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# CENTRAL RETINAL VEIN OCCLUSION - TREATMENT WITH BEVACIZUMAB AND ITS SEASONAL CHARACTERISTICS

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Cover: Left: Extreme ultraviolet image of the sun, Right: Fundus photograph of CRVO
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# ABSTRACT

Central retinal vein occlusion (CRVO) is a retinal vascular disorder that may cause severe visual impairment. When CRVO occurs the venous blood flow is impeded with elevated intraluminal pressure upstream to the occlusion. The increased hydrostatic pressure causes extravasation of fluid to the extracellular space that compromises arterial perfusion inducing retinal hypoxia. This will release vascular endothelial growth factor (VEGF) and inflammatory mediators that in turn induce macular edema (ME) and ocular neovascularization. A seasonal variation in cardiovascular disease onset has been shown previously with a higher incidence during the winter season than in the summer. Serum vitamin D levels are known to have a significant seasonal variation with a peak occurring in the summer. A few studies have suggested a seasonal onset of CRVO with more cases presenting during winter, but no study has investigated vitamin D levels in these patients.

In **paper I** we studied prospectively if repeated intravitreal bevacizumab (IVB) injections every 6 weeks for 6 months could improve visual acuity as compared to sham-treated control patients with ME secondary to CRVO. After 24 weeks 18/30 (60.0%) patients in the study group had gained ≥15 letters compared with 6/30 (20.0%) patients in the control group ($p=0.003$). At the end of follow up patients in the study group improved 14.1 letters compared to a loss of 2.0 letters in the control group ($p<0.01$). We showed for the first time in a randomized prospective study the superiority of IVB treatment compared to sham injections in CRVO.

In **paper II** both groups received treatment with 4 IVB injections every 6 weeks for 6 months. The objectives were to investigate if this regime could maintain visual acuity and to elucidate the impact of delaying treatment for 6 months in patients with ME secondary to CRVO. In the original IVB group a further gain of 2.0 letters was seen with the fixed injection schedule during week 24 to 48. The original sham group, improved to a vision gain of 4.6 letters at week 48 ($p<0.05$). Disease duration seemed to have a critical impact on visual outcome. Patients initially on a sham-regime in paper I improved with IVB treatment after 24 weeks but significantly less than the original IVB group.

In **paper III** we investigated retrospectively the seasonality in CRVO onset and the yearly incidence of CRVO in Stockholm, Sweden. Every year during the 6 year study the highest CRVO onset occurred during the winter-spring period and was significantly higher than during the other seasons reaching its lowest levels in autumn. We found that the CRVO incidence was 2/10000 for persons aged over 40 years. The annual incidence increased from 0.25/10000 at 45 years of age up to 7/10000 above 80 years of age.

In **paper IV** we tested prospectively the hypothesis that patients with CRVO had an increased risk of vitamin D deficiency compared to matched controls. We found that more than 50% of the patients with CRVO had deficient vitamin D levels. In the complete data set no significant differences in vitamin D levels were found between the study groups. Patients less than 75 years old had significantly lower vitamin D levels compared to controls.

# LIST OF SCIENTIFIC PAPERS

I. Epstein DL, Algvere PV, von Wendt G, Seregard S, Kvanta A.
Bevacizumab for macular edema in central retinal vein occlusion: a prospective, randomized, double-masked clinical study.
Ophthalmology. 2012 Jun;119(6):1184-9.

II. Epstein DL, Algvere PV, von Wendt G, Seregard S, Kvanta A.
Benefit from bevacizumab for macular edema in central retinal vein occlusion: twelve-month results of a prospective, randomized study.
Ophthalmology. 2012 Dec;119(12):2587-91.

III. Epstein DL, Kvanta A, Lindqvist P.
Seasonality and incidence of central retinal vein occlusion in Sweden – A 6 year study.
Ophthalmic Epidemiol. 2015 Apr;22(2):94-97.

IV. Epstein DL, Kvanta A, Lindqvist P.
Vitamin D deficiency in patients with central retinal vein occlusion – A prospective case control study.
Submitted.

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| ACE | Angiotensin converting enzyme |
| ARB | Angiotensin II receptor blockers |
| BCVA | Best corrected visual acuity |
| BRVO | Branch retinal vein occlusion |
| CI | Confidence interval |
| CRT | Central retinal thickness |
| CRV | Central retinal vein |
| CRVO | Central retinal vein occlusion |
| CVD | Cardiovascular disease |
| ETDRS | Early treatment diabetic retinopathy study |
| FA | Fluorescein angiography |
| FDA | United States Food and Drug Administration |
| FMD | Flow mediated dilatation |
| HCRVO | Hemicentral retinal vein occlusion |
| HIF | Hypoxia-inducible factor |
| ICD-10 | International statistical classification of disease tenth revision |
| IOP | Intraocular pressure |
| IU | International units |
| IVB | Intravitreal bevacizumab |
| ME | Macular edema |
| NO | Nitric oxide |
| NVA | Neovascularization of the angle |
| NVD | Neovascularization of the disc |
| NVE | Neovascularization elsewhere |
| NVI | Neovascularization of the iris |
| OCT | Optical coherence tomography |
| OR | Odds ratio |
| PlGF | Placental growth factor |
| PM | Particulate matter |
| PRN | Pro renata |

| | |
|---|---|
| PRP | Panretinal photocoagulation |
| PVD | Posterior vitreous detachment |
| RAPD | Relative afferent pupillary defect |
| RAS | Renin-angiotensin system |
| RVO | Retinal vein occlusion |
| TNF-α | Tumor necrosis factor α |
| UVB | Ultraviolet B |
| VDR | Vitamin D receptor |
| VE-cadherin | Vascular endothelial cadherin |
| VEGF | Vascular endothelial growth factor |
| VEGFR | Vascular endothelial growth factor receptor |
| VH | Vitreous hemorrhage |
| VSMC | Vascular smooth muscle cells |
| VTE | Venous thromboembolism |
| 25(OH) D | 25-hydroxyvitamin D3 |

# 1 CRVO - INTRODUCTION

Retinal vein occlusion (RVO) is a common retinal vascular disorder that may cause severe visual impairment. RVO is classified according to the location of the occlusion. In branch retinal vein occlusion (BRVO) a branch of the retinal venous tree is occluded. In central retinal vein occlusion (CRVO) there is a central retinal vein obstruction in the optic nerve. A hemicentral retinal vein occlusion (HCRVO) occurs when of 1 of the 2 trunks of the central retinal vein in the optic nerve is occluded (see anatomy below). A HCRVO could be considered as a variant of CRVO. There are other anatomic subtypes of RVO. A hemispheric retinal vein occlusion is a BRVO with an occlusion at the arteriovenous crossing on or near the optic disk that affects the upper or the lower part of the retina (Hayreh 2012). This occurs when the venous return from approximately one-half of the retina is affected while small occlusions affecting only the posterior pole are described as macular retinal vein occlusion.

## 1.1. ANATOMY

In the embryogenesis of the eye, two trunks of the central retinal vein (CRV) lie on either side of the central retinal artery (Mann, 1969). After birth usually one of the trunks disappears leaving a single central trunk (figure 1). In approximately 20% of eyes both trunks persists (Chodpar 1986) (figure 2). Occlusion of one of those two trunks results in the development of HCRVO. The CRV which receives blood from the retina runs through the optic nerve and after a short distance it drains blood usually into the superior ophthalmic vein and further into the cavernous sinus (Cheung 2003). During its course in the optic nerve numerous collaterals exist with the choroidal circulation (figure 1). A considerable variability has been described in the venous orbital drainage with many collaterals and anatomic variants. The CRV may sometimes join the inferior ophthalmic vein, or even the cavernous sinus directly (Hayreh 2006). Hence differences in the venous orbital anatomy can cause various clinical pictures.

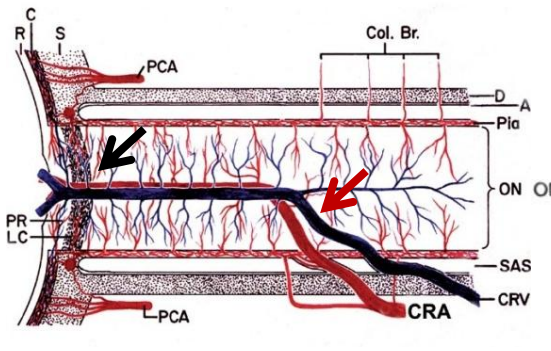


**Figure 1.** Normal anatomy

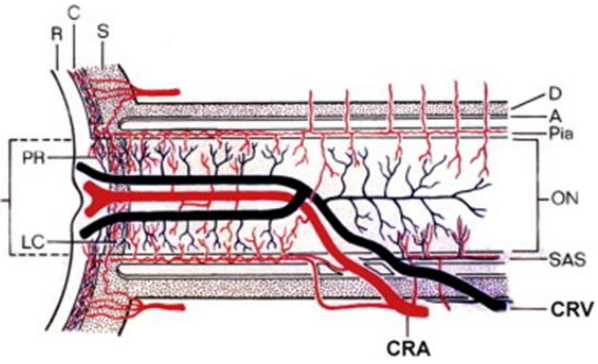


**Figure 2.** Two central retinal vein trunks

CRA = central retinal artery, CRV = central retinal vein, LC = lamina cribrosa, ON = optic nerve; R = retina, S = sclera, Col. Br = collateral branches (Hayreh 2014)

## 1.2. PATHOGENESIS

Degenerative changes of the vessel wall, venous stasis, and blood hypercoagulability may increase the risk of thrombosis (Virchow´s triad). The pathogenesis of CRVO is believed to be associated with arterial disease. The central retinal vein and artery share a common adventitial sheath. Arterial wall changes due to hypertension or atherosclerosis transform the artery into a rigid structure that impinges upon the pliable CRV. The flow through the narrow segment of vein within the lamina cribrosa (figure 1, black arrow) becomes turbulent which may damage the endothelium. This can contribute to stasis, thrombosis, and occlusion (Green 1981). The concept that the occlusion is located at the lamina cribrosa is mainly based on the landmark histopathological studies by Green in eyes with severe ischemic CRVO. It has been argued that in some cases the occlusion may be farther back (figure 1, red arrow) and not at the lamina cribrosa. This could contribute to a milder retinopathy because of the abundant collateral venous outflow in the optic nerve (Hayreh 1994).

When CRVO occurs the venous blood flow is impeded causing an elevated intraluminal pressure upstream to the occlusion. The increased hydrostatic pressure causes extravasation of fluid to the extracellular space, the development of retinal hemorrhage and may compromise arterial perfusion resulting in variable amounts of capillary occlusion. The hypoxia stimulates the release of cytokines, vascular endothelial growth factor (VEGF) and other inflammatory mediators. This may disrupt the blood-retina barrier causing further extravasation of fluid and hemorrhages (Kaur 2008). The effect of VEGF in the pathogenesis of CRVO will be discussed in detail later.

## 1.3. ETIOLOGY

A basic risk factor for CRVO is advancing age. Systemic hypertension and hyperlipidemia are the strongest risk factors associated with the disease (O'Mahoney 2008). There is also a higher risk of CRVO in patients with ocular hypertension and glaucoma (The Eye Disease Case-Control Study Group 1996). It has been argued that venous stasis induced by the raised intraocular pressure may contribute to the occlusion (Hayreh 2004). A low ocular perfusion pressure (calculated as two-thirds of mean arterial pressure minus intra ocular pressure) has also been associated with an increased risk of CRVO. Other significant risk factors are cardiovascular disease and diabetes (Kolar 2014). In young patients coagulopathy and thrombophilia should be considered in the etiology (Yau 2008). Knowledge on thrombophilic abnormalities in CRVO is often based on small retrospective studies and case reports with conflicting results. Increased risk of CRVO has been associated with deficiency of protein C (Tekeli 1999), protein S (Yap 2007) and antithrombin III (Ririe 1979). Furthermore other studies have reported that factor V Leiden mutation (Rehak 2008) , antiphospholipid (Agarwal 2009) and anticardiolipin syndrome (Glueck 2012) hyperhomocysteinaemia (Cahill 2003) and elevated levels of plasminogen activator inhibitor-1 (Marcucci 2001) may play a role in the etiology of CRVO.

