stargardt's disease
	Type II red-green		
	Trichromatic	Mostly between protan/deutan	Usher's, optic nerve disease, chiasmal disorders, choroidal degenerations, RRD, CSR
	Dichromatic	Mostly between protan/deutan	Usher's, optic nerve disease, chiasmal disorders, choroidal degenerations, RRD, CSR
	Type III tritan		
	Trichromatic	Tritan	Vascular retinopathies, papilloedema, glaucoma, dominant optic atrophy
	Dichromatic	Tritan	Vascular retinopathies, papilloedema, glaucoma, dominant optic atrophy

Table 3.1: Summary of congenital and acquired colour vision deficiency, adapted from Simunovic.[313]. The acquired causes are classified as per the Verriest classification.[316]

AD, autosomal dominant; AR, autosomal recessive; CSR, central serous retinopathy; FM-100, Farnsworth Munsell-100; L – long; M – medium; RRD, rhegmatogenous retinal detachment; S – short; XLR, X-linked recessive

Once cone signals are generated, they synapse with bipolar cells and are modulated by retinal ganglion cells.[317] Higher order processing takes place in layers 3-6 of the lateral geniculate nucleus, before arriving for final processing in the occipital visual cortex.[317, 318]

3.1.2 The measurement of colour vision

The testing of colour vision is aimed at identifying errors in chromatic discrimination and colour matching. Colour vision tests can be divided into tests of discrimination and matching.

Tests of discrimination fall into two categories; pseudoisochromatic plate tests and ordering tests. Pseudoisochromatic plate tests consist of a figure composed of coloured dots on a background of differently coloured dots, and are commonly used as screening tests in the general ophthalmology clinic. Examples are the Ishihara, Cambridge Colour, Hardy-Rand-Rittler and Berson tests.[319-321] These are designed to primarily identify those with congenital colour defects, but are less effective at identifying and quantifying acquired colour vision defects, particularly in the early stages of disease.[8]

Ordering assesses colour discrimination using tests where the patient is required to arrange individual coloured samples. The Farnsworth-Munsell 100 (FM-100) hue test assesses fine chromatic discrimination with 84 removable coloured caps, of different hues, divided across 4 rows (Figure 3.3).[322] Each row covers one quarter of the colour circle and has a consistent lightness and saturation. It is quite time consuming and is a test of fine chromatic discrimination. The Farnsworth panel D-15, where the colours differ more widely, can be used as a faster screening assessment. Other examples are the Lanthony Desaturated D-15, Sahlgren's Saturation Test and the Universal Colour Discrimination test.[323-325]

Figure 3.3: Farnsworth-Munsell 100 hue test. The Farnsworth-Munsell 100 test consists of 4 rows of 21 removable coloured tiles, each of different hue. The end tile of each row is a fixed reference point. The tiles are scrambled by the assessor and then presented to the patient to arrange correctly (as shown here).

To perform the FM-100 test, the caps are pre-scrambled by the assessor, and the patient is instructed to rearrange them in an orderly progression of colour. Once the patient is content with their arrangement, the results are analysed by a computer program developed by the manufacturer. This generates a total error score (TES), with a higher TES signifying worse colour discrimination.[326] The normal range of competence for colour discrimination is between 16 and 100; a score less than 16 indicates superior colour discrimination, whilst a score greater than 100 indicates poor colour discrimination. An error pattern map is created to identify the specific nature of the defect (Figure 3.4).

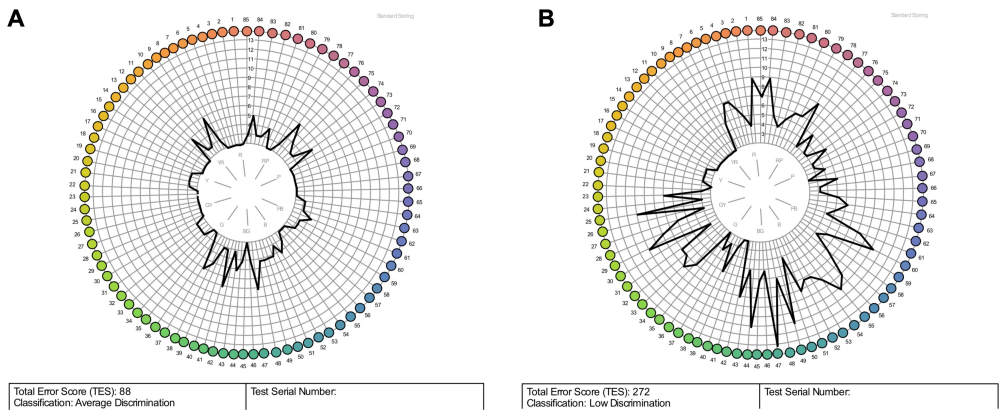


Figure 3.4: Examples of Farnsworth-Munsell 100 test results. A (average discrimination, total error score =88) and B (low discrimination, total error score = 272) show the format of results from the Munsell Colour Services Laboratory computer program by X-Rite Inc (Kentwood, MI, USA). The black line represents the discrimination ability, and its distance from the centre identifies the specific nature (towards the corresponding colours) and degree of the defect present. An error score and classification of discrimination is documented.

The FM-100 test has the ability to detect colour defects at early stages, identify defect type and quantify them in order to assess whether they fall within the normal range. Sequential FM-100 testing can be useful to determine whether a defect is progressing, static or resolving. It is considered to be the test of choice for acquired colour vision deficiency.[313]

Tests of matching are less commonly utilised in clinical practice. They use anomaloscopes to assess how the subject can combine primary colours to match assess colour. The tests involve adjusting two colour lights (e.g. red and green) in order to match to a different colour (e.g. yellow). They are difficult tests to perform and require extensive training. The Rayleigh match test determines the range of red or green primary mixtures that can be matched in colour to the yellow primary, and is useful in the assessment of acquired deficiencies (Figure

3.5).[313] Another example is the Moreland equation which involves matching a mixture of indigo and green to a cyan primary colour.[327]

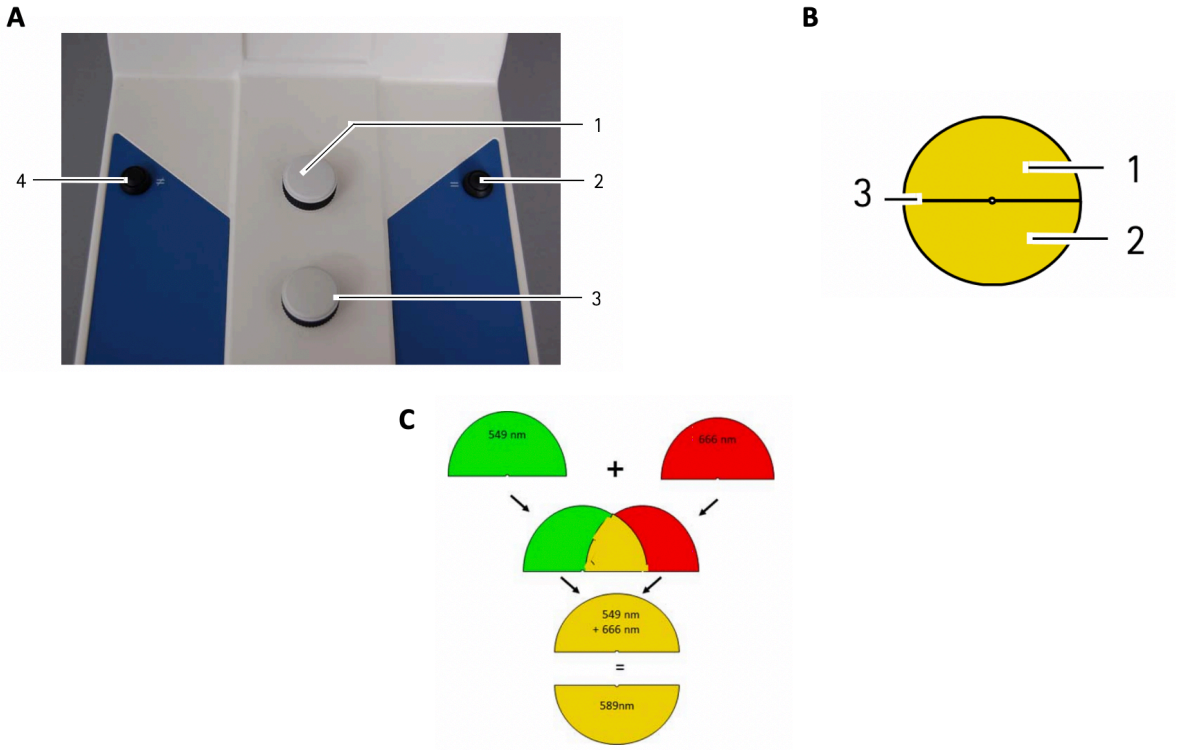


Figure 3.5: An example of colour matching in the Rayleigh test. A: Subject control pad; 1 – "Mixed light" control which adjusts the top test field (Field 1 in B), 2 – "Match button" which is pressed to indicate a match, 3 – "Reference light" control which adjusts the brightness of the reference test field (Field 2 in B), 4 – "No match button" which is pressed if no match can be made. B: The anomaloscope view for the subject; 1 – variable test field, 2 – reference test field, 3 – dividing line. C: The principle of the test showing how a combination of the two primary colours is made to attempt a match with the reference field.[328]

3.2 Methods

This retrospective consecutive case series included all patients who elected to receive an intravitreal injection of 125 µg ocriplasmin for the treatment of symptomatic VMA (sVMA) at King's College Hospital, London between July 2014 and July 2015. The assessment of colour hue discrimination was performed to objectively measure and detect any change in colour vision as part of routine clinical care and therefore did not require ethical review. This was confirmed by the local research and development team. The work adhered to the tenets of the Declaration of Helsinki.

Patients were identified from the hospital electronic medical records and all additional patient paper records were available for review when requested. All patients had a diagnosis of sVMA made by clinical examination and spectral domain OCT (Spectralis, Heidelberg Engineering, Heidelberg, Germany). Patient eligibility was in accordance with technology appraisal TA297 issued by the UK's National Institute of Health and Care Excellence (NICE). This states that ocriplasmin is suitable for patients with vitreomacular traction (VMT) causing either severe sight problems or a MH up to 400 µm, in the absence of epiretinal membrane.[65]

Ocriplasmin treatment was given in accordance with its marketing authorisation via a pars plana injection at 3.5 to 4.0 mm from the limbus, using a standard 30-gauge needle aimed towards the optic nerve and inserted up to the hub.[64] Clinical examination and all tests were performed on both the injected eye and the fellow uninjected eye at baseline, one week, one month and one year after ocriplasmin injection. Each visit included assessment of best-corrected visual acuity (BCVA) using a standard Early Treatment Diabetic Retinopathy

(ETDRS) visual acuity chart at 4 m, colour vision testing, full ocular examination including dilated fundoscopy, and OCT.

Colour vision was assessed using the FM-100 test according to the manufacturers' instructions, as described earlier, under standard overhead fluorescent lighting conditions.[329] FM-100 testing was conducted under normal ambient room lighting conditions. Patients used their regular method of refractive correction for near tasks (e.g. spectacles). The right eye was always tested before the left eye, and there were no time restrictions. The results were calculated using the Munsell Color Services Laboratory computer programme (X-Rite Inc. Kenwood, MI, USA).

Whilst the primary outcome measure was FM-100 TES, secondary outcomes included BCVA and anatomic success, defined as complete release of VMA on OCT, in the absence of MH. We also related any changes in FM-100 TES to OCT features, to look for any association.

This study was not designed to formally test a hypothesis, but after testing for normality of data, non-parametric Wilcoxon matched-pairs signed ranks tests were used to compare FM-100 and BCVA scores at various time points in both the treated eye and fellow eye, without multiplicity adjustments. Two-tailed p-values <0.05 were considered statistically significant.

3.3 Results

Thirteen patients received an intravitreal injection of 125 µg ocriplasmin, with a mean age of 74.8 years (range: 48 to 89 years). Five injections were performed in the right eye and eight in the left eye. There were no intraoperative complications. None had anatomic success at one week, but two patients (2/13, 15.4%) had anatomic success at one month. Subsequently, five out of 11 patients chose to undergo pars plana vitrectomy (PPV) for surgical release of VMT, after ocriplasmin had been unsuccessful.

The mean BCVA in the entire cohort of injected eyes reduced from 60.8 letters (range: 19 to 76; Snellen equivalent 20/60) at baseline to 57.4 letters (range: 16 to 86, p=0.11; Snellen equivalent 20/70) at one week and to 56.3 letters (range: 11 to 86, p=0.24; Snellen equivalent 20/70) at one month. However, mean BCVA improved to 63.8 letters (range: 14 to 86, p=0.32; Snellen equivalent 20/50) at one year. In fellow eyes, mean BCVA was 67.5 letters at baseline (range: 31 to 86; Snellen equivalent 20/50), 71.4 letters at one week (range: 36 to 90, p=0.17; Snellen equivalent 20/40), 64.2 letters at one month (range: 33 to 78, p=0.27; Snellen equivalent 20/50) and 66.2 letters at one year (range: 39 to 86, p=0.95; Snellen equivalent 20/50).

Table 3.2 shows the results of FM-100 testing on both the injected and fellow eyes (Table 3.2). Two patients did not undergo FM-100 testing at one week or one month, whilst one year data were only obtained on five patients due to follow-up losses. The mean TES at baseline was similar in the injected eye and fellow eye (331.4 vs 336.8). In injected eyes, mean TES worsened from 331.4 (range: 128 to 656) to 371.6 (range: 156 to 740) at one week following ocriplasmin, but this did not reach significance (p=0.29). The mean TES at one month

reduced further, to 397.1 (range: 104 to 1124) before recovering to 349.6 at one year (range: 100 to 692) but neither result was statistically significant (p=0.40 and *p*=0.19, respectively versus baseline) (Figure 3.6). In fellow eyes, the mean TES was similar comparing baseline (336.8) to one week and one month (327.6 and 325.1; ranges: 64 – 840, and 104 – 844; *p*=0.29 and *p*=0.38, respectively). The TES at one year in the fellow eye was slightly worse at 343.2 (range: 92 to 656; p=0.81) (Figure 3.7).

Case	**Diagnosis**	**Anatomical success with ocriplasmin**	**Vitrectomy (timing, months)**	**Injected eye**				**Fellow eye**			
				B/L	**W1**	**M1**	**Y1**	**B/L**	**W1**	**M1**	**Y1**
1	VMT	Yes	No	208	-	-	100	244	-	-	92
2	VMT	Yes	No	228	164	300	-	200	196	264	-
3	VMT	No	No	128	192	104	-	172	152	108	-
4	VMT	No	No	296	240	292	268	274	264	260	224
5	VMT	No	No	656	740	1124	-	872	840	844	-
6	VMT	No	No	596	-	-	424	320	-	-	656
7	MH	No	Yes (1.5)	284	336	272	-	148	64	104	-
8	VMT	No	Yes (6)	464	348	356	264	400	276	272	196
9	VMT	No	No	176	512	432	-	220	380	268	-
10	VMT	No	Yes (3)	272	428	324	-	616	516	504	-
11	MH	No	Yes (2)	192	376	404	-	160	180	140	-
12	MH	No	Yes (2)	168	156	164	-	252	156	168	-
13	VMT	No	No	640	596	596	692	500	580	644	548
Mean	-	-	-	331.4	371.6	397.1	349.6	336.8	327.6	325.1	343.2

Table 3.2: Farnsworth-Munsell 100 total error score following intravitreal ocriplasmin injection in injected and fellow eyes

B/L, baseline; FM-100, Farnsworth-Munsell 100; M1, month 1; MH, macular hole; VMT,

vitreomacular traction; W1, one week; Y1, one year. Missing data denoted with hyphens.

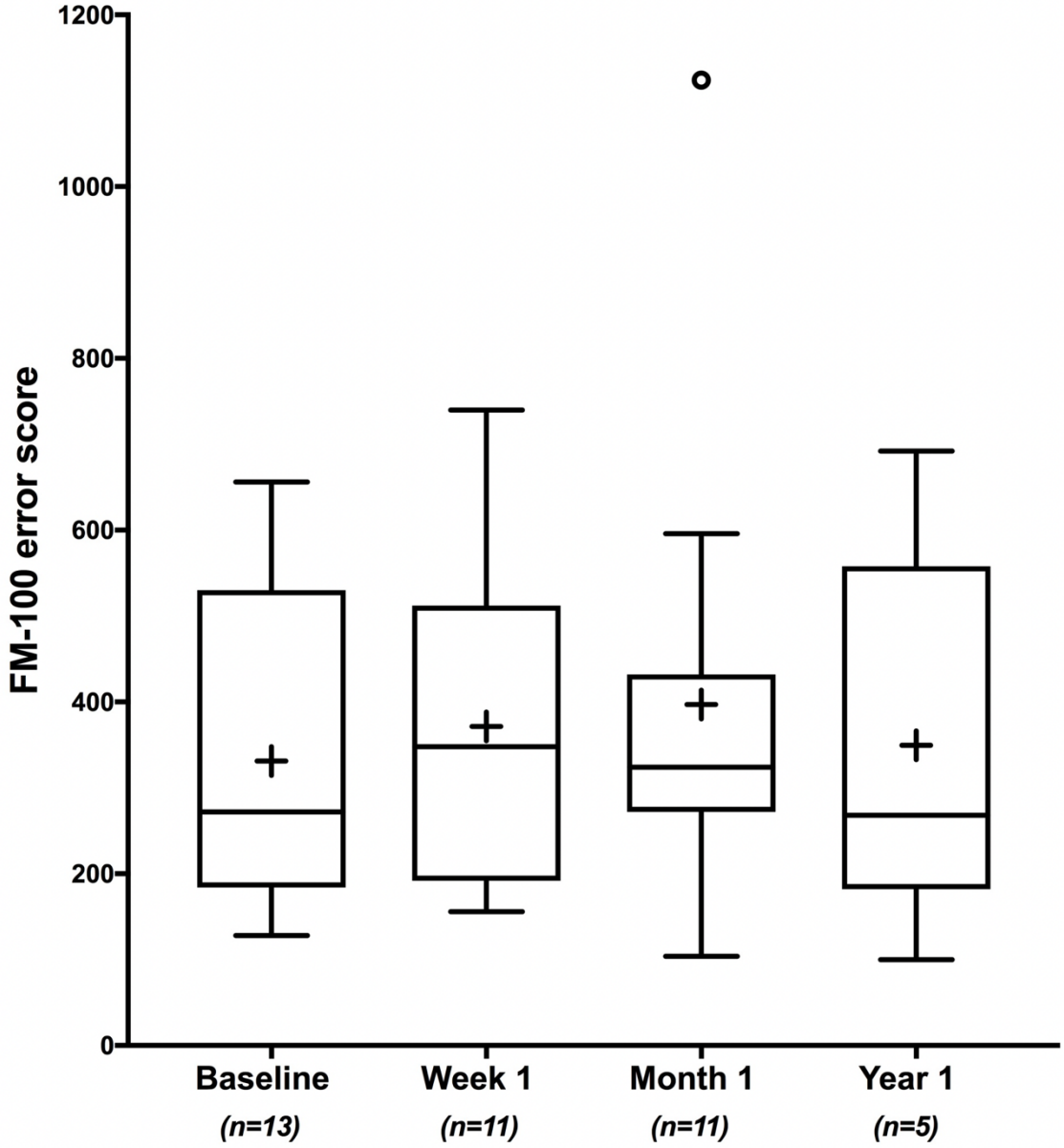


Figure 3.6: Farnsworth-Munsell 100 total error score over 12 months in ocriplasmin injected eyes. Box-and-whisker plot of Farnsworth-Munsell 100 scores in injected eyes, at baseline, one week, one month and one year post-injection.

The mean is shown as a cross inside the box. The median is shown as a band inside the box and the top and bottom of the box represent the 75th and 25th quartiles, respectively. A small circle represents an outlier.

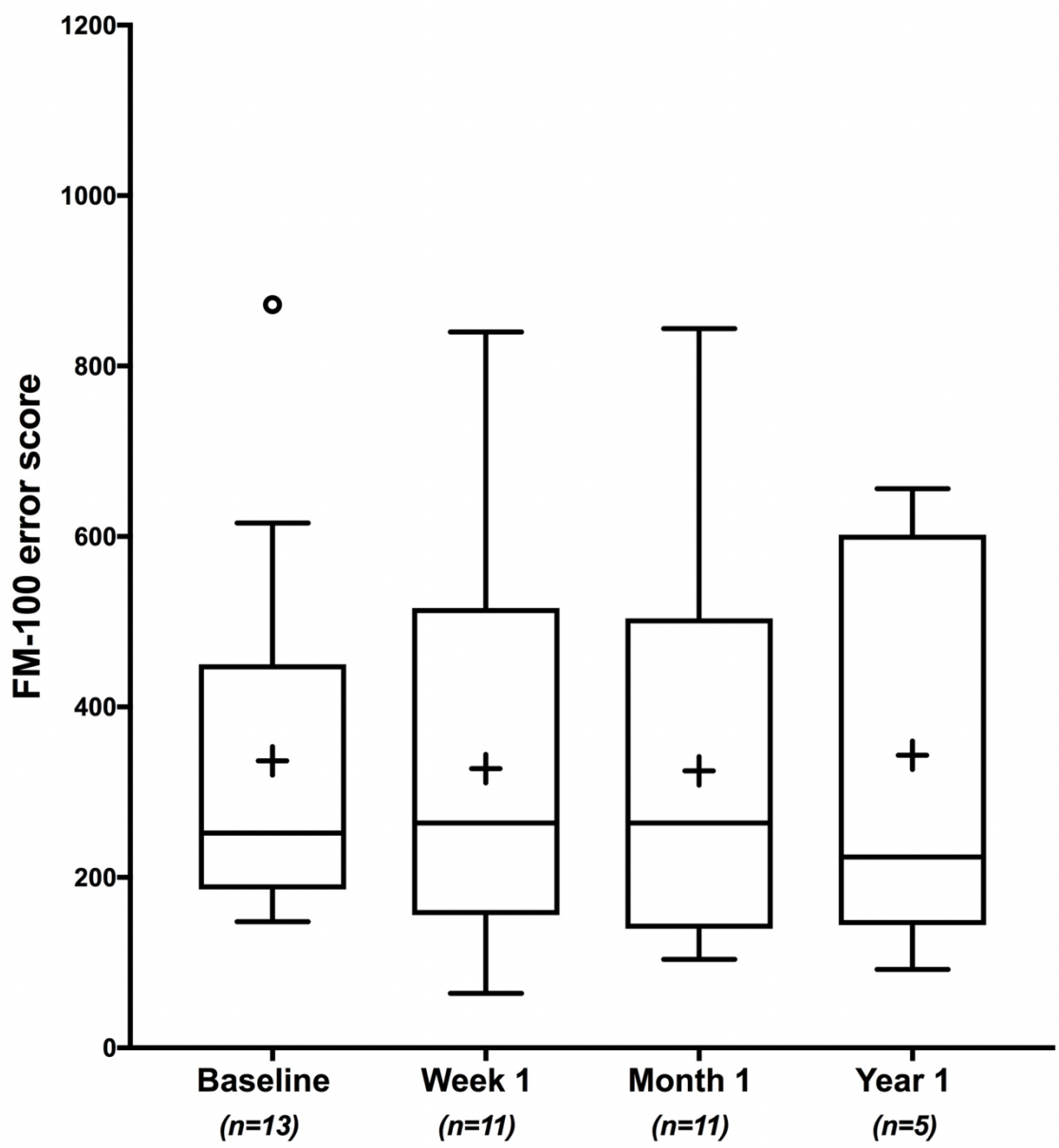


Figure 3.7: Farnsworth-Munsell 100 total error score over 12 months in fellow eyes.

Box-and-whisker plot of FM-100 scores in fellow eyes, at baseline, one week, one month and one year. The figure conventions are the same as those described for Figure 3.6.

Cases 1 and 2 achieved successful release of VMT by one month. In Case 1, TES was not recorded at one week or one month, but at one year had improved to 100 from 208 at baseline, whilst BCVA improved from 71 to 86 letters (Snellen equivalent 20/40 to 20/20). In Case 2, TES improved from 228 at baseline to 164 at one week, but then worsened to 300 at one month. Best corrected visual acuity was 70 letters at baseline (Snellen equivalent 20/40), and remained relatively stable at one week (69 letters; Snellen equivalent 20/40) and one month (70 letters; Snellen equivalent 20/40) but reduced to 60 letters (Snellen equivalent 20/60) at one year.

The OCT findings at baseline, one week and one month are shown in Table 3.3. Aside from the two patients (Cases 1 and 2) with VMT release, there were six patients who had OCT changes at either one week or one month, including a pseudohole, new or resolved intraretinal cysts (IRC) and new SRF. A subgroup analysis of patients with OCT changes in whom one month FM-100 data were available (n = 6) showed a deterioration in TES from 198.0 at baseline to 282.7 at one month (p = 0.34). Those without OCT changes (n = 5) also had a deterioration in TES from 463.2 at baseline to 534.4 at one month (p = 0.89).

Case	Diagnosis	Additional OCT findings		
		B/L	W1	M1
1	VMT	IRC	No change	VMT release
2	VMT	IRC	No IRC	VMT release, new IRC
3	VMT	IRC	Pseudohole	No pseudohole
4	VMT	IRC	No change	No IRC
5	VMT	Nil	New IRC	No change
6	VMT	IRC	No IRC	No IRC
7	MH	Nil	No change	No change
8	VMT	IRC	No change	No change
9	VMT	Nil	New SRF and IRC	No change
10	VMT	IRC	No change	No change
11	MH	Nil	New IRC	No change
12	MH	Nil	No change	New IRC
13	VMT	IRC	No change	No change

Table 3.3: Optical coherence tomography features in patients at baseline, one week and one month. 'No change' relates to a comparison with previous time point scan.

B/L, baseline; IRC, intraretinal cysts; MH, macular hole; M1, one month; OCT, optical coherence tomography; SRF, subretinal fluid; VMT, vitreomacular traction, W1; one week.

One patient (case 9) developed new SRF after ocriplasmin which persisted at the one month visit, but had resolved by one year. In association with the OCT changes, the TES reduced from 172 at baseline to 512 and 432 at one week and one month, respectively, and the BCVA similarly reduced from 56 letters (Snellen equivalent 20/70) at baseline to 49 letters (Snellen equivalent 20/100) and 47 letters (Snellen equivalent 20/100) at one week and one month, respectively.

The only subjective report of colour vision change was case 8, who reported a mild colour vision abnormality at one year post-ocriplasmin. Paradoxically, his TES improved to 264, from 464 at baseline.

Five patients underwent vitrectomy for surgical release of VMT, between one and six months after ocriplasmin injection. The mean BCVA in these patients improved from 58.8 letters at baseline to 72.8 letters at one year ($p = 0.07$). Eight patients did not undergo any vitrectomy during the study. In these patients, mean BCVA was 62.0 letters at baseline, and 57.8 letters at one year ($p = 0.69$).

3.4 Discussion

This study assessing hue discrimination did not find a statistically significant reduction following ocriplasmin at one year, but there was a trend for worsening colour vision at both one week and one month. Fellow eyes showed relatively stable colour vision. FM-100 testing can identify subtypes of dyschromatopsia such as protanomaly and deuteranomaly, but our work did not identify any specific colour defect attributable to ocriplasmin.

The therapeutic target site for ocriplasmin is the vitreomacular interface, and it has been suggested that the drug may also penetrate the adjacent retina (with direct access in those with MH) and thereby affect structural laminins in the interphotoreceptor matrix.[330, 331] An animal model has shown laminin in this region is potentially susceptible to ocriplasmin degradation.[332] Adverse events of SRF accumulation after ocriplasmin are well described, which are thought to be due to structural disruption of the retina following the enzymatic response.[268, 299] In addition, it has been shown that the edges of a macula hole can be lifted (resulting in larger basal diameter) following ocriplasmin, potentially due to drug effect on the surrounding interphotoreceptor matrix.[333]

Sub-retinal fluid and associated photoreceptor misalignment may potentially be the cause of dyschromatopsia after ocriplasmin, particularly as this has been described as the mechanism for pseudoprotanomaly sometimes seen in central serous retinopathy.[334] Furthermore, patients with macula-off retinal detachments have been shown to have a persisting loss of generalised colour hue discrimation.[335] One case in our study (case 9) developed sub-retinal fluid and reduced BCVA after ocriplasmin, which was accompanied by a worsening of the FM-100 TES from 176 at baseline to 512 and 432 at one week and one month, respectively.

Analysis of OCT scans at baseline, one week and one month showed some changes after ocriplasmin including new IRCs and SRF. It is not possible to determine whether these structural alterations were due to ocriplasmin or the natural disease processes. In five cases, a deterioration of the macular architecture was associated with a reduction in FM-100 TES, although others had FM-100 TES variation that did not correlate with OCT appearance. Interestingly, all but one patient reported no change in their colour vision at any of the study visits, which raises the possibility that subclinical dyschromatopsia may be more common than previously reported.

None of our three patients with MH had anatomical success with ocriplasmin, and therefore we are unable to say whether closure of MH would have resulted in an improvement in FM-100 TES. Colour vision has been shown to improve following successful MH surgery.[336] Poon et al previously showed a strong correlation between the red-green axis and BCVA improvement after MH surgery.[337]Another possible issue can be the use of indocyanine green (ICG) as a dye during MH surgery, which has been reported to confer a toxic effect resulting in dyschromatopsia.[338, 339] The effect of VMT release after vitrectomy on colour vision has not been described.

In terms of vision, the mean BCVA showed a trend for worsening vision following injection, but this did not reach statistical significance. Given the small numbers, this is perhaps unsurprising, although it was reassuring to see there were no cases of sudden loss of vision, as has been reported previously.[299, 302] In the two patients who had successful release of VMA, concurrent BCVA was stable, but it is well known that visual function questionnaires often show an improvement independent of BCVA when sVMA resolves.[303] It is also

important to note that five patients underwent PPV, all at least one month following ocriplasmin, which may have influenced the one year visual acuity results. In patients who did not undergo vitrectomy, visual acuity remained stable at one year compared to baseline. Another limitation of this study was the use of fluorescent lighting conditions for FM-100 testing which is a potential source of variability.

This is the first report of the effect of ocriplasmin on color hue discrimination measured by FM-100. A limitation of our case series is the low number of cases. The trend for worsening of the FM-100 TES may have been significant with a larger cohort, but conversely the apparent trend may be due to chance. A larger cohort may also be able to determine if any changes in colour vision relate to anatomic success. Further studies of colour vision appear warranted, with larger numbers and longer follow-up.

4 Intravitreal gas for symptomatic vitreomacular adhesion: a synthesis of the literature

This chapter is the published literature synthesis of intravitreal gas for symptomatic vitreomacular adhesion. The same text appears in the final published pdf version (Appendix 14).

Citation

Neffendorf JE, Simpson ARH, Steel DHW, Desai R, McHugh DA, Pringle E, Jackson TL. Intravitreal gas for symptomatic vitreomacular adhesion: a synthesis of the literature. Acta Ophthalmologica. 2018 Nov;96(7):685-691. DOI:10.1111/aos.13547.

4.1 **Abstract**

Symptomatic vitreomacular adhesion (sVMA) is defined as visual loss secondary to foveal damage from vitreomacular traction (VMT) and includes isolated VMT, impending macular hole (MH), and full-thickness MH with persisting vitreous attachment. Management options include pars plana vitrectomy (PPV), intravitreal ocriplasmin, intravitreal gas injection or observation. This synthesis of the literature aimed to assess the safety and efficacy of intravitreal gas for sVMA. Articles describing patients with VMT or MH treated with intravitreal expansile gas were selected by systematic literature review using MEDLINE, EMBASE, and the Cochrane Database of Controlled Trials (CENTRAL) up to September 2016. The main outcomes at one month and final review were logarithm of the minimum angle of resolution (logMAR) visual acuity (VA), anatomical success (absence of both VMT and MH, without PPV) and adverse events (AEs). The intended comparator was observation. Nine of 106 identified articles were eligible, and none were randomized controlled trials. The mean VA of 91 eyes improved from 0.55 (Snellen equivalent 6/21) to 0.48 (6/18) logMAR at one month and to 0.35 (6/13) logMAR at final review. The mean VA at final review, prior to a vitrectomy, was 0.42 (6/16). Anatomic success was 48% at one month and 57% at final review. The reported AEs comprised retinal detachment in two highly myopic eyes. Intravitreal gas injection can relieve sVMA. Larger controlled studies are needed to determine safety and efficacy relative to observation, ocriplasmin, or vitrectomy.

4.2 **Background**

Perifoveal vitreous separation may occur as part of normal ageing, or as part of a disease spectrum ranging from vitreomacular traction (VMT) to macular hole (MH). Symptomatic vitreomacular adhesion (sVMA) is defined as visual loss secondary to foveal damage as a result of VMT, and includes isolated VMT, impending MH, and full thickness MH with persisting vitreous attachment.[37, 41]

Treatment strategies for VMA depend on disease severity. Asymptomatic VMT can be observed, since vitreofoveal separation may occur spontaneously without sequelae. However, persisting VMT may result in foveal damage, thus prompting treatment if symptoms are significant or visual acuity (VA) is reduced.[42, 47, 48] For many years, pars plana vitrectomy (PPV) was the standard approach for VMT.[1] More recently, pharmacological vitreolysis with ocriplasmin (Jetrea; Thrombogenics, Leuven, Belgium) has emerged as an alternative that may avoid the need for PPV.[60-65, 284, 340]

Another treatment modality for sVMA is pneumatic displacement with an intravitreal expansile gas bubble, potentially avoiding the need for vitrectomy or enzymatic vitreolysis. The potential advantage of an intravitreal gas injection includes its low cost and ease of adoption. For example, the cost of ocriplasmin and vitrectomy are estimated at $3 950 (jetrea.com/JETRAOrderinginfo.pdf) and $3 147 in the USA, respectively, and £3 000 and £1 634, respectively, in the UK.[341, 342] The cost of ocriplasmin is magnified by the fact that many cases fail to respond and therefore still need to progress to vitrectomy. Gases such as C_3F_8 and SF_6 cost as little as £1 if taken from large medical gas cylinders, or typically less than £100 from single use canisters licensed for intraocular use. Intravitreal gas is easy to

store and administer, and does not require the capital costs or surgical expertise needed to undertake PPV. In addition, intravitreal gas injection may potentially be a safer procedure compared to the more invasive PPV.

Given these potential advantages of intravitreal gas we undertook a review of the safety and efficacy of intravitreal gas for sVMA, to guide clinical care or future studies. Specifically, we aimed to determine the benefit of intravitreal gas in terms of releasing VMT or closing MHs, the effect on VA, and the risk in terms of intra- and postoperative complications.

4.3 **Methods**

4.3.1 Eligibility criteria for considering studies for this review

The population was patients with sVMA, namely VMT with or without MH, to include stage 1, 2 and 3 MH. The intervention was a single intravitreal expansile gas injection. The intended control was natural history. The main efficacy outcomes were VA and anatomic success, defined as an absence of VMT or MH without recourse to PPV. Both outcomes were assessed at one month and final follow up. Safety outcomes included all reported surgical complications or adverse events attributed to intravitreal gas. The study protocol was registered with the international prospective register of systematic reviews (2015:CRD42015017338, National Institute of Health Research Centre for Reviews and Dissemination, University of York, UK) and conducted in accordance with Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidance (http://www.prisma-statement.org/, accessed 28 May 2015).

There were no restrictions with regards to gender or ethnicity of patients or language of article. In the anticipated absence of any randomized controlled trials and to maximise safety data, prospective, retrospective, controlled and uncontrolled studies, including case reports, were eligible. Inclusion criteria were: studies of VMT or stage 1-3 MHs[44]; at least 28 days follow up; VA outcomes reported; either MH closure or VMT release rates; reporting results in adults over 18 years of age. We excluded editorials and expert opinions, and articles appearing as abstract only. Eyes with prior treatment of VMA were excluded, including PPV, intravitreal gas, and pharmacologic vitreolysis. Eyes being treated for myopic macular hole retinal detachment were excluded.

4.3.2 Search methods for identifying studies

PubMed MEDLINE, EMBASE, and Cochrane Database of Controlled Trials (CENTRAL) searches were performed including all articles up to and including September 2016 using Boolean operators with the following keywords (and corresponding MESH headings if they were available): SF6, sulfur hexafluoride, sulphur hexafluoride, C2F6, hexafluoroethane, C3F8, octafluoropropane, perfluoropropane, gas, intravitreal, macular hole, sulphur hexafluoride, vitreomacular adhesion, and vitreomacular traction.

4.3.3 Study Selection

Abstracts were retrieved from the search and further articles were identified in the reference lists of the retrieved articles. Two clinicians (JN and TJ) independently assessed articles for provisional eligibility based on their abstract. Full-text copies of all possibly relevant manuscripts were obtained, to determine final eligibility. Any discrepancy in eligibility was resolved by consensus following discussion.

4.3.4 Data collection and risk of bias assessment

Two reviewers (JN and TJ) extracted the relevant information into a database, including: 1) overview of the study (aim and key findings); 2) methodological details (study design, study population, inclusion criteria, exclusion criteria, intervention, comparator if available, study period); 3) VA before and after gas; 4) anatomic success after gas; 5) need for vitrectomy; 6) safety outcomes. To compare across studies, VA was converted to logarithm of the minimum angle of resolution (logMAR) units.[286]

4.3.5 Data synthesis and analysis

Where necessary, authors were contacted to obtain unpublished raw data. Two-sided, paired t-tests were used to compare mean VA before and after interventions. Safety was assessed by adverse events (AEs) and serious adverse events (SAEs) reported. Safety data were pooled across all studies, using individual data where available or study means otherwise. Sub-group analysis was performed for those with diagnoses of MH or VMT.

4.4 **Results**

Of 106 articles, 106 abstracts were assessed as potentially eligible, from which nine articles were deemed eligible after full text review. A total of 91 eyes from 90 patients with sVMA were included from one non-randomized controlled study, seven uncontrolled studies and two individual case reports (Table 4.1).[57-59, 343-348] Additional, anonymous participant-level VA data were obtained from one study author as this information was not available in his report, in accordance with PRISMA guidance.[59] A risk of bias tool was not used as the literature search found no eligible randomised controlled trials.

Article (first author)	Year	Methodology	Number of eyes	Mean age	Male (%)	Gas used	Posturing	Number with VMT	Number with Stage I MH	Number with Stage 2 MH	Number with Stage 3 MH
Chan[57]	1995	Prospective case series	19	70	32	0.3-0.5ml C_3F_8	Face down (4d)	0	11	6	2
Costa[343]	2001	Case report	1	65	NS	0.4 ml C_3F_8	Face down (5d)	1	0	0	0
Jorge[345]	2006	Prospective case series	6	NS	NS	0.4ml C_3F_8	Face down (14d)	0	0	6	0
Mori[58]	2007	Prospective case series	20	64	30	0.5ml SF_6	Face down (3-5d)	0	0	20	0
Chen[344]	2011	Prospective case series	12	59	17	0.2ml C_3F_8	Face down (5d)	0	0	12	0
Gupta[346]	2011	Case report	1	55	0	0.3ml SF_6	Upright daytime	0	1	0	0
Rodrigues[59]	2013	Retrospective case series	15	72	53	0.3ml C_3F_8	None	15	0	0	0
Day[347]	2015	Retrospective case series	9	73	11	0.3ml SF_6	None	7	2	0	0
Yu[348]	2016	Retrospective case series	8	68.1	12.5	0.3ml C_3F_8	Face down (2d)	7	0	1	0
All			**91**	**67.3**	**28.6**			**30**	**14**	**45**	**2**

Table 4.1: Demographic information on studies deemed eligible for synthesis of the literature.

C_3F_8, perfluoropropane; d, days; MH, macular hole; NS, not specified; SF_6, sulphur hexafluoride; VMT, vitreomacular traction.

There were 24 males and 59 females, with a mean age of 67.3 years (range 36 to 91, n = 85). Gender and age data were missing from one study of six eyes and the gender of a patient was not stated in one case report. There were 44 eyes (44 patients) with a baseline diagnosis of VMT, including 14 with stage 1 MH. Stage 2 MH was present in 45 eyes (45 patients), and stage 3 MH in 2 eyes (2 patients). One patient underwent bilateral treatment for a stage 3 MH in the right eye and a stage 2 MH in the left eye. Perfluoropropane gas was used in 62 eyes, with the volume injected varying from 0.2ml to 0.5ml. Sulphur hexafluoride 0.5 ml was used in the other 29 eyes. Post-operative posturing techniques were not consistent between studies, varying from 14 days of face down posturing to no posturing. A PPV was performed in 31 of 91 eyes (34%) for varying reasons: persisting MH despite VMT release with gas in 14 eyes (45%), persisting VMT and MH despite gas injection in eleven eyes (36%), retinal detachment in two eyes (7%), new MH following successful VMT release with gas in two eyes (7%), persisting isolated VMT in one eye (3%) and vitreous haemorrhage secondary to proliferative diabetic retinopathy in one eye (3%).

At one month following gas injection, 44 of 91 eyes (48%) had anatomic success, defined as no VMT or MH and without recourse to PPV. At a mean final follow up period of 14.5 months (range: 1 to 48 months), anatomic success was achieved in 52 eyes (57%). Twenty six eyes underwent PPV specifically for failure of gas, 14 for persisting MH, 11 for persisting combined VMT/MH, 1 for persisting isolated VMT, and all responded with anatomic success.

The mean pre-intervention logMAR VA was 0.55 (n = 91; range: 0 to 2.00; Snellen equivalent 6/21). In the 62 eyes (68%) with VA documented at one month the mean VA improved from 0.57 logMAR by 0.09 units to 0.48 logMAR (range: 0 to 2.00; 6/18;

p=0.036). No eyes had undergone PPV by month one. Mean VA at final follow up was 0.35 logMAR (n = 88; range: -0.09 to 2.00; 6/13), which was significantly better than baseline (p<0.001)(Table 4.2). A post hoc analysis of the final VA outcome prior to any PPV revealed a VA of 0.42 logMAR (n=78; 6/16), significantly better than baseline (p=0.001). Three patients did not have a post-gas VA documented.

Article (first author)	Number of eyes	Mean follow up period (months)	Mean initial visual acuity (logMAR)	Mean month 1 visual acuity (logMAR)	Mean final visual acuity (logMAR)	Anatomic success at month 1 (n, %)	Anatomic success at final review (n, %)
Chan[57]	19	15.6	0.41	0.32	0.30	9 (47.4)	13 (68.4)
Costa[343]	1	10	0.60	NS	0.10	0 (0)	1 (100)
Jorge[345]	6	40.7	0.68	0.22	0.22	5 (83.3)	5 (83.3)
Mori[58]	20	19.5	0.38	NS	0.19	10 (50)	10 (50)
Chen[344]	12	8.2	0.94	0.82	0.46	3 (25)	3 (25)
Gupta[346]	1	1	1.00	0.3	0.3	1 (100)	1 (100)
Rodrigues[59]	15	11.5	0.52	0.64	0.49	6 (40)	9 (60)
Day[347]	9	1	0.39	0.30	0.30	5 (55.5)	5 (55.5)
Yu[348]	8	6	0.82	NS	0.72	5 (62.5)	5 (62.5)
All	**91**	**14.5**	**0.55**	**0.48**	**0.35**	**44 (48.4)**	**52 (57.1)**

Table 4.2: Data displaying anatomic success and visual acuity change following intravitreal gas injection.

logMAR, logarithm of the minimum angle of resolution; NS, not specified; VA, visual acuity.

In the 30 eyes (33%) with a baseline diagnosis of isolated VMT, the mean VA was 0.55 logMAR (range: 0.1 to 2.00; 6/21) at baseline and remained unchanged at 0.55 (range: 0.00 to 2.00; 6/21) at month one (n = 22; p=0.226), before subsequently improving to 0.49 (range: 0.00 to 2.00; 6/19) at a mean follow up of 7.7 months (n=28; p = 0.096) (Figure 4.1). Anatomic success was achieved in fourteen eyes (47%) at month one and eighteen eyes (60%) at final follow up (Figure 4.2). Eight of 30 (27%) eyes with VMT underwent PPV, all after month one. The indication in one case was vitreous haemorrhage secondary to proliferative diabetic retinopathy in which the initial gas injection had previously resulted in a complete posterior vitreous detachment (PVD) at month one. In two eyes, PPV was

performed for a full-thickness MH following earlier successful VMT release with gas. The other five PPVs were carried out to treat persistent VMT despite intravitreal gas injection.

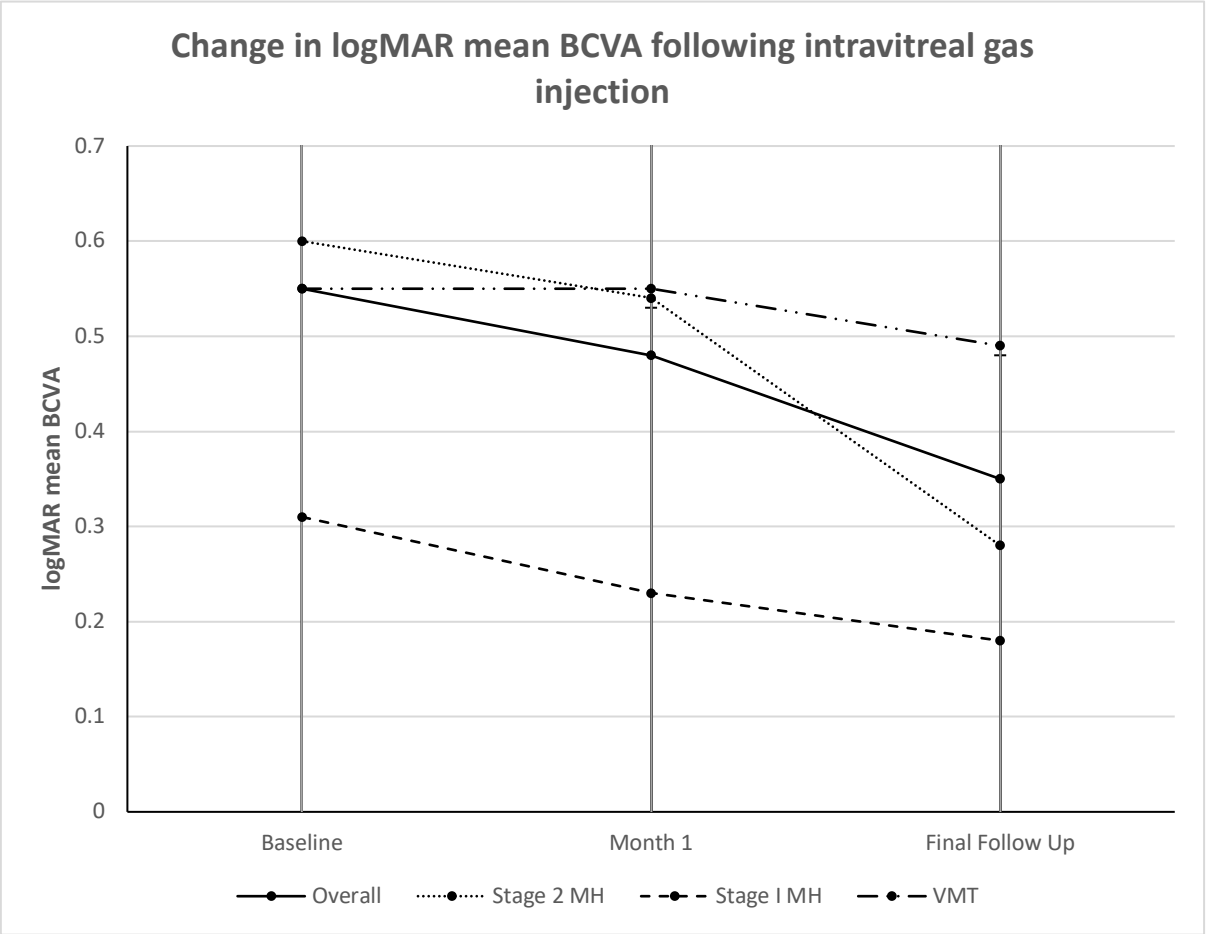


Figure 4.1: Visual acuity. The graph shows the mean logMAR visual acuity at baseline, one month after intravitreal gas injection, and at final follow-up prior to vitrectomy (if carried out).

