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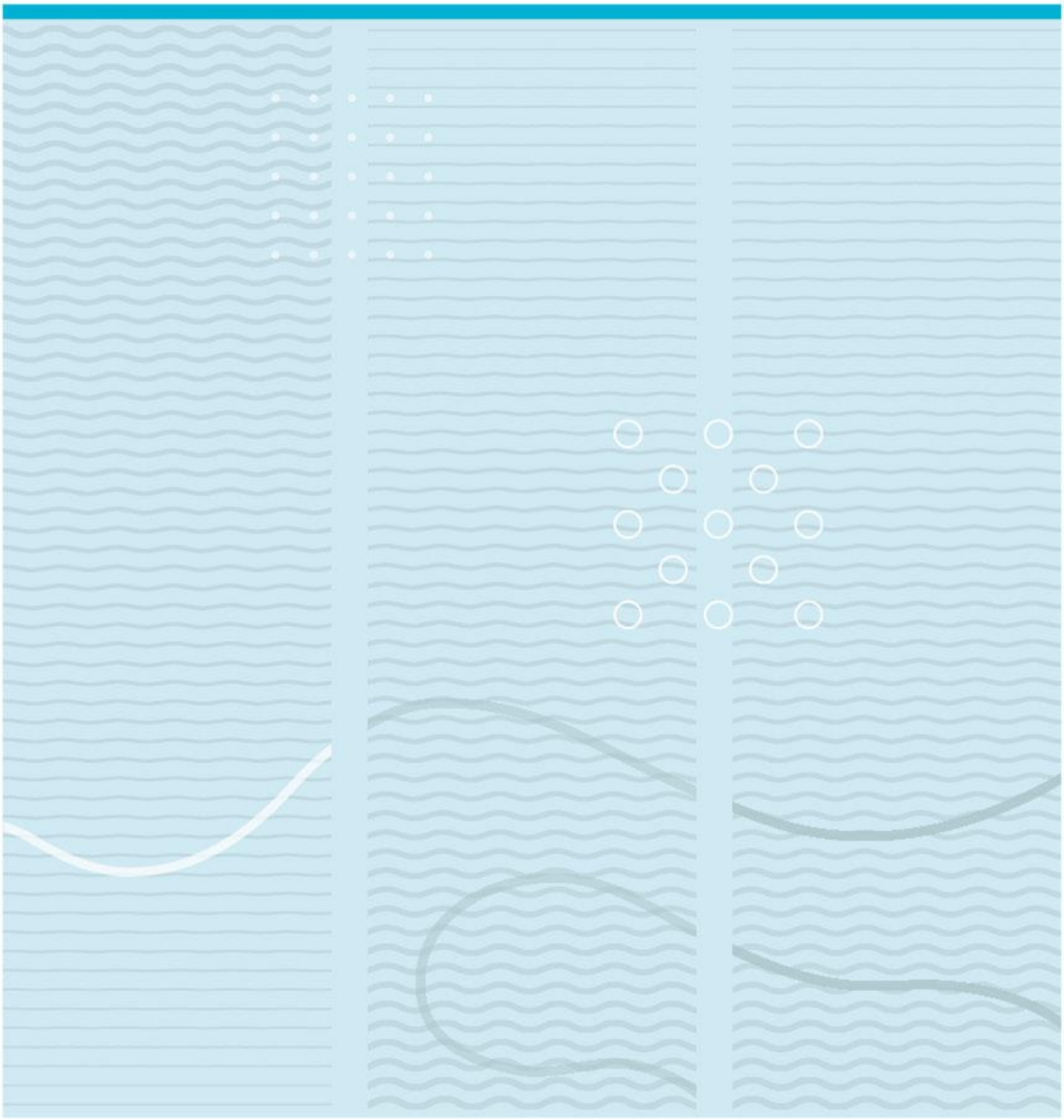
Association between saccadic reaction time obtained with BulbiCAM® and the estimated risk of developing Primary Open-Angle Glaucoma



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This thesis is worth 30 study points

Abstract

Background

Primary Open-Angle glaucoma (POAG) causes structural changes leading to irreversible loss of visual function. Finding the balance between identifying early cases of glaucoma and avoiding unnecessary referrals is a growing challenge in primary eye care. Early reports indicate that eye movement perimetry (EMP) has the potential of detecting development of POAG when functional damage has not yet occurred. BulbiCam® is an instrument developed with an aim to map visual function based on EMP.

Objective

The purpose of this study was to investigate the association between saccadic reaction time (SRT) obtained with BulbiCam® and the estimated risk of developing POAG. SRT has been reported increased in areas of the visual fields without functional damage, in people with POAG (Thepass, Lemij, Vermeer, van der Steen, & Pel, 2021). Secondary aim was to investigate the correlation between SRT and age to provide useful information to the clinical assessment of POAG.

Methods

A cross-sectional study with prospective data from three months was conducted. Patients aged 40 – 70 years who visited an optometric practice in Norway to conduct an eye examination were consecutively recruited into the study. Optometric examination included assessment of ocular structure and function based on subjective evaluation of optic nerve head, visual fields, structure-function-relationship and intraocular pressure, along with major risk factors, and resulted in an overall estimated risk graded as low, medium and high. Auxiliary measurements with BulbiCam® was conducted to obtain SRT. SRT for 26 points in the visual fields were used for statistical analyses retrieved from R and SAS. Continuously variables, pairwise difference between groups, probability of risk and correlation between SRT and age was analysed. Statistical significance was set to $p < 0.05$.

Results

48 participants aged 56.3 (± 9.27, 62.5% female, 37.5% male) years were included in the study. Increased SRT was associated with increased risk of POAG ($p<0.001$). Significant difference between

left ($p < 0.0001$) and right eye ($p = 0.08$) when considering difference in mean SRT between low (n=32) and higher (medium (n=10) /high (n=6)) risk groups. Predicted probability of having medium or high risk in people aged ≥60 years was close to 100% for an SRT of 450 ms. Secondly, a weak positive correlation ($r = 0.15$, $p = 0.33$) between SRT and age was found.

Conclusion

It has for the first time been demonstrated that SRT obtained with BulbiCam® is related with the risk of developing POAG as assessed by evidence based clinical methods. An increase in SRT was found strongly associated with an increase in estimated risk. The probability of having higher estimated risk of POAG was found to increase with both age and mean SRT. Secondly, weak positive correlation between SRT and age was found. Further research is warranted to further investigate whether SRT obtained with BulbiCam® add useful diagnostic information to the clinical assessment of glaucoma and how SRT changes with age.

Key words

POAG, eye movement perimetry, BulbiCAM®, saccadic reaction time, optometrist

Abstrakt

Bakgrunn

Primær åpenvinklet glaukom (POAG) medfører strukturelle forandringer som fører til irreversibelt tap av visuell funksjon. Å finne balansen mellom å identifisere tidlige tilfelle av glaukom og å unngå unødvendige henvisninger er i økende grad en utfordring i primær øyehelsetjeneste. Tidlige studier indikerer at øye bevegelses perimetri (EMP) har potensial for å oppdage utvikling av POAG når funksjonell skade ennå ikke har oppstått. BulbiCam® er et instrument utviklet med et mål om å kartlegge visuell funksjon basert på EMP.

Formål

Formålet med studien var å undersøke assosiasjonen mellom sakkade reaksjonstid (SRT) målt med BulbiCam®, og den estimerte risikoen for å utvikle POAG. Det er funnet at SRT er økt i områder av synsfeltet uten funksjonell skade hos personer med POAG. Sekundært mål var å undersøke korrelasjonen mellom SRT og alder for å gi nyttig informasjon til den kliniske vurderingen av POAG.

Metoder

En tverrsnittstudie med prospektive data fra tre måneder ble gjennomført. Pasienter mellom 40 og 70 år som besøkte en optometrisk praksis i Norge for å gjennomføre en synsundersøkelse, ble fortløpende rekruttert inn i studien. Optometrisk undersøkelse inkluderte vurdering av okulær struktur og funksjon basert på subjektiv evaluering av optisk nervehode, synsfelt, struktur-funksjon-forhold og intraokulært trykk, samt store risikofaktorer, og resulterte i en total estimert risiko gradert som lav, medium og høy. Tilleggsundersøkelser med BulbiCam® ble gjennomført for å innhente SRT. SRT for 26 punkter i synsfeltet ble brukt i statistiske analyser i R og SAS. Kontinuerlige variabler, parvise forskjeller mellom grupper, sannsynlighet for risiko og korrelasjon mellom SRT og alder ble analysert. Statistisk signifikans var satt til $p < 0.05$.

Resultater

48 pasienter i alderen 56.3 (± 9.27, 62.5% kvinner, 37.5% menn) år var inkludert i studien. Økt SRT var assosiert med økt risiko for POAG ($p<0.001$). Signifikant forskjell mellom venstre ($p < 0.0001$) og høyre ($p= 0.08$) øye ved vurdering av forskjell i gjennomsnittlig SRT mellom lav (n =32) og høyere (medium (n=10) /høy (n=6) risikogrupper. Predikert sannsynlighet for å ha medium eller høy risiko

for personer ≥60 år var nær 100% for en SRT på 450 ms. Sekundært, en svak positiv korrelasjon (r = 0.15, p = 0.33) mellom SRT og alder ble funnet.

Konklusjon

Det har for første gang blitt demonstrert at SRT fra BulbiCam® ver relatert til risikoen for utvikling av POAG basert på evidensbaserte kliniske metoder. En økning i SRT ble funnet å være sterkt assosiert med en økning i estimert risiko. Sannsynligheten for å ha høyere estimert risiko for POAG ble funnet å øke med både alder og gjennomsnittlig SRT. Sekundært, svak positiv korrelasjon mellom SRT og alder ble funnet. Videre forskning er berettiget for å videre undersøke om SRT med BulbiCam® tilfører nyttig diagnostisk informasjon til den kliniske vurderingen av glaukom og hvordan SRT forandres med alder.

Nøkkelord

POAG, øyebevegelses perimetri, BulbiCAM®, sakkade reaksjonstid, optometrist

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Foreword

Glaucoma is a very complex disease and from experience, dealing with this condition in optometric practice can be very challenging. This study was conducted because I am sincerely interested in finding out if there are alternative methods to detect glaucoma earlier than we do today because the condition affects so many people and can potentially lead to severe vision loss. For me personally, it was important to carry out a study that could contribute to increased knowledge about glaucoma so that I can work more evidence – based when meeting patients at pronounced risk of developing glaucoma. This both to be more confident in my job as an optometrist, and to reduce my share of false positive referrals to the specialist health service. In addition, I am very curious about the development of the profession of optometrists and I think it is crucial to both want and be receptive to new knowledge. This in order to offer patients the best treatment available. BulbiTech was originally developed in Norway and even in Trondheim, and thus I find it extra rewarding to be able to contribute with research that sheds light on the usefulness of BulbiCam® in an optometric practice. The work with this study has given me increased knowledge about glaucoma and the use of eye movement perimetry, and I now feel more secure in my job as an optometrist in meeting with patients at risk of developing glaucoma. Furthermore, the completion of the study has made me, if possible, even more curious about glaucoma and I am very interested in continuing with research on this topic. I would like to thank my supervisor Per Olof Lundmark for guiding me through this study and contributing with both inspiration and motivation, as well as constructive criticism and help along the way. I would also like to thank BulbiTech for lending me a BulbiCam® so that the study could be carried out , as well as Bård Dalhøi and Johan Pel for all the help using the instrument and tips and tricks for writing the thesis. I am forever greatful for all help an guidance from Stig Larsen and Hans Fagertun regarding statistical analysis and understanding these. Lastly, I would like to thank my partner, bonus – daughter, friends, family, colleagues and fellow students for supporting me through this process.

