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Venous Circulation in Glaucoma

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Chapter 1. Introduction

Glaucoma is the second most common cause of blindness in the developed world. Nearly 9 million people worldwide are bilaterally blind from this disease, with up to 1% of all population over the age of 40 presenting with some form of the disease (1). Unfortunately, around 50% of these patients go undetected, as most of this permanent vision loss is asymptomatic until the very late stages of the disease (2).

Worldwide, the most common type of the disease is primary open-angle glaucoma (POAG). As the name implies, no cause can be detected for this optic neuropathy. In most cases, the only identifiable risk fact is an increased intraocular pressure (IOP). However, there are patients in which IOP has never been detected to be above the normal range, in which case they are considered to have normal-tension glaucoma (NTG). Importantly, this form of the disease can represent up to 50% of glaucoma population in the western hemisphere (3), while in Japan this figure can go up to 90% (4). Thus, and while IOP is the main risk factor and IOP lowering remains the cornerstone for glaucoma management, IOP cannot explain all the mechanisms involved in glaucoma pathogenesis. This claim is widely supported by the established fact that a significant number of patients still progress despite an otherwise successful reduction in IOP (5) (6).
As such, the quest for the identification of other non-IOP related pathogenic mechanisms has been the work of countless research groups worldwide. The vast majority of the studies regarding non-IOP related risk factors, such as vascular disturbances, have been focused on the arterial component, with an increasing accumulation of data suggesting the existence of defective autoregulation mechanisms (7) (8) (9).

The emphasis on ocular blood flow studies in glaucoma has been particularly appealing for researchers in glaucoma pathogenic mechanisms, as these changes in ocular blood flow seem to precede glaucomatous damage (10) (11).

Despite these efforts to understand the ocular blood flow in glaucoma, very little is still known about the venous circulation in the eye and its potential role in glaucoma pathogenesis. Venous circulation may have an impact on glaucoma by two different mechanisms: on the one hand, it is responsible for the drainage of the aqueous humour and thus could play a role in modulating the main risk factor for the disease (IOP). Additionally, as elsewhere in the body, any change in retinal venous pressure can significantly impact the ocular perfusion pressure – another important factor in glaucoma progression.

A wider knowledge of what influences venous output is therefore important not only to better understand the ocular physiology, but also to better understand how these physiological mechanisms
might be impaired in glaucoma patients. This information could be particularly important in the interpretation of ocular blood flow studies in glaucoma, especially in the more vascular-prone NTG patients.
I. Glaucoma

The disorder now identified as glaucoma has been known to man throughout the ages. The oldest description to have survived until nowadays was made in Greece around 400 BC (12), where a Hippocratic text states a condition where ‘once the pupil has the colour of the sea – eyesight is destroyed and you will often find that the other eye is also blind’. While this description was used to label a number of diseases throughout history, idea of a distinct disease associated with an increased stiffness of the eyeball was first identified by McKenzie in 1830, branding it with the name Glaucoma (13). However, the emphasis on a pressure-related disease was questioned by Jaeger already in 1858, suggesting other intrinsic non-IOP related causes for the disease (14) and in 1885, Smith suggested that vascular factors were likely involved in the process (15). Since then, the controversy has remained between the relevance of either the mechanic or vascular theories to the pathogenesis of open-angle glaucomas.

In fact, other than the widely recognized risk factor of increased IOP, a number of other variables have been identified as risk factors for disease progression. The severity of damage at the time of diagnosis, increasing age, low central corneal thickness (CCT), but also cardiovascular variables such as low ocular perfusion pressure and a history of cardiovascular disease have all been associated with worsening of the disease (16). While IOP remains the most easily modifiable risk factor, the impact of the other
variables may be more significant when all IOP measurements are found to be within the normal range.

The distinction of NTG and POAG patients has been determined as patients with the former having a maximum registered IOP of equal or below 21mmHg. This may, however, be an arbitrary division as they may represent two points in a larger spectrum of optic neuropathies of variable sensitiveness to IOP (16). Nevertheless, and despite patients with NTG being more prone to show signs of vascular dysfunction, from migraine (17), peripheral vasospasm (18), systemic hypotension (19) to cerebral microvascular ischemia (20), they still present a significant relation to IOP. In fact, even in these otherwise normal-range IOP patients, lowering this variable has been shown to slow disease progression (21).

The modern definition of glaucoma thus reflects the acknowledgment that this disease is a progressive neurodegenerative process, where an elevated IOP is only one of the involved mechanisms. Indeed, the European Glaucoma Society currently defines primary open-angle glaucomas as chronic, progressive optic neuropathies that have in common characteristic morphologic changes at the optic nerve head and retinal nerve fiber layer, in the absence of other ocular disease or congenital abnormalities (16).
While the relentless retinal ganglion cell death and progressive visual field loss can be detected by modern structural and functional tests, respectively, the ocular circulation intricate anatomy and physiology makes its study (and interpretation) a complex issue. A number of technologies have emerged to assess the normal ocular blood flow and its change in glaucoma patients (22) (23) (24) (25). One of the more popular techniques is the study of the retrobulbar arteries’ flow velocities by color Doppler Imaging (CDI). Glaucoma patients have been consistently demonstrated to have different patterns in the Doppler waveform, from decreased velocities to increased resistivity index (10) (26) (27) (28). However, the interpretation of these variables has been limited compared to other medical specialities. Glaucoma ocular blood flow studies have put the emphasis on the arterial peak and trough blood velocities and on the resistance index that can be calculated from them. As cardiologic and neurologic studies have demonstrated, a significant number of additional information can be retrieved from the arterial Doppler Waveform (29) (30). As such, by integrating these knowledge into ocular blood flow studies, our current understanding of the arterial blood flow in glaucoma is evolving at a fast pace. However, little is known about venous flow patterns in the eye, its correlation with other cardiovascular or ocular variables or even whether glaucoma patients have any detectable venous dysfunction.
II. **Ocular Circulation**

In order to address any question regarding ocular blood flow, one must be aware of the particular vascular anatomy of the eye, particularly around the disease’s primary location: the optic nerve head (ONH). Henceforth, an anatomic description of the vascular anatomy involved in glaucoma (both venous and arterial) will be depicted, as well as a summary of its complex physiology.

II. **A) Arterial Circulation**

All blood to the optic nerve comes from the carotid artery through its ophthalmic artery (OA) branch. This OA follows a tortuous path inside the orbit towards the anterior nasal orbital wall, crossing the optic nerve as the short posterior ciliary arteries, the long posterior ciliary arteries and the central retinal artery (CRA) branch off.

The short ciliary arteries are responsible for the supply of most of the choroid, while the long posterior ciliary arteries enter the eye next to the optic nerve and travel anteriorly between the sclera and the choroid, until the ciliary body. By creating the iris major arterial circle, these long ciliary arteries supply most of the anterior segment of the eye.

The OA gives additional branches to the lacrimal artery, the ethmoidal arteries, the muscular arteries and medial palpebral arteries before dividing into two terminal branches (frontal and nasal dorsal arteries). Considering this ocular and extra-ocular
branching, the amount of blood from the OA going into the ocular structures does not exceed 25% of the total (31).

The neuron cell most affected by glaucoma, the ganglion cells and their axons along the retinal nerve fiber layer, are exclusively supplied by the CRA. This artery penetrates the optic nerve sheath, crossing a layer of cerebrospinal fluid before reaching the lamina cribrosa already within the optic nerve mass. After a short pathway in the optic nerve head, it divides into 4 smaller arteries, forming the two vascular arcades which are responsible for supplying the inner retinal layers.

The blood supply to the ONH is conditioned by this structure’s complex anatomy and its relation with the lamina cribrosa. Its four different vascularization compartments reflect the unique pathway of the ganglion cells’ axons as they move from the innermost part of the retina towards the outside of the eye. The most anterior part, named surface nerve fiber layer, is supplied by the retinal arteries. At a more posterior level, the prelaminar and laminar compartments are supplied by branches of the short posterior ciliary artery, which sometimes encircle the ONH, creating the Zinn-Haller ring. This functional anastomosis could theoretically protect the optic nerve from occlusion or hypoperfusion of a single short posterior ciliary artery. The most posterior compartment, the retrolaminar region, is mostly supplied by pial vessels that give off centripetal branches into the septa of the optic nerve.
II. B) Venous Circulation

The terminal pathways for most of the venous drainage of the orbit are the ophthalmic veins. However, for practical purposes, this process can be divided into the venous drainage of the retina on one hand and the venous drainage of the rest of the ocular structures on the other.

Blood flows from the retinal capillaries towards the retinal venules in a centripetal pattern towards the ONH. This pathway resembles the arterial branching, by forming two arcades around the macula with increasing diameters as they approach the optic disc. At this stage, the two hemiveins merge into a central retinal vein (CRV). This vessel is always located temporal to the corresponding CRA (32). From this point, the CRV leaves the eye through the lamina cribrosa. After leaving the eye within the optic nerve, the CRV pierces the optic nerve, crossing the space between the optic nerve and its sheath. This sheath is separated from the optic nerve by a laminar layer of cerebrospinal fluid, which is in continuity with the rest of the central nervous system. Having passed the sheath, the CRV most frequently merges itself with the superior ophthalmic vein (33), although it can also drain directly into the cavernous sinus (34) and then onward to the right atrium through the central nervous venous system.

The other aspect in ocular venous circulation of particular importance in glaucoma is the one involved in the aqueous
humour drainage. Aqueous humour is secreted into the eye’s posterior chamber through the ciliary processes. This aqueous humour is produced by both active secretion of solutes and water diffusion, obtained from blood circulation. The function of this solution is to nurture the inner segments of the non-vascularized cornea, to provide the eye with appropriate tonus, and to remove metabolic end-products, among others. The aqueous humour travels into the anterior chamber through the pupil and exits the eye by passing through the trabecular meshwork into Schlemm’s canal. After entering Schlemm’s canal, the aqueous humour enters the collector channels that communicate with the aqueous veins. These four to five aqueous veins are sparsely visible near the limbus and have a short length before merging with the episcleral veins (35). In turn, these episcleral veins then connect with the anterior ciliary veins (responsible for drainage of the eye’s anterior segment), which, together with the vortex veins (responsible for the drainage of the choroid), drain into the ophthalmic veins. While the main part of the blood exiting the ocular tissues enters directly the superior ophthalmic vein, a much smaller portion that arises from the lower part of the eye enters the inferior ophthalmic vein. The latter takes a pathway through the floor of the orbit before merging with the superior ophthalmic vein near the annulus of Zinn (34).

This system is based on passive diffusion. As such, any change in pressure throughout the venous pathway (from the aqueous vein
up to the cavernous sinus and the right atrium) is theoretically able to interfere with aqueous humour drainage and therefore influence IOP (36) (37).
II. C) Ocular Blood flow regulation

The regulation of ocular blood flow differs between the various ocular compartments. As elsewhere in the body, blood flow in the eye should be under the control of the autonomic nervous system. However, as this innervation stops at the level of the lamina cribrosa, the retinal circulation is not regulated by sympathetic output. Instead, retinal vessels have the ability, through not completely understood mechanisms, to constrict or dilate in response to changes in oxygen or pH, thus maintaining a constant metabolic environment despite exposure to conditions that might upset this equilibrium.

The choroidal circulation, on the contrary, is under the control of the autonomic nervous system and has no intrinsic ability to adapt to these stimuli. It is able to decrease or increase blood flow in response to cervical sympathetic stimulation, but it cannot adapt to sudden changes in IOP, for example. A clinical consequence, for instance, of this inability to self-regulate its flow is the uveal effusion that can be seen when opening the eye during surgery. As a consequence of these differences in vasoreactive mechanisms, the response to medical therapy also differs between these vascular beds.

Much like their retinal counterparts, the ONH capillaries lack pre-capillary sphincters; they have pericytes instead. As in the retinal circulation, these pericytes respond to metabolic and
neuroendocrine factors that regulate their contractility. However, while there is no consistent evidence of an autonomic innervation directly regulating ONH blood flow, the lack of a cellular barrier separating the ONH from the choroid tissues could make the ONH susceptible to autonomic stimulations. As both are supplied by the same vessels, imbalances in the choroidal blood flow could redirect blood flow away from the ONH.

In the eye, the vascular endothelium plays a key role in the regulation of vessel tone. It regulates the blood flow in the retina, ONH and choroid by releasing agents that are responsible for vasodilation and vasoconstriction and by modifying this release in response to local metabolic needs. In vitro and in vivo studies have provided evidence for the role of endothelium-derived vasoactive substances in the control of blood flow in the retina, ONH and choroid (38) (39). Nitric oxide is involved in the control of basal blood flow in the choroid, optic nerve and retina by maintaining the basal vasodilator tone. A sufficient blood supply to the ocular circulation requires the maintenance of a basal vasodilator tone in ocular arteries. As in many other arterial beds, this is largely provided by a constant formation of nitric oxide (NO) by NO synthase 1 and 3 (40) at basal conditions. Hence, systemic administration of an NO synthase inhibitor reduces blood flow in the choroid, retina and ONH (41) (42) (43), indicating a contribution of NO to vascular tone in human ocular vasculature. In addition, a number of agonist-induced vasodilatory effects have
been shown to be NO-dependent, including those to histamine
(44), insulin (45) and carbon dioxide (CO2) (46). Another key
regulator of ocular blood flow is endothelin. Exogenous endothelin
dose-dependently reduces retinal, choroidal and ONH blood flow
(47) (48) (49) which is reversible by blockade of the endothelin A
receptor subtype. Blockade of this receptor also modifies the
choroidal blood flow response to isometric exercise, which strongly
indicates a role of endothelin in choroidal blood flow regulation
during changes in perfusion pressure (50). In addition, the potent
vasoconstrictive effects of hyperoxia in the retina appear to be at
least partially mediated via this endothelin receptor (51).
Endothelial cell dysfunction produces an imbalance between
vasodilator and vasoconstrictor pathways, most notably the NO
and endothelin systems (52). Endothelin has been implicated as a
contributory factor in many vasospastic disease processes,
including vasospastic coronary artery disease (53), cerebral
vasospasm (54) and Raynaud’s disease (55). Vasospasm,
characterized by exaggerated vascular responses to various stimuli
such as temperature and stress (56) (57), is a transient, reversible
vasoconstriction that results from impaired endothelium-
dependent regulation of vascular tone (58). The possibility that
endothelin contributes to vasospasm in ocular diseases such as
glaucoma is supported by the demonstration of elevated basal
plasma endothelin concentrations in patients (59), combined with
an abnormal response of plasma endothelin concentrations to
postural (60) and temperature changes (61).
III. Ocular Blood Flow in Glaucoma

The above described mechanisms of blood flow regulation may be impaired in glaucoma patients, with a growing number of publications showing disturbances in these patients’ ocular blood flow. These changes extend beyond the ocular circulation, with systemic circulation also showing signs of regulatory impairment, resulting in glaucoma being called “a sick eye in a sick body” (62). However, the nature of these disturbances is still under discussion, including weather such changes are part of the pathogenic mechanisms or secondary to the underlying disease.

When considering ocular diseases in which vascular mechanisms are involved, such as diabetic retinopathy or occlusion of either of the central retinal vessels, none of these diseases produce a characteristic glaucomatous cupping of the disc (63) (64). Therefore, hypoperfusion, as seen in those diseases, does not provide a full explanation for glaucomatous neuropathy. Should hypoperfusion alone represent the vascular risk factor for glaucoma (progression), one would expect a stronger relationship between glaucoma and known risk factors for atherosclerosis, such as C-reactive protein or dyslipidaemias. However, data on these variables is far from consistently pointing them out as risk factors for glaucoma (65). Alternatively, or in addition to hypoperfusion, there may be a vascular dysfunction that impairs the normal self-regulating mechanisms response to fluctuations in perfusion (66). Interestingly, the ONH, due to its unique anatomical condition, is
exposed to circulating hormones in a way that the rest of the central nervous system is not. Not only is the barrier function decreased in the capillaries in this region (67), but there is also diffusion from the choroid, where these adaptive mechanisms do not exist (68). Vasoconstrictive agents, such as angiotensin II and endothelin, can therefore have a greater impact in this region than elsewhere in the nervous system. Available data suggest that glaucoma patients may have an endothelial dysfunction, with increased levels of endothelin (69) and vasodilation impairment (70), thus increasing the risk for ONH injury. Such dysfunction, as suggested by Flammer, consists of an instability of ocular perfusion (rather than a stable reduction in ocular blood flow), leading to a repeated mild reperfusion injury (71) (72). This mechanism of ischaemia-reperfusion injury is associated with the generation of reactive oxygen species and cellular apoptosis. The resulting increase in vasoconstriction and oxidative stress can lead to an activation of several apoptotic pathways through cellular dysfunction (73). It has been suggested that this oxidative stress could also occur as a result of elevated IOP, in what seems to be an IOP-related mechanical stress event (74). In humans, the role of this cellular hypoxia in glaucoma is further supported by increased staining of hypoxia-induced factor (HIF-1α) in the retina and optic nerve of patients with glaucoma compared with non-glaucomatous individuals (75). This impaired self-regulation capacity of the vessels supplying the ONH may be clinically relevant. Recent studies have shown that fluctuations in perfusion pressure are
particularly important in glaucoma progression, especially in patients with NTG (76). Glaucoma patients are also more prone to show abnormal circadian vascular rhythms that can tip the balance of perfusion pressure into pathogenic levels. These abnormal circadian cardiovascular responses may be due to an underlying dysautonomic disturbance, as it is the autonomic nervous system’s responsibility to regulate these daily rhythms (77). Glaucoma patients seem to have a number of signs of autonomic dysfunction, from dysregulation of aqueous humour production and drainage (78) to abnormal heart rate and blood pressure variability, both of which are associated with increased cardiovascular risk (79) (80). This blood pressure variability is particularly prominent in patients with NTG, and a correlation between the extent of autonomic nervous system dysfunction and the severity of the disease has been suggested (78).

A number of studies have shown that this vascular dysfunction and impairment in normal blood flow associated with glaucoma is not restricted to the eye. Indeed, there are also indications of slower flow in peripheral capillary beds (81) and signs of microvascular encephalopathy, such as white matter lesions (20). This association between ocular circulation and systemic cardiovascular disease has been reinforced by epidemiological findings. Pooled data from the Beaver Dam Eye Study and the Blue Mountains Eye Study have demonstrated that smaller retinal arterial diameters and larger retinal venous diameters are associated with increased risk for
stroke mortality (82) (83). One of the most striking disturbances in ocular vessels, the splinter optic disk haemorrhages, are an important clinical feature that has been associated with glaucoma progression. These haemorrhages occur more frequently in patients with NTG glaucoma and are strongly associated with altered circulation within the optic disc (84) (85). Some authors have suggested that these splinter haemorrhages may represent distressed small venules (86), as these thinner veins may reflect earlier lamina cribrosa changes than their thicker arterial counterparts (87).

The changes in ocular blood flow are not restricted to the retinal vessels, as changes of clinical significance have also been found in the retrobulbar circulation. A number of CDI studies have found reduced peak systolic and diastolic velocities and increased resistivity indices in the retrobulbar vessels of glaucoma patients compared to healthy normal controls (88) (89) (26) (27). Interestingly, patients that progress seem to have a more important alteration in blood flow, namely, reduced PSV and EDV in short posterior ciliary arteries (90). Moreover, a prospective study showed that within glaucomatous individuals, the eye with the more pronounced blood flow impairment also showed a faster progression (91).
The extent of the damage induced by ocular blood flow impairment alone is difficult to determine. Blood flow changes per se might lead to glaucoma damage, but they could also synergistically act with other risk factors. For example, blood flow disturbances might act as a sensitizer to IOP, making it possible for normal-range values of IOP to produce glaucomatous damage.
IV. Venous dysfunction in glaucoma

Although studies on ocular venous changes in glaucoma are few when compared to their arterial counterparts, venous circulation seems to differ between glaucoma patients and healthy individuals.

Aqueous veins in glaucoma patients have a significant change in an otherwise normal pulsatility pattern (92) (93). Normal blood flow fluctuations during the cardiac cycle in the arterial-vein transition around the trabecular meshwork creates a pressure gradient acting as a Venturi-like pump, bringing an aqueous humour pulse wave inside the aqueous vein (94). This cyclic wave absorption of aqueous humour into vessels containing blood can be seen as a pulsating pattern. Glaucoma patients, however, have a much lesser frequency of this pattern. While the increased trabecular meshwork resistance could account for a smaller aqueous humour wave, the downstream change in venous pressure could also dampen this wave (95). In fact, agents such as brimonidine (96) and epinephrine (97) have been demonstrated to increase stroke volume and flow velocity in these veins. As both these drugs do not interfere with trabecular meshwork physiology directly, but can interfere directly and indirectly with episcleral venous pressure, these data reinforce the importance of venous function in an adequate aqueous humour drainage.
Retinal venous circulation is not involved in aqueous humour drainage. Instead, it is responsible for the drainage of the blood supply of the retina and the ONH. As in any other part of the organism, venous pressure is involved in perfusion pressure. The perfusion pressure of an organ is the difference between arterial input pressure and venous output pressure in relation to the organ’s vascular resistance. This vascular resistance is nevertheless dependent not only on the pressure difference of the vessel’s pressure but also on variables such as blood viscosity and vascular diameter. As venous circulation is a low pressure system in which blood flows into the lowest pressure point (the right atrium), its systemic value (central venous pressure) is usually in the range of 2-3 mmHg (98). In the eye however, venous pressure has to at least match IOP (99) (100). Any other possibility would lead to a constant vessel collapse and no blood flow (101). As such, the classical calculations of ocular perfusion pressure have used the assumption that venous pressure resembles IOP and uses this latter easily verifiable variable as a surrogate.

This assumption has been, however, increasingly questioned by a number of authors. In experimental conditions, venous pressure has been consistently demonstrated to be at least 5mmHg higher than IOP (102) (103). While this difference would be small enough to force a change in paradigm, a number of publications have shown retinal venous pressure to be significantly higher in glaucoma patients (87) (104) (105). While the nature of this
increase in venous pressure is under debate, it would still mean that the calculations of ocular perfusion pressure in such patients could be significantly overestimated. This is particularly important as low perfusion pressures have been consistently proven to be associated with disease progression (16).

