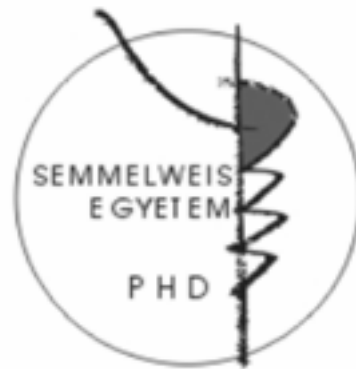# Detection of glaucomatous retinal ganglion cell loss with scanning laser polarimetry

Ph.D. thesis

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INTRODUCTION

Glaucoma is a progressive apoptotic disease of the retinal ganglion cells. The process leads to irreversible axonal and optic nerve head damage and, as a consequence, to a characteristic pattern of visual field deterioration. In the elderly glaucoma is a frequent disease worldwide and it is also a leading causes of blindness. According to epidemiological projections, in 2020 the estimated number of people suffering from primary glaucoma will be around 80 million; of them 11.2 million will deteriorate to bilateral blindness.

In case of primary open angle glaucoma, which is the most common form of the disease in Caucasian populations, progression remains free from symptoms for long time, which allows development of severe damage before the patients realize the visual impairment. Since glaucomatous functional damage is irreversible, early diagnosis and early initiated efficient treatment, that prevents significant visual impairment, is crucial. In addition to the traditional diagnostic methods, modern glaucoma diagnostics and management use computerized structural methods such as scanning laser polarimetry (SLP) and scanning laser tomography (SLT). SLP is a non-invasive clinical diagnostic method for the measurement of the retinal nerve fibre layer thickness.

SLP has been developed and introduced in clinical practice fifteen years ago, and since that time it has been under continuous development. Although the sensitivity and specificity of the commercially available SLP instrument (GDx-VCC) is good in a hospital-based environment, its value in long-term follow-up and detection of mild progression remains to be specified. Similarly, limited data has been available on the screening accuracy of the GDx-VCC. In 15-30% of the eyes, the commercially available GDx-VCC software exhibits atypical retardation pattern, which represents measurement noise originating from the tissues around the retina. Atypical retardation is difficult to interpret. The new software version (GDx-ECC) currently under clinical testing was developed to neutralize this artefact; however, prior to our publications, no data was available on its clinical usefulness.

AIMS

1. To investigate whether the GDx-ECC software reduces the atypicality of the SLP measurements compared to the GDx-VCC software, and to determine whether this possible effect is similar in glaucomatous and healthy eyes. We also investigated if in case of atypical retardation pattern the GDx-ECC is more suitable for classification of the eyes than the GDx-VCC.

2. To investigate if GDx-ECC is able to neutralise measurement noise originating from the anterior segment of the eye, and to compare this effect between GDx-VCC and GDx-ECC. In our study anterior segment measurement noise was induced by alteration of corneal birefringence with laser-assisted in situ keratomileusis (LASIK).

3. To investigate the effect of peripapillary myelinated retinal nerve fibres on the GDx-VCC and GDx-ECC measurements, and to clarify if this effect is different with the different softwares.

4. To investigate the correlation between the structural damage measured with GDx-VCC and the functional damage as measured with standard automated perimetry (SAP), and a selective test, frequency doubling perimetry (M-FDT). Selective tests stimulate only a non-redundant subpopulation of retinal ganglion cells. Also, we wanted to investigate the correlation between SAP and M-FDT on glaucomatous and healthy eyes and to evaluate whether the selective or the non-selective test is more suitable for differentiating glaucomatous and normal groups.

5. The purpose of our mass glaucoma screening trial was to assess the screening ability of GDx-VCC and M-FDT (both used both with screening and full programs). We also investigated which parameters and which combinations of the different parameters provide the best screening accuracy. In addition we tested whether combined assessment of GDx-VCC and M-FDT provides better screening accuracy than the individual use of the devices.

6. In our next screening trial we investigated the screening performance of GDx-VCC and GDx-ECC (both the screening mode and the full examination) as well as that of scanning laser tomography (SLT). We determined the criteria providing the highest screening accuracy for both devices. Another aim of this study was to evaluate if combined assessment of SLP and SLT measurements increases the screening accuracy as compared to the individual use of the different instruments. We compared the screening accuracy achieved with combined evaluation of SLP and SLT to that found by us using combined evaluation of SLP and M-FDT, in the previous study.