Several other studies have failed to prove this relationship. In a case-controlled study no association was found between antithrombin, Protein C, Protein S and homocysteine levels, lupus anticoagulant and anticardiolipin antibodies and risk of CRVO (Di Capua 2010).

## 1.4. EPIDEMIOLOGY

Studies have found prevalence rates of CRVO ranging from 0.08-0.2% (Mitchell 1996, Klein 2000, Yasuda 2010). The Beaver Dam Eye Study reported a 15-year cumulative CRVO incidence of 0.5%. Arakawa reported a 9-year cumulative CRVO incidence of 0.3% (Arakawa 2011).

## 1.5. CLINICAL PICTURE

Patients usually present with a sudden unilateral blurred vision. The retinal veins are tortuous and dilated. Dot/blot and flame-shaped hemorrhages, throughout all four quadrants out in the periphery are seen. Optic disc and macular edema (ME) are usually present. Soft exudates as a sign of retinal ischemia may be present (figure 3). Classically CRVO is divided into

ischemic and non-ischemic types (Hayreh 1983). The disease is classified as non-ischemic when less than 10 disc areas of non-perfusion are seen as assessed by fluorescein angiography (FA) and ischemic with more than 10 disc areas of non-perfusion (The Central Vein Occlusion Study Group 1995). With standard FA mainly the posterior portion of the eye is visualized and large portions of ischemia in the peripheral retina may be missed. Recent technological advances have made high-resolution peripheral angiographic visualization possible. Ultra wide field FA with the Optos C200 MA scanning laser ophthalmoscope (Optos PLC, Dunfermline, United Kingdom), provides visualization up to 200° peripherally (Friberg 2008). This can contribute to a more adequate evaluation of the degree of ischemia. A recent report investigated the peripheral ischemia in patients with non-ischemic CRVO (classified with standard FA visualization). When evaluated with Optos- FA all eyes had significant peripheral ischemia averaging 92 disc areas of non-perfusion (Spaide 2011). Hence the assumption that findings in the posterior portion of the eye are representative of the whole eye can be erroneous and the concept of 10 disc areas of non-perfusion is probably insufficient. Patient classified as non-ischemic with standard FA may have extensive peripheral ischemia. Extensive hemorrhages at presentation may make angiographic evaluation impossible. Functional tests such as visual acuity, relative afferent pupillary defect (RAPD), visual fields and electroretinography may help in differentiating the types of CRVO (Hayreh 1990). Non-ischemic CRVO is the most common form accounting for approximately 80% of cases (Hayreh 1994). Patients usually present with mild to moderate visual loss, commonly 20/200 or better, and an absent RAPD. Non-ischemic CRVO may in a few cases resolve fully with good visual outcome. However frequently it results in significant, permanent visual loss and may progress to the ischemic type (Chen 1995). Eyes with ischemic CRVO present with severe visual loss, extensive retinal hemorrhages, soft exudates and presence of a RAPD (figure 3). Commonly there is extensive macular ischemia contributing to a poor visual prognosis.

### 1.5.1 Natural course

Resolution of the retinopathy often takes a long time and is in most cases associated with substantial vison loss. One year after disease onset 30 % of non-ischemic CRVO cases have resolved while only 6 % of the ischemic cases show no retinopathy (Hayreh 2015). The mechanism by which CRVO resolves is complex and not completely understood. Recanalization of the thrombus occurs commonly during the first year of disease but involves in most cases only a minor part of the lumen (Green 1981). Compensatory collaterals that bypass the occlusion may contribute to the resolution of the disease. Collateral vessels shunt

venous blood from the retinal circulation to the choroidal vasculature thus improving the venous retinal blood flow. The site of occlusion determines how efficient the collateral outflow can be. Occlusions located downstream to the lamina cribrosa have an abundancy of vessels that can develop into prominent collaterals while occlusions at the lamina cribrosa only have a few vessels from the prelaminal region that can develop into retinociliary shunts. These retinociliary collaterals are seen at the optic disc on clinical examination, while deeper collaterals in the optic nerve cannot be observed. The appearance of retinociliary vessels is not necessarily an indicator of a good prognosis since occlusions closer to the lamina cribrosa are associated with a more severe retinopathy. It has been shown that eyes with non-ischemic CRVO that developed retinociliary shunt vessels had a slower resolution of macular edema and a worse visual outcome (Hayreh 2011).

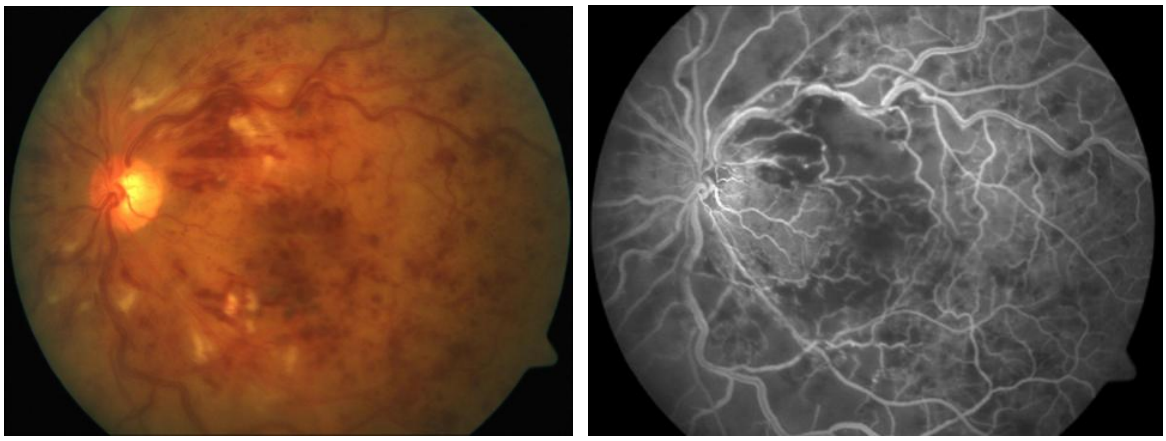

**Figure 3**. Left: Fundus photograph of CRVO showing torturous veins with extensive hemorrhages and soft exudates. Right: Fundus angiogram with severe retinal ischemia.

### 1.6. VEGF IN CRVO

In humans the different VEGF molecules include, VEGF-A, VEGF-B, VEGF-C, VEGF-D and placenta growth factor (PlGF) (Holmes 2007). The retinal hypoxia in CRVO causes the release of VEGF-A and inflammatory mediators. This can induce ME and ocular neovascularization.

ME is the most common cause of visual impairment in CRVO (McIntosh 2010). When the retina becomes hypoxic the cells produce hypoxia-inducible factor (HIF). HIF stimulates the production of VEGF-A that in turn binds to VEGF receptors (VEGFR-1 and -2) on the endothelial cells. VEGFR-2 regulates vascular endothelial function and mediates most of the cellular response to VEGF-A (Holmes 2007). VEGFR-2 is a tyrosine kinase receptor that is

activated by transphosphorylation. This activates a cellular signaling pathway that ultimately causes the phosphorylation of the intracellular tail of vascular endothelial (VE)-cadherin (Gavard 2006). VE-cadherin plays an important role in cell to cell adhesion at the adherens junctions and protects the integrity of the endothelial barrier. The phosphorylation causes endocytosis and internalization of VE-cadherin, thereby disrupting the endothelial barrier and promoting macular edema by allowing passage of macromolecules and fluid. Contraction of the endothelial actin/myosin cytoskeleton also plays a part in the pathogenesis of ME. Myosin phosphorylation creates a contractile force that pulls VE-cadherin inward, forcing dissociation of the adherens junction thereby producing interendothelial gaps (Vandenbroucke 2008) (figure 4). The pathways by which VEGF-A can cause ME are not fully understood and there may be other important mechanisms involved.

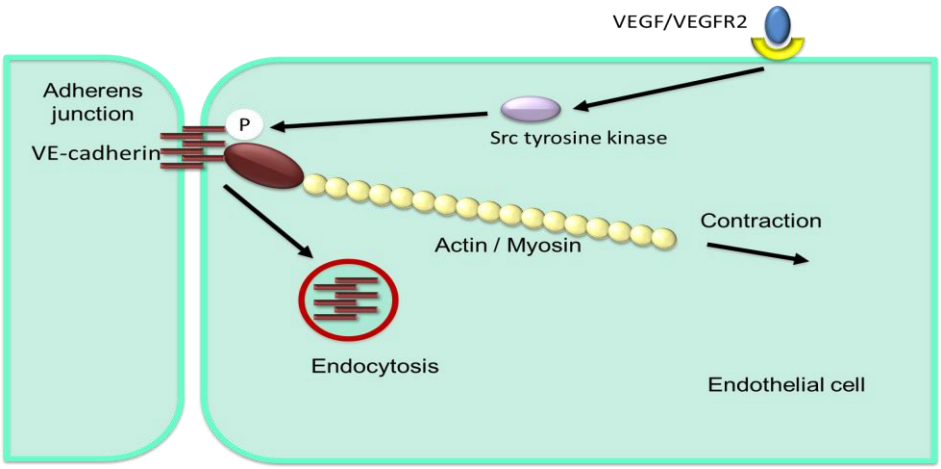


**Figure 4.** VEGF-induced disruption of cell−cell adhesion. VEGF-A binding to VEGFR-2 activates an intercellular signaling pathway causing the phosphorylation of VE-cadherin and the actin/myosin complex promoting endocytosis and internalization of VE-cadherin. (Adapted by D Epstein from figure courtesy A Kvanta)

Ocular neovascularization is usually only seen in ischemic CRVO and is most common in the anterior part of the eye causing iris rubeosis and neovascular glaucoma. Anterior segment neovascularization typically occurs within 1-4 months and virtually all cases occur within 1 year (Hayreh 1983). Neovascularizton of the iris (NVI) and the angle (NVA) can cause the development of fibrovascular tissue and subsequently anterior synechiae obstructing aqueous outflow through the trabecular meshwork resulting in neovascular glaucoma. Early treatment of the ischemic retina with panretinal photocoagulation (PRP) is important in order to reduce the VEGF- induced angiogenesis and possibly avoid the anterior synechiae formation

(Browning 2012). Adjuvant anti-VEGF therapy could be a useful tool to efficiently reduce the VEGF load before the effect of the PRP treatment is seen (Wakabayashi 2008, Lüke 2013). Neovascularization of the posterior segment is uncommon and typically occurs later than development of iris rubeosis with an average time until onset up to 12 months (Murdoch 1991). Histopathological evaluation of eyes with CRVO has shown an absence of retinal capillary endothelial cells possibly complicating the process of retinal angiogenesis (Little 1979). Neovascularization of the optic disc (NVD) is seen more often than neovascularization of the retina (NVE). Vitreous hemorrhage (VH) may be caused by NVD and NVE but is more commonly due to a break-through bleeding from the hemorrhagic retina especially when occurring in the early stages of the disease when retinal neovascularization is uncommon (Hayreh 2015). Thus, the occurrence of VH does not necessarily indicate retinal neovascularization. Several studies have shown that patients with a posterior vitreous detachment (PVD) of the vitreous have a much lower risk of retinal neovascularization (Akiba 1991, Hikichi 1995). It has been argued that the vitreous serves as a scaffold necessary for the development of the retinal neovascularization (Browning 2012). It has been suggested that a PVD may share physiological features with a vitrectomized eye (Stefánsson 2009). Several studies have shown that the retinal oxygenation improves after vitrectomy (Stefánsson 1990, Williamson 2009) and this process may also occur after a PVD. As a result of the improved retinal oxygenation the VEGF production is reduced. Additionally VEGF may be cleared better into the vitreous cavity after vitrectomy or PVD reducing the preretinal VEGF concentration and risk for ischemic complications (Stefánsson 2009).