Trondheim, April 29th, 2022

Maria Lorentzen

1 Introduction

1.1 Definition of glaucoma

The concrete, formal definition of glaucoma is as follows: “Glaucoma describes a group of ocular disorders of multi-factorial aetiology united by a clinically characteristic optic neuropathy with potentially progressive, clinically visible changes at the optic nerve head (ONH), comprising focal or generalized thinning of the neuroretinal rim with excavation and enlargement of the optic cup, representing neurodegeneration of retinal ganglion cells axons and deformation of the lamina cribrosa; corresponding diffuse and localized nerve-fibre-bundle pattern visual field loss may not be detectable in early stages; while visual acuity is initially spared, progression can lead to complete loss of vision; the constellation of clinical features is diagnostic” (Foster, Buhrmann, Quigley, & Johnson, 2002). It is hereby understood that glaucoma is a potentially blinding condition that might be challenging to detect at early stages. This study aims to investigate whether measurements of saccadic reaction time (SRT) obtained in eye-movement perimetry provides diagnostic information that may be useful for the risk assessment of glaucoma in an optometric practice.

1.2 Epidemiology

Glaucoma is known to be asymptomatic until late stages of the condition and it has been shown that about half of all cases of glaucoma are undiagnosed (Nayak, Maskati, & Parikh, 2011). Further, it has also been reported that about one third of patients have advanced glaucoma at the time of diagnosis, and the functional loss seen in POAG is proven preventable if detected at an early stage (Kastner & King, 2020; Weinreb & Khaw, 2004). In a large meta-analysis performed by Zhang and colleagues (2021), hundred and fifty studies from the last twenty years were reviewed with an aim to provide an overview of the prevalence of POAG among the general population aged forty years and more. The study showed a worldwide prevalence of 2.4% (95% confidence interval (CI); 2.0 – 2.8%) and that the prevalence was increasing with age. Furthermore, it was estimated that the global number of cases of POAG is 68.56 million in people above 40 years of age (Zhang, Wang, Li, & Jiang, 2021). Thus, POAG is a vision threatening condition affecting the worldwide population and the prevalence numbers are high, and expected to increase in the future (Tham et al., 2014) . These epidemiological data combined with the knowledge of glaucoma being a potentially vision

threatening disease, highlight the need for early case detection in order to be able to offer legitimate follow – up and treatment.

1.3 Characteristics of glaucoma

Glaucoma is a collective term for a number of conditions that can cause both structural damage and reduced visual function (Foster et al., 2002). The subgroups of glaucoma have different characteristics and are traditionally classified according to how the condition involves the anterior segment of the eye (Weinreb & Khaw, 2004). In angle closure glaucoma, the anterior chamber angle is blocked by the iris which further prevents the drainage of aqueous humor, and IOP consequently increases (Schuster, Erb, Hoffmann, Dietlein, & Pfeiffer, 2020; Weinreb & Khaw, 2004). In comparison, in POAG this chamber angle is open. So why is the drainage reduced and IOP elevated, while the chamber angle is open? The complete answer is not fully known, but the main theory addressing this concern is that drainage is impaired due to a dysfunction in the trabecular meshwork, which accounts for most of the drainage of aqueous humor, that further leads to an elevation of the IOP (Mahabadi, Foris, & Tripathy, 2022). Normal tension glaucoma (NTG) has similarities to POAG in that the condition is also considered an optic neuropathy and the anterior chamber angle is open. On the other hand, NTG presents with intraocular pressure within the considered normal range, i.e., $\leq$ 21 mmHg (Quon et al., 2016). In secondary open angle glaucoma, reduced drainage and consequently increased IOP occur as a result of an external influence. Examples of such influence can be trauma, injury or inflammation (Schuster, Erb, et al., 2020; Weinreb & Khaw, 2004).

In this study the risk of POAG development was assessed, i.e., a condition where the chamber angle is open. As mentioned, the complete pathophysiology of POAG is not known, but a common denominator for all subgroups of glaucoma is that retinal ganglion cells (RGC) die, either as a result of elevated IOP, decreased blood flow, or a combination of these (Gardiner, Cull, Fortune, & Wang, 2019; Mahabadi et al., 2022; Rao et al., 2020). RGCs are located in the retina and its axons run through the optic nerve (Weinreb, Aung, & Medeiros, 2014; Weinreb & Khaw, 2004). The location of RGCs helps to explain why structural changes occur in ONH in glaucoma. In the event of extensive RGC loss, eventually functional damage will be constituted. Functional damage is known as irreversible, reduced sensitivity in visual fields (Kanjee, Yucel, Steinbach, Gonzalez, & Gupta,

2012). This explains the importance of assessing the structure-function-relationship when handling patients suspected of developing POAG. That is, assessing whether functional changes consistent with glaucoma, are associated with the structural changes in ONH and RNFL (Malik, Swanson, & Garway-Heath, 2012; Weinreb et al., 2014). Hereby it is understood that glaucoma has the potential to cause severe loss of visual function if left untreated, and the importance of identifying cases at true risk of developing POAG in an optometric practice, is further specified.

1.4 Early case detection

The advantages of early case detection of glaucoma is that it enables adequate follow – up and treatment at a stage in the course of the disease which could potentially limit the negative effect on visual function (Chang & Singh, 2016; Weinreb & Khaw, 2004). Furthermore, it is necessary for optometrists to work in a way that enables distinguishment between people who are likely to develop glaucoma in the future, from those where this is less likely. That is because people with suspected glaucoma should be referred to a specialist to be diagnosed and offered treatment. If optometrists were able to reliably identify pathology and severity of disease development, to further suggest diagnosis at an appropriate time in the course of the disease, it could potentially lead to a reduced number of referrals to specialists (Chang & Singh, 2016; Gordon & Kass, 2018) and consequently unnecessary society and patient load. On the other hand, an important challenge with the management of POAG is that there are no specific findings for grading risk, and the risk assessment is therefore based on a comprehensive, subjective assessment of glaucoma-suspected findings. Further, the financial costs both to the patient, the clinicians and the society is substantial and it is necessary for optometrists to prepare reliable and sensitive working routines that ensures evidence-based evaluation of the degree of risk of developing POAG and further, whether it is necessary to refer to a specialist for secondary assessment and possible determination of diagnosis.

1.5 Risk assessment and diagnosis of POAG

Many of the referrals to ophthalmologists regarding suspected glaucoma are false positive referrals (Thomas et al., 2014). This knowledge not only implicates that the specificity and sensitivity in clinical work used for assessing patients at risk of developing glaucoma in optometric practice are weak, but additionally highlights how challenging it can be for an optometrist in primary eye care to

detect cases of true glaucoma. A combination of a variety of examination methods aiming to increase the sensitivity while maintaining a high specificity is described in clinical guidelines for the management of glaucoma-suspected patients (Norges Optikerforbund, 2020; National Institute for Health and Care Excellence, 2017).

1.5.1 Risk factors

Risk factors for the development of glaucoma are a much discussed, and not least debated topic in previous studies. What is repeated in the majority of these studies is that the risk of developing glaucoma increases with increasing age, elevated IOP, with high myopia and with a positive family history. The latter applies in particular if the previous generation has confirmed diagnosis (Schuster, Erb, et al., 2020; Schuster, Wagner, Pfeiffer, & Hoff Mann, 2020). Accordingly, age, ocular status, family history and evaluation of IOP was included in the risk assessment of POAG in this study, in addition to subjective grading of clinical findings typically seen in POAG. This in order to provide an overall estimated risk of POAG of the participants, which was used to address the main purpose of this study, that is investigation of the association between estimated risk of POAG-development and SRT.

1.5.2 Clinical findings

It is known that loss of RGCs is a prominent feature in glaucoma. A problem associated with handling glaucoma patients is that the structural changes leading to impaired visual function is not detectable until many of the RGCs are lost (Weinreb et al., 2014). In early stages the condition is thus asymptomatic, but with an ever-decreasing number of RGCs, the sensitivity of the visual fields will be limited and this deterioration is irreversible (Hood, 2019; Hood et al., 2019). Moreover, clinical features might be detectable before the condition becomes symptomatic. Thinning of neuroretinal rim is a cardinal diagnostic finding of glaucoma which occurs due to axonal deterioration and RGC loss. This change is normally initiated in the inferior part of the neuroretinal rim (Mahabadi et al., 2022), meaning that the ISNT-rule is no longer followed. The ISNT – rule of an optic nerve considered as healthy, is defined as an appearance of the neuroretinal rim as follows; the inferior part is wider than the superior part which further is wider than the nasal part, and last the temporal part is the narrowest (Lee, Ro, Yi, & Choi, 2021). Other changes possibly detectable

are thinning of parapapillary retinal nerve fiber layer (RNFL), enlargement of excavation of ONH and asymmetry between the eyes (Schuster, Erb, et al., 2020; Schuster, Wagner, Pfeiffer, & Hoffmann, 2021). In this study where estimating the risk of POAG-development is important, clinical assessment of ONH, RNFL, visual fields as well as the structure-function-relationship were included in the overall estimated risk. The latter included assessment of whether structural damage consistent with glaucoma was related to functional impairment consistent with glaucoma.

1.5.3 Patient course

Standard routine optometric eye examination performed by an optometrist includes a battery of tests aiming to ensure any suspicious eye conditions being detected. In the event of detected suspicious eye condition, a diagnose is suggested. Further, the patient in question is recommended to be referred to a specialist for confirmation of possible diagnosis and treatment by an ophthalmologist. In case of pronounced suspicion of glaucoma, an extended examination must be performed in order to be able to offer correct follow-up.

1.6 Eye movement perimetry

One of the many clinical findings of POAG is the functional loss in visual fields, described as an impairment in visual fields which are not correctable and consequently reduces visual function (Tham et al., 2014). Assessment of visual fields has high clinical utility in cases where it is challenging to distinguish glaucoma-suspected patients from those at low risk of developing POAG (Chang & Singh, 2016). Standard Automated Perimetry (SAP) is referred to as the gold standard in most countries for assessment of visual fields in the clinical assessment of glaucoma. Despite this, there are some disadvantages with SAP. These include, among other factors, requirements for stable fixation, fatigue and reduced reliability due to the patient learning the test procedure during repeated measurements (Tiwari, Aishwarya, & Bhale, 2018), as well as the fact that impaired visual function detected with SAP is usually irreversible (Hood, 2019; Hood et al., 2019). The disadvantages with SAP has evoked curiosity among several researchers to whether there are alternative methods to SAP, measuring functional damage with a higher degree of sensitivity. Eye movement perimetry (EMP) is presented as such an alternative method (Kadavath Meethal et al., 2018; Meethal et al., 2019). EMP bases its measurements on the natural movement of the eyes and

are by eye tracking able to measure both the visual fields, similar to SAP, and SRT using eye tracking (Thepass et al., 2021). SRT is known as the time it takes from a peripheral stimuli is presented, until an eye movement towards the peripheral stimuli is initiated, obtained using eye tracking (Kadavath Meethal et al., 2018). The relation between sensitivity loss in the visual fields and the SRT has been investigated by Thepass and colleagues (2021). The researchers confirmed that SRT is slower in areas of the visual field with detected visual field loss using SAP, compared to areas without reduced sensitivity. Interestingly, they also reported that SRT is significantly slower in areas *without* reduced visual field sensitivity, in patients *with* POAG (Thepass et al., 2021). That is, it is known that SRT increases with an increased proportion of visual field loss, and that SRT is slower in areas without reported visual field loss in people with POAG compared to healthy controls. However, what is not known, which this study aimed to investigate, is whether SRT was slower in people without POAG, but with an estimated increased risk of developing POAG, compared to those with a lower estimated risk.

1.6.1 BulbiCam®

BulbiCam® is an instrument that uses EMP and vision physiology to map visual function (BulbiTech AS, 2020). In a BulbiCam®, EMP is used to assess visual function, and other features, both quantitatively and qualitatively (BulbiTech AS, 2020). In this way, such an instrument can potentially increase the sensitivity without compromising the specificity for identifying POAG development. Saccadic eye movements (SEM) are part of the measurements with EMP and BulbiCam®, and an evaluation of oculomotor function can thus also be performed using BulbiCam®. As mentioned, SRT is an important parameter of measurements obtained with EMP, and thereby also of parameters obtained with BulbiCam®. In a study performed by Mazumdar and colleagues (2019) where the purpose was to investigate the effect of, among others, age and gender on SRT obtained with EMP, it was found that SRT was significantly slower with increasing age, but no significant difference in gender was reported (Mazumdar et al., 2019). Further, in a study performed by Kadavath Meethal and colleagues (2018), an increase in SRT in early stages of glaucoma compared to healthy controls was found (Kadavath Meethal et al., 2018). It is therefore reason to believe that BulbiCam® may have potential of becoming an important instrument in the clinical work of detecting patients at increased risk of developing POAG, especially in early stages of the condition where there are a limited number of clinical findings related to POAG present.