7. In our long-term follow-up study we investigated if glaucoma progression is detectable with GDx-VCC or GDx-ECC in a 2.5-year time-period. To that, we investigated if there was any significant long-term change of the polarimetric parameters in eyes with visual field progression, and also, if the long-term variability of the polarimetric parameters is different in progressing glaucoma as compared to the healthy and stable glaucomatous eyes. Our further aim was to compare the usefulness of the GDx-VCC and the GDx-ECC in detecting progression.

METHODS

All participants of the studies have signed an informed consent form and were voluntarily taking part. The research protocols were approved by the Institutional Review Board for Human Research of the Semmelweis University. In the statistical analyses, p-values of less than 0.05 were considered significant.

1. In our first study, of all eyes imaged with GDx-VCC and at the same time with GDx-ECC between May 2004 and January 2005 at the Glaucoma Unit of the Department of Ophthalmology of Semmelweis University, the eyes showing atypical pattern with the VCC software were selected for the investigation. Altogether 27 glaucomatous and 19 healthy individuals were enrolled. Retinal thickness parameters and the typicality of the images (Typical Scan Score, TSS) were compared between the VCC and the ECC techniques. The SPSS 10.0 program package was used for statistical analysis.

2. In our second study, one eye of 15 healthy ametropic subjects was imaged immediately before and 7 days after LASIK surgery with GDx-VCC and GDx-ECC. Corneal retardation was assessed at each measurement session. For post-LASIK calculations, either actual post-LASIK corneal retardation, or the pre-LASIK corneal retardation (VCC* and ECC* groups) was used. VCC* and ECC* images contained intentional artificial measurement noise. During statistical analysis, corneal retardation, the TSS values of the images and the retina thickness values at the 64 plots along the measuring ellipse were assessed. The Statistica 6.0 program package was used for statistical analysis.

3. In our third study, using GDx-VCC and GDx-ECC we investigated the polarimetric effect of myelinated retinal nerve fibres in 5 eyes of 4 individuals. The locations of the maximal retardation induced by myelinated retinal nerve fibres on the VCC images were used to characterise the effect of myelinated retinal nerve fibres for both compensation techniques.

4. Twenty-two glaucoma patients and 16 healthy individuals were enrolled in our fourth study. GDx-VCC, standard automated threshold perimetry (SAP) and matrix frequency-doubling perimetry (M-FDT) were performed by all participants who were divided into groups in two different ways. The glaucoma group and the normal group were established

according to detailed clinical examinations. The other categorization was based on the standard classification of the Nerve Fibre Indicator (NFI) parameter of SLP: we had groups with healthy polarisation pattern (NFI<30), borderline (NFI 30-40) and pathological patterns (NFI>40). During statistical analysis, we investigated the possible correlation between the diagnostic techniques for all subjects and by groups. The Statistica 6.0 program package was used for statistical analysis.

5. The mass glaucoma screening was publicized in a radio program and a newspaper, the publicity also giving background information on risk factors for the disease. After registration of the ophthalmic and general medical history, the following examination protocol was performed for each participant: (1) best corrected visual acuity testing, (2) detailed slit-lamp examination; (3) GDx-VCC tests (screening and full examination); (4) M-FDT tests (screening and threshold examination); (5) IOP measurement with Goldmann-applanation tonometry; (6) stereoscopic evaluation of the optic nerve head after pupil dilatation. Each participant with possible glaucoma was scheduled for detailed clinical examination at the Glaucoma Unit of the Department of Ophthalmology of Semmelweis University. Several alternative criteria for glaucomatous damage were defined for each test method. Sensitivity, specificity, accuracy, positive predictive value and positive likelihood ratio were calculated for each criterion and for their combinations. The Statistica 6.1 program package was used for statistical analysis.

6. Our second mass glaucoma screening was publicized similarly to the first screening. The protocol of the study was similar to that shown in point 5, but SLT examination was performed instead of M-FDT test. The eyes showing atypical polarisation pattern with the VCC technique were imaged with the ECC as well. Evaluation and analysis of the results were the same as described in point 5. The Statistica 7.0 program package was used for statistical analysis.