Additionally, the notion that retinal vein physiology may be disturbed in glaucoma patients is reinforced by the suggestion that optic nerve haemorrhages, a major clinical sign of disease deterioration, may represent distressed venules. In these patients, these venules would, for still undetermined reasons, be unable to meet the increased intravascular pressure and rupture (106). Another aspect which shows a link between retinal vein dysfunction and glaucoma is the fact that glaucoma is a major risk factor for CRV occlusion (107). The nature of this link is, again, incompletely understood. Some authors have suggested that changes in the lamina cribrosa associated with increased IOP in glaucoma patients may increase extramural pressure, thus increasing the chances of a thrombotic event (108) (109).

In both conditions, the questions remains as to whether this apparently deleterious increase in retinal venous pressure is a consequence of glaucoma or whether it may be associated with the pathogenesis of the disease (by decreasing perfusion pressure).
V. Spontaneous Venous Pulsation Phenomenon

Perhaps the most clinically appealing sign of venous changes in glaucoma patients is the significant decrease in frequency of the CRVs spontaneous venous pulsation (SVP) phenomenon over the optic disc. From up to 98% in the healthy population, this easily, clinically verifiable phenomenon decreases to nearly half in glaucoma patients (110) (111).

Importantly, especially when considering the detection of this variable in a disease such as glaucoma, the visualization of this phenomenon seems to be dependent on the optic disc characteristics and the linearity of vessel’s pathway in the disc (112). However, little is known on how morphometric variables such as depth or cup/disc ratios influence such pulsatility detection. Whether the changes in the ONH associated with glaucoma damage have any impact on the SVP phenomenon remains unknown.

Independently on the threshold of detection, the mechanisms behind this intriguing phenomenon are not completely understood. Described in 1853 (113), two theories exist as to why the retinal veins pulsate near the optic disc.
The classical theories, presented in the early years of the XX century (114) (115), propose that retinal vein pulsations occur because of a periodic reversal of the pressure gradient across the wall of the retinal vein. The blood pressure inside the vein – retinal venous pressure – and the fluid pressure of the eye outside the vein – intraocular pressure (IOP) – each vary during the cardiac cycle. During the part of the cardiac cycle when the IOP exceeds the retinal pressure, the vein would collapse. When that retinal pressure again becomes higher than the IOP, the vein would re-expand. This theory would suggest that vein collapse would happen during systole. The increased input of blood during systole inside the relatively unexpandable eyeball would lead to a relatively higher increase in IOP. As retinal pressure would fluctuate in a retrograde transmission from the right atrium, it would be higher during diastole. It would be during this period that retinal vein pressure would be higher and the vein would therefore reexpand.

The previous theories assume there is no connection between the fluctuations in IOP and the retinal pressure. However, this is not possible. IOP fluctuates only because the input of blood during a certain period of the cardiac cycle is not matched by a similar increase in the blood output (venous flow). Should venous flow increase in a similar magnitude as the arterial input, no change in blood volume would occur and accordingly, the ocular pulse amplitude would be zero. This rise in IOP from the excess arterial
inflow must, therefore, be transmitted instantaneously to the contents of the capillaries and the veins. The only possibility for this increase in outside pressure not to change retinal venous pressure would be if the walls of these veins were rigid enough to maintain a similar transmural gradient throughout the cardiac cycle. This is not the case in the eye (116). Therefore, and since IOP does fluctuate, both IOP and retinal pressure must fluctuate in phase (116).

A new theory thus emerged that takes into account the fact that IOP is always lower than retinal venous pressure (116). This new approach takes into account the pulse pressures in the different compartments involved in the venous circulation. In fact, as CRV traverses the lamina cribrosa, the extravascular pressure drops from IOP to the cerebrospinal fluid pressure over a distance less than 0.5mm (117). In this hypothesis, the fluctuation of IOP (and therefore retinal venous pressure) during a cardiac cycle is greater than the fluctuation in the cerebrospinal fluid pressure (118). Although these fluctuations are small enough not to reverse the pressure gradient, the prelaminar to postlaminar gradient is higher than the mean value during systole and below its mean in the diastole. If it is assumed that vein diameters throughout the retina are not changed, the blood flow at the retinal vein exit will follow the pressure gradient, rising in systole and falling in diastole. Since flow into the retinal veins is constant, but flow velocities fluctuate, pulsations must occur.
Despite addressing some of the inconsistencies of the classical theory, this latter approach assumes that the collapse of the vessels happens during the diastolic component of the cardiac cycle. This, however, has been challenged recently (105), with authors suggesting the opposite pattern. This would raise the possibility for this phenomenon to occur when IOP and cerebrospinal pressure pulses have different amplitudes or despite an out-of-phase synchronicity.

Independently of the theories used, both agree on the fundamentals of the clinical use of this pulsating vein variable. Since its original description, this sign has been used to exclude the existence of an increased intracranial pressure (113) (119). The loss of this pulsating sign has been additionally described to identify ocular hypotony and retinal venous occlusion (117) (120). Because both theories rely on the amplitude of fluctuation in the ocular, extraocular and retinal vessel compartments, all of the described conditions (high ICP, low IOP and higher retinal venous pressure) would buffer the translaminar gradient and decrease the likelihood of SVP detection. The importance for this balance in fluctuating compartments has been further supported by the suggestion of a minimal ocular pulse amplitude threshold to exist (1.2mmHg), below which no SVP would be visible (121). The level of fluctuations in the other compartments or the existence of a minimal threshold in any of them that condition the SVP
phenomenon remains unanswered, particularly in glaucoma patients.

Glaucoma patients have been suggested to have decreased ICP values (122) (123). Together with higher values of IOP, both these variables would increase the likelihood of SVP detection by synergistically increasing translaminar gradient pressure. As such, glaucoma patients were expected to have higher frequency ratios of SVP (124) (125). However, as previously stated, glaucoma patients have been consistently reported to have much lower frequency ratios of SVP (111) (110). The reason for these results, considering the theories’ premises, remains unclear. Whether the vascular dysfunction known to exist in glaucoma patients has a venous component as well, or whether glaucoma patients have undetermined structural, elastic or morphometric changes in any of the relevant compartments, that would make the detection of this SVP more difficult, is not yet understood.

Interesting, retinal veins are not the only veins in the body that can show pulsation. Either from proximal arterial transmission or downstream changes in heart pressure, veins such as the jugular vein can present with detectable pulsation. The significance of jugular waveform changes has been an important part of medical semiology in the study of heart conditions such as tricuspid insufficiency or pericarditis. Whether SVP determination in retinal veins could have the same impact in glaucoma patients remains to be answered.
Chapter 2. Purpose

I. Global Objective

This project was designed to characterize the ocular venous circulation in glaucoma patients and to determine the anatomical and hemodynamic factors that influence its parameters, specifically their association to the SVP phenomenon.

A case-control study using two sets of glaucoma groups (both high-IOP and more vascular-prone low-IOP patients) was used to detect whether any change in variables would be specific to one group or common to all glaucoma patients. As diagnostic workout, treatment algorithm and risk factors can be different in NTG and POAG diagnosis, our study tried to address whether venous dysfunction, if found in these patients expressing a non-pulsating venous circulation, would be of any additional value to in the daily management of glaucoma patients.

The decision to use tools that are readily available in any hospital reinforces the claim to provide information which can potentially be used in every Ophthalmology unit. Even the highly-expensive ultrasound machines used for color Doppler Imaging are available in radiology departments worldwide.
II. **Main goals**

1. To characterize the frequency of SVP in glaucoma patients (both primary open-angle glaucoma and normal tension glaucoma).

2. To characterize anatomical factors that relate to the SVP phenomenon observed in glaucoma patients.

3. To characterize hemodynamic factors that relate to the venous circulation changes observed in glaucoma patients.

4. To characterize signs of vascular dysfunction and their association to venous circulation variables in glaucoma patients.
Chapter 3. Methods

This study on venous circulation in glaucoma was conducted in the Glaucoma Clinic of the Department of Ophthalmology of the University Hospitals of Leuven, Belgium. The measuring devices that exist in the several centers initially involved (Centro Hospitalar de Lisboa Central, Institute of Molecular Medicine of Lisbon’s Faculty of Medicine and Leuven’s University Hospitals) have different technical specificities which would have prevented any data pooling. As such, and in coordination with both supervisors and the agreement of the Head of the Ophthalmology Department of the Belgian center (Prof. Dr. W. Spileers), the patients recruitment and overall data collection was performed entirely in Leuven.

This prospective, case-control project was in compliance with the existing guidelines for design of glaucoma trials from the World Glaucoma Association (126). The methodology will henceforth be summarily described:
I. **Subject groups**

Three cohorts of individuals over 18 years old were recruited for the study: patients with NTG, patients with POAG and healthy control subjects of comparable age.

Glaucoma was defined as having characteristic optic disc damage (based on cup/disc ratio, thinning of neuroretinal rim, notching, disk hemorrhages, etc.) and visual field defects. The criteria used for this diagnosis have been published elsewhere (127) (128). For the diagnosis of POAG, at least one measurement of IOP of >21mmHg was required, while patients with lower IOP (≤ 21 mmHg) were classified NTG.

Healthy subjects were recruited from the cataract surgery clinic at the University Hospitals Leuven, Belgium. This was designed as to reduce any potential recruiting bias, as both conditions are more common in elderly patients. Ophthalmologists in the Cataract Clinic sent for the study visit individuals that matched both inclusion and exclusion criteria and were willing to participate in the study. These healthy volunteers were furthered screened by a senior member of the glaucoma clinic (IST) and those with a family history of glaucoma, an increased or asymmetrical cup/disc ratio or any other optic disc structural change (notching, disc hemorrhage) or an IOP above 21mmHg were excluded as possible glaucoma suspects.
Only one eye was included per patient. This was done to reduce any potential bias due to systemic conditions affecting both eyes, as any disturbance in heart rate, blood pressure, intracranial pressure or any other systemic condition could impact both eyes simultaneously.

The eye with greater glaucomatous damage was selected in the glaucoma patients, which is a common strategy in glaucoma studies. This is done to increase the likelihood of statistically significant findings in a setting where the number of subjects who did not have SVP was expected to be low, according to the existing literature.

In the healthy individuals, a randomly selected eye was studied. This randomization was done by a third party [in the case, an ophthalmologist not involved in either the screening of patients or in any examination (EDC)]. In case of recent cataract surgery (less than 6 months), the other eye was selected.

In the submitted project, a total number of 100 patients (with a 2:1 ratio of glaucoma/healthy control subjects) were proposed. However, the excellent human and logistic conditions of the UZ Leuven’s Department of Ophthalmology made it possible to enlarge all groups, continuing recruitment for the whole timeframe period.
II. Informed consent

The study was approved by the ethical review committee (Institutional Review Board) at the University Hospitals Leuven and was conducted in accordance with Good Clinical Practice within the tenets of the Helsinki agreement.

Each patient/subject was required to sign an informed consent statement before being enrolled into the study and prior to any study measurements being taken.
III. Exclusion criteria

Other than the unwillingness to sign the informed consent, the following characteristics were defined as exclusion criteria:

III. A) Ocular conditions

Ametropia above 6Dp;

Known history of ocular disease other than primary open-angle glaucoma (eg: uveitic glaucoma, angle-closure glaucoma; juvenile glaucoma);

Media opacification that precluded fundus visualization;

Any optic disc abnormality or arteries’ disposition that precluded the normal observation of the veins over the optic disc was also excluded;

History of ocular vascular pathology in the other eye. Additionally, in the healthy control groups, the diagnosis of glaucoma or the use of topical/intravitreal medications in the other eye;

Ocular surgery (cataract or glaucoma surgery) in the previous 6 months prior to the study visit;

Any previous ocular surgery other than uncomplicated cataract or glaucoma surgery;

Any other eyedrop other than artificial tears (for instance, corticoids, non-steroid anti-inflammatory drugs, etc)
III. B) Systemic conditions

Known significant carotid obstruction or previous endarterectomy;

Known diagnosis of Heart Failure;

Diabetes Mellitus;

History of veno-occlusive disease;

Known diagnosis of orbital or intra-cranial disease;

In the case of glaucoma patients, the intake of systemic carbonic anhydrase inhibitors.
IV. **Measuring devices**

The following list describes the measured devices used in the study. The unit of measurement of each variable provided by each device is depicted in a separate section (see below).

IV. **A) IOP measurement**

IOP was measured using a Goldmann applanation tonometry mounted on a Haag-Streit slit lamp (Haag-Streit BQ 900, Haag-Streit International, Köniz, Switzerland). The eyes were anesthetized with oxybuprocaine 0.4% (Unicaïne 0.4%®, Théa Pharma, Wetteren, Belgium).

IV. **B) Ocular pulse amplitude measurement**

Ocular pulse amplitude (OPA) was assessed using a dynamic contour tonometer (DCT – Pascal® Ziemer Ophthalmic Systems, Switzerland). This device measures continuous IOP providing a pressure curve that is synchronous with the cardiac cycle (129). OPA is calculated from this curve from the difference between the systolic and diastolic IOP. Two consecutive measurements of at least quality-2 readings (quality-1 reading being the most reliable and 5 the least reliable reading) were obtained for each patient, and the average of these two measurements was recorded. These tonometry measurement were taken at least 5 minutes after the Goldmann tonometry, as to reduce any bias arising from corneal deformation made by the Goldman tonometry.
IV. C) Central Corneal Thickness

Central corneal thickness was measured using a contact ultrasonic pachymeter [Pachmate DGH55 (DGH Technology Inc., Exton, PA)].

IV. D) Optic disc tomography

Optic nerve tomography was performed in all eyes using Heidelberg retina tomograph III (HRT; Heidelberg Engineering, Dossenheim, Germany). The optic disc was analysed using HRT version 3.0.3.0 software. The technique is described in detail elsewhere (130). Three high-quality images at 15x15-degree scanning angle were recorded per eye. Subsequent analysis was done on a mean image. All mean images included must have had a mean standard deviation of height measurement ≤ 40µm. The optic disc margin was manually marked at the inner edge of the Elschnig’s ring. The standard reference plane was used for calculations of optic disc topography with the relative and tilted coordinate system turned on. The following variables were obtained: Disc area, Cup area and volume, rim area and volume, cup/disc overall and linear ratios, mean and maximum depth and retinal nerve fiber layer (RNFL) thickness and cross-sectional area.
IV. E) Blood pressure measurement

Blood pressure measurement was taken from the sitting’s subject’s right arm using an electronic sphygmomanometer (Omron, Schaumburg, IL 60173 U.S.A.). The device provided systolic and diastolic pressure readings, as well as peripheral pulse rate. The latter variable was used as a surrogate for the heart rate. Other than systolic and diastolic pressures, calculations for the following parameters were performed off-line:

Blood pressure amplitude:
Systolic BP – diastolic BP

Mean arterial pressure:
2/3 diastolic+1/3 systolic BP

Mean ocular perfusion pressure:
(2/3 diastolic+1/3 systolic BP)*2/3-Goldmann tonometry (131)

IV. F) Visual acuity assessment

The Early treatment diabetic retinopathy study (ETDRS) chart was placed in the same location at the same distance (4 meters) from the patient under the same illumination for all subjects. The logmar scale was chosen to allow a direct statistical analysis, as no logarithmic transformation is needed.
IV. **G) Visual field testing**

Automated perimetry was performed using a Humphrey Field Analyzer, program 24-2, Sita standard strategy (Carl Zeiss, Oberkochen, Germany) or Octopus 301, program G1(30°), dynamic strategy (Interzeag AG, Schlieren, Switzerland). Unreliable visual fields (false positive, false negative or fixation loss values > 20%) were excluded. Visual field analysis was performed using Peridata software (PeriData Software GmbH, Huerth, Germany). Mean defect (MD) values were recorded from these examinations.

IV. **H) SVP assessment**

Optic disc observation was done under pharmacological dilation of the selected eye with tropicamide 0.5% (Tropicol®, Théa Pharma, Wetteren, Belgium). This was confirmed by two observers (LAP, EV), both of them masked to the subject’s diagnosis. In case of disagreement, a second observation by both researchers was made and a consensus was reached. The patient’s discs were observed continuously for at least one minute using a fundus camera for fundoscopy (Topcon TRC-50DX/EX fundus camera). The recording of visible venous pulsation over the disc was done using a binary code: + (present), - (absent).

A validation of the reproducibility of this SVP phenomenon assessment was later performed. As patients returned to either the cataract or Glaucoma clinic (for medical reasons only) during the
recruitment period, they were again observed using the same protocol for SVP detection.

IV. 1) Retrobulbar hemodynamics

Hemodynamics of the retrobulbar vessels were studied using a probe capable of ultra-high frequency sound waves pulsed Doppler (7.5Mhz) from an ultrasound machine (Antares®, Siemens, Munich, Germany). The protocol of data acquisition was performed in accordance to the consensus of CDI technique for ocular blood flow studies (22) and whose reproducibility has been previously validated (132): The examination was performed on a supine patient after a minimum of 10 minutes of rest. The patient was instructed to keep legs uncrossed, arms along the body and to look straight while closing the eye. The examiner was behind the head of the subject, resting the base of his hand on the patient’s forehead to prevent exerting pressure on the globe. The examination started with a B-mode scan with the identification of the optic nerve as a landmark. Then, colour Doppler was applied to visualize the vessels (flow). The appropriate vessel was identified, the sample volume was placed in the center of the vessel, the angle was set parallel to the vessel and several seconds of Doppler waveform were recorded (angle was <60° degrees in all measurements). The CRA and its corresponding vein were taken posterior to the lamina cribrosa. The nasal and temporal short posterior ciliary arteries (NPCA and TPCA were measured at a position that is close to the optic nerve and as anterior as possible. The OA was measured on the nasal side
of the optic nerve, immediately after it crossed the optic nerve (figure 1).

Figure 1. Example from a patients’ CDI examination of the retrobulbar vessels: central retinal artery and vein (CRA, CRV; respectively), ophthalmic artery (OA), nasal and temporal posterior ciliary arteries (NPCA, TPCA; respectively). The anatomic location of the Doppler signal and the characteristic patterns in the Doppler waveform allow for accurate labeling of these vessels.

The following data were retrieved from all the arteries’ Doppler waveform:

Peak Systolic Velocity (PSV) – highest point in waveform curve

End-Diastolic Velocity (EDV) – lowest point in waveform curve

Mean Flow Velocity (MFV) – mean time of the spectral outline

Resistivity index (RI), calculated as RI = (PSV-EDV)/PSV.
In the CRV, the following variables were determined:

Maximal Velocity (Vmax) – highest point in venous waveform

Minimal Velocity (Vmin) – lowest point in venous waveform

Resistivity index (RI), calculated as RI = (Vmax-Vmin)/Vmax
In the OA, the Doppler waveform was further studied for five other variables (figure2):

Early Systolic Acceleration (ESA) – fastest moving portion of systolic component

Acceleration Time (AT) – time period between the beginning and end of the fastest portion

Systolic mean Velocity (Sm) - mean time of the spectral outline from the beginning of the cycle and dicrotic notch

Diastolic mean Velocity (Dm) – mean time of the spectral outline from the dicrotic notch and the EDV

Ratio Sm/Dm was calculated offline

![Image](image.png)

Figure 2. OA Doppler wavefront analyzed characteristics. ESA (early systolic acceleration) represents the fastest moving portion of the systolic component. Early systolic compliance peak (ESP), corresponds to the acute angle in the wavefront just after the first peak; AT, acceleration time; Sm, systolic mean blood flow velocity; Dm, diastolic mean flow velocity; PSV, peak-systolic velocity; EDV, end-diastolic velocity.
Additionally, the vascular cutpoints in glaucoma patients associated with the upper limit of the vessel regulation as previously published by our group (133) were also determined. Patients were labeled as being above or below the pre-determined value:

POAG patients:
RI of CRA 0.77

NTG patients:
RI of CRA 0.61
RI of OA 0.82

In the case of NTG patients, due to the existence of two cutpoints (for CRA and OA respectively), calculations were made to each artery separately and to the combination of both (CRA, OA and CRA+OA).
IV. **J) Optic Nerve Sheath Diameter measurement**

The same ultrasound device and probe as used for the Doppler studied was further used to assess the Optic Nerve Sheath Diameter (ONSD), using the previously published protocol (134). Using mode B, the insonation depth was set to 5–8 cm with the transducer placed over the upper eyelid in an axial plane. The ONSD was calculated perpendicular to the vertical axis of the scanning place 3 mm behind the globe (figure 3), where the optic nerve sheath structure is more prone to expansion due to increases in ICP (135).

![Figure 3. Optic nerve sonography. The optic nerve complex is shown as a sharply-defined hypoechoic stripe in between the echogenic retrobulbar fat](image)

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V. Experimental design

Patients from both the Glaucoma Clinic and subjects from the general ophthalmology consultation were asked to participate in the study. Inclusion/exclusion criteria in both glaucoma patients and healthy subjects were screened by the head of the glaucoma clinic (IST). If conditions were met, each patient/subject was required to sign an informed consent statement before being enrolled into the study and prior to any study measurements being taken.

In this screening visit, best corrected visual acuity and prior medical history including topical medication intake was recorded. All subjects underwent a visual field as well as disc tomography on the day of the study visit. For the healthy individuals, randomization for the eye to be included in the study was made by a third person, blind to any of the subject characteristics or examination results.
Subjects from all groups were taken to a different observation room with another examiner (LAP). This examiner was masked to the subject diagnosis or any other clinical variable and whose only indication was a paper indicating which eye to perform the following examinations in a pre-determined order:

(1) OPA reading;

(2) CCT measurement

(3) blood pressure and pulse rate measurement;

(4) retrobulbar hemodynamic assessment;

(5) ONSD measurements;

(6) SVP assessment.

Of note, 20 minutes were taken between step 5 and 6 in order to allow proper optic disc observation through adequate pupil dilation.

The recruitment of patients and the previously described methodology was performed during two periods, totalizing 4 months (January-February 2011, and September-October 2011).
VI. Statistical analysis

Chi-square tests (for 3x2 contingency tables) were used to analyze the present/absent spontaneous venous pulsation ratios. When 2x2 contingency tables were performed, a Fisher exact test was used. In all cases, the tests will be performed using absolute number of the variables. However, for graphical purposes, the figures will be designed as percentage of total.