We now know that glaucoma is a condition that potentially causes blindness and can be difficult for an optometrist to detect. The introduction has shown why early case detection of the condition in optometric practice is appropriate and why evidence – based work is necessary. Furthermore, it has shown that SRT has been found to be significantly different in people with POAG compared to healthy controls. Therefore, the purpose of this study was to investigate whether SRT measured with EMP from BulbiCam® was different in the different stages of estimated risk of POAG-development, which was obtained from clinical assessment of risk in an optometrisk practice. Such finding would indicate that SRT measured with BulbiCam® could potentially be considered a useful measurement for optometrists in the clinical work of detecting development of POAG.

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2 Research question

2.1 Research and significance

The main purpose of this study was to investigate the association between SRT obtained with BulbiCam® and the assessed risk of POAG. This in order to investigate whether SRT provided diagnostic information that could be useful for the clinical risk assessment of glaucoma in an optometric practice. The secondary objective was to investigate the correlation between SRT and age in a clinical population aged 40 – 70 years to provide useful information to the clinical assessment of POAG.

2.2 Research questions

Research questions used as a basis of answering the purpose of the study was as follows;

- what is the association between SRT obtained with BulbiCAM® and the estimated risk of developing POAG in adults who undergo a routine examination in an optometric practice in Norway?
- What is the predicted probability of having estimated higher risk of developing POAG with increased SRT?

The research questions forming the basis of the main purpose of this study was based on the following hypotheses;

- Measurements of SRT obtained with BulbiCam® are associated with estimated risk of developing POAG graded as low, medium or high, based on the clinical assessment of ocular structure and visual function, along with major risk factors like age and ocular status in a clinical population aged 40 – 70 years who underwent a routine examination in an optometric practice in Norway.
- Predicted probability of having an estimated higher risk of developing POAG with increased SRT, obtained with BulbiCam®, was higher in elderly people in a clinical population aged 40 – 70 years who underwent a routine examination in an optometric practice in Norway.

Secondary objective was to investigate the correlation between SRT and age and it was hypothesized that SRT would increase with increasing age.

2.3 Study significance

The purpose of this study was to assess whether there was an association between SRT measured with BulbiCam® and the estimated risk of POAG. Results were expected to contribute knowledge about to what extent SRT provides diagnostic information to the clinical risk assessment of developing POAG in an optometric practice. The introduction has shown why early case detection of POAG is appropriate and why evidence-based work is crucial. Furthermore, it has shown that SRT has been found to be significantly slower in participants with POAG compared to healthy controls. Therefore, the results from this study was used to predict whether SRT obtained with BulbiCam® could be considered a useful measurement method for optometrists in the clinical work of detecting development of POAG.

3 Methods

Parts of this thesis has been presented in the project protocol as the final exam in MRES019 Research methods and project description (M, Lorentzen, 2021) at USN (unpublished).

The study was a cross – sectional study with prospective data over a period of three months. Invited participants to the study were people between the age of 40 and 70 years who visited Koren Optikk (optometric practice in Trondheim, Norway) to conduct an optometric eye examination in the period September 2021 to February 2022. It was chosen to only invite people who have sought the optometric practice on their own initiative. In other words, there was no system for identifying and inviting potential participants as the invitation took place continuously. Here, all participants had the same starting point, i.e., they needed an eye examination. People who were invited to participate was asked by one of the project manager's colleagues at the time they made an appointment for an eye examination. Information- and consent-form (appendix 2) was also given at this time. Upon attendance at the appointment, invited people had the opportunity to ask questions about the study to the project manager's colleague, who was carefully informed about the study prior to its start-up. Secondly, the requested people were given as long period of reflection as they wished, as no response deadline was specified for the request. The persons in question was recruited when any question was answered and understood, and consent-form had been signed. The participants were then placed within the correct age group, either 40 – 50 (age group 1), 51 – 60 (age group 2) or 61 – 70 (age group 3) years with a unique, randomized identification number. Deidentification was done to ensure the participant's privacy, as well as to allow the collected data to be used in publications. Only people not understanding Norwegian were excluded from the study due to the risk of misunderstanding instructions, which were given in Norwegian. This study was approved by the Regional Committee for Medical Research Ethics (REK) (Case number: 274755, date: 01/11/21) and Norwegian center of research data (NSD) (Case number 613252 (26/11/21). The study also follows the Helsinki – declaration.

3.1 Study measurement

All participants underwent a standard routine eye examination in agreement with clinical guidelines prepared by the Norwegian Optician´s Association (Norges Opikerforbund, 2005). The examination

was performed on both eyes by an experienced optometrist (M.L.). For every measurement included in this study the right eye was measured first. The examination included patient history, assessment of visual and oculomotor functions, refraction, assessment of accommodation and binocular vision, assessment of IOP with noncontact tonometer (NCT) and assessment of retinal status with fundus photography and OCT. In the clinic where the data collection was performed, OCT is considered a standard routine examination despite this not being the case in the majority of optometric practices. These examinations resulted in an overall estimated risk of the development of POAG. The participants underwent auxiliary measurement of SRT with BulbiCam® after assessment of estimated risk of developing POAG was completed. Both examinations was performed by the project manager and hence the results from the subjective assessment of estimated risk was not blind from the results from assessment of SRT.

3.1.1 Assessment of optic nerve head and retinal nerve fiber layer thickness

ONH was assessed using fundus photography. Participants graded at low risk of POAG-development in this study had no features that would indicate an increased risk of glaucoma. That is, a well – defined disc, normal appearance of blood vessels, a cup/disc ration within normal range when adjusted for disc size and ISNT rule was followed. Classification as either medium or high estimated risk was a result of subjective evaluation based on an overall assessment of glaucoma-suspected findings.

Assessment of the parapapillary RNFL thickness was obtained with OCT. The measurement was performed with standard procedure using Topcon OCT (Topcon 3D OCT-1) with a 3D wide-scan. The scan included 50, 000 a-scans per second and both macula and ONH was captured at the same time. The length of the scan was 12.0 x 9.0 mm and the scan resolution was 512 x 128. Parapapillary RNFL thickness was obtained by a circular scan measuring ONH with a diameter of 3.4 mm yielding an overall mean parapapillary RNFL thickness and sectoral means. The participants in this study was graded according to the reference database used in the software of Topcon OCT (Topcon Healthcare, 2021, p. 1-8).

3.1.2 Assessment of visual fields

Assessment of visual fields was measured with SAP using standard procedure in a Medmont Standard Automated perimeter (Medmont, M700) with central 22A fast threshold test. A demonstration of the measurement procedure was conducted before the original examination. Participants estimated at low risk of POAG-development in this study had no features that would indicate an increased risk of POAG. Classification as either medium or high estimated risk was a result of subjective assessments based on any discrepancies from normative values, i.e., size, location and depth, as well as global clues. Measurements considered insufficient, with regards to amount of false negative and positive responses, was repeated in line with recommendations in national and international clinical guidelines.

3.1.3 Assessment of structure-function-relationship

In assessment of the relationship between structure and function it was investigated whether functional damage discovered when assessing visual fields was related to structural changes discovered when assessing ONH and parapapillary RNFL thickness. An example could be if there was discovered parapapillary RNFL thinning in inferior section combined with narrowing of rim in inferior section, a functional damage located in superior part of the visual fields were likely or possibly, depending on subjectively assessed severity, related to the structural damage detected.

3.1.4 Assessment of intraocular pressure

Measurement of the IOP was performed with Nidek Tonoref (Nidek Tonoref III). Goldmann applanation tonometer (GAT) is considered the gold standard method of measuring IOP (Parrish, 2006). In this study IOP was assessed using a non-contact tonometer (NC), in which the cornea is deformed with an air pulse. Hence, the method was similar to GAT with mean deviation of 0.2 [95% CI: -0.1 – 0.6] mmHg (Cook et al., 2012). The grading of risk of developing POAG was based on the average IOP of several measurements, measured in mmHg and graded as low, medium or high. The grading was performed in line with national guidelines where an IOP ≥ 21mmHg is considered elevated (Norges Optikerforbund, 2020).

3.1.5 Important findings in patient history

Patient history was included in the standard routine examination and revealed whether the participants had confirmed diagnosis and was recorded as negative or positive. Positive in this case referred to information given in a medical report from an ophthalmologist where the diagnosis was confirmed. Participants further was asked about their knowledge of POAG in the family. In this study the participants were asked whether they were familiar with confirmed diagnosis with POAG in first degree relatives, meaning their parents, siblings or children and results were recorded as positive or negative. Age was used to divide participants into subgroups by age. Information about ocular status was retrieved from refraction. A spherical equivalent was calculated based on their spherical ametropia and astigmatism and classified as either myopia or hyperopia. A spherical equivalent (SE) of a myopic eye $\leq$ 5.0 diopters was set to be graded as high myopia.

3.1.6 Risk assessment of developing POAG

The estimated risk was performed in accordance with the criteria presented in table 1, which was based on clinical guidelines (Norges Optikerforbund, 2020; National Institute for Health and Care Excellence, 2017), and recorded as 1 (low risk), 2 (medium risk) or 3 (high risk). The use of "AND" and "OR" in table 1 refers to the overall risk assessment. Participants with findings deviating from the considered normal according to national and international guidelines were recommended follow-up at an optometrist. This in order to comply with the recommendation of repeatable measurements and clinical findings before a possible referral is to be carried out. Accordingly, none of the levels of estimated risk of POAG used in this study equaled referral to specialists, as it was recommended conducting repeatable measurements before considering referral. The type of clinical findings and degree of assessed risk determined both what measurements were recommended re -tested, and at what frequency. Further, the recommendation for follow – up also applied to participants with one or more risk factors of developing POAG (Norges Optikerforbund, 2020; National Institute for Health and Care Excellence, 2017).

		1	2	3
		LOW	MEDIUM	HIGH
Diagnosed POAG	Negative	X		
	Positive			X
ONH & RNFL	Normal	X		
	Suspect		X	
	Glaucomatous			X
		AND	OR/AND	OR/AND
Visual fields	Normal	X		
	Suspect		X	
	Glaucomatous			X
Structure-function-relationship	None	X		
	Possibly		X	X
	Likely		X	X
IOP	Low	X		
	Medium	X		
	High	X		
		OR/AND		
Family history	Negative	X		
	Positive	X		

Table 1: Criteria for estimated risk of POAG. the variables that form the basis for the risk assessment are carefully described above (3.1.1 – 3.1.5).

3.2 Measurements obtained using BulbiCam®

In addition to the ordinary examination, the participants agreed to carry out auxiliary measurements of the visual fields and SRT using BulbiCAM®. BulbiCam® is a multi-test instrument developed by BulbiTech AS (BulbiTech AS, certificate no. M-139) with an aim to map visual function based on EMP and vision physiology. The instrument can contribute with a number of different examinations based on a measurement method where SEM responses are used. Examples of examinations that might be obtained with BulbiCam® are perimetry, dynamic pupillometry, intuitive nystagmography and ptosis grading. Results from BulbiCam® are thus a measure of oculomotor function (BulbiTech AS, 2020). SEM refers to fast eye movements used both to perceive a central stimulus and to move the eye towards a new, static, stimulus (Kanjee et al., 2012; Yang, Bucci, & Kapoula, 2002). The instrument (figure 1) is head – mounted and has two digital screens and an

infrared camera which makes it possible to track SEMs both binocularly and monocularly at the same time (BulbiTech AS, 2020).

Figure 1: BulbiCam®. The instrument uses SEM responses to assess visual and oculomotor function. SRT is an important parameter obtained with BulbiCam® (Illustration image used with permission from BulbiTech AS, 2022).

