

**Structural evaluation of retinal ganglion cell damage:  
Fourier-domain optical coherence tomography and scanning  
laser polarimetry in glaucoma and acute optic neuritis**

PhD Thesis

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## INTRODUCTION

Glaucoma is caused by progressive apoptotic loss of the retinal ganglion cells, which results in irreversible damage of the retinal nerve fiber layer (RNFL) and the optic nerve head (ONH). It is one of the leading causes of severe visual impairment and blindness. Since most forms of glaucoma develop without clinical symptoms, it is not surprising that 50 % of glaucoma sufferers remain undetected, even in the developed countries. The prevalence of chronic open-angle glaucoma (OAG) is 1.5 to 3 % in Caucasians over 40 years of age, and increases up to approximately 7 % in advanced age. According to epidemiological estimations, there will be 79.6 million people with OAG and angle closure glaucoma (ACG) in 2020, and of them 74 % will suffer from OAG. In 2020, 5.9 million people with OAG and 5.3 million people with ACG will suffer from bilateral blindness.

In addition to the traditional diagnostic methods computerized structural investigation of the retinal nerve fiber thickness (RNFLT) has become an important part of modern glaucoma diagnostics. Optical coherence tomography (OCT) and scanning laser polarimetry (SLP) are such structural diagnostic methods. The new methods allow quantitative analysis of morphological alterations of the RNFL and the ONH, both of which are known to precede the development of clinically detectable visual field defects, in many cases.

In the last decade, time-domain optical coherence tomography (TD-OCT) became a standard method for glaucoma diagnostics. The recently developed Fourier-domain OCT (FD-OCT) technology, however, provides several technical innovations compared to TD-OCT technology. SLP with variable cornea compensation (GDx-VCC) is also a well-established imaging method for detection of glaucoma. The recently released enhanced cornea compensation software (GDx-ECC) was developed to improve image quality in case of atypical retardation pattern, which is difficult to classify with the GDx-VCC software.

The RTVue-100 Fourier-domain OCT (RTVue-100 OCT) is one of the new, commercially available FD-OCT instruments. The role of RTVue-100 OCT in glaucoma diagnostics was not evaluated in detail before my PhD period. Since in future both FD-OCT technology and SLP may be used to detect glaucoma, comparative evaluation of their diagnostic usefulness for real-life clinical decision making situations is of practical importance for ophthalmologists.

## OBJECTIVES

1. To investigate intrasession and intersession reproducibility of the RNFLT and ganglion cell complex (GCC) measurements in normal and glaucomatous eyes; and to investigate the role of several patient-related factors in measurement reproducibility: cooperation (previous patient experience in imaging examinations) and 3 factors with potential influence on the instrument's signal-to-noise ratio (glaucoma severity, pupil size and age of the patients).
2. To compare repeatability of RNFLT measurements made using the RTVue-100 OCT against repeatability of those made using GDx-VCC and GDx-ECC.
3. To evaluate the diagnostic accuracy of RNFLT, GCC and ONH measurements made with the RTVue-100 OCT to detect glaucoma in a Caucasian referral population.
4. To compare the diagnostic accuracy of the RTVue-100 OCT and the GDx-VCC and GDx-ECC to detect glaucoma in a Caucasian referral population.
5. To investigate whether early/intermediate age-related macular degeneration (AMD), untreated choroidal neovascularization (CNV) and CNV after intravitreal antiangiogenic treatment influence the different average and pattern-based GCC parameters and their software-provided classification for detection of glaucoma, in non-glaucomatous eyes.
6. To investigate the dynamics of RNFLT decrease and macular thinning, using RTVue-100 OCT and GDx-VCC/ECC in acute optic neuritis in multiple sclerosis; and to evaluate and compare the usefulness of the different devices, for the detection of thickness changes during the follow-up.

## METHODS

The research protocols were approved by the Institutional Review Board for Human Research of Semmelweis University, Budapest, Hungary. Informed consent was obtained from all participants before enrollment. The SPSS program package version 15.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis.