Kruskal-Wallis tests were used to compare the three diagnostic groups on different variables. When statistical differences were detected, Mann-Whitney test was used in pairwise group comparisons.

Normal distribution of the data was verified with the D’Agostino and Pearson omnibus normality test. If the data were normal distributed, the existence of correlation between variables was tested using Pearson’s correlation. Otherwise, the Spearman correlation was used.

For practical purposes, acquisition of the data from a clinical setting could not match sample calculations based on previous publications from CDI technology alone for instance (136). In order to address this, the projected work aimed at 100 patients. Not only would it still be one of the largest works done in the subject of ocular blood flow in retinal venous studies, but also the division in three groups would still comply with the central limit theorem. This theorem implies that groups of independent variables that are larger than a certain number (usually above 30) tend to have a
normal distribution (137). Our group samples, by being at least twice these numbers, try to account for the practical limitations of clinical research in reaching sample calculations.

Statistical significance was considered when p<0.05.

Values depicted as mean±SD unless otherwise indicated.

Analyses were performed using Graphpad Prism® ver. 5.0; (Graphpad Software Inc, La Jolla, CA).
Chapter 4. Results

Results will be presented as separate sets, according to the analyzed variables. This was done to allow a proper discussion of each variable/correlation.

As such, the following subchapters will be presented:

I) Experimental group characteristics

II) SVP frequency

III) Patients’ overall characteristics by SVP status

IV) SVP phenomenon frequency and functional damage

V) Optic Disc Tomography and SVP status

VI) IOP and SVP phenomenon

VII) OPA, ONSD and SVP phenomenon

VIII) Cardiovascular parameters and SVP phenomenon

IX) Ocular blood flow and SVP phenomenon
I. **Experimental groups’ characteristics**

A thorough analysis of the characteristics of the different experimental groups is crucial to a correct interpretation of any further correlations. As such, in addition to the complete description of the variables in each group, the agreement of these results with the existing literature is discussed.

I. **A) Overall characteristics**

Table 1 summarizes the patients’ characteristics in the different diagnostic groups. Kruskal-Wallis test indicated no overall age differences between the studied groups (p=0.13). The best corrected visual acuity was significantly higher in the healthy group (p<0.01), although no difference was detected between the glaucoma groups (NTG vs POAG, p=0.11). Both CCT and IOP were also statistically different (p=0.03, p<0.001, respectively), with again the healthy individuals presenting higher IOP values as well as a higher pachymetry. In a pairwise comparison between NTG and POAG however, only IOP was detected to be different (p<0.001), with CCT presenting a significant overlap (p=0.73).

OPA readings were not significantly different (p=0.32). Additionally, ONSD difference between the three groups was borderline significant (p=0.05). However, comparison between NTG and POAG group showed a greater similarity of ONSD values (p=0.21).
As expected, there was a significant difference in functional and structural parameters of glaucoma damage between the three groups (MD and RNFL thickness: \( p<0.001 \), respectively), with lesser damage values seen in healthy individuals. However, when comparing the two glaucoma groups, no difference was detected (MD: \( p=0.79 \); RNFL thickness: \( p=0.90 \)).

<table>
<thead>
<tr>
<th>Table 1. Patients characteristics</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>N</td>
</tr>
<tr>
<td>Age</td>
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<tr>
<td>Visual acuity</td>
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<tr>
<td>IOP</td>
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<td>CCT</td>
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<td>OPA</td>
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<td>ONSD</td>
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<tr>
<td>MD</td>
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<tr>
<td>RNFL thickness</td>
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<tr>
<td>Systolic BP</td>
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<tr>
<td>Diastolic BP</td>
</tr>
<tr>
<td>MOPP</td>
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<tr>
<td>MAP</td>
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<tr>
<td>BP amplitude</td>
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<tr>
<td>Pulse rate</td>
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</tbody>
</table>

Mean values (and SD) are depicted. SVP refers to number of patients who presented that phenomenon compared to the total number of patients. Kruskal-Wallis indicates \( P \) values of overall differences between the diagnostic groups. NTG vs POAG comparison was made using Mann-Whitney test.
The analysis of the cardiovascular parameters revealed systolic BP, median ocular perfusion pressure (MOPP), mean arterial pressure (MAP) and BP amplitude comparison not to be statistically different (p=0.91, p=0.16, p=15 and p=0.38; respectively). There were however differences in diastolic BP and pulse rate (p<0.01), with healthy individuals presenting lowest diastolic values and NTG patients presenting the lowest pulse rate. Pairwise comparison between NTG and POAG group demonstrated a significant difference in pulse rate, with NTG patients again having a lower value (p=0.01). The diastolic BP between these two later groups was not significant (p=0.12).

Topical medications in both glaucoma groups are summarized in table 2. The fixed combinations were documented according to the number of active drugs. Fisher exact-test detected no difference in the proportion of patients under beta-blockers, alpha-agonists and carbonic anhydrase inhibitors therapy between the two groups (p=0.19, p=1.00, p=0.21, respectively). A larger portion of the POAG group was taking prostaglandin analogs (p=0.01).

<table>
<thead>
<tr>
<th>Table 2. Topical medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-blockers</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>POAG</td>
</tr>
<tr>
<td>NTG</td>
</tr>
</tbody>
</table>

Number of patients and percentage (between brackets) are depicted.
I. B) Optic disc tomography

The variables extracted from the optic disc tomography performed using the HRT are described in table 3. As expected, there were significant differences in the overall comparison between the three groups. Both cup area and volume were smaller in the healthy group ($p<0.001$), although no difference was detected between the NTG and POAG groups ($p=0.70$; $p=0.76$; respectively). Conversely, rim area and volume showed the reverse pattern, with lower values in the glaucoma groups ($p<0.001$), with again no difference being seen between NTG and POAG ($p=0.09$; $p=0.20$; respectively). In regard to the relation between disc and cup areas (cup/disc ratio), glaucoma patients did present with higher values at both overall relation and linear vertical ratio ($p<0.001$).

Regarding mean and maximum cup depth, the mean values between the groups were statistically different, with lower values in the healthy group ($p<0.001$; $p=0.02$; respectively). No significant differences were found between the glaucoma groups ($p=0.42$).

Again, as expected, glaucoma patients had lower mean values of RNFL thickness and cross-sectional area ($p<0.001$). The two glaucoma groups revealed a high degree of similarity in these two variables ($p=0.90$; $p=0.94$; respectively).

These results were observed in the setting of an overall similarity of the disc areas ($p=0.24$).
### Table 3. Data from optic disc tomography

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>NTG</th>
<th>POAG</th>
<th>Overall comparison</th>
<th>NTG vs POAG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Disc area</strong></td>
<td>2.27(0.6)</td>
<td>2.34(0.6)</td>
<td>2.45(0.6)</td>
<td>0.24</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>Cup area</strong></td>
<td>0.62(0.5)</td>
<td>1.23(0.6)</td>
<td>1.22(0.6)</td>
<td>&lt;0.001</td>
<td>0.70</td>
</tr>
<tr>
<td><strong>Cup volume</strong></td>
<td>0.17(0.2)</td>
<td>0.39(0.3)</td>
<td>0.39(0.4)</td>
<td>&lt;0.001</td>
<td>0.76</td>
</tr>
<tr>
<td><strong>Rim area</strong></td>
<td>1.65(0.4)</td>
<td>1.10(0.6)</td>
<td>1.23(0.5)</td>
<td>&lt;0.001</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Rim volume</strong></td>
<td>0.43(0.2)</td>
<td>0.25(0.3)</td>
<td>0.27(0.2)</td>
<td>&lt;0.001</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Cup/disc ratio</strong></td>
<td>0.25(0.2)</td>
<td>0.53(0.2)</td>
<td>0.49(0.2)</td>
<td>&lt;0.001</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>Linear cup/disc ratio</strong></td>
<td>0.47(0.2)</td>
<td>0.71(0.2)</td>
<td>0.69(0.1)</td>
<td>&lt;0.001</td>
<td>0.32</td>
</tr>
<tr>
<td><strong>Mean cup depth</strong></td>
<td>0.21(0.1)</td>
<td>0.33(0.1)</td>
<td>0.31(0.2)</td>
<td>&lt;0.001</td>
<td>0.30</td>
</tr>
<tr>
<td><strong>Maximum cup depth</strong></td>
<td>0.59(0.2)</td>
<td>0.74(0.2)</td>
<td>0.71(0.3)</td>
<td>0.02</td>
<td>0.42</td>
</tr>
<tr>
<td><strong>RNFL thickness</strong></td>
<td>0.24(0.1)</td>
<td>0.15(0.1)</td>
<td>0.15(0.1)</td>
<td>&lt;0.001</td>
<td>0.90</td>
</tr>
<tr>
<td><strong>RNFL cross-sectional area</strong></td>
<td>1.24(0.3)</td>
<td>0.83(0.7)</td>
<td>0.82(0.6)</td>
<td>&lt;0.001</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Mean values (and SD) are depicted. Kruskal-Wallis indicates P values of overall differences between the diagnostic groups. NTG vs POAG comparison was made using Mann-Whitney test.
I. C) Retrobulbar hemodynamics

The blood flow velocities obtained by CDI of the retrobulbar vessels (CRA, NPCA, TPCA, OA and the CRV) are described in table 4.

The CRAs from the glaucoma groups had lower PSV, EDV and MFV than their healthy group’s counterparts (p=0.04; p=0.01; p=0.04; respectively). RI values, however, were not significantly different between any groups (p=0.70). None of these four variables were statistically different between the two glaucoma groups (p>0.11 in all pairwise comparisons).

Both the maximum and minimal velocities of the CRV were different when comparing the three groups. Healthy patients had higher velocities than glaucoma patients (Vmax p<0.01; Vmin p<0.01). No statistical significance was found when overall comparing RI (p=0.08).

In both NPCA and TPCA analysis no differences were found in any of the four variables between the three groups, nor in pairwise comparisons between NTG and POAG groups (p values between 0.06 and 0.88).

OA’s PSV were higher in the healthy group (p=0.04). Glaucoma groups had however non-different PSV values (p=0.99). None of the other variables was different between any of the groups (p values between 0.25 and 0.78 for overall and glaucoma pairwise comparisons).
Table 4. CDI variables of the retrobulbar vessels in the experimental groups

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>POAG</th>
<th>NTG</th>
<th>Overall</th>
<th>POAG vs NTG</th>
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<tbody>
<tr>
<td><strong>CRA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSV</td>
<td>11.9(4.3)</td>
<td>10.1(3.1)</td>
<td>10.7(3.4)</td>
<td>0.04</td>
<td>0.56</td>
</tr>
<tr>
<td>EDV</td>
<td>3.20(1.2)</td>
<td>2.70(0.9)</td>
<td>2.96(1.1)</td>
<td>0.01</td>
<td>0.11</td>
</tr>
<tr>
<td>RI</td>
<td>0.72(0.1)</td>
<td>0.73(0.1)</td>
<td>0.71(0.1)</td>
<td>0.70</td>
<td>0.43</td>
</tr>
<tr>
<td>MFV</td>
<td>6.42(2.3)</td>
<td>5.50(1.7)</td>
<td>5.75(1.9)</td>
<td>0.04</td>
<td>0.60</td>
</tr>
<tr>
<td><strong>CRV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vmax</td>
<td>5.89(1.6)</td>
<td>5.13(1.3)</td>
<td>5.32(1.5)</td>
<td>&lt;0.01</td>
<td>0.47</td>
</tr>
<tr>
<td>Vmin</td>
<td>3.40(0.8)</td>
<td>3.09(0.6)</td>
<td>3.08(0.7)</td>
<td>&lt;0.01</td>
<td>0.42</td>
</tr>
<tr>
<td>RI</td>
<td>0.41(0.1)</td>
<td>0.38(1.0)</td>
<td>0.41(0.1)</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>NPCA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSV</td>
<td>9.29(2.7)</td>
<td>9.51(2.9)</td>
<td>9.51(2.8)</td>
<td>0.73</td>
<td>0.88</td>
</tr>
<tr>
<td>EDV</td>
<td>3.13(1.1)</td>
<td>3.15(1.0)</td>
<td>3.2(1.0)</td>
<td>0.77</td>
<td>0.65</td>
</tr>
<tr>
<td>RI</td>
<td>0.66(0.1)</td>
<td>0.70(0.4)</td>
<td>0.66(0.1)</td>
<td>0.84</td>
<td>0.69</td>
</tr>
<tr>
<td>MFV</td>
<td>5.50(1.8)</td>
<td>5.59(1.8)</td>
<td>5.69(1.7)</td>
<td>0.68</td>
<td>0.74</td>
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<tr>
<td><strong>TPCA</strong></td>
<td></td>
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<tr>
<td>PSV</td>
<td>8.97(2.4)</td>
<td>9.90(1.8)</td>
<td>9.20(2.7)</td>
<td>0.06</td>
<td>0.09</td>
</tr>
<tr>
<td>EDV</td>
<td>3.08(0.9)</td>
<td>3.37(1.1)</td>
<td>3.06(0.9)</td>
<td>0.16</td>
<td>0.12</td>
</tr>
<tr>
<td>RI</td>
<td>0.65(0.1)</td>
<td>0.65(0.1)</td>
<td>0.66(0.1)</td>
<td>0.78</td>
<td>0.64</td>
</tr>
<tr>
<td>MFV</td>
<td>5.40(1.4)</td>
<td>6.00(1.8)</td>
<td>5.44(1.5)</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>OA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSV</td>
<td>37.7(17)</td>
<td>31.7(11)</td>
<td>31.6(10)</td>
<td>0.04</td>
<td>0.99</td>
</tr>
<tr>
<td>EDV</td>
<td>6.85(3.9)</td>
<td>5.93(3.0)</td>
<td>5.81(3.7)</td>
<td>0.26</td>
<td>0.53</td>
</tr>
<tr>
<td>RI</td>
<td>0.82(0.1)</td>
<td>0.81(0.1)</td>
<td>0.82(0.1)</td>
<td>0.52</td>
<td>0.27</td>
</tr>
<tr>
<td>MFV</td>
<td>16.8(8.7)</td>
<td>14.2(5.2)</td>
<td>14.4(6.1)</td>
<td>0.25</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Data shown as mean(SD). Kruskal-Wallis indicates P values of overall differences between the diagnostic groups. NTG vs POAG comparison was made using Mann-Whitney test.
Further analysis of the OA Doppler waveform provided 4 more variables that are described in table 5.

ESA was significantly higher in the healthy group (p=0.04). This variable was not different between the two glaucoma groups (p=0.45). AT was not significantly different either between the groups, nor in a glaucoma pairwise comparison (p=0.14; p=0.54; respectively). Both Sm and Dm, were not different between the groups (p values between 0.57 and 0.96). The comparison between the Sm/Dm ratios was also not statistically significant (p=0.67 in overall comparison; p=0.37 in glaucoma pairwise comparison).

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>POAG</th>
<th>NTG</th>
<th>Overall</th>
<th>POAG vs NTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESA</td>
<td>628(174)</td>
<td>501(184)</td>
<td>565(254)</td>
<td>0.04</td>
<td>0.45</td>
</tr>
<tr>
<td>AT</td>
<td>0.04(0.01)</td>
<td>0.05(0.01)</td>
<td>0.05(0.01)</td>
<td>0.14</td>
<td>0.54</td>
</tr>
<tr>
<td>Sm</td>
<td>25.3(8.04)</td>
<td>25.2(8.39)</td>
<td>25.7(8.64)</td>
<td>0.96</td>
<td>0.86</td>
</tr>
<tr>
<td>Dm</td>
<td>9.04(4.54)</td>
<td>9.26(4.76)</td>
<td>8.91(5.69)</td>
<td>0.82</td>
<td>0.57</td>
</tr>
<tr>
<td>Sm/Dm ratio</td>
<td>3.20(1.21)</td>
<td>3.15(1.37)</td>
<td>3.34(1.36)</td>
<td>0.67</td>
<td>0.37</td>
</tr>
</tbody>
</table>

Data shown as mean(SD). Kruskal-Wallis indicates P values of overall differences between the diagnostic groups. NTG vs POAG comparison was made using Mann-Whitney test.
I. **D) Discussion**

The validation of this case-control study depends on control individuals presenting no characteristics of eye disease. Healthy control group patients showed no signs of glaucomatous damage by neither structural nor functional criteria. Mean IOP in healthy individuals, although higher than glaucoma groups, is similar to the mean IOP in population-based studies (138) (139).

The case/control recruiting process seems to have produced an effective pairing, as most of the non-disease related variables are similar between the groups. Age for instance, shows a remarkably similar range between groups. Nevertheless, in view of the fact that this is an elderly population, the question remains how “healthy” these subjects were, particularly as we were dependent on information provided by the patient. Despite our best efforts to thoroughly inquire for any of the exclusion/exclusion criteria, this is a key limitation to our project, as it is for every project dependent on self-reported information. This is particularly true in silently progressing conditions such as cardiovascular diseases, which are usually underestimated by the patient (140).

Further focusing on systemic variables, the similar values in cardiovascular parameters such as systolic BP, MOPP, MAP and BP amplitude between the three groups are in line with the published literature, including previous reports from the UZ Leuven’s Glaucoma department (133) (141). Diastolic BP, however, was higher in glaucoma groups. While most studies report no statistical
differences between glaucoma patients and healthy individuals, there have been reports of increased diastolic pressures in glaucoma patients (142) (143). This is not unexpected, as not only higher values of diastolic BP are associated with an increase in glaucoma incidence (144), but also glaucoma patients, and particularly NTG patients, have increased BP variability (78). These fluctuations have been suggested to reflect endothelial dysfunction known to exist in glaucoma and a dysautonomic control over the cardiovascular system, respectively (9) (78).

In the analysis of all these cardiovascular variables, the fact that no data regarding the BP medications were recorded must be taken into account. As some classes of anti-hypertensives can have an impact on heart rate or have different impacts on diastolic or systolic BP (145), including the blood flow at the ocular circulation level (146), this important bias must be reported. However, the similar age and overall BP parameters, along with the large number of patients should have lessen this bias. Additionally, a previous paper on ocular blood flow originating from the same sample population (Ophthalmology, UZ Leuven), using the same recruitment methodology, has shown a similar distribution of systemic BP medication (133).

IOP values are lower in glaucoma groups, with NTG patients presenting lower IOP levels than POAG. As IOP-lowering strategies intend to induce a 30% reduction in this variable (either by surgical or medical intervention) (16), the IOP values in treated glaucoma
patients can be anticipated to be lower than the average IOP in an untreated population. Average IOP levels in treated POAG patients of nearly 15mmHg are likely to represent a treatment intended to reach or surpass the 30% reduction (if considered an IOP above 21mmHg needed to a POAG diagnosis). In NTG, target IOP is often very low (the limit being the episcleral venous pressure) and often a combination of surgical and medical intervention is required (147). NTG IOP levels below 13 mmHg would comply with the lower incidence of progression known to exist should IOP be consistently below 14mmHg (148). As such, it can be argued that both glaucoma groups have IOP levels within the expected range for their condition.

Both glaucoma groups were observed while under topical (but not systemic) IOP lowering medications. All the classes of medications and their possible impact on ocular blood flow have been extensively studied. Literature on topical Beta-blockers have suggested these agents are likely to cause either increased, decreased or no change in vascular resistance (149) (150). Carbonic acid inhibitors on the other hand, are well known for their impact on ocular blood flow, that extends the normal improvement of ocular perfusion pressure (i.e., that cannot be accounted for the IOP decrease) (151). Topical alpha-agonists have been controversial about their impact on the arterial ocular blood flow, with authors suggesting either a neutral impact or a constrictive effect (152) (153) (154). Nevertheless, and despite the lack of input
from the autonomic nervous system to stimulate the retinal vessels, there have been descriptions of the presence of alpha-receptors in these vessels, including retinal veins (155). While this latter finding could suggest a possible effect on vascular tone, both NTG and POAG had a similar frequency of patients under treatment with this class of drugs, thereby decreasing any possible bias. There was, however, a difference in prostaglandin analogs administration. In spite of this asymmetry, this class of drugs seems to have a neutral effect on ocular blood flow (156) (157).

The homogeneity between the two glaucoma groups has also apparently been achieved. Considering the modified Hoddap classification (158), both groups can be classified as presenting mostly patients with moderate glaucoma, with similar degrees of functional and structural damage, thus making these groups suitable for pairing for further analysis.

The ONSD, similar between the groups, is in line with the previous literature on the subject [from our group] (134). This variable has been suggested to indirectly reflect intracranial pressure, as a higher volume of cerebrospinal fluid would increase the ONSD (159). As translaminar pressure gradient has been suggested to be altered in glaucoma patients (123) (122), any tool that can provide a quantifiable variable could prove to be a valuable asset. It is important however to note that the p value is borderline significant, and although the study is more than twice the size of the original approved project, the question of the study being
underpowered to detect minor changes could be considered to be a study limitation.

CCT variable was also in accordance to the existing literature. While healthy groups presented CCT values similar to the expected to population-based studies (160), the glaucoma patients presented lower CCT values. Corneal thickness has been extensively studied as a possible measuring bias in a Goldman tonometry, as thicker corneas would need an extra force to produce the same applanation area than a thinner one (161). This assumption has been challenged, and evidence is cumulating against correcting IOP for CCT values (162). The current EGS guidelines no longer support such correction (16), which is the reason we did not perform such calculations in the present study. However, it remains a valid variable as a risk factor for developing glaucoma and POAG progression (163). While it has been reported that NTG patients could have lower CCT values (164), there have been reports where no difference has been found, as was the case in our study (134) (165). Additionally, it has also been suggested that CCT possibly reflects the lamina cribrosa thickness (166), thus making it particularly important in the study of the translaminar gradient pressures.
Optic disc morphometric analysis revealed similar disc areas between the groups, in what can be classified as moderate optic disc diameters (16). This is particularly important as significant ametropias are associated with different optic disc characteristics (167). High myopia, for instance, would have a larger disc area and larger cup/disc ratios, thus introducing a bias to any tomography analysis (168). As such, these similar disc areas would reinforce the effective pairing between these groups as far as ocular basic anatomy is concerned. As expected, glaucoma patients did show the expected change in glaucoma structural damage variables (such as cup/disc ratios and RNFL thickness) and, importantly, the degree of functional glaucomatous damage was similar between the groups.