3.2.1 Visual fields and SRT with BulbiCam®

Assessment of visual fields was one of the measurements obtained with BulbiCam® in this study. The method was used for detecting impaired sensitivity of visual fields, based on EMP. The instrument was adjusted for pupil distance and placed on the participants head and he or she was asked to look straight ahead towards the screen inside the instrument. The examination of visual fields was conducted by first introducing the participant to a central target (colored green). Furthermore, peripheral targets (colored white) were presented in different areas of the visual field within 30 degrees horizontally and 25 degrees vertically with a fixed intensity corresponding to 16.3 dB ± 1 dB. The participant was asked to focus on the green target that was presented first. Later on, the participant was instructed to, physically with eye movement, move his or hers gaze towards the with target, and then back to the green target. The presentation of the peripheral target (white) provides the eye with stimulus which triggers light sensitive cells in the retina, in which an interaction of the neural system is enhanced. Further, stimulation of reflexive and controlled eye movements follows due to neural integration in the brain towards the specific peripheral target (Luna, Velanova, & Geier, 2008). The participants eye movement was observed in three phases; i.) departure from the central visual target, ii.) fast eye movement towards the specific peripheral target and iii.) arrival back to the central visual target.

The assessment of visual fields included measurement of 26 points and SRT for the same points for each eye. On average, the examination took about 2 minutes per eye. Examination of visual fields resulted in the parameters “seen” and “unseen”, which refers to whether measured points in the visual field were registered as seen or not. If the majority of the 26 points tested were marked “unseen”, the examination was repeated. The instrument captured the points tested by assessing the participant’s eye movements at 400 frames per second (BulbiTech AS, 2020). The instrument relies on continuous tracking of the participant’s pupils in order to obtain information about the person’s eye movements.

SRT is known as the time it takes from a peripheral stimuli is presented, until an eye movement towards the peripheral stimuli is initiated, using eye tracking. In measurement with BulbiCam® that is, the presentation of a peripheral (white) target initiated measurement of SRT. As soon as the instrument perceived that the eye in question was moving away from the central (green) target, the SRT measurement was terminated. In this way, a measurement was obtained of the time it took from the white stimuli was presented until the brain processing was completed, the necessary signals to relevant eye muscles had emerged and an eye movement was initiated. Mean SRT for all the 26 points tested was the parameter used for statistical analysis in this study and only points marked as “seen” was included in the calculation of mean SRT. Figure 2 (A) shows the SRT for one of the 26 measured points, as well as how the eye movement to the relevant point was. Figure 2 (B) shows the same as figure 2 (A), except that the point is registered as “unseen”. From the eye movement, it appears that this result was due to the eye movement not reaching the point. According to Kadavath Meethal (2018) SRT is affected both by the angle distance towards the peripheral stimuli and the positioning in the visual fields.

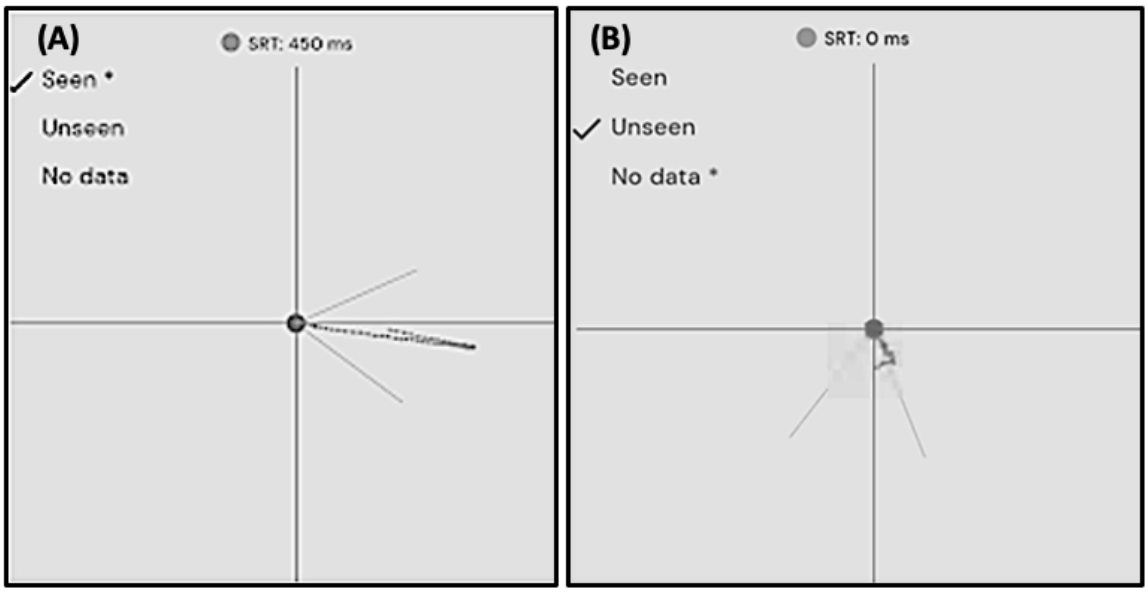

Figure 2: Example of assessment of SRT for one point obtained from visual field assessment in BulbiCam®, recorded in BulbiHub. (A): Example of point registered as "seen" from assessment of eye movement. (B): Example of point registered as "unseen". (Illustration image used with permission from BulbiTech AS, 2022).

BulbiHub (BulbiTech AS) is the software associated with the instrument. If the eye movement towards the presented peripheral stimuli was detected and followed all the three phases mentioned earlier, an algorithm in BulbiHub marked the point as “seen”. If the presented stimuli was not registered as detected, or that the point was detected but the eye movement towards it was considered too imprecise by the algorithms in BulbiHub, the point was marked as “unseen”. Imprecise movement refers to the eyes wandering a lot before noticing the target. The software assess results from assessment of visual fields and the points are coded by color according to results from SRT-measurements (< 200 ms (dark blue), 200 – 300 ms (light blue), 300 – 400 ms (green), 400-500 (light green), 500 – 600 (yellow), 600 – 800 (dark yellow), 800 – 1000 (orange) and > 1000 ms (red)), as visualized in figure 3. Figure 3 further shows what measurement of visual fields and SRT with BulbiCam® might look like.

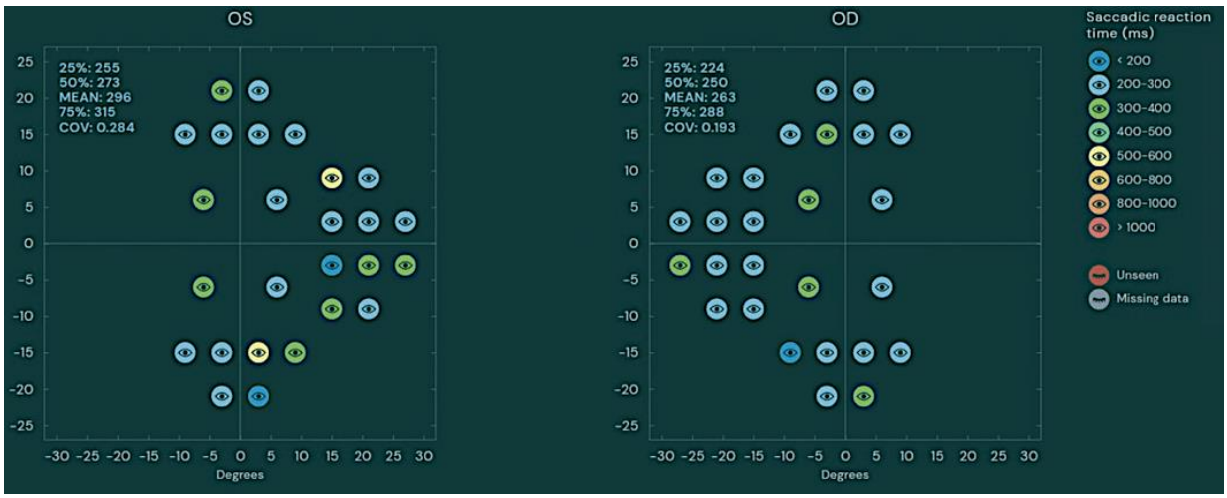

Figure 3: Example of assessment of visual fields and SRT obtained with BulbiCam®, recorded in BulbiHub (Illustration image used with permission from BulbiTech AS, 2022)

3.3 Statistical analyses

Results from estimated risk was organized in a database (Microsoft Office Excel) together with mean SRT from both eyes. From all measurements included in the standard routine examination, the eye considered the "worst" was used for statistical analysis. When both eyes were considered equal a random choice was made. All data were checked and evaluated by the project manager to avoid unrealistic values and missing data. Statistical analysis was performed using R (4.1.1 GUI 1.77) and Statistical analysis system (SAS, version 9.4). With regards to the conclusive estimated risk of developing POAG, the participants were graded as low, medium or high. For statistical analyses, the two participants with confirmed diagnosis of POAG were included in the group at high risk of developing POAG, as the results from these participants were not considered as major outliers. Further, medium and high risk group were combined for some of the analysis to enlarge sample size. Participants were divided at 60 years of age to enlarge sample size for analysis of predicted probability. Continuously distributed variables were expressed by mean values with 95% CI. Differences between groups of estimated risk were analyzed using Analysis of Variance (ANOVA) (Altman, 1999). Pairwise difference between groups were expressed with Forest plots (Li, Zeng, Tian, Levine, & Thabane, 2020). Probability of risk was analyzed using Logistic regression analysis (Agresti, 2002; Anderson, 2003). Correlation analysis was analyzed with Pearson's correlation coefficient and results were visualized using simple linear regression with intercept, slope and 95% CI. Statistical significance is set to $p < 0.05$ and the hypothesis test were two – sided.

4 Results

A total of 52 people were asked to participate in the study, and 50 of them agreed to the terms of participation. Two participants were excluded due to insufficient data, this being that they fell asleep during examination with BulbiCam® and did not wish to repeat the measurement on a later occasion. That leaves 48 included participants in which age ranged from 40 to 70 years with a mean age of 56.3 (± 9.27) years with 1 standard deviation (SD) in parenthesis. The study population included 30 women and 18 men in which 27 and 21 of them were found to have hyperopia and myopia, respectively. Among the 27 participants with myopia, seven of them were classified as having high myopia. Mean age in risk groups was 55.1 (± 8.75) years, 56.9 (± 10.42) years and 61.2 (± 10.0) years in estimated low, medium and high risk, respectively.

4.1 Main results from measurement of SRT

MEAN SRT BY AGE AND ESTIMATED RISK OF POAG			
	Mean SRT (ms)	1 SD	n =
Entire population	325,1	± 44.34	48
Age group 1 (40 - 50 years)	312,1	± 21,79	14
Age group 2 (51 - 60 years)	332,2	± 53,86	17
Age group 3 (61 - 70 years)	328,8	± 47,68	17
Low	313,6	± 43,38	32
Medium	332,8	± 46,89	10
High	373,9	± 58,08	6

Table 2: Main results from measurement of SRT obtained with BulbiCam®. Mean SRT with 1 SD and number of participants is presented for age groups, risk groups and the entire population.

Table 2 showed a trend of increasing mean SRT with increasing estimated risk of developing POAG, with mean SRT in high risk group being largest, despite relatively large SDs. Further, there appeared

to be an increase in mean SRT with age when considering difference in mean SRT between age group 1 and 3, but mean SRT in age group 2 was found larger than in both the other age groups. A substantial SD was found in both age group 2 and 3, while SD in age group 1 was a lot smaller. Sample size in risk group low was a lot larger than the higher risk groups.

4.2 Association between estimated risk of POAG and SRT

Mean SRT with 95% CI for low, medium and high risk of POAG development is displayed in figure 4. Table 2 shows that mean SRT seemed to be increasing with an increase in estimated risk. This association was found highly statistically significant ($p < 0.001$), meaning that an increase in SRT was strongly associated with having higher estimated risk of POAG. The results are presented in figure 4.