BCVA, best-corrected visual acuity; logMAR, logarithm of the minimum angle of resolution; MH, macular hole; VA, visual acuity; VMT, vitreomacular traction

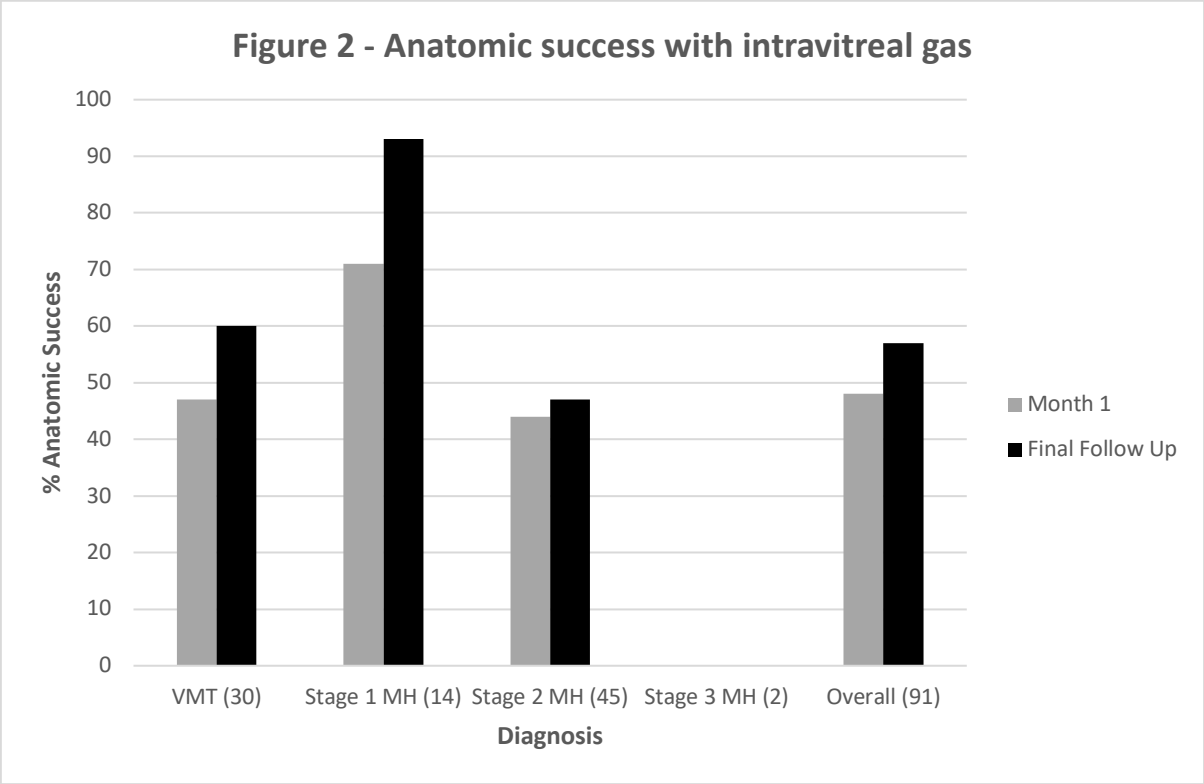


Figure 4. 2: Anatomic success. The chart shows anatomic success, over time, of intravitreal gas injection for each subset of symptomatic vitreomacular adhesion. Anatomic success was defined as an absence of VMT and MH, without recourse to vitrectomy.

MH, macular hole; VMT, vitreomacular traction

A stage 1 MH was present at baseline in 14 eyes. In these eyes, VA improved from 0.31 logMAR (range: 0.18 to 0.48; 6/12) to 0.23 (range: 0.00 to 1.00; 6/10) at month one (p=0.338), and significantly to 0.18 (range: 0.00 to 0.30; 6/9) at a mean final follow up of 12.9 months (p=0.015) (Figure 4.1). Anatomic success occurred in 10 of 14 eyes (71%) at one month post-gas, and 13 of 14 eyes (93%) at final follow up (Figure 4.2).

The distinction between stage 1 (impending) MH and advanced VMT relies on the investigator's judgement and did not appear to be standardised in the literature. Further,

impending MH is often now grouped together with VMT. We therefore undertook a post hoc analysis combining VMT and stage 1 MH. In this group, VA improved from 0.45 logMAR (range: 0.00 to 2.00; 6/17) to 0.43 (range: 0.00 to 2.00; 6/16) at month one (p=0.382), and then improved significantly, relative to baseline, to 0.39 (range: 0.00 to 2.00; 6/15) at a mean follow up of 9.4 months (p=0.019). Anatomic success occurred in 24 of 37 eyes (65%) at one month, and 31 of 37 eyes (84%) at final follow up.

There were 45 eyes treated with intravitreal gas for a stage 2 MH, with a mean baseline VA of 0.60 (range: 0.00 to 1.52; 6/24). In the 24 eyes with month one VA data, the mean logMAR improved to 0.54 (range: 0.10 to 2.00; n = 24; 6/21). At final follow up (mean = 17.9 months), mean VA significantly improved to 0.28 logMAR (range: -0.09 to 1.00; 6/11) compared to baseline (p<0.001)(Figure 4.1). Anatomic success occurred in 20 of 45 eyes (44%) at month one, and 21 of 45 eyes (47%) at final follow up (Figure 4.2). A PPV was undertaken in 22 eyes. In 20, the indication was failure of MH closure with gas (although 17/20 had resulted in PVD), and all PPVs were successful in closing the MH. The other two PPVs were performed successfully to treat retinal detachment.

Two intravitreal gas procedures were performed for stage 3 MH, but neither was successful anatomically either at month one or by a final mean follow up of 33 months.

The diameter of MH was only documented in one study of 20 stage 2 MH.[58] Successful release of vitreous traction and closure of MH at both month one and at an average final follow up of 20 months in patients with a MH diameter <250μm was 78% (7/9). Those with larger holes (>250μm) had successful anatomical resolution in 27% of cases (3/11) at one

month. All those with failed anatomical resolution at one month underwent PPV which resulted in successful MH closure.

Adverse events (AEs) included two retinal detachments. Both occurred in myopic eyes (-5.75D and -8.50D) with stage 2 MH. In two patients with VMT at baseline, intravitreal gas resulted in PVD at one month and development of a full-thickness MH which was successfully closed with PPV. One eye with an impending MH developed a full thickness MH 10 months after failed gas injection, and was successfully closed with PPV. Two eyes with stage 1 MH were diagnosed with macular pseudohole at month 13. There was one patient who was diagnosed with a retinal tear at one month following gas, and underwent successful laser retinopexy. No other AEs were reported.

4.5 **Discussion**

We undertook a review to evaluate the safety and efficacy of intravitreal gas as a treatment for sVMA. We found a lack of high quality evidence. A series of uncontrolled, before/after studies found that 57% of eyes had anatomic success following intravitreal gas, defined as an absence of VMT and MH, without recourse to PPV. There was also a VA gain of 0.13 logMAR units (approximately 1 Snellen line), without the need for PPV. This modest gain in VA may not fully capture the potential symptomatic benefit achieved in this patient group, given that metamorphopsia may be at least as important as VA. The good presenting VA may also impose a ceiling on any VA improvement that can be detected following gas injection. Studies of ocriplasmin and PPV for symptomatic VMA also show modest VA gains, although the visual improvements are often better in the MH subset, compared to those with isolated VMT.[63, 349] We also found better VA gains in those with a baseline diagnosis of MH compared to isolated VMT when treated with gas.

Our literature search found one study of 20 eyes of 17 patients with VMT that underwent an 0.2ml intravitreal injection of either SF_6 or $C2F_6$.[350] This was a retrospective case series which reported an 85% (17/20) overall release of VMT, favourable visual acuity outcomes and no major safety concerns. However, we excluded this study from our analysis because there was insufficient information regarding when VMT release occurred and when post-operative visual acuities were measured.[350]

The management of symptomatic VMA does not currently have a gold standard, with options including observation, intravitreal gas, ocriplasmin, and PPV. Observation of VMT may lead

to spontaneous separation in 17-34% of eyes, but conversely some may progress to MH, and prolonged disease may result in loss of vision.[275, 277]

A combined analysis of two randomized controlled trials of ocriplasmin reported that 26.5% of eyes responded within one month, with no further response after this time point. Despite using a somewhat stricter definition of success (absence of both VMT and MH, not just an absence of VMT) the rate of release in our review of intravitreal gas appears higher, at 48.4% by month one (and 57.1% at final review). However, without direct comparison this conclusion needs to be interpreted with considerable caution, as the difference could reflect patient selection, chance, publication bias, and differences in OCT interpretation, amongst other reasons.

In terms of safety, there were three cases of impending MH that progressed to full-thickness MH. In two cases, the gas injection resulted in PVD and full-thickness MH at one month, but the other occurred ten months after gas injection so causation is unclear. A retinal tear occurred in one case, at month one following gas injection, which was successfully treated with laser retinopexy. Most of the studies did not comment whether the patients were phakic or pseudophakic at baseline. Excluding cases undergoing PPV, two eyes were noted to have progression of nuclear sclerosis but neither required cataract surgery. The most clinically important AEs were two cases of retinal detachment in myopic patients (2%). This suggests that myopic eyes may be best excluded from future studies of intravitreal gas for symptomatic VMA. By extension it may also be reasonable to exclude other risk factors for retinal detachment, such as lattice degeneration or treated retinal breaks, although the risk in these patients in assumed rather than proven. The small number of eyes treated means it is not possible to quantify the overall clinical impact of retinal detachment, however, any such

risks needs to be balanced against the risk of PPV or ocriplasmin. A recent literature review of PPV undertaken for VMT found a retinal detachment rate of 4.6%.[349] The retinal detachment rate in the pivotal studies of ocriplasmin was 0.4%, vs 1.6% in the placebo group (p=0.16), although several cases of retinal detachment following ocriplasmin have now been published and the true rate of RRD after ocriplasmn with longer follow up may be higher than in the phase 3 trials.[278, 351]

The majority of adverse events associated with ocriplasmin have been considered mild, non-serious and transient such as vitreous floaters, eye pain, photopsia and reduced VA.[352] However, concerns remain about dyschromatopsia, ERG changes and severe loss of vision, and there have been isolated case reports of ellipsoid zone changes on OCT and RPE-photoreceptor adhesion release potentially due to the enzymatic activity of the drug.[269, 300, 353-355]

Only one study reported MH diameter and found a higher success rate of stage 2 MH closure in small diameter holes (<250μm) as opposed to those larger than 250μm (78% vs 27%). This greater efficacy with smaller diameter is consistent with a sub-group analysis of the data from the pivotal ocriplasmin trial.[278, 279] The influence of ERM on anatomic success is hard to determine as most studies excluded ERM, with only four cases included across all studies.[57, 347] Rodrigues et al reported that high reflectivity of the inner retinal surface, a possible precursor of ERM, was associated with a lower rate of VMT release, which is also consistent with the sub-group analysis of the pivotal ocriplasmin trial.[59, 278, 279] It has been shown that phakic patients have a higher likelihood of successful sVMA release following ocriplasmin injection than pseudophakic patients.[278, 279, 356] In our analysis, only 2 of 9 articles documented whether patients were phakic or pseudophakic at baseline and therefore due to

missing data, we did not perform a subgroup analysis to further investigate whether this trend is also seen with intravitreal gas.

A strength of our study is that we have pooled data in a standardised method with predefined outcome measures. However, there are several important weakness. Most importantly the number of patients is low, and only one of the studies had a control group (and in that in turn was not randomised). Accordingly, many studies may be subject to bias. Furthermore, diagnostic criteria varied across studies, as did the type and volume of gas injected and the posturing regimen. Our findings may underestimate VMT release in non-diabetic patients as our group contained 8% (7/91) diabetics, who might be expected to have firmer VMA. In addition, some studies did not report the duration of disease prior to treatment, and others had significant variability in duration (1-7 months). One study was conducted in the pre-OCT era, however, it provided relatively rigorous assessment of VMA including B-scan ultrasonography.[57] It is also not clear which gas offers the best efficacy.

In conclusion, our synthesis of the literature suggests that there is insufficient evidence to conclude on the safety and efficacy of an intravitreal expansile gas injection for the treatment of sVMA. The limited results available do however appear to justify further research, most helpfully as a comparative study versus other management options such as observation, ocriplasmin, or vitrectomy. Diagnostic inclusion criteria can be defined using recognized photographic standards or agreed classification systems, and outcome measures could be expanded to include cataract progression, validated quality of life questionnaires and assessment of metamorphopsia.[25, 38, 357-360] An economic evaluation comparing different treatments of symptomatic VMA also appears warranted, given the potential cost advantage of intravitreal gas.

4.6 **Supplementary Material**

After publication of the gas for symptomatic VMA review article, there were substantial clinical developments which prompted inclusion of this supplementary information. On the basis of the encouraging results from the various case series', the diabetic retinopathy clinical research (DRCR) network designed two studies to determine the effect of pneumatic vitreolysis for vitreomacular traction (VMT).[361-363]

Protocol AG was devised as a randomised controlled trial to compare an intravitreal injection of 0.3 ml perfluoropropane (C_3F_8) with a sham injection (pressing the hub of a needleless syringe against the conjunctiva) for symptomatic VMT without macular hole (MH). The primary outcome measure was the proportion of eyes with central VMT release without rescue pars plana vitrectomy (PPV) at 24 weeks. Secondary outcomes included the number requiring rescue treatment or PPV up to 24 weeks, and mean change in Early Treatment Diabetic Retinopathy Study (ETDRS) best corrected visual acuity (BCVA) from baseline to 24 weeks. Those with co-existing significant retinal disease (e.g. neovascular age-related macular degeneration), high myopes (defined as spherical eqivalent of -8.00 dioptres or more myopic if phakic or retinal abnormalities consistent with pathologic myopia if phakic or pseudophakic), glaucoma, previous intravitreal injections or vitrectomy, and untreated retinal tears were part of the exclusion criteria.

Protocol AH was an observational, single arm, prospective study assessing the use of intravitreal 0.3 ml C_3F_8 for full thickness MH (FTMH) less than 250 μm at its narrowest point. The primary outcome measure was the proportion of eyes with MH closure without rescue vitrectomy at eight weeks. Secondary outcomes included ETDRS visual acuity at eight

weeks, as well as the number requiring rescue PPV, and the success rate of MH closure with rescue PPV. Exclusion criteria were similar to Protocol AG (above). In contrast to Protocol AG, participants in Protocol AH were required to position face-down for 50% of the time for at least four days after the injection.

The results were published in Ophthalmology in 2021.[364] Both studies were discontinued early due to unacceptably high rates of retinal detachment and retinal tears. By this point, Protocol AG had recruited 46 participants (24 with gas, 22 with sham injection), and Protocol AH had recruited 35 participants. Seven of 59 (12%) eyes which had received an intravitreal gas injection developed a rhegmatogenous retinal detachment (RRD, 6 eyes) or retinal tear (1 eye), and all underwent PPV.

In Protocol AG, VMT release occurred in 78% (18/23) without rescue vitrectomy versus 9% (2/22) in the sham group (adjusted risk difference, 66%, 95% CI, 44 – 88%, p <0.001). Two participants underwent PPV for RRD, all in the gas group. Mean change in BCVA from baseline to 24 weeks was 6.7 ETDRS letters in the gas group versus 6.1 letters in the sham group (n = 22; adjusted difference, -0.8, 95% CI, -6.1 to 4.5 letters, p = 0.77).

For Protocol AH, MH closure without rescue vitrectomy occurred in 29% of eyes (10/35) by eight weeks. Twelve patients underwent rescue vitrectomy for persisting MH, and was successful in 83% (10/12). Five patients required PPV for RRD (4) or retinal tear without RRD (1). By 24 weeks, VMT released without PPV in 94% (33/35) of eyes. The BCVA change at eight weeks was -1.5 ETDRS letters (95% CI, -10.3 to 7.3 letters).

In conclusion, the anatomical results (VMT release) of the studies were encouraging and in line with those from the earlier case studies.[361] However, the MH closure rate did not reflect the earlier studies. Visual acuity outcomes were also not supportive of a benefit of using intravitreal gas for symptomatic VMT. The safety concerns regarding RRD were the rationale for early cessation of the studies. The rate of RRD/retinal tear in Protocols AG and AH (12%) were higher than that in our literature synthesis (2.2%, 2/91).[361]

It is important to consider why the Protocol AG and AH patients suffered an unacceptably high RRD rate. A review of the baseline characteristics showed 5 of 59 patients receiving gas had a diagnosis of retinal lattice degeneration. There is insufficient information provided in the publication to determine if these patients were at a higher risk of RRD compared with those with a normal peripheral retina.[364] In our publication and review, we suggested that those with risk factors for retinal detachment, such as lattice degeneration and treated retinal breaks, might represent a contraindication for pneumatic vitreolysis. It is unclear whether this would improve the safety profile of the treatment. Given the results of the Protocol AG and AH studies, gas is not currently recommended for the treatment of symptomatic VMT.

5 The VITCLEAR Study: the systemic safety of anti-vascular endothelial growth factor therapy based on vitreous status and agent

In this chapter, the background, methods, results and discussion of the VITCLEAR study are reported.

5.1 **Background**

Anti-vascular endothelial growth factor (anti-VEGF) drugs are the treatment of choice for neovascular age-related macular degeneration (nAMD). Dosing strategies have evolved from fixed monthly regimens, to *pro re nata (PRN)*, and more recently treat-and-extend (T&E), whereby the treatment interval is determined by disease activity, or the occasional off-label use of bimonthly dosing.[365] A T&E approach has been shown to produce improved visual outcomes compared to *PRN* regimens, and may conserve clinic capacity due to the requirement for fewer patient visits than fixed monthly or PRN strategies.[366, 367] There are, however, cost implications when increasing the number of injections despite the reduction in clinical visits, and potentially increased risk of rare, injection-related complications such as endophthalmitis.

Retreatment decisions and dosing interval planning in T&E regimens are based on visual acuity (VA) and signs of disease activity such as the presence of haemorrhage and macular oedema seen usually via optical coherence tomography (OCT).[365] Despite the fact vitreomacular status has been shown to influence the clinical course of nAMD, it does not tend to influence treatment decision making.[2] As discussed in the thesis introduction, patients with a posterior vitreous detachment (PVD) require fewer retreatments and are significantly more likely to be successfully extended that those without a PVD.[368]

There are various reasons why vitreomacular status may contribute to nAMD and its response to treatment. When vitreomacular adhesion (VMA) is present, direct tractional force may influence disease activity or result in chronic low-grade inflammation.[79, 369] Posterior vitreous detachment increases retinal oxygenation and therefore may be protective as hypoxia is a

potent stimulus for vascular endothelial growth factor (VEGF) production.[37] Furthermore, contact of aqueous with the macula in PVD could alter the diffusion of disease modifying cytokines.[81]

Another way in which vitreomacular status may impact the clinical course of nAMD is the effect it might have on anti-VEGF drug pharmacokinetics. It is possible that anti-VEGF drugs diffuse out of the eye faster, and thus exert less activity, in a vitrectomised eye or when a PVD is present. If clearance of anti-VEGF drugs varies depending on vitreous status, it may also potentially affect the systemic drug concentration and therefore influence systemic safety. These are the areas which the VITCLEAR study is designed to investigate.

5.1.1 Effect of vitreous status on the pharmacokinetics of intravitreal anti-vascular endothelial growth factor drugs

A detailed review of anti-VEGF pharmacokinetics is provided earlier in the thesis. With regards to vitreomacular status, pharmacokinetic studies have shown ranibizumab has a shorter ocular half-life in rabbits when the eye is vitrectomised (2.1 versus 2.8 days).[171] A similar finding has been reported in rabbit eyes receiving bevacizumab (2.3 versus 4.2 days) and monkey eyes receiving ranibizumab (1.4 versus 2.3 days).[173, 195] A monkey animal model has also shown reduced ocular half-life in vitrectomised eyes receiving aflibercept (1.5 versus 2.2 days).[173] However, another study did not find any statistically significant difference between vitrectomised and non-vitrectomised rabbit eyes receiving ranibizumab (2.5 versus 2.8 days).[181]

Given this trend of faster anti-VEGF drug clearance in vitrectomised eyes, it is conceivable that a liquified vitreous in PVD could have a similar effect due to increased convection compared to an eye without a PVD (nPVD).[370] If this is the case, then it may suggest eyes with a PVD require a shorter retreatment interval. This contradicts with some findings, described earlier, that eyes with a PVD require fewer retreatments.[368] It is likely that the mechanisms behind this are multifactorial.

There have been no human pharmacokinetic studies assessing the effect of vitrectomy or vitreomacular status (PVD versus nPVD) on anti-VEGF drug half-life. This is most likely due to the impracticality of repeated ocular sampling. A previous study on cynomolgus monkeys showed that ranibizumab clears in parallel from all ocular compartments (vitreous, aqueous and retina) after intravitreal injection.[189] Retinal concentration was approximately one third of the vitreous concentration. Serial serum measurements showed a similar half-life to the ocular measurements, with all compartments reaching peak concentration at 6 hours, suggesting serum samples could be used to interpret ocular pharmacokinetics.[189] It is important to note, however, that it is not possible to calculate the ocular half-life based on serum sampling alone.

5.1.2 Renal safety

The systemic safety of anti-VEGF drugs continue to be debated.[270] When they were first introduced, the Food and Drug Administration (FDA) data suggested the serum drug concentrations after intravitreal injection for nAMD were below clinically relevant levels.[371] Specifically, the detected serum level for ranibizumab and aflibercept were reported as 0.05 nmol/L and 0.2 nmol/L, respectively.[371, 372] Subsequently, studies showed the serum levels

were far higher, and equivalent to the 50% half-maximal inhibitory concentration for VEGF inhibition (IC_{50}).[198, 229]

As serum anti-VEGF concentrations are higher than originally thought, attention has turned to their potential risk of causing systemic side effects such as arteriothrombotic events and nephrotoxicity.[242, 373] Both are well recognised after systemically administered anti-VEGF.[374] A recent study investigated the effect of ranbizumab and aflibercept on systemic concentrations of thromboembolism markers (platelet count, fibrinogen, platelet/lymphocyte ratio) and reassuringly did not find any change one day after the third injection of the loading dose.[375] The same study did however find statistically significant decreases in neutrophils, monocytes and low density lipoprotein-cholesterol after aflibercept, suggesting an interaction between systemic VEGF blockade and inflammation.[375]

Disruption of the normal VEGF signalling cascade has been shown to cause hypertension, glomerular disease, and worsening of proteinuria.[373] There have also been numerous case reports of nephrotoxicity, some with biopsy confirmation, presumed to be caused by intravitreal anti-VEGF drugs (Table 5.1).[254-263, 376-384] This includes development or worsening of hypertension and proteinuria, nephrotic syndrome, and thrombotic microangiopathy. These findings suggest systemic absorption of intravitreally administered anti-VEGF may affect endothelial cells and podocytes in the renal glomeruli.[262]

Author	Proposed renal injury	Anti-vascular endothelial growth factor agent(s)	Number of patients	Study Type
Hanna et al.[262]	Proteinuria, worsening HTN, decreased eGFR, minimal change disease	Bevacizumab	3	Case series
Khneizer et al.[257]	Decreased eGFR	Bevacizumab	1	Case report
Sato et al.[261]	Relapse of nephrotic syndrome	Bevacizumab	1	Case report
Morales et al.[258]	Decreased eGFR, proteinuria	Ranibizumab	1	Case report
Pelle et al.[259]	TMA	Ranibizumab	1	Case report
Perez-Valdivia et al.[260]	Relapse of minimal change disease	No details available	1	Case report
Jamrozy-Witkowska et al.[256]	Decreased eGFR	No details available	1	Case report
Cheungpasitporn et al.[376]	MGN, proteinuria, decreased eGFR	1 – Bevacizumab/Aflibercept 2 – Ranibizumab/Aflibercept	2	Case series
Scott et al.[254]	Decreased eGFR	Bevacizumab	3	RCT data
Georgalas et al.[255]	Decreased eGFR, end-stage renal disease	No details available	2	Case series
Kenworthy et al.[377]	Worsening proteinuria	No details available	1	Case report
Nobakht et al.[378]	Worsening proteinuria, decreased eGFR, GS	Bevacizumab/ Ranibizumab/ Aflibercept	1	Case report
Shye et al.[379]	1 - Worsening proteinuria, decreased eGFR 2 – Decreased eGFR, GS, interstitial nephritis 3 – Decreased eGFR, worsening proteinuria and HTN, GS, interstitial nephritis	1 – Bevacizumab/Ranibizumab 2 – Bevacizumab 3 – Bevacizumab/Ranibizumab	3	Case series
Hanna et al.[263]	Worsening HTN and proteinuria	Bevacizumab/Ranibizumab	1	Case series
Hanna et al.[380]	1 - Worsening HTN and proteinuria, GS, decreased eGFR, TMA 2 – Worsening HTN and proteinuria, GS, decreased eGFR 3 – Worsening HTN and proteinuria, GS, decreased eGFR, TMA	1 - Bevacizumab 2 – Bevacizumab 3 – Ranibizumab/Aflibercept	3	Case series
Valsan and Kazi[381]	AIN	No details available	1	Case report
Touzani et al.[382]	TMA, proteinuria, decreased eGFR	Bevacizumab	1	Case report
Yen and Zhang[383]	Endotheliosis, TMA	No details available	1	Case report
Kakeshita et al.[384]	GS	Aflibercept	1	Case report

Table 5.1: Documented cases of renal toxicity presumed secondary to intravitreal anti-vascular endothelial growth factor.

AIN, allergic interstitial nephritis; HTN, hypertension; eGFR, estimated glomerular filtration rate; GS, glomerulosclerosis; MGN, membranous glomerulonephritis; RCT, randomised controlled trial; TMA, thrombotic microangiopathy

There have been no studies in nAMD patients receiving anti-VEGF therapy that specifically investigates these renal safety concerns. This has been raised by renal medicine specialists as a priority for investigation.[373]

5.1.3 Systemic C-reactive protein

There is strong evidence that chronic inflammation is involved in nAMD pathogenesis.[385] The combination of inflammation with oxidative stress results in outer blood-retinal-barrier breakdown, retinal pigment epithelium dysfunction and photoreceptor damage. In nAMD, the complement system is thought to be dysfunctional, and various genetic complement defects show disease association with nAMD.[386] These include variants on the complement factor H, factor B, C2, C3, C5 and ARMS2 genes.[387] In particular, variants of the complement factor H gene have shown major association with age-related macular degeneration (AMD), increasing the risk by approximately 2-4 fold in heterozygous patients and 7-fold in homozygous patients.[388-392] Furthermore, genetic defects in modulators of neovascularisation such as VEGF, TIMP-3 and lipoproteins (e.g. ApoE) have been association with nAMD.[103] A large genome-wide association study found 52 single-nucleotide polymorphisms which were independently associated with the risk of developing AMD.[104]

C-reactive protein (CRP) is an acute phase reactant protein of the pentraxin group, which modulates the complement system.[393] It is manufactured in the liver in response to inflammation, and is frequently used as biomarker or measure of response to treatment for diseases involving inflammation.[394] Cross-sectional and case-control studies have investigated CRP in nAMD. Some groups have shown serum CRP concentration is higher in nAMD patients than controls.[395-401] Aqueous CRP concentration has also been found to be

higher in nAMD patients than cataract control groups.[402] Furthermore, a large meta-analysis of 41,690 patients found an elevated CRP level (> 3 mg/L) conferred a two-fold increased likelihood of developing AMD versus low CRP levels (< 1 mg/L).[403] In contrast, other studies have demonstrated no association between systemic circulating CRP levels and AMD.[404-407] A cohort study of 2,868 patients found baseline CRP concentration was not associated with an increased 5-year incidence of any type of AMD.[408]

This divided opinion resulted in groups questioning whether a higher sensitivity CRP test, referred to as 'high sensitivity CRP' (hsCRP), which can better differentiate low concentrations of CRP, would be a more accurate analyte. The Rotterdam study found elevated baseline serum hsCRP concentration was associated with the development of early and late AMD at a mean follow-up of 7.7 years in 4,914 participants.[409] This was supported by the Women's Health Study of 27,687 patients, which suggested elevated serum hsCRP concentration was a positive predictive biomarker for the development of AMD at 10 years.[410] There was also modest evidence that higher baseline hsCRP concentration increased the 20 year cumulative incidence of early AMD in the Beaver Dam Eye Study.[411] Furthermore, a case-control study found higher hsCRP levels in nAMD patients versus controls.[412] However, Klein *et al* found a lack of association between hsCRP and the 2 year risk of nAMD development in a cross-sectional study of 5,887 patients.[413]

More recently, a UK biobank study of mendelian randomization has shown genetic variants which predict elevated serum CRP concentrations are strongly associated with nAMD.[414] Whether a high CRP level and nAMD development is causal is difficult to determine from the currently available literature. In addition, whether circulating CRP represents a biomarker for disease, or if CRP is directly implicated in the local disease process remains unknown.[393]

How serum hsCRP concentration changes in response to nAMD treatment, and in particular over the period immediately after an anti-VEGF injection, has not been investigated. Change may be due to the reduction in pro-inflammatory activity of macular bound CRP, a reflection of reduced inflammation and disease activity, or related to drug absorption directly affecting systemic CRP.

We hypothesise that serum hsCRP may decrease following an anti-VEGF injection, in response to reduced disease activity, before rising again when drug effect wears off prior to the next injection. If this is the case, hsCRP could potentially be used as an acute marker of disease activity and help clinicians with dosing decisions, for example, to refine selection of suitable dosing intervals, or to predict response to different anti-VEGF agents and inform choice.[271] In addition, this could improve our understanding of the disease process, systemic risks, and help with the development of future therapeutics.

5.1.4 Systemic cytokines

Cytokines are modulators of the immune system, and have been suggested to have a substantial role in the pathogenesis of nAMD.[271] There have been numerous studies assessing nAMD cytokine biomarkers, primarily comparing disease cases with controls, or the change in ocular biomarker profile one month following anti-VEGF treatment (Table 5.2). In addition, cytokines have been shown to be biomarkers of response to anti-VEGF treatment. For example, higher interleukin-6 (IL-6) and interleukin-8 (IL-8) aqueous concentrations have been shown to be positively correlated with volume of macular oedema in nAMD.[415] Also, aqueous concentrations of IL-6 and monocyte chemoattractant protein (MCP-1) were found to decrease over a 1 year period of anti-VEGF treatment for nAMD.[416] These findings

suggest cytokine concentration and modulation may be related to disease development, activity and response to anti-VEGF treatment.

Cytokine	Function	Findings in nAMD patients
Angiogenin (Ang)	Pro-angiogenic; activates vessel endothelium and smooth muscle cells[417]	Increased [aqueous] in nAMD patients[a,f] versus controls[418, 419] Increased [plasma] in nAMD patients[f] versus controls[419]
Angiopoietin 2 (Ang-2)	Pro-angiogenic; loss of endothelial cell junction integrity, and angiogenic sprouting[420]	Increased [aqueous] post-bevacizumab[b,a,d] for nAMD[421-423]
Angiopoietin-like 4 (ANGPTL4)	Pro-angiogenic; increases vasopermeability[424]	Increased [aqueous] in nAMD patients[a] versus controls[425]
Basic fibroblast growth factor (bFGF)	Pro-angiogenic, involved in tissue repair[426]	Decreased [aqueous] in nAMD patients[f] versus controls[426]
Chemokine ligand 1 (CXCL-1)	Activates and recruits neutrophils[427]	Decreased [aqueous] post 2nd ranibizumab[d] for nAMD[428]
Chemokine ligand 9 (CXCL-9) or MIG	Chemoattractant; causes tissue extravasation[429]	Increased [aqueous] in nAMD patients[e,c,a] versus controls[418, 429-431]
Chemokine ligand 10 (CXCL-10) or IP-10	Pro-angiogenic; Chemoattractant for immune cells[271]	Increased [aqueous] in nAMD patients[a,e,c] versus controls[402, 428, 432-435] Decreased [aqueous] post 2nd ranibizumab[d] for nAMD[428] Decreased [aqueous] in nAMD patients[a] versus controls[436]
Chemokine ligand 12 (CXCL-12) or SDF1	Pro-angiogenic; Pro-inflammatory; recruits endothelial progenitor cells[271]	Increased [aqueous] in nAMD patients[a] versus controls[428] Decreased [aqueous] post 2nd ranibizumab[d] for nAMD[428]
Chemokine ligand 13 (CXCL-13) or BLC or BCA-1	Pro-angiogenic[437]	Increased [aqueous] in nAMD patients[a] versus controls[428] Decreased [aqueous] post 2nd ranibizumab[d] for nAMD[428]
Endostatin	Anti-angiogenic[438]	Increased [aqueous] in nAMD patients[a] versus controls[438]
Epidermal growth factor (EGF)	Modulates cell proliferation and differentiation[271]	Increased [aqueous] in nAMD patients[e] versus controls[430]
Hepatocyte growth factor (HGF)	Pro-angiogenic; stimulates cell proliferation and migration[439]	Increased [aqueous] in nAMD patients[e,a] versus controls[422, 430] Increased [aqueous] post-bevacizumab[b,d] for nAMD[421, 423] Decreased [aqueous] in nAMD patients[a] versus controls[436]
Insulin-like growth factor 1 (IGF-1)	Pro-angiogenic[440]	Increased [aqueous] in nAMD patients[a] versus controls[441]
Intercellular adhesion molecule 1 (ICAM-1)	Pro-angiogenic; activates vascular endothelial cells[430]	Increased [aqueous] in nAMD patients[c,e] versus controls[430, 442, 443]
Interferon gamma (IFN-γ)	Activates macrophages, and immunomodulator[271]	Increased [aqueous] in nAMD patients[a] versus controls[418]
Interleukin 1-alpha (IL-1α)	Proinflammatory; stimulates fibroblast proliferation[271]	Decreased [aqueous] post 2nd bevacizumab[d] for nAMD[444] Increased [aqueous] in nAMD patients[e] versus controls[430]
Interleukin 1-beta (IL-1β)	Proinflammatory; produced by T lymphocytes[428]	Increased [vitreous] in nAMD patients[f] versus controls[445]
Interleukin 2 (IL-2)	Pro-angiogenic; proinflammatory[446]	Decreased [aqueous] in nAMD patients[c] versus controls[447] Increased [aqueous] in nAMD patients[c] versus controls[434]
Interleukin 3 (IL-3)	Pro-angiogenic; pro-inflammatory[448]	Increased [aqueous] in nAMD patients[e] versus controls[430]
Interleukin 6 (IL-6)	Pro-angiogenic; pro-inflammatory[436]	Decreased [aqueous] in nAMD patients[a] versus controls[435, 449] Increased [aqueous] in nAMD patients[e,g,f] versus controls[430, 450, 451] Increased [aqueous] 2 days post 1st bevacizumab for nAMD compared to baseline[418] Decreased [aqueous] post 2nd ranibizumab[d] for nAMD[428]

		Decreased [aqueous] in nAMD patients[d] over 1 year course of ranibizumab[416]
Interleukin 8 (IL-8) or CXCL-8	Pro-angiogenic; regulate immune response[452]	Increased [aqueous] in nAMD patients[e,c,a,f] versus controls[422, 430, 434, 451, 453] Increased [aqueous] 2 days post 1st bevacizumab for nAMD compared to baseline[418] Increased [aqueous] post-bevacizumab[b,d] for nAMD[421, 423]
Interleukin 10 (IL-10)	Pro-angiogenic; anti-inflammatory[454]	Increased [aqueous] in nAMD patients[a] versus controls[428] Decreased [aqueous] post 2nd ranibizumab[d] for nAMD[428]
Interleukin 12 (IL-12)	Anti-angiogenic[271]	Increased [aqueous] in nAMD patients[e,a] versus controls[430, 432]
Interleukin 13 (IL-13)	Regulates IgE synthesis and mediates allergic inflammation[455]	Decreased [aqueous] in nAMD patients[a] versus controls[436]
Monocyte chemoattractant protein 1 (MCP-1) or CCL2	Pro-angiogenic; recruits inflammatory cells (e.g. monocytes and macrophages) to sites of inflammation[456, 457]	Increased [aqueous] in nAMD patients[a,c,e,f] versus controls[419, 428-430, 434, 442, 451, 453, 458] Decreased [aqueous] in nAMD patients[d,a] versus controls[436, 459] Decreased [aqueous] post aflibercept[d] for nAMD[453] Decreased [aqueous] post ranibizumab[d] for nAMD[428] Decreased [aqueous] in nAMD patients[d] over a 1 year course of ranibizumab[416]
Pigment epithelium-derived factor (PEDF)	Anti-angiogenic[460]	Decreased [vitreous] in nAMD patients[a] versus controls[460, 461] Increased [aqueous] in nAMD patients[a] versus controls[462, 463] Increased [aqueous] post-bevacizumab[h] for nAMD[464] Decreased [aqueous] post-ranibizumab[d] for nAMD[465]
Placental Growth Factor (PlGF)	Pro-angiogenic; pro-inflammatory[271]	Increased [aqueous] in nAMD patients[a] versus controls[466] Decreased [aqueous] post-ranibizumab[d] for nAMD[466]
Platelet Derived Growth Factor-AA (PDGF-AA)	Pro-angiogenic; chemoattractant and activates fibroblasts[467]	Decreased [aqueous] post-aflibercept[d] for nAMD[453, 459] Increased [aqueous] in nAMD patients[f] versus controls[453]
Transforming growth factor beta (TGF-β)	Pro-angiogenic; induces VEGF expression[468]	Increased [aqueous] in nAMD patients[a] versus controls[463] Increased [vitreous] in nAMD patients[f] versus controls[469] Decreased [aqueous] in nAMD patients[d] over a 1 year course of ranibizumab[416]
Tumour necrosis factor alpha(TNF-α)	Pro-angiogenic; pro-inflammatory[447]	Decreased [aqueous] in nAMD patients[c] versus controls[429, 447]
Vascular cell adhesion protein 1 (VCAM-1)	Pro-inflammatory; mediates adhesion of white cells to vascular endothelium[470]	Increased [aqueous] in nAMD patients[c,a,f] versus controls[434, 442, 443, 470]
Vascular endothelial growth factor (VEGF)	Pro-angiogenic; increases vascular permeability and vasodilation[271]	Increased [vitreous] in nAMD patients[a] versus controls[461] Increased [aqueous] in nAMD patients[a,g,c,f] versus controls[419, 425, 432, 434, 436, 441, 450, 451, 453, 462, 466, 471] Decreased [aqueous] post bevacizumab[d] for nAMD[444, 445, 464, 471] Decreased [vitreous] post bevacizumab[b] for nAMD[472] Decreased [aqueous] post ranibizumab[d] for nAMD[425, 465, 473, 474] Increased [plasma] in nAMD[a,f] patients versus controls[419, 449] Decreased [aqueous] in nAMD[a] patients versus controls[449] Decreased [aqueous] post bevacizumab[d] for nAMD[471] Decreased [aqueous] 2 days post bevacizumab for nAMD compared to baseline[418] Decreased [aqueous] at numerous time points post-aflibercept[f,h] for nAMD [453, 459, 475-477] Decreased [aqueous] at numerous time points post-bevacizumab[a,c] for nAMD[421, 423, 478] Decreased [aqueous] at numerous time points post-ranibizumab[d,f] for nAMD[453, 459, 466, 470] Decreased [plasma] at numerous time points post-ranibizumab[d] for nAMD[470]

Table 5.2: Studies investigating change in cytokine concentrations in neovascular age-related macular degeneration.

[a] – treatment naïve; [b] – post-preop bevacizumab, duration undocumented and multiple timepoints thereafter; [c] – previous anti-vascular endothelial growth factor, unknown duration; [d] – one month since previous anti-VEGF; [e] – at least 6 months since previous anti-vascular endothelial growth factor; [f] – insufficient detail about whether previous anti-vascular endothelial growth factor received; [g] – at least 3 months since previous anti-vascular endothelial growth factor; [h] – at least 2 months since previous anti-vascular endothelial growth factor.
[aqueous], aqueous concentration; nAMD, neovascular age-related macular degeneration; [plasma], plasma concentration; [vitreous], vitreous concentration

A recent meta-analysis of 95 studies investigated ocular cytokine concentrations in nAMD patients and attempted to establish their role as diagnostic or prognostic biomarkers.[271] Vascular endothelial growth factor, MCP-1, monokine induced by interferon-gamma (MIG) and transforming growth factor beta (TGF- β) were found to be elevated in nAMD patients compared to controls. These cytokines have pro-inflammatory, angiogenic and cell regulation roles. Many other cytokines were investigated in the meta-analysis, but none were found to have a statistically significant difference between nAMD cases and controls.

Vascular endothelial growth factor is produced by endothelial cells, activated T-cells, macrophages, and other cell types.[479] When hypoxia occurs, the expression of hypoxia-inducible factor-1 increases the production of VEGF.[480] The major isoform of VEGF is VEGF-A, which is relatively specific for endothelial cells.[480] This isoform exerts its pro-angiogenic functions of endothelial cell proliferation and increasing vascular permeability by binding primarily to two tyrosine kinase receptors (VEGFR-1 and VEGFR-2).[480] In contrast, VEGF-B has a wider tissue distribution with high concentration in skeletal muscle. It binds to

VEGFR-1 and activates apoptotic pathways. Another member of the VEGF family is platelet derived growth factor (PlGF), which is expressed widely in the body.[481] It has a similar structure to VEGF-A, but only binds to VEGFR-1.[482] Ranibizumab and brolucizumab bind to VEGF-A only, whereas aflibercept binds to VEGF-A, VEGF-B and PlGF.

The other members of the VEGF group are VEGF-C, VEGF-D, and VEGF-E.[480] The ligands VEGF-C and VEGF-D bind to VEGFR-2, as well as VEGFR-3 which is also known to produce pro-angiogenic functions when activated.[483] Patients with nAMD have been shown to have higher expression of VEGF-C and VEGF-D in the retinal pigment epithelium (RPE), and higher systemic circulating concentrations of VEGF-C compared to healthy controls.[484, 485] In addition, both VEGF-C and VEGF-D can be upregulated when VEGF-A is inhibited.[486] A therapeutic agent, OPT-302, binds to and blocks the activity of VEGF-C and VEGF-D, is currently under investigation as a treatment for nAMD.[486] The Phase 2b clinical trial of monthly intravitreal OPT-302 in combination with intravitreal ranibizumab versus monthly ranibizumab alone, met its primary endpoint of statistical superiority over ranibizumab monotherapy for mean visual acuity at 24 weeks.[486] Two large phase 3 studies (Study of OPT-302 in Combination with Ranibizumab (ShORe) and Combination of OPT-302 with Aflibercept Study (COAST), using OPT-302 in combination with ranibizumab and aflibercept, respectively) are underway to further assess its efficacy and safety.[487, 488]

The influence of intravitreal anti-VEGF agents on intraocular VEGF concentration has been extensively studied. The vast majority of studies, including a meta-analysis, have shown VEGF is elevated in nAMD patients versus controls, significantly reducing one month after an intravitreal anti-VEGF injection.[271]

There has also been a focus on systemic VEGF concentration after intravitreal anti-VEGF. Wang et al. noted significantly reduced serum and plasma VEGF concentrations one month following aflibercept, but no change after ranibizumab.[272] Another study found reduced systemic VEGF concentration after intravitreal aflibercept or bevacizumab, but not ranibizumab, attributing their findings to drug size and speed of clearance.[198, 229] Similar results have been described in patients with diabetic macular oedema (DMO).[489] Aside from VEGF, systemic cytokine profiles in nAMD have been less well investigated. Cytokine concentrations are known to be influenced by other systemic disease, age and ethnicity, which causes difficulty when forming a control group and attributing disease association.

It is possible that systemic cytokine concentrations change within the one month period of an intravitreal anti-VEGF injection. This intra-individual temporal observation bypasses the problem of comparison with a 'normal' patient cytokine profile, and has the potential to give further insight into their role in nAMD, and the effect of anti-VEGF treatment. Repeated ocular samples over a one month period are unethical, and therefore a systemic cytokine profile, assessed by venous blood sampling, is more practical.

5.1.5 Objectives of the study

- To assess the serum pharmacokinetics of intravitreal ranibizumab and aflibercept.
- To determine whether the serum pharmacokinetics of intravitreal ranibizumab and aflibercept vary according to vitreous status.
- To investigate the effect of intravitreal ranibizumab and aflibercept on systemic markers of renal function.

- To investigate whether those with pre-existing renal dysfunction or associated diseases (e.g. hypertension) are at an increased risk of nephropathy after intravitreal ranibizumab and aflibercept.
- To establish whether serum hsCRP concentration fluctuates after intravitreal ranibizumab or aflibercept.
- To determine whether the systemic serum cytokine profile changes after intravitreal ranibizumab or aflibercept.

5.2 **Methods**

5.2.1 Study Design

Ethical approval for the study was obtained from the National Research Ethics Service (NRES) Committee London – Central, NHS Health Research Authority. The research protocol and documentation were approved by the King's College London Research and Development department. Although ranibizumab and aflibercept were used within their marketing authorisation, this study was classified as a Clinical Trial of an Investigational Medicinal Product (CTIMP), as is often the case for studies gathering pharmacokinetics data on licensed medicines.

This was a single centre, prospective, pharmacokinetic and pharmacodynamic study comparing ranibizumab and aflibercept clearance in two groups of participants; those with and without prior vitrectomy (Figure 5.1). It compared ranibizumab clearance to aflibercept clearance as well as determining their effect on systemic renal and inflammatory markers. Participants were already receiving ranibizumab 0.5 mg or aflibercept 2 mg therapy irrespective of their participation and therefore this study did not alter medical management or the choice of drug.