The degree of similarity between our subjects and the characteristics published in the literature for these diagnostic groups extend to the ocular flow velocities of the retrobulbar velocities. Along with the numerous literature on the subject, including previous reports by Prof. Stalmans’ group (169) (170) (171), we measured lower velocities in the CRA (both peak systolic and end-diastolic velocities) and lower peak systolic velocities in the OA in the glaucoma groups when compared to the healthy individuals. Again, the lack of difference between NTG and POAG patients’ blood flow velocities is in accordance with the existing literature (including the same reports from patients from UZ Leuven).
The analysis of the Doppler waveform of the OA in glaucoma and healthy individuals has been recently presented by our group: a relation between waveform pattern and glaucomatous damage was reported to exist (172). Our current data agree with ESA to be lower in the glaucoma groups. This ESA variable has been described in vascularization trees elsewhere to be associated with the vessel distal resistance and compliance (173) (174). At the same pressure, higher ESA values represent rigid non-compliant vessel. However, lower ESA values can represent not only a more compliant artery, but also simply a significant decrease in perfusion pressure (regardless of a better arterial compliance status).

The other Doppler waveform variables match the existing literature, except for the Sm/Dm ratios. We detected no difference in these ratios in the current study, contrary to our previous findings (172). These lower ratios in glaucoma patients have been attributed to a restrictive pattern in blood flow and diastolic dysfunction (172), as Sm/Dm ratio has been suggested to reflect systemic arterial compliance (175). One reason for the lack of differences in the current study is that the groups were more effectively age-paired than our previous waveform report (172), where control patients were older than the glaucoma groups. As compliance decreases with age (176), more advanced age is associated with more rigid vessels and consequently a potentially higher Sm/Dm ratio. Additionally, there have been additional
reports of systemic arterial compliance and glaucoma, where no association was found (65) (177).

As explained, the criteria for a case control study, where the differences between control and glaucoma individuals rest solely on the glaucoma diagnosis and no other characteristics, was respected. Additionally, the overall characteristics of our groups seem to reflect the existing literature on the subject, on demographic, cardiovascular and ocular anatomic variables. Furthermore, the pairing between glaucoma groups showed a similar damage degree between NTG and POAG group. This demonstration of the validation of the studied groups was a fundamental step before any valid conclusions could be retrieved from any further analysis.
II. **SVP frequency**

The detection of SVP phenomenon has been part of the clinical assessment of patients with neurological conditions, with few studies performed in other diseases not directly related to changes in intracranial pressure. Despite some reports on glaucoma patients, to the best of our knowledge, it had never been determined whether NTG patients had a different frequency ratio. As the underlying vascular dysfunctions have more emphasis in the former group, our study was the first to try to address this question.

II. **A) Overall Frequency**

A statistical significance between the SVP status in the three groups existed, with the healthy group having a higher frequency of this phenomenon [66 out of 81(82%)] when compared to both POAG [43 out of 86(50%)] and NTG groups [35 out of 69(51%)]. This difference in overall comparison is statistically significant (Chi-square test: p<0.001). No difference was detected when comparing both glaucoma groups (Fisher exact test: p=0.75)(table 6).

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>NTG</th>
<th>POAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVP +</td>
<td>66(82)</td>
<td>35(51)</td>
<td>43(50)</td>
</tr>
<tr>
<td>SVP -</td>
<td>15(18)</td>
<td>34(49)</td>
<td>43(50)</td>
</tr>
</tbody>
</table>

Number of patients and percentage (between brackets) are depicted.
II. B) Reproducibility

One important limitation of our work would be the reproducibility of the SVP phenomenon. As such, we have conducted a sideline study to assess this reproducibility, as some of the variables known to have a possible impact on SVP can have fluctuations (78). As such, a phenomenon observed in one day at a specific hour could be undetected in the following visit.

As previously explained under Methods, we tried to compensate for this limitation by re-assessing the SVP phenomenon in a number of random patients from all three groups. In this second evaluation, only the protocol for SVP phenomenon detection was performed.

<table>
<thead>
<tr>
<th>Table 7. Reproducibility of SVP assessment by experimental group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>SVP +</td>
</tr>
<tr>
<td>1st Visit Assessment</td>
</tr>
<tr>
<td>2nd Visit Assessment</td>
</tr>
</tbody>
</table>

Number of patients in each group according to SVP status in the two visits

In the healthy population, from the 9 who previously presented with SVP, 8 (89%) also had it in the second evaluation. Both glaucoma groups also revealed a high level of reproducibility with 10 out of 12 POAG patients and 8 out of 9 NTG patients still having the SVP phenomenon (83% and 89%, respectively)(table 7). All the patients who did not have a visible SVP phenomenon in the first visit, independently of the diagnosis, also did not have it in the second observation.
II. C) Discussion

Our data agree with the reports on glaucoma patients that have demonstrated these subjects to have a significant decrease in SVP frequency when compared to healthy individuals. In fact, several authors have described decreases from 98% and 75% in healthy subjects to 54% and 64% in glaucoma patients, respectively (110) (111).

However, at least in theory, glaucoma patients and particularly the ones with high-IOP would be expected to have a higher-than-normal SVP frequency (124). According to some authors, the absence of a visible SVP phenomenon in these patients could even be used in the follow-up of glaucoma patients, as it would mean a decrease in IOP (125). This apparent contradiction in concepts seems to derive from the assumption that IOP would be the only factor to be different between glaucoma and healthy individuals. Higher IOP (with the according higher pulse pressures) would only present a direct connection to a higher venous pulsation frequency if all the other factors involved in ocular blood output would remain unchanged. Different translaminar gradients, lamina cribrosa properties, vessel curve radius and particularly vessel stiffness could provide a partial explanation for this apparent controversial data. In fact, all of the above described variables have been described to be altered in glaucoma patients, particularly NTG patients (123) (178) (179) (180).
The current work has tried to address this question and to provide a better understanding of the impact of each of these variables in the detection of this phenomenon. Additionally, as nearly all of these variables seem to be most prominently altered in NTG patients, it was particularly interesting to assess whether these patients had the same influence from these variables as the other open-angle-glaucomas. In fact, our data is, to our best knowledge, the first to address this question in this type of glaucoma patients, confirming that at least in SVP detection, no difference exist between the two glaucoma populations.
III. Patients’ overall characteristics by SVP status

The reports of SVP in glaucoma patients have been scarce and have only correlated this phenomenon with a very small number of variables. We have therefore combined the largest-yet number of variables with the largest-yet number of patients in recorded literature on the subject to try to uncover any previously undetected differences.

Each of the three groups was divided according to their SVP status. Table 8 depicts the pairwise comparisons of the patients’ characteristics in each of the three groups.

| Table 8. Characteristics of the experimental groups according to SVP status |
|-----------------|----------------|----------------|----------------|----------------|----------------|
|                 | Healthy        | POAG           | NTG            |
|                 | SVP +          | SVP -          | SVP +          | SVP -          | SVP +          | SVP -          |
| N               | 66             | 15             | 43             | 43             | 35             | 34             |
| Age             | 65.8(14)       | 59.3(14)       | 67.6(13)       | 67.3(12)       | 69.0(11)       | 70.0(11)       |
| Visual acuity   | 0.06(0.2)      | 0.21(0.3)      | 0.28(0.3)      | 0.15(0.2)      | 0.30(0.3)      | 0.22(0.3)      |
| IOP             | 16.1(4.9)      | 15.3(4.8)      | 15.9(5.8)      | 13.9(4.1)*     | 12.3(3.1)      | 12.4(2.5)      |
| CCT             | 583(55)        | 564(62)        | 558(32)        | 553(39)        | 547(31)        | 563(35)        |
| MD              | 1.21(4.1)      | 0.48(2.6)      | -9.86(9.4)     | -8.76(7.8)     | 6.92(7.8)      | -11.1(8.7)*    |
| RNFL thickness  | 0.23(0.1)      | 0.24(0.1)      | 0.16(0.1)      | 0.15(0.1)      | 0.16(0.1)      | 0.15(0.1)      |

Data shown as mean(SD). Mann-Whitney tests were used in pairwise comparison between SVP+/SVP- subgroups. The symbol * represent p values of <0.05.
In any of the groups, age and visual acuity were not different (Healthy: p=0.08, p=0.19; POAG: p=0.73, p=0.18; NTG: p=0.95, p=0.39; respectively). CCT was also not different between any of the two subgroups (Healthy: p=0.28; POAG: p=0.44; NTG: p=0.16). In the POAG group, patients with a visible SVP had a higher IOP value (p=0.04), while in the other two groups, there was no significant difference in IOP between SVP positive and negative patients (Healthy: p=0.97; NTG: p=0.55).

Regarding the degree of glaucoma damage (functional or structural), NTG patients with SVP phenomenon had a worse visual field defect (p=0.04), despite non-different RNFL thickness values (p=0.72). No statistical differences in both variables were detected neither in the healthy controls nor in the POAG group (Healthy: MD p=0.29, RNFL thickness p=0.44; POAG: MD p=0.92, RNFL thickness p=0.63).

Table 9. Topical medications

<table>
<thead>
<tr>
<th></th>
<th>POAG</th>
<th>NTG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SVP +</td>
<td>SVP -</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>20(47)</td>
<td>22(51)</td>
</tr>
<tr>
<td>Alpha-agonists</td>
<td>5(12)</td>
<td>3(7)</td>
</tr>
<tr>
<td>CAI</td>
<td>10(23)</td>
<td>11(26)</td>
</tr>
<tr>
<td>Prostaglandins</td>
<td>26(60)</td>
<td>27(63)</td>
</tr>
</tbody>
</table>

Number of patients and percentage (between brackets) are depicted.
Topical medications in both glaucoma groups are summarized in table 9. The fixed combinations were documented according to the number of active ingredients. Fisher exact-test detected no difference in the proportion of patients under beta-blockers, alpha-agonists, carbonic anhydrase inhibitors and prostaglandin analogues therapy between the subgroups of both glaucoma groups (POAG: p=0.89, p=0.71, p=1.00, p=1.00; NTG: p=1.00, p=1.00, p=0.33; respectively).
III. A) Discussion

Interestingly, age does not appear to impact the frequency of SVP, as in none of the three groups a difference was detected. Since age is a non-modifiable risk factor for glaucoma progression (181), any change in glaucoma characteristics could have been attributed to the change in age. Additionally, considering the number of age-related changes known to exist in both cardiovascular and ocular structures, from increased vascular rigidity (182) to increased thickness of the lamina cribrosa (183) and decreased compliance (184), detecting any age difference would account as a confounding factor. It could be however, that within the age range of our groups, no statistical difference could be detected, but that could have been observed should the groups had more patients in age extremes. This emphasis on possible changes in the lamina cribrosa is particularly important when considering a possible pressure gradient, as any gradient depends not only on the pressures in two compartments but also on the nature of the wall separating them [Fick’s Law of Diffusion Coefficient] (185). Information on the behavior of the lamina cribrosa and whether it can be inferred from other ocular structures has been scarce and controversial, with authors suggesting or refuting a correlation with CCT (186) (187) (188). Nevertheless, we reported no difference in CCT between any of the subgroups.

SVP relation to IOP changes and glaucoma damage will be addressed in the following chapters.
IV. SVP phenomenon frequency and functional damage

Our data demonstrated that there was a significant difference in functional damage between NTG patients who did or did not present a visible SVP. These results lead to a further analysis that tried to determine whether in fact this SVP phenomenon could be used to assess the level of glaucoma damage in NTG patients, and whether POAG patients also had any such pattern.

Despite an overall similarity in SVP frequency in both POAG and NTG groups (50% and 51%, respectively; \( p=0.72 \)), further analysis of this frequency according to the degree of glaucoma damage revealed differences to exist between these two groups (figure 4). In the NTG group, SVP frequency decreased with increasing functional damage, from 73% (11 out of 15) in patients between +2 and -2dB, to 56% (15 out of 27) in patients between -2 and -10dB and 33% (9 out of 27) in patients with MD worse than -10dB (\( p=0.04 \)). In the POAG group, no differences in SVP frequency were detected [MD between +2 and -2dB: 10 out of 18(56%); MD between -2 and -10dB: 15 out of 35(43%); MD above –10dB: 18 out of 33(55%); \( p=0.55 \)].
Figure 4. Spontaneous venous pulsation frequency in the three diagnostic groups. Glaucoma groups are divided into three categories according to an increased functional damage criteria: I – MD between +2 and -2dB; II – MD between -2 and -10dB; III – MD above -10dB. Values presented in percentage. Statistical comparisons between categories performed with Chi-Square contingency tables (3x2).
IV. A) Discussion

A different pattern of SVP status with increasing functional damage was observed in NTG and POAG. In NTG, but not in POAG patients, higher levels of functional damage were associated with a lower frequency of the SVP phenomenon. Interestingly, despite a higher degree of functional damage, there was no difference in degree of structural damage. The link between structural and functional damage has been controversial, with a number of authors suggesting a non-linear relationship (189) (190). If such, our data would suggest that the lack of the SVP phenomenon in NTG patients could signal that this specific individual could be on the steep part of this relationship slope. Accordingly, a significant mismatch between functional and structural damage was seen in these non-SVP patients, as an apparently similar RNFL was associated with twice the functional damage of their SVP+ counterparts.

Should the lack of SVP phenomenon be a sign of a vascular dysfunction, including the possibility of a lesser-than-expected perfusion pressure or an increase in vasoconstrictor agents, there is a possibility of a metabolic impairment at the ganglion cell level to exist in these patients. There has been one report claiming deeper glaucomatous visual field defects to be associated with increased oxygen saturation in venules and decreased arteriovenous difference in retinal oxygen saturation (191). This paper, however, did not address any correlation with structural defects.
As such, the possibility remains that oxygen metabolism is affected in the glaucomatous retina, thereby decreasing the function but not the size of the fiber layer.

This could also help explain as to why this relation was not seen in POAG patients, as the vein morphology changes may be less important in these high-IOP glaucomas (180). Further studies on retinal veins using oximetry methods are needed to further explore this intriguing concept.
V. Optic Disc Tomography by SVP status

Existing reports suggesting that SVP detection could be related to disc morphology have led to our work focusing on this segment of glaucoma evaluation. As structural analysis is part of the routine evaluation of glaucoma patients, knowing whether the variables provided by a confocal laser scanning microscopy can affect the SVP detection can be invaluable when interpreting this phenomenon.

| Table 10. Characteristics of the experimental groups according to SVP status |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                               | Healthy POAG NTG |
|                                                | SVP + | SVP - | SVP + | SVP - | SVP + | SVP - |
|                                                | 66    | 15    | 43    | 43    | 35    | 34    |
| Disc area                                       | 2.25(0.49) | 2.34(0.75) | 2.40(0.71) | 2.50(0.55) | 2.33(0.47) | 2.34(0.75) |
| Cup area                                        | 0.61(0.53) | 0.71(0.59) | 1.13(0.62) | 1.31(0.50) | 1.19(0.45) | 1.29(0.64) |
| Cup volume                                      | 0.15(0.21) | 0.20(0.24) | 0.41(0.47) | 0.38(0.21) | 0.35(0.25) | 0.44(0.34) |
| Rim area                                        | 1.64(0.43) | 1.63(0.45) | 1.27(0.60) | 1.19(0.43) | 1.14(0.49) | 1.06(0.74) |
| Rim volume                                      | 0.42(0.18) | 0.45(0.17) | 0.29(0.20) | 0.25(0.20) | 0.25(0.16) | 0.26(0.34) |
| Cup/disc ratio                                  | 0.26(0.19) | 0.28(0.18) | 0.46(0.21) | 0.51(0.15) | 0.51(0.18) | 0.56(0.23) |
| Linear cup/disc ratio                           | 0.47(0.20) | 0.50(0.17) | 0.67(0.15) | 0.71(0.10) | 0.70(0.13) | 0.72(0.18) |
| Mean cup depth                                  | 0.20(0.10) | 0.24(0.12) | 0.31(0.20) | 0.31(0.10) | 0.31(0.18) | 0.34(0.12) |
| Maximum cup depth                               | 0.58(0.19) | 0.63(0.24) | 0.70(0.29) | 0.73(0.21) | 0.75(0.23) | 0.73(0.22) |
| RNFL thickness                                  | 0.23(0.08) | 0.25(0.06) | 0.16(0.10) | 0.15(0.11) | 0.16(0.09) | 0.15(0.13) |
| RNFL cross-sectional area                       | 1.17(0.33) | 1.32(0.35) | 0.82(0.55) | 0.83(0.63) | 0.84(0.51) | 0.82(0.79) |

Data shown as mean(SD). Data shown as mean(SD). Mann-Whitney test were used in pairwise comparison.
Table 10 depicts the optic disc tomography variables divided according to the experimental group by SVP status.

In all of the described variables (areas, volumes and ratios of cup, disc, depth or RNFL measurements), no differences were found between SVP+ and SVP- patients in any of the three experimental groups (p>0.05 in all pairwise comparisons).
V.  A) Discussion

Optic disc morphology was not different between SVP+ and SVP-subgroups of any of the three diagnostic groups. Levine has designed a mathematical model explaining that collapse and expansion of the venous retinal system would be most marked near the point of origin of these fluctuations in blood flow, i.e., in the immediate prelaminar portion of the central vein (116). While there have been few reports on the possible effect of cup morphology on the visualization of the SVP phenomenon (112) (179), we did not observe any difference in optic disc morphology. As such, our data would suggest that this prelaminar point is not dependent on the overall disc morphology. Venous outflow at the CRV formation reflects the entire retinal venous output, with the venous flow of the optic nerve head itself making very little contribution to the overall output. Accordingly, more advanced cupping or increased depth would primarily affect local mechanic factors (for instance, with a steep cup conditioning a more turbulent, more curved retinal vein). Flow in a curved tube correlates positively with the pressure gradient and vessel diameter but negatively with the radius curvature of the bent vessel (192). As the curving of a tube increases, so does the fragmentation of flow patterns inside the tube. Interestingly, a wavy wall, i.e., capable of collapse/expand its diameter along the cardiac cycle, helps disperse this change of flow induced by a curved pathway (193). Our data would suggest that the inevitable
increase in the vein’s curvature radius - resulting from the increased cupping, larger depth and lesser rim area - is not associated with that collapsing/expanding vein.

There are several possibilities as to why this does not happen: either there is an increase in the translaminar pressure gradient that compensates for the curvature radius and smaller vessel diameter (194), or there is an intrinsic change on the wall properties of the glaucoma patient’s veins. Considering the fact these reports on associations of SVP and disc morphology were performed in healthy individuals, both hypotheses may apply to glaucoma patients. These theories are far from mutually exclusive. Indeed, there have been reports of ocular venous endothelial cells to show arterial receptors when in a high translaminar gradient (195), as well as publications on a tendency for veins to show signs of hypertrophy when in the presence of higher endothelin-1 concentrations (196), with both conditions known to exist in glaucoma patients (197) (69). Healthy individuals without SVP have small, non-significant increases in disc cupping, cup depth and cup area when comparing to the ones with a positive SVP phenomenon. Considering the large variability and the large asymmetry in the number of individuals between the two healthy subgroups, the possibility of the study to be underpowered to detect these small changes cannot be excluded.
VI. Intra-ocular pressure in SVP phenomenon

IOP has a major significance for this analysis for two main reasons. Being the main risk factor for the disease - and thus the target for current glaucoma therapy - whether or not IOP levels influence SVP detection is mandatory when interpreting this sign. Moreover, whether this influence is different in healthy and glaucoma patients remains undetermined.

An analysis of the SVP frequency taking into account the level of IOP was performed (figure 5).

Sorting patients according to IOP level (categories I – IOP ≤ 13mmHg; II – IOP between 14 and 17mmHg; III – IOP ≥ 18mmHg) detected a very significant increase in the proportion of patients with SVP phenomenon with higher levels of IOP in the POAG group (p<0.001). This association was not seen in neither healthy nor NTG groups (p=0.59; p=0.39; respectively).
Figure 5. Spontaneous venous pulsation frequency in the three diagnostic groups. Glaucoma groups are divided into three categories according to an increased IOP level criteria: I – IOP ≤ 13mmHg; II – IOP between 14 and 17mmHg; III – IOP ≥ 18mmHg. Values presented in percentage. Statistical comparisons between categories performed with Chi-Square contingency tables (3x2).

Additionally, comparing the frequency of SVP in each category (I, II, and III) between the three groups, a significant difference existed in all three categories (p<0.01 in all comparisons). However, when making a pairwise comparison between the two glaucoma groups, no statistical difference between the two was detected (I: p=0.05; II: p=0.85; III: p=0.18).
VI. A) Discussion

The existing literature suggests that patients with higher IOP values are more likely to have a detectable SVP phenomenon than patients with lower IOPs (125). In healthy individuals, this SVP frequency remained the same throughout the considered IOP-range. The significant decrease in the SVP frequency in the glaucoma groups is in line with the published data (111) (110).

Interestingly, for all the levels of IOP, the SVP frequency in healthy individuals was higher than in glaucoma patients. Our results suggest that the threshold in the fluctuations in the blood flow leaving the eye that create the SVP is set to a higher value in glaucoma patients than in healthy individuals. As previously explained, part of that blood flow fluctuations relies on the transmission of the intraocular pressure to the intravascular pressure. Should the vessels be more rigid, they would potentially decrease this transmission and therefore higher IOPs would be needed to have the same effect on the venous blood flow. Indeed, it has been demonstrated that NTG patients do have stiffer, more rigid retinal vessels (180), in a process that could be related to either an increase in vasoconstrictors (as endothelin) or an impairment in vasodilation/compliance (70) (9). This would help explain why the behavior of NTG patients is different from the POAG individuals. According to this hypothesis, IOP values that would be otherwise able to generate the necessary pulse for SVP,
in NTG patients that same level may be insufficient to help create the minimal pulse necessary for SVP to occur.