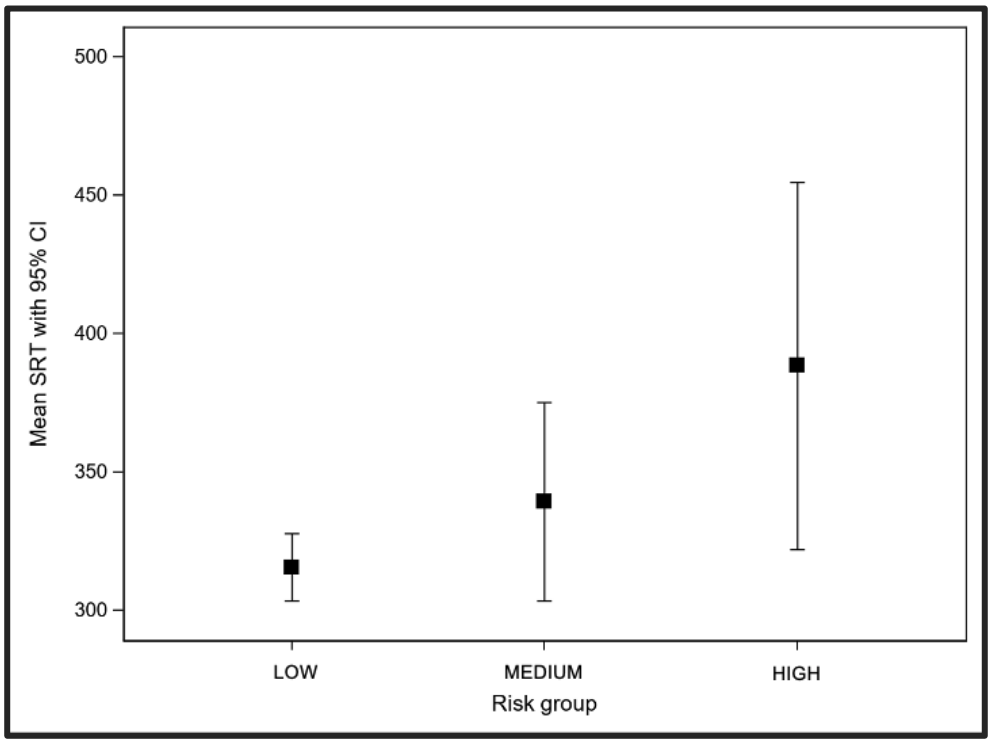

Figure 4: Association between estimated risk of POAG - development and SRT. Mean SRT with 95% CI in each risk group is presented.

The plot shows mean SRT with 95% CI from both eyes combined and visualize how SRT changed with estimated risk. Mean SRT of participants with estimated high risk was substantially increased compared to the other risk groups, and it was interesting to conduct additional analysis

investigating whether there was also significant differences between low and high, and between low and either medium or high estimated risk, the latter was to enlarge the sample size. In addition, both 95% CI and SD was larger in estimated high risk group compared to both medium and low. These findings evoked curiosity to investigate whether there was differences between the eyes that accounted for the large spread of data. The pairwise difference in mean SRT between low and higher (medium and high combined) risk groups was analyzed and the results are presented in a Forest plot in figure 5.

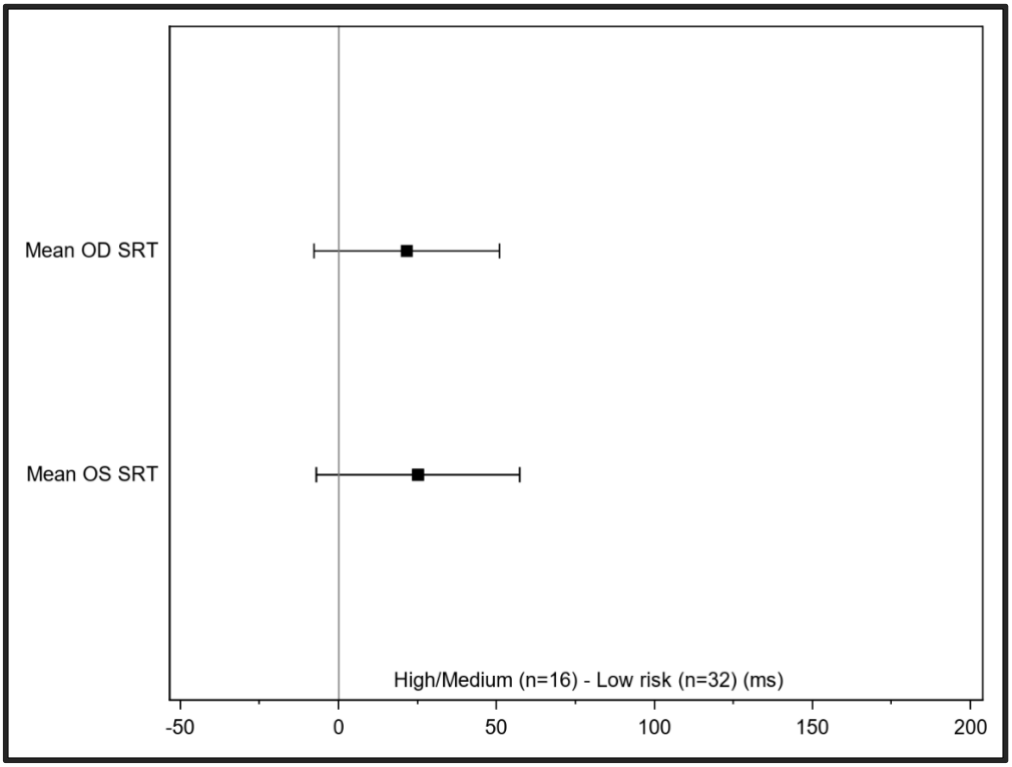

Figure 5: Difference in mean SRT with 95% CI (line through point) between low and medium/high estimated risk of developing POAG for right and left eye. A CI including the value zero indicates an insignificant difference.

Figure 5 shows the Forest plot of difference in mean SRT with 95% CI between low estimated risk and higher (medium and high) risk, in each eye individually. X-axis represents the difference in mean SRT (ms) between the two risk groups, and hence the plot visualizes how mean SRT changed with estimated risk in each eye. From the plot it was understood that the difference in mean SRT between low and higher overall estimated risk was not found significant in right eye (Oculus Dexter, OD) ($p = 0.15$) or left eye (Oculus Sinister, OS) ($p = 0.12$). The significance level is visualized in the

plot by the fact that the CI does include the value zero for both eyes, which indicates insignificant results. Nevertheless, the plot showed a slightly larger difference in mean SRT in OS compared to OD. Analysis of the two eyes together showed similar results, i.e., no significant difference in mean SRT between low and higher risk groups (ANOVA: df = 1, f = 0.65, p = 0.43). On the other hand, very interesting findings were made when analyzing the pairwise difference in mean SRT between low and high risk group, in each eye individually. The results were presented in a Forest plot in figure 6.

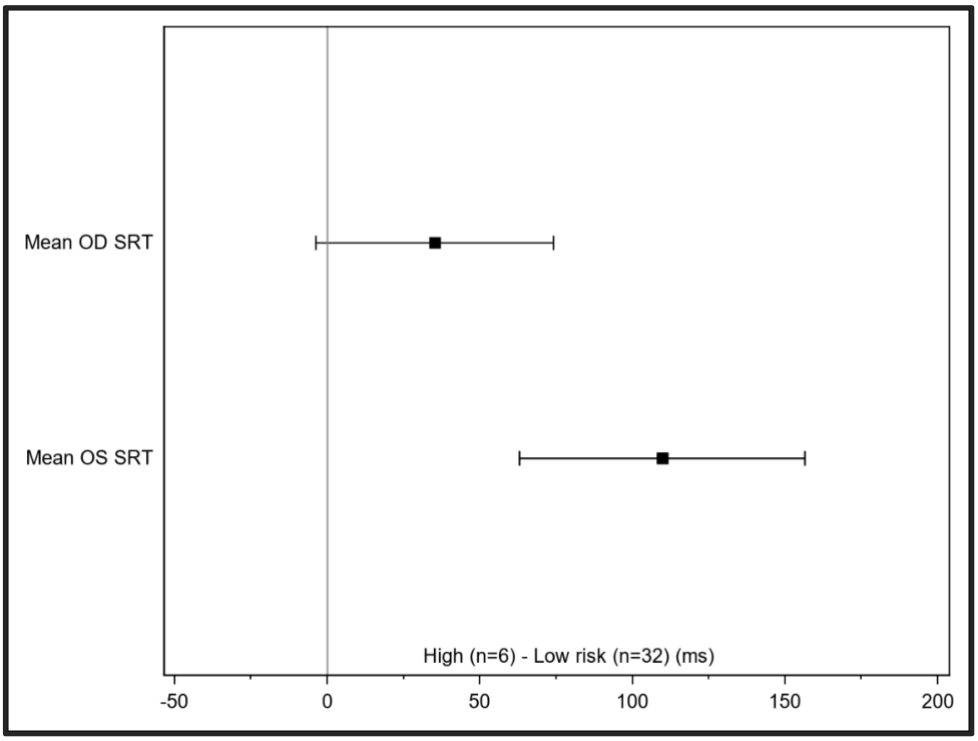

Figure 6: Difference in mean SRT with 95% CI between low and high estimated risk of developing POAG for right and left eye. A CI not including the value zero indicated significant results.

Figure 6 shows the Forest plot of difference in mean SRT with 95% CI between low and high estimated risk in each eye individually. From the plot it was understood that the difference in mean SRT between low and high estimated risk was highly significant in left eye ($p < 0.0001$), compared to right eye ($p = 0.08$), since 95% CI of OS does not include the value zero which indicates significant findings. Further, analysis of the two eyes together showed no significant difference in mean SRT between low and high risk group (ANOVA: df = 1, f = 0.482, p = 0.49). Additional interesting information related to this plot was that three participants estimated at high risk had mean SRT values a lot larger for their left eye, compared to their right eye, potentially causing outliers explaining the results in figure 4 and 6.

4.3 Predicted probabilities for estimated risk

Logistic regression was used to provide a preliminary model of predicted probability of having medium or high estimated risk of developing POAG and the results are presented in figure 7.

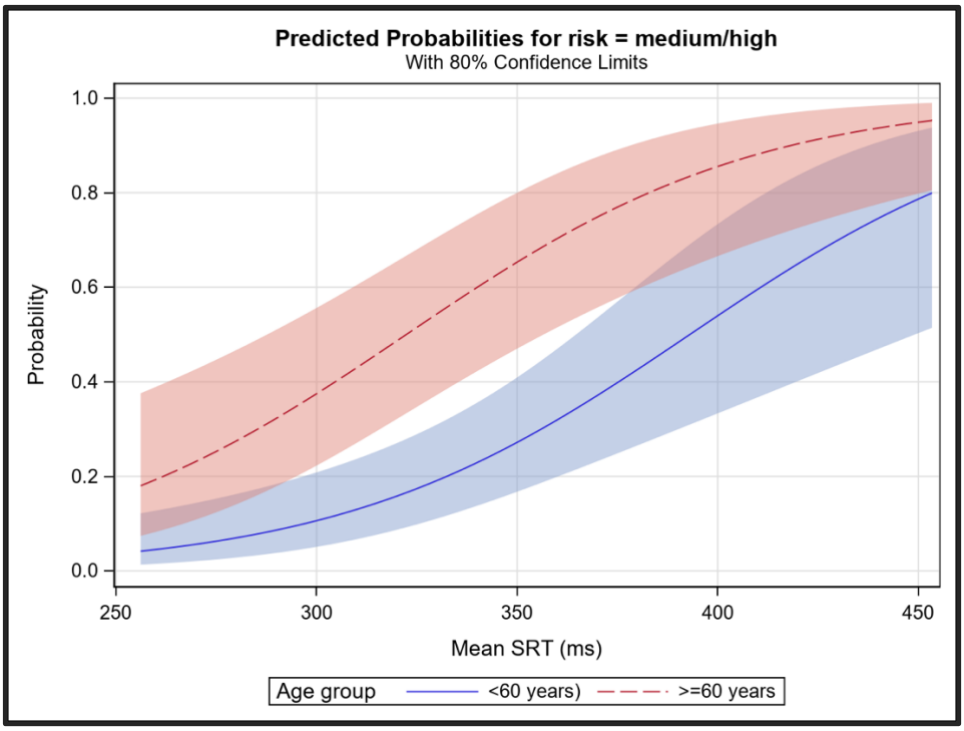

Figure 7: Possibility of having medium or high estimated risk of developing POAG when aged below 60 years, or 60 years or above.