7. Our long-term follow-up study was conducted between May 2004 and June 2007. Data of the participants who completed a minimum 2-year study period with a minimum of 5 visits (1 baseline and 4 follow-up visits) were analysed. According to this, data of 27 control subjects and 52 glaucoma patients were statistically evaluated. After a detailed baseline examination, the following measurements were performed at 6-month intervals: (1) determination of best-corrected visual acuity, (2) GDx-VCC and ECC measurements, (3) standard automated perimetry, (4) detailed ophthalmic examination, and (5) Goldmann-

applanation tonometry. At the end of the follow-up period, each study eye was assigned to one of 3 groups: control, stable glaucoma and progressing glaucoma. We compared the demographic characteristics and the distribution of the number of eyes showing atypical polarisation pattern between the groups as well as the distribution of the baseline glaucoma stages between the two glaucoma groups. We investigated the influence of time on the different polarimetric parameters in each group. Also, long term variability of each SLP parameter was compared between the groups. The Statistica 8.1 program package was used for statistical analysis.

RESULTS

1. All 46 eyes selected for this study showed clinically significant atypical polarisation pattern with GDx-VCC. The typical scan score (TSS), which automatically calculates the degree of typicality, increased significantly when using ECC both in the normal and in the glaucoma group. When using ECC, the retinal polarisation became more real: in the glaucoma group neutralization of atypical retardation with ECC resulted in lower thickness values and increased TSNIT standard deviation, in the healthy group the retinal polarisation pattern became more 'healthy'.

2. The polarimetric effect of the cornea changed significantly after LASIK. The LASIK had no effect on the retinal thickness data either with VCC or with ECC, when the actual polarimetric data of the cornea were used for the post-LASIK GDx measurements. On the contrary, when the pre-LASIK cornea values were used for the post-LASIK GDx measurements, an uncompensated retardation pattern was seen in all cases with the VCC software (VCC*). Also, the polarimetric retinal nerve fibre layer thickness values changed clinically significantly in many sectors. In contrast to this, using the ECC software (ECC*), the post-LASIK SLP images did not show any uncompensated polarimetric pattern, and the polarimetric retinal nerve fibre layer thickness did not change after LASIK. The number of significantly altered sectors did not show a significant difference between VCC, ECC (using the actual cornea values) and ECC*.

3. Our cases suggest that myelinated retinal nerve fibres have clinically significant influence on the polarimetric retinal nerve fibre layer thickness. Using the VCC technique, the myelinated retinal nerve fibre areas adjacent to the optic nerve head appeared as areas of increased polarization. In contrast, using the ECC mode, the same retinal nerve fibre areas showed low signal areas in the superior and inferior quadrants, and high signal areas in the temporal and nasal quadrants. When the wedge-shaped area of myelinated retinal nerve fibres was not continuous to the optic nerve head and followed a retinal nerve fibre bundle, its increased retardation was not neutralized by either compensation technique.

4. Visual field indices of both the selective perimetry (M-FDT) and the non-selective perimetry (SAP) differentiated the healthy and the glaucoma groups. Both functional methods differentiated eyes with normal and pathologic polarisation pattern. The glaucoma group and the normal group were also differentiated by the NFI parameter of the SLP.

Correlation between the NFI and the functional examinations was general when evaluating the healthy and glaucomatous eyes together. In contrast, evaluating only the glaucoma group, the structure-function correlation was different according to the visual field test. There was no correlation using the conventional non-selective perimetry, while using the M-FDT both Mean Sensitivity and Mean Deviation correlated with the NFI.

5. Two-hundred and thirty-three attendees visited our first screening, and of them, 345 eyes of 181 participants were eligible for the GDx and M-FDT examinations. The suitability of GDx-VCC was good, high quality images were not successfully obtained only in 43 eyes of 466 (11%). M-FDT had a poorer suitability rate (21%). Independent use of the different tests and criteria was not optimal for mass glaucoma screening: the positive likelihood ratio of 10 (optimal) was not reached, and the highest positive predictive value was 52.6%. When the criteria of NFI (cut off value 30), GDx-VCC 'nerve fibre bundle type defect' (a parameter developed by us, characterizing the localized thinning) and M-FDT screening classification were combined, the positive likelihood ratio was over 10, with a specificity, accuracy and sensitivity of 96.9%, 91.7% and 41.7%, respectively.