In POAG patients, as the levels of endothelin are less significant than in NTG, but nevertheless still higher than in the normal healthy individuals (69), the threshold for IOP in SVP generation could probably be found somewhere between the low-IOP healthy and high-IOP NTG patients.
VII. OPA and ONSD in SVP phenomenon

Considering that both theories regarding the formation of the SVP phenomenon involved pulse pressures and translaminary gradients, we tried to address these variables using everyday tools in Ophthalmology clinic. Ocular pulse amplitude (OPA) was assessed using the Pascal® dynamic contour tonometer device. The extraocular pressure assessment, on the other hand, was made using ONSD as a clinical surrogate for cerebrospinal fluid pressure acting behind the *lamina cribrosa*.

VII. A) Ocular pulse amplitude

As mentioned above, no significant difference was found between OPA measurements of the three experimental groups (p=0.32). Table 11 describes the pairwise comparison and statistical significance between SVP subgroups of the healthy, POAG or NTG patients.

<table>
<thead>
<tr>
<th>Table 11. OPA values according to SVP status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
</tr>
<tr>
<td>SVP +</td>
</tr>
<tr>
<td>OPA</td>
</tr>
</tbody>
</table>

Data shown as mean(SD). Mann-Whitney test were used in pairwise comparison. * indicates p values <0.05.

The differences between OPA values of SVP+ and SVP- did not reach statistical significance in both healthy and POAG groups (p=0.12 on both pairwise comparisons). However, in the NTG population, patients with the SVP phenomenon had higher OPA values than their SVP- counterparts (p=0.023).
A further analysis of the SVP frequency taking into account the level of OPA was performed (figure 6).

Sorting patients into OPA level categories (I – OPA ≤ 2 mmHg; II – OPA between 2 and 3 mmHg; III – OPA ≥ 3 mmHg) was able to detect a statistical difference in the overall proportion of patients with or without SVP in each of the three OPA categories between the three diagnostic groups (p=0.01). Pairwise comparison between POAG and NTG group was non-significant (p=0.69).

Figure 6. Spontaneous venous pulsation frequency in the three diagnostic groups. Glaucoma groups are divided into three categories according to an increased OPA level criteria: I – OPA ≤ 2 mmHg; II – OPA between 2 and 3; III – OPA ≥ 3mmHg. Values presented in percentage. Statistical comparisons between categories performed with Chi-Square contingency tables (3x2).
In the two lower categories, a statistical difference was seen between the three groups (p<0.01), but not between POAG and NTG groups (I: p=0.49; II: p=0.91). However, in the category with higher OPA values, the proportion of patients with SVP was not different, neither between the three groups (p=0.14) nor between the two glaucoma groups (p=0.98).

VII. B) Optic Nerve Sheath Diameter

As previously described, a borderline significant difference was found between ONSD measurements of the three experimental groups (p=0.05). Table 12 describes the pairwise values and statistical significance (if any) between SVP subgroups of the healthy, POAG or NTG patients.

<table>
<thead>
<tr>
<th>Table 12. ONSD measurements according to SVP status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
</tr>
<tr>
<td>SVP +</td>
</tr>
<tr>
<td>ONSD 6.40(0.88)   6.14(0.78)     5.91(0.89)     5.75(0.76)     6.12(0.64)     5.88(0.90)</td>
</tr>
</tbody>
</table>

Data shown as mean(SD). Mann-Whitney test were used in pairwise comparison.

No differences were found in any of the pairwise analyses (Healthy: p=0.24; POAG: p=0.40; NTG: p=0.26).
Figure 7. Spontaneous venous pulsation frequency in the three diagnostic groups. Glaucoma groups are divided into three categories according to an increased ONSD level criteria: I – ONSD 4-5.5 mm; II – ONSD between 5.5 and 7mm; III – ONSD ≥ 7mm. Values presented in percentage. Statistical comparisons between categories performed with Chi-Square contingency tables (3x2).

Similarly to the analysis done for IOP and OPA, we sorted patients through different ONSD categories (Category I – 4-5.5mm; Category II – 5.5-7mm; Category III - >7mm). In healthy patients, those with higher ONSD had lesser frequency of SVP phenomenon (p<0.001). In glaucoma groups, both NTG and POAG, no relation was detected between higher ONSD values and SVP detection (NTG: p=0.18; POAG: p=0.69)(figure 7).

An analysis of the association between ONSD and IOP was additionally performed. In Healthy and POAG patients, no statistically significant correlation was detected (p range between
0.18 and 0.81). In the NTG population however, a nearly significant association was seen when analyzing the total number of NTG patients (p=0.05, r=0.27). When analyzing the SVP- subgroup specifically, a strong positive correlation was observed (p=0.04, r=0.42). The full description of the statistical tests is depicted in table 13.

<table>
<thead>
<tr>
<th>Table 13. Correlation between IOP and ONSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-value</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Healthy Overall</td>
</tr>
<tr>
<td>Healthy SVP+</td>
</tr>
<tr>
<td>Healthy SVP-</td>
</tr>
<tr>
<td>POAG Overall</td>
</tr>
<tr>
<td>POAG SVP+</td>
</tr>
<tr>
<td>POAG SVP-</td>
</tr>
<tr>
<td>NTG Overall</td>
</tr>
<tr>
<td>NTG SVP+</td>
</tr>
<tr>
<td>NTG SVP-</td>
</tr>
</tbody>
</table>

P values and coefficient of correlation of Spearman’s correlation test are indicated.

The analysis of the correlation between OPA and ONSD did not detect any significant association in any of the healthy and POAG groups (p range between 0.15 and 0.99). In the NTG patients, however, a strong negative correlation was seen in the SVP- patients (p=0.01, r=-0.74), but not in any other NTG group (overall: p=0.22; SVP+: p=0.35)(table14).
VII. C) Discussion

Higher OPA values are associated with higher degree of SVP detection in all groups. OPA was lower in the NTG patients’ subgroup that did not have a visible SVP. As a lower ocular pulse pressure can have a direct influence on the SVP generation, it is reasonable to assume a lower SVP detection could simply reflect this lower pulse amplitude. OPA has been described to reflect the change in ocular blood volume between the two phases of the cardiac cycle. As the bulk of such volume is directed into the choroid compartment, differences in OPA readings can either reflect decreases in choroid blood flow or may imply different ocular structural properties. This latter proposition is based on the fact that pulse amplitude is read as a pressure measurement and
thus is also dependent not only on the volume but also the ocular compliance to that change in volume. Not only have glaucoma patients been known to have more elastosis areas than healthy individuals, but also extracellular matrix changes at the scleral level (198) (199). This distortion in the normal architecture of the sclera has been suggested to affect its stiffness and how the eye adapts to a certain IOP value (200). However, choroidal blood has been suggested to be decreased in glaucoma patients as well (201), with subfoveal decreases in choroidal blood predating functional damage (202). As such, and although the proportional contribution of each factor to the overall OPA reading is unknown, our data suggests this to be remarkably important in NTG patients. In line with the propositions made in previous chapters, increases in vasoconstrictor agents could be related to both activation of fibroblasts (203) and a decrease in choroidal flow (204) (205), thus potentially lowering OPA. However, because patients with increased glaucomatous damage tend to have a lower OPA value than patients with a less advanced disease (206) (207), the question of whether this lower reading is primary or secondary remains unanswered.

A negative correlation between OPA and ONSD was detected only in NTG SVP negative patients. This is apparently a contradiction, as ONSD has a positive correlation with IOP (134). OPA depends not only on the globe stiffness, IOP value and also the amount of blood entering the eye. Considering this OPA/ONSD relationship was only
seen in these patients who are more likely to have vascular changes, it is reasonable to hypothesize this relationship to be vascular-based. However, OPA is mostly dependent on choroidal blood flow and not the blood circulating in the retinal vessels. As such, in apparently low choroidal blood flow conditions (as seen in the lower OPA values in these NTG patients without SVP), this variable is inversely related to the retrolaminar pressure. Because choroidal flow is not autoregulated, an increase in pressures outside the eye, could theoretically decrease the flow in the choroidal tissues. The reason this may only affect NTG patients could be related to anatomic changes behind the lamina cribrosa, as eyes with more advanced glaucoma may have a different posterior curve radius that can displace the surrounding tissues thereby exposing the peripheral structures to the changes in CSF pressure (197). This upholds our current rationale, as decreases in OPA have been associated with higher degrees of glaucomatous disease, especially in NTG patients (202).

Another aspect relevant to the translaminar gradient pressure is the extraocular component, namely the retrolaminar pressure. We indirectly assessed it through the ONSD, which is been increasingly used as a surrogate of ICP (159). In healthy conditions, an increase in ONSD (and expectedly, an increase in the retrolaminar pressure component), is associated with a decrease in the SVP detection. One possible explanation is that by decreasing the translaminar gradient, it could affect the output flow fluctuations in these
vessels. Another possible explanation is that increasing the retrolaminar pressure can affect the retinal venous drainage itself, as part of the central vein pathway is through the subarachnoid space, and as such, this vessel may be influenced by that outside pressure. Accordingly, as the difference in pressure between the intra and extraocular segments of the lamina cribrosa would be lower, that could decrease the flow rate, and consequently, the visibility of the SVP phenomenon.

Interestingly, in glaucoma patients, no association was detected between changes in ONSD and SVP frequency. One possibility is that the microenvironment of vasoactive agents has an impact on the vein outside the eye as well. As previously stated, authors have found extraocular (retrolaminar) segments of the CRVs to have a wall hypertrophy and an endothelial metaplasia-like process, by which it expresses arterial receptors (195). This process has been suggested to be a response to a high pressure gradient, which would be of greater importance immediately after its lumen reduction by the lamina cribrosa. Additionally, the influences of outside forces acting only downstream of that major constriction zone would be ultimately less important.
However, the optic nerve sheath cul-de-sac in glaucoma patients may have a different behavior from the otherwise healthy individuals. Authors have suggested an insufficient CSF reabsorption to occur at this level, with CSF selectively accumulating on this segment (208). More interestingly, these structures are also likely targets of a number of molecules known to exist in greater concentrations in glaucoma patients. Orbital fibroblasts, for instance, are sensitive to endothelin concentrations (203). Additionally, these changes in structure of the ONSD could lead to accumulation of lipocalin-type prostaglandin D2 synthase (L-PGDS), which could have a toxic effect on the optic nerve itself (209). One possibility for this lack of association is that a stiffer, less compliant sheath can interfere with the CSF pulse, thereby decreasing the translaminar pulse. This suspected change in structural organization of this tissue, while possibility not interfering with the overall CSF pressure assessment, could potentially affect the CSF pulse and subsequently interfere with the SVP phenomenon. Again, this hypothesis could help to understand this widely unknown disease-mechanism, particularly in patients with NTG.

Additionally, this ONSD has a correlation with IOP only in this more advanced form of the disease (NTG patients without SVP phenomenon), which agrees with the previous reports from our group (134). Because IOP is not related to the central retinal vessels, the only possible relation would be some connection to
exist between ocular aqueous humour drainage and ONSD. One suggestion is that NTG would have a lesser degree of upstream obstruction (possibly at the trabecular meshwork), which would make these patients more sensitive to downstream fluctuations. As such, smaller or higher ICP values would have an impact on aqueous humour drainage with subsequent increases or decreases in IOP (134). Should the superior ophthalmic vein structure be also affected by vasoconstrictive-prone agents as suggested to be the case in the CRV, then a stiffer superior ophthalmic vein would also be less compliant to the changes in ICP. Accordingly, this lack of the vein’s buffer ability would have repercussions upstream at the aqueous humour drainage. Interestingly, this line of thought could also imply that in NTG patients, the IOP – the main modifiable risk factor – can actually indirectly reflect the degree of the extraocular changes occurring behind the lamina cribrosa.
VIII. Cardiovascular parameters by SVP status

As vascular aspects have been associated with glaucoma, cardiovascular-related have been associated with a number of ocular blood flow parameters (210) (211). However, whether variables such as blood pressure and heart rate have any influence on the SVP phenomenon remains undetermined. Additionally, any cardiovascular differences between the diagnostic subgroups would preclude any conclusions from the scheduled blood flow analysis.

No differences in cardiovascular parameters were observed between the Healthy controls’ subgroups (systolic BP p= 0.95; diastolic BP p= 0.89; MOPP p= 0.37; BP amplitude p=1.00; pulse rate p= 0.24). NTG patients’ subgroups differed in pulse rate, with patients without a visible SVP having a lower pulse rate (NTG: p=0.03; POAG: p=0.22). POAG patients that did not have a visible SVP had both higher diastolic pressures and MOPP, but not NTG patients (POAG: p=0.01; p=0.01; NTG: p=0.32; p=0.37; respectively). Systolic BPs were not different between any of the glaucoma subpopulations (POAG: p=0.50; NTG: p= 0.95). MAP was found to be higher in POAG patients without SVP (p=0.03), but no difference was found in the healthy nor NTG pairwise comparisons (p=0.41, p=0.50, respectively). An overall description is made in table 15.
Table 15. Cardiovascular parameters according to groups’ SVP status

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>POAG</th>
<th>NTG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SVP +</td>
<td>SVP -</td>
<td>SVP +</td>
</tr>
<tr>
<td>N</td>
<td>66</td>
<td>15</td>
<td>43</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>150(19.2)</td>
<td>154(28.2)</td>
<td>148(19.8)</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>81.3(10.3)</td>
<td>84.4(17.9)</td>
<td>83.5(11.6)</td>
</tr>
<tr>
<td>Amplitude BP</td>
<td>68.5(18.8)</td>
<td>69.6(18.6)</td>
<td>64.7(16.9)</td>
</tr>
<tr>
<td>MAP</td>
<td>104(10.7)</td>
<td>108(20.2)</td>
<td>105(12.5)</td>
</tr>
<tr>
<td>MOPP</td>
<td>54.9(8.58)</td>
<td>57.8(14.2)</td>
<td>54.5(8.56)</td>
</tr>
<tr>
<td>HR</td>
<td>69.0(10.5)</td>
<td>73.6(13.1)</td>
<td>67.7(14.0)</td>
</tr>
</tbody>
</table>

Data shown as mean(SD). Mann-Whitney test were used in pairwise comparisons. The symbols θ, * and Δ were used to represent p-values of <0.01, <0.02 and <0.05, respectively.
To our best knowledge, there is only one paper that assessed whether retinal venous pressure correlated with similar cardiovascular blood pressure variables (212). As no associations were found, it can be reasoned that as arterial blood pressure is dampened at the retinal capillary level, no transmission to the venous circulation would theoretically occur (180). Nevertheless, blood pressure may interfere with the downstream venous circulation. As retinal venous blood flows into the heart depending on a pressure gradient, any arterial-related increase in central venous pressure could interfere with ocular venous drainage. While this is not the case in healthy individuals, conditions associated with right ventricular dysfunction can interfere with left ventricular function, potentially impacting systemic blood pressure and increasing right atrium filling pressure (213). This is the reason why any known heart condition was an exclusion criteria, as interpretation of venous pulsation in such settings would not be possible. While it is possible that some patient would be unaware of or fail to report this condition, only a severe right-heart failure would significantly affect venous circulation above the heart (214). Symptoms such as dyspnea, fatigue, weakness and pronounced jugular veins would likely be noticed at the time of examinations.

Blood pressure plays an important role in glaucoma. While low ocular perfusion pressure has been consistently implied in the list of risk factors for glaucoma (215) (216), arterial hypertension per
se has been demonstrated to reduce the incidence of the disease (216), increase its frequency (217) and to have no significant effect (218), depending on the considered epidemiological studies. The large epidemiological study that focused in a population that is most likely similar our groups (Rotterdam Eye Study, in The Netherlands – around 100km from Leuven) have shown low diastolic pressure to be associated with POAG only in patients under blood pressure medication (219). While we do not know the patient’s systemic medication status, this factor can have influenced our findings and as such, should be carefully interpret in the light of our important study limitation.

Nevertheless, POAG patients with an absent SVP phenomenon had higher diastolic pressures than their SVP positive counterparts. As diastolic pressure have been described as an independent risk factor for CRV occlusion (220), the lack of a visible SVP in POAG patients could potentially be used as a marker for underlying risk factors for developing this retinal vascular disease. This hypothesis, however, needs to be confirmed by further studies.

We did however notice a heart rate difference between the NTG patient subgroups. In physiological terms, increases in heart rate mostly occur at the expense of diastolic time. As such, fluctuations between the normal systolic peak and the time-shortened end-diastolic flow are diminished (221). Accordingly, a lower frequency of SVP phenomenon was to be expected in more tachycardic patients. This would also be in line with previous reports from our
group, where we described a negative correlation between heart rate and OPA (222). While topical glaucoma medications (namely beta-blockers) have been suggested to decrease heart rate (223), a potential bias could have taken place as a number of patients in all glaucoma groups were being treated with drugs from this pharmacological class. However, as previously seen, there was no difference in the proportion of the medication classes in any glaucoma subgroup. As differences in heart rate have not been suggested to be a risk factor for glaucoma progression (224), these changes could be related to an underlying autonomic dysfunction. As previously explained, glaucoma patients have been widely reported to have cardiovascular signs of a dysfunction in the homeostatic mechanism of autonomic nerve system, from baroreflex dysfunction (225) to wider-than-expected blood pressure fluctuations (226).

In fact, the degree of autonomic dysfunction has been suggested to correlate with glaucoma progression, at least in NTG patients (227).
IX. Ocular Blood Flow and SVP phenomenon

This final part represents much of the novelty in our study, as no ocular blood flow study had been previously performed when assessing the SVP phenomenon. Not only were flow velocities retrieved from the Doppler studies, but also variables from the recent contributions by our group to the Ocular blood flow Doppler studies (autoregulation cutpoints and Waveform analysis) (172) (133).

IX. A) CDI examination of retrobulbar vessels

Table 16 depicts the data from CDI examinations according to the SVP status of the three experimental groups. Healthy patients without SVP had a higher Vmin than their SVP positive counterpart (p<0.05). POAG patients who didn’t show SVP, on the other hand, had both lower CRV Vmin and RI than their SVP+ counterparts (p<0.02; p<0.05 respectively). NTG patients had a more pronounced difference in hemodynamic pattern between both subgroups, with differences in both arterial and venous central retinal vessels. The NTG SVP absent subgroup had significantly lower central retinal artery PSV and MFV (p<0.01; p<0.05, respectively) as well as lower Vmax and RI of the CRV (p<0.02; p<0.05, respectively) than the NTG patients with SVP phenomenon. Flow variables such as PSV, EDV, RI and MFV were not significantly different in any of the other arteries (NPCA, TPCA and OA) between the SVP subgroups of neither healthy, nor NTG or POAG populations (p>0.05 in all accounts).
| Table  16. CDI variables of the retrobulbar vessels in the experimental groups |
|-------------------------------|------------------|------------------|------------------|------------------|------------------|
|                              | Healthy          | POAG             | NTG              | SVP +            | SVP -            |
| CRA                          |                  |                  |                  |                  |                  |
| PSV                          | 12.1(4.4)        | 11.1(3.8)        | 10.2(3.6)        | 10.0(2.6)        | 11.8(3.4)        | 9.46(3.0)*       |
| EDV                          | 3.21(1.1)        | 3.14(1.4)        | 2.73(1.0)        | 2.67(0.8)        | 3.10(1.0)        | 2.81(1.2)        |
| RI                           | 0.72(0.1)        | 0.73(0.1)        | 0.72(0.1)        | 0.72(0.1)        | 0.73(0.1)        | 0.70(0.1)        |
| MFV                          | 6.45(2.3)        | 6.23(2.5)        | 5.52(2.0)        | 5.47(1.4)        | 6.19(1.8)        | 5.27(1.9)*       |
| CRV                          |                  |                  |                  |                  |                  |                  |
| PSV                          | 5.77(1.5)        | 6.54(1.9)        | 5.31(1.3)        | 4.94(1.4)        | 5.68(1.7)        | 4.78(0.9)Δ       |
| EDV                          | 3.32(0.7)        | 3.84(1.1)*       | 3.07(0.6)        | 2.67(0.83)Δ      | 3.13(0.7)        | 2.90(0.4)        |
| RI                           | 0.41(0.1)        | 0.40(0.1)        | 0.40(0.1)        | 0.35(0.1)*       | 0.43(0.1)        | 0.38(0.1)*       |
| NPCA                         |                  |                  |                  |                  |                  |                  |
| PSV                          | 9.31(2.8)        | 9.22(2.7)        | 9.30(2.6)        | 9.71(3.2)        | 9.43(2.8)        | 9.62(2.8)        |
| EDV                          | 3.11(1.0)        | 3.23(1.3)        | 2.98(0.8)        | 3.30(1.2)        | 3.07(0.9)        | 3.35(1.1)        |
| RI                           | 0.66(0.1)        | 0.65(0.1)        | 0.67(0.1)        | 0.66(0.1)        | 0.66(0.1)        | 0.65(0.1)        |
| MFV                          | 5.46(1.8)        | 5.83(1.7)        | 5.31(1.6)        | 5.85(2.0)        | 5.52(1.5)        | 5.88(1.9)        |
| TPCA                         |                  |                  |                  |                  |                  |                  |
| PSV                          | 8.80(2.5)        | 9.75(2.0)        | 10.2(3.1)        | 9.64(2.4)        | 9.44(2.7)        | 8.93(2.6)        |
| EDV                          | 3.01(0.8)        | 3.42(1.1)        | 3.27(0.9)        | 3.47(1.2)        | 3.01(0.8)        | 3.06(0.9)        |
| RI                           | 0.65(0.1)        | 0.64(0.1)        | 0.67(0.1)        | 0.63(0.1)        | 0.66(0.1)        | 0.65(0.1)        |
| MFV                          | 5.28(1.4)        | 5.94(1.4)        | 6.02(1.8)        | 5.98(1.8)        | 5.54(1.5)        | 5.33(1.5)        |
| OA                           |                  |                  |                  |                  |                  |                  |
| PSV                          | 38.5(17)         | 33.1(9.9)        | 32.0(12)         | 31.6(8.8)        | 31.2(10)         | 32.1(11)         |
| EDV                          | 7.04(4.0)        | 5.86(3.6)        | 5.34(2.7)        | 6.44(3.2)        | 5.30(2.3)        | 6.34(4.7)        |
| RI                           | 0.82(0.1)        | 0.82(0.1)        | 0.83(0.1)        | 0.80(0.1)        | 0.83(0.1)        | 0.81(0.1)        |
| MFV                          | 17.4(9.1)        | 13.8(5.7)        | 13.8(5.6)        | 14.7(4.9)        | 13.7(5.2)        | 15.1(7.0)        |

Data shown as mean(SD). Comparison between the subgroups were made using Mann-Whitney tests. *, Δ, ø indicated statistically significant differences in pairwise comparison with SVP+ counterpart (p<0.05; p<0.02; p<0.01, respectively).
**IX. B) Doppler Waveform analysis by SVP status**

Table 17 illustrates the OA waveform characteristics according to the SVP status of the experimental groups.