## 1.7. TREATMENT OF MACULAR EDEMA

The last decade has witnessed a revolution in the treatment of ME. Potent anti-VEGF drugs and corticosteroids have dramatically improved the visual outcomes.

Anti-VEGF treatment: Intravitreal treatment with ranibizumab, aflibercept and bevacizumab have all shown superiority to the natural history of the disease. Intravitreal injections can be given monthly due to the relative long intravitreal half-life (t½) of anti-VEGF drugs. Bevacizumab, ranibizumab and aflibercept have a t½ of between 7-10 days (Meyer 2011, Krohne 2012 and Stewart 2014).

Ranibizumab: Ranibizumab (Lucentis®, Genetech Inc.) is a monoclonal antibody Fab fragment (48kDa) that by binding to VEGF-A prevents the interaction of VEGF-A with its receptors on the surface of endothelial cells. It is FDA approved for treating age- related macular degeneration, pathologic myopia, diabetic ME and ME secondary to RVO. Best

corrected visual acuity (BCVA) is measured with an ETDRS (Early treatment diabetic retinopathy study) chart where every line represents 5 letters. The CRUISE study evaluated monthly treatment with ranibizumab versus sham injections in patients with CRVO. After 6 months patients the ranibizumab group had gained a mean of 14.9 letters, compared with 0.8 letters in the sham group (Brown 2010). The visual gain was maintained after 12 months when treated on a pro renata regimen (PRN) improving 13.9 letters compared to baseline. The sham group received active treatment after 6 months improving 7.3 letters (Campochiaro 2011). Concomitant with the rapid improvement in visual acuity there was a fast reduction of the ME. The central retinal thickness (CRT) decreased 452 µm compared to 168µm in the sham arm.

Aflibercept: Aflibercept (Eylea®, Bayer HealthCare Pharmaceuticals) is a recombinant fusion protein (97kDa) consisting of VEGF-binding portions from the extracellular domains of human VEGFR-1 and -2, fused to the Fc portion of the human IgG1 immunoglobulin. Aflibercept binds to circulating VEGFs thereby inhibiting the activity of the VEGF subtypes VEGF-A and VEGF-B, as well as to PlGF. It is FDA approved for treating age-related macular degeneration, diabetic ME and ME secondary to RVO. The COPERNICUS study investigated the treatment of monthly aflibercept injections compared to sham in CRVO. After 6 months patients the aflibercept group had gained a mean of 17.3 letters, compared with a loss of 4.0 letters in the sham group. The CRT decreased 457 µm compared to 145µm in the sham arm. No patients in the aflibercept arm developed neovascularization versus 7% in the sham group (Boyer 2012). The visual gain was maintained with PRN treatment at 12 months. The aflibercept group improved 16.2 letters compared to a 3.8 letter gain in the previous sham group (Brown 2013).

Bevacizumab: Bevacizumab (Avastin®, Genetech Inc.) is a recombinant humanized monoclonal antibody (149kDa) that inhibits VEGF-A. It has a similar mode of action as ranibizumab (see above). It was originally developed as a drug for various metastatic cancers. It has also gained extensive off-label use in treating various eye diseases especially age related macular degeneration and vascular retinopathies. When initiating this thesis in 2010 no prospective randomized sham-controlled studies had been conducted in treating CRVO patients with bevacizumab. However several case series had shown good effect on visual acuity improvement and reduction of ME (Prager 2009, Figueroa 2010).

Corticosteroid treatment: Ozurdex® is a slow release dexamethasone implant that is given as an intravitreal injection. The implant achieves higher and more stable levels of dexamethasone than other routes of corticosteroid administration and has shown a peak intravitreal concentration at 60 days after administration. The intravitreal levels of the drug

then start to decline and at 90 days low concentrations are found (Chang-Lin 2011). The GENEVA study evaluated the treatment with Ozurdex® compared to sham injections in patients with ME due to RVO. After 60 days the study group improved 9.7 letters compared to a visual loss of 1.3 letters in the control group. After 90 and 180 days the study group improved 5.0 and 0.8 letters respectively compared to baseline (Haller 2010). After 6 months all patients received open label Ozurdex® treatment. After 60 days the study group improved 8.0 letters compared to 4.0 letters in the control group. At 12 months 30% of the retreated phakic patients developed cataract. A third of the retreated patients had at least a 10-mmHg increase in intraocular pressure (IOP) from baseline at some point in the 12-month study but in virtually all cases the IOP was normal 180 days after the last treatment (Haller 2011). The results from the CRUISE, COPERNICUS and GENEVA studies suggest that early treatment is important in order to optimize the visual outcome in patients with CRVO.

# 2 SEASONALITY IN CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD) is the leading cause of death worldwide. In 2008 30% of all global deaths were caused by CVDs (Mendis 2011). A seasonal variation in CVD onset has been shown in several studies with a higher incidence during the winter season than in the summer. This relationship applies to both arterial disease and venous thromboembolism (VTE). Seasonal variations in the arterial blood pressure have been seen in many studies. Both systolic and diastolic blood pressures show a peak during the winter and a nadir in the summer (Fujiwara 1995, Minami 1996, Sinha 2010). Similarly seasonality has been described in numerous studies in the onset of aortic dissection (Lasica 2006), stroke (Khan 1996), heart failure (Boulay 1999), myocardial infarction (Hopstock 2011), and heart arrhythmias (Frost 2002). In VTE a similar pattern is seen both in deep vein thrombosis and pulmonary embolism (Gallerani 2004, Manfredini 2004). A meta-analysis of the seasonal onset of VTE showed an increased risk of 14% in disease onset during the winter (Dentali 2011).

## 2.1. SEASONALITY IN CENTRAL RETINAL VEIN OCCLUSION

The literature is relatively sparse in information regarding seasonality in CRVO. A large retrospective population-based study from Taiwan including over 20000 patients showed a significant seasonality with a peak in January (Ho 2008). Lavin et al found a more frequent disease onset in the period September to February (Lavin 1987). Other studies have failed to find a seasonal relationship (Malayan 1999). In a large study including more than 1000 patients most patients had a disease onset between January and April, however this was not statistically significant (Hayreh 1992). No information is available on the seasonality in the Nordic countries.

## 2.2. MECHANISMS - SEASONALITY

The seasonality of CVDs could possibly be multifactorial. Suggested mechanisms include:

### 2.2.1 Hypertension

Raised systolic blood pressure is a significant risk factor for CVD and CRVO. Numerous studies have shown an increased blood pressure during the winter (Marti-Soler 2014).

Important mechanisms are peripheral vasoconstriction, decreased sweating and salt retention at colder temperatures (Cuspidi 2012). Several other risk factors for hypertension such as catecholamine, cholesterol and vasopressin have a seasonal variation with higher serum levels in the winter (Gordon 1988, Wittert 1992, Radke 2007).

### 2.2.2 Temperature

Both reduced indoor and outdoor temperature as recorded during winter have been shown to significantly increase blood pressure (Barnett 2007). Beside the direct effect on blood pressure an association has also been seen between cold temperature and increased blood viscosity. A cold environment may increase the platelet and the red blood cell counts, rise fibrinogen and cause a decrease in antithrombin III (Bull 1979, Keatinge 1984, Neild 1984).

### 2.2.3 Air pollution

Epidemiological studies have shown an increased risk for CVDs in relation to ambient particulate matter (PM) exposure. Higher PM concentrations have been observed during the winter months. Peters et al showed that elevated PM concentrations in the air may transiently increase the risk of MIs within 2 hours and 1 day after exposure (Peters 2001).

### 2.2.4 Physical activity

Physical inactivity is strongly correlated with CVD (Salo 2013). Physical activity is significantly higher in summer than in winter. It is believed that physical exercise improves the endothelial function (Black 2009). Exercise causes an increase in shear stress on the blood vessel endothelium. Blood vessels exposed to more shear stress are usually found to be free of atherosclerotic changes. Increased shear stress as a result of exercise may improve the endothelial function and can prevent CVD (Sherman 2000).

### 2.2.5 Latitude

The seasonality in CVD onset is affected by the distance from the Equator. In countries close to the Equator no significant seasonality in CVD presentation is seen while populations living in the northern or southern hemisphere show a significant seasonality (Marti-Soler 2014).

### 2.2.6 Vitamin D deficiency

Serum vitamin D levels have a marked seasonal variation, with peaking serum levels in the summer and a nadir during winter (Brot 2001). Data from the Framingham Offspring Study suggested that hypertensive patients with deficient vitamin D levels had a 60% increased risk of a cardiovascular event compared to patients with better vitamin D status (Wang 2008). Two recent meta-analyses have demonstrated an association between 25(OH)-vitamin D deficiency with both hypertension (Burgaz 2011) and risk of CVD (Wang 2012). In type 2 diabetes mellitus patients with low 25-OH vitamin D3 levels, supplementation with vitamin D reduced blood pressure and significantly increased blood vessel vasodilatation, a sign of improved endothelial function (Sugden 2008). Furthermore it has been shown that women with a more active sun exposure have a significantly lower risk of venous thromboembolism (Lindqvist 2009).

# 3 VITAMIN D - INTRODUCTION

Vitamin D has long been known to regulate serum calcium and phosphate metabolism and promoting bone mineralization. During the last decades numerous publications have shown that vitamin D may also have an impact on several other physiological functions. Vitamin D deficiency has been associated with CVD, autoimmune diseases, type 2 diabetes mellitus, cancer and infectious diseases (Holick 2011).

## 3.1. METABOLISM

Vitamin D, the sunshine vitamin, is actually a hormone. During exposure to ultraviolet B (UVB) irradiation in sunlight the 7-dehydrocholesterol in the skin is converted to previtamin D3. It is isomerized by the body heat to vitamin D3. In the liver it is hydroxylated by the vitamin D-25-hydroxylase to 25-hydroxyvitamin D3 (25(OH) D). This is the metabolite that is used to clinically measure vitamin D status. In order to become biologically active it is converted mainly in the kidneys by the 25-hydroxyvitamin D-1α-hydroxylase to its biologically active form 1,25-dihydroxyvitamin D3 (Holick 2011) (figure 5). 1,25-dihydroxyvitamin D3 acts by binding to the vitamin D receptor (VDR), which is present in many cells throughout the body, including vascular smooth muscle cells (VSMC), endothelium and cardiomyocytes (Judd 2008). Normal levels of vitamin D have not been firmly established, but a level <50 nmol/l (20 ng/ml) is considered deficient and a threshold to avoid vitamin D deficiency associated complications (Ross 2011).

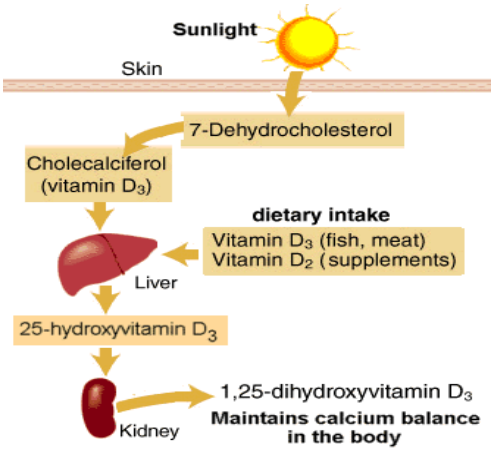


**Figure 5.** Vitamin D synthesis (courtesy John Berardi, www.precisionnutrition.com)

### 3.2. FACTORS INFLUENCING VITAMIN D SYNTHESIS

Vitamin D synthesis is dependent on the intensity of UVB irradiation which varies with season and latitude (Webb 1988). The solar zenith angle plays a crucial role in this process (figure 6). In the winter and at higher latitudes the zenith angle increases. The UVB irradiation has a longer path through the stratospheric ozone layer and less UVB light reach the earth's surface (Holick 2007). In Nordic countries it is not possible to synthesize vitamin D at sufficient levels in winter and the exposure time required to reach a standard dose is impractical from at least September through April (Burgaz 2009, Webb 2006). Similarly during the morning and in the late afternoon, when the zenith angle is large, the incoming UVB light is not sufficient for vitamin D synthesis.