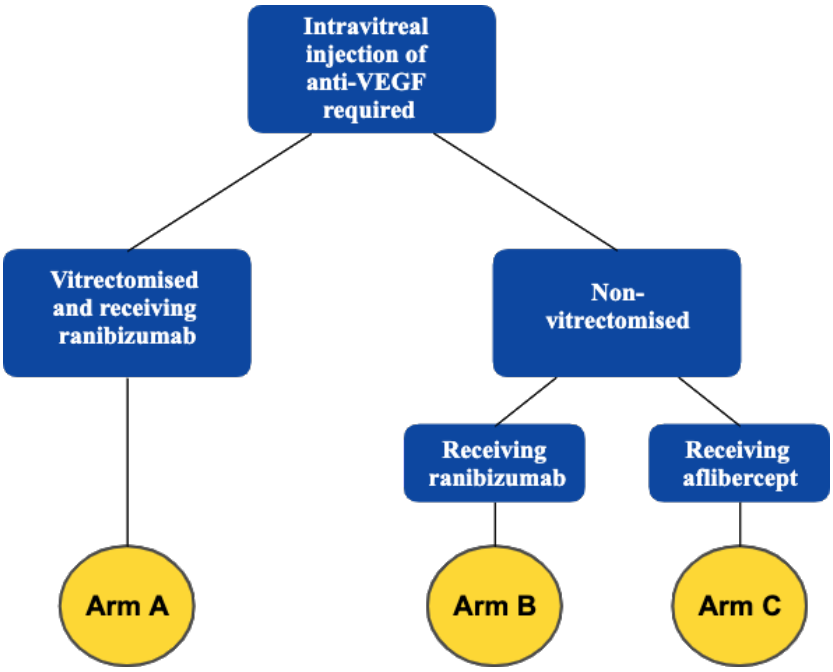


Figure 5.1: Study design and allocation to three arms of study

5.2.2 Participant eligibility

The following criteria were used to determine participant eligibility:

Inclusion Criteria

1. Adults of either sex aged 55 years and older
2. Active nAMD in the study eye
3. Intravitreal dose of ranibizumab 0.5 mg or aflibercept 2 mg required, as per current clinical guidelines
4. Venous access that was sufficient to allow easy blood sampling on a frequent basis
5. Able to give written consent
6. Willingness to comply with all study procedures

Exclusion Criteria

1. Myopia greater than 8 dioptres in the study eye
2. Axial length of eye under 20 mm or over 26 mm
3. Aphakia in the study eye
4. Pseudophakia with a defect in the posterior capsule
5. Glaucoma in study eye
6. Current renal dialysis
7. Presence of inflammatory eye conditions, such as uveitis, or systemic conditions likely to elevate hsCRP
8. Intraocular surgery within 6 months of enrolment, except for routine phacoemulsification cataract surgery that could occur within 4 months of enrolment
9. Current treatment for nAMD with an intravitreal agent other than ranibizumab or aflibercept in the study eye. Expected to change their anti-VEGF agent during the study period.
10. Known significant allergy to ranibizumab or aflibercept
11. Participants who, in the opinion of the investigator, would not be willing or able to comply with the study protocol or provide informed consent
12. Participants with severe anaemia
13. Participants who had received anti-VEGF therapy in either eye within 8 weeks of enrolment, or who were likely to require anti-VEGF treatment in the fellow eye during the course of venous sampling.
14. Participants taking any topical (skin or eye), periocular, intraocular, local or systemic treatment with immunosuppressive or anti-inflammatory agents, such as steroids, steroid sparing agents, and non-steroidal anti-inflammatory drugs. Participants who had received any of these agents within 2 months prior to enrolment were also

excluded, as were those thought likely to receive these medications during the course of venous sampling.

5.2.3 Sample Size

The sample size power calculations were based on the pharmacokinetic component of the study. A rabbit model study showed the half-life of triamcinolone acetonide in a vitrectomised eye was 55% of that in a non-vitrectomised eye (1.57 versus 2.89 days).[490] Another study in rabbits calculated the vitreous half-life of ranibizumab at 2.88 days.[192] The rabbit has a vitreous volume of approximately 1.5 ml.[188] The human vitreous volume measures approximately 4.5 ml, which would be expected to reduce the half-life, but by how much is not known. However, a mathematical model of ranibizumab clearance estimated the half-life of ranibizumab in humans as 4.75 days.[211]

In the absence of much data on systemic drug levels post-intravitreal injection, we assumed that systemic levels would approximately mirror intraocular half-lives. Therefore, we assumed that the intravitreal half-life of ranibizumab in non-vitrectomised eyes was 4.75 days, reducing to 55% of this value in vitrectomised eyes. We assumed the standard deviation to be half the mean value for both arms, as variance was not published. Assuming a half-life of 4.75 ± 2.375 days in non-vitrectomised eyes, 2.61 ± 1.305 in vitrectomised eyes, a power of 90%, and significance at the 0.05 level, the study required a sample size of 14 participants in Arm A and Arm B.[211]

A study in rabbits showed the vitreous half-life of aflibercept to be 4.58 days.[208] Using the same percentage increase in half-life from rabbits to humans (as described above) of 64.9%,

we estimated the vitreous half-life of aflibercept in humans to be 7.83 days. For power calculations we therefore assumed a mean half-life of 4.75 ± 2.375 days in Arm B and 7.83 ± 3.91 days in Arm C, with power set at 90%, and significance at 0.05. This produced a sample size of 24 participants in each of Arms B and C.

Therefore, the study aimed to recruit 62 participants; fourteen participants in Arm A, 24 in Arm B, and 24 in Arm C. We planned to replace participants in the study if they failed to complete the follow-up, until target recruitment levels were met. We recognised that Arm A would be the hardest to recruit to, as there are far fewer patients who have had a vitrectomy than have not. Since Arms B and C were both larger than necessary for the vitrectomised versus non-vitrectomised endpoint comparison, under-recruitment to Arm A would not necessarily result in insufficient power.

5.2.4 Study visits and baseline assessment

All participants who met the inclusion and exclusion criteria underwent a full history and ophthalmic examination to determine baseline characteristics. A best-corrected Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity was measured for both eyes.

An important aspect of the study was determining the vitreous status. This was required for allocating vitrectomised patients to Arm A, as well as deciding whether a PVD was present in those within Arms B and C. Slit-lamp biomicroscopy, OCT and B-scan ultrasonography were used to confirm the presence or not of a complete PVD. If there was any uncertainty or disagreement of vitreous status, the participant was excluded from the study. Participants

with peri-foveal vitreous detachment that were attached at both the disc and macula were assigned to the nPVD group.

After history and examination were complete, a baseline blood sample was taken followed by the anti-VEGF intravitreal injection. Patient care was then transferred to the NIHR Wellcome King's Clinical Research Facility (CRF) at King's College Hospital for serial blood testing. No other ophthalmology clinic visits were required for the study, but routine care continued as necessary.

5.2.5 Venous blood sampling

A study measuring the ranibizumab concentration in the vitreous and serum of monkeys showed that the peak serum concentration occurred 6 hours following the intravitreal injection.[189] To estimate ranibizumab and aflibercept clearance therefore, it was important to measure serum drug concentrations at several intervals within the first 24 hours. Participants were scheduled to have venous blood sampling at baseline (prior to injection) as well as the following times after their ranibizumab or aflibercept injection (Figure 5.2):

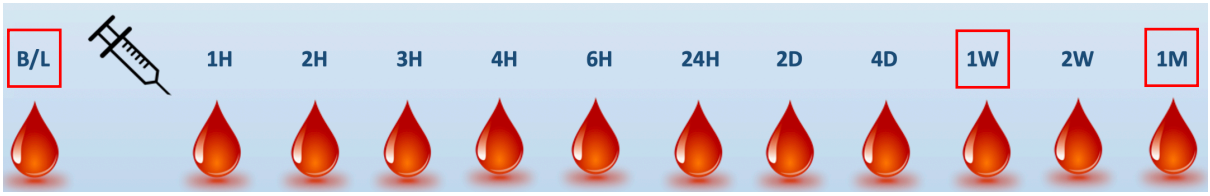


Figure 5.2: Timing of blood samples.

The syringe indicates the timing of anti-vascular endothelial growth factor injection. Red boxes indicate time points when cytokines, renal markers and inflammatory markers were measured.
B/L, baseline; D, days; H, hours; W, weeks; M, month.

The drug level (ranibizumab or aflibercept) was measured at every time point. The following renal markers, inflammatory markers and cytokines were measured at the red boxed time points (baseline, one week and one month) shown above;

Renal Markers: urea, creatinine, sodium, potassium, urate
Inflammatory Markers: high sensitivity c-reactive protein (hsCRP)
Cytokines: epidermal growth factor (EGF), interleukin-1a (IL-1a), interleukin-1b (IL-1b), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), interferon-gamma (IFN-γ), tumour necrosis factor-alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1), platelet-derived growth factor-AA (PDGF-AA), VEGF (vascular endothelial growth factor).

For pragmatic reasons, we recognised that blood samples might not be possible at the exact time points after the injection. We therefore used the above time points as a guide, but aimed for all samples to be taken ± 10% margin. We recorded the exact time that the blood sample was taken to ensure accurate pharmacokinetic data.

Venous blood sampling was performed and processed by the CRF. On day one, samples were drawn from a cannula, preventing the need for multiple venepunctures. Subsequent day blood sampling was performed using standard venepuncture technique. One 5 ml Serum Separator

Tube (SST) II tube and one 4 ml ethylenediaminetetraacetic acid (EDTA) tube were collected at every timepoint.

After collection, blood samples were left to rest for 15 minutes before undergoing centrifugation at 3000 rpm for 15 minutes at room temperature. Aliquots of serum and plasma were then stored at -80° Celsius in the pathology department (ViaPath) at King's College Hospital, London.

5.2.6 Anti-vascular endothelial growth factor injections

As previously discussed, participation in the study did not influence clinical decision making on the type or timing of the anti-VEGF injection delivered prior to sampling. Administration of intravitreal ranibizumab 0.5mg or aflibercept 2mg was performed using standard technique, via a pars plana injection.

5.2.7 Assays

The Advia chemistry system (Siemens Healthcare Diagnostics Inc., Munich, Germany) was used to test blood samples for urea and electrolytes. Sodium and potassium concentration were based on indirect potentiometric procedures using ion selective electrodes (ISE).[491, 492] The urea concentration method was based on the Roch-Ramel enzymatic reaction using urease and glutamate dehydrogenase.[493] Creatinine was measured using a standard method based on the reaction of picric acid with creatinine in an alkaline medium.[494]

The aflibercept assay (Eagle Biosciences. Inc., Nashua, NH, USA) utilised a sandwich type enzyme-linked immunosorbent assay (ELISA).[495] The ranibizumab assay (Krishgen BioSystems, Mumbai, India) also utilised a sandwich type ELISA.[496]

All twelve cytokines were simultaneously quantified using the Randox Evidence Investigator™ Biochip Array technology (Randox Laboratories, Crumlin, UK), which incorporates the principles of a sandwich chemiluminescent immunoassay using a solid state device containing antibodies specific to the different cytokines and growth factors.[497] Human PDGF-AA measurement utilised a solid phase ELISA which incorporates a quantitative sandwich enzyme immunoassay technique (R&D systems, Minneapolis, MN, USA).[498]

The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, following advice from a renal medicine specialist.[499] This is considered the optimal method for estimating eGFR in normal adults. The equation uses serum creatinine concentration, age, sex and race in its calculation; [500]

$eGFR = 141 \times \min(S_{Cr}/\kappa,1)^{\alpha} \times \max(S_{Cr}/\kappa,1)^{-1.209} \times 0.993^{Age} \times 1.018$ (if female) x 1.159 (if black)

Where:

eGFR is estimated glomerular filtration rate (ml/min/1.73m^2)

S_{Cr} is standardised serum creatinine (μmol/L)

κ is 61.9 for females and 79.6 for males

α is -0.329 for females and -0.411 for males

min indicates the minimum of S_{Cr}/κ or 1

max indicates the maximum of S_{Cr}/κ or 1

5.2.8 Outcomes

1. Serum pharmacokinetics of intravitreal ranibizumab in vitrectomised, PVD and nPVD eyes.
2. Serum pharmacokinetics of intravitreal aflibercept in PVD and nPVD eyes.
3. Serum urea, urate, creatinine, eGFR and electrolyte concentrations
4. Serum hsCRP concentration
5. Serum cytokine concentrations (EGF, IL-1a, IL-1b, IL-2, IL-4, IL-6, IL-8, IL-10, IFN-γ, TNF-α, MCP-1, VEGF in ng/L; PDGF-AA in ng/ml).

5.2.9 Statistical plan

Statistical comparison of baseline characteristics was performed between arm A and B, and between arm B and C, in line with the objectives of the study. All means were documented plus or minus one standard error of the mean. A p-value of ≤ 0.05 was considered significant. The comparison of numerical baseline characteristics was performed using a 2-tailed t-test with 95% confidence intervals of the difference for independent samples. Fisher's exact test was used to compare baseline characteristics of categorical variables. Summary measures for categorical baseline characteristics were frequencies and percentages. Normality assessment was performed using histograms to assess for any significant skewness of data, with confirmation obtained from a local expert statistician.

Non-compartmental analysis (NCA) of serum drug concentration-time data was used to calculate the following ocular parameters: area under curve (AUC); maximum observed concentration (C_{max}); time to maximum concentration (t_{max}) and systemic half-life ($t_{1/2}$)

assuming first-order elimination. Non-compartmental analysis is a standard technique for assessing pharmacokinetic data with fewer model assumptions and is an alternative to using nonlinear regression analysis.[501] It estimates AUC using the trapezoidal rule, whereby each segment of the serum-time curve is determined by multiplying the average concentration by the segment width. Total AUC is then calculated by adding all segments to give an approximation of degree of exposure from a drug. For half-life calculations, a log-linear scale with line of best fit was used to identify and remove outliers ($R^2 < 0.95$).

An independent samples 2-tailed t-test was used to evaluate differences in baseline serum concentrations of two groups for normally distributed data. A one-way analysis of variance (ANOVA) was used to compare baseline serum markers for three subgroups. If a significant difference in baseline markers across groups was noted on ANOVA, then Tukey's test was planned for post-hoc analysis.

The statistical plan for assessing changes in serum concentrations over time was as follows. For normally distributed data, a paired sample t-test was used to compare across two time points. For the analysis of change across three time points, for different subgroups (e.g. drug, vitreous status), a factorial analysis (two-way mixed ANOVA) was used.

Microsoft Excel (Version 16.54, Microsoft Corporation, Redmond, WA, USA) was used for pharmacokinetic analysis. IBM SPSS Statistics (Version 26, IBM, Armonk, NY, USA) was used for all other statistical analysis.

Adverse and serious adverse events were coded using Medical Dictionary for Regulatory Activities (MedDRA) and reported for the entire cohort in a descriptive manner.

5.3 **Results**

5.3.1 Recruitment

Recruitment commenced in June 2014 and finished in June 2019. A decision was taken to terminate the study early. Arms B and C had fully recruited by this stage, but Arm A remained short of participants. It was agreed by the trial team to stop recruitment at this point and end the study early due to the low likelihood of being able to further recruit to Arm A, despite extensive effort.

Pre-screening of participants was performed using the hospital electronic medical record to identify those who were likely to meet the criteria for enrolment. In total, 59 participants were invited for screening. Of these, the inclusion and exclusion criteria were met by 57. Table 5.3 shows the reasons for screen failure.

Reason	**Number of participants**
Taking steroid or non-steroidal anti-inflammatory medication	1
Aphakia	1

Table 5.3: Reasons for screen failure in VITCLEAR study.

5.3.2 Early withdrawal

Four participants did not complete the study. In case one, it was not possible to site an intravenous cannula or perform venepuncture practically, and therefore they were withdrawn from the study. Case two withdrew consent following recruitment due to an unwillingness to

attend all study visits. Case three was recruited into Arm A, but it was subsequently realised that they had received aflibercept rather than ranibizumab which invalidated their participation, and was therefore withdrawn from the study. Case four had already been recruited and completed the study at an earlier date, and therefore her second attendance was deemed invalid.

5.3.3 Demographics

Fifty three participants completed the study. The baseline characteristics were well matched between arms (Table 5.4). The mean ages were 76.4, 79.3, and 77.5 years for arms A, B and C, respectively. Arm A had a longer disease duration (months) than Arm B, although this did not quite reach statistical significance (60.4 ± 12.7 vs 33.2 ± 5.8; p = 0.06). There was no statistically significant difference in disease duration (months) between arms B and C (33.2 ± 5.8 vs 38.5 ± 6.7; p = 0.55). The number of previous anti-VEGF injections was 14.4 ± 3.9, 10.8 ± 1.9 and 17.7 ± 2.9 for arms A, B, and C, respectively, with no significant difference between arms A and B (p = 0.43). There was a significant difference in previous anti-VEGF injections between arms B and C (10.8 ± 1.9 vs 17.7 ± 2.9; p = 0.05).

Baseline visual acuity (ETDRS letters) was similar between arms B and C (59.0 ± 3.3 vs 61.7 ± 2.3; p = 0.49), but was significant worse in arm A than arm B (34.6 ± 8.9 vs 59.0 ± 3.3; p = 0.01). There was a significantly larger proportion of pseudophakia patients in the vitrectomy arm compared to Arm B (100% vs 37.5%; p = 0.02). This would be expected due to the high likelihood of cataract formation, and subsequent cataract surgery, after vitrectomy surgery.[502]

	Arm A (Vitrectomy + Ranibizumab) n = 5	Arm B (Ranibizumab) n = 24	Arm C (Aflibercept) n = 24	p-value (95% C.I.) Arm A vs Arm B	p-value (95% C.I.) Arm B vs Arm C
Age (years)					
Range	72 - 81	68 - 96	62 – 96		
Mean ± SEM	76.4 ± 2.0	79.3 ± 1.5	77.5 ± 1.7	*0.40 (-9.9, 4.1)*	*0.44 (-2.8, 6.4)*
Gender					
Male (n, %)	1 (20.0%)	8 (33.3%)	10 (41.7%)		
Female (n, %)	4 (80.0%)	16 (66.7%)	14 (58.3%)	*1.00*	*0.77*
Study Eye					
Right (n, %)	2 (40.0%)	15 (62.5%)	15 (62.5%)		
Left (n, %)	3 (60.0%)	9 (37.5%)	9 (37.5%)	*0.62*	*1.00*
Duration of Disease (months)					
Range	12 - 84	1 - 99	4 - 105		
Mean ± SEM	60.4 ± 12.7	33.2 ± 5.8	38.5 ± 6.7	*0.06 (-1.5, 56.0)*	*0.55 (-23.2, 12.6)*
No. of prev. anti-VEGF injections					
Range	5 - 25	0 - 40	0 - 46		
Mean ± SEM	14.4 ± 3.9	10.8 ± 1.9	17.7 ± 2.9	*0.43 (-5.7, 13.0)*	***0.05** (-13.9, 0.1)*
Refractive Status					
Emmetropic (n, %)	4 (80.0%)	17 (70.8%)	14 (58.3%)		
Myopic (n, %)	0 (0.0%)	3 (12.5%)	5 (20.8%)		
Hypermetropic (n, %)	1 (20.0%)	4 (16.7%)	5 (20.8%)	*0.71*	*0.64*
Visual Acuity					
Range	11 - 52	28 - 83	42 - 75		
Mean ± SEM	34.6 ± 8.9	59.0 ± 3.3	61.7 ± 2.3	***0.01** (-41.1, -7.6)*	*0.49 (-10.7, 5.2)*
Lens Status					
Phakic (n, %)	0 (0.0%)	15 (62.5%)	13 (54.2%)		
Pseudophakic (n, %)	5 (100.0%)	9 (37.5%)	11 (45.8%)	***0.02***	*0.77*
Axial Length					
Range	22.7 – 24.7	21.5 – 25.5	21.9 – 24.9		
Mean ± SEM	23.5 ± 0.5	23.1 ± 0.2	23.5 ± 0.2	*0.49 (-0.7, 1.4)*	*0.20 (-0.9, 0.2)*
Vitreous Status					
PVD (n, %)	N/A	12 (50.0%)	10 (41.7%)		
No PVD (n, %)	N/A	12 (50.0%)	14 (58.3%)	-	*0.77*

Table 5.4: Baseline characteristics of participants who completed the study. 95% Confidence intervals of the difference are displayed for 2-tailed t-tests of continuous variables. Categorical variables p-values are calculated using Fisher's exact test.

PVD, posterior vitreous detachment; SEM, standard error of the mean; VEGF, vascular endothelial growth factor.

5.3.4 Pharmacokinetic analysis

Mean serum drug concentrations are displayed in Table 5.5, and Figures 5.3 - 5.5 show concentration versus time curves for each study arm. Missing samples were due to missed participant visits or insufficient serum quantity for assay analysis.

	Arm A **Mean concentration of ranibizumab in ng/ml** (n; range)	**Arm B** **Mean concentration of ranibizumab in ng/ml** (*n; range*)	**Arm C** **Mean concentration of aflibercept in ng/ml** (*n; range*)
Time			
B/L	1086 (4; 300 – 2708)	881 (19; 179 – 2101)	8.29 (23; 3.31 – 17.65)
1 Hour	1057 (4; 346 – 2680)	866 (21; 183 – 2058)	8.34 (23; 1.12 – 19.00)
2 Hours	1082 (4; 321 – 2705)	892 (21; 190 – 2223)	9.97 (23; 1.87 – 26.30)
3 Hours	1102 (4; 308 – 2832)	892 (21; 173 – 2233)	13.15 (23; 2.20 – 31.70)
4 Hours	1071 (4; 292 – 2748)	889 (21; 172 – 2473)	15.77 (22; 3.65 – 37.78)
6 Hours	1059 (4; 293 – 2614)	895 (21; 162 – 2590)	18.28 (23; 3.45 – 40.39)
24 Hours	1093 (4; 286 – 2733)	822 (21; 173 – 1781)	21.72 (22; 7.43 – 39.42)
2 Days	1061 (4; 293 – 2578)	837 (21; 192 – 1641)	19.83 (22; 9.26 – 33.33)
4 Days	1305 (3; 373 – 2588)	831 (19; 174 – 1633)	17.38 (21; 9.32 – 28.03)
1 Week	1109 (4; 323 – 2739)	841 (21; 205 – 1718)	15.14 (22; 9.22 – 25.97)
2 Weeks	1083 (4; 302 – 2581)	829 (18; 351 – 1659)	11.87 (21; 4.68 – 21.88)
4 Weeks	1155 (4; 262 – 2775)	887 (21; 183 – 2558)	9.44 (22; 3.33 – 19.73)

Table 5.5: Mean serum drug concentration at all time points for participants in each study arm.

B/L, baseline.

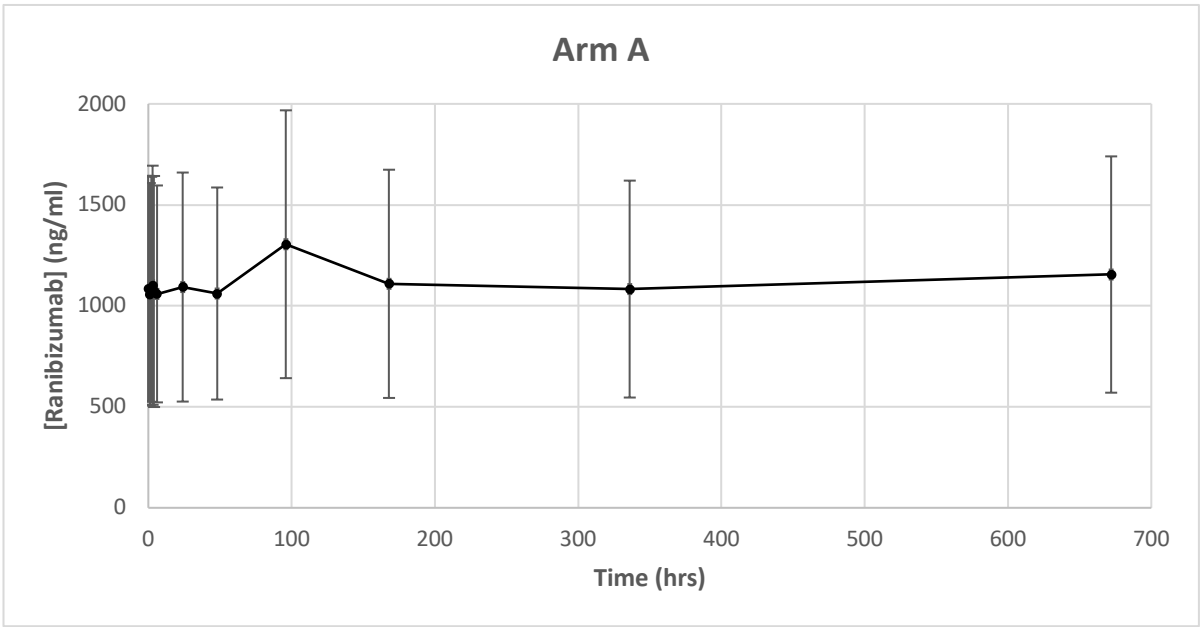


Figure 5.3: Ranibizumab concentration versus time curve for participants in Arm A.

Error bars indicate 1 standard error of the mean.

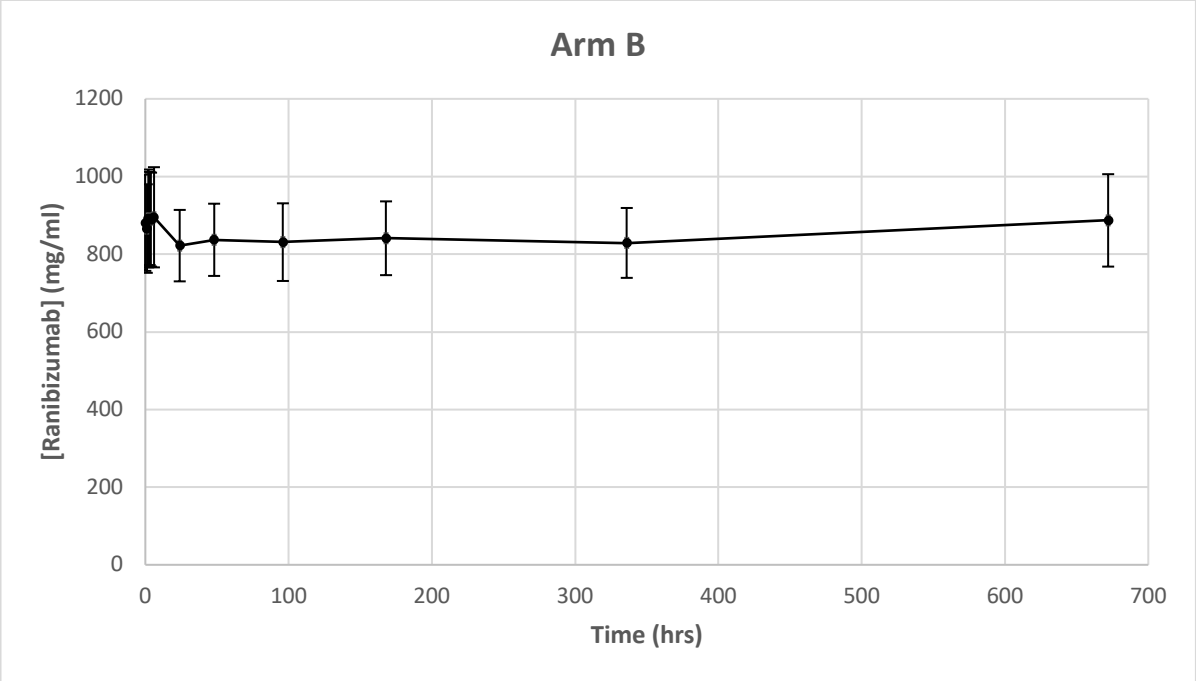


Figure 5.4: Ranibizumab concentration versus time curve for participants in Arm B.

Error bars indicate 1 standard error of the mean.

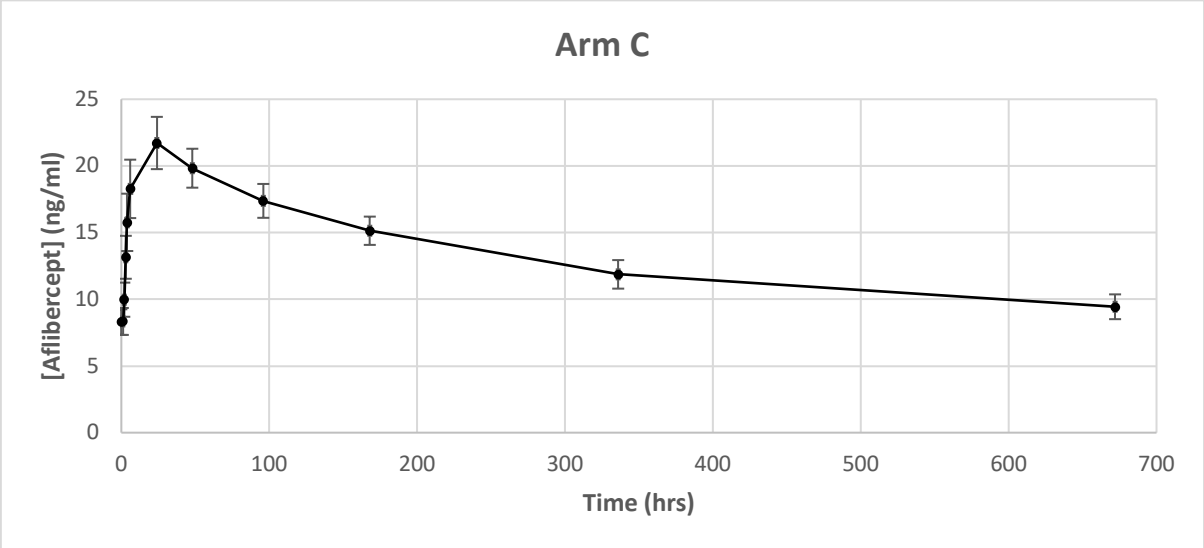


Figure 5.5: Aflibercept concentration versus time curve for participants in Arm C.

Error bars indicate 1 standard error of the mean.

At this point, a decision was taken to investigate the ranibizumab assay due to the uncharacteristic shape of the Arm A and Arm B pharmacokinetic curves and the unexpectedly high concentrations compared to those previously reported in the peer-reviewed literature.[198, 229, 372] An internal verification report for the ranibizumab analysis was conducted which initially did not find any specific kit performance problems. However, it was noted that two participants (001-004 and 001-008) were treatment naïve at the start of the trial. The baseline drug concentrations for these participants were 655 ng/ml and 400 ng/ml, respectively, meaning the negative control had failed.

The manufacturer of the ranibizumab assay was contacted to discuss the assay performance. They performed an internal investigation and review of the data, confirming that we had followed the assay instructions correctly. Their investigation concluded that there was a failure of the assay kits for unknown reason, and replacement kits were issued. Review of the replacement kits showed the calibrator concentrations and standard curve ranges had increased from 0 – 10 ng/ml to 0 – 320 ng/ml. This resulted in the limit of detection (LOD) changing from the original kit value of 0.125 ng/ml to 18.5 ng/ml, meaning the replacement assays were approximately 150 times less sensitive. The company was again contacted for clarification, which resulted in further replacement kits being sent. These assays had a calibration curve range of 0 - 10 ng/ml, and an LOD at the original value of 0.125 ng/ml.

Using the new assay kits, a standard curve was produced which confirmed the assay was performing appropriately when tested with known ranibizumab concentrations (Figure 5.6). Repeated standards used as quality controls showed all results fell within 25% of the known standard concentrations. On the basis of this, further quality control was performed using serum samples from twenty known negative control patients from outside of the VITCLEAR

trial, none of whom had ever received ranibizumab treatment or any other anti-VEGF agent. These assays produced results which were far higher than the LOD, meaning the negative controls had again failed (Table 5.5). The assay appeared to be picking up some interference, either in the form of non-specific binding or cross-reactivity with other substances. One possibility was that the assay might have been influenced by the baseline VEGF concentration, although this was discounted when comparing VEGF concentration (for those who had a paired measurement) with the calculated ranibizumab concentration in the healthy controls (Table 5.6).

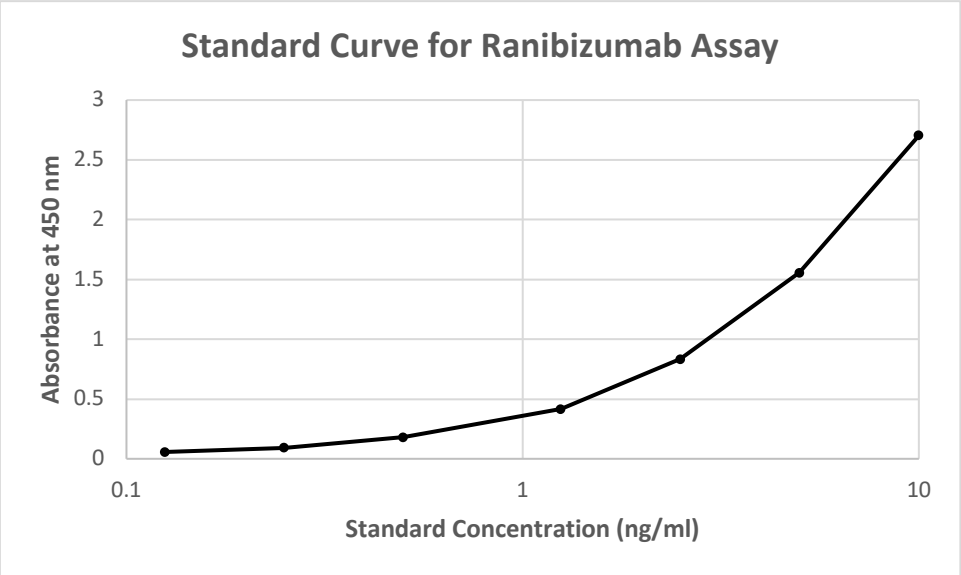


Figure 5.6: Standard curve produced as part of quality control assessment of ranibizumab assay.

Healthy Control	[Ranibizumab] (ng/ml)	[VEGF] (ng/L)
HS 1	4.60	58.30
HS 2	2.30	136.12
HS 3	1.77	61.52
HS 4	3.86	107.30
HS 5	19.75	14.49
HS 6	3.12	46.50
HS 7	2.48	105.50
HS 8	2.29	23.63
HS 9	1.57	138.61
HS 10	1.06	-
HS 11	5.17	56.38
HS12	4.10	84.61
HS 13	4.71	21.93
HS 14	1.37	34.21
HS 15	7.94	-
HS 16	2.92	-
HS 17	8.03	-
HS 18	3.62	-
HS 19	2.31	-
HS 20	9.38	93.2
Mean	4.62	70.2

Table 5.6: Negative control serum samples (HS1 – HS20) used to test assay performance. Vascular endothelial growth factor concentrations are shown for those who had a paired measurement.

VEGF, vascular endothelial growth factor.

We did not continue with this assay on our participant samples due to the limited volume of sample available and the lack of confidence in its accuracy. At the time of thesis submission, no further analysis of the ranibizumab drug concentrations have been performed, and we are in the process of sourcing new assays from a different supplier.

Pharmacokinetic analysis of Arm C was performed. The highest mean concentration observed in Arm C was 24 hours after intravitreal administration (C_{max} = 21.7 ng/ml). Systemic aflibercept half-life was calculated for each participant. The mean half-life was 22.2

days (range: 4.3 – 63.3; SEM 3.0 days). The mean AUC was 6725 hours x ng/ml (range 3324 – 10040; SEM 411 hours x ng/ml).

A subgroup analysis of those receiving aflibercept (Arm C) was performed to assess the pharmacokinetics depending on vitreous status (PVD vs nPVD). Both groups had a highest mean concentration (T_{max}) at 24 hours after intravitreal administration. Those with a PVD (n = 9) had a mean systemic half-life of 20.6 days (range: 4.3 – 39.4; SEM 3.3 days) compared to 23.3 days (range: 8.5 – 63.3; SEM 4.5 days) in those (n=13) with no PVD (p=0.66). The AUC in participants with a PVD was 6277 hours x ng/ml (range: 4729 – 10040; SEM 573 hours x ng/ml) compared to 7035 hours x ng/ml (range: 3324 – 9669; SEM 573 hours x ng/ml) in those with no PVD (p = 0.36).

Figure 5.7 shows the mean concentration versus time curves for both vitreous status subgroups and Figure 5.8 shows individual pharmacokinetic curves for all participants.

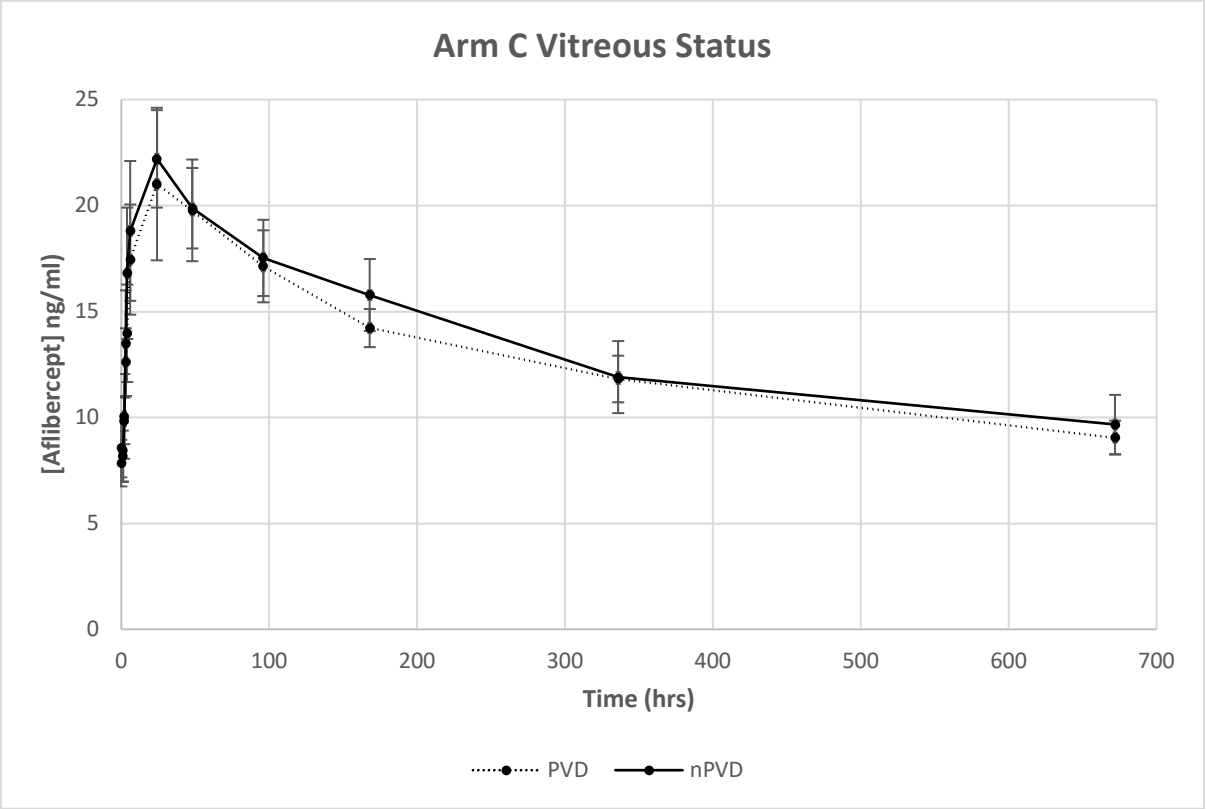
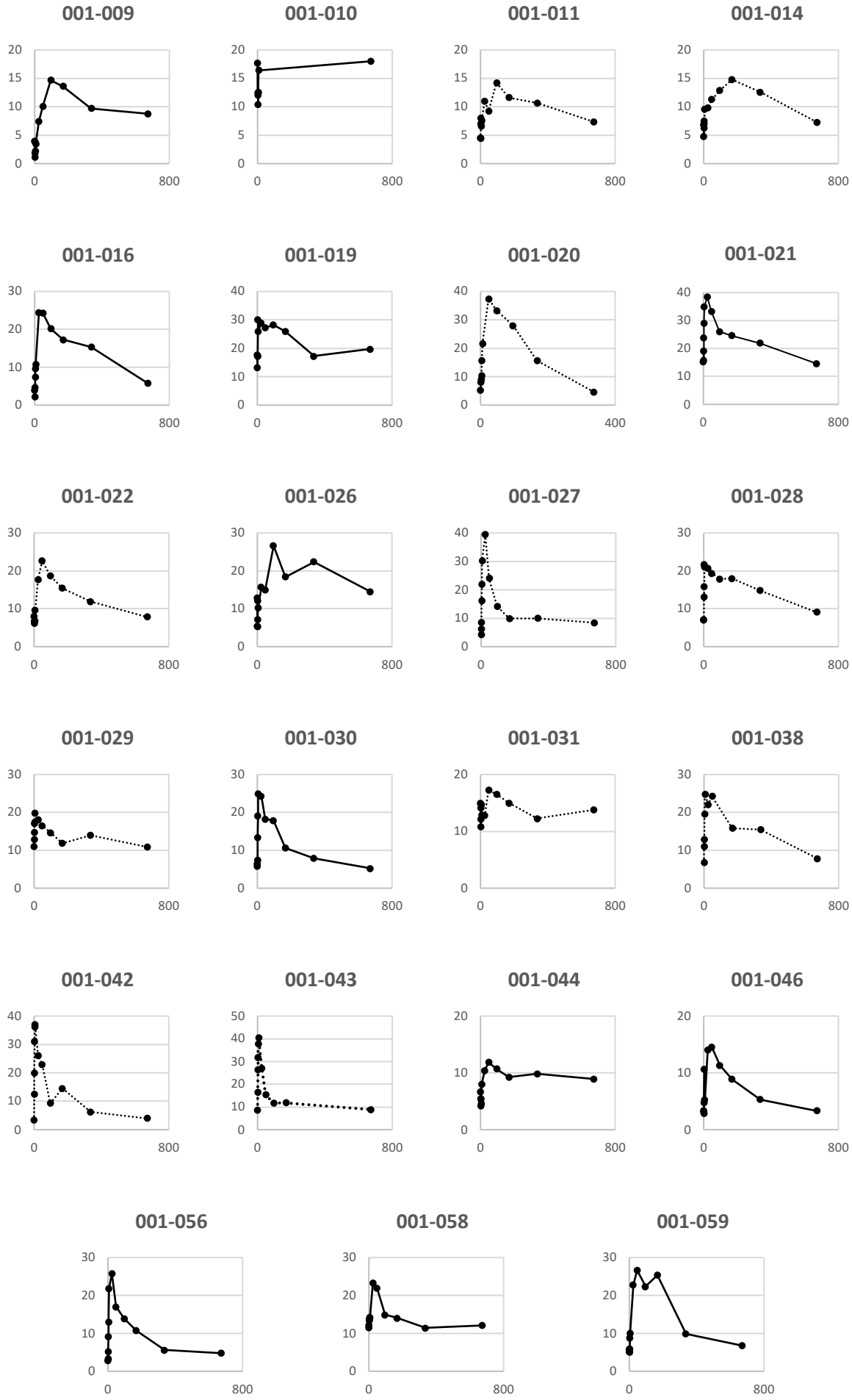


Figure 5.7: Aflibercept concentration versus time curves for participants in Arm C based on vitreous status.

Error bars indicate 1 standard error of the mean.

nPVD, no posterior vitreous detachment; PVD, posterior vitreous detachment.


001-009
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001-058
001-059

Figure 5.8: Composite showing aflibercept concentration versus time curve for individual participants.

X-axes represent time (hours) and y-axes represent aflibercept concentration (ng/ml). Solid lines indicate patients with no posterior vitreous detachment, and dotted lines indicate patients with a posterior vitreous detachment. Patient identification numbers are displayed above each graph.

5.3.5 Analysis of renal function

Serum renal function markers were tested on all participants, with the exception of one participant at one week, due to a missed study visit. Ten baseline potassium measurements were not analysed due to blood sample haemolysis. The baseline, one week and one month electrolytes, urea, urate, creatinine and eGFR measurements are displayed in Table 5.7 and Figure 5.9.

	B/L	W1	M1	*p-value (95% C.I.) B/L v M1
Sodium (mmol/L)				
n	53	52	53	
Range	129.0 – 144.0	128.0 – 144.0	128.0 – 145.0	
Mean (SEM)	139.1 (0.4)	139.3 (0.4)	139.5 (0.4)	*0.28 (-1.1, 0.3)*
Potassium (mmol/L)				
n	43	52	53	
Range	3.1 – 5.5	3.3 – 5.4	3.3 – 6.0	
Mean (SEM)	4.3 (0.1)	4.5 (0.1)	4.4 (0.1)	*0.19 (-0.2, 0.0)*
Urea (mmol/L)				
n	53	52	53	
Range	3.3 – 16.4	2.9 – 16.7	3.1 – 12.7	
Mean (SEM)	6.5 (0.3)	6.6 (0.4)	6.7 (0.3)	*0.51 (-0.6, 0.3)*
Urate (µmol/L)				
n	53	52	53	
Range	150.0 – 550.0	150.0 – 560.0	140.0 – 560.0	
Mean (SEM)	326.8 (12.0)	331.8 (12.3)	331.2 (12.2)	*0.34 (-13.4, 4.7)*
Creatinine (µmol/L)				
n	53	52	53	
Range	40.0 – 245.0	42 – 211.0	44 – 147.0	
Mean (SEM)	78.3 (4.2)	78.4 (3.7)	79.1 (2.9)	*0.70 (-5.1, 3.5)*
eGFR (ml/min/1.73m^2)				
n	53	52	53	
Range	20.0 – 121.0	24.0 – 100.0	31.0 – 102.0	
Mean (SEM)	73.6 (2.5)	72.3 (2.4)	71.6 (2.2)	***0.04*** *(0.1, 3.8)*

Table 5.7: Serum renal function for all participants at baseline, week one and month one.

** Paired t-test comparing baseline with month one values, 95% confidence interval of the difference. Bold type denotes statistical significance.*

B/L, baseline; C.I., confidence interval; eGFR, estimated glomerular filtration rate; M1, one month; SEM, standard error of the mean; W1, one week.

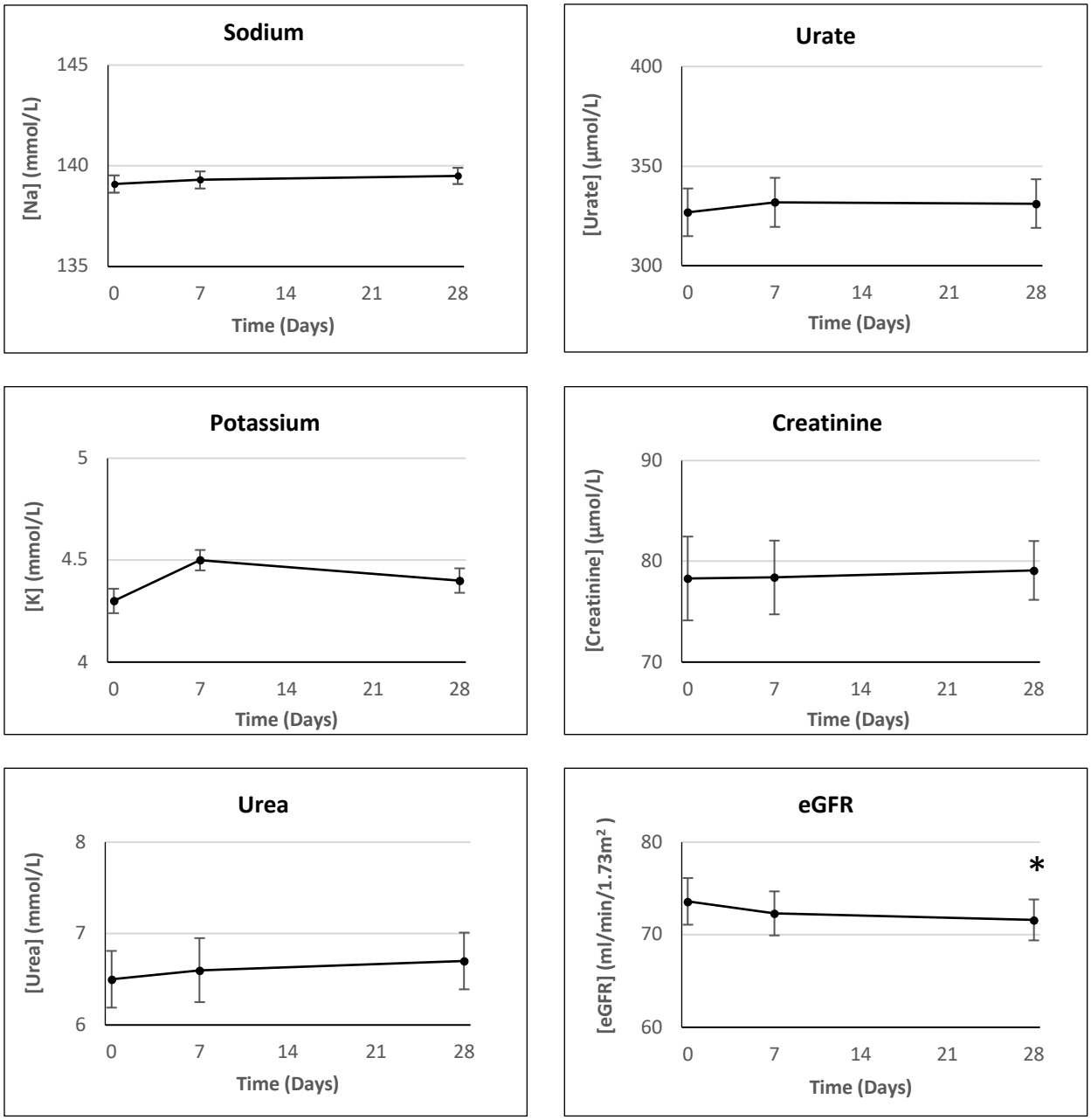

Figure 5.9: Line graphs showing the baseline, one week and one month mean serum renal function for all participants.