No differences were found in ESA, AT or Dm between the subgroups of either the healthy, POAG and NTG populations.

However, there was a statistically significant difference in the Sm between the SVP subgroups of the NTG patients (p<0.05), with higher velocities in the SVP- population. No differences were found between SVP+ and SVP- patients in neither the healthy nor the POAG groups.

Another difference was found in the ratio between the Sm/Dm in the POAG SVP subgroups, with lower ratios in the SVP- patients (p<0.05).

| Table 17. OA waveform characteristics according to groups’ SVP status |
|---|---|---|---|---|---|---|
| Healthy | SVP + | SVP - | POAG | SVP + | SVP - | NTG |
| N | 66 | 15 | 43 | 43 | 35 | 34 |
| ESA | 607(183) | 701(130) | 504(195) | 497(178) | 539(251) | 576(263) |
| AT | 0.05(0.01) | 0.04(0.01) | 0.05(0.01) | 0.05(0.01) | 0.05(0.01) | 0.05(0.01) |
| Sm | 25.0(8.53) | 26.4(6.66) | 25.8(8.97) | 24.4(7.83) | 20.9(5.86) | 27.9(9.21) |
| Dm | 8.61(4.00) | 10.6(6.48) | 8.14(3.91) | 10.6(5.52) | 6.78(2.65) | 10.3(6.73) |
| Sm/Dm | 3.17(0.97) | 3.31(2.01) | 3.55(1.44) | 2.65(1.13) | 3.42(1.27) | 3.28(1.44) |

Data shown as mean(SD). Mann-Whitney test were used in pairwise comparison. Δ stands for p values <0.05.
IX. C) Correlation between central retinal arterial and venous flow

A study was performed on the correlation of flow velocities between the central retinal vessels in the SVP subgroups of each diagnostic group. A complete description of the statistical findings is made on tables 18, 19 and 20, each concerning one diagnostic subgroup analysis.

In the healthy population, there were significant differences between the SVP+ and SVP- subgroups. In subjects with SVP+, both PSV and MFV had a positive correlation with the CRV’s Vmax and RI (PSV x Vmax: p=0.04, r=0.27; PSV x RI: p=0.01, r=0.36; MFV x Vmax: p=0.02, r=0.31; MFV x RI: p=0.04, r=0.27), while in the SVP-subgroup, no correlations were detected (p range between 0.89 and 0.97). The CRA RI had a positive correlation with the CRV RI (p<0.01, r=0.55) of SVP+ individuals, while it had a negative correlation with CRV Vmax and Vmin of the SVP- healthy controls (x Vmax: p=0.04, r=-0.64; x Vmin: p=0.02, r=-0.67). The CRA EDV had no impact on CRV variables on neither subgroup (p range between 0.11 and 0.98).

On the relation between flow variables in the CRV itself, in both subgroups a strong degree of correlation was seen between Vmax and Vmin (p <0.01 on both correlations; SVP+: r=0.78, SVP-: r=0.82). Additionally, in the SVP+ subgroup, there was also a strong correlation between CRV’s Vmax and RI (p<0.01, r=0.60), which
was not observed in the SVP- population (p=0.78). In neither of the subgroups, Vmin had no correlation with RI (p range between 0.19 and 0.99).

Table 18. Correlation between central retinal vessel's velocities

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>SVP+</th>
<th>SVP-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CRV Vmax</td>
<td>CRV Vmin</td>
<td>CRV RI</td>
</tr>
<tr>
<td>PSV</td>
<td>0.04 (0.27)</td>
<td>0.58</td>
<td>0.01 (0.36)</td>
</tr>
<tr>
<td>EDV</td>
<td>0.21</td>
<td>0.11</td>
<td>0.91</td>
</tr>
<tr>
<td>MFV</td>
<td>0.02 (0.31)</td>
<td>0.15</td>
<td>0.04 (0.27)</td>
</tr>
<tr>
<td>RI</td>
<td>0.19</td>
<td>0.19</td>
<td>&lt;0.01 (0.55)</td>
</tr>
</tbody>
</table>

P values of Spearman’s correlation test are indicated. R values (between brackets) are shown when p<0.05.
Table 19. Correlation between central retinal vessel’s velocities

<table>
<thead>
<tr>
<th></th>
<th>SVP+</th>
<th>SVP-</th>
</tr>
</thead>
<tbody>
<tr>
<td>POAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vmax</td>
<td>0.02 (0.35)</td>
<td>0.04 (0.25)</td>
</tr>
<tr>
<td>Vmin</td>
<td>0.10</td>
<td>0.16</td>
</tr>
<tr>
<td>RI</td>
<td>0.15</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSV</td>
<td>0.28</td>
<td>0.20</td>
</tr>
<tr>
<td>EDV</td>
<td>0.05 (0.30)</td>
<td>0.64</td>
</tr>
<tr>
<td>MFV</td>
<td>0.04 (0.31)</td>
<td>0.44 (0.34)</td>
</tr>
<tr>
<td>RI</td>
<td>0.19</td>
<td>0.04 (0.30)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01 (0.42)</td>
</tr>
</tbody>
</table>

P values of Spearman’s correlation test are indicated. R values (between brackets) are shown when p<0.05.

In the POAG, in similarity with the healthy group, SVP+ patients had a statistically significant correlation between CRA’s PSV and MFV with CRV Vmax (PSV x Vmax: p=0.02, r=0.35; MFV x Vmax: p=0.04, r=0.31). Additionally, CRA EDV had a borderline significant correlation with CRV Vmin (p=0.05, r=0.30). Similar pattern of correlations were seen in the POAG SVP- counterpart (PSV x Vmax: p=0.04, r=0.25; MFV x Vmax: p=0.01, r=0.38). In both subgroups, there was a correlation between both vessels’ RI (SVP+: p=0.04, r=0.30; SVP-: p=0.01, r=0.42).
Table 20. Correlation between central retinal vessel’s velocities

<table>
<thead>
<tr>
<th></th>
<th>SVP+</th>
<th></th>
<th>SVP-</th>
<th></th>
<th>NTG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CRV Vmax</td>
<td>CRV Vmin</td>
<td>CRV RI</td>
<td>CRV Vmax</td>
<td>CRV Vmin</td>
</tr>
<tr>
<td>PSV</td>
<td>0.51</td>
<td>0.99</td>
<td>0.65</td>
<td>0.01 (0.51)</td>
<td>0.12</td>
</tr>
<tr>
<td>EDV</td>
<td>0.33</td>
<td>0.40</td>
<td>0.04</td>
<td>0.02 (0.43)</td>
<td>0.01</td>
</tr>
<tr>
<td>MFV</td>
<td>0.20</td>
<td>0.90</td>
<td>0.16</td>
<td>0.06</td>
<td>0.05 (0.36)</td>
</tr>
<tr>
<td>RI</td>
<td>0.57</td>
<td>0.31</td>
<td>0.06</td>
<td>0.29</td>
<td>0.06</td>
</tr>
<tr>
<td>Vmax</td>
<td>-</td>
<td>&lt;0.01 (0.65)</td>
<td>&lt;0.01 (0.63)</td>
<td>-</td>
<td>&lt;0.01 (0.73)</td>
</tr>
<tr>
<td>Vmin</td>
<td>&lt;0.01 (0.65)</td>
<td>-</td>
<td>0.50</td>
<td>&lt;0.01 (0.73)</td>
<td>-</td>
</tr>
<tr>
<td>RI</td>
<td>&lt;0.01 (0.63)</td>
<td>0.50</td>
<td>-</td>
<td>&lt;0.01 (0.65)</td>
<td>0.89</td>
</tr>
</tbody>
</table>

P values of Spearman’s correlation test are indicated. R values (between brackets) are shown when p<0.05.

Both subgroups revealed a strong correlation between Vmax and both Vmin and RI (p<0.01 in all correlations; r range between 0.65 and 0.86). POAG SVP+ had a similar correlation pattern with the healthy SVP+ on what CRV variables’ correlation was concerned. While in POAG SVP+ patients, Vmin was not associated with CRV’s RI (p=0.51), a borderline significant association was detected between these variables in the SVP- subgroup (p=0.05, r=0.31).
IX. C) Central retinal vessels variables and glaucomatous damage

This analysis was made, as expected, only in the glaucoma groups. In this statistical analysis, both overall and subgroup correlations were looked upon. The full description of the statistical analysis correlating the venous flow parameters is depicted in table 21.

In the POAG group, Vmax had a positive correlation with MD in the SVP+ patients as well as in the overall POAG population (p=0.03, r=0.33; p=0.01, r=0.31; respectively), while in the SVP- population, that association was only borderline significant (p=0.05, r=0.30). Vmin and RI had a positive correlation with MD in the overall POAG population (p=0.02, r=0.26; p=0.04, r=0.22; respectively), but not in any of the subgroups (p range between 0.07 and 0.14). RNFL thickness had no correlation with any of the CRV variables in any of the studied populations (p range between 0.18 and 0.93).

In the NTG group, no correlations between CDI parameters and functional or structural glaucomatous damage were found in the overall population, nor in the SVP+ population (p range between 0.13 and 0.97). However, in the SVP- subgroup, a negative association was detected between RNFL thickness and RI (p=0.04, r=-0.37), and a borderline negative association with Vmax (p=0.05, r=-0.35). In this subgroup, functional damage was not associated with any of the CRV variables (p range between 0.41 and 0.73).
Table 21. Correlation between glaucomatous damage and CRV variables

<table>
<thead>
<tr>
<th></th>
<th>Vmax</th>
<th></th>
<th></th>
<th>Vmin</th>
<th></th>
<th></th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MD</td>
<td>RNFL</td>
<td>MD</td>
<td>RNFL</td>
<td>MD</td>
<td>RNFL</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>0.01</td>
<td>0.76</td>
<td>0.02</td>
<td>0.28</td>
<td>0.04</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>(0.31)</td>
<td></td>
<td></td>
<td>(0.26)</td>
<td></td>
<td>(0.22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>POAG</td>
<td>0.03</td>
<td>0.39</td>
<td>0.14</td>
<td>0.18</td>
<td>0.14</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>SVP+</td>
<td>(0.33)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SVP-</td>
<td>0.05</td>
<td>0.91</td>
<td>0.07</td>
<td>0.56</td>
<td>0.12</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.30)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>0.13</td>
<td>0.20</td>
<td>0.28</td>
<td>0.33</td>
<td>0.18</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>SVP+</td>
<td>0.23</td>
<td>0.97</td>
<td>0.65</td>
<td>0.92</td>
<td>0.08</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>NTG</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SVP-</td>
<td>0.71</td>
<td>0.05</td>
<td>0.41</td>
<td>0.28</td>
<td>0.73</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(-0.35)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(-0.37)</td>
<td></td>
</tr>
</tbody>
</table>

*P values of Spearman’s correlation test are indicated. R values (between brackets) are shown when *p* < 0.05.

In the analysis concerning CRA (table 22), both PSV and MFV had a positive association with the functional damage in the POAG overall patient group and SVP+ subgroup (Overall: *p*=0.01, *r*=0.28; *p*=0.02, *r*=0.25; SVP+: *p*=0.02, *r*=0.37; *p*=0.03, *r*=0.34; respectively). MFV also had an association with RNFL thickness in both these populations (Overall: *p*=0.03, *r*=0.24; SVP+: 0.01, *r*=0.33). RNFL was also positively correlated with PSV and EDV in this POAG SVP+ subgroup (*p*=0.04, *r*=0.26; *p*=0.03, *r*=0.27; respectively). In the overall POAG population, CRA’s RI was also significantly associated with the MD values (*p*=0.03, *r*=0.24). No correlation between any of the glaucomatous damage variables and CRA hemodynamic parameters was detected in the POAG SVP- subgroup (*p* range between 0.31 and 0.98).
Table 22. Correlation between glaucomatous damage and CRA variables

<table>
<thead>
<tr>
<th></th>
<th>PSV</th>
<th>EDV</th>
<th>MFV</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MD</td>
<td>RNFL</td>
<td>MD</td>
<td>RNFL</td>
</tr>
<tr>
<td>Overall</td>
<td>0.01 (0.28)</td>
<td>0.07</td>
<td>0.30</td>
<td>0.11</td>
</tr>
<tr>
<td>POAG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SVP+</td>
<td>0.02 (0.37)</td>
<td>0.04 (0.26)</td>
<td>0.22</td>
<td>0.03 (0.27)</td>
</tr>
<tr>
<td>SVP-</td>
<td>0.38</td>
<td>0.65</td>
<td>0.98</td>
<td>0.74</td>
</tr>
<tr>
<td>NTG</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>SVP+</td>
<td>0.75</td>
<td>0.59</td>
<td>0.42</td>
<td>0.64</td>
</tr>
<tr>
<td>SVP-</td>
<td>0.57</td>
<td>0.89</td>
<td>0.04 (0.36)</td>
<td>0.86</td>
</tr>
</tbody>
</table>

P values of Spearman’s correlation test are indicated. R values (between brackets) are shown when p<0.05.

In the NTG patients, no statistical correlations were observed in either overall population or SVP+ subgroup (p range between 0.16 and 0.92). The only significant association observed in the NTG patients was between the functional damage and CRA’s EDV in the SVP- subgroup (p=0.04, r=0.36).

Of note, considering that MD values are expressed as minus (below 0), a positive coefficient of correlation (r value) indicates that higher velocities are associated with a lesser glaucomatous damage.
IX. D) Central retinal vessels and IOP

An analysis of possible associations between central retinal vessels’ blood flow velocities and IOP was performed. Only the association between CRA’s RI and IOP reached p values below 0.05 and only in the POAG overall patients (p=0.02, r=0.25). No other associations – including in every NTG group - were of statistical significance (p range between 0.08 and 0.96). These statistical analyses are depicted in table 23.

<table>
<thead>
<tr>
<th></th>
<th>CRA</th>
<th>CRV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PSV</td>
<td>EDV</td>
</tr>
<tr>
<td>Overall</td>
<td>0.13</td>
<td>0.34</td>
</tr>
<tr>
<td>Healthy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SVP+</td>
<td>0.95</td>
<td>0.54</td>
</tr>
<tr>
<td>SVP-</td>
<td>0.44</td>
<td>0.22</td>
</tr>
<tr>
<td>Overall</td>
<td>0.89</td>
<td>0.08</td>
</tr>
<tr>
<td>POAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SVP+</td>
<td>0.61</td>
<td>0.21</td>
</tr>
<tr>
<td>SVP-</td>
<td>0.57</td>
<td>0.20</td>
</tr>
<tr>
<td>Overall</td>
<td>0.74</td>
<td>0.16</td>
</tr>
<tr>
<td>NTG</td>
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<td>SVP+</td>
<td>0.59</td>
<td>0.36</td>
</tr>
<tr>
<td>SVP-</td>
<td>0.15</td>
<td>0.33</td>
</tr>
</tbody>
</table>

P values of Spearman’s correlation test are indicated. R values (between brackets) are shown when p<0.05.
IX. E) Central retinal vessels and ONSD

An analysis of the correlation between these ONSD measurements and flow velocities in the central retinal vessels is described in table 24.

In the healthy population, there was a strong negative association between venous maximal velocity and ONSD in the SVP- subgroup (p=0.02, r=-0.61). No other statistical associations were found in any of the healthy subjects’ analysis (p range between 0.18 and 0.97).

POAG patients demonstrated several associations with ONSD. Both the overall POAG patient group and the SVP- subgroup had negative associations between CRA’s EDV and MFV and ONSD (Overall: p=0.02, r=-0.27; p=0.03, r=-0.27; SVP-: p=0.40, r=-0.40; p=0.01, r=-0.42; respectively). The venous Vmin was also negatively correlated with ONSD in these two groups (Overall: p=0.04, r=-0.25; SVP-: p=0.03, r=-0.36). Additionally, venous vmax negatively correlated with ONSD in the overall patients, and arterial PSV in the overall patients as well as the SVP- subgroups (Vmax: p=0.04, r=-0.24; PSV: p=0.03, r=-0.37). No correlations were seen in the POAG SVP+ subgroup (p range between 0.13 and 0.54).

Furthermore, no statistical associations were observed between central retinal flow velocities and ONSD in any of the NTG patient groups (p range between 0.06 and 0.99).
<table>
<thead>
<tr>
<th></th>
<th>CRA</th>
<th></th>
<th></th>
<th></th>
<th>CRV</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PSV</td>
<td>EDV</td>
<td>MFV</td>
<td>RI</td>
<td>Vmax</td>
<td>Vmin</td>
</tr>
<tr>
<td>Healthy</td>
<td>Overall</td>
<td>0.65</td>
<td>0.75</td>
<td>0.56</td>
<td>0.82</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>SVP+</td>
<td>0.27</td>
<td>0.26</td>
<td>0.21</td>
<td>0.97</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>SVP-</td>
<td>0.37</td>
<td>0.34</td>
<td>0.42</td>
<td>0.38</td>
<td>0.02 (-0.69)</td>
</tr>
<tr>
<td>Healthy</td>
<td>SVP+</td>
<td>0.06</td>
<td>0.02 (-0.27)</td>
<td>0.03 (-0.25)</td>
<td>0.29</td>
<td>0.04 (-0.24)</td>
</tr>
<tr>
<td></td>
<td>SVP-</td>
<td>0.03 (-0.37)</td>
<td>0.02 (-0.40)</td>
<td>0.01 (-0.42)</td>
<td>0.68</td>
<td>0.11</td>
</tr>
<tr>
<td>Healthy</td>
<td>Overall</td>
<td>0.46</td>
<td>0.65</td>
<td>0.35</td>
<td>0.29</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>SVP+</td>
<td>0.96</td>
<td>0.52</td>
<td>0.59</td>
<td>0.45</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>SVP-</td>
<td>0.08</td>
<td>0.72</td>
<td>0.19</td>
<td>0.06</td>
<td>0.45</td>
</tr>
</tbody>
</table>

P values of Spearman’s correlation test are indicated. R values (between brackets) are shown when p<0.05.
IX.  F) Central retinal vessels and OPA

As with ONSD, an analysis on the association between OPA and other variables was made, including the central retinal vessels’ blood flow. Tables 25 illustrate such analysis.

<table>
<thead>
<tr>
<th>Table 25. Correlation between central retinal vessels and OPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRA</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td>Overall</td>
</tr>
<tr>
<td>Healthy</td>
</tr>
<tr>
<td>SVP+</td>
</tr>
<tr>
<td>SVP-</td>
</tr>
<tr>
<td>POAG</td>
</tr>
<tr>
<td>Overall</td>
</tr>
<tr>
<td>SVP+</td>
</tr>
<tr>
<td>SVP-</td>
</tr>
<tr>
<td>NTG</td>
</tr>
<tr>
<td>Overall</td>
</tr>
<tr>
<td>SVP+</td>
</tr>
<tr>
<td>SVP-</td>
</tr>
</tbody>
</table>

P values of Spearman’s correlation test are indicated. R values (between brackets) are shown when p<0.05.

In healthy subjects, no correlations were detected between OPA and central flow velocities in either of the two subgroups (p range between 0.13 and 0.96).

In the POAG patients, only the venous Vmin was associated with OPA and only in the overall patient group and in the SVP+ subgroup (p=0.03, r=-0.35; p=0.01, r=-0.57; respectively). Every other association in any of the POAG patient groups did not reach statistical significance (p range between 0.13 and 0.95).
In NTG patients, a strong negative correlation was seen between OPA and CRV RI in the SVP+ patients (p=0.02, r=-0.63). Other correlations were not statistically significant (p range between 0.08 and 0.99).

IX. G) Doppler signs of dysregulation by SVP status

An analysis of the retrobulbar hemodynamic pattern was made using the cutpoints in vascular resistance recently described in the literature (133). In the POAG group, 8 out of the 43 SVP+ patients (18.6%) had a CRA with a RI > 0.77. In the 43 SVP- patients, 13 of them had CRA RI values above this cutpoint (30.2%). No difference was seen between these proportions in the two subgroups (p=0.31, OR=0.53, CI=0.19-1.44). Additional Chi-square tests between the POAG overall population, SVP+ and SVP- populations demonstrated no differences between these three population sets (p=0.46). These results are illustrated in table 26 and figure 8.

<table>
<thead>
<tr>
<th>CRA cutpoints by SVP status</th>
<th>POAG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SVP +</td>
</tr>
<tr>
<td>CRA &gt; 0.77</td>
<td>8</td>
</tr>
<tr>
<td>CRA &lt; 0.77</td>
<td>35</td>
</tr>
</tbody>
</table>

Data presented as absolute number of patients with RI above and below 0.77 in the considered subgroup. Fisher-exact test was used in this analysis.
In the NTG population, the two cutpoints previously described were considered for analysis (CRA RI > 0.61, OA > 0.82). Additionally, the existences of the two combined cutpoints were also examined. The CRA analysis revealed no difference in the proportion of patients above or below that cutpoint when overall, SVP+ and SVP- groups were considered (p=0.11). No differences were found in the OA cutpoints, neither in the comparison of three sets of patients nor in the SVP+/SVP- head-to-head comparison (Chi-Square: p=0.70; Fisher test: p=0.46, OR=1.52, CI=0.57-4.00; respectively). The analysis of the combined cutpoints (CRA+OA) again revealed no differences between any of the three groups or SVR+/SVP- subgroups (Chi-Square: p=0.56; Fisher test: p=0.33 OR=1.70 CI=0.64-4.50, respectively). Table 27 to 29 and figure 9 illustrate these findings.
Table 27. NTG CRA cutpoints by SVP status

<table>
<thead>
<tr>
<th>NTG</th>
<th>SVP +</th>
<th>SVP -</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRA &gt; 0.61</td>
<td>30</td>
<td>25</td>
<td>0.11</td>
</tr>
<tr>
<td>CRA &lt; 0.61</td>
<td>5</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

Data presented as absolute number of patients with RI above and below 0.61 in the considered subgroup. Fisher-exact test was used in this analysis.