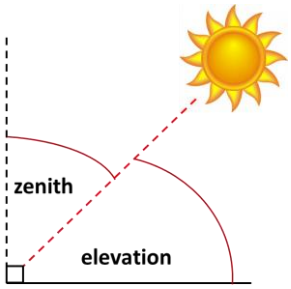


**Figure 6.** Solar zenith angle (D Epstein)

Skin pigmentation has also a major impact on the vitamin D synthesis. Melanin absorbs efficiently the UVB light reducing the vitamin D production in the skin. People with dark pigmentation need substantially longer sun exposure compared to individuals with fair skin to synthesize the same amount of vitamin D (Armas 2007). Aging reduces vitamin D production in skin. The aging skin becomes thinner and the 7-dehydrocholesterol concentration in the epidermis decreases. This causes a reduced response to UVB light and a more than twofold decrease in the formation of previtamin D3 (MacLaughlin 1985). Vitamin D is fat soluble and is found in oily fish like salmon and herring. Cod liver oil is another excellent source of vitamin D. Mushrooms are good sources of vitamin D but only if they are exposed to UVB irradiation. Fortified dairy products also contain vitamin D.

### 3.3. VITAMIN D AND CARDIOVASCULAR MORBIDITY -MECHANISMS

The mechanism for how vitamin D can affect cardiovascular disease outcomes has not yet been completely elucidated. Two of the central proposed mechanisms are:

### 3.3.1 Suppression of the renin-angiotensin-system

The renin–angiotensin system (RAS) plays a key role in regulating blood pressure and in controlling water and sodium balance. Elevated RAS activity is an important mechanism in the pathogenesis of hypertension. Inhibitors of RAS as angiotensin converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARB), and renin inhibitors are therefore widely used in the clinic (Skov 2014). Studies have shown that 1,25-dihydroxyvitamin D3 can decrease renin and angiotensin II thereby reducing blood pressure. Vitamin D deficient individuals may also have an attenuated reduction in renal plasma flow in response to angiotensin II stimulus. This supports the thesis that vitamin D deficiency may be associated with an upregulated RAS (Forman 2010).

### 3.3.2 Enhancing endothelial and vascular function

The endothelium plays an important role in the maintenance of vascular homeostasis. Deficient endothelial function has been suggested to provide a final common pathway by which multiple risk factors exert their deleterious effects on cardiovascular health. In normal vascular physiology, nitric oxide (NO) plays a crucial role in maintaining vascular tone by silencing oxidative processes potentially harmful for the endothelium (Gimbrone 1999). VDRs and 1-alpha-hydroxylase that convert vitamin D to its active form are found in the vascular endothelium (Zehnder 2002). In vitro studies have shown that vitamin D interaction with VDR increases NO formation thereby reducing the oxidative stress in the endothelium (Molinari 2011). Vitamin D also induces VSMC proliferation (Cardús 2006). It has been hypothesized that this can reduce vascular calcification by reducing the space for apoptotic bodies that are inducers of vascular calcium deposition (Proudfoot 2000). Endothelial function can be assessed in vivo by measuring brachial artery flow-mediated dilation (FMD). Vitamin D deficiency may cause a reduced FMD and an increased arterial stiffness (Al Mheid 2011). Vitamin D has also been shown to have anti-inflammatory properties important for the endothelial function by regulating the production of various cytokines. In vitro, vitamin D can reduce the production of tumor necrosis factor α (TNF-α) and other pro inflammatory cytokines (Zhang 2012).

# 4 GENERAL AIMS

1. To investigate if repeated intravitreal bevacizumab (IVB) injections every 6 weeks for 6 months could improve visual acuity as compared to sham-treated control patients with ME secondary to CRVO and to evaluate the change in foveal thickness and the number of patients developing neovascular glaucoma in the study groups (paper I).

2. To test if continuous injection every 6 weeks for 6 months could maintain visual acuity and to elucidate the impact of delaying treatment for 6 months in patients with ME secondary to CRVO (paper II).

3. To investigate the seasonality in CRVO onset and the yearly incidence of CRVO in Stockholm Sweden (paper III).

4. To test the hypothesis that patients with CRVO have an increased risk of vitamin D deficiency compared to matched controls (paper IV).

# 5 MATERIAL AND METHODS

## 5.1 PAPER I - II

### 5.1.1 Inclusion/exclusion criteria

The main inclusion and exclusion criteria are summarized in table 1. Study II included patients that had completed study I**.**

| Key Inclusion Criteria | Key Exclusion Criteria |
|---|---|
| CRVO with a duration of 6 months or less | CRVO with neovascularisation. |
| Best corrected visual acuity (BCVA) between 15-65 ETDRS letters (Snellen equivalent approximately 20/50 to 20/500). | Any previous treatment for CRVO. |
| Mean central subfield thickness ≥ 300µm as measured by OCT (Cirrus OCT) | Intraocular surgery during the previous 3 months. |
| | Vascular retinopathy of other causes. |
| | Glaucoma with advanced visual field defect or uncontrolled ocular hypertension >25mmHg despite full therapy. |
| | Myocardial infarction or stroke during the last 12 months. |
| CRVO= central retinal vein occlusion, ETDRS= Early Treatment Diabetic Retinopathy Study, OCT = Optical coherence tomography | |

**Table 1:** Main inclusion/exclusion criteria

### 5.1.2 Method

From April 2009 to December 2010 60 eyes of 60 patients with CRVO were consecutively enrolled. Study patients were randomized with equal probability to IVB injections (study group) or sham injections (control group). At baseline and at each follow-up visit, BCVA was measured at a distance of 4 m (or at 1 m if needed) by a certified tester using an ETDRS chart. Gonioscopy was performed prior to dilation at all visits. After dilation, optical coherence tomography (OCT) images were obtained by a certified technician using the Zeiss Cirrus OCT machine (Carl Zeiss Meditec, Inc.,Dublin, Ca). In study I each patient received 4 injections (IVB or sham) every 6 weeks. The total follow–up period was 24 weeks. Study patients were masked to the treatment given. Staff performing visual acuity

testing, OCT, fundus photographs and follow-up investigators were masked to treatment group. In study II both groups received 4 IVB injections every 6 weeks. The total follow–up period was an additional 24 weeks. Primary outcome measure: The proportion of patients gaining 15 ETDRS letters (3 lines) or more at 6 months (study I) and 12 months (study 2). Secondary outcome measures: Change in BCVA, change in CRT as measured by OCT and the number of patients with neovascular glaucoma defined as increased IOP due to the forming of new vessels in the angle as diagnosed by gonioscopy.

### 5.1.3 Statistical analysis

For the power calculation we assumed that 35% of the patients treated with bevacizumab and 5% of the sham-treated patients would achieve the primary endpoint (gain of at least 15 ETDRS letters). With a statistical power of 80% and the level of statistical significance set at $p<0.05$ we estimated that a minimum of 24 patients would be required in each group (MedCalc Software, Mariakerke, Belgium). For statistical analyses, the independent Student's t-test and the Fisher’s Exact Test (to compare differences in distributions between the groups) were used.

## 5.2 PAPER III

### 5.2.1 Inclusion/ exclusion criteria

The study population consisted of 854 consecutive patients with CRVO visiting the St. Erik Eye Hospital between January 2008 and December 2013. Patients that presented with neovascular glaucoma or with an uncertain date of onset of symptoms (n=278) were excluded from the analysis of seasonal onset but were included in the incidence calculation. Thus 576 patients were included in the analysis of seasonal distribution.

### 5.2.2 Method

Using the hospitals database the month of disease presentation was recorded in patients with CRVO and International Statistical Classification of Diseases Tenth Revision (ICD-10) code H34.8a. The seasons were divided accordingly: Winter from December to February, spring from March to May, summer from June through August and autumn from September until November.

### 5.2.3 Statistical analysis

Chi-square test and cross tabulations were used to compare differences in distributions between the seasons.

## 5.3 PAPER IV

### 5.3.1 Inclusion/ exclusion criteria

During the study period every new patient presenting with CRVO with a maximum duration of 3 months was offered to participate. The control patients were chosen randomly by Statistics Sweden and matched for age and gender.

### 5.3.2 Method

The study included 72 patients with CRVO and 144 controls recruited between December 2012 and August 2014. All the patients underwent a blood sample measuring the 25(OH)D level. Every season 120 patients in the control group were invited to participate in the study. They received a letter with information of the study, informed consent form and details about the blood sampling procedure. Every season 36 controls were included in the study. Similarly 18 patients with a new CRVO were included in the study group each season.

### 5.3.3 Statistical analysis

The power calculation was based on a difference in the vitamin D serum concentration between the groups of 10 nmol/l. With a statistical power of 80% and the level of statistical significance set at $P<0.05$, we estimated that a minimum of 50-60 patients would be required in the study group (MedCalc Software, Mariakerke, Belgium). For statistical analyses, the independent Student t test was used for continuous variables. For categorical data Pearson's chi-squared test and Fisher's exact test were used.

# 6 RESULTS

## 6.1 PAPER I

### 6.1.1 Visual outcomes

After 24 weeks 18/30 (60.0%) patients in the IVB group had gained ≥15 letters compared with 6/30 (20.0%) patients in the control group (p=0.003). At the end of follow up patients in the IVB group improved 14.1 letters compared to a loss of 2.0 letters in the control group (p<0.01) (figure 6). An analysis of the impact of disease duration on visual outcome was done. Patients with a disease duration of less than 90 days improved 18.3 letters (p<0.001) compared with patients with disease duration of more than 90 days who gained 9.1 letters (p=0.039).

### 6.1.2 Anatomical outcomes

The IVB group had a significantly greater reduction of the CRT (426 μm) than in the control group (102 μm) at all-time points up to week 24 (p<0.001) (figure 7). No ME, defined as CRT of less than 300μm at 24 weeks, was found in 26/30 (86.7%) patients in the treatment group as compared with 6/30 (20%) patients in the control group (p<0.001).

### 6.1.3 Neovascularization

No patients developed retinal neovascularization. At week 24 5/30 (16,7%) of the patients in the sham group had developed iris rubeosis. No patients in the IVB group had iris rubeosis at week 24 (p=0.052).

## 6.2 PAPER II

### 6.2.1 Visual outcomes

At the end of follow up, 18/30 (60.0%) patients in the original IVB group had gained ≥15 letters compared with 10/30 (33.3%) patients in the original sham group (p<0.05). In the original IVB group a further gain of 2.0 letters was seen with the fixed injection schedule during week 24 to 48. The original sham group, improved to a vision gain of 4.6 letters at

week 48 ($p<0.05$) (figure 6). A retrospective analysis of the impact of age and response to therapy was done. Younger patients had a better visual outcome. Overall patients <70 years improved 14.2 letters (95% confidence interval (CI), 6.3-22.0) compared with 7.4 letters (95% CI, 0.2-14.6) in the age group >70 years ($p>0.05$). Patients >70 years had a significantly worse outcome when receiving delayed treatment losing 1.4 letters (95% CI, -9.7-8.4) in the original sham group compared with a gain of 20.1 letters (95% CI, 13.9-26.3) the original IVB group ($p=0.003$).