** indicates p < 0.05 (baseline versus one month). Error bars indicate 1 standard error of the mean.*

eGFR, estimated glomerular filtration rate

The mean serum concentration of sodium and potassium remained unchanged at one month following anti-VEGF compared to baseline. The mean serum concentration of urea and urate increased at one month (6.5 – 6.7 mmol/L and 326.8 – 331.2 μmol/L, respectively), although this difference was not statistically significant ($p = 0.51$ and $p = 0.54$, respectively). Mean serum creatinine, an estimate of kidney filtration ability, remained stable from 78.3 μmol/L at baseline to 78.4 μmol/L at one week and 79.1 μmol/L at one month ($p = 0.70$). The mean eGFR significantly reduced from 73.6 ml/min/1.73m^2 at baseline to 71.6 ml/min/1.73m^2 at one month ($p = 0.04$, 95% C.I. 0.1 – 3.8).

A subgroup analysis of renal function in different anti-VEGF groups was performed (Table 5.8 and Figure 5.10). There was no statistically significant difference between ranibizumab and aflibercept participants for any serum renal marker at baseline (sodium, $p = 0.25$; potassium, $p = 0.85$; urea, $p = 0.40$; urate, $p = 0.35$; creatinine, $p = 0.20$; eGFR, $p = 0.14$). Mean serum electrolyte concentrations at one month compared to baseline remained stable for ranibizumab and aflibercept patients. There was no significant change from baseline to one month in mean serum urea or urate concentrations for either anti-VEGF. Participants receiving ranibizumab had a small reduction in mean serum creatinine at one month compared to baseline (83.1 to 82.5 μmol/L; $p = 0.85$). Those receiving aflibercept had a non-significant rise in their mean serum creatinine (72.4 to 75.0 μmol/L; $p = 0.11$).

	B/L		M1		*p-value (95% C.I.) B/L - M1	*p-value (95% C.I.) B/L - M1
	Ranibizumab	**Aflibercept**	**Ranibizumab**	**Aflibercept**	**Ranibizumab**	**Aflibercept**
Sodium (mmol/L)						
n	29	24	29	24		
Range	132.0 – 144.0	129.0 – 142.0	130.5 – 142.4	128.0 – 145.0		
Mean (SEM)	139.5 (0.6)	138.5 (0.6)	139.7 (0.5)	139.2 (0.7)	*0.74 (-1.2, 0.8)*	*0.20 (-1.6, 0.4)*
Potassium (mmol/L)						
n	23	20	29	24		
Range	3.1 – 5.0	3.7 – 5.5	3.8 – 5.4	3.3 – 6.0		
Mean (SEM)	4.3 (0.1)	4.4 (0.1)	4.5 (0.1)	4.3 (0.1)	*0.08 (-0.4, 0.0)*	*0.96 (-0.2, 0.2)*
Urea (mmol/L)						
n	29	24	29	24		
Range	3.4 – 16.4	3.3 – 11.4	3.1 – 12.7	4.2 – 12.0		
Mean (SEM)	6.8 (0.5)	6.2 (0.4)	6.9 (0.4)	6.4 (0.5)	*0.59 (-0.6, 0.4)*	*0.69 (-0.9, 0.6)*
Urate (µmol/L)						
n	29	24	29	24		
Range	150.0 – 550.0	171.0 – 427.0	140.0 - 560.0	173.0 – 473.0		
Mean (SEM)	337.2 (19.0)	314.3 (13.2)	340.1 (18.8)	319.3 (14.6)	*0.52 (-15.6, 8.0)*	*0.49 (-19.9, 9.9)*
Creatinine (µmol/L)						
n	29	24	29	24		
Range	52.0 – 245.3	40.0 – 122.0	59.3 – 147.1	44.0 – 119.0		
Mean (SEM)	83.1 (6.6)	72.4 (4.4)	82.5 (4.1)	75.0 (4.0)	*0.85 (-6.9, 8.3)*	*0.11 (-6.0, 0.7)*
eGFR (ml/min/1.73m^2)						
n	29	24	29	24		
Range	20.0 – 107.0	47.0 – 121.0	31.0 – 102.0	48.0 – 98.0		
Mean (SEM)	70.1 (3.5)	77.7 (3.5)	68.6 (3.1)	75.3 (3.0)	*0.23 (-1.0, 4.2)*	*0.08 (-0.26, 5.1)*

Table 5.8: Subgroup analysis of serum renal function in ranibizumab and aflibercept participants at baseline and one month.

** Paired t-test comparing baseline with month one values, 95% confidence interval of the difference.*

B/L, baseline; C.I., confidence interval; eGFR, estimated glomerular filtration rate; M1, one month; SEM, standard error of the mean.

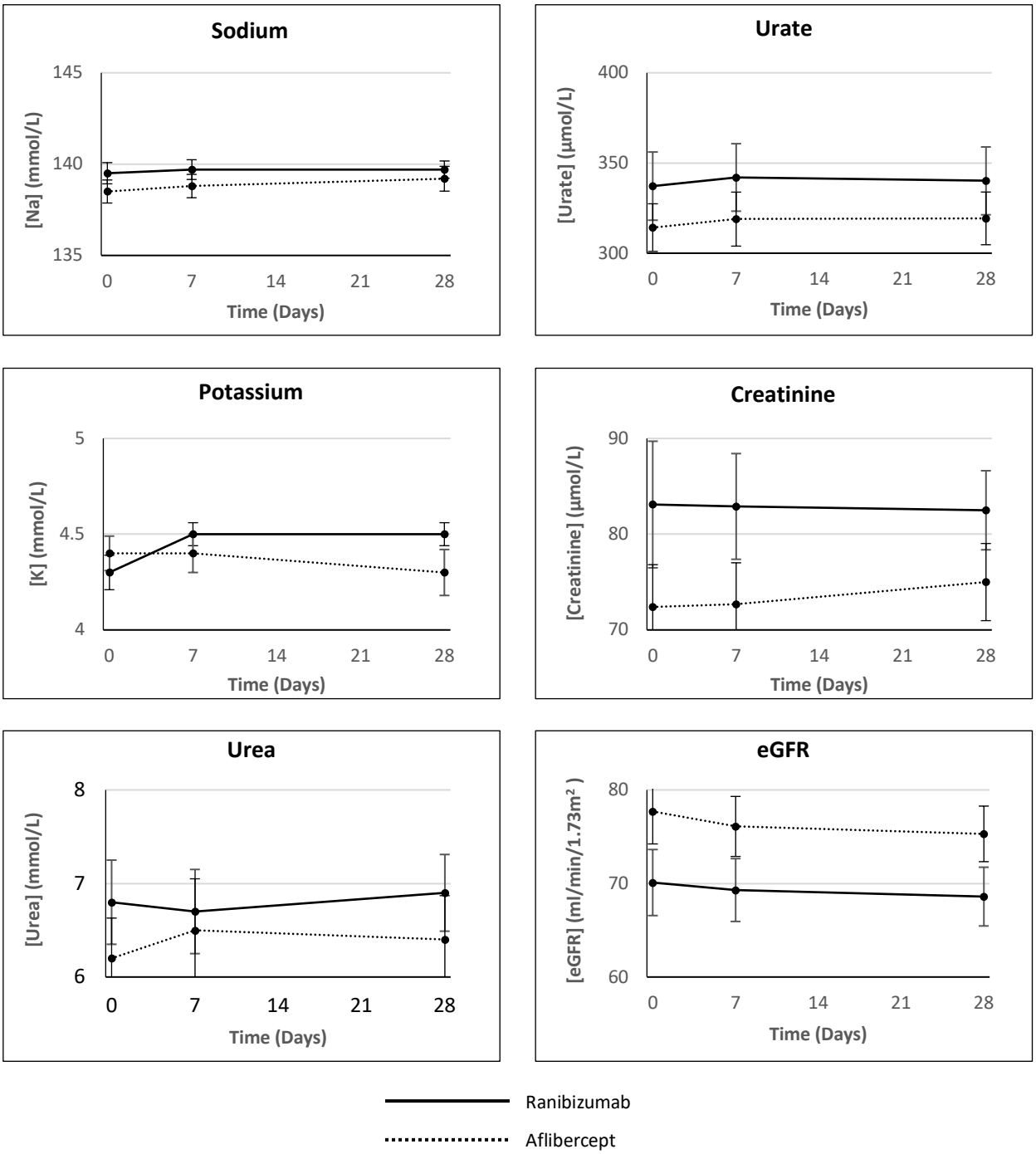

Figure 5.10: Line graphs showing the baseline, one week and one month serum renal function in participants receiving ranibizumab and aflibercept.

Error bars indicate 1 standard error of the mean.

eGFR, estimated glomerular filtration rate

A subgroup analysis of change in renal function based on baseline renal function (as measured by eGFR) was performed (Table 5.9). Participants with an eGFR < 60 ml/min/1.73m^2 (moderate and severe Chronic Kidney Disease (CKD), levels 3 - 5) were compared to those with an eGFR > 60 ml/min/1.73m^2 (normal and mild CKD levels, 1 - 2). The mean eGFR at baseline in the CKD 1-2 participants was 81.0 ml/min/1.73m^2, compared to 45.4 ml/min/1.73m^2 in the CKD 3-5 participants. There was no significant change over time in mean serum electrolytes in either renal function group. Mean serum urea and urate concentrations were also similar at baseline and one month for both groups. There was a statistically significant increase in mean serum creatinine concentration in participants with CKD 1-2 from 67.9 µmol/L at baseline to 71.5 µmol/L at one month ($p < 0.01$). Participants with CKD 3-5 had a non-significant decrease in mean serum creatinine concentration from 117.8 µmol/L at baseline to 108.1 µmol/L at one month ($p = 0.22$). Table 5.9 and Figure 5.11 show the mean serum renal function measures for each eGFR group.

	B/L		M1		*p-value (95% C.I.) B/L v M1	*p-value (95% C.I.) B/L v M1
	eGFR > 60	**eGFR < 60**	**eGFR > 60**	**eGFR < 60**	**eGFR > 60**	**eGFR < 60**
Sodium (mmol/L)						
n	42	11	42	11		
Mean (SEM)	138.9 (0.5)	139.8 (0.8)	139.3 (0.5)	140.0 (0.4)	*0.29 (-1.3, 0.4)*	*0.83 (-1.4, 1.2)*
Potassium (mmol/L)						
n	33	10	33	10		
Mean (SEM)	4.3 (0.1)	4.6 (0.2)	4.4 (0.8)	4.7 (0.2)	*0.32 (-0.3, 0.1)*	*0.24 (-0.3, 0.1)*
Urea (mmol/L)						
n	42	11	42	11		
Mean (SEM)	5.8 (0.2)	9.4 (0.9)	6.1 (0.3)	8.8 (0.9)	*0.11 (-0.8, 0.1)*	*0.27 (-0.6, 1.9)*
Urate (µmol/L)						
n	42	11	42	11		
Mean (SEM)	310.1 (12.1)	390.5 (28.0)	318.9 (13.1)	377.8 (27.7)	*0.09 (-18.9, 1.3)*	*0.18 (-6.8, 32.1)*
Creatinine (µmol/L)						
n	42	11	42	11		
Mean (SEM)	67.9 (2.0)	117.8 (13.3)	71.5 (1.9)	108.1 (7.0)	***< 0.01*** *(-5.5, -1.6)*	*0.33 (-11.2, 30.5)*
eGFR (ml/min/1.73m^2)						
n	42	11	42	11		
Mean (SEM)	81.0 (1.8)	45.4 (3.1)	77.6 (1.7)	48.6 (3.4)	***< 0.01*** *(1.6, 5.0)*	*0.22 (-8.8, 2.3)*

Table 5.9: Serum renal function comparison in participants with estimated glomerular filtration rate greater or less than 60 ml/min/1.73m^2, at baseline and one month.

** Paired t-test comparing baseline with month one values, 95% confidence interval of the difference. Bold type denotes statistical significance.*

B/L, baseline; C.I., confidence interval; eGFR, estimated glomerular filtration rate; M1, one month; SEM, standard error of the mean.

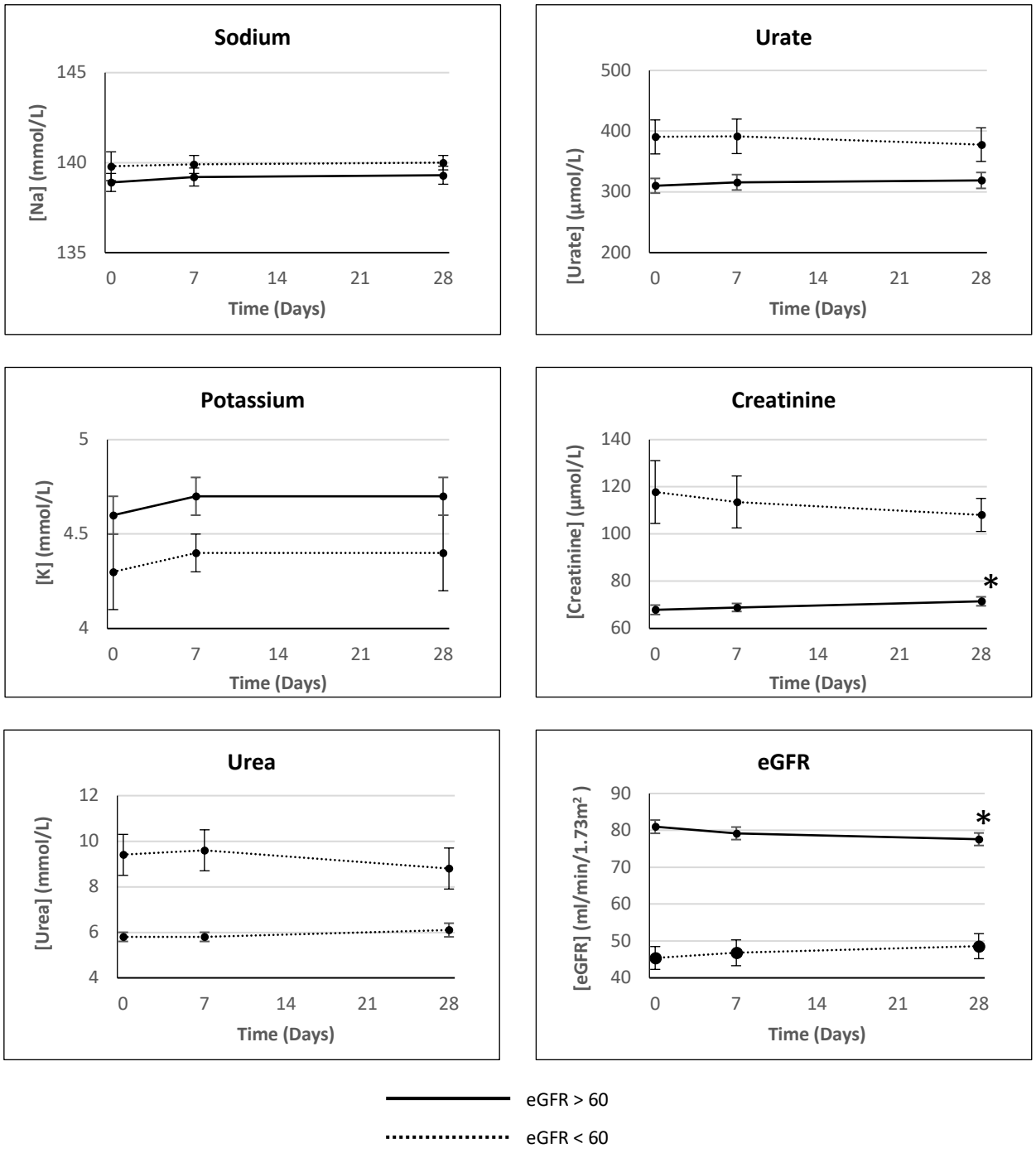

Figure 5.11: Line graphs showing the baseline, one week and one month serum renal function in participants with estimated glomerular filtration rate above and below 60 ml/min/1.73m^2.

** indicates p < 0.05 (baseline versus one month). Error bars indicate 1 standard error of the mean.*

eGFR, estimated glomerular filtration rate

Participants with or without hypertension were compared in a subgroup analysis. No significant change was found in mean serum electrolytes, urea or urate concentration from baseline to one month in participants with or without hypertension (Table 5.10 and Figure 5.12). The mean serum creatinine concentration in participants with hypertension remained stable from 85.0 µmol/L at baseline to 84.0 µmol/L at one month ($p = 0.81$). Participants without hypertension had an increase in their creatinine level from 71.8 µmol/L at baseline to 74.4 µmol/L at one month ($p < 0.01$). These participants also saw a statistically significant reduction in eGFR over the same time period (76.7 ml/min/1.73m^2 versus 74.5 ml/min/1.73m^2; $p = 0.007$). Those with hypertension did not have any change in mean serum creatinine concentration or eGFR measurement during the study.

	B/L		M1		*p-value (95% C.I.) B/L v M1	*p-value (95% C.I.) B/L v M1
	HTN	**No HTN**	**HTN**	**No HTN**	**HTN**	**No HTN**
Sodium (mmol/L)						
n	26	27	26	27		
Mean (SEM)	138.9 (0.6)	139.3 (0.6)	139.3 (0.5)	139.7 (0.6)	*0.50 (-1.5, 0.7)*	*0.40 (-1.3, 0.5)*
Potassium (mmol/L)						
n	21	22	21	22		
Mean (SEM)	4.4 (0.1)	4.3 (0.1)	4.6 (0.1)	4.3 (0.1)	*0.09 (-0.4, 0.0)*	*0.86 (-0.1, 0.2)*
Urea (mmol/L)						
n	26	27	26	27		
Mean (SEM)	7.3 (0.5)	5.8 (0.3)	7.2 (0.6)	6.1 (0.2)	*0.86 (-0.7, 0.9)*	*0.07 (-0.7, 0.0)*
Urate (µmol/L)						
n	26	27	26	27		
Mean (SEM)	337.2 (18.5)	316.7 (15.4)	336.7 (18.9)	325.9 (15.8)	*0.94 (-15.3, 16.5)*	*0.07 (-18.9, 0.6)*
Creatinine (µmol/L)						
n	26	27	26	27		
Mean (SEM)	85.0 (7.7)	71.8 (3.0)	84.0 (4.9)	74.4 (3.0)	*0.81 (-7.8, 9.9)*	***< 0.01** (-4.4, 0.9)*
eGFR (ml/min/1.73m^2)						
n	26	27	26	27		
Mean (SEM)	70.3 (4.3)	76.7 (2.6)	68.6 (3.6)	74.5 (2.6)	*0.34 (-1.8, 5.1)*	***0.007** (0.7, 3.8)*

Table 5.10: Serum renal function in participants with and without a baseline diagnosis of hypertension, at baseline and one month.

**Paired t-test comparing baseline with month one values, 95% confidence interval of the difference. Bold type denotes statistical significance.*

B/L, baseline; C.I., confidence interval; eGFR, estimated glomerular filtration rate; HTN, hypertension; M1, one month; SEM, standard error of the mean.

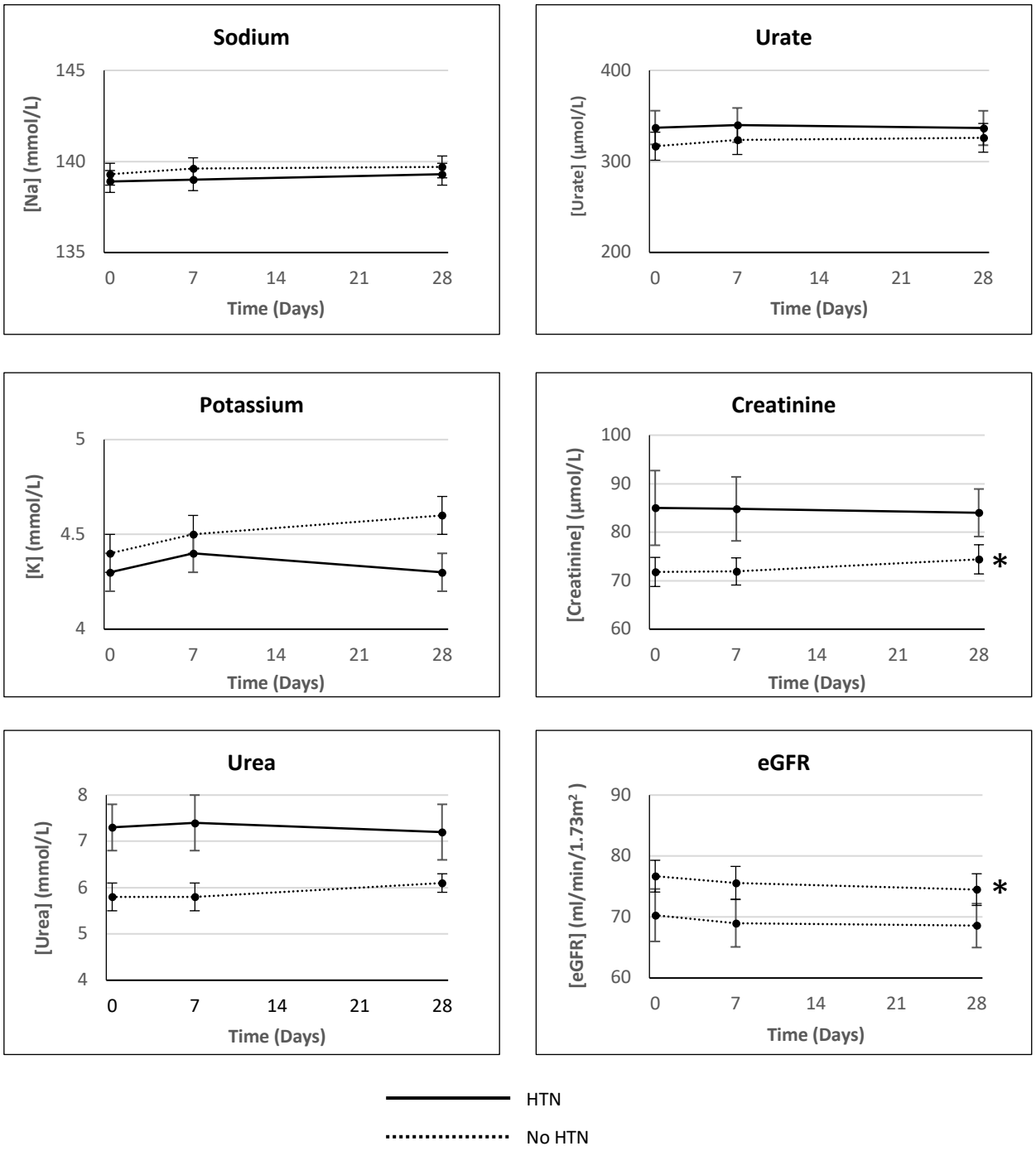

Figure 5.12: Line graphs showing the baseline, one week and one month serum renal function in participants with and without a diagnosis of hypertension.

** indicates p < 0.05 (baseline versus one month). Error bars indicate 1 standard error of the mean.*

eGFR, estimated glomerular filtration rate; HTN, hypertension.

5.3.6 Analysis of high sensitivity C-reactive protein

All participants except one were considered eligible for the analysis of serum hsCRP concentration. The excluded participant suffered a traumatic haemarthrosis during the study which was deemed to potentially affect serum hsCRP concentration. Analysis showed the serum hsCRP concentration at one month in this case to be a severe outlier (studentized residual value = 5.03) and therefore the participant was excluded from hsCRP analysis.

The mean serum hsCRP concentration at baseline was 3.82 mg/L, which increased to 5.38 mg/L at one week, and 6.13 mg/L at one month (Figure 5.13). There was no statistically significant change in serum hsCRP concentration at one week compared to baseline ($p = 0.315$), one month compared to baseline ($p = 0.14$), or one week compared to one month ($p = 0.653$).

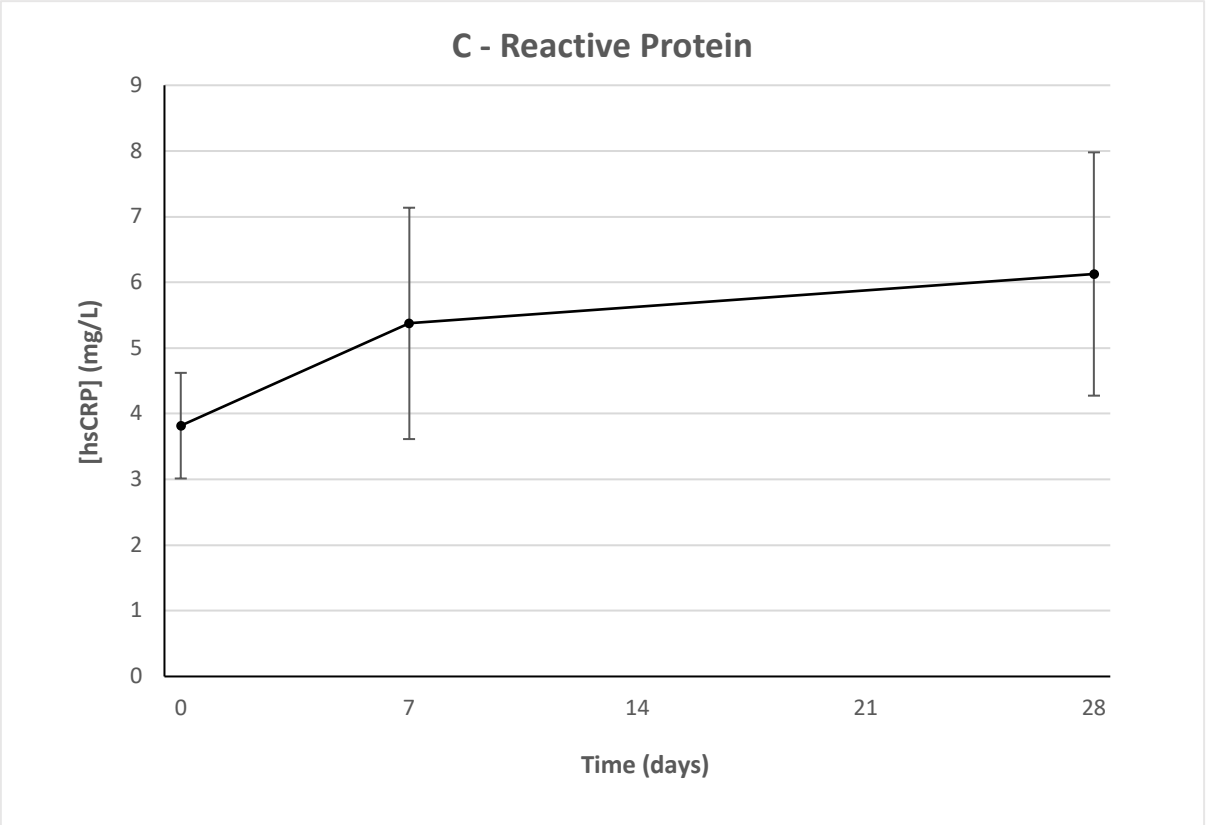

Figure 5.13: Change in high sensitivity C-reactive protein.

Error bars indicate 1 standard error of the mean.

[hsCRP], concentration of high sensitivity C-reactive protein

There was no statistically significant difference in mean baseline hsCRP in patients receiving ranibizumab or aflibercept (2.9 ± 0.6 v 5.1 ± 1.5 mg/L; p = 0.20). The serum hsCRP concentrations for ranibizumab and aflibercept eyes at baseline, one week and one month, were as follows; 2.7 ± 1.1 vs 2.7 ± 2.3 vs 2.7 ± 2.4 mg/L and 5.1 ± 1.2 vs 8.6 ± 2.6 vs 10.3 ± 2.7 mg/L, respectively (Figure 5.14).

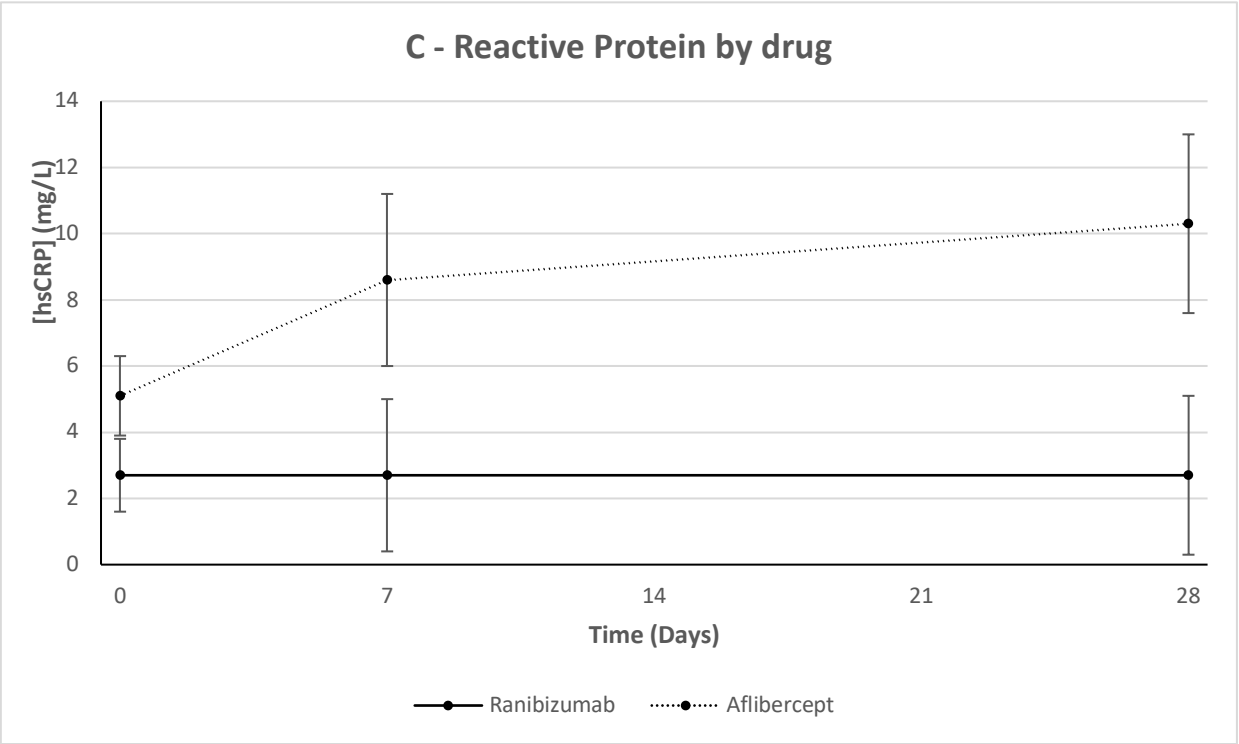

Figure 5.14: Mean serum high sensitivity C-reactive protein dependent on drug.

Error bars indicate 1 standard error of the mean.

hsCRP, high sensitivity C-reactive protein

A subgroup analysis of change in mean hsCRP concentration depending on anti-VEGF was performed, using a two-way mixed ANOVA. There was no statistically significant interaction between anti-VEGF and time on mean hsCRP concentration, $F(2, 98) = 1.42$, $p = 0.25$, partial $\eta^2 = 0.03$. The main effect of time did not show a statistically significant difference in mean serum hsCRP concentration at the different time points, $F(1, 49) = 2.85$, $p = 0.10$, partial $\eta^2 = 0.06$. The main effect of group showed that there was a statistically significant difference in mean serum hsCRP concentration between anti-VEGF drugs $F(1,49) = 4.91$, $p = 0.03$, partial $\eta^2 = 0.091$.

Another subgroup analysis was performed to determine the effect of vitreous status. There was no difference in baseline mean hsCRP depending on vitreous status (vitrectomised, 3.3 ± 1.4 mg/L vs PVD, 3.8 ± 1.2 mg/L vs nPVD 1.3 ± 0.4 mg/L; $p = 0.169$, one-way ANOVA). The serum hsCRP concentrations for vitrectomised, PVD and nPVD eyes receiving ranibizumab at baseline, one week and one month, were as follows; 3.3 vs 4.7 vs 5.1 mg/L, 3.8 vs 3.2 vs 3.0 mg/L and 1.3 vs 1.2 vs 1.2 mg/L, respectively (Figure 5.15).

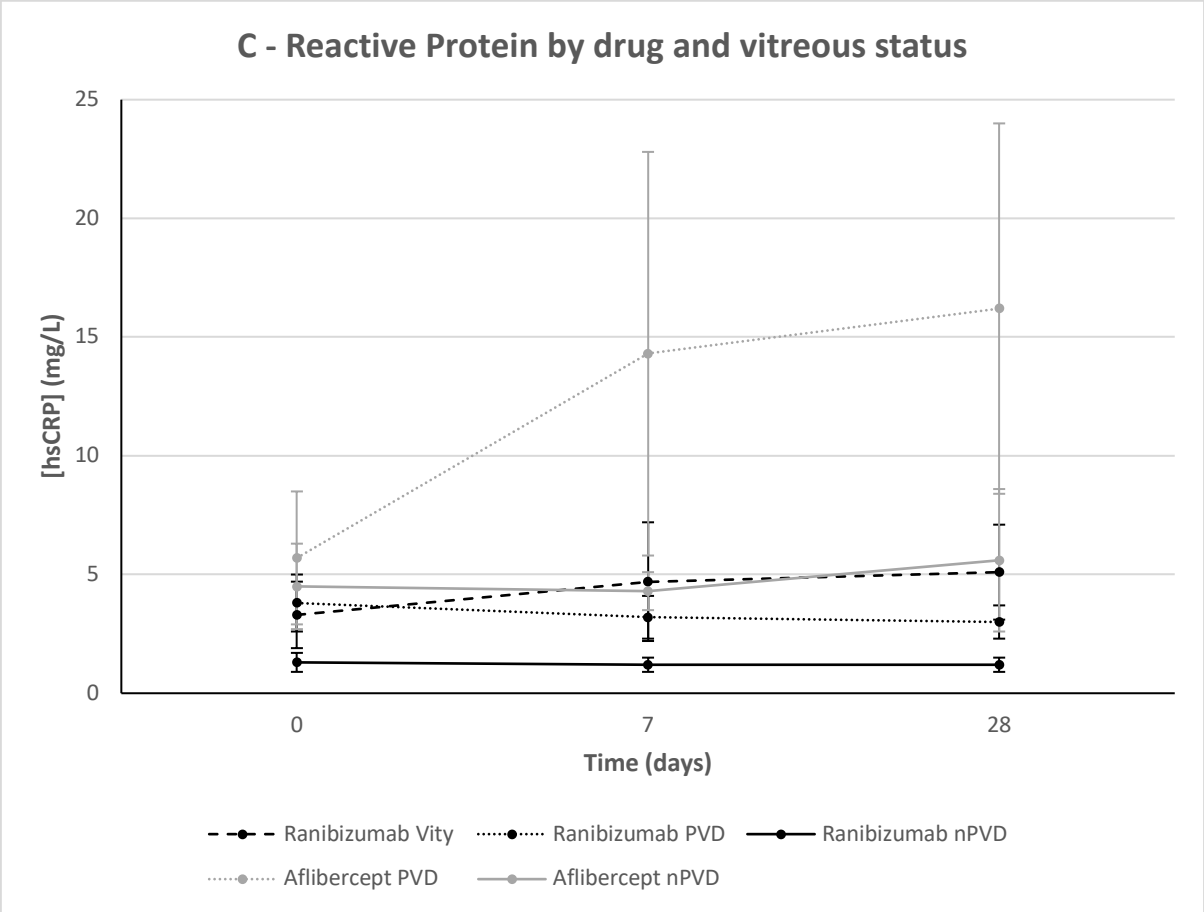

Figure 5.15: Effect of vitreous status for ranibizumab and aflibercept participants on high sensitivity C-reactive protein.

Error bars indicate 1 standard error of the mean.

hsCRP, high sensitivity C-reactive protein; nPVD, no posterior vitreous detachment; PVD, posterior vitreous detachment; vity, vitrectomy

A two-way mixed ANOVA was performed to assess for a relationship between vitreous status at baseline and change in serum hsCRP for ranibizumab participants. There was no statistically significant interaction between vitreous status and time on hsCRP concentration, $F(4, 50) = 0.71$, $p = 0.59$, partial $\eta^2 = 0.05$. The main effect of time did not show a statistically significant difference in mean serum hsCRP concentration at the different time

points, F(2, 50) = 0.15, p = 0.86, partial η^2 = 0.01. The main effect of group showed that there was a statistically significant difference in mean serum hsCRP concentration between vitreous status groups F(2,25) = 3.96, p = 0.03, partial η^2 = 0.241. Post-hoc testing (Games-Howell test) found a significant mean difference between PVD and nPVD participants (2.09 ± 0.78, p = 0.04). No significant difference was found for vitrectomised versus PVD participants, or vitrectomised versus nPVD participants (1.02 ± 1.78, p = 0.84, and 3.11 ± 1.67, p = 0.26, respectively).

For participants receiving aflibercept, there was no difference in baseline mean hsCRP depending on vitreous status (PVD, 5.7 ± 2.8 mg/L vs nPVD 4.5 ± 1.8 mg/L; p = 0.712, one-independent two-tailed t-test). The serum hsCRP concentrations for PVD and nPVD eyes at baseline, one week and one month, were as follows; 5.7 vs 14.3 vs 16.2 mg/L, and 4.5 vs 4.3 vs 5.6 mg/L, respectively (Figure 5.15le

A two-way mixed ANOVA was performed to assess for a relationship between vitreous status at baseline and change in serum hsCRP for aflibercept participants. There was no statistically significant interaction between vitreous status and time on hsCRP concentration, F(2, 42) = 1.18, p = 0.32, partial η^2 = 0.05. The main effect of time did not show a statistically significant difference in mean serum hsCRP concentration at the different time points, F(1, 21) = 3.20, p = 0.09, partial η^2 = 0.13. The main effect of group showed that there was a statistically significant difference in mean serum hsCRP concentration between vitreous status groups F(1,21) = 1.91, p = 0.18, partial η^2 = 0.083.

5.3.7 Analysis of cytokines

Serum cytokine testing was performed on all participants, with the exception of one participant at one week (missed study visit), two patients at three time points (insufficient serum available) and two patients at one time point (insufficient serum available). Table 5.11 displays the mean cytokine concentrations at baseline, one week and one month. A small but significant change was detected in mean IL-4 concentration from baseline to one week (1.8 ng/L vs 1.7 ng/L; $p = 0.04$), and baseline compared to one month (1.8 ng/L vs 1.7 ng/L, $p = 0.04$). Mean VEGF concentration significantly reduced from baseline to one week (122.9 ng/L vs 79.1 ng/L; $p<0.01$) and from baseline compared to one month (122.9 ng/L vs 91.1 ng/L; $p<0.01$). The mean concentration of MCP-1 reduced significantly from baseline to one week and one month (295.6 ng/L vs 268.5 ng/L vs 275.4 ng/L; $p <0.01$ and $p = 0.01$, respectively).

	B/L	W1	M1	p-value (95% C.I.)	
				B/L vs W1	B/L vs M1
EGF (ng/L)					
n	49	50	51		
Range	2.9 – 39.8	2.9 – 46.7	2.9 – 76.5		
Mean (SEM)	15.0 (1.29)	7.46 (1.20)	10.2 (2.04)	***<0.01*** *(4.1, 10.7)*	*0.08 (-0.6, 9.9)*
IFN-γ (ng/L)					
n	49	50	51		
Range	3.5 – 16.8	3.5 – 16.2	3.5 – 13.8		
Mean (SEM)	3.8 (0.27)	3.8 (0.25)	3.7 (0.21)	*0.10 (0.0, 0.1)*	*0.22 (-0.1, 0.2)*
IL-1α (ng/L)					
n	49	50	51		
Range	0.8 – 147.7	0.8 – 139.1	0.8 – 202.2		
Mean (SEM)	4.2 (3.00)	3.9 (2.77)	5.1 (3.95)	*0.14 (-0.1, 0.7)*	*0.36 (-3.3, 1.2)*
IL-1β (ng/L)					
n	49	50	51		
Range	1.6 – 44.1	1.6 – 30.2	1.6 – 30.7		
Mean (SEM)	4.5 (1.27)	3.8 (0.93)	3.9 (0.91)	*0.09 (-0.1, 1.5)*	*0.27 (-0.4, 1.4)*
IL-2 (ng/L)					
n	49	50	51		
Range	4.8 – 37.0	3.0 – 27.2	4.8 – 27.9		
Mean (SEM)	6.4 (0.88)	6.1 (0.65)	5.9 (0.57)	*0.20 (-0.1, 0.8)*	*0.19 (-0.2, 1.1)*
IL-4 (ng/L)					
n	49	50	51		
Range	1.2 – 9.8	1.2 – 8.6	1.2 – 8.3		
Mean (SEM)	1.8 (0.19)	1.7 (0.15)	1.7 (0.15)	***0.04*** *(0.0, 0.2)*	***0.04*** *(0.0, 0.2)*
IL-6 (ng/L)					
N	49	50	51		
Range	1.2 – 16.9	1.2 – 25.0	1.2 – 24.7		
Mean (SEM)	3.3 (0.47)	3.6 (0.62)	3.7 (0.64)	*0.28 (-1.1, 0.3)*	*0.16 (-1.2, 0.2)*
IL-8 (ng/L)					
n	49	50	51		
Range	7.9 – 175.1	7.9 – 281.4	7.8 – 190.2		
Mean (SEM)	14.8 (3.41)	17.2 (5.42)	15.6 (3.56)	*0.29 (-6.9, 2.1)*	*0.14 (-1.8, 0.3)*
IL-10 (ng/L)					
n	49	50	51		
Range	1.8 – 21.1	1.8 – 15.0	1.8 – 15.9		
Mean (SEM)	2.8 (0.48)	2.4 (0.30)	2.5 (0.31)	*0.06 (0.0, 0.8)*	*0.19 (-0.1, 0.7)*
MCP-1 (ng/L)					
n	49	50	51		
Range	33.5 – 612.8	54.4 – 555.1	54.0 – 543.8		
Mean (SEM)	295.6 (17.5)	268.5 (14.6)	275.4 (14.83)	***<0.01*** *(14.2, 40.3)*	***0.01*** *(4.6, 35.8)*
PDGF-AA (ng/ml)					
n	49	50	51		
Range	7.0 – 52.6	7.0 – 41.4	6.7 – 44.7		
Mean (SEM)	15.1 (1.12)	14.3 (0.90)	14.3 (0.99)	***0.03*** *(0.1, 1.4)*	*0.09 (-0.1, 1.3)*
TNF-α (ng/L)					
n	49	50	51		
Range	4.4 – 4.4	4.4 – 5.6	4.4 – 4.4		
Mean (SEM)	4.4 (0.00)	4.4 (0.02)	4.4 (0.00)	*0.32 (-0.1, 0.0)*	*n/a*
VEGF (ng/L)					
n	49	50	51		
Range	21.9 – 381.9	14.6 – 340.69	14.6 – 371.0		
Mean (SEM)	122.9 (12.26)	79.1 (11.37)	91.1 (11.6)	***<0.01*** *(25.5, 63.4)*	***<0.01*** *(15.3, 49.7)*

Table 5.11: Cytokine results for entire cohort at baseline, one week and one month.

Bold type denotes statistical significance.

B/L, baseline; C.I, confidence interval; EGF, epidermal growth factor; IFN-γ, interferon gamma; IL, interleukin; M1, one month; MCP-1, monocyte chemoattractant protein-1; PDGF-AA, platelet derived growth factor-AA; SEM, standard error of the mean; TNF-α, tumour necrosis factor-alpha; VEGF, vascular endothelial growth factor; W1, one week.

A subgroup analysis of change in cytokine concentration depending on anti-VEGF drug was performed (Tables 5.12 and 5.13). In participants who received ranibizumab, a decrease was seen in mean EGF from baseline to one week (16.2 vs 8.0 ng/L; $p < 0.01$). A decrease in mean MCP-1 was also seen over the same time period (279.7 vs 258.7 ng/L; $p = 0.01$). There was no statistically significant change in mean VEGF concentration from baseline to one week or one month (139.3 vs 125.9 vs 133.4 ng/L, respectively; $p = 0.10$ and $p = 0.61$). In participants who received aflibercept, mean VEGF statistically significantly decreased from baseline to one week (104.5 vs 19.5 ng/L; $p < 0.01$) and from baseline compared to one month (104.5 vs 39.6 ng/L; $p < 0.01$). There was also a decrease at one week compared to baseline in EGF, IL-4, MCP-1, and PDGF-AA, and at one month compared to baseline in MCP-1 (Table 5.13).

	B/L	W1	M1	p-value (95% C.I.)	
				B/L vs W1	B/L vs M1
EGF (ng/L)					
n	26	28	28		
Range	2.9 – 39.8	2.9 – 46.7	2.9 – 79.5		
Mean (SEM)	16.2 (2.03)	8.0 (1.71)	10.6 (3.05)	***>0.01*** *(2.9, 13.3)*	*0.20 (-3.0, 13.9)*
IFN-γ (ng/L)					
n	26	28	28		
Range	3.5 – 16.8	3.5 – 16.2	3.5 – 13.8		
Mean (SEM)	4.1 (0.51)	4.0 (0.45)	3.9 (0.37)	*0.10 (0.0, 0.2)*	*0.22 (-0.1, 0.4)*
IL-1α (ng/L)					
n	26	28	28		
Range	0.8 – 147.7	0.8 – 139.1	0.8 – 202.2		
Mean (SEM)	7.1 (5.63)	6.2 (4.93)	8.5 (7.18)	*0.19 (-0.2, 1.2)*	*0.35 (-6.3, 2.3)*
IL-1β (ng/L)					
n	26	28	28		
Range	1.6 – 44.1	1.6 – 30.2	1.6 – 30.7		
Mean (SEM)	6.7 (2.32)	5.2 (1.61)	5.4 (1.60)	*0.11 (-0.3, 2.6)*	*0.25 (-0.7, 2.7)*
IL-2 (ng/L)					
n	26	28	28		
Range	4.8 – 37.0	3.0 – 27.2	4.8 – 27.9		
Mean (SEM)	7.5 (1.61)	6.8 (1.11)	6.5 (0.97)	*0.19 (-0.3, 1.6)*	*0.18 (-0.4, 2.2)*
IL-4 (ng/L)					
n	26	28	28		
Range	1.2 – 9.8	1.2 – 8.6	1.2 – 8.3		
Mean (SEM)	2.1 (0.34)	1.9 (0.27)	1.9 (0.26)	*0.19 (-0.1, 0.3)*	*0.08 (0.0, 0.3)*
IL-6 (ng/L)					
N	26	28	28		
Range	1.2 – 16.9	1.2 – 18.8	1.2 – 20.2		
Mean (SEM)	3.5 (0.74)	3.5 (0.71)	3.5 (0.76)	*0.76 (-0.9, 0.7)*	*0.87 (-0.8, 0.7)*
IL-8 (ng/L)					
n	26	28	28		
Range	7.9 – 22.9	7.9 – 19.7	7.9 – 22.9		
Mean (SEM)	11.5 (0.74)	11.8 (0.70)	12.6 (0.88)	*0.71 (-1.2, 0.8)*	*0.23 (-2.3, 0.6)*
IL-10 (ng/L)					
n	26	28	28		
Range	1.8 – 21.1	1.8 – 15.0	1.8 – 15.9		
Mean (SEM)	3.6 (0.87)	2.8 (0.53)	2.9 (0.56)	*0.07 (-0.1, 1.5)*	*0.14 (-0.2, 1.4)*
MCP-1 (ng/L)					
n	26	28	28		
Range	33.5 – 481.3	54.4 – 402.7	54.0 – 499.7		
Mean (SEM)	279.7 (20.6)	258.7 (17.0)	276.9 (19.3)	***0.01*** *(5.6, 38.4)*	*0.74 (-12.3, 17.1)*
PDGF-AA (ng/ml)					
n	26	28	28		
Range	7.0 – 52.6	7.2 – 41.4	7.4 – 44.7		
Mean (SEM)	15.7 (1.97)	14.8 (1.50)	15.1 (1.63)	*0.28 (-0.6, 1.8)*	*0.48 (-0.7, 1.4)*
VEGF (ng/L)					
n	26	28	28		
Range	21.9 – 381.9	36.3 – 340.7	26.2 – 371.0		
Mean (SEM)	139.3 (18.4)	125.9 (15.3)	133.4 (16.7)	*0.10 (-2.2, 25.3)*	*0.61 (-11.5, 19.1)*

Table 5.12: Cytokine results for participants receiving ranibizumab at baseline, one week and one month. Bold type denotes statistical significance.