Table 28. NTG OA cutpoints by SVP status

<table>
<thead>
<tr>
<th>NTG</th>
<th>SVP +</th>
<th>SVP -</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OA &gt; 0.82</td>
<td>23</td>
<td>19</td>
<td>0.46</td>
</tr>
<tr>
<td>OA &lt; 0.82</td>
<td>12</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

Data presented as absolute number of patients with RI above and below 0.82 in the considered subgroup. Fisher-exact test was used in this analysis.

Table 29. NTG OA + CRA cutpoints by SVP status

<table>
<thead>
<tr>
<th>NTG</th>
<th>SVP +</th>
<th>SVP -</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRA &gt;0.61 ∧ OA &gt; 0.82</td>
<td>23</td>
<td>18</td>
<td>0.33</td>
</tr>
<tr>
<td>CRA &lt;0.61 ∨ OA &lt; 0.82</td>
<td>12</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

Data presented as absolute number of patients with RI above cutpoints in CRA and OA compared to the rest of the considered subgroup. Fisher-exact test was used in this analysis.

Figure 9. NTG patients distribution by SVP status and CRA, OA and CRA+OA RI cutpoints
IX. 

H) Discussion

This section is particularly interesting as it’s the first section where differences between healthy individuals with and without a SVP phenomenon are seen. SVP+ subjects had a higher venous Vmin than their SVP- counterparts. This increase in Vmin without the consequent increase in Vmax should theoretically decrease venous pulsatility. Such decreases are seen in venous stasis conditions, from lower limb vein to portal vein thrombosis (228) (229). In our results, the lack of difference in RI could be related to the fact this variable depends on the fluctuations of both Vmax and Vmin, and as such, more reliant on the differences in proportion than on the isolated values of each. However, the asymmetry in numbers between the two subgroups could, as previously explained, have made this study underpowered to address this issue. It is thus possible that RI differences could have reached statistical significance should the number of patients been higher.

The nearly 18% individuals we found not to have a visible SVP, while healthy in ocular terms to the best of our knowledge, may represent patients with a number of conditions which can interfere with venous drainage at some point between the retinal veins and the right atrium. Increases in the cavernous sinus pressure or in the jugular veins have been related to increases in IOP (230) (231), in what has been linked to increases in downstream pressure with the subsequent venous stasis. Additionally, our own results point to a negative correlation between venous velocities and the ONSD.
This could be caused by an increase in downstream pressure related to the intracranial pressure ability to interfere with the venous drainage (232). However, and despite reports stating that healthy individuals’ ON sheath does correlate with the cerebral ventricular fluid pressure, the hypothesis involving a more localized effect with an increase in external compression of the CRV walls during its pathway around the optic nerve cannot be ruled out. Furthermore, other systemic variables can interfere with venous stasis. Blood viscosity for instance, has been related to CRV occlusion (233). In this context, decreases in red cell deformability have been correlated with similar Doppler central retinal venous measurements (234). Interestingly, as CDI measurements are performed in the retrobulbar portion of the CRV, the stasis pattern would not be seen should the major obstruction point be upstream. Of note, while our results point to the fact that this lack of SVP phenomenon is probably related to an extra-ocular mechanism, it has been suggested that patients whose retinal veins do not pulsate may have an increased venous pressure and therefore can be at an increased risk for central vein occlusions (235). Further longitudinal studies would be needed to assess this theory.

There were, however, additional differences in the correlations seen in SVP+ and SVP- healthy individuals. While in SVP+ healthy individuals higher CRA velocities correlated, although weakly, with the venous velocities, no such correlation was detected in the
individuals in whom this phenomenon was absent. Additionally, in these subjects lacking SVP, the higher the central arterial resistivity, the lower the venous velocities were. Apparently, our results could suggest that, in the absence of SVP, an increase in arterial velocities would not be matched by an increase in venous velocities. This could be related to the inability of the venous flow to accommodate that arterial increase in face of an increased venous stasis. For instance, some authors have suggested arterial flow to affect post-capillary retinal venous flow (236), raising the possibility this could be a factor involved in the SVP phenomenon in healthy individuals. In SVP- healthy individuals, however, the proposed intraluminal pressure increase could dampen this arterial propagation, as seen in increased venous pressure conditions, for instance in patients with central vein occlusion (236). This negative correlation between arterial resistance and venous velocities could be related to an adaptation to the low perfusion pressure at the level of the retinal capillaries. In other vascular trees capable of auto-regulation, a decrease in the arteries’ perfusion pressure is compensated by a dilation in more distal, smaller arteries (237) (238). This increase in the cross-section vascular area attempts to lessen distal resistance and keep blood flow velocities within normal range (173). Only when these small vessels reach their maximal dilation capability, does blood flow velocity decay. Our results thus suggest that, according to this hypothesis, the ocular circulation correlations in healthy SVP- individuals could just reflect an adaptation to a decrease in ocular perfusion pressure (239).
Another possible explanation for this association between CRA and CRV velocities could relate to the common pathway these vessels share. The common adventitia between these two vessels at the optic nerve head (240) and within the optic nerve mass can help propagate the arterial wave into the CRV (241). This ability for this anatomically close artery to interfere with venous physiology has been widely described, being credited as one of possible factors promoting branch retinal vein occlusion (242).

Concerning the OA Doppler waveform characteristics, our data suggest that differences between healthy individuals who did and did not present a visible SVP did not relate to wall properties. As these variables are suggested to reflect arterial compliance and overall stiffness (175), our results thus reinforce our hypothesis that SVP status refers to a difference in intraluminal pressures, rather than wall structures.

Regarding the glaucoma populations, the differences in Doppler variables or the correlations between them were not the same in NTG and POAG patients.

In both glaucoma groups, we have identified differences in blood velocities and resistance indices between those with and without a visible SVP at the CRV level. As glaucoma patients have retinal vessels with a smaller diameter (194), lower blood velocities are expected if perfusion pressure is not proportionally increased. Considering the existence of a throttle of the retinal vein as it
leaves the eye at the *lamina cribrosa* level, this upstream stenosis could affect flow at the level of CDI measurements (i.e. behind the lamina cribosa). As *lamina cribrosa* structural properties are changed in glaucoma patients (198), its pores can have a significant impact on the arterial and venous flow in the vessels that pass through them. Should this pore-related stenosis be hemodynamically significant, this could significantly decrease flow velocities, as is elsewhere seen in post-stenotic flow assessments throughout the body (for instance at the carotid or renal level) (243) (244). Additionally, the decrease in RI could represent a further extent in the loss of pulsatility of non-pulsating veins, as previously explained (see above). These smaller differences between Vmax and Vmin are common in vascular systems with a significant decrease in perfusion pressure and/or significant decrease in vessel compliance (174). Considering that these recordings were taken after a widely established obstruction point, these readings are *de facto* taken at a post-stenotic level, where the pressure would significantly decay (245). If, as suggested, the veins in glaucoma are exposed to a number of agents known to promote vessel hypertrophy and increase in vessel stiffness, then the ability of the post-stenotic vessel to accommodate outside modulation might be additionally impaired.

In POAG patients, a higher ONSD negatively impacted flow velocities in both central vessels in SVP negative patients. By acting outside the eye, an increased ONSD (and the implied retrobulbar
pressure) would be effectively changing the translaminar gradient. As previously explained, the gradient between any compartments depends on the nature of the structure separating them. As glaucoma patients have been suggested to have morphologic changes at the lamina cribosa level, even small changes in each compartment could lead to significant changes at that gradient. Therefore, it is possible that increases in ONSD could lead to a significant lowering of the otherwise elevated pressure gradient. Because velocity measurements are done behind the lamina cribrosa, this decrease in gradients would decrease flow turbulence. The observed blood velocity would still be dependent on the degree of vessel dilation that is usually seen after a significant obstruction. As this latter variable (vessel width) is not accurately measured by CDI, any interpretation of flow output based on the blood velocity is limited by this important technological limitation. However, and should in fact a hemodynamically significant obstruction exist, then the translaminar gradient would be maintained at a higher level despite the pressure difference between the two compartments becoming smaller. This would mean that ONSD would affect a post-stenotic vessel section, where a significant decrease in intraluminal pressure is likely to occur. As such, these flow patterns could be more easily modulated by outside forces. Summarizing, our results could suggest that SVP negative POAG patients could have different lamina cribrosa properties than their
SVP positive counterparts, which might make downstream flow more sensitive to outside compression.

Increases in OPA, on the other hand, lead to an increase in pulse pressure inside the eye. This increases the pressure gradient between the two compartments. If the venous outflow is already impaired by a severe downstream obstruction, then the additional increase in pulse pressure would not produce any change in venous output as measured after the obstruction (at least in our result range). As such, these changes were only seen in SVP+ patients.

In NTG patients however, retro-ocular pressure has been described to be lower, and therefore the translaminar pressure to be higher (246). As previously explained, we have used ONSD as a surrogate for such retro-ocular pressure, and have not verified such decrease (134). Nevertheless, within our results range, no correlations were found between ONSD and blood velocity in any NTG patient. Our working-hypothesis that NTG patients possess more rigid, less compliant veins are therefore less likely to propagate the external exerting pressure into an already small intraluminal pressure, thus providing a rationale as to why no association is detected. As to the OPA, it apparently negatively affects venous resistance only in SVP+ patients. Increases in OPA would increase the amount of blood pressure in the eye, and therefore – either in phase or not, i.e. with a delay in time or not – more blood pressure would be seen in the vessels leaving the eye. If a significant obstruction
exists, then it is possible that no downstream flow change would occur. In this scenario however, the upstream increase in pressure would have to be compensated by either an increased retinal vessel diameter or a significant increase in wall stress. While our experimental setting did not allow for vessel width determination, it remains an important limitation to the interpretation of our data regarding these SVP-absent NTG patients. As for NTG patients with a visible SVP, there is a visible fluctuation of flow leaving the eye. Increases in pulse pressure can therefore be reasoned to affect this outward flow. It can be argued that the increased outward pressure leads to a decrease in retrobulbar CRV pulsatility. Again, diameter calculations would be unvaluable to assess whether any kind of post-stenotic vessel dilation would interfere significantly with blood velocity. However this inability to accurately measure diameters remains one of the major limitations of any CDI study of the retrobulbar vessels.

Our results have shown that NTG patients also showed significant arterial velocity changes. It can be argued that one of two possible mechanisms may be involved. Either the hypothetical increase in retinal venous pressure increases downstream resistance to arterial flow (thus decreasing perfusion pressure) or, alternatively, those same pores that are responsible for the venous obstruction could also affect the arterial inflow into the eye by compressing the CRA. Interestingly, only these patients with both arterial and venous decrease in blood velocities (NTG patients with an absent
SVP phenomenon) had a more advanced stage of the disease. Existing data support the association between a lower PSV and higher glaucomatous damage (90), which would agree with our results. One possibility is that these NTG patients may have a more severe vascular dysfunction, in which increased venous stiffness and increased retinal venous pressure can affect perfusion pressure. As decreased perfusion has been consistently associated with disease progression (163), it can be argued that the lack of SVP phenomenon in NTG patients can be used as an indirect sign of a severe vascular dysfunction (affecting both arterial and venous flow) and a more advanced form of the disease.

In POAG patients, however, it could be argued that SVP-absent patients, even though showing signs of venous dysfunction, these may not be severe enough to condition arterial flow impairment. Endothelin, for instance, has been suggested to play a greater role in NTG patients, not only by having a potentially higher concentration, but also because NTG patients may have different endothelin-receptor polymorphisms (69) (247).

Endothelin, a well-known vasoconstrictor agent with a likely link to glaucoma (248) has a strong impact on the venous endothelium (196). In other vascular beds, such as in the lungs, veins have a nearly tenfold higher sensibility to endothelin than the corresponding arteries (249). In conditions of ischemia and hypoxia, there is a known effect of hypertrophy and increased resistance of an otherwise very low resistance vascular bed (250).
In the ocular circulation, a recent report has even suggested that this venous wall stress caused by such a high pressure gradient in the post lamina cribrosa segment can lead to a pseudo-arterialization of the veins’ endothelium (195). Several studies have reinforced the role of endothelin in significantly modulating venous flow, as endothelin receptor antagonists have a positive impact on retinal venous flow (204) (251). Interestingly, this peptide has been shown to be also increased in patients with ocular venous diseases such as retinal central vein occlusions (252) and retinal branch venous occlusions (253).

Taken together, this literature review regarding NTG related to our data, suggests that changes in venous drainage seen in NTG patients could be related to an underlying mechanism that can potentially affect glaucoma pathogenesis while aggravating structural venous changes. The most likely candidate so far is endothelin. The stiffer, thicker (and probably smaller diameter) CRV resulting from exposure to such vasoactive agent would be less likely to be influenced by external sources, potentially raising the threshold for SVP generation in glaucoma patients to a much higher level.

Interestingly, POAG patients without SVP had a lower Sm/Dm ratio, which could suggest that these patients had a more compliant vessel. Converting a central pulsatile flow into a steady flow in the peripheral tissues (Windkessel function) depends on the arteries’ compliance status. During the cardiac cycle, a portion of the kinetic
energy from each systolic pulse is stored within the compliance of the vascular tree by distension of the vessel walls. When intravascular pressure decreases towards the end of systole, this potential energy is released as compliance flow. In low distal resistance settings, this anterograde compliance flow prolongs systole and provides anterograde flow in diastole. When distal resistance is high, the compliance flow is now retrograde and does not contribute to the diastolic pulse (174) (254). Our results would thus reflect a lower distal vascular resistance, an otherwise adaptive response by a vascular territory capable of autoregulation to a state of low-perfusion.

The studies that analyzed OA waveforms to detect arterial compliance were performed in otherwise ocular healthy individuals (175), and as such neglects to take into account local effects of the disease. While the interpretation of their results is valid for our healthy individuals (see above), our interpretation of the observations in glaucoma patients was based on one recently published study by our group (172). In that study, we argued that OA waveform patterns were different according to the disease stage. Interestingly, the pattern change we found was similar the pattern changes seen heart failure. Transmitral flow analysis for ventricular diastolic dysfunction has remarkable similarities to our data. A normal relaxed ventricle has a left-upward waveform. A stiffer, less compliant ventricle prolongs relaxation time, reducing early velocities and increasing late diastolic velocities, producing a
rightward movement. However, when the ventricular dysfunction is high enough there is a “pseudo-normalization” of the waveform. The very high ventricular stiffness, coupled with a secondary upstream increase in pressure returns the blood flow pattern into an otherwise normal left-upward waveform. In the last stages of diastolic dysfunction, a restrictive pattern develops where flow is almost abolished in this last part of the cardiac cycle (255) (256). This waveform progression pattern is very interesting, as the more advanced glaucoma waveform’s shift to the left could be compared to this pseudo-normalization phenomenon (172). This may help explain the differences in our waveform pattern results. The NTG pattern in SVP-absent patient resembles this more restrictive pattern (higher Sm), in accordance to the more advanced disease, while the lower Sm/Dm ratio in POAG can resemble an earlier stage of this dysfunction.

Unlike healthy individuals, where a higher arterial RI was associated with lower venous velocities, in SVP-glaucoma patients, the correlation between arterial and venous Doppler, although of small magnitude, was a positive one. If the limit of auto-regulation has been reached (or surpassed) then arterial flow could have a direct impact on venous velocity. The lack of buffer capacity could lead to higher arterial velocities to condition a higher venous velocity. However, this limit does not relate to the existence/absence of the SVP phenomenon in POAG patients, as similar (although weak) correlation patterns are seen in both
subpopulations. In NTG patients, however, a distinct pattern is seen in patients without a visible SVP. Considering our autoregulation hypothesis, the lack of SVP would be associated with a significant microvascular poor adaptation to an already impaired arterial function. These assumptions of the autoregulatory ability of these patients are in accordance with our data correlating blood vessel velocity and the degree of glaucomatous damage. In POAG patients, no differences were seen between SVP+ and SVP- patients in the correlations between flow velocities and glaucomatous damage. On the other hand, in NTG patients, only SVP- patients showed such an association.

As expected from the recent literature, the IOP levels did not correlate with flow velocities in glaucoma patients nor healthy individuals (257). This is particularly important as it supports the notion that retrobulbar flow changes detected in glaucoma patients by Doppler technology are not dependent on IOP and that accordingly, targeting IOP per se as a therapy would not change retrobulbar hemodynamics. Accordingly, this line of thought would suggest that topical IOP-lowering glaucoma medication would be unlikely candidates to change the patients’ SVP status. This hypothesis, however, does not take into account any non-IOP related vascular effect some of the medications are known to have, particularly carbonic anhydrase inhibitors (see above).

Our assessment of the autoregulation cutpoints did not detect any differences between any subpopulations of both glaucoma groups.
Our cutpoints were designed to detect changes in the vessel pulsatility patterns when faced with increased arterial resistance. However, and despite its potential use to detect the resistance point at which this vessel starts to develop a different pattern, no study so far has detected the clinical usefulness of these cutpoints (i.e. whether abnormally pulsatile vessels are associated with increased glaucomatous damage or progressing disease). Our results suggest that there may be differences in structural wall patterns in SVP+ and SVP- patients that could potentially change that pulsatility pattern. Accordingly, new cutpoints would need to be determined in each of these SVP+ and SVP- subgroups in order to detect the amount of arterial resistance associated with this change in pulsatility patterns.
Chapter 5. General Discussion

This work tried to assess through a case-control study the significance of the SVP in the clinical evaluation of a glaucoma patient. By characterizing this pulsating phenomenon in accordance to the ocular anatomic, clinical and hemodynamic variables of both healthy and glaucoma patients, we aimed at clarifying the potential usefulness of identifying SVP in this disease. Of additional interest, the logistics of the center where this study was conducted allowed for the recruitment of more than the double of patients originally planned. This large-scale increase allowed for the addition of a specific glaucoma group – NTG patients. Study of this phenomenon in this set of glaucoma patients had not been performed before, nor whether the differences or associations between variables were similar to the rest of the glaucoma populations. This provided the opportunity to study the relevance of this vascular variable in the set of glaucoma patients that are more liable to vascular dysfunctions.

After a thorough validation of the conditions of our case-control study (including its reproducibility), we verified whether both glaucoma groups had a significantly lower frequency of the SVP phenomenon, thus answering our first research question. Literature on this subject has been conflicting. Both of the theories regarding SVP would suggest it to be higher in glaucoma patients (114) (116). This hypothesis, however, does not match our results, nor the clinical studies performed in glaucoma patients (111) (258),
in which these patients show a significant decrease in SVP when compared to healthy controls.

Accordingly, the existence of SVP in glaucoma patients may be dependent on conditions different from what would be expected from healthy ocular physiology. As much of the differences between the groups and SVP subgroups have been discussed earlier in their proper chapter, we will try to henceforth summarize our findings, clarify how we addressed our proposed goals and discuss our study limitations.

Our results have failed to detect any optic disc head differences between healthy and glaucoma patients who did and did not present a visible SVP. Despite reports claiming that some optic disc features may help improve SVP visualization in healthy eyes (112), we have failed to detect any asymmetry in cup/disc ratio, cup depth or disc area between SVP+ and SVP- subjects in any of the three groups. As increased disc cupping, for instance, would change the CRV pathway by altering its curve radius (192) – and therefore affecting flow patterns inside the vessel (193) – it could be argued that topography would have played a role in whether SVP could be visualized or not. The large disproportion between healthy patients with and without SVP may have made this study underpowered to fully address this morphology questions in otherwise healthy eyes. However, as far as glaucoma patients are concerned - from both POAG and NTG groups alike -, there are several possibilities as to why morphologically induced flow
changes have not apparently interfered with the SVP. Either there is an increase in intraluminal pressure related to a downstream obstruction that compensates for the curvature radius and smaller vessel diameter, or there is an intrinsic change in the wall properties of the glaucoma patients’ veins. One important limitation for these anatomic considerations is that we have not measured one important variable: the thickness of the lamina cribrosa. As interesting as this could have been – as the thickness and structural properties of any membrane separating two compartments can affect the pressure gradient (185) - technological instruments capable of accurate in vivo measurements are still under validation process (186). Therefore, there is a possibility that later studies that look into this very important aspect may prove anatomical considerations to be relevant for the development of a visible SVP. Our answer (and goal) would, however, remain untarnished, as from the clinical observation point of view, SVP phenomenon in glaucoma patients cannot be attributed to currently-assessed morphometric variables.

IOP measurements are an essential part of any ophthalmology evaluation, but of paramount importance in glaucoma patients. Moreover, it has been implicated in the generation of SVP (114) (116). In fact, the higher the IOP, the higher the value of intraocular pulse pressure and therefore, an increased likelihood of a higher translaminar pulse difference. Interestingly, and to the
same IOP level, we observed a much lower SVP frequency in glaucoma patients than in healthy individuals. It can thus be argued that either transmural propagation of the IOP-induced pulse pressure is more difficult, or that there is a more significant downstream obstruction to this propagation. The more likely alternative is that both situations exist in glaucoma patients, where both the otherwise normal CRV throttle may be exacerbated by a distorted *lamina cribrosa* (259), but where the vein wall may be hypertrophied and less compliant as well (250) (195).

However, we did detect lower IOP levels in SVP- POAG than in their SVP+ counterparts. This is supported by the existing data that suggest the lack of a visible SVP to be an indirect sign of effective IOP-lowering (125). The lack of differences if IOP between the two NTG subgroup, coupled with the feeble to non-existing correlations between IOP and blood velocities in the CRV, supports the vascular approach to the phenomenon rather than just external (mechanical) transmission of pulse by IOP. In this sense, the threshold for SVP formation in NTG patients is most likely set to a higher IOP value than in healthy individuals.

Perhaps the most interesting aspect of our study is the different correlation between glaucomatous damage and SVP frequency in both glaucoma groups. While in POAG patients, there is no change in SVP detection as the functional damage increases, in NTG patients there was a relevant, statistically significant decrease in the frequency of the SVP phenomenon with a worsening MD. In
fact, the overall MD was almost twice as high in SVP- than in SVP+ NTG patients. This apparent link between functional damage and what appears to be a sign of vascular venous dysfunction may prove invaluable in the clinical setting. This quick, easily assessable sign can, at first glance, potentially label those patients with a more advanced condition.