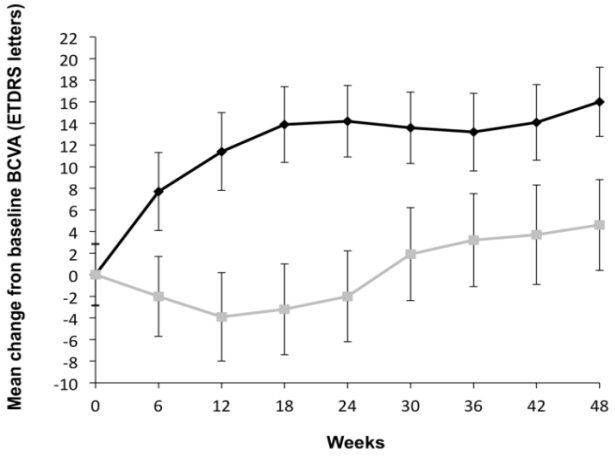


**Figure 6.** Mean change from baseline BCVA over time to month 12. Grey line represent original sham group and black line original IVB group.

6.2.2 Anatomical Outcomes

At 48 weeks the mean change in CRT was similar in both groups with a mean decrease of 435 µm (original IVB group) and 404 µm (original sham group) at week 48 ($p>0.05$, Figure 7).

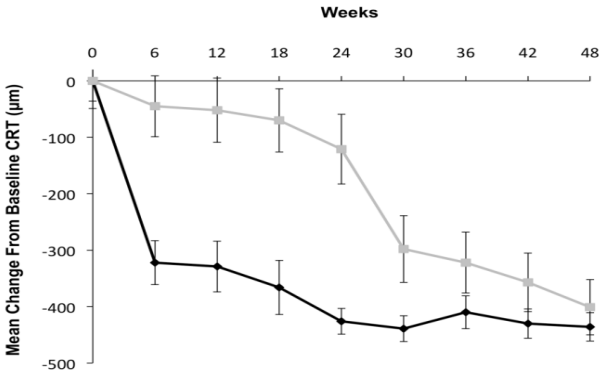


**Figure 7.** Mean change from baseline CRT over time to month 12. Grey line represent original sham group and black line original IVB group.

### 6.2.3 Neovascularization

No new cases of neovascularization were seen between week 24 and 48 in either group.

### 6.2.4 Ocular safety and systemic adverse events (study I-II)

There were no events of endophthalmitis, retinal tear or retinal detachment during the 48 weeks treatment period. No serious non-ocular adverse events were reported. These studies were not powered to provide definite data on safety.

## 6.3 PAPER III

### 6.3.1 Seasonality of CRVO onset

Every year during the 6 year study most patients had a CRVO onset during the winter-spring period that was significantly higher than during the other seasons reaching its lowest levels in autumn. The number of patients presenting in the different seasons were 166 (28.8% CI 95% 25.3-32.6) in winter, 172 (29.9% CI 95% 26.3-33.7) in spring, 131 (22.7% CI 95% 19.5-26.3) in summer and 107 (18.9% CI 95% 15.6-22.0) in autumn ($p<0.0002$ winter/spring compared with summer/ autumn) (figure 8). There was an increased risk of over 40 % of developing CRVO in winter/spring compared with summer/ autumn. Odds Ratio (OR) 1.4 (95% CI 1.20-1.68).

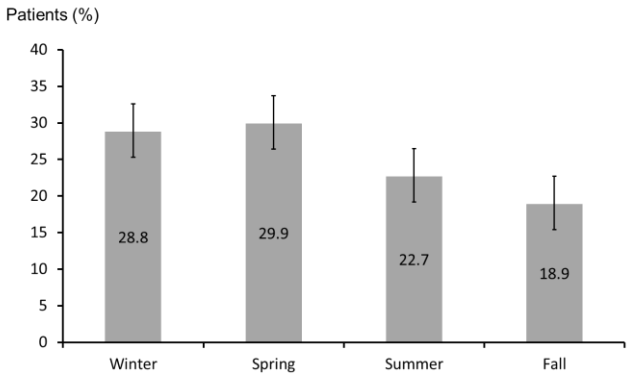


**Figure 8.** Overall central retinal vein occlusion onset (%) according to season.

### 6.3.2 CRVO incidence

During the study period 854 subjects developed CRVO for an annual incidence of 2/10000 for persons aged over 40 years. The annual incidence was increasing from 0.25/10000 at 45 years of age up to 7/10000 above 80 (figure 9). Age was significantly associated with the incidence of CRVO (p<0.001).

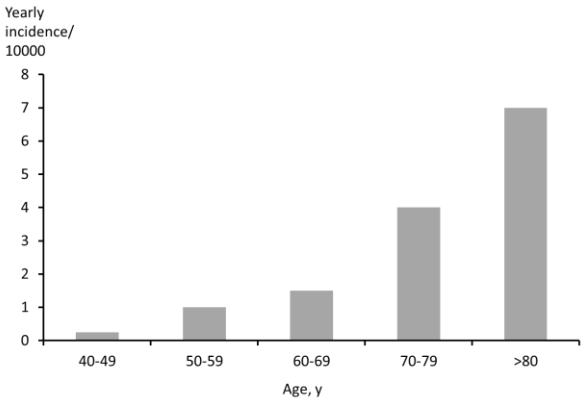


**Figure 9:** Yearly incidence of central retinal vein occlusion (CRVO) stratified in age groups.

## 6.4 PAPER IV

### 6.4.1 Vitamin D deficiency

A total of 216 patients were included in the study. Eight patients did not have eligible blood samples. Thus the final analysis included 208 subjects (68 patients in the study group and 140 controls).

More than half the patients 35/68 (51.4%) in the CRVO group had vitamin D deficiency (serum 25(OH)D <50 nmol/l) compared to 55/140 (39.3%) in the control group. OR 1.64, (95% CI 0.91-2.94, p=0.10). The mean concentration of serum 25(OH)D was 55.3 nmol/L (95% CI 48.4-62.2) in the study group compared to 59.8 nmol/L (95% CI 55.4-64.2) in the control group (p=0.28). There was a significant seasonal variation of the serum 25(OH)D levels in both groups peaking during summer (p=0.04) (figure 10).

An ad-hoc analysis of the impact of age on vitamin D levels was done. In the CRVO group of patients less than 75 years old 62.1% (23/37) had vitamin D deficiency compared to 43.2% (38/88) in the control group. Among subjects less than 75 years old the 25(OH)D

levels were significantly lower in the CRVO group 47.8 nmol/l (95% CI 40.6-55.0) compared to the control group with 59.0 nmol/l (95% CI 53.6-64.4) (p=0.02).

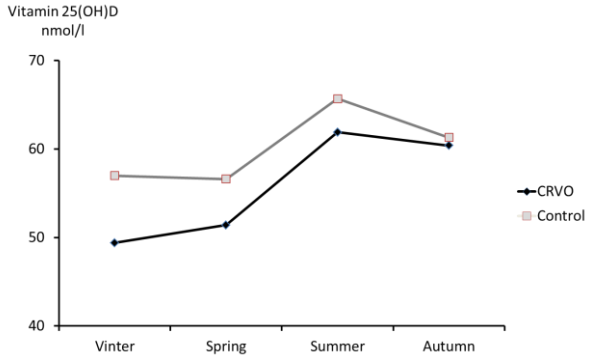


**Figure 10:** Seasonal distribution of vitamin 25 (OH) D levels.

# 7 DISCUSSION

## 7.1 PAPER I-II

In study I it was shown for the first time in a randomized prospective study the superiority of IVB treatment compared to sham injections in CRVO. More than 60% of the patients improved more than 3 lines on the ETDRS chart compared to 20% in the control group. The results showed comparable results to randomized trials with ranibizumab and aflibercept. Neovascular glaucoma may be a devastating complication of CRVO with additional vision loss and patient suffering. We found that no patients with active IVB treatment developed neovascular glaucoma compared to 16% in the control group. This is very good news for patients with ischemic CRVO. However, it is important to consider that by injecting anti-VEGF we are changing the natural course of the disease. This may in turn delay the onset of neovascular complications, especially when anti-VEGF treatment is withdrawn. Disease duration seems to have a critical impact on visual outcome. Patients initially on a sham-regime in study I improved with IVB treatment after 24 weeks but significantly less than the original IVB group. Both the CRUISE study evaluating ranibizumab and the COPERNICUS study investigating aflibercept showed similar results (Campochiaro 2011, Boyer 2012). ME causes disorganization of the retinal cellular structure and may cause severe damage to the neural cells. Long-lasting edema may lead to irreversible macular damage, which cannot be restored solely by resolution of the ME. Therefore, early therapeutic intervention to reduce the ME is important to optimize the patients' visual outcome. Bevacizumab is an off-label treatment but has a widespread use over the world mainly because its lower price compared to other anti-VEGF agents. Bevacizumab may be a good treatment option especially in resource-poor regions due to price issues.

## 7.2 PAPER III

We found an increased risk of over 40% in the onset of CRVO during winter/ spring as compared with summer/autumn in Stockholm. Similar findings have been seen in some previous reports (Lavin 1987, Ho 2008) while others failed to show a seasonal relationship (Hayreh 1992). Several casual mechanisms may be involved in the seasonal onset of CRVO. A cold temperature may increase blood viscosity by boosting fibrinogen levels and raising the platelet and blood red counts (Bull 1979, Keatinge 1984, Neild 1984). Several

studies have shown that blood pressure is higher during the winter (Marti-Soler 2014). This may be caused by peripheral blood vessel vasoconstriction and higher levels of catecholamine and vasopressin during winter (Gordon 1988, Wittert 1992, Radke 2007). Vitamin D has a marked seasonal variation with lower levels during the winter. In vitro studies have shown that vitamin D is important for the endothelial function by inducing NO formation and reducing oxidative stress (Gimbrone 1999). Vitamin D is also known to have a lowering effect on the blood pressure by decreasing serum levels of renin and angiotensin II (Forman 2010). In the current study we found a CRVO incidence of 2/10000. In previous studies the incidence of CRVO has been between 3.3-4/10000 (Klein 2000, Cugati 2006). The higher incidence in these studies may be explained by the inclusion of asymptomatic patients diagnosed by fundus photography. In our study only symptomatic patients actively seeking medical care were included. Our symptomatic CRVO incidence could be useful when estimating the actual number of patients that will seek medical care every year.

### 7.3 PAPER IV

In this study we found that more than 50% of the patients with CRVO had deficient vitamin D levels. In the complete data set no significant differences in vitamin D levels were found between the study groups. A non-planned sub group analysis showed that patients younger than 75 years old with CRVO had significantly lower vitamin D levels compared to controls. This result should be interpreted with caution because of the ad-hoc design and the smaller subgroup size. Interestingly patients older than 75 years had better vitamin D status than younger subjects. This is somewhat surprising considering that older individuals are at risk for vitamin D deficiency due to reduced dietary intake and decreased synthesis. This could possibly be explained by the increased focus on vitamin D supplementation lately in the elderly. In the recent Nordic Nutrition Recommendations ($5^{th}$ edition 2012) individuals over 75 years are recommended to take 800 international units (IU) vitamin D. This hypothesis is strengthen by the fact that significantly more patients in the CRVO group above 75 years old received vitamin D supplementation. Our study suggests that vitamin D deficiency is prevalent in patients with CRVO. If vitamin D deficiency is involved in the causal pathway of cardiovascular disease or the result of confounding bias has not yet been determined. We cannot exclude that we did not have the statistical power to show that vitamin D deficiency may increase the risk for CRVO. Thus, larger studies are needed to establish if vitamin D deficiency is a risk factor in developing CRVO.

# 8 MAIN CONCLUSIONS

1. In treating ME in CRVO, IVB injections are superior to sham injections in improving visual and anatomical outcomes.

2. Early treatment is important to optimize the visual outcome in CRVO patients.

3. There is an increased risk in the onset of CRVO during winter/ spring as compared with summer/autumn in Stockholm. We found a CRVO incidence of 2/10000.