B/L, baseline; C.I, confidence interval; EGF, epidermal growth factor; IFN-γ, interferon gamma; IL, interleukin; M1, one month; MCP-1, monocyte chemoattractant protein-1;

PDGF-AA, platelet derived growth factor-AA; SEM, standard error of the mean; VEGF, vascular endothelial growth factor; W1, one week.

	B/L	W1	M1	p-value (95% C.I.)	
				B/L vs W1	B/L vs M1
EGF (ng/L)					
n	23	22	23		
Range	4.3 – 27.6	2.9 – 35.7	2.9 – 40.0		
Mean (SEM)	13.6 (1.50)	6.8 (1.67)	9.8 (2.67)	***<0.01*** *(2.5, 10.6)*	*0.25 (-2.8, 10.3)*
IFN-γ (ng/L)					
n	23	22	23		
Range	3.5 – 3.5[a]	3.5 – 3.5[a]	3.5 – 3.5[a]		
Mean (SEM)	3.5 (0.00)[a]	3.5 (0.00)[a]	3.5 (0.00)[a]	-	-
IL-1α (ng/L)					
n	23	22	23		
Range	0.8 – 2.4	0.8 – 2.5	0.8 – 2.5		
Mean (SEM)	1.0 (0.09)	0.9 (0.08)	0.9 (0.07)	*0.40 (-0.1, 0.2)*	*0.44 (-0.1, 0.2)*
IL-1β (ng/L)					
n	23	22	23		
Range	1.6 – 5.3	1.6 – 4.5	1.6 – 6.1		
Mean (SEM)	2.0 (0.20)	1.9 (0.19)	2.0 (0.26)	*0.40 (-0.1, 0.2)*	*0.66 (-0.3, 0.2)*
IL-2 (ng/L)					
n	23	22	23		
Range	4.8 – 13.6	4.8 – 14.2	4.8 – 14.4		
Mean (SEM)	5.2 (0.38)	5.2 (0.43)	5.2 (0.42)	*0.33 (-0.1, 0.0)*	*0.33 (-0.1, 0.0)*
IL-4 (ng/L)					
n	23	22	23		
Range	1.2 – 2.6	1.2 – 2.5	1.2 – 2.4		
Mean (SEM)	1.5 (0.08)	1.5 (0.07)	1.5 (0.08)	***0.03*** *(0.0, 0.2)*	*0.32 (-0.1, 0.2)*
IL-6 (ng/L)					
N	23	22	23		
Range	1.2 – 12.5	1.2 – 25.0	1.2 – 24.7		
Mean (SEM)	3.1 (0.58)	3.7 (1.11)	4.1 (1.09)	*0.27 (-1.9, 0.6)*	*0.12 (-2.3, 0.3)*
IL-8 (ng/L)					
n	23	22	23		
Range	7.9 – 175.1	7.9 – 281.35	7.8 – 190.2		
Mean (SEM)	18.5 (7.22)	24.0 (12.31)	19.1 (7.85)	*0.31 (-15.1, 5.0)*	*0.41 (-2.4, 1.0)*
IL-10 (ng/L)					
n	23	22	23		
Range	1.8 – 2.4	1.8 – 2.5	1.8 – 3.4		
Mean (SEM)	1.8 (0.03)	1.8 (0.02)	1.9 (0.07)	*0.47 (0.0, 0.1)*	*0.10 (-0.2, 0.0)*
MCP-1 (ng/L)					
n	23	22	23		
Range	84.2 – 612.8	68.7 – 555.1	75.7 – 543.8		
Mean (SEM)	313.7 (29.02)	281.0 (25.2)	273.5 (23.44)	***0.01*** *(11.4, 55.6)*	***0.01*** *(12.4, 68.1)*
PDGF-AA (ng/ml)					
n	23	22	23		
Range	7.0 – 26.5	7.0 – 23.9	6.7 – 22.9		
Mean (SEM)	14.3 (0.88)	13.6 (0.78)	13.4 (0.93)	***<0.01*** *(0.3, 1.4)*	*0.09 (-0.2, 2.0)*
TNF-α (ng/L)					
n	23	22	23		
Range	4.4 – 4.4	4.4 – 5.6	4.4 – 4.4		
Mean (SEM)	4.4 (0.00)	4.5 (0.05)	4.4 (0.00)	*0.33 (-0.2, 0.1)*	-
VEGF (ng/L)					
n	23	22	23		
Range	36.3 – 337.6	14.6 – 33.5	14.6 – 148.4		
Mean (SEM)	104.5 (15.26)	19.5 (1.30)	39.6 (5.94)	***<0.01*** *(51.4, 115.5)*	***<0.01*** *(37.2, 92.7)*

Table 5.13: Cytokine results for participants receiving aflibercept at baseline, one week and one month. Bold type denotes statistical significance.

[a] *all values below the lower limit of quantification*

B/L, baseline; C.I, confidence interval; EGF, epidermal growth factor; IFN-γ, interferon gamma; IL, interleukin; M1, one month; MCP-1, monocyte chemoattractant protein-1; PDGF-AA, platelet derived growth factor-AA; SEM, standard error of the mean; VEGF, vascular endothelial growth factor; W1, one week.

A subgroup analysis of change in VEGF concentration over time for each anti-VEGF drug was performed, using a two-way mixed ANOVA. There was a statistically significant interaction between the anti-VEGF drug and time on VEGF concentration, $F(2,92) = 15.93$, $p<0.001$, partial $\eta^2 = 0.091$. There was no statistically significant difference in VEGF concentration between ranibizumab and aflibercept at baseline (139.3 ± 16.7 ng/L vs 104.5 ± 17.7 ng/L, respectively; $p = 0.16$). The VEGF concentration was statistically significantly different between ranibizumab and aflibercept at one week (125.9 ± 11.5 ng/L vs 19.5 ± 12.9 ng/L, respectively; $p < 0.001$). In addition, the VEGF concentration was statistically significantly different at one month (133.4 ± 12.9 ng/L vs 39.6 ±14.3 ng/L, respectively; $p < 0.001$). There was no statistically significant effect of time on VEGF concentration for those receiving ranbizumab ($F(2,50) = 1.47$, $p = 0.24$, partial $\eta^2 = 0.0056$). There was a statistically significant effect of time on VEGF concentration for those receiving aflibercept ($F(2,42) = 24.8$, $p < 0.001$, partial $\eta^2 = 0.541$). Figure 5.16 shows the change in mean VEGF concentration over time, depending on anti-VEGF drug injected.

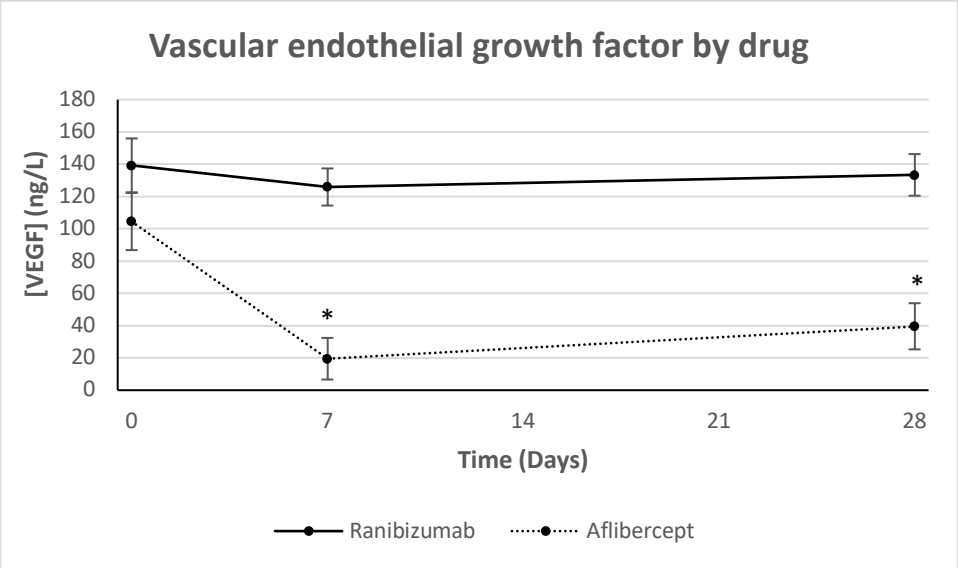

Figure 5.16: Effect of drug type on vascular endothelial growth factor concentration.

** indicates p< 0.05 (versus baseline). Error bars indicate 1 standard error of the mean. SE, standard error. VEGF, vascular endothelial growth factor.*

5.3.8 Clinical outcomes

The study was not designed to address visual or anatomic outcomes, and therefore did not mandate, nor formally collect, data at a clinical visit one or two months after anti-VEGF injection. Information was, however, gathered retrospectively, and a significant amount of data were not retrievable due to transfer of clinical records from a paper based system to electronic records. The majority of patients receiving aflibercept did not have any clinical assessment until two months following intravitreal injection.

Participants who received ranibizumab, with one month VA data available had a mean (± SEM) baseline of 55.8 (± 5.3) letters, which improved to 56.9 (± 5.4) letters at one month (n=13). Optical coherence tomography information was available for 20 patients, and showed

a reduction in central subfield thickness from 346.1 (± 19.1) μm to 302.9 (± 16.3) μm at one month. Similarly, macular volume reduced from 8.47 (± 0.15) mm^3 to 8.19 (± 0.12) mm^3.

Those receiving aflibercept had a reduction in VA from 62.2 (± 2.3) letters at baseline to 60.2 (± 2.9) letters at two months (n = 21). Central subfield thickness reduced from 318 (± 12.8) μm to 293.5 (± 13.6) μm, and macular volume reduced from 8.32 (± 0.16) mm^3 to 8.06 (± 0.17) mm^3 (n = 20).

5.3.9 Adverse events

Table 5.14 shows the ocular and non-ocular adverse events which occurred. No participants required anterior chamber paracentesis following the anti-VEGF injection. There were no cases of endophthalmitis or retinal detachment. There were three ocular events, which were minor and related to the injection procedure. One participant suffered a corneal abrasion immediately following the injection, likely related to the placement of the eyelid speculum. This was treated with chloramphenicol ointment. One participant presented one day following their injection with a mild punctate keratopathy, which required no further intervention. One participant had an intra-ocular pressure rise immediately after the injection, which was successfully managed with an anterior chamber paracentesis and a *stat* dose of topical apraclonidine.

n (%)	Arm A (n = 5)	Arm B (n = 24)	Arm C (n = 24)
Ocular			
Corneal abrasion			1 (4)
Keratopathy		1 (4)	
Intraocular pressure increased			1 (4)
Non-ocular			
Fall	1 (20)		
Haematuria		1 (4)	
Head injury	1 (20)		
Skin infection		2 (8)	
Haemarthrosis, site unspecified		1 (4)	
Respiratory tract infection			2 (8)

Table 5.14: Adverse events in the study, classified by medical dictionary for regulatory activities codes.

There were no deaths and no serious adverse events related to the study anti-VEGF injection or venepuncture. One participant developed haematuria, was investigated, and found to have a bladder stone. There were no suspected unexpected serious adverse reactions (SUSARs).

5.4 Discussion

This chapter investigated the pharmacokinetics and pharmacodynamics of ranibizumab and aflibercept in nAMD. The current failure of the ranibizumab assay prevents an analysis of the effect of vitrectomy on anti-VEGF pharmacokinetics. Multiple attempts were made with different assays from the same manufacturer, but in all cases we experienced failure of the negative controls. At the time of thesis submission, repeat analysis of the samples is planned with an assay from a different manufacturer, however, through much of the analysis phase, timelines have been greatly impacted by the COVID-19 pandemic. Although, at this stage, it is not possible to compare ocular clearance of ranibizumab and aflibercept, other insights arose via collection of serum systemic renal function and cytokine assays.

The aflibercept assay produced a serum concentration versus time curve which followed characteristic distribution and elimination phases. Once cleared from the eye, aflibercept predominantly exists in its inactive VEGF-bound form.[503] The assay in our study measured free aflibercept levels which acts as a better estimation of active drug concentration than total aflibercept. This enables a better understanding of how it may exert unwanted systemic side effects. The maximum concentration of free aflibercept occurred 24 hours following intravitreal injection.

The systemic half-life of aflibercept in our study was higher than we had expected. During the study design there was limited pharmacokinetic information about aflibercept available, aside from animal model studies which had calculated a vitreous half-life of 4.58 days.[208] After intravenous injection, the systemic half-life of aflibercept has been measured at 6 days.[504] We calculated the aflibercept systemic half-life to be 22.2 days after intravitreal

injection. This likely reflects the fact the eye acts as a depot, slowly releasing the drug into the systemic circulation. For ethical reasons, our study did not include ocular sampling and therefore we cannot directly compare our half-life against either systemic half-lives after intravenous injection, or ocular half-lives after intravitreal injection. However, we can use our calculations to infer trends on ocular clearance, for example in different vitreous states. Furthermore, our technique of serum sampling is less invasive than ocular sampling and therefore allows for multiple time points to be assessed over a short period.

Part of the conception of this work was to compare anti-VEGF ocular clearance depending on whether a PVD was present. For patients receiving aflibercept, the tmax occurred at 24 hours post-injection in those with PVD and without PVD. It may be that more frequent blood sampling is required around 24-48 hrs to detect a difference between vitreous status, but this would be challenging practically. We found a lower systemic half-life in PVD compared to no PVD, but this did not reach statistical significance. These results may suggest that aflibercept is cleared from the eye faster when a PVD is present, but further research would be required to confirm this. Due to the technical problem with the ranibizumab assay, it is not possible at this stage to analyse the effect of vitreous status on ranibizumab pharmacokinetics.

A significant reduction in serum VEGF concentration occurred one week after aflibercept. It is, however, important to note that no VEGF assays were performed in the sample period between baseline and one week. It is therefore possible that serum VEGF concentration changes occur earlier in the post-injection phase. The reduced serum VEGF concentration remained present at one month post-injection. In contrast, there was no significant change in serum VEGF concentration after ranibizumab. Given that both drugs are considered to have

similar ophthalmic efficacy, a reduction in serum VEGF concentration is therefore likely due to systemic drug binding rather than a secondary effect of a reduction in ocular disease activity. Systemic VEGF concentration is an important and tightly regulated process. It is crucial for physiological angiogenesis, widely produced by various cell types, and is upregulated in the presence of hypoxia, as described in the introduction.[480]

Vascular endothelial growth factor also acts as a pro-inflammatory cytokine, via its mechanisms of increasing endothelial cell permeability, acting as a monocyte chemoattractant and inducing expression of endothelial adhesion molecules.[505, 506] There is substantial overlap, therefore, between VEGF and inflammation in the pathogenesis of nAMD in addition to its pro-angiogenic function.[507] Local inflammation contributes to retinal pigment epithelium and photoreceptor degeneration.[508] Choroidal neovascularisation is associated with complement system activation and anti-VEGF intravitreal injections have been shown to increase the concentration of intraocular inflammatory cytokines, MCP-1 and TGF- β.[271] In our study, we sought to investigate how systemic inflammatory markers changed following an intravitreal anti-VEGF injection using a hsCRP assay and cytokine panel.

Our detailed screen identified changes in cytokine concentrations at baseline, one week and one month. For the entire cohort and both anti-VEGF subgroups, EGF significantly reduced from baseline to one week before returning to a similar concentration at one month compared to baseline. Jonas et al. previously found aqueous EGF concentrations to be higher at baseline in a nAMD cohort compared to a control group.[430] This is a pro-inflammatory cytokine, which might indicate that control of macular disease results in a lower serum inflammatory signal, for example after anti-VEGF injection.

Similarly, PDGF-AA followed a trajectory of a significant decrease at one week, but not at one month. Subgroup analysis found the PDGF-AA change occurred in the aflibercept patients only. PDGF-AA is a growth factor which stimulates cellular proliferation and directs cellular movement.[509] A previous study has shown increased vitreous levels of PDGF-AA in non-proliferative diabetic retinopathy, but serum levels were similar between patients and controls, suggesting the findings were due to intraocular synthesis rather than serum diffusion.[509] The lack of any change in our ranibizumab group would support this theory, and instead the serum findings we identified after aflibercept may be due to the systemic effect of the drug as per the VEGF findings.

Interleukin-4 and MCP-1 were both significantly reduced at one week and one month, compared to baseline. Those receiving ranibizumab saw a reduction in MCP-1 concentration at one week only, before returning to baseline at one month. This is in contrast to aflibercept patients who had a significantly lower concentration of MCP-1 at one week and one month, compared to baseline. Previous studies have shown aqueous concentrations of MCP-1 significantly reduce at one month following the second ranibizumab or aflibercept injection of a loading dose for nAMD compared to baseline.[428, 453] Monocyte protein-1 recruits monocytes to sites of inflammation, which results in a major cascade of cytokine upregulation (including VEGF and IL-6) and animal laser induced CNV models have demonstrated its involvement in macrophage infiltration.[457, 510] Our serum findings may therefore indicate control of localised macular inflammation, particularly in the early phase after anti-VEGF.

No significant changes were seen in the serum concentrations of the other measured cytokines. This may be due to how localised the nAMD disease process is, with disease

confined to the RPE and retina at the macula. In contrast, patients with diabetic maculopathy or macular oedema secondary to vein occlusion, have a larger area of ischaemic disruption, and therefore a further study using these patients might result in a greater effect on systemic cytokine concentrations.

Overall, it is challenging to assign mechanistic reasons for the cytokine changes in our study, particularly as we used serum profiles. Either the changes we did observe support a link between angiogenesis and inflammation in nAMD, or reflect a change in the serum cytokine profile, possibly as a result of systemic drug interactions, which at present are unknown.

We did not detect a significant change in serum hsCRP concentration from baseline to either one week or one month when the entire cohort was analysed together. However, subgroup analysis revealed those receiving aflibercept had a significant rise in systemic hsCRP concentration from baseline to one week and one month. No such change was seen with ranibizumab. This did not support our original hypothesis, that serum hsCRP concentration might reflect macular disease activity and therefore reduce following an anti-VEGF injection. Our work suggests that hsCRP is not a good marker for disease activity and response to treatment. Similar to the cytokine findings, it suggests that systemic drug binding may be responsible for changing the systemic inflammatory environment.

Patients receiving aflibercept were also found to have an increase in serum creatinine from baseline to one week and one month, although this did not reach statistical significance. In contrast, those receiving ranibizumab had minimal change in creatinine. On the basis of this study, it is uncertain whether aflibercept truly affects serum creatinine, and if it does, whether this is clinically relevant to renal function. Aflibercept has been shown in monkey studies to

bind to renal glomeruli and cause reduction in the number of endothelial fenestrations.[252] No similar binding was observed with ranibizumab, which might suggest ranibizumab confers a lower potential risk of nephrotoxicity. This may be due to its more rapid clearance from the systemic circulation. Aptamers have shorter half-lives and therefore may be safer in at risk groups. Further studies of renal function with larger numbers of patients receiving anti-VEGF agents could further investigate this.

There are numerous descriptions of acute kidney injury after anti-VEGF, as described earlier. In the kidney, VEGF has been shown to be highly expressed by renal podocytes and responsible for activating VEGF receptor 2 on glomerular endothelial cells to maintain structure and function. Dysregulation could conceivably therefore affect renal function, but this was not strongly suggested in our study. It may be that some patients have a genetic susceptibility to anti-VEGF induced renal scarring, and therefore it may be prudent for patients receiving frequent anti-VEGF treatment to undergo periodic monitoring of their renal function. A small retrospective study of 85 patients receiving anti-VEGF for DMO did not find any treatment related change in eGFR over a mean duration of 2.6 years.[511]

Our study also included calculating eGFR, which is a measure of chronic renal failure after scarring. Whilst there was a small decrease in eGFR with both aflibercept and ranibizumab, neither reached statistical significance. It is also difficult to interpret the significance of eGFR change over a small time interval since scarring would usually be expected to occur at least three months after kidney injury rather than over our one month study period.[512] However, the vast majority of patients in this study had received numerous previous anti-VEGF injections and may have already had subclinical renal scarring which could have been

potentiated by the study anti-VEGF injection. A future avenue for research could measure serial eGFR measurements in patients receiving anti-VEGF over a longer period of time.

We performed a subgroup analysis to investigate whether worse baseline renal function, as measured by eGFR status, conferred an increased risk for renal function deterioration. We found those with better baseline renal function (eGFR > 60 ml/min/1.73m^2) were more likely to have an increase in creatinine than those with chronic kidney disease (eGFR < 60 ml/min/1.73m^2). Similarly, those with a known diagnosis of hypertension did not have an increased risk of renal dysfunction. It is difficult to explain these findings physiologically, as we might expect those with impaired kidney function or co-pathology to be more susceptible to systemic drug side effects. However, it is reassuring that these at-risk groups did not undergo worsening of their renal function. Kameda et al. who found no deterioration in eGFR after aflibercept, bevacizumab or ranibizumab at one week or one month.[513] Furthermore, their study focused on patients receiving anti-VEGF for diabetic retinopathy, which is a group who could conceivably be at higher risk with coexisting nephropathy.

There are a number of limitations with our study. Firstly, no ocular fluid samples were taken. This was due to the ethical issues of repeated aqueous or vitreous sampling in such a short space of time, which would have exposed the participants to unnecessary risk. The lack of paired ocular drug levels prevents the calculation of an ocular half-life, despite this being the basis of our power calculations. It is also important to note that aqueous concentration does not necessarily correlate with vitreous concentration. This was evident in work by Zapata et al. who found the aqueous concentration of VEGF to be lower in treatment naïve nAMD patients compared to controls, despite the majority of studies showing higher VEGF concentrations in the vitreous cavity when nAMD is present.[449] Repeat vitreous sampling is

impractical, and therefore our work represents a new method of identifying trends in ocular clearance at multiple time points by analysing serum samples.

Another limitation of our work was the accuracy of the assays. As described above, there were substantial issues with the reliability of the ranibizumab assay. Drug assays are known to have measurement variability, and ideally should be run with duplicate or triplicate measurements. We did not have the ability for multiple testing of each sample due to the high cost of the drug assays and the volume of sample stored. Furthermore, our samples were stored at -80 degrees Celsius until batch testing. We assumed the drug remained stable at this temperature. Due to the nature of the multi-sample testing, it would not have been feasible to test each sample immediately post-collection.

There were also limitations in the power calculations and statistical plan. Our power calculations were based on a number of assumptions based on anti-VEGF pharmacokinetic information at that time. Due to the relatively recent introduction of aflibercept, we based our power calculations on animal model studies of ocular half-life. This means we may have been underpowered to detect cytokine concentration changes, which were a secondary outcome measure and did not feature in the power calculations. In addition, the significant under recruitment to Arm A for clinical reasons will underpower our assessment of the effect of vitrectomy. Furthermore, we also noticed some differences in the baseline characteristics between groups, such as previous number of anti-VEGF injections, which may have confounded our analysis. It is important to note, however, that our sample size was relatively small, which impacts the relevance of baseline characteristic comparison.

A weakness of our study design was that it did not include a mandated VA and macular assessment at one month post treatment. With hindsight, this information would have been beneficial in order to look for a correlation between cytokine change and anatomical or visual benefit. Patients with nAMD-associated subretinal fluid have been shown to have a different vitreous cytokine footprint to those without subretinal fluid.[514] Furthermore, IL-6 has been shown to be a biomarker for treatment response, with concentrations correlating with macular central subfield thickness change.[450]

In conclusion, the main finding of the study was that patients receiving aflibercept had a significant reduction in the serum VEGF concentration at one week and one month, which was in contrast to those receiving ranibizumab where the concentration was unchanged. This supports the literature that shows aflibercept is cleared more slowly than ranibizumab, and could also explain the trends in our study towards potential systemic side effects affecting the renal system and the cytokine concentration profile. Further studies to identify the systemic risk of aflibercept are warranted in all conditions which are managed with anti-VEGF agents and with an emphasis on at risk groups such as diabetics or those with pre-existing renal disease.

Another finding was the trend to decreased systemic half-life of aflibercept when a PVD was present compared to an attached vitreous. The study was not powered to answer this question, which might explain why the difference was not statistically significant. However, it is an interesting observation, which warrants further investigation in an appropriately powered study. The analysis in this thesis cannot currently determine whether vitreous status has a clinically significant effect on anti-VEGF clearance and inflammatory markers. Studies have shown the aqueous concentrations of many cytokines including VEGF are lower if a PVD is

present, which suggests that vitreous status may play a role in the disease course.[515] Patients with complete PVD have been measured as having lower aqueous concentrations of VEGF than their attached vitreous counterparts.[516] Our work showed some signals which suggest the presence of a PVD may alter the pharmacokinetics of aflibercept, but further investigation is needed to understand whether this is clinically relevant and whether it could suggest a benefit in changing dosing schedules.

6 Conclusion

As William Osler stated, *"The good physician treats the disease; the great physician treats the patient who has the disease."* We must never lose sight of the importance of ensuring the treatment options are acceptable for our patients. One aspect of this is ensuring systemic safety and appropriate dosing.

Symptomatic vitreomacular adhesion

The first two aims of this thesis were to assess the likelihood of successful vitreous separation with intravitreal ocriplasmin and determine its safety. A meta-analysis was performed of randomised controlled trials (RCTs) which had been published before February 2017. The headline result was that ocriplasmin treatment, compared to control, was more likely to result in vitreomacular adhesion (VMA) release within 28 days (risk ratio (RR) 3.46, 95% confidence interval (CI) 2.00 to 6.00; 859 eyes across 4 RCTs).[302] This work was published by Cochrane, and as per convention, will be reappraised at 5 year intervals.[302]

Since the publication of our meta-analysis, there has been further research on the efficacy and safety of ocriplasmin. A very recent systematic review using individual participant data for the meta-analysis of RCT data was performed by Jackson et al.[517] This technique of analysis has the benefit of providing subgroup analysis using patient-level data, to identify which patient characteristics might increase the likelihood of successful treatment, as well as a more in depth look at any adverse or serious adverse events. The review confirmed the findings of our Cochrane review in terms of likelihood of VMA release, macular hole (MH) closure, and visual benefit. Importantly, it also highlighted that increasing age, male gender and the

presence of broad VMA were associated with decreased treatment response. Regarding safety, early-onset temporary visual impairment was common, but did not affect final visual outcome.

The third aim of the thesis was to determine the risk of dyschromatopsia after ocriplasmin. There was a trend towards worsening of hue discrimination at one week and one month after ocriplasmin, with a return to baseline at one year. This is the first study to primarily investigate hue discrimination after ocriplasmin treatment, but is limited in its conclusions due to the small sample size. Interestingly, Jackson et al. found 4.5% of ocriplasmin injected patients suffered dyschromatopsia, although there is limited information about duration or severity.[517] Subjective reports of changes in colour vision coupled with our findings suggest a larger study is warranted to further investigate the effect of ocriplasmin on colour vision.

There is still debate on whether ocriplasmin should be used as a first line treatment for symptomatic vitreomacular adhesion (sVMA).[299] It has been shown that prompt pars plana vitrectomy (PPV) for sVMA does not provide better outcomes in comparison with PPV, if required, after ocriplasmin injection.[518] Therefore, there may be a role for ocriplasmin as a less invasive first line treatment option for sVMA.

Chapter 4 of the thesis addressed another potential treatment option for sVMA; an intravitreal gas bubble. Our literature synthesis was encouraging for the potential benefit of gas, and concluded that a definitive RCT would be of benefit.[361] The Protocol AG and Protocol AH studies were designed by the Diabetic Retinopathy Clinical Research Network to determine the safety and efficacy of an intravitreal gas injection for symptomatic vitreomacular traction (VMT) and full-thickness macular hole (FTMH), respectively.[362, 363] The results of the studies

were published in November 2021.[364] They found that gas resolved VMT in most eyes, and FTMH, if present, closed in 33% of cases. However, the studies were terminated early due to a high rate of retinal detachment.

At this stage, there is insufficient information to know whether these studies represent a moratorium for gas as a treatment option for sVMA. The use of gas as part of pneumatic retinopexy for rhegmatogenous retinal detachment (RRD) is established, but its use is determined by pre-operative disease characteristics such as the position of the retinal breaks.[519] Although there are differences between RRD and sVMA, the increasing interest in pneumatic retinopexy might suggest gas for sVMA could be an option if the inclusion and exclusion criteria can be better delineated to improve its safety profile.

Vitreomacular interface and age-related macular degeneration

Chapter 5 reported the findings of the VITCLEAR study. This looked at the effect of the vitreomacular status on anti-vascular endothelial growth factor drugs (anti-VEGF) and their systemic safety. At the time of thesis submission, the ranibizumb assay has not been performed due to multiple assay technical failures, and this is explained in detail within the chapter.

There was a trend towards a decreased systemic half-life of aflibercept in eyes where a posterior vitreous detachment (PVD) was present, compared to those with an attached vitreous. Larger numbers are required to power a study specifically to answer this question with statistical significance. However, the findings do suggest anti-VEGF agents might be cleared faster from an eye with a PVD, and therefore our work adds weight to the

multifactorial argument that vitreomacular status influences neovascular age-related macular degeneration (nAMD) and its treatment. At this stage, it is too early to tell if vitreomacular status should influence dosing regimens or treatment intervals.

There is substantial research occurring into new therapeutic options for nAMD, many of which are addressing treatment interval. The port delivery system (PDS; Genentech, South San Francisco, CA, USA) with ranibizumab recently obtained approval from the United States Food and Drug Administration (FDA).[520] The PDS is a surgically implanted reservoir, filled 6-monthly with ranibizumab to provide a continuous supply of anti-VEGF treatment without the need for frequent intravitreal injections. The phase 3 trial of the PDS met its primary endpoint of non-inferiority and equivalence in terms of visual acuity when compared to monthly ranibizumab.[521] By the first 24 week interval, 98.4% did not require supplementary rescue treatment. Furthermore, compliance and acceptability was favourable, with 93% preferring the PDS to regular intravitreal injections.[522] Longer term safety and efficacy is awaited.

There are other nAMD therapeutics in development which are attempting to reduce the treatment burden. Faricimab (Roche, Basel, Switzerland) is a bispecific antibody against VEGF-A and angiopoietin-2 (ang-2).[523] The targeting of ang-2 is designed to stabilise vessels, reduce vascular leakage and reduce inflammation.[523] Two multicentre phase 3 trials are underway (TENAYA and LUCERNE) to investigate the efficacy, safety and durability of faricimab.[524, 525] Early data have been promising, and show noninferiority of faricimab (given monthly for 4 months, and then T&E) versus mandated biomonthly aflibercept at the week 48 endpoint.[526] Nearly 80% (79.7% for TENAYA, and 77.8% for LUCERNE) of patients have required 12-weekly or less frequent dosing at week 48. The data are yet to be fully analysed,

but there was a numerical increase in intraocular inflammation in the faricimab group.[526] It remains to be seen whether ang-2 pathway modification has any effect on systemic homeostasis, as we suggested in our work on aflibercept.

Aside from new therapeutic agents, research continues to determine whether extending the interval of existing therapies might still maintain efficacy. Less frequent anti-VEGF treatment would likely be beneficial for reducing any potential systemic risk. The efficacy and safety of intravitreal aflibercept (IVT-AFL) T&E compared with fixed dosing (q8) neovascular age-related macular degeneration (AZURE) study has found non-inferior visual outcomes with T&E at week 52. Furthermore, 37% of patients achieved an interval of over 12 weeks. This reduction in dosing frequency would be reassuring when considering our findings of systemic VEGF suppression and the concerns of renal dysfunction.

Finally, as with all new treatments, it remains to be seen whether trial data efficacy would be replicated in real world usage. The safety signal can also change substantially, as was seen with intraocular inflammation and brolucizumab.[527] Due to the volume of cases required for rare systemic adverse events to occur, it is also possible that systemic safety of anti-VEGF, as discussed in this thesis, would take even longer to establish.

The landscape of nAMD therapeutics continues to develop. We continue to strive for the most efficient and cost-effective therapeutic options, whilst ensuring their ocular and systemic side effect profile is acceptable.

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Appendix 1

Cochrane Library

Cochrane Database of Systematic Reviews

Ocriplasmin for symptomatic vitreomacular adhesion (Review)

Neffendorf JE, Kirthi V, Pringle E, Jackson TL

Neffendorf JE, Kirthi V, Pringle E, Jackson TL.
Ocriplasmin for symptomatic vitreomacular adhesion.
Cochrane Database of Systematic Reviews 2017, Issue 10. Art. No.: CD011874.
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[Intervention Review]

Ocriplasmin for symptomatic vitreomacular adhesion

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ABSTRACT

Background

Symptomatic vitreomacular adhesion (sVMA) is a recognised cause of visual loss and by tradition has been managed by pars plana vitrectomy (PPV). A less invasive alternative to surgery in some people is enzymatic vitreolysis, using an intravitreal injection of ocriplasmin.

Objectives

To assess the efficacy and safety of ocriplasmin compared to no treatment, sham or placebo for the treatment of sVMA.

Search methods

We searched the Cochrane Central Register of Controlled Trials (CENTRAL) (which contains the Cochrane Eyes and Vision Trials Register) (2017, Issue 1), MEDLINE Ovid (1946 to 24 February 2017), Embase Ovid (1947 to 24 February 2017), PubMed (1946 to 24 February 2017), the ISRCTN registry (www.isrctn.com/editAdvancedSearch); searched 24 February 2017, ClinicalTrials.gov (www.clinicaltrials.gov); searched 24 February 2017 and the World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) (www.who.int/ictrp/search/en); searched 24 February 2017. We did not use any date or language restrictions in the electronic searches for trials.

Selection criteria

We included randomised controlled trials (RCTs) of people with sVMA. The intervention was intravitreal ocriplasmin 125 µg injection, and this was compared to placebo or sham injection (control). Placebo was defined as a single intravitreal injection of 0.10 mL placebo with identical drug vehicle diluted with saline. A sham injection was defined as the syringe hub or blunt needle touching the conjunctiva to simulate an injection.

Data collection and analysis

Two authors independently selected relevant trials, assessed methodological quality and extracted data. We graded the certainty of the evidence using the GRADE approach.

Main results

This review included four RCTs conducted in Europe and the USA with a total of 932 eyes of 932 participants. Participants were 18 to 97 years of age, with evidence of focal vitreomacular adhesion (VMA) on optical coherence tomography (OCT) imaging, with a best corrected visual acuity (BCVA) of 20/25 or worse in the study eye and 20/400 or better in the fellow eye. The interventions compared were intravitreal ocriplasmin versus sham (two RCTs) or placebo (two RCTs) injection. Both sham and placebo injection were classified as the control group. The main outcome measures were assessed at 28 days and six months. Overall, we judged the studies to have a low or unclear risk of bias. All four RCTs were sponsored by the manufacturers of ocriplasmin.

Compared with control, ocriplasmin treatment was more likely to result in VMA release within 28 days (risk ratio (RR) 3.46, 95% confidence interval (CI) 2.00 to 6.00; 859 eyes, 4 RCTs, high-certainty evidence). Approximately 97/1000 eyes will have VMA release within 28 days

without treatment. An additional 237 eyes will have VMA release within 28 days for every 1000 eyes treated with ocriplasmin (95% CI 96 more to 482 more).

Treatment with ocriplasmin was also more likely to result in macular hole closure (RR 2.87, 95% CI 1.50 to 5.51; 229 eyes, 3 RCTs, high-certainty evidence). Approximately 123/1000 eyes with macular holes will have closure with no treatment. An additional 231 eyes will have macular hole closure for every 1000 eyes treated with ocriplasmin (95% CI 62 more to 556 more).

Eyes receiving ocriplasmin were also more likely to have complete posterior vitreous detachment (PVD) within 28 days (RR 2.94, 95% CI 1.39 to 6.24; 689 eyes, 3 RCTs, high-certainty evidence). Approximately 40/1000 eyes will have complete PVD within 28 days without treatment. An additional 78 eyes will have complete PVD within 28 days for every 1000 eyes treated with ocriplasmin (95% CI 16 more to 210 more).

Eyes receiving ocriplasmin were more likely to achieve 3-line or greater improvement in BCVA at six months (RR 1.95, 95% CI 1.07 to 3.53; 674 eyes, 3 RCTs, moderate-certainty evidence). Approximately 61/1000 eyes will have a 3-line or greater improvement in BCVA at six months without treatment. An additional 58 eyes will have 3-line or greater improvement in BCVA at six months for every 1000 eyes treated with ocriplasmin (95% CI 9 more to 154 more).

Receiving ocriplasmin also reduced the requirement for vitrectomy at six months (RR 0.67, 95% CI 0.50 to 0.91; 689 eyes, 3 RCTs, moderate-certainty evidence). Approximately 265/1000 eyes will require vitrectomy at six months without treatment and 87 fewer eyes will require vitrectomy for every 1000 eyes treated with ocriplasmin (95% CI 24 fewer to 132 fewer).

Treatment with ocriplasmin resulted in a greater improvement in validated Visual Function Questionnaire form score at six months (mean improvement difference 2.7 points, 95% CI 0.8 to 4.6; 652 eyes, 2 RCTs, moderate-certainty evidence).

Eyes receiving ocriplasmin were more likely to have an adverse event (RR 1.22, 95% CI 1.09 to 1.37, 909 eyes, 4 RCTs, moderate-certainty evidence). Approximately 571/1000 eyes will have an adverse event with sham or placebo injection and 106 more eyes will have an adverse event for every 1000 eyes treated with ocriplasmin (95% CI 52 more to 212 more).

Authors' conclusions

Evidence from a limited number of RCTs suggests that ocriplasmin is useful in the treatment of sVMA. However, up to 20% of eyes treated with ocriplasmin will still require additional treatment with PPV within six months. There were more ocular adverse events in eyes treated with ocriplasmin than control (sham or placebo injection) treatment. Many of these adverse events, particularly vitreous floaters and photopsia, are known to be associated with posterior vitreous detachment. At present however, there is minimal published long-term safety data on eyes treated with ocriplasmin. Further large RCTs comparing ocriplasmin with other management options for sVMA would be beneficial.

PLAIN LANGUAGE SUMMARY

Ocriplasmin for symptomatic vitreomacular adhesion

What is the aim of this review?
The aim of this Cochrane Review was to find out how well ocriplasmin works in the treatment of symptomatic vitreomacular adhesion (sVMA). Cochrane Review authors collected and analysed all relevant studies to answer this question and found four studies.

Key messages
People with sVMA treated with ocriplasmin have an increased chance of release of sVMA and improved vision compared with people who are not treated with ocriplasmin (high-certainty evidence). They are also probably less likely to require surgery, but one in five people with sVMA treated with ocriplasmin will probably still require surgery at a later date to treat sVMA (moderate-certainty evidence).

What was studied in the review?
With age, the gel-like substance (vitreous) that fills the eye begins to pull away from the back of the eye (retina). Sometimes the vitreous remains attached to the retina and causes damage to the retina as it pulls away, leading to visual loss. This is known as symptomatic vitreomacular adhesion or sVMA. sVMA includes two related conditions, vitreomacular traction and macular hole.

The standard treatment for sVMA is surgery. Ocriplasmin is an alternative, less invasive, treatment. This is an enzyme that can be injected directly into the eye to release the vitreous from the retina.

What are the main results of the review?
Cochrane Review authors found four studies that compared ocriplasmin with control (sham or placebo treatment) for the treatment of sVMA. All four studies were sponsored by the manufacturers of ocriplasmin.

The review showed that:

• ocriplasmin increases the chance of sVMA resolution compared with no treatment (high-certainty evidence);

• people with sVMA treated with ocriplasmin have improved vision compared with people who are not treated with ocriplasmin (high-certainty evidence);
• treatment with ocriplasmin probably reduces the requirement for surgery, but approximately one in five people treated with ocriplasmin may require further surgery at a later date (moderate-certainty evidence);
• there were more ocular adverse events in eyes treated with ocriplasmin than control (sham or placebo injection) treatment.

How up-to-date is this review?
Cochrane Review authors searched for studies that had been published up to 24 February 2017.

SUMMARY OF FINDINGS

Summary of findings for the main comparison. Ocriplasmin injection compared with control for symptomatic vitreomacular adhesion

Ocriplasmin injection compared with control for symptomatic vitreomacular adhesion

Patient or population: people with symptomatic vitreomacular adhesion

Settings: eye hospital

Intervention: ocriplasmin injection

Comparison: sham or placebo injection

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of eyes (studies)	Certainty of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Sham or placebo injection	Ocriplasmin injection				
Complete release of vitreous adhesion Follow-up: 28 days	**97 per 1000**	**334 per 1000** (193 to 579)	**RR 3.46** (2.00 to 6.00)	859 (4 studies)	⊕⊕⊕⊕ **High**	-
Closure of macular hole Follow-up: 28 days to 24 months	**123 per 1000**	**354 per 1000** (185 to 679)	**RR 2.87** (1.50 to 5.51)	229 (3 studies)	⊕⊕⊕⊕ **High**	-
Complete posterior vitreous detachment Follow-up: 28 days	**40 per 1000**	**118 per 1000** (56 to 250)	**RR 2.94** (1.39 to 6.24)	689 (3 studies)	⊕⊕⊕⊕ **High**	-
3-line or greater improvement in best-corrected visual acuity Follow-up: 6 months	**61 per 1000**	**119 per 1000** (70 to 215)	**RR 1.95** (1.07 to 3.53)	674 (3 studies)	⊕⊕⊕⊖ **Moderate**[a]	-
Requirement for vitrectomy Follow-up: 6 months	**265 per 1000**	**178 per 1000** (133 to 241)	**RR 0.67** (0.50 to 0.91)	689 (3 studies)	⊕⊕⊕⊖ **Moderate**[a]	-
Mean change in validated visual function questionnaire score from baseline	**Mean change in NEI-VFQ score was 0.7**	**NEI-VFQ score was 2.7 higher** (0.8 higher to 4.6 higher)	-	652 (2 studies)	⊕⊕⊕⊖ **Moderate**[a]	-

Score ranges from 0 to 100, higher scores are better visual function Follow-up: 6 months						
Any ocular adverse event Follow-up: 6 months	**571 per 1000**	**697 per 1000** (623 to 783)	**RR 1.22** (1.09 to 1.37)	909 (4 studies)	⊕⊕⊕⊖ **Moderate**[a]	-

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: confidence interval; **NEI-VFQ:** National Eye Institute Visual Function Questionnaire; **RCT:** randomised controlled trial; **RR:** risk ratio.

GRADE Working Group grades of evidence
High-certainty: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate-certainty: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low-certainty: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low-certainty: We are very uncertain about the estimate.

[a]Downgraded one level for imprecision (-1).

BACKGROUND

Description of the condition

In healthy eyes, the posterior vitreous face lies in contact with the internal limiting membrane (ILM) of the retina with various points of stronger adhesion such as the macula, vasculature and optic disc. Over time, the structure of the vitreous liquefies in a process known as synchysis, with reduction in the adhesive forces between vitreous and ILM. This often results in the vitreous gel detaching from all parts of the retina, except at the vitreous base anteriorly, in a normal process known as posterior vitreous detachment (PVD) (Foos 1982). The process usually starts with focal detachment in the perifovea of the superior quadrant and then extends slowly for years until eventually resulting in a complete PVD with release of vitreopapillary adhesion (Ito 2003; Johnson 2010; Uchino 2001). However, in certain cases, incomplete PVD may occur, leaving the vitreous in contact with the macula or optic disc, or both.

Although, anatomically, vitreomacular adhesion (VMA) may refer to a normal asymptomatic state, clinically, the term is used when VMA occurs in the context of an incomplete PVD. There is a spectrum of VMA associated with incomplete PVD, which ranges from asymptomatic, non-tractional VMA to extensive distortion of the retinal structure due to vitreomacular traction (VMT) which may result in loss of visual function. These distinctions tend to be based on optical coherence tomography (OCT), sometimes in reference to defined photographic standards (Simpson 2012). However, it is important to note that the OCT changes, which may include retinal thickening and intraretinal oedema, do not always correlate with visual function and symptoms.

Symptomatic vitreomacular adhesion (sVMA) is defined as visual loss secondary to foveal damage caused by abnormal VMT. sVMA includes isolated VMT, impending macular hole (MH) and MH with persisting vitreous attachment (Jackson 2013a). Impending MH is often grouped with VMT. Epiretinal membrane (ERM) often coexists with sVMA. It is possible that VMA influences the clinical course of, or may be associated with, other diseases such as diabetic macular oedema, retinal vein occlusion or neovascular age-related macular degeneration, although the data are sometimes conflicting (Jackson 2013a; Jackson 2013b; Nomura 2014; Simpson 2012; Terao 2014; Waldstein 2014; Yoon 2014). Whilst there may be an association between sVMA and these other diseases, it is not certain that this is causal (Simpson 2012). Consequently, it is difficult to define the prevalence of sVMA. One study reported that VMA may occur in isolation or in association with other eye disease in approximately 1.5% of the population (Jackson 2013a). However, the majority of these cases occurred alongside ERM, and thus the VMA may not be responsible for visual loss. Excluding cases associated with ERM reduced the prevalence to 0.35% in the same population-based study; however, this figure also included cases with other diseases, such as wet age-related macular degeneration and diabetic macular oedema (Jackson 2013a). If only cases of isolated VMA/VMT with or without MH were considered, then the prevalence of sVMA was 171.5 per 100,000 population (Jackson 2013a).

The natural history of sVMA varies. sVMA may spontaneously resolve, with detachment of the posterior vitreous face from the ILM (Steel 2013). One study of 53 eyes showed a complete PVD occurred in 11% of eyes over 60 months' follow-up (Hikichi 1995). Weinard and colleagues reported that approximately 10% of cases of VMT syndrome resolve spontaneously (Weinard 2009). Other studies have found spontaneous resolution in 17% to 35% of cases with VMT (Almeida 2015; Theodossiadis 2014; Zhang 2015). Eyes with VMT and isolated inner retinal distortion, as well as those receiving vitreous injections, have an increased likelihood of VMT release (Almeida 2015). Poor prognostic indicators for spontaneous release include the presence of ERM and large horizontal adhesion diameter (Haller 2015; Jackson 2016; Theodossiadis 2014; Zhang 2015). It has been shown that many, if not most, MHs result from persistent VMT which either fully detaches from the retina causing an MH, or remains attached at the edge of the hole (Chauhan 2000; Gass 1988; Gaudric 1999; La Cour 2002; Tanner 2001).