In this figure participants was divided at 60 years of age to enlarge sample size. The blue line represents participants aged below 60 years and the red line represents participants aged ≥ 60 years. The band around the lines refers to the 80% confidence limits. Figure 7 showed that the predicted probability of having medium or high estimated risk of developing POAG if SRT was increasing, increased in both groups. Further, in participants aged below 60 years the predicted probability of having medium or high estimated risk was 0.8, meaning 80% if mean SRT was found to be 450 ms. In participants aged ≥ 60 years, the predicted probability of having medium or high estimated risk was close to 1.0, meaning close to 100% if mean SRT was 450 ms.

4.4 Correlation between age and SRT

The secondary objective of this study was to investigate the correlation between SRT and age to provide information on how SRT changed with age in a population of healthy participants aged 40 – 70 years who conducted a standard routine eye examination in an optometric practice. Results of this correlation analysis is presented in figure 8, using simple linear regression.

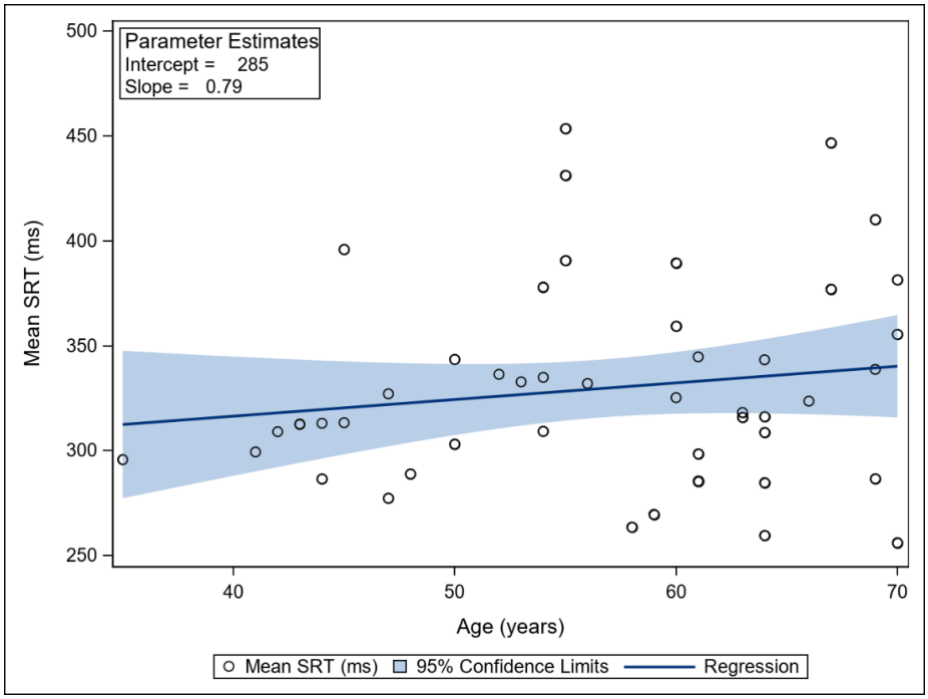

Figure 8: Correlation between age in years and SRT (ms) for all participants.

Intercept in the plot in figure 8 refers to the point on the y-axis in which the x-value equaled zero, while slope refers to the slope coefficient of the curve. The plot showed a slightly positive correlation (r = 0.15, p = 0.33) between age and mean SRT, this meaning the SRT appeared to increase with increasing age. Further the plot showed that quite many of the SRT – values were outside the fitted area (95% CI of mean SRT), indicating large spread of data. It was known from the introduction that age is a major risk factor of developing POAG and that SRT are thought to be increased in POAG. It was therefore expected that mean SRT would be positively correlated with estimated risk of POAG. The results showed a positive correlation, but it was not particularly strong, nor statistically significant. These results provoked a desire to investigate how the correlation between SRT and age was in the different risk groups. Correlation between SRT and age in participants at low risk is visualized in figure 9, while correlation between SRT and age in participants graded at both medium and high risk is visualized in figure 10.

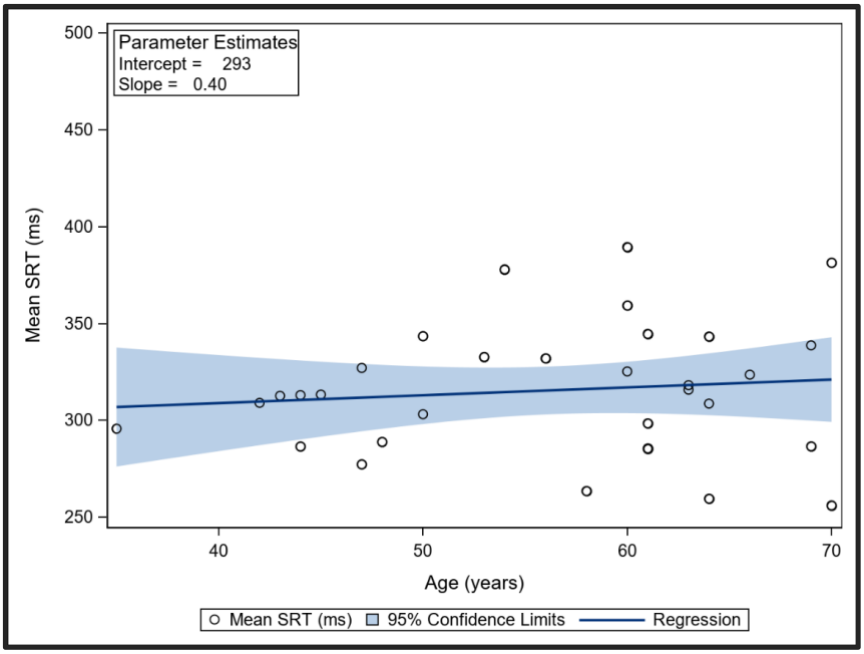

Figure 9: Correlation between age in years and SRT (ms) for participants at estimated low risk

The plot presented in figure 9 showed a correlation close to zero (r = 0.11, p = 0.54) between age and mean SRT, this meaning a slight, close to no increase in mean SRT with increasing age was found. Further, the plot shows that some of the SRT-values were outside the fitted area, indicating large spread of data.

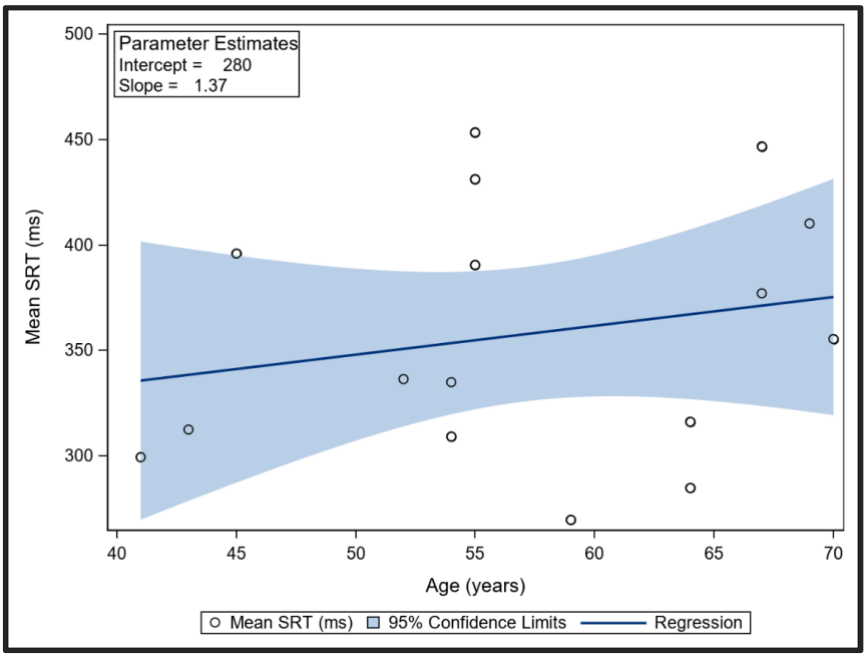

Figure 10: Correlation between age in years and SRT (ms) for participants at estimated medium/high risk

The plot presented in figure 10 showed a fair positive correlation (r = 0.21, p = 0.43) between age and mean SRT, this meaning the SRT appeared to increase with increasing age in participants estimated at medium/high risk. Further, the plot shows that some of the values were outside the fitted area, indicating spread of data. This correlation was found to be the strongest among the correlation analysis conducted. Moreover, results from none of the correlation analysis performed was found significant.

4.5 Additional analyses

Additional analysis was performed in order to investigate whether the association reported between SRT and estimated risk, was also the case when major risk factors was accounted for. In the introduction, it has been shown that age and myopia are strong risk factors of POAG and these variables thus have a substantial expected impact on estimated risk. It was investigated whether the variable also were related to SRT and consequently could be possible confounders that could help explain the relationship shown between estimated risk and SRT. The plot of mean SRT in each risk group by age and ocular status is presented in figure 11 (A) and (B), respectively.

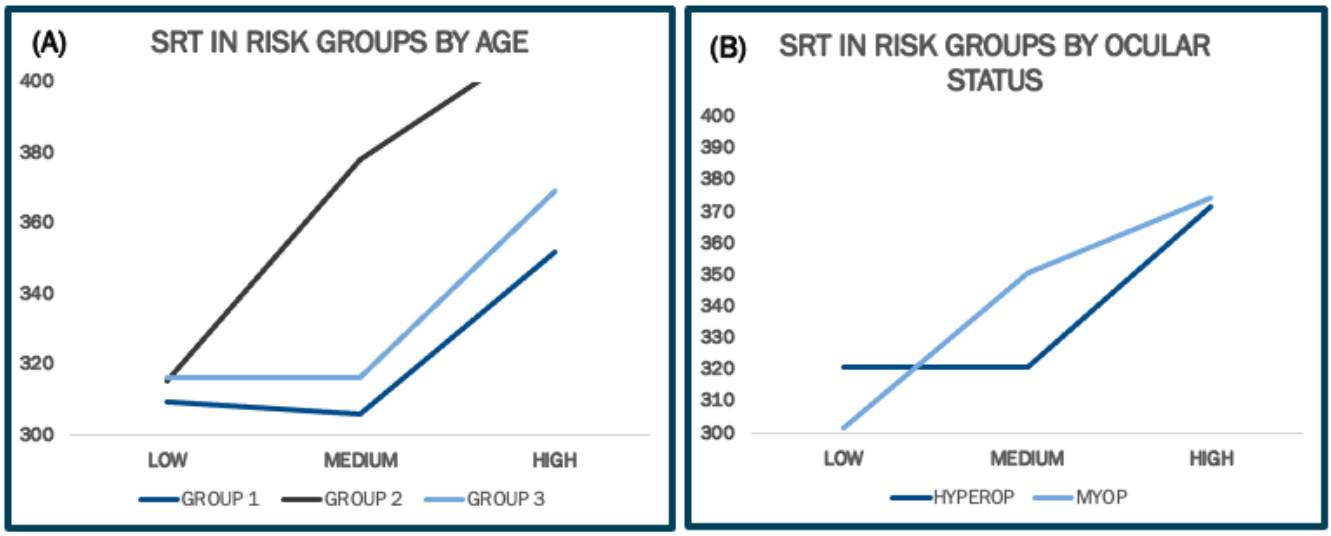

Figure 11: Distribution of mean SRT within each group of estimated risk of POAG - development when major risk factors is accounted for. (A): distribution of SRT in risk groups by age. (B): ocular status.