1. Between August and September 2008, 37 white individuals were enrolled in our first study. These comprised 14 healthy and ocular hypertensive (OHT) subjects; 11 patients with glaucoma of moderate severity; and 12 patients with advanced glaucoma. All were experienced in imaging examinations. In November 2008, 40 consecutive unselected participants in a free glaucoma screening trial with no previous experience in imaging tests were also enrolled in the study. The screening participants were classified as normal based on the results of a detailed clinical investigation. One eye per participant was selected for the investigation. Each participant completed the measurements for determination of intrasession variability and the measurements via dilated pupil on the same day. For determination of intersession reproducibility, 34 of the 37 hospital-based patients repeated the measurement series at 3 months after the initial examination. Five ONH scans and 5 GCC scans were obtained with the RTVue-100 OCT. During the image acquisition procedure, the head of the participant was moved from the headrest after each image was obtained and the head was repositioned for the following measurement. During the first visit, for the 37 hospital-based participants, pupil dilation was performed after the first measurement series. The p-values of  $<0.05$  were considered to indicate statistical significance.
2. The same 37 individuals, described in our first study participated in the current study. The same eyes and groups were used. Each participant completed the study within one day. Image acquisition was started with the RTVue-100 OCT by obtaining 5 high-quality ONH scans. Subsequently, 5 separate GDx measurements were taken in the VCC mode, followed by 5 separate GDx measurements in the ECC mode. For all procedures, the participant's head was moved from the headrest after each image was obtained, and repositioned for the following measurement. All eyes were classified with all the 3 methods investigated. The p-values of  $<0.01$  were considered to indicate statistical significance.

3. One randomly selected eye of each of 316 consecutive Caucasian individuals referred for detection of glaucoma in the Glaucoma Center of the Semmelweis University in Budapest, who all underwent RNFLT measurements made with the RTVue-100 OCT between 1<sup>st</sup> January and 30<sup>th</sup> November 2009, was enrolled in our third study. The patient population comprised 93 healthy subjects; 36 OHT subjects; 46 preperimetric glaucoma patients; and 111 perimetric glaucoma patients. The final clinical classification based on a detailed clinical investigation was made by the head of the glaucoma team. The RTVue-100 OCT examinations were not used for the clinical classification of the patients. The p-values of <0.05 were considered to indicate statistical significance.
  
4. In our fourth study one randomly selected eye of each of 177 consecutive Caucasian individuals referred for detection of glaucoma in the Glaucoma Center of the Semmelweis University in Budapest, who all underwent RNFLT measurements made with the RTVue-100 OCT, GDx-VCC and GDx-ECC between 1<sup>st</sup> January and 31<sup>st</sup> October 2009, was enrolled. The final clinical classification based on a detailed clinical investigation was made by the head of the glaucoma team. The RTVue-100 OCT and GDx-VCC/ECC examinations were not used for the clinical classification of the patients. The patient population comprised 50 healthy subjects; 28 OHT subjects; 33 preperimetric glaucoma patients; and 66 perimetric glaucoma patients. For each instrument, the software-provided automatic comparison with the corresponding age-corrected normative database was used for classification. The p-values of <0.01 were considered to indicate statistical significance.
  
5. Between April and June 2010, 79 consecutive Caucasian persons with no glaucoma and diabetes mellitus in their history were enrolled in our fifth study (one eye of each participant). Of these, 25 eyes had no AMD (age-matched healthy controls), 19 eyes suffered from early or intermediate AMD (drusen group) with mild RPE abnormalities but without other pathology, 16 eyes had untreated subfoveal CNV scheduled for intravitreal antiangiogenic treatment (CNV group), and 19 eyes had juxtafoveal or subfoveal CNV previously treated with at least one intravitreal antiangiogenic injection (all vascular endothelial growth factor [VEGF] blocking agents included; CNV-anti-VEGF group). All CNV membranes were caused by AMD. The RTVue-100 OCT examinations were not used for the clinical classification of the patients.