4. More than half the CRVO patients had deficient vitamin D levels. Patients younger than 75 years old with CRVO had significantly lower vitamin D levels compared to controls.

# 9 FUTURE PERSPECTIVES

Intravitreal injections have fundamentally changed the treatment and outcomes in patients with CRVO. Several different anti-VEGF molecules and steroid implants are available on the market but the optimal treatment has yet to be established. Future head-to-head trials could answer this question. Different treatment algorithms could also have an impact on the visual outcome. Traditionally patients with CRVO have been treated with an initial loading dose followed by a PRN (as needed) regimen. This allows frequent relapses of the ME which may deteriorate visual outcome. More proactive regimens such as treat-and-extend where the patient is treated at each visit with extending intervals as long as the macula is dry may improve the outcome in these patients. Different treatment algorithms should be evaluated in future prospective studies.

Today we are unfortunately not able to treat the basic pathology. None of the new intravitreal injections have any known effect on the occluded vessel. New treatment modalities directed at the site of occlusion could dramatically change the natural course of the disease and reduce the risk of chronicity which is a common problem today.

The diagnosis of CRVO is usually straightforward due to the distinct fundus appearance. The presenting clinical picture and visual acuity can be quite various. Sometimes a spontaneous recovery can be seen but usually the natural course is not favourable. The reason for this variation is unclear. It could be hypothesized that processes in the clot or various collateral blood flow may influence this heterogeneity. However little is known about the clot, the size of the occlusion and if some parts of the blood vessel lumen have a patent blood flow. Newer diagnostic imaging tools visualizing the site of occlusion could help us increase our knowledge of the pathogenesis of CRVO and the variable natural course in these patients.

The role of seasonality in CVD and RVO is intriguing.  A deeper understanding of the processes involved in the seasonal disease onset could help in preventing some future cases.

Vitamin D deficiency is a common problem that may have an influence on global health. Whether vitamin D deficiency is involved in the causal pathway of cardiovascular disease has not yet been firmly determined. Large randomized studies on vitamin D supplementation are needed to answer this question.

In the meantime we can rejoice in the thought that sunshine may be the easiest way to improve our physical and mental health.

# 10 SAMMANFATTNING PÅ SVENSKA

Centralventrombos i näthinnan (CRVO) innebär att en blodpropp bildas i en ven som för blod ut från ögat. Blodcirkulationen försämras i näthinnan som svullnar av vätska och blod vilket kan leda till grav synnedsättning. Den försämrade blodcirkulationen orsakar syrebrist i näthinnan vilket leder till att olika tillväktfaktorer (VEGF) utsöndras. VEGF försvagar blodkärlens väggar och kan orsaka att en svullnad i gula fläcken uppstår. VEGF kan även orsaka att nya sjuka blodkärl bildas som kan orsaka grön starr med högt tryck.
Fram till för några år sedan har det inte funnits någon behandling för denna sjukdom. Genom att injicera en antikropp till VEGF i glaskroppen kan man behandla svullnaden i gula fläcken samt minska risken för att patienten utvecklar grön starr.

I **delarbete I** undersökte vi hur behandling med tillväxthämmaren bevacizumab påverkade synskärpan, svullnaden i gula fläcken samt risken för uppkomst av grön starr. Patienter som lottades till aktiv behandling fick bevacizumab injektioner med 6 veckors intervall. Patienter i kontrollgruppen fick simulerade injektioner med 6 veckors intervall. Sammanlagt 60 patienter deltog i studien med 30 deltagare i varje grupp. Studien pågick i 24 veckor. Resultaten visade att patienter som fick behandling med bevacizumab förbättrades nästan 3 rader på syntavlan jämfört med en liten synförsämring hos patienterna i kontrollgruppen. Svullnaden i gula fläcken försvann hos nästan alla som fick bevacizumab behandling till skillnad mot kontrollgruppen där svullnaden kvarstod i de flesta fallen. Inga patienter i bevacizumab gruppen utvecklade grön starr.

I **delarbete II** studerade vi hur effekten av fördröjd behandling påverkade synskärpan. Vi undersökte även om de goda synresultaten från delarbete I kvarstod. Alla 60 patienterna fick bevacizumab injektioner med 6 veckors intervall under 24 veckors tid. Patienterna i den ursprungliga bevacizumab gruppen hade fortsatt bra synskärpa. De patienterna som fick bevacizumab behandling med 24 veckors fördröjning förbättrade synen dock var deras synvinst 2 rader sämre än den ursprungliga bevacizumab gruppen. Båda grupperna hade likvärdiga förbättringar av svullnaden i gula fläcken. Inga patienter utvecklade grön starr.
*Dessa båda arbeten visar för första gången i en prospektiv studie att bevacizumab förbättrar synskärpan vid CRVO i jämförelse med naturalförloppet samt att tidig behandling är viktig för att få bästa möjliga synprognos. Risken för grön starr minskar med bevacizumab behandling.*

Tidigare studier har visat att insjuknandet i hjärt och kärlsjukdomar ökar under vinterhalvåret. Enstaka arbeten har visat ett liknande mönster även vid CRVO. Orsaken till detta är inte klarlagt. Det har spekulerats i att detta kan bero på olika säsongsrelaterade faktorer som förändras beroende på årstid såsom blodtryck, temperatur, luftföroreningar, fysisk aktivitet samt vitamin D brist. Vitamin D erhålls från kost eller från solljus. Då de flesta födoämnen har låga vitamin D nivåer är den viktigaste källan ultraviolett ljus som stimulerar hudens produktion av vitamin D. För att vitamin D skall produceras måste solen stå högt över horisonten vilket endast sker i Stockholm mellan maj och augusti. Under vinterhalvåret kan därför vitamin D brist uppstå. Vitamin D har visats ha en viktig roll för blodkärlens funktion samt blodtrycket. Det är möjligt att låga vitamin D nivåer kan öka risken för tromboser.

I **delarbete III** utvärderade vi när på året patienter insjuknade i CRVO samt hur vanlig sjukdomen var. Vi undersökte 854 patienter med CRVO mellan 2008 – 2013. Under varje år insjuknade flest patienter under vintern samt våren. 2 nya fall per 10000 invånare registrerades i genomsnitt varje år. Äldre patienter hade en ökad risk att få CRVO. *Detta är den första studien som undersöker säsongsvariationen samt incidensen av CRVO i Sverige.*

I **delarbete IV** jämförde vi i en prospektiv studie vitamin D nivåerna hos CRVO patienter med slumpvis utvalda kontrollpatienter. Detta för att utvärdera om vitamin D brist kan vara en riskfaktor för insjuknandet i CRVO. Sammanlagt 216 patienter deltog i studien. Mer än hälften av CRVO patienterna hade vitamin D brist jämfört med en tredjedel i kontrollgruppen. Dessa skillnader var inte signifikanta när hela patientmaterialet analyserades dock hade patienter yngre än 75 år statistiskt lägre vitamin D nivåer. Större studier behövs för att säkert utvärdera om vitamin D brist är en riskfaktor för CRVO. *Denna studie visar för första gången att vitamin D brist är vanligt hos CRVO patienter.*

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# 12 REFERENCES


Agarwal M, Biswas J. Central retinal vein occlusion in primary antiphospholipid antibody syndrome. Retin Cases Brief Rep. 2009 Summer;3(3):293-5.

Akiba J, Kado M, Kakehashi A, Trempe CL. Role of the vitreous in posterior segment neovascularization in central retinal vein occlusion. Ophthalmic Surg. 1991 Sep;22(9):498-502.

Al Mheid I, Patel R, Murrow J, Morris A, Rahman A, Fike L, Kavtaradze N, Uphoff I, Hooper C, Tangpricha V, Alexander RW, Brigham K, Quyyumi AA. Vitamin D status is associated with arterial stiffness and vascular dysfunction in healthy humans. J Am Coll Cardiol. 2011 Jul 5;58(2):186-92.

Arakawa S, Yasuda M, Nagata M, Ninomiya T, Hirakawa Y, Doi Y, Kiyohara Y, Ishibashi T. Nine-year incidence and risk factors for retinal vein occlusion in a general Japanese population: the Hisayama Study. Invest Ophthalmol Vis Sci. 2011 Jul 29;52(8):5905-9.

Armas LA, Dowell S, Akhter M, Duthuluru S, Huerter C, Hollis BW, Lund R, Heaney RP. Ultraviolet-B radiation increases serum 25-hydroxyvitamin D levels: the effect of UVB dose and skin color. J Am Acad Dermatol. 2007 Oct;57(4):588-93.

Barnett AG, Sans S, Salomaa V, Kuulasmaa K, Dobson AJ; WHO MONICA Project. The effect of temperature on systolic blood pressure. Blood Press Monit. 2007 Jun;12(3):195-203.

Beer PM, Wong SJ, Hammad AM, Falk NS, O'Malley MR, Khan S. Vitreous levels of unbound bevacizumab and unbound vascular endothelial growth factor in two patients. Retina. 2006 Oct;26(8):871-6.

Black MA, Cable NT, Thijssen DH, Green DJ. Impact of age, sex, and exercise on brachial artery flow-mediated dilatation. Am J Physiol Heart Circ Physiol. 2009 Sep;297(3):H1109-16.

Boulay F, Berthier F, Sisteron O, Gendreike Y, Gibelin P. Seasonal variation in chronic heart failure hospitalizations and mortality in France. Circulation. 1999 Jul 20;100(3):280-6.

Boyer D, Heier J, Brown DM, Clark WL, Vitti R, Berliner AJ, Groetzbach G, Zeitz O, Sandbrink R, Zhu X, Beckmann K, Haller JA. Vascular endothelial growth factor Trap-Eye for macular edema secondary to central retinal vein occlusion: six-month results of the phase 3 COPERNICUS study. Ophthalmology. 2012 May;119(5):1024-32.

Brot C, Vestergaard P, Kolthoff N, Gram J, Hermann AP, Sørensen OH. Vitamin D status and its adequacy in healthy Danish perimenopausal women: relationships to dietary intake, sun exposure and serum parathyroid hormone. Br J Nutr. 2001 Aug;86 Suppl 1:S97-103.

Brown DM, Campochiaro PA, Singh RP, Li Z, Gray S, Saroj N, Rundle AC, Rubio RG, Murahashi WY; CRUISE Investigators. Ranibizumab for macular edema following central retinal vein occlusion: six-month primary end point results of a phase III study. Ophthalmology. 2010 Jun;117(6):1124-1133.

Brown DM, Heier JS, Clark WL, Boyer DS, Vitti R, Berliner AJ, Zeitz O, Sandbrink R, Zhu X, Haller JA. Intravitreal aflibercept injection for macular edema secondary to central retinal vein occlusion: 1-year results from the phase 3 COPERNICUS study. Am J Ophthalmol. 2013 Mar;155(3):429-437.

Browning D. Retinal Vein Occlusions Evidence-Based Management. Springer Science. New York 2012 p243.

Bull GM, Brozovic M, Chakrabarti R, Meade TW, Morton J, North WR, Stirling Y. Relationship of air temperature to various chemical, haematological, and haemostatic variables. J Clin Pathol. 1979 Jan;32(1):16-20.

Burgaz A, Akesson A, Michaëlsson K, Wolk A. 25-hydroxyvitamin D accumulation during summer in elderly women at latitude 60 degrees N. J Intern Med 2009;266:476-83.

Burgaz A, Orsini N, Larsson SC, Wolk A. Blood 25-hydroxyvitamin D concentration and hypertension: a meta-analysis. J Hypertens. 2011;29:636-45.

Cahill MT, Stinnett SS, Fekrat S. Meta-analysis of plasma homocysteine, serum folate, serum vitamin B(12), and thermolabile MTHFR genotype as risk factors for retinal vascular occlusive disease. Am J Ophthalmol. 2003 Dec;136(6):1136-50.