Description of the intervention

Treatment strategies for VMA vary depending on disease severity. Asymptomatic VMT can be observed, since separation of the posterior vitreous face may occur spontaneously and without sequelae. However, a longer duration of VMT may lead to loss of vision and possibly lower efficacy of any subsequent intervention, and therefore treatment is often considered if symptoms are significant or visual acuity is reduced (Hikichi 1995; Melberg 1995; Sonmez 2008). If VMT progresses to MH then intervention is usually advised, and an evolving VMT/impending MH may likewise necessitate intervention.

If intervention is considered for sVMA, various strategies may be considered. Traditionally, pars plana vitrectomy (PPV) is the standard approach for VMT or MH (Steel 2013). Small uncontrolled studies reported that an intravitreal gas bubble can pneumatically release VMT, without the need for PPV, with success rates varying from 71% to 95% (Chan 1995; Mori 2007; Rodrigues 2013).

Pharmacological vitreolysis has been investigated as an alternative treatment for VMT, and for MH with persisting VMA (Benz 2010; De Smet 2009; Stalmans 2010; Stalmans 2012). Autologous plasmin, an enzyme that breaks down the laminin and fibronectin bonds maintaining vitreous adhesion, has been used perioperatively to induce a PVD during vitrectomy (Margherio 1998; Sakuma 2006; Williams 2001). However, autologous plasmin is not suited to the treatment of VMT due to its autolytic instability (Gandorfer 2008). Based on autologous plasmin, a recombinant DNA molecule, initially referred to as microplasmin, and more recently ocriplasmin (Jetrea; ThromboGenics, Leuven, Belgium), was developed to provide the same catalytic properties but with greater stability.

Ocriplasmin is administered as a single intravitreal injection of 125 µg in 0.1 mL. It has marketing authorisation for the treatment of VMT, including when associated with MH of diameter of 400 µm or less (SmPC 2013). In the UK, the National Institute for Health and Care Excellence (NICE) supports the use of ocriplasmin for adults with VMT causing severe sight problems or a macula hole up to 400 µm, in the absence of ERM (NICE 2013).

How the intervention might work

Ocriplasmin is a proteolytic enzyme which targets laminin and fibronectin, both of which are important structural components of the interface between the vitreous and the retina. It is a truncated form of the human serine protease plasmin which functions in a two-stage mechanism; liquefaction of the vitreous and vitreoretinal separation (Kuppermann 2012).

Why it is important to do this review

Ocriplasmin has marketing authorisation in Europe and the USA and is the only licensed, non-surgical treatment for sVMA. MH is the second most common indication for PPV, and both MH and VMT can cause substantial visual problems (Jackson 2013c). This review is important as it assessed the efficacy and safety of ocriplasmin treatment.

OBJECTIVES

To assess the efficacy and safety of ocriplasmin compared to no treatment, sham or placebo for the treatment of sVMA.

METHODS

Criteria for considering studies for this review

Types of studies

We included randomised controlled trials (RCTs) only.

Types of participants

We included participants with a diagnosis of sVMA, including VMT and MH of 400 µm or less with persisting VMA. There were no restrictions with regards to gender, age or ethnicity.

Types of interventions

We included any RCT in which intravitreal ocriplasmin was compared to no treatment, sham injection or placebo.

Types of outcome measures

Primary outcomes

- Proportion of eyes with complete release of vitreous adhesion as determined by analysis of OCT images captured 28 days after ocriplasmin, sham or placebo treatment.

Secondary outcomes

- Proportion of eyes with closure of MH as determined by analysis of OCT images captured 28 days after ocriplasmin, sham or placebo treatment.
- Proportion of eyes with complete PVD as measured by clinical examination or B-scan ultrasonography 28 days after ocriplasmin, sham or placebo treatment.
- Proportion of eyes with 3-line or greater improvement in best corrected visual acuity (BCVA) from baseline, measured using Early Treatment Diabetic Retinopathy Study (ETDRS) at 4 m or Snellen chart, at six months after ocriplasmin, sham or placebo treatment.
- Proportion of eyes requiring PPV within six months of ocriplasmin, sham or placebo treatment (as recommended by the investigator if the underlying condition deteriorated, BCVA worsened by more than 2 lines on ETDRS or Snellen chart, or if the underlying condition had not improved within 28 days after treatment).
- Mean change in validated Visual Function Questionnaire (VFQ) score from baseline, measured at six months after ocriplasmin, sham or placebo treatment.

Safety outcomes

- Description of ocular adverse events and serious adverse events, and any non-ocular serious adverse events attributed to ocriplasmin or no treatment/sham/placebo.

Search methods for identification of studies

Electronic searches

The Cochrane Eyes and Vision Information Specialist conducted systematic searches in the following databases for randomised controlled trial and controlled clinical trials. There were no language or publication year restrictions. The date of the search was 24 February 2017.

- Cochrane Central Register of Controlled Trials (CENTRAL; 2017, Issue 1) (which contains the Cochrane Eyes and Vision Trials Register) in the Cochrane Library (searched 24 February 2017) (Appendix 1);
- MEDLINE Ovid (1946 to 24 February 2017) (Appendix 2);
- Embase Ovid (1980 to 24 February 2017) (Appendix 3);
- PubMed (1946 to 24 February 2017) (Appendix 4);
- ISRCTN registry (www.isrctn.com/editAdvancedSearch; searched 24 February 2017) (Appendix 5);
- US National Institutes of Health Ongoing Trials Register ClinicalTrials.gov (www.clinicaltrials.gov; searched 24 February 2017) (Appendix 6);
- World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) (www.who.int/ictrp; searched 24 February 2017) (Appendix 7).

Searching other resources

We searched the reference lists of included studies for other possible studies. We did not search proceedings from conferences specifically, because such RCTs presented at these meetings were searched by Cochrane Eyes and Vision and included in CENTRAL.

Data collection and analysis

Selection of studies

Three authors (JN, VK and TJ) independently assessed the results identified by the searches and classified each record as either possibly relevant or definitely not relevant. We then obtained full-text copies of all possibly relevant records, and three authors (JN, VK and TJ) classified them as definitely include, unsure or definitely exclude based on the criteria for inclusion. In the event of any difficulty in classification due to lack of clarity or data, we contacted study investigators for further information. All contacted authors responded to our requests. We resolved discrepancies by consensus following discussion between authors (JN, VK and TJ) and documented this in the review. All excluded records were documented.

Data extraction and management

Two authors (JN and VK) independently extracted trial data for the primary and secondary outcomes onto paper data extraction forms developed by Cochrane Eyes and Vision. Subsequently, data were transcribed into Review Manager 5 (RevMan 2014) by one author (JN) and verified by a second author (VK). Any discrepancies were resolved by consensus between authors (JN, VK and TJ) and documented in the review.

We collected the following information on study characteristics (see Appendix 8):

- study design: parallel group RCT/within-person RCT/one or both eyes reported;
- participants: country, total number of participants, age, sex, inclusion and exclusion criteria;
- intervention and comparator details: including number of people (eyes) randomised to each group;
- primary and secondary outcomes as measured and reported in the trials, adverse events;
- length of follow-up;
- date study conducted;
- funding and conflicts of interest.

We extracted the following data from each included study for intervention and comparator groups separately:

- number of events and number of participants for outcome data collected for dichotomous variables (release of vitreous adhesion at 28 days, closure of MH at 28 days and complete PVD at 28 days);
- mean, standard deviation and number of participants for outcome data measured for continuous variables (change in BCVA at six months and change in validated VFQ at six months). To compare visual acuity across studies, the mean BCVA was converted to logarithm of the minimum angle of resolution units (logMAR). Counting fingers vision was assigned a logMAR acuity of 1.6, hand movements 1.9, light perception 2.2 and no light perception 2.5 (Westheimer 1979). The default VFQ assessed was the National Eye Institute Visual Functioning Questionnaire - 25 (NEI-VFQ25).

We collected evidence of harm from RCTs only.

Assessment of risk of bias in included studies

Two authors (JN and VK) independently assessed the included trials for bias using the methods and grades described in Chapter 8 of the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011a). We assessed the following: methods of sequence generation used for randomisation; allocation concealment; masking (blinding) of outcome assessors; masking of participants and personnel; incomplete outcome data; selective outcome reporting; other bias. We considered the use, or not, of independent masked OCT image analysis assessors in the assessment of bias. We then classified each item as 'low,' 'high' or 'unclear' risk of bias.

Measures of treatment effect

We presented dichotomous data as risk ratios (RR) with 95% confidence intervals (CI);

- Primary outcome:
  - resolution of VMA.
- Secondary outcomes:
  - closure of MH;
  - complete PVD;
  - proportion of eyes with 3-line or greater gain in BCVA;
  - requirement for PPV.

We presented continuous data as mean differences with 95% CIs:

- change in validated VFQ measure.

Unit of analysis issues

Trials randomised one or both eyes to the intervention or comparator. If people were randomly allocated to treatment but only one eye per person was included in the trial then there was no unit of analysis issue. In these cases, we documented how the eye was selected and if this was done before randomisation. If people were randomly allocated to treatment but both eyes were included and reported, we planned to analyse as 'clustered data,' that is, adjust for within-person correlation. If the study was a within-person study, that is, one eye was randomly allocated to intervention and the other eye received the comparator, then we planned to analyse as paired data. We planned to contact the trial investigators for further information to do this if necessary.

Dealing with missing data

In the event of missing trial outcome data, we contacted the authors of the trial to understand why the data were missing. If no response was received within four weeks, we used the information provided in the published articles. Missing data were handled in accordance with the guidelines given in Chapter 16 of the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011b). We planned to perform sensitivity analyses on the impact of missing data and comment on the findings in the discussion of the review.

Assessment of heterogeneity

We assessed heterogeneity and inconsistency among trials statistically using an I^2 value (> 50%) to assess if variability in effect was due to sampling error. We also planned to assess diversity among studies by reviewing participant characteristics and trial methodology.

Assessment of reporting biases

We assessed selective outcome reporting by comparing intended outcomes in published protocols, published methods papers and clinical trial registries to reported outcomes in the results sections of trial reports. If there were 10 or more eligible RCTs, we planned to use a funnel plot to assess for study-reporting bias.

Data synthesis

If there were three or fewer eligible RCTs then we planned to use a fixed-effect model for the meta-analyses. If there were more than three included trials, we planned to use a random-effects model instead. If we had evidence of high heterogeneity (e.g. I^2 > 50%), it would not be sensible to pool the data from different trials; in which case, we planned to do a narrative summary of the results.

Subgroup analysis and investigation of heterogeneity

If trials demonstrated clinical heterogeneity and sufficient data were available, including age (< 65 years, 65 years and over), presence of ERM, size of adhesion (less than 1500 µm, 1500 µm or greater) and sVMA subtype (isolated VMT, and MH with persisting vitreous attachment), we planned to perform subgroup analyses for the primary outcome.

Sensitivity analysis

We planned to conduct one sensitivity analysis, excluding studies that were at high risk of bias in one or more domains.

'Summary of findings' table

We prepared a 'Summary of findings' table for the following outcomes:

- resolution of VMA at 28 days;
- complete PVD at 28 days;
- closure of MH at 28 days;
- proportion gaining 3-line or greater improvement in BCVA at six months;
- requirement of PPV at six months;
- change in validated VFQ measure at six months;
- adverse and serious adverse events.

Two authors (JN and VK) independently graded the overall certainty of the evidence for each outcome using the GRADE Working Group classification (GRADEpro 2014).

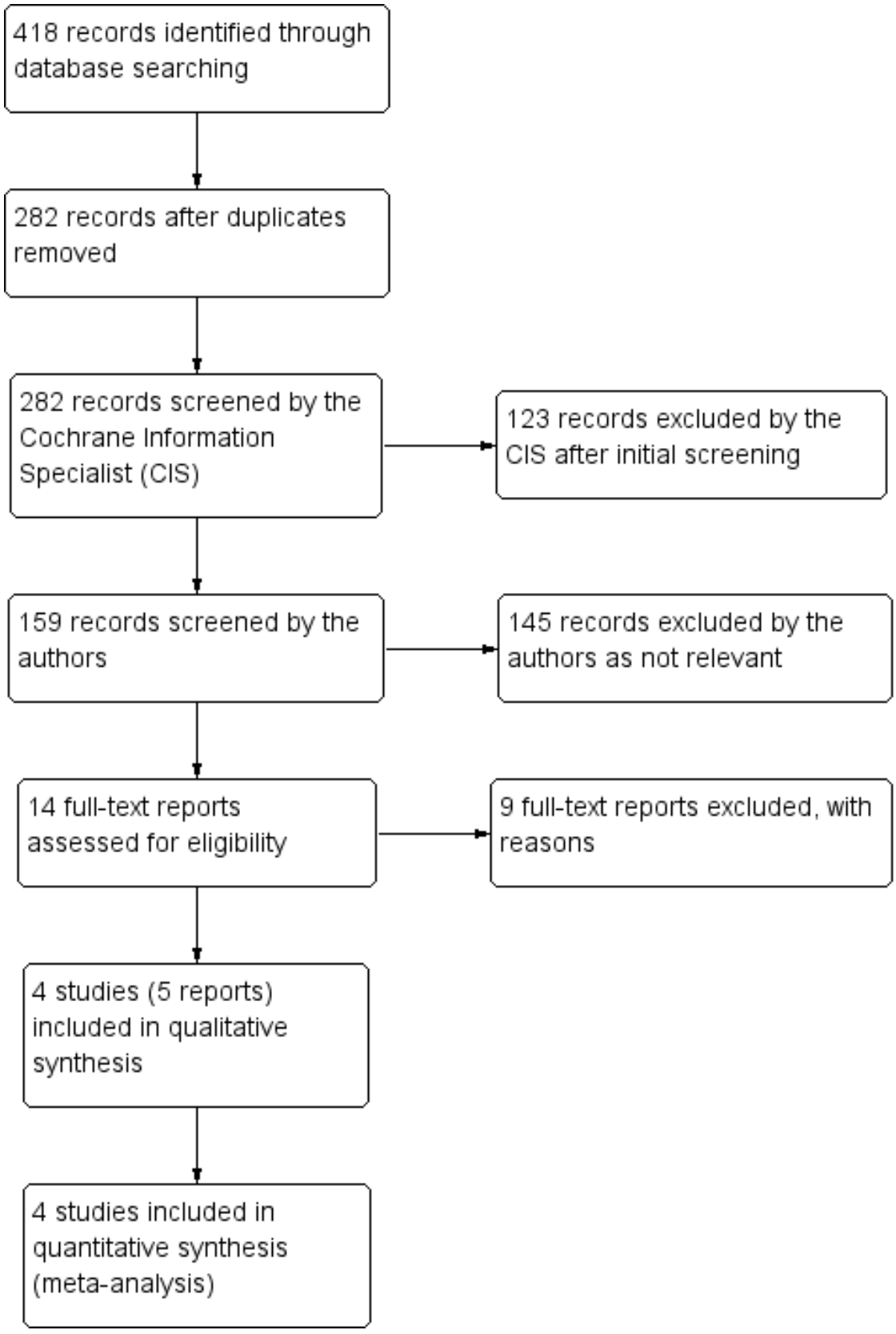
RESULTS

Description of studies

Results of the search

The electronic searches yielded 418 records (Figure 1). The Cochrane Information Specialist scanned the search results, removed 136 duplicates and then removed 123 references which were irrelevant to the scope of the review. We screened the remaining 159 reports and obtained 14 full-text reports for further assessment. We included five reports of four RCTs, three reports (Haller 2015; Stalmans 2012; Varma 2015) analysed separate outcomes from the same two RCTs (TG-MV-006 2012; TG-MV-007 2012). We excluded nine reports of nine studies (see Characteristics of excluded studies for details). We did not identify any ongoing studies from our searches of clinical trials registries.

Figure 1. Study flow diagram.



Included studies

The following is a summary of the characteristics of the four RCTs that met the review inclusion criteria (MIVI-IIT 2010; OASIS 2016; TG-MV-006 2012; TG-MV-007 2012). All data were initially obtained from published literature, then verified for discrepancies using the clinical trials registries described in the Methods section. See the Characteristics of included studies table for further information.

Types of participants

The four RCTs included enrolled 932 participants (932 eyes). All participants received individually randomised, parallel group treatment to a single eye. The age range of all included participants was 18 to 97 years. All included participants had evidence of focal VMA on OCT, BCVA of 20/25 or worse in the study eye and 20/400 or better in the fellow eye (ETDRS acuity chart). Exclusion criteria were: active proliferative diabetic retinopathy, high myopia (axial length greater than 26 mm or more than -8 dioptres), previous vitrectomy or uncontrolled glaucoma, previous intravitreal injections within the past three months in the study eye, intraocular surgery or laser photocoagulation within the past three months in the study eye or rhegmatogenous retinal detachment in either eye. Additional exclusion criteria in TG-MV-007 2012 were: neovascular age-related macular degeneration, retinal vascular occlusion, aphakia, MH greater than 400 µm in diameter, vitreous opacification or lenticular or zonular instability. In OASIS 2016, eyes with an ERM were also excluded from enrolment.

Types of interventions

MIVI-IIT 2010 compared a single injection of ocriplasmin 75 µg, ocriplasmin 125 µg or ocriplasmin 175 µg with sham injection (conjunctiva touched with a blunt needle to simulate an injection) to establish the optimal dose. A fourth cohort of participants underwent an initial injection of ocriplasmin 125 µg, but also a repeat injection at four and eight weeks if VMA was still present on OCT. Therefore, only data from participants receiving ocriplasmin 125 µg in this study were extracted and pooled for analysis. TG-MV-006 2012 and TG-MV-007 2012 both compared a single injection of ocriplasmin 125 µg with placebo injection (of the same vehicle used in the ocriplasmin injection). OASIS 2016 compared a single injection of ocriplasmin 125 µg with sham injection (syringe hub pressed into conjunctiva to simulate an injection).

Types of outcome measures

All four studies reported data for some of our primary and secondary outcome measures. No trial reported data for every outcome measure. Two trial reports (OASIS 2016; Varma 2015) provided data on participant-reported outcome measures using the NEI-VFQ25.

Data synthesis, subgroup and sensitivity analyses

As the search identified four trials, we used a random-effects model (see Data synthesis). As there was no evidence of significant heterogeneity for the primary outcomes ($I^2 < 50\%$), we pooled data and performed no subgroup analyses of the primary outcome. Since no studies had a high risk of bias in any domain, we did not conduct a sensitivity analysis.

Excluded studies

We excluded nine articles after reviewing full-text copies (Benz 2010; De Smet 2009; Dugel 2015; Elbendary 2011; Lanzetta 2014a; Lanzetta 2014b; Lescrauwaet 2016; Jackson 2017; Novack 2015). See Characteristics of excluded studies table for details.

Risk of bias in included studies

See Figure 2 and Figure 3.

Figure 2. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

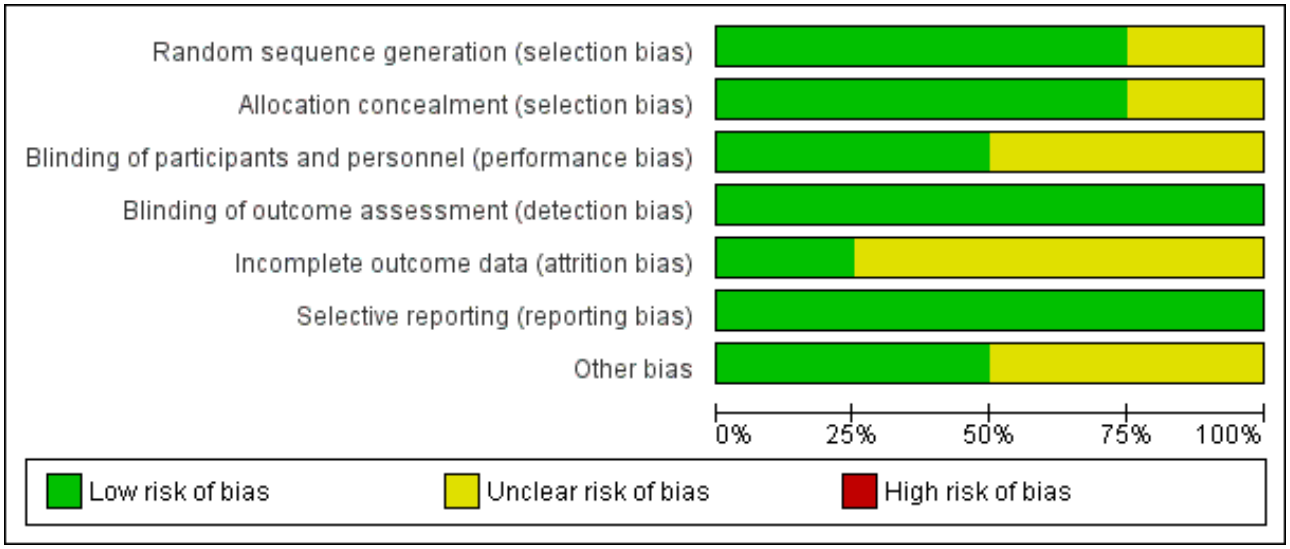
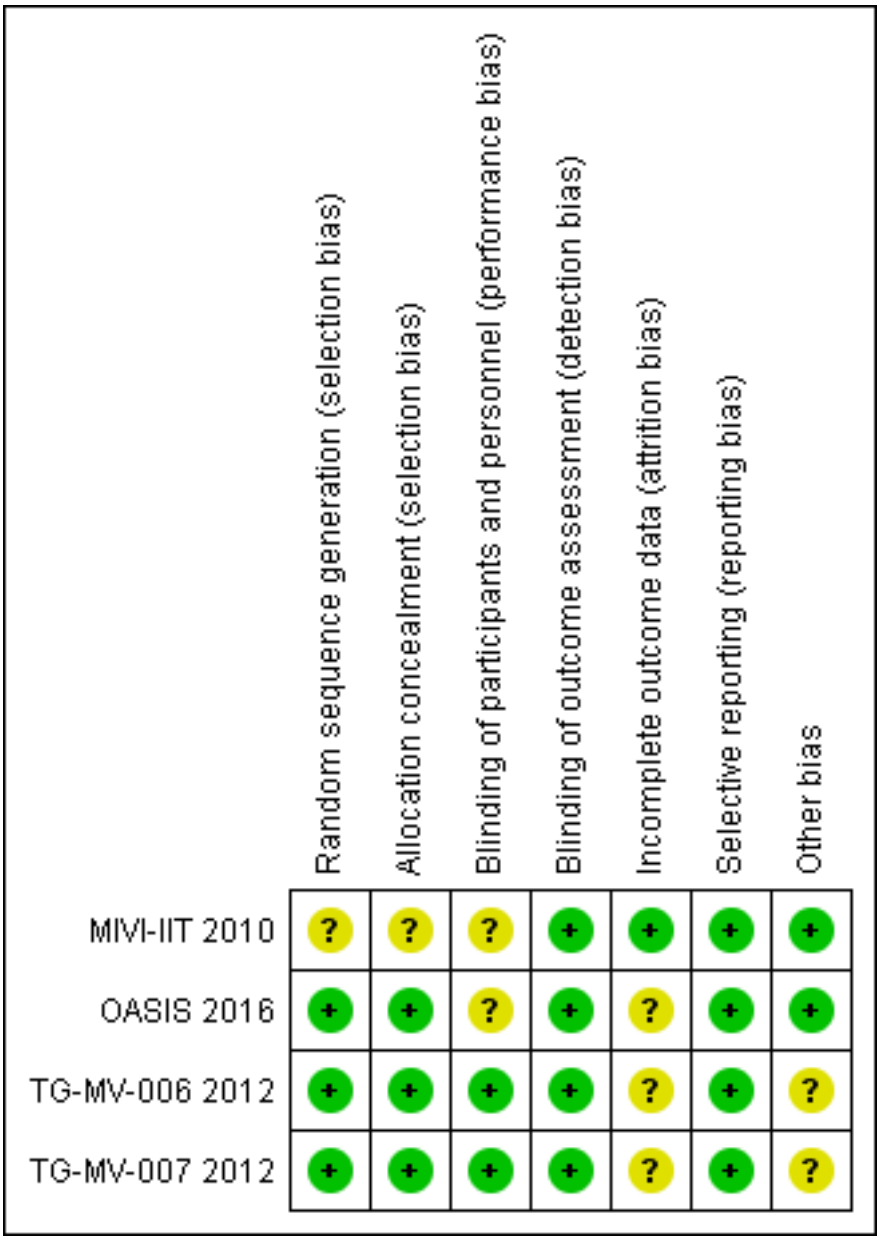


Figure 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.



Allocation

MIVI-IIT 2010 did not describe the method of sequence generation, and provided insufficient information to also assess allocation concealment (Stalmans 2010). TG-MV-006 2012 and TG-MV-007 2012 clearly described randomisation and allocation concealment, which as a centralised telephone-based system with blocks of treatment assigned to sites (Haller 2015; Stalmans 2012; Varma 2015). OASIS 2016 clearly described the method of randomisation, which used a centralised interactive voice response system.

Blinding

Two trials adequately masked participants and investigators (TG-MV-006 2012; TG-MV-007 2012). However, two trials did not mask investigators to sham injections (MIVI-IIT 2010; OASIS 2016), which may have induced a different sensation to a true injection. The risk of performance bias was graded as unclear for both studies.

Incomplete outcome data

We graded risk of bias as low in one study (MIVI-IIT 2010), and unclear in the other three studies (OASIS 2016; TG-MV-006 2012; TG-MV-007 2012). Unclear risk was due to losses to follow-up not being reported and being unequal in different study groups. In addition, OASIS 2016 randomised 200 participants, but 50 participants were later found to be incorrectly enrolled by the central reading centre for a variety of reasons including MH greater than 400 µm, presence of ERM or no VMA at baseline. A subgroup analysis of this smaller cohort of participants, who met the inclusion and exclusion criteria, was performed, but only on outcome data for VMA release.

One trial reported a dilution error, which resulted in an extra participant treated in the ocriplasmin 125 µg cohort and one less participant in the ocriplasmin 175 µg cohort (MIVI-IIT 2010).

Selective reporting

All studies reported on all prespecified primary and secondary outcomes (MIVI-IIT 2010; OASIS 2016; TG-MV-006 2012; TG-MV-007 2012).

Other potential sources of bias

Two studies reported a baseline imbalance between study groups as pseudophakia was more common in the ocriplasmin group than in the placebo group and there were more women in the ocriplasmin group than in the placebo group (TG-MV-006 2012; TG-MV-007 2012). Therefore, this was at unclear risk of bias.

Effects of interventions

See: **Summary of findings for the main comparison** Ocriplasmin injection compared with control for symptomatic vitreomacular adhesion

See Summary of findings for the main comparison.

1. Proportion of eyes with complete release of vitreous adhesion

All four RCTs provided data for proportion of eyes with complete release of vitreous adhesion as determined by analysis of OCT images captured 28 days after ocriplasmin, sham or placebo treatment (MIVI-IIT 2010; OASIS 2016; TG-MV-006 2012; TG-MV-007 2012). After excluding participants with protocol violations from OASIS 2016, analysis of the pooled data showed higher complete release of vitreous adhesion in the ocriplasmin group compared with control (placebo or sham) treatment (RR 3.46, 95% CI 2.00 to 6.00; 859 eyes; 4 studies; high-certainty evidence; Analysis 1.1). A total of 97/1000 eyes had VMA release within 28 days without treatment. An additional 237 eyes had VMA release within 28 days for every 1000 eyes treated with ocriplasmin (95% CI 96 more to 482 more).

2. Proportion of eyes with closure of macular hole

Three studies provided data for proportion of eyes with closure of MH as determined by analysis of OCT images captured 28 days after ocriplasmin, sham or placebo treatment (OASIS 2016; TG-MV-006 2012; TG-MV-007 2012); data from MIVI-IIT 2010 could not be included in this analysis as the original paper did not provide a breakdown of the ocriplasmin doses used to treat MH. OASIS 2016 measured MH closure at three months and the closure rate remained the same to the end of the study at 24 months. After excluding 14 participants incorrectly enrolled in OASIS 2016 due to MH being greater than 400 µm, analysis of the pooled data showed higher closure of MH in the ocriplasmin group compared with control (placebo or sham) treatment (RR 2.87, 95% CI 1.50 to 5.51; 229 eyes; 3 studies; high-certainty evidence; Analysis 1.2). A total of 123/1000 eyes with MHs had closure with no treatment. An additional 231 eyes had MH closure for every 1000 eyes treated with ocriplasmin (95% CI 62 more to 556 more).

3. Proportion of eyes with complete posterior vitreous detachment

Three studies provided data for proportion of eyes with complete PVD as measured by clinical examination or B-scan ultrasonography 28 days after ocriplasmin, sham or placebo treatment (MIVI-IIT 2010; TG-MV-006 2012; TG-MV-007 2012). Analysis revealed a higher incidence of complete PVD at 28 days in eyes treated with ocriplasmin compared with control (placebo or sham) treatment (RR 2.94, 95% CI 1.39 to 6.24; 689 eyes; 3 studies; high-certainty evidence; Analysis 1.3). A total of 40/1000 eyes had complete PVD within 28 days without treatment. An additional 78 eyes had complete PVD within 28 days for every 1000 eyes treated with ocriplasmin (95% CI 16 more to 210 more).

4. Proportion of eyes with 3-line or greater improvement in best corrected visual acuity

Three studies provided data for proportion of eyes with 3-line or greater improvement in BCVA measured using the ETDRS scale, at six months after ocriplasmin, sham or placebo treatment (MIVI-IIT 2010; TG-MV-006 2012; TG-MV-007 2012). Due to separate outcomes reported for eyes with and without full-thickness MH, and large numbers of participants not meeting eligibility criteria, data were not included from OASIS 2016. Eyes that had undergone PPV in MIVI-IIT 2010 during this six-month period were also excluded. Analysis of the pooled data revealed that eyes treated with ocriplasmin without PPV were more likely to achieve 3-line or greater improvement in BCVA than control (sham or placebo) eyes (RR 1.95, 95% CI 1.07 to 3.53; 674 eyes; 3 studies; moderate-certainty evidence; Analysis 1.4). A total of 61/1000 eyes had 3-line or greater improvement in BCVA at six months without treatment. An additional 58 eyes had 3-line or greater improvement in BCVA at six months for every 1000 eyes treated with ocriplasmin (95% CI 9 more to 154 more).

5. Proportion of eyes requiring vitrectomy within six months of ocriplasmin, sham or placebo treatment

Three studies provided data for proportion of eyes requiring vitrectomy (MIVI-IIT 2010; TG-MV-006 2012; TG-MV-007 2012). All three RCTs defined the requirement for vitrectomy as "recommended by the investigator if the underlying condition deteriorated, BCVA worsened by more than two lines on ETDRS or Snellen chart, or if the underlying condition had not improved within 28 days after treatment." Due to separate outcomes reported for eyes with and without full-thickness MH, and large numbers of participants not meeting eligibility criteria, data were not included from OASIS 2016. Analysis revealed a lower requirement for vitrectomy in eyes treated with ocriplasmin compared with control (placebo or sham) treatment (RR 0.67, 95% CI 0.50 to 0.91; 689 eyes; 3 studies; moderate-certainty evidence; Analysis 1.5). A total of 265/1000 eyes required vitrectomy at six months without treatment and 87 fewer eyes required vitrectomy for every 1000 eyes treated with ocriplasmin (95% CI 24 fewer to 132 fewer).

6. Mean change in validated Visual Function Questionnaire score from baseline measured at six months after ocriplasmin, sham or placebo treatment

One trial reported data for mean change in validated VFQ score from baseline (Varma 2015), which analysed pooled participant-reported visual function outcomes for TG-MV-006 2012 and TG-MV-007 2012. In all eyes across both studies, mean increases in

the composite NEI-VFQ25 score at six months from baseline were greater in eyes treated with ocriplasmin (464 eyes) than placebo (188 eyes) (mean change: 3.4 with ocriplasmin versus 0.7 with placebo; P = 0.005). We calculated the mean difference as 2.7 (95% CI 0.8 to 4.6). Visual function data was also reported in OASIS 2016, but this was not reported for the subgroup who met the inclusion and exclusion criteria following central reading centre analysis.

7. Adverse effects

Due to inconsistencies between the studies and differences in control groups (placebo injection versus sham injection), we did not perform a pooled analysis of adverse events. Instead, a descriptive account of the types of ocular adverse event is provided below, based on data from three studies (OASIS 2016; TG-MV-006 2012; TG-MV-007 2012). Although a large number of participants were incorrectly enrolled in OASIS 2016, safety data are presented for all participants who underwent intervention with ocriplasmin or control treatment.

7.1. Any ocular adverse events

These were defined as any ocular adverse event that did not meet the criteria for a serious ocular adverse event (see '7.2. Any serious ocular adverse events'). All four RCTs provided data for any ocular adverse event (MIVI-IIT 2010; OASIS 2016; TG-MV-006 2012; TG-MV-007 2012). Analysis revealed more ocular adverse events in eyes treated with ocriplasmin compared with placebo or sham-treated eyes (RR 1.22, 95% CI 1.09 to 1.37; 909 eyes; 4 studies; moderate-certainty evidence; Analysis 1.6).

A breakdown of the most frequently reported ocular adverse events is listed in the table below (n = number of eyes affected, **not** total number of events). The first five ocular adverse events were participant-reported. The most commonly reported ocular adverse events following ocriplasmin treatment were vitreous floaters (affecting 133/611 eyes or 21.8%), photopsia (affecting 98/611 eyes or 16.0%) and injection-related eye pain (affecting 83/611 eyes or 13.6%). The incidence of vitreous floaters, photopsia, injection-related eye pain, blurred vision and visual impairment was significantly greater in eyes treated with ocriplasmin than those treated with sham or placebo injection.

Study	MIVI-IIT 2010		TG-MV-006 2012		TG-MV-007 2012		OASIS 2016	
	Ocriplasmin (n = 25)	Control (n = 12)	Ocriplasmin (n = 220)	Control (n = 106)	Ocriplasmin (n = 245)	Control (n = 81)	Ocriplasmin (n = 146)	Control (n = 74)
Any ocular adverse event	21	9	159	62	159	38	106	47
Vitreous floaters[a]	-	-	42	9	36	5	55	6
Photopsia[a]	-	-	36	4	19	1	43	5
Injection-related eye pain[a]	-	-	33	6	30	5	20	6
Blurred vision[a]	-	-	24	4	16	2	27	4
Visual impairment[a]	-	-	21	3	4	0	21	4
Conjunctival haemorrhage	8	3	34	14	34	10	14	1
Increased intraocular pressure[a]	-	-	9	10	9	0	10	10
Retinal tear[a]	-	-	5	2	1	3	2	5
Cataract[a]	-	-	14	12	12	5	19	10
Anterior chamber cells[b]	1	0	-	-	-	-	-	-
Iridocyclitis[b]	1	0	-	-	-	-	-	-
Vitritis[b]	3	0	-	-	-	-	-	-

[a]Ocular adverse events not reported in MIVI-IIT.

[b]Ocular adverse events not reported in OASIS 2016, TG-MV-006 2012, or TG-MV-007 2012.

Note: the control group in MIVI-IIT 2010 and OASIS 2016 was sham injection. The control group in TG-MV-007 2012 and TG-MV-006 2012 was placebo injection.

7.2. Any serious ocular adverse events

Two studies defined serious ocular adverse event as: an event resulting in persistent or clinically significant disability, incapacity or both; an event requiring inpatient hospitalisation or prolongation of an existing hospital stay; or an event that was considered to be medically important (TG-MV-006 2012; TG-MV-007 2012). One study did not provide a definition of a serious ocular adverse event (OASIS 2016). MIVI-IIT 2010 reported no instances of serious ocular adverse events.

A breakdown of the most frequently reported serious ocular adverse events is listed in the table below (n = number of eyes affected, **not** total number of events). The total incidence of serious ocular adverse events was 66/611 (10.8%) in eyes treated with ocriplasmin compared with 35/261 (13.4%) treated with sham or placebo injection. Most frequently reported was an increased or new macular hole, which occurred in 47/611 (7.7%) of eyes treated with ocriplasmin compared with 26/261 (9.9%) of eyes treated with sham or placebo injection. None of the included studies reported any cases of endophthalmitis.

Study	MIVI-IIT 2010		TG-MV-006 2012		TG-MV-007 2012		OASIS 2016	
	Ocriplasmin (n = 25)	Control (n = 12)	Ocriplasmin (n = 220)	Control (n = 106)	Ocriplasmin (n = 245)	Control (n = 81)	Ocriplasmin (n = 146)	Control (n = 74)
Any serious ocular adverse event	0	0	21	11	15	9	30	15
Macular hole (increased or new)	-	-	15	11	9	5	23	10
Retinal detachment	-	-	2	2	0	1	1	1
Reduced visual acuity	-	-	1	0	2	1	18	18
Endophthalmitis	0	0	0	0	0	0	0	0

Note: the control group in MIVI-IIT 2010 and OASIS 2016 was sham injection. The control group in TG-MV-007 2012 and TG-MV-006 2012 was placebo injection.

DISCUSSION

Summary of main results

We identified four RCTs, with 932 eyes, comparing ocriplasmin with control (placebo or sham injection) treatment. On full-text analysis, we excluded 50 participants due to breaches of our inclusion criteria, and 23 participants because they received a different dose of ocriplasmin, giving 859 eyes for outcome analysis. The studies were conducted in Europe and the USA. We found that treatment with ocriplasmin increased the likelihood of complete release of vitreous traction compared to control (sham or placebo injection) treatment. Ocriplasmin was also associated with a 3-line or greater improvement in BCVA and improvement in participant-reported visual function.

There were however, more ocular adverse events in eyes treated with ocriplasmin than control (placebo or sham injection) treatment. Many of these adverse events, particularly vitreous floaters and photopsia, are known to be associated with posterior vitreous detachment. Of the serious ocular adverse events, increased or new macular hole was the most frequently reported. Given the high incidence in all eyes regardless of treatment, this most likely represents the natural history of VMT in a significant proportion of patients.

Overall completeness and applicability of evidence

Three of the included studies were large and contributed the majority of included participants (834) for our analysis (OASIS 2016; TG-MV-006 2012; TG-MV-007 2012). The other study, designed to determine the appropriate dose, contributed a relatively small number (25) of participants (MIVI-IIT 2010). The control groups in the trials also varied, with participants in TG-MV-006 2012 and TG-MV-007 2012 receiving a placebo injection, and participants in MIVI-IIT 2010 and OASIS 2016 receiving a sham injection. Due to the mechanical nature of the primary outcome, the variation in control group intervention could impact on the validity of the results, particularly adverse events. All four trials reported the same primary outcome and follow-up periods were identical. One trial reported additional secondary outcome data at 24-months (OASIS 2016).

It is important to note that OASIS 2016 initially randomised and treated 220 participants, but subsequent central reading centre analysis revealed 50 participants were ineligible due to lack of sVMA, presence of ERM or presence of MH greater than 400 µm. To comply with the inclusion and exclusion criteria of this review, we used only data from this smaller, central reading centre verified cohort of participants. Despite this attrition bias, sufficient pooled data were available, hence the impact of this bias was deemed small.

Quality of the evidence

Generally, we graded the risk of bias as low. However, two studies reported cases that did not complete the study on the ClinicalTrials.gov database (see Characteristics of included studies table) but the publications did not describe these losses to follow-up (TG-MV-006 2012; TG-MV-007 2012). The authors confirmed using the last-observation-carried-forward (LOCF) method for their missing outcome data, assuming the outcome was unlikely to change after discontinuation of treatment and likely to improve spontaneously over time. As these losses to follow-up were not described in the original papers, we judged the risk of bias for incomplete outcome data as unclear.

Potential biases in the review process

We followed a standard Cochrane protocol (Neffendorf 2015), to minimise potential methodological biases in the review process.

Agreements and disagreements with other studies or reviews

In the UK, NICE recommends the use of ocriplasmin for adults with VMT causing severe sight problems or a MH up to 400 µm, in the absence of ERM. Our findings support this.

Subsequent publications and postmarket surveillance studies have addressed the safety of ocriplasmin. One large postmarket surveillance study found lower rates of adverse events than were reported in the registration studies, but noted that under-reporting is common in post-market surveillance studies (Hahn 2015). Members of the British and Eire Association of VitreoRetinal Surgeons (BEAVRS) have reported their experience with ocriplasmin in comparison to the MIVI-TRUST trial data (Haynes 2017). They found a lower rate of MH closure and increased incidence of adverse events with ocriplasmin compared to the registration studies, but there is an uncertain risk of reporting bias.

Our review found a higher rate of vitreous floaters and photopsia with ocriplasmin, but no increased risk of loss in visual acuity and retinal detachment. There have been reports of acute reduction in visual acuity, electroretinography changes, dyschromatopsia, phacodonesis and OCT ellipsoid zone alteration, but the majority have been transient (Khan 2016; Neffendorf 2016).

Various studies and reviews have suggested certain subgroups of sVMA participants may be more likely to respond successfully to ocriplasmin treatment based on baseline characteristics such as adhesion diameter, lack of coexisting ERM, and the angle between the posterior vitreous cortex and the ILM (Haller 2015; Jackson 2016; Paul 2017). However, such analyses are exploratory, and without confirmatory prospective RCTs they are beyond the scope of this review.

There are different approaches to potentially manage sVMA including PPV, intravitreal gas injection, ocriplasmin and observation. Further research, ideally in a head-to-head trial, would be beneficial.

AUTHORS' CONCLUSIONS

Implications for practice

We found evidence to support the use of ocriplasmin for the treatment of symptomatic vitreomacular adhesion (sVMA), although the number of studies was low. There are reported concerns about the safety of ocriplasmin treatment and there is debate within the vitreoretinal community regarding the advantages and disadvantages of ocriplasmin.

Implications for research

Further large randomised controlled trials would augment our current understanding of the safety and efficacy of ocriplasmin. Ideally these would compare ocriplasmin with other commonly used management options, in particular observation or pars plana vitrectomy. Randomised controlled trials recruiting participants with baseline characteristics thought to improve the efficacy of ocriplasmin are warranted.

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CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

MIVI-IIT 2010

Methods	**Study design:** RCT, single treated eye. **Number randomised:** 60 total; 48 microplasmin; 12 sham injection. **Exclusions after randomisation:** none. **Number analysed:** at 28 days and 6 months; 60 total; 48 microplasmin; 12 sham injection. **Unit of analysis:** eyes. **Losses to follow-up:** 0 participants total. **How was missing data handled?** no missing data. **Power calculation:** not documented.
Participants	**Country:** Belgium. **Mean age:** 70.0 years overall; 69.9 years for ocriplasmin group; 70.0 years for sham injection group. **Gender:** 33/60 (55%) women, 27/60 (45%) men total; 27/48 (56%) women, 21/48 (44%) men in microplasmin group; 6/12 (50%) women, 6/12 (50%) men in sham injection group. **Inclusion criteria:** aged > 18 years; partial PVD on ultrasound examination; OCT evidence of at least a partial attachment in the foveal area, resulting in a macular thickness of ≥ 250 µm; BCVA ≤ 20/40 in study eye; BCVA ≥ 20/400 in fellow eye. **Exclusion criteria:** active PDR; high myopia (axial length > 26 mm); previous vitrectomy or uncontrolled glaucoma; previous intravitreal injections in the past 3 months in study eye; intraocular surgery or laser photocoagulation in the past 3 months in study eye; rhegmatogenous retinal detachment in either eye. **Equivalence of baseline characteristics:** no; more participants in microplasmin group had tractional diabetic macular oedema compared with sham injection group.
Interventions	**Intervention 1:** single intravitreal injection microplasmin 125 µg.

MIVI-IIT 2010 *(Continued)*

	Intervention 2: single intravitreal injection microplasmin 75 µg. **Intervention 3:** single intravitreal injection microplasmin 175 µg. **Intervention 4:** intravitreal injection of microplasmin 125 µg at baseline, followed by a further microplasmin 125 µg intravitreal injection at 28 days if VMA was still present, followed by a further microplasmin 125 µg intravitreal injection at 56 days after baseline if VMA was still present. **Comparator:** sham injection (conjunctiva touched with a blunt needle by a non-masked investigator and no injection given). **Length of follow-up:** planned 180 days, actual 180 days. As the recommended dose of ocriplasmin is 125 µg, and this is the subject of this review, only data from the first and fourth intervention arms were analysed.
Outcomes	**Primary outcome, as defined in study reports:** "the primary outcome of this study was total PVD induction at Day 14, as assessed by a central reading centre." **Secondary outcomes, as defined in study reports:** total PVD at other time points assessed by the central reading centre and investigators; resolution of index condition (VMA or MH); resolution of VMA; progression of PVD; need for vitrectomy; resolution of macular oedema; change in BCVA; BCVA 5-, 10- and 15-letter improvement. **Adverse events reported:** yes. **Intervals at which outcomes assessed:** 3, 7, 14, 28, 90 and 180 days.
Notes	**Funding sources:** study sponsored by ThromboGenics NV. **Study period:** 2 years; 2007-2009. **Reported subgroup analyses:** yes. Full results of study were presented at EURetina 2009, Nice, France. NCT00435539.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Method of sequence generation for the MIVI-IIT RCT not described. Quote: "Four cohorts of 15 patients were randomised as 4:1 to treatment or sham injection, resulting in 12 patients receiving microplasmin and 3 patients receiving the sham injection in each cohort." p. 1123.
Allocation concealment (selection bias)	Unclear risk	Insufficient information documented to assess allocation concealment. Quote: "Four cohorts of 15 patients were randomised as 4:1 to treatment or sham injection, resulting in 12 patients receiving microplasmin and 3 patients receiving the sham injection in each cohort." p. 1123.
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Sham injection was performed, rather than actual placebo injection. Quote: "In the patients receiving a sham injection, microplasmin was prepared in the same manner, but instead of an intraocular injection, the conjunctiva was touched with a blunt needle by a nonmasked investigator and no injection was given." p. 1123.

MIVI-IIT 2010 *(Continued)*

Blinding of outcome assessment (detection bias) All outcomes	Low risk	Quote: "All patient examinations before drug allocation and in the 6-month follow-up period after the last injection were performed by masked investigators and study personnel." p. 1123. "Posterior vitreous detachment status and macular thickness were assessed by the investigator as well as by a central reading center (CRC), located in Munich, Germany." p. 1124.
Incomplete outcome data (attrition bias) All outcomes	Low risk	No incomplete outcome data.
Selective reporting (reporting bias)	Low risk	All outcomes defined in trial registry were reported.
Other bias	Low risk	

OASIS 2016

Methods	**Study design:** RCT, single treated eye. **Number randomised:** 220 total; 146 ocriplasmin; 74 sham. **Exclusions after randomisation:** 50 participants subsequently deemed ineligible by central reading centre. **Number analysed:** at 28 days: 168 total; 111 ocriplasmin; 59 sham. **Unit of analysis:** eyes. **Losses to follow-up:** 2 participants total; 1 ocriplasmin group (1 lost to follow-up); 1 sham group (1 lost to follow-up). **How was missing data handled?** other than VMA release at 28 days, no data published regarding cohort who met central reading centre eligibility criteria. **Power calculation:** 210 participants for at least 90% power at 2-sided alpha of 0.05 to assume a primary endpoint of 37% in ocriplasmin group and a 14% rate in placebo group.
Participants	**Country:** USA. **Mean age:** 69.1 years overall; 69.4 years for ocriplasmin group; 68.5 years for sham group. **Gender:** 147/218 (67.4%) women, 71/218 (32.6%) men total; 102/145 (70.3%) women, 43/145 (29.7%) men in ocriplasmin group; 45/73 (61.6%) women, 28/73 (38.4%) men in sham group. **Inclusion criteria:** aged > 18 years; presence of VMA; BCVA ≤ 20/32 in study eye; BCVA ≥ 20/800 in non-study eye. **Exclusion criteria:** history or current evidence of proliferative retinopathy, exudative AMD or retinal vein occlusion in the study eye; people with any vitreous haemorrhage or any other vitreous opacification which precludes either visualisation of the posterior pole by visual inspection OR adequate assessment of the macula by OCT; MH > 400 µm in diameter in the study eye; presence of epiretinal membrane; aphakia in study eye; high myopia (> -8 dioptres in study eye); history of rhegmatogenous retinal detachment in either eye; prior vitrectomy in study eye; previous participation in this trial or prior administration of ocriplasmin in study eye. **Equivalence of baseline characteristics:** yes.
Interventions	**Intervention:** single intravitreal injection of ocriplasmin 125 µg in 0.10 mL volume.