Figure 11 (A) shows that SRT appeared to increase with increasing risk in age group 2 (51 – 60 years). Additionally, SRT appeared to only increase with increasing risk from medium to high risk in

age group 1 (40 – 50 years) and 3 (61 – 70) years. It thus appears that when the analysis is stratified in age groups, only mean SRT for participants between 51 – 60 years increases with increasing estimated risk, while the difference between medium and high appears to be real regardless of age group. Mean SRT, which was largest in age group 2, was given in table 2. SD was found to be largest in this age group as well, indicating spread of data. Difference in mean SRT by risk between age groups was not found significant (ANOVA: $f = 0.88$, $df = 2$, $p = 0.42$). Further, figure 11 (B) shows that SRT appeared to increase with increasing estimated risk in both hyperopic and myopic participants, but the difference appeared more prominent in myopic participants. Mean SRT in hyperopic participants was 322.8 (± 34.90, $n = 27$) ms, while the average SRT in myopic participants was 328.2 (± 54.96, $n = 21$). Difference in mean SRT by risk between groups of ocular status was not found significant (ANOVA: 0.35, $df = 3$, $p = 0.79$). On the other hand, seven of the myopic participants was found to have high myopia and in this study it was found that high myopia was associated with increased SRT with increasing estimated risk, compared to the rest of the study population (moderate myopia and hyperopia) (ANOVA: $f = 4.98$, $df = 1$, $p < 0.05$). SD indicated larger spread of data in participants with myopia compared to hyperopia.

5 Discussion

The main purpose of this study was to investigate the association between SRT obtained with BulbiCam® and the assessed risk of POAG development in a clinical, Norwegian population aged 40 – 70 years. Based on previous studies investigating the distribution of SRT in people with confirmed diagnosis of glaucoma, it was hypothesized that the estimated risk of POAG would be related to SRT, meaning it was expected to discover a tendency of increasing SRT with increasing estimated risk. As hypothesized, the results from this study showed that increased SRT was strongly associated with increased estimated risk of POAG. It was known from present research that SRT happens to be increased in POAG compared to healthy controls and that the severity of the disease also affects the SRT – values (Mazumdar et al., 2019; Mazumdar et al., 2014; Thepass et al., 2021). What was added of knowledge about SRT obtained with BulbiCam® through this study, was that SRT was increased in groups of estimated higher risk of POAG, this meaning in people *without* confirmed diagnosis, but at risk of developing POAG in the future. Similar results have not been published before.

The study further aimed to investigate whether measurements of SRT provided diagnostic information that were useful for the risk assessment of glaucoma in an optometric practice. In figure 7 it was illustrated that the predicted probability, with 80% confidence limits, of having an estimated risk of medium or high was about 80% in participants aged below 60 years, while it was close to 100% in participants aged 60 years or older, if their mean SRT was 450 ms. Further, preferably longitudinal, research with larger study population is warranted to substitute these predicted probabilities with a higher level of significance, and to provide knowledge on how the predicted probabilities are in different decades of age. Secondary aim of this study was to investigate the correlation between SRT and age to provide information on how SRT changed with age in a clinical population aged 40 – 70 years. Despite insignificant results, a positive correlation was discovered both when considering SRT and age in the entire population, in participants at either medium or high estimated risk.

5.1 Association between estimated risk of POAG and SRT

As hypothesized, the results from this study showed that increased SRT was associated with higher estimated risk of developing POAG. Further, the results was reported highly significant ($p < 0.001$)

which implies plausible results. However, it was necessary to investigate whether there were confounders to the relationship between estimated risk and SRT. Age was probably one such factor as age is associated with both the risk of POAG and SRT. This was known by the fact that age is stated as a major risk factor for POAG and present studies states that risk of development of POAG increases with increasing age (Czudowska et al., 2010; de Voogd et al., 2005; McMonnies, 2017), combined with knowledge of the relation between SRT and age in POAG (Mazumdar et al., 2019; Mazumdar et al., 2014; Thepass et al., 2021). Figure 4 showed that mean SRT was significantly slower in the high risk group compared with the others. Mean age (65.17 ± 2.73 yrs) was also greatest in this risk group. It has thus been shown that the risk of both having POAG and of developing POAG increases with increasing age, and it was therefore expected that age would also increase with increasing estimated risk, which was the case according to mean age in high risk group. Furthermore, it is known that SRT was slower in cases of confirmed POAG, than without, and that SRT in people with POAG was slower in areas of the visual field where there were no detectable functional damage with SAP. It was thus expected that SRT could be increased also in participants without impaired visual function. From this it was understood that age may have been a substantial contributing factor to the mean SRT being found to be much slower in higher risk groups (medium/high) compared to low risk.

IOP may also be an underlying cause of the study results. Elevated IOP is known as a major risk factor of developing POAG (McCann, Hogg, Fallis, & Azuara-Blanco, 2016) and it is stated that RGC loss and consequently functional impairment might occur as a result of elevated IOP (Rao et al., 2020). We now know that SRT measured with BulbiCam® is a measure of oculomotor function and hence, IOP is likely associated with both the risk of POAG and SRT. It has been shown that elevated IOP constitutes both increased risk of developing POAG and, increased risk of developing structural and functional damage consistent with POAG. Furthermore, it was known that functional damage was associated with increased SRT. It is not known how many of the participants in the medium and high risk group had elevated IOP, but because it has been found that the average SRT was slower at estimated higher risk, it was reasonable to assume that a significant proportion of the participants in these risk groups also had elevated IOP, which in turn might have contributed to the mean SRT being so much slower in the higher risk groups compared to lower. Further, in this study IOP was assessed using NCT which was not in line with the considered god standard assessment of IOP when handling patients at risk of developing POAG (Parrish, 2006). This means that the results of

the estimated risk of developing POAG based on assessment of IOP would probably have been different if GAT had been used as recommended in clinical guidelines (Norges Optikerforbund, 2005; National Institute for Health and Care Excellence, 2017). Research has shown that IOP measured with NCT is consistently higher, compared to IOP measured with GAT, but the reported mean deviation between NCT and GAT was only 0.2 [95% CI: -0.1 – 0.6] mmHg (Cook et al., 2012), meaning the two measurement methods are quite similar. Nevertheless, there is thus a risk that participants in this study have been misclassified at medium or high estimated risk based on measurement of IOP. Accordingly, if these participants was in fact misclassified, it would be reasonable to assume that the severity of structural or functional damage would did not constitute an estimated risk of medium or high, which in turn can help explain the amount data spread in medium and high risk group.

5.1.1 Method related confounders

Results from this study showed that an increase in SRT was strongly associated with increased estimated risk of POAG ($p < 0.001$), which implies plausible results. Nevertheless, the SDs was relatively large in all risk groups indicating a substantial spread of data. On the other hand, high value of the measured parameter indicates a larger SD and SD is consequently both a result of spread in data and the value size. According to figure 4, the 95% CI of the mean SRT was larger in high risk group than the other risk groups meaning that mean SRT in this group was not as representative of the true population mean as the mean SRT in the other risk groups. Further, CI is strongly affected by the sample size which was a lot smaller in high risk groups compared to low and medium. Furthermore, the results from measurements of SRT were not blind from the results of risk assessment and there was therefore a risk that observer biases affected the results. Accordingly, it seemed that both spread in data, sample size and risk assessment may have been contributing factors to the results of the study.

Possible explanations for the spread of data discovered in this study includes the implementation of SRT measurements, division into risk groups, instrument-induced fatigue and size of the study population. There is a risk that information about implementation was misunderstood when using BulbiCam® and assessing SRT, respectively. It was the first time that each participant used such an instrument and also carried out such an examination. This in combination with the fact that the

examination was carried out immediately after the implementation of SAP. When the participants conducted the measurements with SAP, they were instructed to suppress a desire to look at a peripheral stimulus. In comparison, when they conducted measurements of SRT with BulbiCam® they were instructed to alternately look at a central and peripheral stimuli. This means that the implementation of EMP was the opposite of SAP and there is thus a risk that some of the participants may have mixed the instructions of these two measurements. Consequently, this may have led to points either being incorrectly registered as "unseen" or that SRT has potentially been incorrectly increased as a result. If this was the case, then mean SRT and SD was affected in turn with potentially higher values of mean SRT than true mean in the population, and consequently larger SD than true deviation of mean SRT. Furthermore, in contrast to conducting measurements with SAP, a demonstration of the examination with BulbiCam® was not carried out before the counting measurement was performed, and the examination was not repeated unless a majority of the measured points were registered as "unseen". This means that if some participants did not truly understand the examination procedure until late stages of the measurement, it was likely that some of the measured points would be incorrectly registered as "unseen" or increased. As discussed above, this outcome may have affected both mean SRT and the spread of data. There are no present reports covering the learning effect of either EMP or BulbiCam®.

Dividing participants into risk groups may have affected the spread of data. Classifying the participants into bins according to their estimated risk of developing POAG, was based on the subjective assessment performed by a trained examiner combined with the examiners usage of the criteria for risk assessment described in chapter 3. Participants at medium and high estimated risk could therefore have both structural damage, impaired visual function, or a combination of these. For instance, it is known that SRT was a direct measure of oculomotor function and participants with impaired visual function was expected to have a somewhat elevated SRT, compared to those without visual field damage. Furthermore, it was known that research also had reported increased SRT in areas *without* visual field damage in people with POAG, but that these SRT-values was predominantly faster than in areas *with* visual field damage (Thepass et al., 2021).This means that since medium and high risk groups probably included both participants with and without visual field defects, both fast and slow values of mean SRT were likely connected to these risk groups and consequently, the spread of data in these risk groups enlarges.

Confidence intervals are strongly related to sample size and the large CI in medium and high risk visualized in figure 4 was likely due to a small both population size and sample size. A small study population refers to a less stable, and consequently less reliable, analysis of associations compared to a larger study population (Faber & Fonseca, 2014). There is no clear cut-off value to how many participants makes a large enough study population as the size depends on what analysis are performed. In this study, there is a risk that the size of the study population and consequently the sample size in medium and high risk group has been too small to provide plausible results. Nevertheless, significant results from small sample sizes are in fact considered even more powerful than in larger sample sizes (Faber & Fonseca, 2014).

In this study, the results from measurements of SRT obtained with BulbiCam® was not blind from the results of subjective, clinical assessment of estimated risk. This meaning there is a risk of observer biases related to the results from this study. The challenge of reducing amount of potential biases in researches like this study is the risk evaluation of POAG, which was predominantly based on subjective evaluations of abnormal findings potentially consistent with POAG. Therefore, it is conceivable that the credibility of the clinical risk assessment for POAG would have been stronger if the overall estimated risk had been assessed by two experienced optometrists, rather than one. On the other hand, the evaluation of measurements of SRT obtained from BulbiCam® was entirely objective. That is, the SRT-values for each measured point was recorded in BulbiHub and mean SRT from each eye was transcribed into the data recording sheet used in this study (appendix 1), meaning that the project manager was not able to manually adjust the recordings to best fit the results from estimated risk evaluation.

As mentioned, the parameters included in the overall risk assessment of POAG was assessed based on subjective, clinical evaluation performed by one optometrist. Assessment of ONH was conducted with subjective evaluation of fundus photography which compromises with clinical guidelines where stereoscopic assessment with indirect binocular ophthalmoscopy is recommended (Norges Optikerforbund, 2020; National Institute for Health and Care Excellence, 2017). A problem related to changes of ONH being assessed solely based on objective evaluation of a photography is that small discs becomes challenging to assess (Jayasundera, Danesh-Meyer, Donaldson, & Gamble, 2005; Lichter, 1976). In small discs, the cup is consequently small and it can be challenging, even for the trained eye to assess the true presentation of retinal rim (Lichter,

1976) and changes in ONH might have been misjudged to be glaucomatous because the C/D – ratio had a tendency to deviate from the considered normal values. In addition, this might have been the case even for large discs, as very few large discs has a considered normal C/D – ratio and thus do not follow the ISNT – rule (Ikram et al., 2002). It has been reported that stereoscopic assessment of ONH with ophthalmoscopy is a better and more reproducible method to use to circumvent the mentioned problems (Correnti, Wollstein, Price, & Schuman, 2003). In contrast, an advantage of photographic evaluation of ONH is that any examiner can go back on previous examinations and compare the results. This meaning that a combination of stereoscopic assessment by ophthalmoscopy and photography, stereoscopic preferably, of ONH would probably have been a better method to obtain more accurate results of ONH assessment in this study.