Campochiaro PA, Brown DM, Awh CC, Lee SY, Gray S, Saroj N, Murahashi WY, Rubio RG. Sustained benefits from ranibizumab for macular edema following central retinal vein occlusion: twelve-month outcomes of a phase III study. Ophthalmology. 2011 Oct;118(10):2041-9.

Cardús A, Parisi E, Gallego C, Aldea M, Fernández E, Valdivielso JM. 1,25-Dihydroxyvitamin D3 stimulates vascular smooth muscle cell proliferation through a VEGF-mediated pathway. Kidney Int. 2006 Apr;69(8):1377-84.

Chang-Lin JE, Attar M, Acheampong AA, Robinson MR, Whitcup SM, Kuppermann BD, Welty D. Pharmacokinetics and pharmacodynamics of a sustained-release dexamethasone intravitreal implant. Invest Ophthalmol Vis Sci. 2011 Jan 5;52(1):80-6.

Chen JC, Klein ML, Watzke RC, Handelman IL, Robertson JE. Natural course of perfused central retinal vein occlusion. Can J Ophthalmol. 1995 Feb;30(1):21-4.

Cheung N, McNab AA. Venous anatomy of the orbit. Invest Ophthalmol Vis Sci. 2003 Mar;44(3):988-95.

Chopdar A. Hemispheric retinal vein occlusion or hemicentral retinal vein occlusion. Arch Ophthalmol. 1986 Aug;104(8):1128, 1130.

Cuspidi C, Ochoa JE, Parati G. Seasonal variations in blood pressure: a complex phenomenon. J Hypertens 2012;30:1315–20.

Dentali F, Ageno W, Rancan E, Donati AV, Galli L, Squizzato A, Venco A, Mannucci PM, Manfredini R. Seasonal and monthly variability in the incidence of venous

thromboembolism. A systematic review and a meta-analysis of the literature. Thromb Haemost. 2011 Sep;106(3):439-47.

Di Capua M, Coppola A, Albisinni R, Tufano A, Guida A, Di Minno MN, Cirillo F, Loffredo M, Cerbone AM. Cardiovascular risk factors and outcome in patients with retinal vein occlusion. J Thromb Thrombolysis. 2010 Jul;30(1):16-22.

Figueroa MS, Contreras I, Noval S, Arruabarrena C. Results of bevacizumab as the primary treatment for retinal vein occlusions. Br J Ophthalmol 2010;94:1052– 6.

Forman JP, Williams JS, Fisher ND. Plasma 25-hydroxyvitamin D and regulation of the renin-angiotensin system in humans. Hypertension. 2010 May;55(5):1283-8.

Friberg TR, Gupta A, Yu J, Huang L, Suner I, Puliafito CA, Schwartz SD. Ultrawide angle fluorescein angiographic imaging: a comparison to conventional digital acquisition systems. Ophthalmic Surg Lasers Imaging. 2008 Jul-Aug;39(4):304-11.

Frost L, Johnsen SP, Pedersen L, Husted S, Engholm G, Sørensen HT, Rothman KJ. Seasonal variation in hospital discharge diagnosis of atrial fibrillation: a population-based study. Epidemiology. 2002 Mar;13(2):211-5.

Fujiwara T, Kawamura M, Nakajima J, Adachi T, Hiramori K. Seasonal differences in diurnal blood pressure of hypertensive patients living in a stable environmental temperature. J Hypertens. 1995 Dec;13(12 Pt 2):1747-52.

Gallerani M, Boari B, de Toma D, Salmi R, Manfredini R. Seasonal variation in the occurrence of deep vein thrombosis. Med Sci Monit. 2004 May;10(5):CR191-6.

Gavard J, Gutkind JS. VEGF controls endothelial-cell permeability by promoting the beta-arrestin-dependent endocytosis of VE-cadherin. Nat Cell Biol. 2006 Nov;8(11):1223-34.

Gimbrone MA Jr. Vascular endothelium, hemodynamic forces, and atherogenesis. Am J Pathol. 1999 Jul;155(1):1-5.

Glueck CJ, Hutchins RK, Jurantee J, Khan Z, Wang P. Thrombophilia and retinal vascular occlusion. Clin Ophthalmol. 2012;6:1377-84.

Gordon DJ, Hyde J, Trost DC, Whaley FS, Hannan PJ, Jacobs DR, Ekelund LG. Cyclic seasonal variation in plasma lipid and lipoprotein levels: the Lipid Research Clinics Coronary Primary Prevention Trial Placebo Group. J Clin Epidemiol. 1988;41(7):679-89.

Green WR, Chan CC, Hutchins GM, Terry JM. Central retinal vein occlusion: a prospective histopathologic study of 29 eyes in 28 cases. Trans Am Ophthalmol Soc. 1981;79:371-422.

Haller JA, Bandello F, Belfort R Jr, Blumenkranz MS, Gillies M, Heier J, Loewenstein A, Yoon YH, Jacques ML, Jiao J, Li XY, Whitcup SM; OZURDEX GENEVA Study Group. Randomized, sham-controlled trial of dexamethasone intravitreal implant in patients with macular edema due to retinal vein occlusion. Ophthalmology. 2010 Jun;117(6):1134-1146.

Haller JA, Bandello F, Belfort R Jr, Blumenkranz MS, Gillies M, Heier J, Loewenstein A, Yoon YH, Jiao J, Li XY, Whitcup SM; Ozurdex GENEVA Study Group, Li J. Dexamethasone intravitreal implant in patients with macular edema related to branch or central retinal vein occlusion twelve-month study results. Ophthalmology. 2011 Dec;118(12):2453-60.

Hayreh SS. Classification of central retinal vein occlusion. Ophthalmology. 1983 May;90(5):458-74.

Hayreh SS, Klugman MR, Beri M, Kimura AE, Podhajsky P. Differentiation of ischemic from non-ischemic central retinal vein occlusion during the early acute phase. Graefes Arch Clin Exp Ophthalmol. 1990;228(3):201-17.

Hayreh SS, Zimmerman MB, Podhajsky P. Seasonal variations in the onset of retinal vein occlusion. Br J Ophthalmol. 1992 Dec;76(12):706-10.

Hayreh SS, Zimmerman MB, Podhajsky P. Incidence of various types of retinal vein occlusion and their recurrence and demographic characteristics. Am J Ophthalmol. 1994 Apr 15;117(4):429-41.

Hayreh SS. Retinal vein occlusion. Indian J Ophthalmol. 1994 Sep;42(3):109-32. Review.

Hayreh SS, Zimmerman MB, Beri M, Podhajsky P. Intraocular pressure abnormalities associated with central and hemicentral retinal vein occlusion. Ophthalmology. 2004 Jan;111(1):133-41.

Hayreh SS. Orbital vascular anatomy. Eye (Lond). 2006 Oct;20(10):1130-44.

Hayreh SS, Podhajsky PA, Zimmerman MB. Natural history of visual outcome in central retinal vein occlusion. Ophthalmology. 2011 Jan;118(1):119-133.

Hayreh SS, Zimmerman MB. Hemicentral retinal vein occlusion: natural history of visual outcome. Retina. 2012 Jan;32(1):68-76.

Hayreh SS. Ocular vascular occlusive disorders. Springer science. 2015.

Hikichi T, Konno S, Trempe CL. Role of the vitreous in central retinal vein occlusion. Retina. 1995;15(1):29-33.

Ho JD, Tsai CY, Liou SW, Tsai RJ, Lin HC. Seasonal variations in the occurrence of retinal vein occlusion: a five-year nationwide population-based study from Taiwan. Am J Ophthalmol. 2008 Apr;145(4):722-728.

Holick MF, Chen TC, Lu Z, Sauter E. Vitamin D and skin physiology: a D-lightful story. J Bone Miner Res. 2007 Dec;22 Suppl 2:V28-33.

Holick MF. Vitamin D: a d-lightful solution for health. J Investig Med. 2011 Aug;59(6):872-80.

Holmes K, Roberts OL, Thomas AM, Cross MJ. Vascular endothelial growth factor receptor-2: structure, function, intracellular signalling and therapeutic inhibition. Cell Signal. 2007 Oct;19(10):2003-12.

Hopstock LA, Wilsgaard T, Njølstad I, Mannsverk J, Mathiesen EB, Løchen ML, Bønaa KH. Seasonal variation in incidence of acute myocardial infarction in a sub-Arctic population: the Tromsø Study 1974-2004. Eur J Cardiovasc Prev Rehabil. 2011 Apr;18(2):320-5.

Jorde R, Haug E, Figenschau Y, Hansen JB. Serum levels of vitamin D and haemostatic factors in healthy subjects: the Tromsø study. Acta Haematol 2007;117:91–97.

Judd S, Tangpricha V. Vitamin D Deficiency and Risk for Cardiovascular Disease. Circulation. 2008 January 29; 117(4): 503–511.

Kaur C, Foulds WS, Ling EA. Blood-retinal barrier in hypoxic ischaemic conditions: basic concepts, clinical features and management. Prog Retin Eye Res. 2008 Nov;27(6):622-47.

Keatinge WR, Coleshaw SR, Cotter F, Mattock M, Murphy M, Chelliah R. Increases in platelet and red cell counts, blood viscosity, and arterial pressure during mild surface cooling: factors in mortality from coronary and cerebral thrombosis in winter. Br Med J (Clin Res Ed). 1984 Nov 24;289(6456):1405-8.

Khan FA, Engstrom G, Jerntorp I, Pessah-Rasmussen H, Janzon L. Seasonal patterns of incidence and case fatality of stroke in Malmo, Sweden: the STROMA study. Neuroepidemiology. 2005;24(1-2):26-31.

Kivimäki M, Nyberg ST, Fransson EI, Heikkilä K, Alfredsson L, Casini A, Clays E, De Bacquer D, Dragano N, Ferrie JE, Goldberg M, Hamer M, Jokela M, Karasek R, Kittel F, Knutsson A, Koskenvuo M, Nordin M, Oksanen T, Pentti J, Rugulies R, Salo P, Siegrist J, Suominen SB, Theorell T, Vahtera J, Virtanen M, Westerholm PJ, Westerlund H, Zins M, Steptoe A, Singh-Manoux A, Batty GD; IPD-Work Consortium. Associations of job strain and lifestyle risk factors with risk of coronary artery disease: a meta-analysis of individual participant data. CMAJ. 2013 Jun 11;185(9):763-9.

Klein R, Klein BE, Moss SE, Meuer SM. The epidemiology of retinal vein occlusion: the Beaver Dam Eye Study. Trans Am Ophthalmol Soc. 2000;98:133-41; discussion 141-3.

Kolar P. Risk factors for central and branch retinal vein occlusion: a meta-analysis of published clinical data. J Ophthalmol 2014 epub.

Krohne TU, Liu Z, Holz FG, Meyer CH. Intraocular pharmacokinetics of Ranibizumab following a single intravitreal injection in humans. Am J Ophthalmol. 2012 Oct;154(4):682-686.

Lasica RM, Perunicic J, Mrdovic I, Tesic BV, Stojanovic R, Milic N, Simic D, Vasiljevic Z. Temporal variations at the onset of spontaneous acute aortic dissection. Int Heart J. 2006 Jul;47(4):585-95.

Lavin MJ, Dhillon BJ. Cyclic variation in onset of central retinal vein occlusion. Br J Ophthalmol. 1987 Jan;71(1):18-20.

Lindqvist PG, Epstein E, Olsson H. Does an active sun exposure habit lower the risk of venous thrombotic events? A D-lightful hypothesis. J Thromb Haemost. 2009 Apr;7(4):605-10.

Little HL, Chan CC. Infrequency of retinal neovascularization following central retinal vein occlusion attributed to endothelial death. Mod Probl Ophthalmol. 1979;20:121-6.