6. Of the 272 eyes of 136 subjects participating in the second screening, 218 eyes of 118 persons were eligible for statistical evaluation. Of the GDx-VCC tests, 91.9% were successful, while of the SLT measurements, the rate of good quality and successful images was 81.4%. Our results showed that none of the SLT criteria were suitable for glaucoma screening; the positive likelihood ratio was below 5 in each if them. The individual criteria of the GDx test were somewhat better than those of the SLT, but the optimal value of 10 was not reached, either. When using the ECC for eyes showing atypical polarization pattern and the VCC for eyes showing typical pattern, results did not differ in a clinically significant manner from the results calculated only using the VCC measurements. Combining the best criteria within each diagnostic method, the SLT combinations did not provide a clinically significant improvement of the screening efficacy. In contrast, combining the best GDx criteria, the screening efficacy increased. The combined evaluation of SLP and SLT did not improve the screening efficacy compared to the GDx-VCC results alone.

7. During the follow-up time, there was no significant alteration of any polarimetric parameters in any group. The long term variability of ECC-NFI was significantly higher in the progressing glaucoma group than in the stable glaucoma group. In addition, long term

variability of both VCC-NFI and ECC-NFI were significantly higher in the glaucoma groups than in the control group. Also, there was a general tendency for higher long term variability in the glaucoma groups than in the control, and for higher values in progressing than in stable glaucoma. Short term variability of the analyzed SLP parameters was similar in all groups, and it was smaller than the long term variability of the corresponding parameters.

CONCLUSIONS AND SUMMARY OF NEW RESULTS

1. The results of our first study showed that the ECC software significantly reduces atypical polarization pattern for both healthy and glaucomatous eyes. In case of atypical pattern the ECC mode is more suitable for classification than the VCC software.

2. Our second study proved that the ECC software successfully neutralizes measurement noise due to uncompensated corneal polarization, for which the commercially available VCC technique is not capable.

3. The findings of our third study suggest that myelinated retinal nerve fibres around the optic nerve head have an effect on the SLP measurements, both with VCC and ECC. However, this effect varies according to the compensation method, the retinal location of the myelinated retinal nerve fibres and the spatial relationship of the myelinated retinal nerve fibres to the retinal nerve fibre bundles.

4. The results of our fourth study suggest that in case of mild glaucoma, selective perimetry (M-FDT) is more suitable for detection and evaluation of structure-function relationship than conventional standard automated perimetry (SAP).

5. The results of our mass screening trials showed that the screening accuracy with combined use of GDx-VCC full test and M-FDT screening test is satisfactory even in mild glaucoma cases. In contrast, the screening performance of the SLT softwares used by us is rather poor, and their combination with SLP tests provides no further advantage in mass glaucoma screening.

6. The results of our long-term study suggest that in case of mild functional glaucoma progression, long term SLP measurement variability increases before the stage where any change of the polarimetric parameters becomes detectable. For detection of this increased variability GDx-ECC has an advantage over GDx-VCC.

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2. **Tóth M**, Vargha P, Holló G. Comparison of two cornea compensation methods in scanning laser polarimetry on eyes. Ophthalmic Res 2005;37(S1) S:42 EVER, Abstract No 303.

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5. Gunvant P, **Tóth M**, Chang F, Haine CL, Holló G. Diagnostic accuracy of polarimetry images with atypical retardance pattern in diagnosing glaucoma. Invest Ophthalmol Vis Sci 2007;48: ARVO E-Abstract 502.

6. **Tóth M**, Holló G. Glaucoma progression with GDx-VCC. Acta Ophthalmologica Scandinavica 2007; 85, S240 EVER

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1. Gedeon Richter Ph.D. award (2007)
2. 'Scholarship for Young Physicians' (International Glaucoma Symposium 2007)
3. 'MSD Award' for Hungarian ophthalmologists (2005): **I. prize**
4. 'MSD Award' for Hungarian ophthalmologists (2007): **II. prize**
5. National Scientific Students' Associations Conference (2001): **I. prize**

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