5.1.2 Difference between right and left eye

Figure 4 showed that mean SRT of participants with estimated high risk was substantially increased compared to medium and low risk, and it was interesting to perform additional analysis to investigate whether there was also significant differences between low and high risk, and between low and medium and high estimated risk combined. The latter was to increase the sample size. Furthermore, both 95% CI and SD was a lot larger in estimated high risk group compared to the others and these findings evoked curiosity to investigate whether there were differences between the eyes that accounted for the large spread of data. Figure 6 illustrated interesting findings. When distinguishment between left and right eye was made and the difference between low and high estimated risk was analyzed, a highly significant ($p < 0.0001$, $p = 0.08$) difference was reported between left and right eye, respectively. This significant difference was found despite the size of the risk groups, which implies that sample size was in fact not affecting the level of significance and consequently the degree of plausibility of the results from this study. In fact, the discovery of highly significant results despite a small sample size actually strengthens the results (Faber & Fonseca, 2014). When difference between low and medium/high estimated risk was analyzed, just a slight, insignificant ($p = 0.12$, $p = 0.15$), difference between left and right eye, respectively, was reported. It has been stated that asymmetry, i.e., clinical findings consistent with POAG initiated in one eye before the other, is a potential sign of POAG-development (Wang et al., 2004). On the other hand, no studies have indicated a prevalence of POAG-development in one particular eye before the

other and it was therefore an assumed greater risk of the results being related to methods, rather than physiological factors.

Methods used in this study meant that right eye was the first eye assessed, which consequently may have induced a learning effect. A learning effect arises as a result of repeated implementations provoking that one learns how a certain methods is performed and the measurement stability increases (Fogagnolo et al., 2010). If the learning effect was causing the differences between the eyes in this study, one would expect the reaction time of left eye to be faster than in right eye, as right eye was measured first. Nevertheless, a learning effect could potentially indicate that the examination of SRT became more sensitive with repeated measurements and that the results from the assessment of SRT in left eye was therefore more accurate. This statement in light of the results from this study implies a physiological reason for the reported difference between the eyes, but since no previous studies have referred to such a relation, it was still considered that the results from this study could probably be attributed to methods. Furthermore, the examination of SRT using BulbiCam® was performed as the last examination, as well as in dim lighting, and it is conceivable that the participants were less attentive when the left eye was assessed and consequently the reactivity of the left eye was reduced compared to the right. Further research, with preferably randomization of both the order of the examined eye and the conduct of examinations that forms the estimated risk of developing POAG, is necessary to confirm or deny whether the cause of the discussed difference between right and left eye can be attributed to methods.

A significant difference was found between the right and left eye when assessing the difference in mean SRT in the low and high risk group, while only a slight difference between the eyes was found when assessing the difference in mean SRT between low and medium/high risk group. Controversially, when analyzing the difference in mean SRT between low and high risk group without separating the eyes, the results came out different and not significant ($p = 0.49$). Further, the difference in mean SRT between low and medium/high risk group without separating the eyes was also not significant ($p = 0.43$). These results indicated that there was a probability that high risk group included participants with measurements of SRT being substantially larger in left eye compared to right eye. Further investigation of the collected data revealed that this was in fact the case. Three of the participants (50%) estimated at high risk of POAG development was found to

have mean SRT substantially larger in left eye compared to right eye. This knowledge helps explain the large difference discovered between left and right eye when assessing the difference in mean SRT between low and high risk group, as well as why the difference was not as large between the eyes when investigating the difference in mean SRT between low and medium and high risk group combined.

Interesting findings from the three participants discussed above was that their SAP-readings was considered normal, but clinical assessment of ONH and parapapillary RNFL thickness resulted in estimated high risk. It is known that RGC loss causes structural damage which eventually also causes functional impairment, but that about half of the RGCs must be lost for it to become detectable when assessing visual fields (Weinreb et al., 2014). The results from the three participants therefore indicates some very interesting findings, i.e., that SRT obtained with BulbiCam® probably has the potential of detecting functional damage before it becomes visible on assessment of visual fields using SAP, in people *without* POAG. Nevertheless, it must be repeated that two of the participants included in the estimated high risk group actually had confirmed diagnosis of POAG and there is therefore a probability that these two participants were among the three discussed. On the other hand, it would be interesting to perform similar studies with a larger study population to further investigate whether measurements of SRT with BulbiCam® are able to detect functional damage before it becomes visible on assessment of visual fields using SAP, in people without POAG.

5.2 Predicted probabilities

The main purpose of this study was to investigate the association between overall estimated risk of developing POAG and SRT and it can from this study be concluded that SRT was likely to increase with increasing estimated risk. Furthermore, this study aimed to investigate whether measurements of SRT obtained with BulbiCam® were likely to add useful diagnostic information to the risk assessment of glaucoma in an optometric practice. As mentioned earlier, longitudinal studies are required in order to fully investigate whether SRT obtained with BulbiCam® are likely to predict future development of POAG. Nevertheless, this study showed some very interesting findings and a preliminary model was developed to illustrate the predicted probabilities of having a medium or high estimated risk of developing POAG, based on measurements of SRT. Figure 7 showed that, with 80% confidence limits, predicted probability of having an estimated medium or

high risk of developing POAG was close to 1.0 when mean SRT was 450 ms, in participants aged 60 years or above. In participants aged below 60 years, the predicted probability was 0.8. This meaning that SRT obtained with BulbiCam® has the potential of becoming a reliable predictor of estimating risk of POAG-development, at least in participants aged 60 years and above. Nevertheless, further research with a preferably larger study population is necessary to support these findings with a higher level of significance, and to provide knowledge on how the predicted probabilities differs in different decades of age. Furthermore, the graphical presentation of predicted probability clearly shows how the probability of having an estimated higher risk of POAG development increases as SRT increases. This applies to both groups.

5.3 Correlation between age and SRT

The secondary objective of this study was to investigate the correlation between SRT and age in a clinical population aged 40 – 70 years to provide useful information to the clinical assessment of POAG. From the plot in figure 8, a positive correlation between age and mean SRT was visualized. This meaning that there was a tendency of SRT being increased with increasing risk, despite this correlation not being significant and many of the SRT-values was located outside the fitted area, i.e., not included in the 95% CI. Values outside the fitted area implies large spread of data. Potential explanations to spread of data included the implementation of SRT measurements, division into risk groups, instrument-induced fatigue and size of the study population, which was discussed earlier. The positive correlation discovered was not particularly strong. POAG is reported to increase with age, and that the risk increases with each decade after the age of 40 (Czudowska et al., 2010; de Voogd et al., 2005; McMonnies, 2017). This knowledge could be related to the correlation strength and lack of significance and the correlation would probably be stronger if the study included participants aged 70 years and above. When investigating the correlation between age and SRT in the different risk groups, the results came out a bit different. According to figure 9 there was close to no correlation between mean SRT and age in participants estimated at low risk of developing POAG. Further, according to figure 10, the strongest positive correlation was found in participants estimated at medium or high risk. Controversially, none of the correlation analysis discussed was found statistically significant. And the lack of significance was thought to be related to the spread of data, sample size and risk assessment, which were discussed earlier in this section.

Considering the correlation between age and mean SRT in participants at estimated low risk, the results show that regardless of age, the participants included in this risk group had a stable mean SRT of approximately 300 ms, except some outliers that was thought to be related to the spread of data. When considering the correlation between age and mean SRT in participants in medium or high risk, the results show that mean SRT was less stable for the participants included in this risk group, but on the other hand, the correlation was the strongest among the three correlation analysis discussed. It is known that the eye movement in people with POAG tend to wander a lot during measurements of SRT (Lamirel, Milea, Cochereau, Duong, & Lorenceau, 2014), which further causes increased SRT. Mean age in medium and high risk group was higher than in low risk group, and it has been stated that higher age is associated with increased risk of POAG (Czudowska et al., 2010; de Voogd et al., 2005; McMonnies, 2017). This knowledge in combination with the criteria's forming the basis of clinical risk assessment used in this study, contributes to an understanding that medium and high risk group potentially included participants with clinical findings consistent with POAG. This in turn was thought to constitute increased SRT and further help explain why the correlation was strongest in these risk groups.

5.4 Additional analyses

This study showed that increased SRT obtained with BulbiCam® was strongly associated with an increase in estimated risk of POAG. The diagnostic information and clinical value of SRT was considered to be even more powerful if the relation was also present when major risk factors was accounted for. It is known that elderly age and ocular status are considered as major risk factors of developing POAG (Schuster et al., 2021). Figure 11 (A) gave the impression that mean SRT was increasing with increased risk from medium to high in all age groups, but only for age group 2 when considering increase in risk from low to high. It thus appeared that when stratifying the analysis into age groups, much of the difference in mean SRT between low and medium risk group shown in figure 4 was explained by age group, as discussed earlier, while the difference between medium and high seemed to be real, regardless of age group.

Elderly age is a known risk factor of POAG – development and present studies states that risk increases with increasing age (Czudowska et al., 2010; de Voogd et al., 2005; McMonnies, 2017). From this it was expected that age would increase with increasing risk group, but the SRT was not

found significantly different between age groups in this study. The results from this analysis was likely related to methods. Some of the participants could be of an older age, but without clinical findings inducing overall estimated risk of medium or high, and some of the participants could be included in the youngest age group, but with substantial clinical findings consistent with POAG, causing estimated medium or high risk. This in combination with the limitations mentioned earlier regarding population size, have likely affected the analysis discussed. Further, mean SRT was found to be faster in participants aged 40 – 50 (age group 1) years estimated at medium risk compared to the other risk-and age- groups. This meaning that, among the ten participants in medium age group, those who also belonged to age group 1 had faster mean SRT than those in the same risk group, but of younger or older age. This constituted a very small sample size, which has, as discussed earlier, probably had an impact on the average SRT for both medium risk group and age group 1.

Myopia and especially high myopia is known as a risk factor of developing POAG (Chang & Singh, 2016). 44% of the participants was found to have myopia and according to figure 11 (B), SRT appeared to increase with increasing estimated risk to a greater extent in myopic participants compared to hyperopic participants, but the relation was not found significant. The fact that having myopia does not equal having clinical findings consistent with POAG-development was a possible explanation to the lack of significance. On the other hand, the results came out different when distinguishment between moderate and high myopia was made. The association between SRT and high myopia was found significant. This meaning that SRT was found to increase with increased risk in participants with high myopia. Nevertheless, only 7 participants was found to have high myopia which makes a small sample population and significance in such small population is considered a strong significant finding. Another possible explanation to the results may be that the assessment of ONH and RNFL was based on subjective grading which is considered a challenging task, especially in myopic eyes (Chang & Singh, 2016). Optic nerve damage seen in myopia are likely to be misclassified as glaucomatous optic neuropathy. This misclassification normally occurs as a result of a flattened cup due to the axial length, which is normally longer in myopic eyes compared to hyperopic eyes (Jonas, Wang, Dong, & Panda-Jonas, 2020). Taking this knowledge into account provides an understanding that some of the participants with myopia might have been misclassified as eyes with glaucomatous changes of ONH and hence some of the participants in medium or high risk group should potentially be included in the low risk group. The results from this study would be

more accurate if ONH was examined stereoscopically with indirect binocular ophthalmoscopy, as recommended in clinical guidelines (Norges Optikerforbund, 2020; National Institute for Health and Care Excellence, 2017), rather than fundus photography alone.

6 Conclusion

Results from this study has for the first time demonstrated that SRT obtained with BulbiCam® is related with the risk of developing POAG as assessed by evidence based clinical methods. This meaning that an increase in SRT was strongly associated with an increase in estimated risk. Furthermore, the study aimed to investigate whether the measurements of SRT obtained with BulbiCam® were useful for the risk assessment of POAG in an optometric practice. The predicted probability of having higher estimated risk of POAG was found to increase with both age and mean SRT. Secondly, this study aimed to investigate the correlation between SRT and age in people between 40 – 70 years to provide useful information to the clinical assessment of POAG. A weak positive correlation was found. Further research is warranted to further investigate whether SRT obtained with BulbiCam® add useful information to the clinical assessment of glaucoma and how SRT changes with age.

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Appendixes

Appendix 1: Data collection from

Appendix 2: Information and consent form