Image segmentation was reviewed for each completed GCC image, and the presence or absence of segmentation errors was recorded. The p-values of  $<0.05$  were considered to indicate statistical significance.

6. Between August 2008 and August 2009, 9 eyes of 7 individuals experiencing the first episode of acute optic neuritis as the first sign of multiple sclerosis were enrolled in the current study within 2 weeks from the onset of the acute visual deterioration. If a second episode of acute optic neuritis developed in the same eye, data collection was stopped. A control group (9 randomly selected healthy eyes of 9 healthy participants with age similar to that of the patients with multiple sclerosis) was followed for 12 to 19 months, to identify disease-independent measurement variability. The RTVue-100 OCT and GDx-VCC/ECC investigations were performed once a week during the first month, biweekly during the second and third months, once per month between the third and sixth months, and then once every third month until the end of follow-up in the optic neuritis group, and every sixth month in the control group. The mean defect value of the Octopus perimetry report was used to characterize the functional deficit. No statistical analysis was performed.

## RESULTS

1. Before pupil dilation, intrasession coefficient of variation (CV) for average RNFLT, for the different quadrants and for the GCC parameters varied between 1.95 % and 5.69 %. Intrasession ICC varied between 93.9 % and 99.0 %, and intratest variability varied between 3.11  $\mu\text{m}$  and 9.13  $\mu\text{m}$ . When the measurement series was repeated via dilated pupil, only average RNFLT and sector 9 (nasal) RNFLT increased to a statistically significant degree. The pupil dilation did not influence the intrasession CV, intratest variability and the signal strength index for the scans significantly. A significant trend was found for increased CV values in increasing disease severity for most RNFLT and GCC parameters. When the 40 screening participants and the normal subpopulation of the hospital-based patients were compared, no statistically significant difference was seen either for the mean thickness values or for the CV values. No influence of age was found on the measurement reproducibility in the healthy screening trial participants. Intratest variability and intrasession CV represented 79.1 % to 98.6 % and 77.1 % to 95.0 % of test-retest variability and intersession CV, respectively.
2. Mean RNFLT determined with RTVue-100 OCT was consistently different from that measured with either GDx technique. For all participants the CV calculated for the total 360° measuring circle was significantly smaller with RTVue-100 OCT than with GDx-ECC. For all participants the CV for the temporal quadrant was significantly smaller with RTVue-100 OCT than with GDx-ECC, and tended to be smaller than with GDx-VCC. For all participants, repeatability for the inferior quadrant was also significantly better with RTVue-100 OCT than with GDx-VCC. For the other quadrants no difference for measurement repeatability was seen between RTVue-100 OCT and GDx-VCC or GDx-ECC. No significant difference was seen for the narrower sectors when comparing figures calculated for RTVue-100 OCT and either GDx method.
3. When “borderline” and “outside normal limits” classifications were grouped together, specificity of the RTVue-100 OCT was high (94.6 - 100 %) for most RNFLT and GCC parameters, and moderate (72.0 - 76.3 %) for the ONH parameters, in all analyses. Sensitivity for detection of preperimetric glaucoma varied between 73.9 and 76.1 % for the ONH parameters, but only between 6.5 % and 37.0 % for the other parameters.

For detection of perimetric glaucoma, GCC Focal Loss of Variation (FLV) parameter showed the best sensitivity (92.8 %). Considering the whole population, the sensitivity values did not exceed 73.6 % for the ONH parameters, and 62.7 % for the other parameters. Positive likelihood ratio was higher than 10 for average, inferior and superior RNFLT, 12 of the 16 RNFLT sectors, and three of the four GCC parameters.