Lüke J, Nassar K, Lüke M, Grisanti S. Ranibizumab as adjuvant in the treatment of rubeosis iridis and neovascular glaucoma--results from a prospective interventional case series. Graefes Arch Clin Exp Ophthalmol. 2013 Oct;251(10):2403-13.

MacLaughlin J, Holick MF. Aging decreases the capacity of human skin to produce vitamin D3. J Clin Invest. 1985 Oct;76(4):1536-8.

Malayan AS, Shakhsuvaryan ML, Grigoryan GL, Melkonyan AK. Retinal vein occlusion in Armenia. Eur J Ophthalmol. 1999 Jul-Sep;9(3):196-201.

Manfredini R, Gallerani M, Boari B, Salmi R, Mehta RH. Seasonal variation in onset of pulmonary embolism is independent of patients' underlying risk comorbid conditions. Clin Appl Thromb Hemost. 2004 Jan;10(1):39-43.

Mann I. The Development of the Eye. Grune & Stratton Inc., New York 1969 p 228.

Marcucci R, Bertini L, Giusti B, Brunelli T, Fedi S, Cellai AP, Poli D, Pepe G, Abbate R, Prisco D. Thrombophilic risk factors in patients with central retinal vein occlusion. Thromb Haemost. 2001 Sep;86(3):772-6.

Marti-Soler H, Gubelmann C, Aeschbacher S, Alves L, Bobak M, Bongard V, Clays E, de Gaetano G, Di Castelnuovo A, Elosua R, Ferrieres J, Guessous I, Igland J, Jørgensen T, Nikitin Y, O'Doherty MG, Palmieri L, Ramos R, Simons J, Sulo G, Vanuzzo D, Vila J, Barros H, Borglykke A, Conen D, De Bacquer D, Donfrancesco C, Gaspoz JM, Giampaoli S, Giles GG, Iacoviello L, Kee F, Kubinova R, Malyutina S, Marrugat J, Prescott E, Ruidavets JB, Scragg R, Simons LA, Tamosiunas A, Tell GS, Vollenweider P, Marques-Vidal P. Seasonality of cardiovascular risk factors: an analysis including over 230 000 participants in 15 countries. Heart. 2014 Oct;100(19):1517-23.

Marti-Soler H, Gonseth S, Gubelmann C, Stringhini S, Bovet P, Chen PC, Wojtyniak B, Paccaud F, Tsai DH, Zdrojewski T, Marques-Vidal P. Seasonal variation of overall and cardiovascular mortality: a study in 19 countries from different geographic locations. PLoS One. 2014 Nov 24;9(11).

McIntosh RL, Rogers SL, Lim L, Cheung N, Wang JJ, Mitchell P, Kowalski JW, Nguyen HP, Wong TY. Natural history of central retinal vein occlusion: an evidence-based systematic review. Ophthalmology. 2010 Jun;117(6):1113-1123.

Mendis S, Puska P, Norrving B. Geneva: World Health Organization; 2011. Global atlas on cardiovascular disease prevention and control.

Meyer CH, Krohne TU, Holz FG. Intraocular pharmacokinetics after a single intravitreal injection of 1.5 mg versus 3.0 mg of bevacizumab in humans. Retina. 2011 Oct;31(9):1877-84.

Minami J, Kawano Y, Ishimitsu T, Yoshimi H, Takishita S. Seasonal variations in office, home and 24 h ambulatory blood pressure in patients with essential hypertension. J Hypertens. 1996 Dec;14(12):1421-5.

Mitchell P, Smith W, Chang A. Prevalence and associations of retinal vein occlusion in Australia. The Blue Mountains Eye Study. Arch Ophthalmol. 1996 Oct;114(10):1243-7.

Molinari C, Uberti F, Grossini E, Vacca G, Carda S, Invernizzi M, Cisari C. 1α,25-dihydroxycholecalciferol induces nitric oxide production in cultured endothelial cells. Cell Physiol Biochem. 2011;27(6):661-8.

Murdoch IE, Rosen PH, Shilling JS. Neovascular response in ischaemic central retinal vein occlusion after panretinal photocoagulation. Br J Ophthalmol. 1991 Aug;75(8):459-61.

Neild PJ, Syndercombe-Court D, Keatinge WR, Donaldson GC, Mattock M, Caunce M. Cold-induced increases in erythrocyte count, plasma cholesterol and plasma fibrinogen of elderly people without a comparable rise in protein C or factor X. Clin Sci (Lond). 1994 Jan;86(1):43-8.

Nordic Nutrition Recommendations 5th edition 2012 p369. Available at: http://www.norden.org/en/theme/themes-2014/nordic-nutrition-recommendation/nordic-nutrition-recommendations-2012 Accessed March 12 2015.

O'Mahoney P, Wong T, Ray J. Retinal vein occlusion and traditional risk factors for atherosclerosis. Arch Ophthalmol. 2008;126:692–699.

Peters A, Dockery DW, Muller JE, Mittleman MA. Increased particulate air pollution and the triggering of myocardial infarction. Circulation. 2001 Jun 12;103(23):2810-5.

Prager F, Michels S, Kriechbaum K, Georgopoulos M, Funk M, Geitzenauer W, Polak K, Schmidt-Erfurth U. Intravitreal bevacizumab (Avastin) for macular oedema secondary to retinal vein occlusion: 12-month results of a prospective clinical trial. Br J Ophthalmol. 2009 Apr;93(4):452-6.

Proudfoot D, Skepper JN, Hegyi L, Bennett MR, Shanahan CM, Weissberg PL. Apoptosis regulates human vascular calcification in vitro: evidence for initiation of vascular calcification by apoptotic bodies. Circ Res. 2000 Nov 24;87(11):1055-62.

Radke KJ, Izzo JL Jr. Seasonal variation in haemodynamics and blood pressure-regulating hormones. J Hum Hypertens. 2010;24:410-6.

Rehak M, Rehak J, Müller M, Faude S, Faude F, Siegemund A, Krcova V, Slavik L, Hasenclever D, Scholz M, Wiedemann P. The prevalence of activated protein C (APC) resistance and factor V Leiden is significantly higher in patients with retinal vein occlusion without general risk factors. Case-control study and meta-analysis. Thromb Haemost. 2008 May;99(5):925-9.

Ririe DG, Cosgriff TM, Martin B. Central retinal vein occlusion in a patient with familial antithrombin III deficiency: case report. Ann Ophthalmol. 1979 Dec; 11(12):1841-5.

Risk factors for central retinal vein occlusion. The Eye Disease Case-Control Study Group. Arch Ophthalmol. 1996 May;114(5):545-54.

Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, Kovacs CS, Mayne ST, Rosen CJ, Shapses SA. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab. 2011 Jan;96(1):53-8.

Sherman DL. Exercise and endothelial function. Coron Artery Dis. 2000 Mar;11(2):117-22.

Sinha P, Taneja DK, Singh NP, Saha R. Seasonal variation in prevalence of hypertension: Implications for interpretation. Indian J Public Health. 2010 Jan-Mar;54(1):7-10.

Spaide RF. Peripheral areas of nonperfusion in treated central retinal vein occlusion as imaged by wide-field fluorescein angiography. Retina. 2011May;31(5):829-37.

Stefánsson E, Novack RL, Hatchell DL. Vitrectomy prevents retinal hypoxia in branch retinal vein occlusion. Invest Ophthalmol Vis Sci. 1990 Feb;31(2):284-9.

Stefánsson E. Physiology of vitreous surgery. Graefes Arch Clin Exp Ophthalmol. 2009 Feb;247(2):147-63.

Stewart MW. Pharmacokinetics, pharmacodynamics and pre-clinical characteristics of ophthalmic drugs that bind VEGF. Expert Rev Clin Pharmacol. 2014 Mar;7(2):167-80.

Sugden JA, Davies JI, Witham MD, Morris AD, Struthers AD. Vitamin D improves endothelial function in patients with Type 2 diabetes mellitus and low vitamin D levels. Diabet Med. 2008 Mar;25(3):320-5.

Tekeli O, Gürsel E, Buyurgan H. Protein C, protein S and antithrombin III deficiencies in retinal vein occlusion. Acta Ophthalmol Scand. 1999 Dec;77(6):628-30.

The Central Vein Occlusion Study Group N report. A randomized clinical trial of early panretinal photocoagulation for ischemic central vein occlusion. Ophthalmology 1995;102:1434-1444.

Vandenbroucke E, Mehta D, Minshall R, Malik AB. Regulation of endothelial junctional permeability. Ann N Y Acad Sci. 2008 Mar;1123:134-45.

Wakabayashi T, Oshima Y, Sakaguchi H, Ikuno Y, Miki A, Gomi F, Otori Y, Kamei M, Kusaka S, Tano Y. Intravitreal bevacizumab to treat iris neovascularization and neovascular glaucoma secondary to ischemic retinal diseases in 41 consecutive cases. Ophthalmology. 2008 Sep;115(9):1571-80.

Wang L, Song Y, Manson JE, Pilz S, März W, Michaëlsson K, Lundqvist A, Jassal SK, Barrett-Connor E, Zhang C, Eaton CB, May HT, Anderson JL, Sesso HD. Circulating 25-hydroxy-vitamin D and risk of cardiovascular disease: a meta-analysis of prospective studies. Circ Cardiovasc Qual Outcomes. 2012 Nov;5(6):819-29.

Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, Benjamin EJ, D'Agostino RB, Wolf M, Vasan RS. Vitamin D deficiency and risk of cardiovascular disease. Circulation. 2008 Jan 29;117(4):503-11.

Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. J Clin Endocrinol Metab. 1988 Aug;67(2):373-8.

Webb AR, Engelsen O. Calculated ultraviolet exposure levels for a healthy vitamin D status. Photochem Photobiol 2006;82:1697-703.

Williamson TH, Grewal J, Gupta B, Mokete B, Lim M, Fry CH. Measurement of PO2 during vitrectomy for central retinal vein occlusion, a pilot study. Graefes Arch Clin Exp Ophthalmol. 2009 Aug;247(8):1019-23.

Wittert GA, Or HK, Livesey JH, Richards AM, Donald RA, Espiner EA. Vasopressin, corticotrophin-releasing factor, and pituitary adrenal responses to acute cold stress in normal humans. J Clin Endocrinol Metab. 1992 Sep;75(3):750-5.

Yap YC, Barampouti F. Central retinal vein occlusion secondary to protein S deficiency. Ann Ophthalmol (Skokie). 2007 Winter;39(4):343-4.

Yasuda M, Kiyohara Y, Arakawa S, Hata Y, Yonemoto K, Doi Y, Iida M, Ishibashi T. Prevalence and systemic risk factors for retinal vein occlusion in a general Japanese population: the Hisayama study. Invest Ophthalmol Vis Sci. 2010 Jun;51(6):3205-9.

Yau JW, Lee P, Wong TY, Best J, Jenkins A. Retinal vein occlusion: an approach to diagnosis, systemic risk factors and management. Intern Med J. 2008 Dec;38(12):904-10.

Zehnder D, Bland R, Chana RS, Wheeler DC, Howie AJ, Williams MC, Stewart PM, Hewison M. Synthesis of 1,25-dihydroxyvitamin D(3) by human endothelial cells is regulated by inflammatory cytokines: a novel autocrine determinant of vascular cell adhesion. J Am Soc Nephrol. 2002 Mar;13(3):621-9.

Zhang Y, Leung DY, Richers BN, Liu Y, Remigio LK, Riches DW, Goleva E. Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. J Immunol. 2012 Mar 1;188(5):2127-35.

Zhu Q, Ziemssen F, Henke-Fahle S, Tatar O, Szurman P, Aisenbrey S, Schneiderhan-Marra N, Xu X; Tübingen Bevacizumab Study Group, Grisanti S. Vitreous levels of bevacizumab and vascular endothelial growth factor-A in patients with choroidal neovascularization. Ophthalmology. 2008 Oct;115(10):1750-5.