The hemodynamic studies using CDI technology was another novelty in our work, as it had not been previously used to assess this phenomenon. The results of this CDI study enabled the detection of a different pattern in both glaucoma groups. Whereas SVP- NTG patients had both arterial and venous decreased velocities, SVP- POAG patients only presented with venous changes. While a number of possibilities can exist as to why these differences occur, our own data regarding the Doppler analysis of OA waveform can provide a further insight. NTG patients have waveform patterns that are compatible with a less compliant vessel, while POAG patients have waveform patterns that probably resemble a lesser form of wall structure changes (29). Glaucoma patients, and particularly NTG patients, are known to have increased levels of vasoactive agents such as endothelin (69). This peptide has been under scrutiny by a number of authors. For instance, it has been involved in arteriolar vasoconstriction and has been suggested to play a major role in the disease pathogenesis (260) (248). Interestingly, not only have its concentrations been suggested to be higher in progressive glaucoma patients (261), but
also are NTG more prone to endothelin-based vascular responses (59). Despite the small number of publications on ocular venous circulation, endothelin blockers have been shown to modulate venous blood flow in both healthy and glaucoma patients (204). More importantly, veins apparently can present nearly tenfold the sensitivity for this peptide than its corresponding artery (249). In other diseases which are endothelin-related, as in pulmonary hypertension, pulmonary veins have particularly hypertrophied walls (250). In fact, recent studies have supported the hypothesis the same pattern to be seen in glaucoma, as retinal vessels of NTG patients seem to be more rigid (180). As such, our results would agree with glaucoma patients – particularly NTG patients – having increased stiffness and less compliant vessels which would therefore be less responsive to outside modulators of their blood flow.

This hemodynamic study was the only positive finding regarding the healthy group. The higher increase in Vmin in SVP- individuals would raise the possibility of an increase in intraluminal pressure in a pattern that could resemble venous stasis (228). The SVP-patients could therefore be a reflection of some minor obstruction along the venous circulation anywhere from the ocular exit point to the right atrium. Additionally, any increase in viscosity, change in orbital tissue pressure, cavernous sinus, and jugular or heart condition could theoretically interfere with ocular venous output (233) (134) (231). While the possibility of changes in vessel
diameter (either before or after the lamina cribosa) cannot be ruled out, we have found no evidence of structural wall change in these healthy individuals. Nevertheless, while our results raise the hypothesis of this lack of SVP phenomenon in healthy individuals being probably related to an extra-ocular mechanism, it has been suggested that patients whose retinal veins do not pulsate may have an increased venous pressure and therefore can be at an increased risk for central vein occlusions (235). Further longitudinal studies would be needed to assess this theory.

To finalize our hemodynamic study, we tried to determine whether the recent findings of our group regarding resistance cutpoints in autoregulation capacity (133) had any association with the SVP phenomenon. Our lack of positive findings would suggest SVP status not to be determined by the degree of arterial/capillary dysfunction but rather by a more global combined arterial/venous dysfunction. Accordingly, new sets of cutpoints should be sought in each of the glaucoma subgroups.

Our characterization of the hemodynamics in glaucoma patients according to SVP status was far more detailed than any of the CDI studies on venous circulation. Nevertheless, the lack of information regarding these vessels’ diameter has limited the interpretation of our data. As such, and despite fulfilling our goal of characterization of the hemodynamic patterns and associations with SVP, several questions remain unanswered.
The notion that SVP has different meanings in each of the glaucoma groups extends beyond the hemodynamic patterns. Variables associated with pulse pressure changes (such as OPA and ONSD) affect these glaucoma populations differently. Extraocular pressure changes seem to significantly affect venous velocities in POAG rather than NTG patients in our study. This raises the hypothesis that the translaminar gradient might be more important in POAG than in NTG patients, at least as far as generating a SVP phenomenon is concerned. Should we accept the notion that NTG vessels are stiffer and less able to transmit outside compression into its intraluminar flow patterns, then a much higher gradient would be needed to allow for large flow fluctuations. In POAG patients, on the other hand, less vascular wall changes have been documented (180) (including our Waveform analysis), and therefore the difference in SVP status could potentially be determined by a different pressure gradient (when compared to the NTG patients). A significant mechanical “throttle” effect on the CRV by a glaucomatous-changed lamina cribrosa could be more important in these reportedly less-hypertrophied POAG veins. As such, any post-stenotic low flow measurements in SVP- POAG patients taken downstream of this obstruction (CDI measurements are taken behind the lamina cribrosa) could therefore be modulated by outside forces. SVP+ POAG patients had a similar pattern to the healthy individuals, in what could probably account for a less distorted lamina cribrosa. Intraocular pulse pressures, while acting upstream of that
“throttle”, had a similar behavior on both glaucoma subgroups. Nevertheless, our results did suggest that a higher OPA threshold is needed in NTG patients in order to produce a visible SVP.

Although we have addressed all the questions we set out to answer, a number of questions have risen. The limitations (see results section), from the technical limitations in the used tools to the lack of statistical power in analyzing healthy individuals, leaves room for further investigations to take place. Importantly, the fact that all SVP assessments were determined by direct visual detection by observers, allows the possibility that some of the individuals labeled as SVP- have small venous movements that could go undetected by the human eye. Despite a good reproducibility in our own results (see section), new devices are being described to electronically detect these pulsatile vessel movements (262). Additionally, devices such as the retinal oximeter – by its ability to assess retinal metabolism, venous oximetry and vessel diameter (191) – are likely candidates for further studies on this yet undervalued but clinically intriguing subject.

Summarizing, the results of our work indicate that SVP phenomenon might translate as a sign of vascular dysfunction. Interestingly, they also suggest the lack of SVP phenomenon to be related to different variables depending on the patient presenting with either POAG or NTG. While in POAG patients there is likely a mechanical component aggravating an underlying venous
dysfunction, in NTG patients the lack of this SVP could be related to both an additional arterial dysfunction and/or to the underlying dysfunctional venous circulation. Whether the venous dysfunction can condition a decrease in perfusion pressure, thereby altering arterial circulation, or whether both these changes are secondary to increased levels of vasoactive agents, remains undetermined. We report, however, the usefulness of this sign in NTG patients as a possible indication of more advanced glaucomatous disease and more advanced vascular (arterial and venous) dysfunction.
Chapter 6. Summary

Retinal veins spontaneous pulsation has been a well-recognized ophthalmological sign for more than a century. Additional to its usefulness in a number of neurological and ophthalmological conditions, recent papers have suggested it may be relevant as well in the field of glaucoma.

The mechanisms involved in the formation of this ophthalmological sign are not fully consensual, with a number of theories over its genesis. This lack of consensus has led to a creation of several hypothesis as to why the low frequency of SVP in POAG patients when compared to the otherwise healthy population.

Our prospective, case-control study presents the largest series published in this field of study, including using a number of technologies and variables not yet used in this context. Furthermore, for the first time, this was studied in patients where the vascular component may play a significant role in the pathogenesis and progression of the disease (NTG).

Our study confirmed the significant decrease in SVP identification in POAG (50%) and NTG (51%) patients when compared to the control group (82%). No difference was however detected in the frequency of the phenomenon between the two glaucoma groups.

The most interesting fact in our work may be that NTG patients lacking SVP have significantly more advanced functional damage
than NTG patients with a visible SVP. The lack of this sign at the physical examination, by labeling patients with a more advanced condition, may prove extremely useful in the clinical assessment of glaucoma patients with this form of the disease. Our results suggest this difference may be related to a change in the ganglion cell metabolic activity as despite the functional differences, we have not detected any structural differences at the nerve fiber layer thickness.

SVP status did not seem to depend on any morphological variable from optic nerve head topography in both healthy and glaucoma patients. As increased disc cupping necessarily affects the vessel curved pathway and consequently its intraluminal flow patterns, our findings seem to suggest such induced changes may not be sufficient to alter the SVP status.

For the same IOP intervals, glaucoma patients presented a lesser percentage of SVP detection. However, in POAG patients, the increase of this mechanical factor was associated with an increased detection of this phenomenon, while no such relation was seen in NTG patients. This seems to suggest the lack of SVP in NTG patients is not related to intraocular mechanic variables.

Extraocular pressures, examined indirectly by ONSD assessments, seem to have a different impact in the experimental groups. In control subjects, higher ONSD was associated with a lesser
detection of the phenomenon. In glaucoma groups, although with lesser frequency ratios to start with, no change in SVP detection is seen with changing ONSD values. These NTG patients without visible SVP have however an important association with ONSD, in what seems to be related to IOP. Our results would thus suggest that in this type of glaucoma, the drainage of aqueous humour may be particularly sensitive to changes in retrobulbar orbital pressures.

The two glaucoma groups had distinct correlation patterns with this ONSD variable, in only POAG patients without SVP presenting a correlation between this latter variable and central retinal vessel’s flow velocities. Our results would suggest that POAG patients’ CRV may be more susceptible to external compression, particularly if that pressure is exerted downstream to the major throttle point (the lamina cribosa). NTG patients may have a locally vasoactive-rich environment that could lead to a change in vessel structure – probably vascular wall hypertrophy – and consequently be less compliant to external pressures.

Doppler studies of the central retinal vessels have identified NTG patients without SVP to present with lower arterial an venous velocities, while POAG patients only presented with venous change. As the association with a higher degree of glaucomatous
disease was only detected in patients where both arterial and venous changes co-existed, our results seem to suggest that while the lack of SVP may be related to venous changes, only when a simultaneous alteration of arterial flow is seen will a higher glaucomatous damage exist.

Doppler OA waveform analysis has identified changes between the groups, thereby reinforcing our claims regarding the existence of structural vessel wall differences between NTG patients with and without SVP.

Our data does not support SVP status to signal what has been suggested to reflect vascular autoregulation cutpoints in vascular resistance. As our proposed cutpoints would reflect capillary inability to adapt to hypoperfusion status, SVP status may reflect not capillary but a combined, yet undetermined arterial/venous dysfunction.

From a hemodynamic point of view, the only identified difference in healthy individuals was an increase in Vmin in SVP- patients. This pattern has been described in venous stasis elsewhere in the body. Further studies are needed to validate this theory.

Another interesting data from our work is the association between SVP and diastolic blood pressure in POAG patients. As a known risk for CRV occlusion, the lack of this SVP phenomenon may signal the glaucoma patients at higher risk of developing vascular
pathologies. However, longer follow-up studies will be needed to confirm this hypothesis.

Limitations to our study relate to methodological issues and to technological insufficiencies. Our case-control study lacked information regarding systemic medications and relied on self-reported systemic and ocular medical history. Additionally, the limitations imposed by retrobulbar Doppler studies (from which no vessel diameter can be retrieved) as well as a clinical incapacity to directly assess retrobulbar pressures impose a limitation on flow and arterial and venous pressure interpretation. Finally, and considering the high frequency on SVP in healthy controls, and although our study has the largest recorded series on the subject, the small number of controls without SVP may raise concerns considering the relevance of the statistical significance of our findings in these populations.

In summary, this thesis seem to demonstrate that venous pulsations may be particularly important in NTG patients, where their absence may signal not only a wider vascular (arterial and venous) dysfunction but also a more advance stage of the disease. Further studies on the venous circulation will hopefully shed further light on this intriguing yet largely unexplored domain of the venous circulation in glaucoma.
Chapter 7. Sumário

A pulsatilidade venosa espontânea (SVP) da veia central da retina (CRV) é um sinal oftalmológico conhecido há mais de um século. Apesar da sua utilidade ser já reconhecida em várias doenças do foro neurológico e oftalmológico, trabalhos recentes têm sugerido que poderá também ter relevância em doentes com glaucoma.

Os mecanismos envolvidos na formação deste sinal não são consensuais, existindo várias teorias sobre a sua génese. Esta ausência de consensos tem levado a várias hipóteses sobre o porquê da baixa frequência desta SVP em doentes com glaucoma (glaucoma primário de ângulo aberto – POAG) quando comparados com a população em geral.

O nosso estudo prospectivo, caso-controlo apresenta a maior série publicada sobre o tema, incluindo o uso de tecnologias e variáveis ainda não estudadas neste contexto. Mais ainda, pela primeira vez foram estudados doentes em que a componente vascular terá um componente significativo na patogénese e evolução da doença (doentes com glaucoma normotensional – NTG).

O nosso trabalho confirmou a diminuição significativa na identificação da SVP quer em doentes POAG (50%), quer em doentes NTG (51%) quando comparados com a população controlo (82%). Não se verificaram contudo diferenças na prevalência deste fenómeno entre os dois grupos com glaucoma.
O facto mais interessante do nosso trabalho será talvez o facto de
nos doentes com NTG, a ausência de SVP associar-se a danos
funcionais significativamente mais avançados do que a verificada
nos doentes NTG com SVP. A ausência deste sinal ao exame
objectivo, ao indentificar doentes com patologia mais avançada,
poderá ser particularmente útil na avaliação clínica dos doentes
com esta forma da doença. Os nossos resultados sugerem ainda
que esta diferença poderá estar associada a alterações da
atividade metabólica das células ganglionares da retina, uma vez
que apesar das diferenças funcionais, não registámos diferenças
estruturais na camada de fibras nervosas retinianas.

A verificação do fenómeno SVP não se correlacionou com
nenhuma variável morfológica das topografias da cabeça do nervo
óptico, quer em indivíduos saudáveis quer em doentes com
glaucoma. Como um aumento da escavação do disco óptico
influencia necessariamente o trajecto e grau de curvatura dos
vasos papilares (e consequentemente os padrões de fluxo
intraluminal), os nossos resultados parecem sugerir que alterações
de fluxo assim induzidas serão insuficientes para modificar o status
SVP.

Para os mesmos intervalos de IOP, os doentes com glaucoma
apresentam um registo menor de doentes com SVP. No entanto,
nos doentes com POAG, um aumento deste factor mecânico está
associado a uma maior prevalência do fenómeno, enquando que
nos doentes com NTG essa prevalência não é alterada, o que
parece sugerir que a ausência deste fenómeno nesta população não se deverá a factores mecânicos intraoculares.

As pressões extraoculares, avaliadas indirectamente pelo diâmetro da bainha do nervo óptico (ONSD), parecem ter um impacto diferente nos vários grupos experimentais. Em indivíduos controlo, maiores ONSD estiveram associadas a uma menor frequência de detecção do fenómeno. Nos grupos com glaucoma, apesar das menores frequências de registo, essa prevalência não é alterada ao longo dos valores de ONSD. Estes doentes NTG sem SVP têm contudo uma associação importante com o ONSD, na medida em que se parece correlacionar positivamente com a IOP. Os nossos resultados parecem assim sugerir que neste tipo de glaucoma, a drenagem do humor aquoso pelo sistema venoso poderá ser particularmente sensível a alterações das pressões orbitárias retro-oculares.

As duas populações de glaucoma apresentaram ainda correlações distintas com esta variável (ONSD), em que apenas doentes com POAG sem SVP apresentaram uma correlação entre o ONSD e as velocidades de fluxo dos vasos centrais da retina. Os nossos resultados sugerem assim que a veia central da retina (CRV) dos doentes com POAG poderá ser mais susceptível a uma compressão extrínseca, particularmente se esta for aplicada a jusante do ponto major de obstrução do vaso (a passagem pela lamina crivosa). Os doentes com NTG, em que possivelmente se regista uma alteração do microambiente no que diz respeito aos agentes vasoactivos,
poderão apresentar uma alteração estrutural dos vasos – provavelmente hipertrofia da parede vascular – e consequentemente menos maleáveis a pressões externas.

Estes estudos com Eco-Doppler dos vasos centrais da retina identificaram que os doentes NTG sem SVP apresentaram menores velocidades arteriais e venosas, enquanto que os doentes com POAG registaram apenas alterações venosas. Uma vez que a associação a um estádio de doença glaucomatosa mais avançada apenas se observou nos doentes em que co-existiam alterações arteriais e venosas, os nossos resultados parecem sugerir que ainda que a ausência de SVP possa estar associada a alterações venosas, apenas existindo um envolvimento arterial se verifique a associação a com maior dano glaucomatoso.

A análise efectuada das frentes da onda Doppler da artéria oftálmica (OA) identificou diferenças entre os grupos, reforçando a hipótese de existirem variações estruturais ao nível da parede dos vasos entre os doentes NTG com e sem SVP.

Os nossos dados não sustentam que o status SVP identifique limiares de resistência vasculares que têm sido sugerido como limiares de autoregulação vascular nos doentes com glaucoma. Na medida em que os limiares utilizados parecem reflectir uma incapacidade de adaptação por parte dos capilares a condições de hipoperfusão, o status SVP poderá reflectir, não uma disfunção capilar, mas uma disfunção arterial e venosa ainda não esclarecida.
Do ponto de vista hemodinâmico, a única diferença identificada nos indivíduos controlo foi um aumento das velocidades mínimas da veia central da retina (Vmin) nos indivíduos sem SVP. Este padrão tem sido descrito em situações de estase venosa no restante organismo. Estudos adicionais serão ainda necessários para validar esta teoria.

Outro dado interessante do nosso trabalho é a associação nos doentes POAG entre a ausência de SVP e maiores valores de pressão arterial diastólica. Dado este ser um factor de risco conhecido no desenvolvimento da oclusão da CRV, a ausência deste sinal em doentes POAG poderá identificar os doentes com glaucoma em maior risco de desenvolverem esta patologias vascular. Serão contudo necessários estudos com maior follow-up para confirmar esta suspeita.

As limitações do nosso estudo estão condicionadas por falhas metodológicas e insuficiências tecnológicas. O nosso estudo de caso-controlo carece de informação sobre a medicação sistémica e baseia-se na descrição pelo próprio dos seus antecedentes médicos sistémicos e oftalmológicos. Adicionalmente, as limitações referentes a qualquer estudo doppler retrobulbar (em que não é possível extrair dados referentes ao diâmetro dos vasos analisados) bem como a incapacidade de clinicamente avaliar directamente as pressões retrobulbares, impõem limitações sobre a interpretação de fluxos e pressões arteriais e venosas. Por fim, e dado a elevada prevalência de SVP em indivíduos saudáveis, ainda
que com a maior série registada na literatura, o nosso reduzido número de indivíduos controlo sem SVP poderá levantar reservas sobre a significância estatística a atribuir aos nossos resultados nesta população.

Concluindo, e ainda que seja necessário ainda complementar o nosso estudo com outros trabalhos incidindo nesta vertente de circulação venosa no glaucoma, o nosso trabalho parece demonstrar que as pulsatibilidades venosas poderão ser particularmente importantes nos doentes com NTG, onde a ausência das mesmas poderá sinalizar não só uma disfunção vascular (arterial e venosa) como também uma forma mais avançada da doença.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AT-</td>
<td>acceleration time</td>
</tr>
<tr>
<td>BP</td>
<td>blood pressure</td>
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<tr>
<td>CCT</td>
<td>central corneal thickness</td>
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<tr>
<td>CRA</td>
<td>central retinal artery</td>
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<tr>
<td>CRV</td>
<td>central retinal vein</td>
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<tr>
<td>Dm</td>
<td>diastolic mean velocity</td>
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<tr>
<td>Dp</td>
<td>diopter</td>
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<tr>
<td>EDV</td>
<td>end diastolic velocity</td>
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<tr>
<td>ESA</td>
<td>early systolic acceleration</td>
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<tr>
<td>IOP</td>
<td>intraocular pressure</td>
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<tr>
<td>HR</td>
<td>heart rate</td>
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<tr>
<td>MAP</td>
<td>mean arterial pressure</td>
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<td>MD</td>
<td>mean defect</td>
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<td>MFV</td>
<td>mean flow velocity</td>
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<td>MOPP</td>
<td>mean ocular perfusion pressure</td>
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<tr>
<td>NPCA</td>
<td>short posterior ciliary arteries (nasal)</td>
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<td>NTG</td>
<td>normal tension glaucoma</td>
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<tr>
<td>OA</td>
<td>ophthalmic artery</td>
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<td>OPA</td>
<td>ocular pulse amplitude</td>
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<tr>
<td>ONH</td>
<td>optic nerve head</td>
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<tr>
<td>ONSD</td>
<td>Optic nerve sheath diameter</td>
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<tr>
<td>POAG</td>
<td>primary open-angle glaucoma</td>
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<tr>
<td>PSV</td>
<td>peak systolic velocity</td>
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<td>RI</td>
<td>resistivity index</td>
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<td>RNFL</td>
<td>retinal nerve fiber layer</td>
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<td>RVP</td>
<td>retinal venous pressure</td>
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<tr>
<td>Sm</td>
<td>Systolic mean velocity</td>
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<tr>
<td>SVP</td>
<td>spontaneous venous pulsation</td>
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<tr>
<td>TPCA</td>
<td>short posterior ciliary arteries (temporal)</td>
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<tr>
<td>Vmax</td>
<td>maximal venous velocity</td>
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<tr>
<td>Vmin</td>
<td>minimal venous velocity</td>
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**Units of measurement**

The following list associates the variable name with the appropriate unit of measurement (in brackets):

- **Age (years)**: MFV (cm.s⁻¹)
- **AT (seg)**: MD (dB)
- **BP (mmHg)**: ONSD (mm)
- **CCT (µm)**: OPA (mmHg)
- **Cup area (mm²)**: Pulse rate (ppm)
- **Cup Volume (cmm)**: PSV (cm.s⁻¹)
- **Disc area (mm²)**: Retinal cross sectional area (mm²)
- **Dm (cm.s⁻¹)**: Rim area (mm²)
- **EDV (cm.s⁻¹)**: Rim Volume (cmm)
- **ESA (cm.s⁻²)**: RNFL thickness (mm)
- **IOP (mmHg)**: Sm (cm.s⁻¹)
- **Maximum cup depth (mm)**: SVP (+/-)
- **Mean cup depth (mm)**: Visual acuity (logmar)

Variables presented as ratios or indexes (Sm/Dm ratio; cup/disc ratio; linear cup/disc ratio; RI) have no unit of measurement.
References