OASIS 2016 *(Continued)*

	Comparator: sham (the same syringe hub was pressed against the conjunctiva to simulate an injection). **Length of follow-up:** planned 24 months, actual 24 months. Data of central reading centre approved study participants only reported at 28 days.
Outcomes	**Primary outcome, as defined in study reports:** "proportion of subjects with pharmacological vitreomacular adhesion (VMA)/vitreomacular traction (VMT) resolution at day 28. Pharmacological VMA resolution without anatomical defect, based on spectral domain optical coherence tomography and determined by the masked central reading center (CRC), with post-resolution vitrectomy considered as a failure." **Secondary outcomes, as defined in study reports:** "proportion of subjects with a ≥2 line improvement in best-corrected visual acuity (BCVA) from baseline at month 24, irrespective of vitrectomy." **Adverse events reported:** yes. **Intervals at which outcomes assessed:** 7 and 28 days; 3, 6, 9, 12, 15, 18, 21 and 24 months.
Notes	**Funding sources:** study sponsored by ThromboGenics NV. **Study period:** 3 years; 2011-2014. **Reported subgroup analyses:** yes. **Additional information:** large proportion of participants were deemed eligible, recruited and treated by investigators. Retrospective central reading centre review found 50 participants ineligible for following reasons (MH > 400 µm, presence of epiretinal membrane or no sVMA at baseline). Our analysis only included data reported for correctly eligible cohort of participants. NCT01429441.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Method of random sequence generation for the MIVI-IIT RCT described Quote: "Randomization was stratified on the basis of the presence or absence of FTMH at baseline and was centralized through an interactive voice response system." p. 2233.
Allocation concealment (selection bias)	Low risk	Method of allocation concealment for MIVI-IIT RCT described. Quote: "Randomization was stratified on the basis of the presence or absence of FTMH at baseline and was centralized through an interactive voice response system." p. 2233.
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Performance bias explained. Quote: "The trial was conducted in a double-masked manner. To maintain the masking of the investigator, an unmasked injecting physician was assigned to perform the injection and access the interactive voice response system to receive the assigned treatment. The unmasked personnel did not perform or participate in any other trial-related procedures or assessments." p. 2233.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Detection bias appropriately explained. Quote: "The trial was conducted in a double-masked manner. To maintain the masking of the investigator, an unmasked injecting physician was assigned to perform the injection and access the interactive voice response system to

OASIS 2016 *(Continued)*

		receive the assigned treatment. The unmasked personnel did not perform or participate in any other trial-related procedures or assessments." p. 2233.
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Large proportion of participants deemed eligible, recruited and treated by investigators. Retrospective central reading centre review found 50 participants ineligible for following reasons (MH > 400 µm, presence of epiretinal membrane or no sVMA). Outcome data for correct eligible cohort of participants only given for primary outcome. No secondary outcome data described.
Selective reporting (reporting bias)	Low risk	All outcomes defined in trial registry were reported.
Other bias	Low risk	No other source of bias.

TG-MV-006 2012

Methods	**Study design:** RCT, single treated eye. **Number randomised:** 326 total; 219 ocriplasmin; 107 placebo. **Exclusions after randomisation:** 0. **Number analysed:** at 28 days: 326 total; 219 ocriplasmin; 107 placebo. At 180 days: 298 total; 200 ocriplasmin; 98 placebo. **Unit of analysis:** eyes. **Losses to follow-up:** 28 participants total; 19 ocriplasmin group (2 adverse event, 8 withdrawal by participants, 6 lost to follow-up, 3 death); 9 placebo group (2 adverse event, 4 withdrawal by participants, 3 lost to follow-up). **How was missing data handled?** missing data not reported in study publications. **Power calculation:** 320 participants for > 90% power at 2-sided alpha of 0.05 to assume a primary endpoint of 27.5% in ocriplasmin group and 10.0% in placebo group.
Participants	**Country:** USA. **Mean age:** 71.4 years overall; 71.5 years for ocriplasmin group; 71.1 years for placebo group. **Gender:** 207/326 (63.5%) women, 119/326 (36.5%) men total; 148/219 (67.6%) women, 71/219 (32.4%) men in ocriplasmin group; 59/107 (55.1%) women, 52/107 (48.6%) men in placebo group. **Inclusion criteria:** aged > 18 years; focal VMA (vitreous adhesion to macula within 6-mm central retinal field surrounded by elevation of posterior vitreous cortex, as seen on OCT that in the opinion of investigator was related to decreased visual function (e.g. metamorphopsia, decreased visual acuity or other visual complaint); BCVA ≤ 20/25 in study eye; BCVA ≥ 20/800 in non-study eye. **Exclusion criteria:** any evidence of proliferative retinopathy (including PDR or other ischaemic retinopathies involving vitreoretinal vascular proliferation) or exudative AMD or retinal vein occlusion in study eye; people with any vitreous haemorrhage or any other vitreous opacification which precludes either: visualisation of posterior pole by visual inspection OR adequate assessment of macula by either OCT or fluorescein angiogram (or both) in study eye; MH > 400 µm in diameter in study eye; aphakia in study eye; high myopia (> -8 dioptres); uncontrolled glaucoma; lenticular or zonular instability; history of retinal detachment in either eye; prior vitrectomy or prior laser photocoagulation of macula; treatment with ocular surgery, intravitreal injection or retinal laser photocoagulation in the previous 3 months. **Equivalence of baseline characteristics:** no; pseudophakia more common in ocriplasmin group than in placebo group; more women in ocriplasmin group than in placebo group.

TG-MV-006 2012 *(Continued)*

Interventions	**Intervention:** single intravitreal injection of ocriplasmin 125 µg in 0.10 mL volume. **Comparator:** single intravitreal injection of 0.10 mL placebo with identical drug vehicle diluted with saline. **Length of follow-up:** planned 180 days, actual 180 days.
Outcomes	**Primary outcome, as defined in study reports:** "the primary end point was the proportion of subjects with nonsurgical resolution of vitreomacular adhesion at day 28 post-injection, as determined by masked OCT evaluation obtained from the central reading centre." **Secondary outcomes, as defined in study reports:** proportion of participants with total PVD at day 28, as determined by B-scan ultrasound; need for vitrectomy; closure of an MH; gain ≥ 3-lines BCVA without vitrectomy; change from baseline in BCVA and VFQ-25 score at 6 months. **Adverse events reported:** yes. **Intervals at which outcomes assessed:** 7, 14, 28, 90 and 180 days.
Notes	**Funding sources:** study sponsored by ThromboGenics NV. **Study period:** 2 years; 2008-2009. **Reported subgroup analyses:** yes. NCT00781859.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Supplementary material: "subjects will be randomised centralized through a telephone-based Interactive Voice Response System (IVRS) to either microplasmin intravitreal injection or placebo in a 3:1 allocation ratio. Blocks of treatment will be assigned to sites in a manner expected to minimize the potential for imbalance in the desired randomization ratio." Protocol p. 17.
Allocation concealment (selection bias)	Low risk	Supplementary material: "subjects will be randomised centralized through a telephone-based Interactive Voice Response System (IVRS) to either microplasmin intravitreal injection or placebo in a 3:1 allocation ratio. Blocks of treatment will be assigned to sites in a manner expected to minimize the potential for imbalance in the desired randomization ratio." Protocol p. 17.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote: "Patients randomly assigned to the ocriplasmin group received an intravitreal injection of ocriplasmin (125 µg in a 0.10-ml volume) drawn from a vial containing ocriplasmin into which 0.75 ml of commercial saline had been injected (1875 µg of ocriplasmin in a 0.75-ml drug vehicle). Patients randomly assigned to placebo received an intravitreal injection of 0.10 ml of the identical drug vehicle diluted with saline, the method used being the same as that used to prepare ocriplasmin." p. 608.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Quote: "Trained readers at a central reading center (Duke University OCT Reading Center, Durham, NC) who were unaware of the group assignments evaluated the OCT images. All ultrasonographic studies were standardized and performed by certified technicians who underwent special training for the study. Staging of posterior vitreous detachment was based on dynamic ultrasonographic evaluation and performed by an investigator who was unaware of the group assignments." p. 608.
Incomplete outcome data (attrition bias)	Unclear risk	No description found in article, but 28 participants reported as not completing study on ClinicalTrials.gov. The corresponding author of Stalmans and col-

TG-MV-006 2012 *(Continued)*

All outcomes		leagues (2012) was contacted to query this. ThromboGenics NV responded by confirming use of last observation carried forward (LOCF) approach to input missing data for visits postdiscontinuation. Their explanation was: "use of LOCF was appropriate when the outcome is not expected to change after discontinuation and is a conservative method when the outcome is expected to improve spontaneously over time. The primary endpoint, pharmacological VMA resolution in particular, is an outcome of that nature." As these losses to follow-up were not reported in original paper, risk of attrition bias was deemed unclear.
Selective reporting (reporting bias)	Low risk	All defined outcomes in methods were reported.
Other bias	Unclear risk	Baseline imbalance between study groups as pseudophakia was more common in ocriplasmin group than in placebo group and there were more women in ocriplasmin group than in placebo group.

TG-MV-007 2012

Methods	**Study design:** RCT, single treated eye. **Number randomised:** 326 total; 245 ocriplasmin; 81 placebo. **Exclusions after randomisation:** none. **Number analysed:** at 28 days: 326 total; 245 ocriplasmin; 81 placebo. At 180 days: 309 total; 235 ocriplasmin; 74 placebo. **Unit of analysis:** eyes. **Losses to follow-up:** 17 participants total; 10 ocriplasmin group (5 withdrawal by participant, 2 lost to follow-up, 2 adverse event, 1 death); 7 placebo group (1 physician decision, 4 withdrawal by participant, 2 lost to follow-up). **How was missing data handled?** missing data not reported in study publications. **Power calculation:** 320 participants for > 90% power at 2-sided alpha of 0.05 to assume a primary endpoint of 27.5% in ocriplasmin group and 10.0% rate in placebo group.
Participants	**Countries:** Belgium, Czech Republic, Germany, Poland, Spain, UK, USA. **Mean age:** 72.0 years overall; 72.6 years for ocriplasmin group; 70.2 years for placebo group. **Gender:** 222/326 (68.1%) women, 104/326 (31.9%) men total; 166/245 (67.8%) women, 79/245 (32.2%) men in ocriplasmin group; 56/81 (69.1%) women, 25/81 (30.9%) men in placebo group. **Inclusion criteria:** aged > 18 years; focal VMA (vitreous adhesion to macula within 6-mm central retinal field surrounded by elevation of posterior vitreous cortex, as seen on OCT that in the opinion of investigator was related to decreased visual function (e.g. metamorphopsia, decreased visual acuity or other visual complaint); BCVA ≤ 20/25 in study eye; BCVA ≥ 20/800 in non-study eye. **Exclusion criteria:** any evidence of proliferative retinopathy (including PDR or other ischaemic retinopathies involving vitreoretinal vascular proliferation) or exudative AMD or retinal vein occlusion in study eye; people with any vitreous haemorrhage or any other vitreous opacification which precludes either: visualisation of posterior pole by visual inspection OR adequate assessment of macula by either OCT or fluorescein angiogram (or both) in study eye; MH > 400 µm in diameter in study eye; aphakia in study eye; high myopia (> -8 dioptres); uncontrolled glaucoma; lenticular or zonular instability; history of retinal detachment in either eye; prior vitrectomy or prior laser photocoagulation of macula; treatment with ocular surgery, intravitreal injection or retinal laser photocoagulation in the previous 3 months.

TG-MV-007 2012 *(Continued)*

	Equivalence of baseline characteristics: no; pseudophakia more common in ocriplasmin group than in placebo group; more women in ocriplasmin group than in placebo group.
Interventions	**Intervention:** single intravitreal injection of ocriplasmin 125 µg in 0.10 mL volume. **Comparator:** single intravitreal injection of 0.10 mL placebo with identical drug vehicle diluted with saline. **Length of follow-up:** planned 180 days, actual 180 days.
Outcomes	**Primary outcome, as defined in study reports:** "the primary end point was the proportion of subjects with nonsurgical resolution of VMA at day 28 post-injection, as determined by masked OCT evaluation obtained from the central reading centre." **Secondary outcomes, as defined in study reports:** proportion of participants with total PVD at day 28, as determined by B-scan ultrasound; need for vitrectomy; closure of an MH; gain ≥ 3-lines BCVA without vitrectomy; change from baseline in BCVA and VFQ-25 score at 6 months. **Adverse events reported:** yes. **Intervals at which outcomes assessed:** 7, 14, 28, 90 and 180 days.
Notes	**Funding sources:** study sponsored by ThromboGenics NV. **Study period:** 2 years; 2008-2010. **Reported subgroup analyses:** yes. NCT00798317.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Supplementary material: "subjects will be randomised centralized through a telephone-based Interactive Voice Response System (IVRS) to either microplasmin intravitreal injection or placebo in a 3:1 allocation ratio. Blocks of treatment will be assigned to sites in a manner expected to minimize the potential for imbalance in the desired randomization ratio." Protocol p. 17.
Allocation concealment (selection bias)	Low risk	Supplementary material: "subjects will be randomised centralized through a telephone-based Interactive Voice Response System (IVRS) to either microplasmin intravitreal injection or placebo in a 3:1 allocation ratio. Blocks of treatment will be assigned to sites in a manner expected to minimize the potential for imbalance in the desired randomization ratio." Protocol p. 17.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote: "Patients randomly assigned to the ocriplasmin group received an intravitreal injection of ocri- plasmin (125µg in a 0.10-ml volume) drawn from a vial containing ocriplasmin into which 0.75ml of commercial saline had been injected (1875µg of ocriplasmin in a 0.75-ml drug vehicle). Patients randomly assigned to placebo received an intravitreal injection of 0.10 ml of the identical drug vehicle diluted with saline, the method used being the same as that used to prepare ocriplasmin". p. 608.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Quote: "Trained readers at a central reading center (Duke University OCT Reading Center, Durham, NC) who were unaware of the group assignments evaluated the OCT images. All ultrasonographic studies were standardized and performed by certified technicians who underwent special training for the study. Staging of posterior vitreous detachment was based on dynamic ultrasonographic evaluation and performed by an investigator who was unaware of the group assignments." p. 608.

TG-MV-007 2012 *(Continued)*

Incomplete outcome data (attrition bias) All outcomes	Unclear risk	No description found in article, but 17 participants reported as not completing study on ClinicalTrials.gov. The corresponding author of Stalmans and colleagues (2012) was contacted to query this. ThromboGenics NV responded by confirming use of last observation carried forward (LOCF) approach to input missing data for visits postdiscontinuation. Their explanation was: "use of LOCF was appropriate when the outcome is not expected to change after discontinuation and is a conservative method when the outcome is expected to improve spontaneously over time. The primary endpoint, pharmacological VMA resolution in particular, is an outcome of that nature." As these losses to follow-up were not reported in original paper, risk of attrition bias was deemed unclear.
Selective reporting (reporting bias)	Low risk	All defined outcomes in methods were reported.
Other bias	Unclear risk	Baseline imbalance between study groups as pseudophakia was more common in ocriplasmin group than in placebo group and there were more women in ocriplasmin group than in placebo group.

AMD: age-related macular degeneration; BCVA: best corrected visual acuity; FTMH: full-thickness macular hole; MH: macular hole; OCT: optical coherence tomography; PDR: proliferative diabetic retinopathy; PVD: posterior vitreous detachment; RCT: randomised controlled trial; sVMA: symptomatic vitreomacular adhesion; VFQ-25: Visual Function Questionnaire 25; VMA: vitreomacular adhesion.

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Benz 2010	Indication for ocriplasmin was not symptomatic vitreomacular adhesion. It was investigating whether 125 µg microplasmin would induce vitreous release in people scheduled for PPV.
De Smet 2009	Investigated safety and efficacy of 4 different doses of intravitreal microplasmin prior to preplanned PPV. Subsequent PPV occurred either 1-2 hours, 24 hours or 7 days following ocriplasmin, meaning the participant population and outcome measures were not eligible for inclusion in our review.
Dugel 2015	Post hoc analysis of data from studies we already extracted data from (TG-MV-006 and TG-MV-007).
Elbendary 2011	Autologous plasmin injected into participants with diabetic macular oedema associated with vitreomacular traction.
Jackson 2017	Incorrect study design; post hoc analysis.
Lanzetta 2014a	Postmarket surveillance study, not an RCT, therefore not eligible for inclusion.
Lanzetta 2014b	Post-hoc analysis of data, not an RCT, therefore excluded.
Lescrauwaet 2016	Not an RCT.
Novack 2015	Eligible participants for this study required exudative age-related macular degeneration, which did not meet inclusion criteria for our review.

PPV: pars plana vitrectomy; RCT: randomised controlled trial.

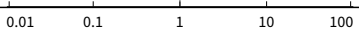
DATA AND ANALYSES

Comparison 1. Ocriplasmin versus sham injection

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Complete release of vitreous adhesion 28 days after treatment	4	859	Risk Ratio (M-H, Random, 95% CI)	3.46 [2.00, 6.00]
2 Closure of macular hole 28 days after treatment	3	229	Risk Ratio (M-H, Random, 95% CI)	2.87 [1.50, 5.51]
3 Complete posterior vitreous detachment 28 days after treatment	3	689	Risk Ratio (M-H, Random, 95% CI)	2.94 [1.39, 6.24]
4 > 3-line improvement in BCVA 6 months after treatment	3	674	Risk Ratio (M-H, Random, 95% CI)	1.95 [1.07, 3.53]
5 Requirement for pars plana vitrectomy at month 6	3	689	Risk Ratio (M-H, Random, 95% CI)	0.67 [0.50, 0.91]
6 Any ocular adverse event	4	909	Risk Ratio (M-H, Random, 95% CI)	1.22 [1.09, 1.37]

Analysis 1.1. Comparison 1 Ocriplasmin versus sham injection, Outcome 1 Complete release of vitreous adhesion 28 days after treatment.

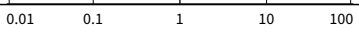
Study or subgroup	Ocriplasmin n/N	Sham n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
MIVI-IIT 2010	11/25	1/12		7.28%	5.28[0.77,36.3]
OASIS 2016	54/111	5/59		25.69%	5.74[2.43,13.57]
TG-MV-006 2012	61/219	14/107		41.92%	2.13[1.25,3.63]
TG-MV-007 2012	62/245	5/81		25.11%	4.1[1.71,9.84]
Total (95% CI)	**600**	**259**		**100%**	**3.46[2,6]**
Total events: 188 (Ocriplasmin), 25 (Sham)					
Heterogeneity: Tau^2=0.11; Chi^2=4.74, df=3(P=0.19); I^2=36.65%					
Test for overall effect: Z=4.42(P<0.0001)					



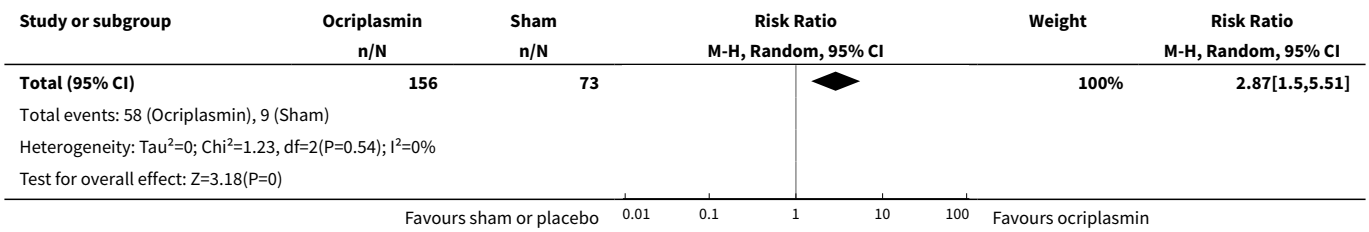
Favours sham or placebo — Favours ocriplasmin

Analysis 1.2. Comparison 1 Ocriplasmin versus sham injection, Outcome 2 Closure of macular hole 28 days after treatment.

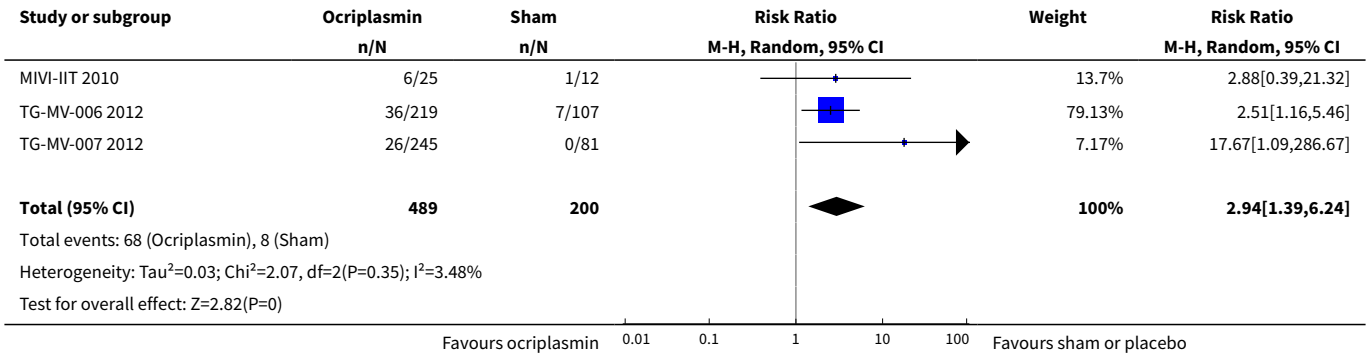
Study or subgroup	Ocriplasmin n/N	Sham n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
OASIS 2016	15/50	4/26		42.79%	1.95[0.72,5.28]
TG-MV-006 2012	25/57	4/32		45.8%	3.51[1.34,9.19]
TG-MV-007 2012	18/49	1/15		11.41%	5.51[0.8,37.92]



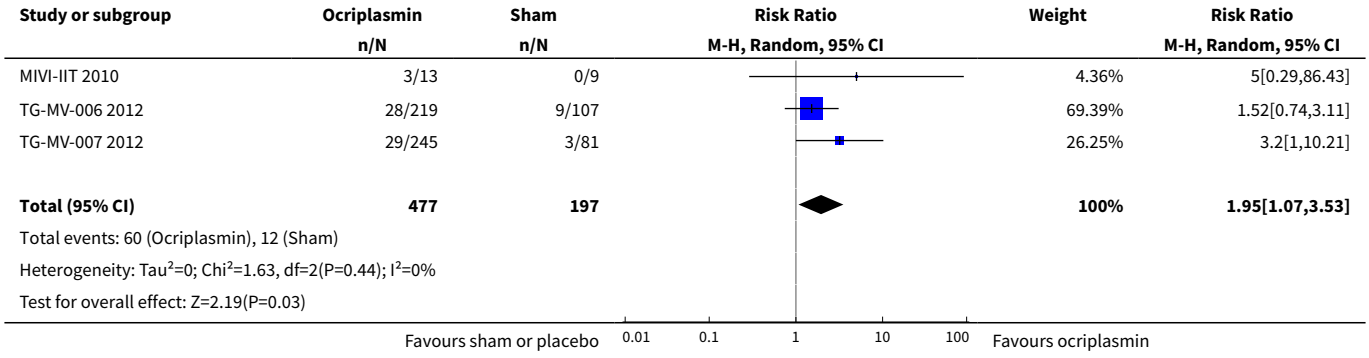
Favours sham or placebo — Favours ocriplasmin



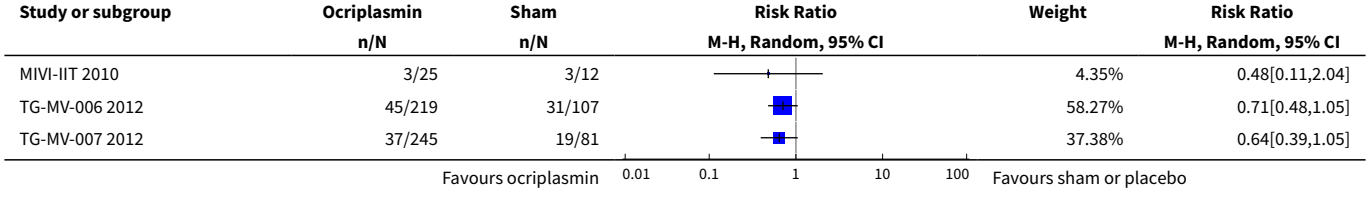
Analysis 1.3. Comparison 1 Ocriplasmin versus sham injection, Outcome 3 Complete posterior vitreous detachment 28 days after treatment.

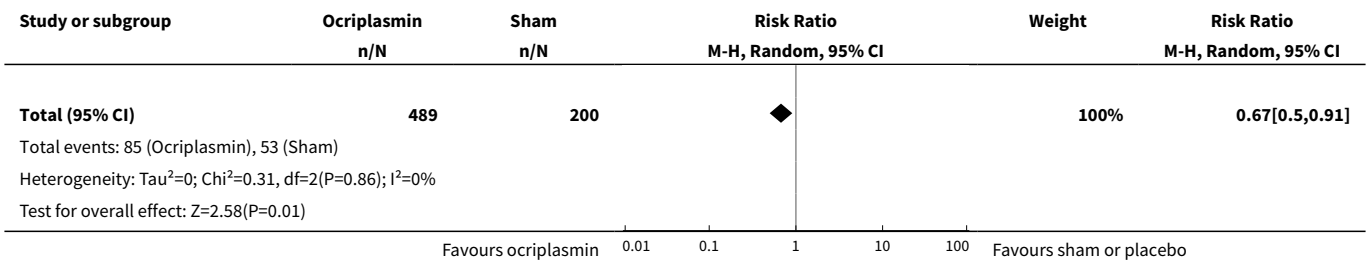


Analysis 1.4. Comparison 1 Ocriplasmin versus sham injection, Outcome 4 > 3-line improvement in BCVA 6 months after treatment.

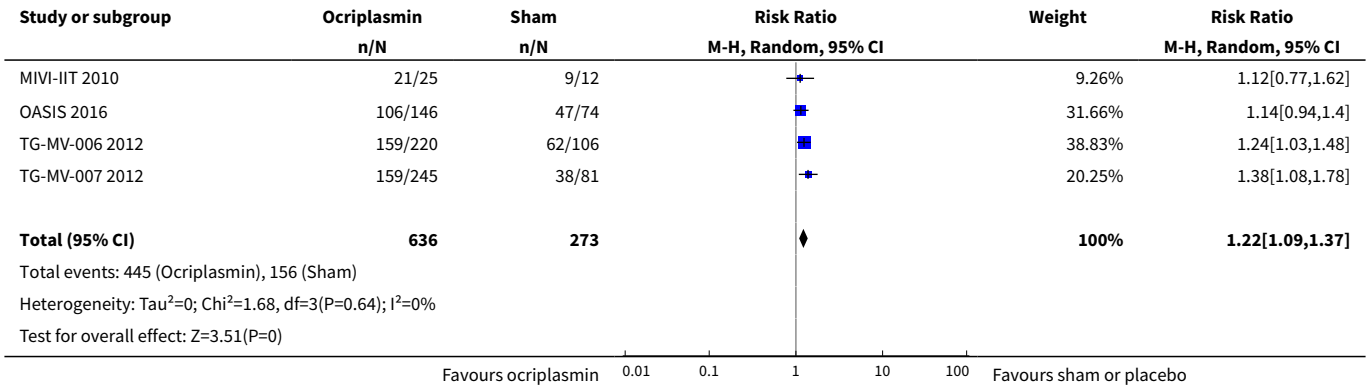


Analysis 1.5. Comparison 1 Ocriplasmin versus sham injection, Outcome 5 Requirement for pars plana vitrectomy at month 6.





Analysis 1.6. Comparison 1 Ocriplasmin versus sham injection, Outcome 6 Any ocular adverse event.



APPENDICES

Appendix 1. CENTRAL search strategy

#1 MeSH descriptor: [Vitreous Body] this term only
#2 MeSH descriptor: [Vitreous Detachment] this term only
#3 MeSH descriptor: [Retinal Perforations] this term only
#4 MeSH descriptor: [Tissue Adhesions] this term only
#5 vitreomacular near/3 (adhesion* or traction*)
#6 VMA* or VMT*
#7 macula* near/2 hole*
#8 #1 or #2 or #3 or #4 or #5 or #6 or #7
#9 MeSH descriptor: [Fibrinolysin] this term only
#10 MeSH descriptor: [Fibrinolytic Agents] this term only
#11 MeSH descriptor: [Proteolysis] this term only
#12 MeSH descriptor: [Peptide Fragments] this term only
#13 ocriplasmin* or Jetrea* or Microplasmin*
#14 #9 or #10 or #11 or #12 or #13
#15 #8 and #14

Appendix 2. MEDLINE Ovid search strategy

1. randomized controlled trial.pt.
2. (randomized or randomised).ab,ti.
3. placebo.ab,ti.
4. dt.fs.
5. randomly.ab,ti.
6. trial.ab,ti.
7. groups.ab,ti.

8. or/1-7
9. exp animals/
10. exp humans/
11. 9 not (9 and 10)
12. 8 not 11
13. Vitreous Body/
14. Vitreous Detachment/
15. Retinal Perforations/
16. Tissue Adhesions/
17. (vitreomacular adj3 (adhesion$ or traction$)).tw.
18. (VMA$ or VMT$).tw.
19. (macula$ adj2 hole$).tw.
20. or/13-19
21. Fibrinolysin/
22. Fibrinolytic Agents/
23. Proteolysis/
24. Peptide Fragments/
25. (ocriplasmin$ or Jetrea$ or Microplasmin$).tw.
26. or/21-25
27. 20 and 26
28. 12 and 27

The search filter for trials at the beginning of the MEDLINE strategy is from the published paper by Glanville 2006.

Appendix 3. Embase Ovid search strategy

1. exp randomized controlled trial/
2. exp randomization/
3. exp double blind procedure/
4. exp single blind procedure/
5. random$.tw.
6. or/1-5
7. (animal or animal experiment).sh.
8. human.sh.
9. 7 and 8
10. 7 not 9
11. 6 not 10
12. exp clinical trial/
13. (clin$ adj3 trial$).tw.
14. ((singl$ or doubl$ or trebl$ or tripl$) adj3 (blind$ or mask$)).tw.
15. exp placebo/
16. placebo$.tw.
17. random$.tw.
18. exp experimental design/
19. exp crossover procedure/
20. exp control group/
21. exp latin square design/
22. or/12-21
23. 22 not 10
24. 23 not 11
25. exp comparative study/
26. exp evaluation/
27. exp prospective study/
28. (control$ or prospectiv$ or volunteer$).tw.
29. or/25-28
30. 29 not 10
31. 30 not (11 or 23)
32. 11 or 24 or 31
33. vitreous body detachment/
34. vitreous disease/
35. retina tear/
36. tissue adhesion/

37. (vitreomacular adj3 (adhesion$ or traction$)).tw.
38. (VMA$ or VMT$).tw.
39. (macula$ adj2 hole$).tw.
40. or/33-39
41. ocriplasmin/
42. fibrinolytic agent/
43. peptide fragment/
44. (ocriplasmin$ or Jetrea$ or Microplasmin$).tw.
45. or/41-44
46. 40 and 45
47. 32 and 46

Appendix 4. PubMed search strategy

(((vitreous body[MeSH Terms]) OR (vitreous detachment[MeSH Terms]) OR (Retinal Perforations[MeSH Terms]) OR (tissue adhesions[MeSH Terms]) OR (vitreomacular adhesion*[Text Word]) OR (vitreomacular traction*[Text Word]) OR (VMA*[Text Word] OR VMT*[Text Word]) OR (macula* AND hole*[Text Word])) AND ((fibrinolysin[MeSH Terms]) OR (fibrinolytic agents[MeSH Terms]) OR (proteolysis[MeSH Terms]) OR (peptide fragments[MeSH Terms]) OR (ocriplasmin*[Text Word] OR Jetrea*[Text Word] OR Microplasmin*[Text Word]))) AND (((randomized controlled trial[Publication Type]) OR (controlled clinical trial[Publication Type]) OR (random*[Text Word] OR placebo*[Text Word] OR trial*[Text Word] OR group*[Text Word])) AND (Medline[sb]))

Appendix 5. ISRCTN search strategy

Ocriplasmin OR Jetrea OR Microplasmin

Appendix 6. ClinicalTrials.gov search strategy

(vitreomacular adhesion OR vitreomacular traction OR macular hole) AND (Ocriplasmin OR Jetrea OR Microplasmin)

Appendix 7. WHO ICTRP search strategy

vitreomacular adhesion OR vitreomacular traction OR macular hole = Intervention AND Ocriplasmin OR Jetrea OR Microplasmin = Condition

Appendix 8. Data on study characteristics

Mandatory items		Optional items
Methods		
Study design	• Parallel group RCT *i.e. people randomised to treatment* • Within-person RCT *i.e. eyes randomised to treatment* • Cluster RCT *i.e. communities randomised to treatment* • Cross-over RCT • Other, specify	Exclusions after randomisation Losses to follow-up Number randomised/analysed How were missing data handled? *e.g. available case analysis, imputation methods* Reported power calculation (Y/N), *if yes, sample size and power* Unusual study design/issues
Eyes *or* Unit of randomisation/unit of analysis	• 1 eye included in study, *specify how eye selected* • 2 eyes included in study, both eyes received same treatment, *briefly specify how analysed (best/worst/mean/both and adjusted for within-person correlation/both and not adjusted for within-person correlation) and specify if mixture 1 eye and 2 eyes* • 2 eyes included in study, eyes received different treatments, *specify if correct pair-matched analysis done*	
Participants		
Country		Setting

(Continued)

Total number of participants	*This information should be collected for total study population recruited into the study. If these data are only reported for the people who were followed up only, please indicate.*	Ethnic group
Number (%) of men and women		Equivalence of baseline characteristics (Y/N)
Mean age and age range		
Inclusion criteria		
Exclusion criteria		
Interventions		
Intervention (n =) Comparator (n =) ***See MECIR 65 and 70***	• Number of people randomised to this group • Drug (or intervention) name • Dose • Frequency • Route of administration	
Outcomes		
Primary and secondary outcomes *as defined in study reports* ***See MECIR R70***	List outcomes Adverse events reported (Y/N) Length of follow-up and intervals at which outcomes assessed	Planned/actual length of follow-up
Notes		
Date conducted	Specify dates of recruitment of participants mm/yr to mm/yr	Full study name:*(if applicable)*
Sources of funding		Reported subgroup analyses (Y/N)
Declaration of interest ***See MECIR 69***		Were trial investigators contacted?

MECIR: Methodological expectations for Cochrane Intervention Reviews; mm: month; n: number of participants; RCT: randomised controlled trial; yr: year.

Appendix 9. Glossary of abbreviations

BCVA: best corrected visual acuity.
BEAVRS: British and Eire vitreoretinal surgeons.
ERM: epiretinal membrane.
ETDRS: Early Treatment Diabetic Retinopathy Study.
GRADE: Grading of Recommendations, Assessment, Development and Evaluation working group.
ILM: internal limiting membrane.
logMAR: logarithm of the minimum angle of resolution.
MH: macular hole.
NEI-VFQ 25: National Eye Institute Visual Function Questionnaire - 25.
NICE: National Institute for Health and Care Excellence.
OCT: optical coherence tomography.
PVD: posterior vitreous detachment.
RCT: randomised controlled trial.
sVMA: symptomatic vitreomacular adhesion.

VFQ: Visual Function Questionnaire.
VMA: vitreomacular adhesion.
VMT: vitreomacular traction.
WHO: World Health Organization.

CONTRIBUTIONS OF AUTHORS

JN: wrote the first draft of the protocol and review and reviewed the electronic search results.
VK: reviewed the electronic search results, carried out the statistical analysis and critically appraised the review.
EP: critically appraised the review.
TJ: reviewed the electronic search results, and critically appraised all drafts of the protocol and review.

DECLARATIONS OF INTEREST

JN: financial support to attend conferences (COPHy 2014 - ThromboGenics; AAO 2016, Retinal World Congress 2017 - VisionCare).
VK: financial support to attend conferences (ARVO 2017 - Bayer).
EP: no disclosures.
TJ: received an honorarium in 2016 to attend the EVER conference in Nice: ThromboGenics; advisory boards: Alcon, Bausch & Lomb, DORC; research grant support (employer): Novartis.

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Internal sources

- No sources of support supplied

External sources

- National Institute for Health Research (NIHR), UK.

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* This review was supported by the NIHR, via Cochrane Infrastructure funding to the CEV UK editorial base.

The views and opinions expressed therein are those of the authors and do not necessarily reflect those of the Systematic Reviews Programme, NIHR, NHS or the Department of Health.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Due to the reporting of BCVA in the included studies, we changed our secondary outcome measure from "mean change in BCVA" to "proportion gaining 3-line or greater improvement in VA, measured using the ETDRS scale".

We added a secondary outcome measure, the requirement of PPV. This gives a good measure of how successful the intervention of ocriplasmin has been (i.e. conventional treatment for sVMA has been PPV, and indeed this remains the treatment modality of choice who fail ocriplasmin therapy).

We added information regarding "other bias" that could not be accurately categorised under the other categories of bias.

INDEX TERMS

Medical Subject Headings (MeSH)

*Vitreous Body; Fibrinolysin [*administration & dosage] [adverse effects]; Fibrinolytic Agents [*administration & dosage] [adverse effects]; Intravitreal Injections; Peptide Fragments [*administration & dosage] [adverse effects]; Randomized Controlled Trials as Topic; Retinal Diseases [*drug therapy]; Time Factors; Tissue Adhesions [drug therapy]; Visual Acuity; Vitrectomy; Vitreous Detachment [drug therapy] [etiology]

MeSH check words

Adult; Aged; Aged, 80 and over; Humans; Middle Aged

Appendix 2

CENTRAL search strategy

#1 MeSH descriptor: [Vitreous Body] this term only

#2 MeSH descriptor: [Vitreous Detachment] this term only

#3 MeSH descriptor: [Retinal Perforations] this term only

#4 MeSH descriptor: [Tissue Adhesions] this term only

#5 vitreomacular near/3 (adhesion* or traction*)

#6 VMA* or VMT*

#7 macula* near/2 hole*

#8 #1 or #2 or #3 or #4 or #5 or #6 or #7

#9 MeSH descriptor: [Fibrinolysin] this term only

#10 MeSH descriptor: [Fibrinolytic Agents] this term only

#11 MeSH descriptor: [Proteolysis] this term only

#12 MeSH descriptor: [Peptide Fragments] this term only

#13 ocriplasmin* or Jetrea* or Microplasmin*

#14 #9 or #10 or #11 or #12 or #13

#15 #8 and #14

Appendix 3

MEDLINE Ovid search strategy

1. randomized controlled trial.pt.

2. (randomized or randomised).ab,ti.

3. placebo.ab,ti.

4. dt.fs.

5. randomly.ab,ti.

6. trial.ab,ti.

7. groups.ab,ti.

8. or/1-7

9. exp animals/

10. exp humans/

11. 9 not (9 and 10)

12. 8 not 11

13. Vitreous Body/

14. Vitreous Detachment/

15. Retinal Perforations/

16. Tissue Adhesions/

17. (vitreomacular adj3 (adhesion$ or traction$)).tw.

18. (VMA$ or VMT$).tw.

19. (macula$ adj2 hole$).tw.

20. or/13-19

21. Fibrinolysin/

22. Fibrinolytic Agents/

23. Proteolysis/

24. Peptide Fragments/

25. (ocriplasmin$ or Jetrea$ or Microplasmin$).tw.

26. or/21-25

27. 20 and 26

28. 12 and 27

Appendix 4

Embase Ovid search strategy

1. exp randomized controlled trial/

2. exp randomization/

3. exp double blind procedure/

4. exp single blind procedure/

5. random$.tw.

6. or/1-5

7. (animal or animal experiment).sh.

8. human.sh.

9. 7 and 8

10. 7 not 9

11. 6 not 10

12. exp clinical trial/

13. (clin$ adj3 trial$).tw.

14. ((singl$ or doubl$ or trebl$ or tripl$) adj3 (blind$ or mask$)).tw.

15. exp placebo/

16. placebo$.tw.

17. random$.tw.

18. exp experimental design/

19. exp crossover procedure/

20. exp control group/

21. exp latin square design/

22. or/12-21

23. 22 not 10

24. 23 not 11

25. exp comparative study/

26. exp evaluation/

27. exp prospective study/

28. (control$ or prospectiv$ or volunteer$).tw.

29. or/25-28

30. 29 not 10

31. 30 not (11 or 23)

32. 11 or 24 or 31

33. vitreous body detachment/

34. vitreous disease/

35. retina tear/

36. tissue adhesion/

37. (vitreomacular adj3 (adhesion$ or traction$)).tw.

38. (VMA$ or VMT$).tw.

39. (macula$ adj2 hole$).tw.

40. or/33-39

41. ocriplasmin/

42. fibrinolytic agent/

43. peptide fragment/

44. (ocriplasmin$ or Jetrea$ or Microplasmin$).tw.

45. or/41-44

46. 40 and 45

47. 32 and 46

Appendix 5

PubMed search strategy

(((vitreous body[MeSH Terms]) OR (vitreous detachment[MeSH Terms]) OR (Retinal Perforations[MeSH Terms]) OR (tissue adhesions[MeSH Terms]) OR (vitreomacular adhesion*[Text Word]) OR (vitreomacular traction*[Text Word]) OR (VMA*[Text Word] OR VMT*[Text Word]) OR (macula* AND hole*[Text Word])) AND ((fibrinolysin[MeSH Terms]) OR (fibrinolytic agents[MeSH Terms]) OR (proteolysis[MeSH Terms]) OR (peptide fragments[MeSH Terms]) OR (ocriplasmin*[Text Word] OR Jetrea*[Text Word] OR Microplasmin*[Text Word]))) AND (((randomized controlled trial[Publication Type]) OR (controlled clinical trial[Publication Type]) OR (random*[Text Word] OR placebo*[Text Word] OR trial*[Text Word] OR group*[Text Word])) AND (Medline[sb]))

Appendix 6

ISRCTN search strategy

Ocriplasmin OR Jetrea OR Microplasmin

Appendix 7

ClinicalTrials.gov search strategy

(vitreomacular adhesion OR vitreomacular traction OR macular hole) AND (Ocriplasmin OR Jetrea OR Microplasmin)

Appendix 8

WHO ICTRP search strategy

vitreomacular adhesion OR vitreomacular traction OR macular hole = Intervention AND Ocriplasmin OR Jetrea OR Microplasmin = Condition

Appendix 9

Mandatory items		**Optional items**
Methods		
Study design	• Parallel group RCT *i.e. people randomised to treatment* • Within-person RCT *i.e. eyes randomised to treatment* • Cluster RCT *i.e. communities randomised to treatment* • Cross-over RCT • Other, specify	Exclusions after randomisation Losses to follow-up Number randomised/analysed How were missing data handled? *e.g. available case analysis, imputation methods* Reported power calculation (Y/N), *if yes, sample size and power* Unusual study design/issues
Eyes *or* Unit of randomisation/unit of analysis	• 1 eye included in study, *specify how eye selected* • 2 eyes included in study, both eyes received same treatment, *briefly specify how analysed (best/worst/mean/both and adjusted for within-person correlation/both and not adjusted for within-person correlation) and specify if mixture 1 eye and 2 eyes* • 2 eyes included in study, eyes received different treatments, *specify if correct pair-matched analysis done*	
Participants		
Country		Setting Ethnic group Equivalence of baseline characteristics (Y/N)
Total number of participants	*This information should be collected for total study population recruited into the study. If these data are only reported for the people who were followed up only, please indicate.*	
Number (%) of men and women		
Mean age and age range		
Inclusion criteria		
Exclusion criteria		
Interventions		
Intervention (n =) Comparator (n =) ***See MECIR 65 and 70***	• Number of people randomised to this group • Drug (or intervention) name • Dose • Frequency • Route of administration	
Outcomes		
Primary and secondary outcomes *as defined in study reports* ***See MECIR R70***	List outcomes Adverse events reported (Y/N) Length of follow-up and intervals at which outcomes assessed	Planned/actual length of follow-up
Notes		
Date conducted	Specify dates of recruitment of participants mm/yr to mm/yr	Full study name: *(if applicable)*
Sources of funding		Reported subgroup analyses (Y/N)
Declaration of interest ***See MECIR 69***		Were trial investigators contacted?

MEICR, Methodological expectations for Cochrane Intervention Reviews; mm, month; n, number of participants; RCT, randomised controlled trial; yr, year.

Appendix 10

Characteristics of included studies: MIVI-IIT

Methods	**Study design:** RCT, single treated eye. **Number randomised:** 60 total; 48 microplasmin; 12 sham injection. **Exclusions after randomisation:** none. **Number analysed:** at 28 days and 6 months; 60 total; 48 microplasmin; 12 sham injection. **Unit of analysis:** eyes. **Losses to follow-up:** 0 participants total. **How was missing data handled?** No missing data. **Power calculation:** none documented.	
Participants	**Country:** Belgium. **Mean age:** 70.0 years overall; 69.9 years for ocriplasmin group; 70.0 years for sham injection group. **Gender:** 33/60 (55%) women, 27/60 (45%) men total; 27/48 (56%) women, 21/48 (44%) men in microplasmin group; 6/12 (50%) women, 6/12 (50%) men in sham injection group. **Inclusion criteria:** aged > 18 years; partial PVD on ultrasound examination; OCT evidence of at least a partial attachment in the foveal area, resulting in a macular thickness of ≥ 250 μm; BCVA ≤ 20/40 in study eye; BCVA ≥ 20/400 in fellow eye. **Exclusion criteria:** active PDR; high myopia (axial length > 26 mm); previous vitrectomy or uncontrolled glaucoma; previous intravitreal injections in the past 3 months in study eye; intraocular surgery or laser photocoagulation in the past 3 months in study eye; rhegmatogenous retinal detachment in either eye. **Equivalence of baseline characteristics:** no; more participants in microplasmin group had tractional diabetic macular oedema compared with sham injection group.	
Interventions	**Intervention 1:** single intravitreal injection microplasmin 125 μg. **Intervention 2:** single intravitreal injection microplasmin 75 μg. **Intervention 3:** single intravitreal injection microplasmin 175 μg. **Intervention 4:** intravitreal injection of microplasmin 125 μg at baseline, followed by a further microplasmin 125 μg intravitreal injection at 28 days if VMA was still present, followed by a further microplasmin 125 μg intravitreal injection at 56 days after baseline if VMA was still present. **Comparator:** sham injection (conjunctiva touched with a blunt needle by a non-masked investigator and no injection given). **Length of follow-up:** planned 180 days, actual 180 days. As the recommended dose of ocriplasmin is 125 μg, and this is the subject of the review, only data from the first and fourth intervention arms were analysed.	
Outcomes	**Primary outcome, as defined in study reports:** "the primary outcome of this study was total PVD induction at Day 14, as assessed by a central reading centre." **Secondary outcomes, as defined in study reports:** total PVD at other time points assessed by the central reading centre and investigators; resolution of index condition (VMA or MH); resolution of VMA; progression of PVD; need for vitrectomy; resolution of macular oedema; change in BCVA; BCVA 5-, 10- and 15-letter improvement. **Adverse events reported:** yes. **Intervals at which outcomes assessed:** 3, 7, 14, 28, 90 and 180 days.	
Notes	**Funding sources:** study sponsored by ThromboGenics NV. **Study Period:** 2 years; 2007 – 2009. **Reported subgroup analyses:** yes. Full results of study were presented at EURetina 2009, Nice, France. NCT00435539.	
Risk of bias		
Bias	**Author's judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Method of sequence generation for the MIVI-IIT RCT not described. Quote: "Four cohorts of 15 patients were randomised as 4:1 to treatment or sham injection, resulting in 12 patients receiving microplasmin and 3 patients receiving the sham injection in each cohort." p. 1123.
Allocation concealment (selection bias)	Unclear risk	Insufficient information documented to assess allocation concealment. Quote: "Four cohorts of 15 patients were randomised as 4:1 to treatment or sham injection, resulting in 12 patients receiving microplasmin and 3 patients receiving the sham injection in each cohort." p. 1123.

Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Sham injection was performed, rather than actual placebo injection. Quote: "In the patients receiving a sham injection, microplasmin was prepared in the same manner, but instead of an intraocular injection, the conjunctiva was touched with a blunt needle by a nonmasked investigator and no injection was given." p. 1123.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Quote: "All patient examinations before drug allocation and in the 6-month follow-up period after the last injection were performed by masked investigators and study personnel." p. 1123. "Posterior vitreous detachment status and macular thickness were assessed by the investigator as well as by a central reading center (CRC), located in Munich, Germany." p. 1124.
Incomplete outcome data (attrition bias) All outcomes	Low risk	No incomplete outcome data.
Selective reporting (reporting bias)	Low risk	All outcomes defined in trial registry were reported.
Other bias	Low risk	

BCVA, best corrected visual acuity; MH, macular hole; OCT, optical coherence tomography; PDR, proliferative diabetic retinopathy; PVD, posterior vitreous detachment; RCT, randomised controlled trial; VMA, vitreomacular adhesion.

Appendix 11

Characteristics of included studies: OASIS

Methods	**Study design:** RCT, single treated eye. **Number randomised:** 220 total; 146 microplasmin; 74 sham injection. **Exclusions after randomisation:** 50 participants subsequently deemed ineligible by central reading centre. **Number analysed:** at 28 days: 168 total; 111 ocriplasmin; 59 sham. **Unit of analysis:** eyes. **Losses to follow-up:** 2 participants total; 1 ocriplasmin group (1 lost to follow-up); 1 sham group (1 lost to follow-up). **How was missing data handled?** Other than VMA release at 28 days, no data published regarding cohort who met central reading centre eligibility. **Power calculation:** 210 participants for at least 90% power at 2-sided alpha of 0.05 to assume a primary endopoint of 37% in ocriplasmin group and a 14% rate in placebo group.

Participants	**Country:** USA. **Mean age:** 69.1 years overall; 69.4 years for ocriplasmin group; 68.5 years for sham group. **Gender:** 147/218 (67.4%) women, 71/218 (32.6%) men total; 102/145 (70.3%) women, 43/145 (29.7%) men in ocriplasmin group; 45/73 (61.6%) women, 28/73 (38.4%) men in sham group. **Inclusion criteria:** aged > 18 years; presence of VMA; BCVA ≤ 20/32 in study eye; BCVA ≥ 20/800 in non-study eye. **Exclusion criteria:** history or current evidence of proliferative retinopathy, exudative AMD or retinal vein occlusion in the study eye; people with any vitreous haemorrhage or any other vitreous opacification which precludes either visualisation of the posterior pole by visual inspection OR adequate assessment of the macula by OCT; MH > 400 μm in diameter in the study eye; presence of epiretinal membrane; aphakia in study eye; high myopia (> -8 dioptres in study eye); history of rhegmatogenous retinal detachment in either eye; prior vitrectomy in study eye; previous participation in this trial or prior administration of ocriplasmin in study eye. **Equivalence of baseline characteristics:** yes.
Interventions	**Intervention 1:** single intravitreal injection of ocriplasmin 125 μg in 0.10 mL volume. **Comparator:** sham (the same syringe hub was pressed against the conjunctiva to simulate an injection). **Length of follow-up:** planned 24 months, actual 24 months. Data of central reading centre approved study participants only reported at 28 days.
Outcomes	**Primary outcome, as defined in study reports:** "proportion of subjects with pharmacological vitreomacular adhesion (VMA)/vitreomacular traction (VMT) resolution at day 28. Pharmacological VMA resolution without anatomical defect, based on spectral domain optical coherence tomography and determined by the masked central reading center (CRC), with post-resolution vitrectomy considered as a failure." **Secondary outcomes, as defined in study reports:** "proportion of subjects with a ≥2 line improvement in best-corrected visual acuity (BCVA) from baseline at month 24, irrespective of vitrectomy." **Adverse events reported:** yes. **Intervals at which outcomes assessed:** 7 and 28 days; 3, 6, 9, 12, 15, 18, 21 and 24 months.
Notes	**Funding sources:** study sponsored by ThromboGenics NV. **Study Period:** 3 years; 2011 – 2014. **Reported subgroup analyses:** yes. **Additional information:** large proportion of patients were deemed eligible, recruited and treated by investigators. Retrospective central reading centre review found 50 particpants ineligible for following reasons (MH > 400 μm, presence of epiretinal membrane or no sVMA at baseline). Our analysis only included data reported for correctly eligible cohort of participants. NCT01429441.

Risk of bias

Bias	**Author's judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Method of random sequence generation for the MIVI-IIT RCT described. Quote: "Randomization was stratified on the basis of the presence or absence of FTMH at baseline and was centralized through an interactive voice response system." p. 2233.
Allocation concealment (selection bias)	Low risk	Method of allocation concealment for MIVI-IIT RCT described. Quote: "Randomization was stratified on the basis of the presence or absence of FTMH at baseline and was centralized through an interactive voice response system." p. 2233.
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Performance bias explained. Quote: "The trial was conducted in a double-masked manner. To maintain the masking of the investigator, an unmasked injecting physician was assigned to perform the injection and access the interactive voice response system to receive the assigned treatment. The unmasked personnel did not perform or participate in any other trial-related procedures or assessments." p. 2233.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Detection bias appropriately explained. Quote: "The trial was conducted in a double-masked manner. To maintain the masking of the investigator, an unmasked injecting physician was assigned to perform the injection and access the interactive voice response system to receive the assigned treatment. The unmasked personnel did not perform or participate in any other trial-related procedures or assessments." p. 2233.

Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Large proportion of participants deemed eligible, recruited and treated by investigators. Retrospective central reading centre review found 50 participants ineligible for following reasons (MH > 400 µm, presence of epiretinal membrane or no sVMA). Outcome data for correct eligible cohort of participants only given for primary outcome. No secondary outcome data described.
Selective reporting (reporting bias)	Low risk	All outcomes defined in trial registry were reported.
Other bias	Low risk	No other source of bias.

AMD, age-related macular degeneration; BCVA, best corrected visual acuity; CRC, central reading centre; FTMH, full thickness macular hole; MH, macular hole; OCT, optical coherence tomography; RCT, randomised controlled trial; sVMA, symptomatic vitreomacular adhesion; VMA, vitreomacular adhesion; VMT, vitreomacular traction.

Appendix 12

Characteristics of included studies: TG-MV-006

Methods	**Study design:** RCT, single treated eye. **Number randomised:** 326 total; 219 ocriplasmin; 107 placebo. **Exclusions after randomisation:** 0. **Number analysed:** at 28 days: 326 total; 219 ocriplasmin; 107 placebo. At 180 days: 298 total; 200 ocriplasmin; 98 placebo. **Unit of analysis:** eyes. **Losses to follow-up:** 28 participants total; 19 ocriplasmin group (2 adverse event, 8 withdrawal by participants, 6 lost to follow-up, 3 death); 9 placebo group (2 adverse event, 4 withdrawal by participants, 3 lost to follow-up). **How was missing data handled?** missing data not reported in study publications. **Power calculation:** 320 participants for > 90% power at 2-sided alpha of 0.05 to assume a primary end-point of 27.5% in ocriplasmin group and 10.0% in placebo group.
Participants	**Country:** USA. **Mean age:** 71.4 years overall; 71.5 years for ocriplasmin group; 71.1 years for placebo group. **Gender:** 207/326 (63.5%) women, 119/326 (36.5%) men total; 148/219 (67.6%) women, 71/219 (32.4%) men in ocriplasmin group; 59/107 (55.1%) women, 52/107 (48.6%) men in placebo group. **Inclusion criteria:** aged > 18 years; focal VMA (vitreous adhesion to macula within 6-mm central retinal field surrounded by elevation of posterior vitreous cortex, as seen on OCT that in the opinion of investigator was related to decreased visual function (e.g. metamorphopsia, decreased visual acuity or other visual complaint); BCVA ≤ 20/25 in study eye; BCVA ≥ 20/800 in non-study eye. **Exclusion criteria:** any evidence of proliferative retinopathy (including PDR or other ischaemic retinopathies involving vitreoretinal vascular proliferation) or exudative AMD or retinal vein occlusion in study eye; people with any vitreous haemorrhage or any other vitreous opacification which precludes either: visualisation of posterior pole by visual inspection OR adequate assessment of macula by either OCT or fluorescein angiogram (or both) in study eye; MH > 400 µm in diameter in study eye; aphakia in study eye; high myopia (> -8 dioptres); uncontrolled glaucoma; lenticular or zonular instability; history of retinal detachment in either eye; prior vitrectomy or prior laser photocoagulation of macula; treatment with ocular surgery, intravitreal injection or retinal laser photocoagulation in the previous 3 months. **Equivalence of baseline characteristics:** no; pseudophakia more common in ocriplasmin group than in placebo group; more women in ocriplasmin group than in placebo group.

Interventions	**Intervention:** single intravitreal injection of ocriplasmin 125 µg in 0.10 mL volume. **Comparator:** single intravitreal injection of 0.10 mL placebo with identical drug vehicle diluted with saline. **Length of follow-up:** planned 180 days, actual 180 days.
Outcomes	**Primary outcome, as defined in study reports:** "the primary end point was the proportion of subjects with nonsurgical resolution of vitreomacular adhesion at day 28 post-injection, as determined by masked OCT evaluation obtained from the central reading centre." **Secondary outcomes, as defined in study reports:** proportion of participants with total PVD at day 28, as determined by B-scan ultrasound; need for vitrectomy; closure of an MH; gain ≥ 3-lines BCVA without vitrectomy; change from baseline in BCVA and VFQ-25 score at 6 months. **Adverse events reported:** yes. **Intervals at which outcomes assessed:** 7, 14, 28, 90 and 180 days.
Notes	**Funding sources:** study sponsored by ThromboGenics NV. **Study period:** 2 years; 2008-2009. **Reported subgroup analyses:** yes. NCT00781859.

Risk of bias

Bias	**Author's judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Supplementary material: "subjects will be randomised centralized through a telephone-based Interactive Voice Response System (IVRS) to either microplasmin intravitreal injection or placebo in a 3:1 allocation ratio. Blocks of treatment will be assigned to sites in a manner expected to minimize the potential for imbalance in the desired randomization ratio." Protocol p. 17.
Allocation concealment (selection bias)	Low risk	Supplementary material: "subjects will be randomised centralized through a telephone-based Interactive Voice Response System (IVRS) to either microplasmin intravitreal injection or placebo in a 3:1 allocation ratio. Blocks of treatment will be assigned to sites in a manner expected to minimize the potential for imbalance in the desired randomization ratio." Protocol p. 17.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote: "Patients randomly assigned to the ocriplasmin group received an intravitreal injection of ocriplasmin (125 µg in a 0.10-ml volume) drawn from a vial containing ocriplasmin into which 0.75 ml of commercial saline had been injected (1875 µg of ocriplasmin in a 0.75-ml drug vehicle). Patients randomly assigned to placebo received an intravitreal injection of 0.10 ml of the identical drug vehicle diluted with saline, the method used being the same as that used to prepare ocriplasmin." p. 608.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Quote: "Trained readers at a central reading center (Duke University OCT Reading Center, Durham, NC) who were unaware of the group assignments evaluated the OCT images. All ultrasonographic studies were standardized and performed by certified technicians who underwent special training for the study. Staging of posterior vitreous detachment was based on dynamic ultrasonographic evaluation and performed by an investigator who was unaware of the group assignments." p. 608.
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	No description found in article, but 28 participants reported as not completing study on ClinicalTrials.gov. The corresponding author of Stalmans and colleagues (2012) was contacted to query this. ThromboGenics NV responded by confirming use of last observation carried forward (LOCF) approach to input missing data for visits postdiscontinuation. Their explanation was: "use of LOCF was appropriate when the outcome is not expected to change after discontinuation and is a conservative method when the outcome is expected to improve spontaneously over time. The primary endpoint, pharmacological VMA resolution in particular, is an outcome of that nature." As these losses to follow-up were not reported in original paper, risk of attrition bias was deemed unclear.
Selective reporting (reporting bias)	Low risk	All outcomes defined in methods were reported.
Other bias	Unclear risk	Baseline imbalance between study groups as pseudophakia was more common in ocriplasmin group than in placebo group and there were more women in ocriplasmin group than in placebo group.

AMD, age-related macular degeneration; BCVA, best corrected visual acuity; IVRS, interactive voice response system; LOCF, last observation carried forward; MH, macular hole; OCT, optical coherence tomography; PDR, proliferative diabetic retinopathy; PVD, posterior vitreous detachment; RCT, randomised controlled trial; VMA, vitreomacular adhesion; VFQ-25, visual function questionnaire-25; VMT, vitreomacular traction.

Appendix 13

Characteristics of included studies: TG-MV-007

Methods	**Study design**: RCT, single treated eye. **Number randomised:** 326 total; 245 ocriplasmin; 81 placebo. **Exclusions after randomisation:** none. **Number analysed**: at 28 days: 326 total; 245 ocriplasmin; 81 placebo. At 180 days: 309 total; 235 ocriplasmin; 74 placebo. **Unit of analysis:** eyes. **Losses to follow-up:** 17 participants total; 10 ocriplasmin group (5 withdrawal by participant, 2 lost to follow-up, 2 adverse event, 1 death); 7 placebo group (1 physician decision, 4 withdrawal by participant, 2 lost to follow-up). **How was missing data handled?** missing data not reported in study publications. **Power calculation:** 320 participants for > 90% power at 2-sided alpha of 0.05 to assume a primary end-point of 27.5% in ocriplasmin group and 10.0% rate in placebo group.
Participants	**Countries:** Belgium, Czech Republic, Germany, Poland, Spain, UK, USA. **Mean age:** 72.0 years overall; 72.6 years for ocriplasmin group; 70.2 years for placebo group. **Gender:** 222/326 (68.1%) women, 104/326 (31.9%) men total; 166/245 (67.8%) women, 79/245 (32.2%) men in ocriplasmin group; 56/81 (69.1%) women, 25/81 (30.9%) men in placebo group. **Inclusion criteria:** aged > 18 years; focal VMA (vitreous adhesion to macula within 6-mm central retinal field surrounded by elevation of posterior vitreous cortex, as seen on OCT that in the opinion of investigator was related to decreased visual function (e.g. metamorphopsia, decreased visual acuity or other visual complaint); BCVA ≤ 20/25 in study eye; BCVA ≥ 20/800 in non-study eye. **Exclusion criteria:** any evidence of proliferative retinopathy (including PDR or other ischaemic retinopathies involving vitreoretinal vascular proliferation) or exudative AMD or retinal vein occlusion in study eye; people with any vitreous haemorrhage or any other vitreous opacification which precludes either: visualisation of posterior pole by visual inspection OR adequate assessment of macula by either OCT or fluorescein angiogram (or both) in study eye; MH > 400 µm in diameter in study eye; aphakia in study eye; high myopia (> -8 dioptres); uncontrolled glaucoma; lenticular or zonular instability; history of retinal detachment in either eye; prior vitrectomy or prior laser photocoagulation of macula; treatment with ocular surgery, intravitreal injection or retinal laser photocoagulation in the previous 3 months. **Equivalence of baseline characteristics:** no; pseudophakia more common in ocriplasmin group than in placebo group; more women in ocriplasmin group than in placebo group.
Interventions	**Intervention:** single intravitreal injection of ocriplasmin 125 µg in 0.10 mL volume. **Comparator:** single intravitreal injection of 0.10 mL placebo with identical drug vehicle diluted with saline. **Length of follow-up:** planned 180 days, actual 180 days.
Outcomes	**Primary outcome, as defined in study reports:** "the primary end point was the proportion of subjects with nonsurgical resolution of VMA at day 28 post-injection, as determined by masked OCT evaluation obtained from the central reading centre." **Secondary outcomes, as defined in study reports:** proportion of participants with total PVD at day 28, as determined by B-scan ultrasound; need for vitrectomy; closure of an MH; gain ≥ 3-lines BCVA without vitrectomy; change from baseline in BCVA and VFQ-25 score at 6 months. **Adverse events reported:** yes. **Intervals at which outcomes assessed:** 7, 14, 28, 90 and 180 days.

Notes	**Funding sources:** study sponsored by ThromboGenics NV. **Study period:** 2 years; 2008-2010. **Reported subgroup analyses:** yes. NCT00798317.	
Risk of bias		
Bias	**Author's judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Supplementary material: "subjects will be randomised centralized through a telephone-based Interactive Voice Response System (IVRS) to either microplasmin intravitreal injection or placebo in a 3:1 allocation ratio. Blocks of treatment will be assigned to sites in a manner expected to minimize the potential for imbalance in the desired randomization ratio." Protocol p. 17.
Allocation concealment (selection bias)	Low risk	Supplementary material: "subjects will be randomised centralized through a telephone-based Interactive Voice Response System (IVRS) to either microplasmin intravitreal injection or placebo in a 3:1 allocation ratio. Blocks of treatment will be assigned to sites in a manner expected to minimize the potential for imbalance in the desired randomization ratio." Protocol p. 17.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote: "Patients randomly assigned to the ocriplasmin group received an intravitreal injection of ocriplasmin (125µg in a 0.10-ml volume) drawn from a vial containing ocriplasmin into which 0.75ml of commercial saline had been injected (1875µg of ocriplasmin in a 0.75-ml drug vehicle). Patients randomly assigned to placebo received an intravitreal injection of 0.10 ml of the identical drug vehicle diluted with saline, the method used being the same as that used to prepare ocriplasmin". p. 608.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Quote: "Trained readers at a central reading center (Duke University OCT Reading Center, Durham, NC) who were unaware of the group assignments evaluated the OCT images. All ultrasonographic studies were standardized and performed by certified technicians who underwent special training for the study. Staging of posterior vitreous detachment was based on dynamic ultrasonographic evaluation and performed by an investigator who was unaware of the group assignments." p. 608.
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	No description found in article, but 17 participants reported as not completing study on ClinicalTrials.gov. The corresponding author of Stalmans and colleagues (2012) was contacted to query this. ThromboGenics NV responded by confirming use of last observation carried forward (LOCF) approach to input missing data for visits postdiscontinuation. Their explanation was: "use of LOCF was appropriate when the outcome is not expected to change after discontinuation and is a conservative method when the outcome is expected to improve spontaneously over time. The primary endpoint, pharmacological VMA resolution in particular, is an outcome of that nature." As these losses to follow-up were not reported in original paper, risk of attrition bias was deemed unclear.
Selective reporting (reporting bias)	Low risk	All defined outcomes in methods were reported.
Other bias	Unclear risk	Baseline imbalance between study groups as pseudophakia was more common in ocriplasmin group than in placebo group and there were more women in ocriplasmin group than in placebo group.

AMD, age-related macular degeneration; BCVA, best corrected visual acuity; IVRS, interactive voice response system; LOCF, last observation carried forward; MH, macular hole; OCT, optical coherence tomography; PDR, proliferative diabetic retinopathy; PVD, posterior

vitreous detachment; RCT, randomised controlled trial; VMA, vitreomacular adhesion; VFQ-25, visual function questionnaire-25; VMT, vitreomacular traction.

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Appendix 14

Review Article

Intravitreal gas for symptomatic vitreomacular adhesion: a synthesis of the literature

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ABSTRACT.

Symptomatic vitreomacular adhesion (sVMA) is defined as visual loss secondary to foveal damage from vitreomacular traction (VMT) and includes isolated VMT, impending macular hole (MH), and full-thickness MH with persisting vitreous attachment. Management options include pars plana vitrectomy (PPV), intravitreal ocriplasmin, intravitreal gas injection or observation. This synthesis of the literature aimed to assess the safety and efficacy of intravitreal gas for sVMA. Articles describing patients with VMT or MH treated with intravitreal expansile gas were selected by systematic literature review using MEDLINE, EMBASE, and the Cochrane Database of Controlled Trials (CENTRAL) up to September 2016. The main outcomes at 1 month and final review were logarithm of the minimum angle of resolution (logMAR) visual acuity (VA), anatomical success (absence of both VMT and MH, without PPV) and adverse events (AEs). The intended comparator was observation. Nine of 106 identified articles were eligible, and none were randomized controlled trials. The mean VA of 91 eyes improved from 0.55 (Snellen equivalent 6/21) to 0.48 (6/18) logMAR at 1 month and to 0.35 (6/13) logMAR at final review. The mean VA at final review, prior to a vitrectomy, was 0.42 (6/16). Anatomic success was 48% at 1 month and 57% at final review. The reported AEs comprised retinal detachment in two highly myopic eyes. Intravitreal gas injection can relieve sVMA. Larger controlled studies are needed to determine safety and efficacy relative to observation, ocriplasmin, or vitrectomy.

Key words: gas – macula – vitreomacular adhesion – vitreomacular traction – vitreous

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Introduction

Perifoveal vitreous separation may occur as part of normal ageing, or as part of a disease spectrum ranging from VMT to MH. Symptomatic vitreomacular adhesion (sVMA) is defined as visual loss secondary to foveal damage as a result of VMT and includes isolated VMT, impending MH and full thickness MH with persisting vitreous attachment (Simpson et al. 2012; Jackson et al. 2013c).

Treatment strategies for vitreomacular adhesion (VMA) depend on disease severity. Asymptomatic VMT can be observed, as vitreofoveal separation may occur spontaneously without sequelae. However, persisting VMT may result in foveal damage, thus prompting treatment if symptoms are significant or VA is reduced (Hikichi et al. 1995; Melberg et al. 1995; Sonmez et al. 2008). For many years, PPV was the standard approach for VMT (Steel & Lotery 2013). More recently, pharmacological vitreolysis with ocriplasmin (Jetrea; Thrombogenics, Leuven, Belgium) has emerged as an alternative that may avoid the need for PPV (Gandorfer 2008; De Smet et al. 2009; Benz et al. 2010; Stalmans et al. 2010, 2012; Jetrea Summary of Product Characteristics 2013; NICE technology appraisal guidance 2013; Maier et al. 2015).

Another treatment modality for sVMA is pneumatic displacement with an intravitreal expansile gas bubble, potentially avoiding the need for vitrectomy or enzymatic vitreolysis. The potential advantage of an intravitreal gas injection includes its low cost and ease of adoption. For example, the cost of ocriplasmin and vitrectomy is estimated at $3950 (jetrea.com/JETRAOrderinginfo.pdf) and $3147 in the USA, respectively, and £3000 and £1634, respectively, in the UK

(Gupta et al. 2008; Nicod et al. 2016). The cost of ocriplasmin is magnified by the fact that many cases fail to respond and therefore still need to progress to vitrectomy. Gases such as C_3F_8 and SF_6 cost as little as £1 if taken from large medical gas cylinders, or typically less than £100 from single use canisters licensed for intraocular use. Intravitreal gas is easy to store and administer and does not require the capital costs or surgical expertise needed to undertake PPV. In addition, intravitreal gas injection may potentially be a safer procedure compared to the more invasive PPV.

Given these potential advantages of intravitreal gas, we undertook a review of the safety and efficacy of intravitreal gas for sVMA, to guide clinical care or future studies. Specifically, we aimed to determine the benefit of intravitreal gas in terms of releasing VMT or closing MHs, the effect on VA and the risk in terms of intra- and postoperative complications.

Materials and Methods

Eligibility criteria for considering studies for this review

The population was patients with sVMA, namely VMT with or without MH, to include stages 1, 2 and 3 MH. The intervention was a single intravitreal expansile gas injection. The intended control was natural history. The main efficacy outcomes were VA and anatomic success, defined as an absence of VMT or MH without recourse to PPV. Both outcomes were assessed at 1 month and final follow-up. Safety outcomes included all reported surgical complications or AEs attributed to intravitreal gas. The study protocol was registered with the international prospective register of systematic reviews (2015:CRD42015017338, National Institute of Health Research Centre for Reviews and Dissemination, University of York, UK) and conducted in accordance with Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidance (http://www.prisma-statement.org/, accessed 28 May 2015).

There were no restrictions with regard to gender or ethnicity of patients or language of article. In the anticipated absence of any randomized controlled trials and to maximize safety data, prospective, retrospective, controlled, and uncontrolled studies, including case reports, were eligible. Inclusion criteria were as follows: studies of VMT or stage 1–3 MHs (Gass 1988); at least 28 days follow-up; VA outcomes reported; either MH closure or VMT release rates; reporting results in adults over 18 years of age. We excluded editorials and expert opinions, and articles appearing as abstract only. Eyes with prior treatment of VMA were excluded, including PPV, intravitreal gas and pharmacologic vitreolysis. Eyes being treated for myopic MH retinal detachment were excluded.

Search methods for identifying studies

PubMed MEDLINE, EMBASE and CENTRAL searches were performed including all articles up to and including September 2016 using Boolean operators with the following keywords (and corresponding MESH headings if they were available): SF6, sulfur hexafluoride, sulphur hexafluoride, C2F6, hexafluoroethane, C3F8, octafluoropropane, perfluoropropane, gas, intravitreal, MH, sulphur hexafluoride, vitreomacular adhesion and VMT. An example search is shown in Appendix 1.

Study selection

Abstracts were retrieved from the search, and further articles were identified in the reference lists of the retrieved articles. Two clinicians (JN and TJ) independently assessed articles for provisional eligibility based on their abstract. Full-text copies of all possibly relevant manuscripts were obtained, to determine final eligibility. Any discrepancy in eligibility was resolved by consensus following discussion.

Data collection and risk of bias assessment

Two reviewers (JN and TJ) extracted the relevant information into a database, including: (1) overview of the study (aim and key findings); (2) methodological details (study design, study population, inclusion criteria, exclusion criteria, intervention, comparator if available, study period); (3) VA before and after gas; (4) anatomic success after gas; (5) need for vitrectomy; (6) safety outcomes. To compare across studies, VA was converted to logMAR units (Jackson et al. 2013a).

Data synthesis and analysis

Where necessary, authors were contacted to obtain unpublished raw data. Two-sided, paired *t*-tests were used to compare mean VA before and after interventions. Safety was assessed by AEs and serious adverse events (SAEs) reported. Safety data were pooled across all studies, using individual data where available or study means otherwise. Subgroup analysis was performed for those with diagnoses of MH or VMT.

Results

Of 106 articles, 106 abstracts were assessed as potentially eligible, from which nine articles were deemed eligible after full-text review. A total of 91 eyes from 90 patients with sVMA were included from one nonrandomized controlled study, six uncontrolled studies and two individual case reports (Table 1) (Chan et al. 1995; Costa et al. 2001; Jorge et al. 2006; Mori et al. 2007; Gupta & McHugh 2011; Chen et al. 2012; Rodrigues et al. 2013; Day et al. 2016; Yu et al. 2016). Additional, anonymous participant-level VA data were obtained from one study author as this information was not available in his report, in accordance with PRISMA guidance (Rodrigues et al. 2013). A risk of bias tool was not used as the literature search found no eligible randomized controlled trials.

There were 24 males and 59 females, with a mean age of 67.3 years (range 36–91, $n = 83$). Gender and age data were missing from one study of six eyes, and the gender of a patient was not stated in one case report. There were 44 eyes (44 patients) with a baseline diagnosis of VMT, including 14 with stage 1 MH. Stage 2 MH was present in 45 eyes (45 patients), and stage 3 MH in two eyes (two patients). One patient underwent bilateral treatment for a stage 3 MH in the right eye and a stage 2 MH in the left eye. Perfluoropropane gas was used in 62 eyes, with the volume injected varying from 0.2 ml to 0.5 ml. Sulphur hexafluoride 0.5 ml was used in the other 29 eyes. Postoperative posturing techniques were not consistent between studies, varying from 14 days of face

Table 1. Demographics.

Article (First Author)	Year	Methodology	Number of Eyes	Mean Age	Male (%)	Gas Used	Posturing (days)	Number with VMT	Number with Stage 1 MH	Number with Stage 2 MH	Number with Stage 3 MH
Chan	1995	Prospective Case Series	19	70	32	0.3–0.5 ml C_3F_8	Face down (4)	0	11	6	2
Costa	2001	Case Report	1	65	NS	0.4 ml C_3F_8	Face down (5)	1	0	0	0
Jorge	2006	Prospective Case Series	6	NS	NS	0.4 ml C_3F_8	Face down (14)	0	0	6	0
Mori	2007	Prospective Case Series	20	64	30	0.5 ml SF_6	Face down (3–5)	0	0	20	0
Chen	2011	Prospective Case Series	12	59	17	0.2 ml C_3F_8	Face down (5)	0	0	12	0
Gupta	2011	Case Report	1	55	0	0.3 ml SF_6	Upright daytime	0	1	0	0
Rodrigues	2013	Retrospective Case Series	15	72	53	0.3 ml C_3F_8	None	15	0	0	0
Day	2015	Retrospective Case Series	9	73	11	0.3 ml SF_6	None	7	2	0	0
Yu	2016	Nonrandomized controlled study	8	68	12	0.3 ml C_3F_8	Face down (2)	7	0	1	0
All			91	67.3	28.6			30	14	45	2

C_3F_8 = perfluoropropane, MH = macular hole, NS = not specified, SF_6 = sulphur hexafluoride, VMT = vitreomacular traction.
Demographic information on studies deemed eligible for synthesis of the literature.

down posturing to no posturing. A PPV was performed in 31 of 91 eyes (34%) for varying reasons: persisting MH despite VMT release with gas in 14 eyes (45%), persisting VMT and MH despite gas injection in eleven eyes (36%), retinal detachment in two eyes (7%), new MH following successful VMT release with gas in two eyes (7%), persisting isolated VMT in one eye (3%) and vitreous haemorrhage secondary to proliferative diabetic retinopathy (DR) in one eye (3%).

At 1 month following gas injection, 44 of 91 eyes (48%) had anatomic success, defined as no VMT or MH and without recourse to PPV. At a mean final follow-up period of 14.5 months (range: 1–48 months), anatomic success was achieved in 52 eyes (57%). Twenty-six eyes underwent PPV specifically for failure of gas, 14 for persisting MH, 11 for persisting combined VMT/MH, 1 for persisting isolated VMT and all responded with anatomic success.

The mean preintervention logMAR VA was 0.55 (n = 91; range: 0–2.00; Snellen equivalent 6/21). In the 62 eyes (68%) with VA documented at 1 month, the mean VA improved from 0.57 logMAR by 0.09 units to 0.48 logMAR (range: 0–2.00; 6/18; p = 0.036). No eyes had undergone PPV by month 1. Mean VA at final follow-up was 0.35 logMAR (n = 88; range: −0.09 to 2.00; 6/13), which was significantly better than baseline (p < 0.001) (Table 2). A *post hoc* analysis of the final VA outcome prior to any PPV revealed a VA of 0.42 logMAR (n = 78; 6/16), significantly better than baseline (p = 0.001). Three patients did not have a postgas VA documented.

In the 30 eyes (33%) with a baseline diagnosis of isolated VMT, the mean VA was 0.55 logMAR (range: 0.10–2.00; 6/21) at baseline and remained unchanged at 0.55 (range: 0.00–2.00; 6/21) at month 1 (n = 22; p = 0.226), before subsequently improving to 0.49 (range: 0.00–2.00; 6/19) at a mean follow-up of 7.7 months (n = 28; p = 0.096) (Fig. 1). Anatomic success was achieved in fourteen eyes (47%) at month 1 and eighteen eyes (60%) at final follow-up (Fig. 2). Eight of 30 (27%) eyes with VMT underwent PPV, all after month 1. The indication in one case was vitreous haemorrhage secondary to proliferative DR in which the initial gas injection had resulted in a complete posterior vitreous detachment (PVD) at month 1. In two eyes, PPV was performed for a full-thickness MH following earlier successful VMT release with gas. The other five PPVs were carried out to treat persistent VMT despite intravitreal gas injection.

A stage 1 MH was present at baseline in 14 eyes. In these eyes, VA improved from 0.31 logMAR (range: 0.18–0.48; 6/12) to 0.23 (range: 0.00–1.00; 6/10) at month 1 (p = 0.338), and significantly to 0.18 (range: 0.00–0.30; 6/9) at a mean final follow-up of 12.9 months (p = 0.015) (Fig. 1). Anatomic success occurred in 10 of 14 eyes (71%) at 1 month postgas, and 13 of 14 eyes (93%) at final follow-up (Fig. 2).

The distinction between stage 1 (impending) MH and advanced VMT relies on the investigator's judgement and did not appear to be standardized in the literature. Further, impending MH is often now grouped together with VMT. We therefore undertook a *post hoc* analysis combining VMT and stage 1 MH. In this group, VA improved from 0.45 logMAR (range: 0.00–2.00; 6/17) to 0.43 (range: 0.00–2.00; 6/16) at month 1 (p = 0.382), and then improved significantly, relative to baseline, to 0.39 (range: 0.00–2.00; 6/15) at a mean follow-up of 9.4 months (p = 0.019). Anatomic success occurred in 24 of 37 eyes (65%) at 1 month, and 31 of 37 eyes (84%) at final follow-up.

There were 45 eyes treated with intravitreal gas for a stage 2 MH, with a mean baseline VA of 0.60 (range: 0.00–1.52; 6/24). In the 24 eyes with

Table 2. Overall VA and anatomic success.

Article (First Author)	Number of eyes	Mean follow up period (months)	Mean initial VA (logMAR)	Mean month 1 VA (logMAR)	Mean final VA (logMAR)	Anatomic success at month 1 (*n*, (%))	Anatomic success at final review (*n*, (%))
Chan	19	15.6	0.41	0.32	0.30	9 (47.4)	13 (68.4)
Costa	1	10	0.60	NS	0.10	0 (0)	1 (100)
Jorge	6	40.7	0.68	0.22	0.22	5 (83.3)	5 (83.3)
Mori	20	19.5	0.38	NS	0.19	10 (50)	10 (50)
Chen	12	8.2	0.94	0.82	0.46	3 (25)	3 (25)
Gupta	1	1	1.00	0.3	0.3	1 (100)	1 (100)
Rodrigues	15	11.5	0.52	0.64	0.49	6 (40)	9 (60)
Day	9	1	0.39	0.30	0.30	5 (55.5)	5 (55.5)
Yu	8	6	0.82	NS	0.72	5 (62.5)	5 (62.5)
All	91	14.5	0.55	0.48	0.35	44 (48.4)	52 (57.1)

logMAR = logarithm of the minimum angle of resolution, NS = not specified, VA = visual acuity.
Data displaying anatomic success and visual acuity change following intravitreal gas injection.

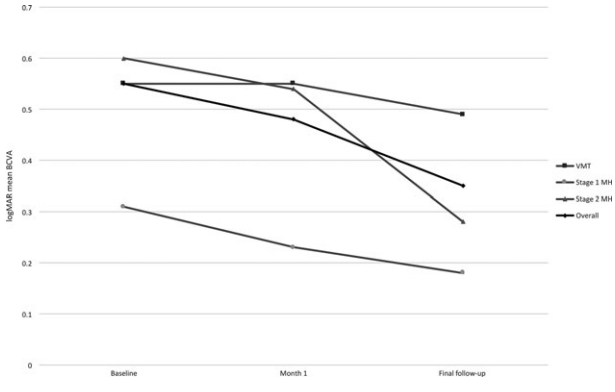

Fig. 1. Visual acuity. The graph shows the mean logMAR VA at baseline, 1 month after intravitreal gas injection, and at final follow-up prior to vitrectomy (if carried out). BCVA = best corrected visual acuity, logMAR = logarithm of the minimum angle of resolution, MH = macular hole, VA = visual acuity, VMT = vitreomacular traction.

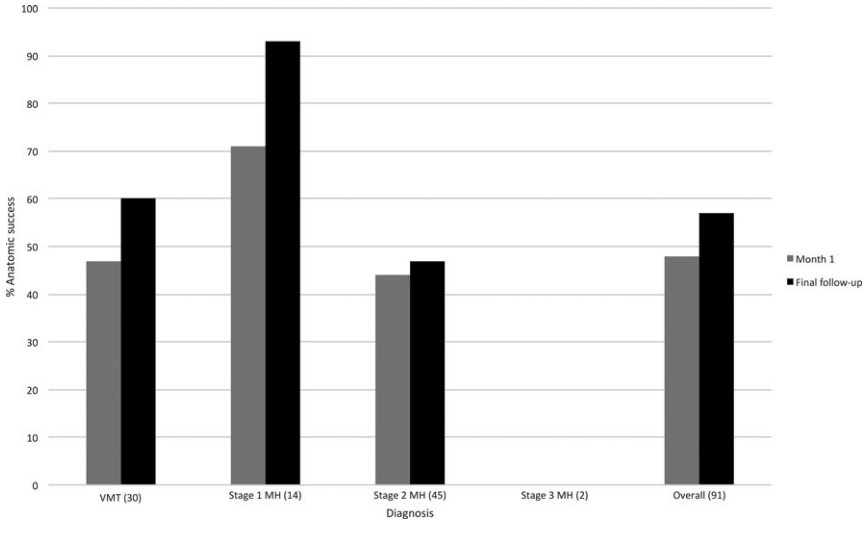

Fig. 2. Anatomic success. The chart shows anatomic success, over time, of intravitreal gas injection for each subset of symptomatic vitreomacular adhesion. Anatomic success was defined as an absence of VMT and MH, without recourse to vitrectomy. MH = macular hole, VMT = vitreomacular traction.

month 1 VA data, the mean logMAR improved to 0.54 (range: 0.10–2.00; $n = 24$; 6/21). At final follow-up (mean = 17.9 months), mean VA significantly improved to 0.28 logMAR (range: −0.09 to 1.00; 6/11) compared to baseline ($p < 0.001$) (Fig. 1). Anatomic success occurred in 20 of 45 eyes (44%) at month 1, and 21 of 45 eyes (47%) at final follow-up (Fig. 2). A PPV was undertaken in 22 eyes. In 20, the indication was failure of MH closure with gas (although 17/20 had resulted in PVD), and all PPVs were successful in closing the MH. The other two PPVs were performed successfully to treat retinal detachment.

Two intravitreal gas procedures were performed for stage 3 MH, but neither was successful anatomically either at month 1 or by a final mean follow-up of 33 months.

The diameter of MH was only documented in one study of 20 stage 2 MH (Mori et al. 2007). Successful release of vitreous traction and closure of MH at both month 1 and at an average final follow-up of 20 months in patients with a MH diameter <250 μm was 78% (7/9). Those with larger holes (>250 μm) had successful anatomical resolution in 27% of cases (3/11) at 1 month. All those with failed anatomical resolution at one month underwent PPV which resulted in successful MH closure.

Adverse events (AEs) included two retinal detachments. Both occurred in myopic eyes (−5.75D and −8.50D) with stage 2 MH. In two patients with VMT at baseline, intravitreal gas resulted in PVD at 1 month and development of a full-thickness MH which was successfully closed with PPV. One eye with an impending MH developed a full thickness MH 10 months after failed gas injection and was successfully closed with PPV. Two eyes with stage 1 MH were diagnosed with macular

pseudohole at month 13. There was one patient who was diagnosed with a retinal tear at 1 month following gas and underwent successful laser retinopexy. No other AEs were reported.

Discussion

We undertook a review to evaluate the safety and efficacy of intravitreal gas as a treatment for sVMA. We found a lack of high quality evidence. A series of uncontrolled, before/after studies found that 57% of eyes had anatomic success following intravitreal gas, defined as an absence of VMT and MH, without recourse to PPV. There was also a VA gain of 0.13 logMAR units (approximately 1 Snellen line), without the need for PPV. This modest gain in VA may not fully capture the potential symptomatic benefit achieved in this patient group, given that metamorphopsia may be at least as important as VA. The good presenting VA may also impose a ceiling on any VA improvement that can be detected following gas injection. Studies of ocriplasmin and PPV for symptomatic VMA also show modest VA gains, although the visual improvements are often better in the MH subset, compared to those with isolated VMT (Stalmans et al. 2012; Jackson et al. 2013b). We also found better VA gains in those with a baseline diagnosis of MH compared to isolated VMT when treated with gas.

Our literature search found one study of 20 eyes of 17 patients with VMT that underwent an 0.2 ml intravitreal injection of either SF_6 or $C2F_6$ (Claus et al. 2016). This was a retrospective case series which reported an 85% (17/20) overall release of VMT, favourable VA outcomes and no major safety concerns. However, we excluded this study from our analysis because there was insufficient information regarding when VMT release occurred and when postoperative visual acuities were measured (Claus et al. 2016).

The management of symptomatic VMA does not currently have a gold standard, with options including observation, intravitreal gas, ocriplasmin and PPV. Observation of VMT may lead to spontaneous separation in 17–34% of eyes, but conversely some may progress to MH, and prolonged disease may result in loss of vision (Almeida et al. 2015; Zhang et al. 2015).

A combined analysis of two randomized controlled trials of ocriplasmin reported that 26.5% of eyes responded within 1 month, with no further response after this time-point. Despite using a somewhat stricter definition of success (absence of both VMT and MH, not just an absence of VMT), the rate of release in our review of intravitreal gas appears higher, at 48.4% by month 1 (and 57.1% at final review). However, without direct comparison, this conclusion needs to be interpreted with considerable caution, as the difference could reflect patient selection, chance, publication bias and differences in optical coherence tomography (OCT) interpretation, amongst other reasons.

In terms of safety, there were three cases of impending MH that progressed to full-thickness MH. In two cases, the gas injection resulted in PVD and full-thickness MH at one month, but the other occurred 10 months after gas injection so causation is unclear. A retinal tear occurred in one case, at month 1 following gas injection, which was successfully treated with laser retinopexy. Most of the studies did not comment whether the patients were phakic or pseudophakic at baseline. Excluding cases undergoing PPV, two eyes were noted to have progression of nuclear sclerosis but neither required cataract surgery. The most clinically important AEs were two cases of retinal detachment in myopic patients (2%). This suggests that myopic eyes may be best excluded from future studies of intravitreal gas for symptomatic VMA. By extension it may also be reasonable to exclude other risk factors for retinal detachment, such as lattice degeneration or treated retinal breaks, although the risk in these patients in assumed rather than proven. The small number of eyes treated means it is not possible to quantify the overall clinical impact of retinal detachment; however, any such risks needs to be balanced against the risk of PPV or ocriplasmin. A recent literature review of PPV undertaken for VMT found a retinal detachment rate of 4.6% (Jackson et al. 2013b). The retinal detachment rate in the pivotal studies of ocriplasmin was 0.4%, versus 1.6% in the placebo group ($p = 0.16$), although several cases of retinal detachment following ocriplasmin have now been published and the true rate of RRD after ocriplasmin with longer follow-up may be higher than in the phase 3 trials (Haller et al. 2015; Madi et al. 2016).

The majority of AEs associated with ocriplasmin have been considered mild, nonserious and transient such as vitreous floaters, eye pain, photopsia and reduced VA (Kaiser et al. 2015). However, concerns remain about dyschromatopsia, ERG changes and severe loss of vision, and there have been isolated case reports of ellipsoid zone changes on OCT and RPE-photoreceptor adhesion release potentially due to the enzymatic activity of the drug (Hager et al. 2015; Johnson et al. 2015; Quezada Ruiz et al. 2015; Abraham et al. 2016; Neffendorf et al. 2016).

Only one study reported MH diameter and found a higher success rate of stage 2 MH closure in small diameter holes (<250 μm) as opposed to those larger than 250 μm (78% versus 27%). This greater efficacy with smaller diameter is consistent with a subgroup analysis of the data from the pivotal ocriplasmin trial (Haller et al. 2015; Jackson et al. 2016). The influence of ERM on anatomic success is hard to determine as most studies excluded ERM, with only four cases included across all studies (Chan et al. 1995; Day et al. 2016). Rodrigues et al. (2013) reported that high reflectivity of the inner retinal surface, a possible precursor of ERM, was associated with a lower rate of VMT release, which is also consistent with the subgroup analysis of the pivotal ocriplasmin trial (Haller et al. 2015; Jackson et al. 2016). It has been shown that phakic patients have a higher likelihood of successful sVMA release following ocriplasmin injection than pseudophakic patients (Haller et al. 2015; Jackson et al. 2016; Feng et al. 2017). In our analysis, only two of nine articles documented whether patients were phakic or pseudophakic at baseline and therefore due to missing data, we did not perform a subgroup analysis to further investigate whether this trend is also seen with intravitreal gas.

A strength of our study is that we have pooled data in a standardized method with predefined outcome measures. However, there are several important weakness. Most

importantly, the number of patients is low, and only one of the studies had a control group (not randomized). Accordingly, many studies may be subject to bias. Furthermore, diagnostic criteria varied across studies, as did the type and volume of gas injected and the posturing regimen. Our findings may underestimate VMT release in nondiabetic patients as our group contained 8% (7/91) diabetics, who might be expected to have firmer VMA. In addition, some studies did not report the duration of disease prior to treatment, and others had significant variability in duration (1–7 months). One study was conducted in the pre-OCT era; however, it provided relatively rigorous assessment of VMA including B-scan ultrasonography (Chan et al. 1995). It is also not clear which gas offers the best efficacy.

In conclusion, our synthesis of the literature suggests that there is insufficient evidence to conclude on the safety and efficacy of an intravitreal expansile gas injection for the treatment of sVMA. The limited results available do, however, appear to justify further research, most helpfully as a comparative study versus other management options such as observation, ocriplasmin or vitrectomy. Diagnostic inclusion criteria could be defined using recognized photographic standards or agreed classification systems (Duker et al. 2013; Steel et al. 2016), and outcome measures could be expanded to include cataract progression, validated quality of life questionnaires and assessment of metamorphopsia (Tanner & Williamson 2000; Khadka et al. 2013; Nomoto et al. 2013; Ugarte et al. 2013). An economic evaluation comparing different treatments of symptomatic VMA also appears warranted, given the potential cost advantage of intravitreal gas.

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Appendix 1: Search strategy on MEDLINE

(1) vitreomacular traction
(2) vitreomacular adhesion
(3) macular hole
(4) or/1–3
(5) perfluoropropane
(6) C3F8
(7) Octafluoropropane
(8) Sulphur hexafluoride
(9) Sulfur hexafluoride
(10) SF6
(11) Hexafluoroethane
(12) C2F6
(13) Gas
(14) or/5–13
(15) Intravitreal
(16) 4 and 14 and 15

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