4. When the software-provided “borderline” and “outside normal limits” classifications were grouped together against “within normal limits” classification, the RTVue-100 OCT was more sensitive (65.7 %) for average RNFLT, than GDx-VCC (49.5 %). For the different localized nerve fiber bundle defect parameters, sensitivity of the RTVue-100 OCT was higher than of the GDx-VCC/ECC. Agreement of classification between the average RNFLT of the RTVue-100 OCT and the Nerve Fiber Indicator (NFI; the summary parameter indicating the probability of glaucoma on the GDx-VCC/ECC report) of the GDx-VCC/ECC was high. Of the 99 glaucoma eyes 73.7 % were identified with at least one technique. Of these 78.1 % were detected by all methods, 87.7 % by at least one of the GDx techniques and RTVue-100 OCT, 1.4 % by RTVue-100 OCT only, and 11.0 % by at least one of the GDx methods only. The kappa measure of agreement between RTVue-100 OCT average RNFLT and NFI was 0.84 for GDx-VCC and 0.85 for GDx-ECC.
5. Compared to the age-matched controls, no difference in any RNFLT and ONH parameter value and their classification was seen for any AMD group. Inner retinal image segmentation errors were detected in 8 of the 19 eyes with drusen, all 16 CNV eyes and 17 of the 19 eyes in the CNV-anti-VEGF group. Superior, inferior and average GCC values did not differ significantly between the healthy control eyes and the various AMD groups. In contrast, all pattern-based GCC parameters were significantly greater (more abnormal) in the CNV and CNV-anti-VEGF group than in the control eyes. For GCC FLV, a similar, significant difference was found between the control eyes and the drusen group. Of the four GCC parameters classified by the software, classification of superior, inferior and average GCC thickness did not differ significantly between the normal eyes and the different AMD groups for all but one comparison. In contrast, for GCC FLV, the only pattern-based GCC parameter classified by the instrument, the frequency of “borderline” and “outside normal limits” classifications was significantly greater in each AMD group than in the control group.

6. Using the RTVue-100 OCT, in the first month of follow-up, average RNFLT increased considerably in all eyes with diffuse optic disc edema in the acute phase. Temporal RNFLT measured with RTVue-100 OCT increased in 2 of these eyes. In contrast, peripapillary RNFLT measured both with GDx-VCC and GDx-ECC decreased in the acute phase of the disease, in all eyes. Temporal sector RNFLT measured with either GDx method showed a large variability during the follow-up. After the second month of follow-up, the initial increase of RTVue-100 OCT RNFLT disappeared, and peripapillary RNFLT decreased until a stable value was reached at 2 to 5 months. Average GCC decreased from the initial visit, for all eyes. Stability of average GCC appeared when stability of RNFLT appeared during the follow-up.

## CONCLUSIONS AND SUMMARY OF THE NEW RESULTS

1. We have shown that intra-, and intersession reproducibility of the RNFLT and GCC measurements with the RTVue-100 OCT is satisfactory for clinical diagnostic purposes both in healthy and glaucomatous eyes with moderate to severe damage. Pupil dilation, patient's age, and previous experience in imaging examinations have no clinically significant influence on the intrasession reproducibility.
2. We have shown that for the total 360° measuring circle and for the temporal quadrant, RTVue-100 OCT's repeatability is better than that with either GDx-VCC or GDx-ECC. For the other quadrants the repeatability was similar to that of GDx-VCC and GDx-ECC.
3. We have shown that in a Caucasian referral population the RNFLT and GCC parameters of the RTVue-100 OCT have moderate sensitive but high specificity, positive predictive value and PLR for detection of glaucoma. The optic disc parameters had lower diagnostic accuracy than the RNFLT and GCC parameters.
4. We have shown that a "borderline" or "outside normal limits" classification on the RTVue-100 OCT, GDx-VCC or GDx-ECC report represents clinically important and similarly specific information on RNFL damage. However, for localized nerve fiber bundle defects the sensitivity of RTVue-100 OCT is superior to that of either GDx technique.
5. We have shown that in non-glaucomatous eyes, AMD significantly influences the pattern-based inner macular thickness parameters of the RTVue-100 OCT and the software-provided classification of GCC FLV, for detection of glaucoma. Our results suggest that a detailed examination of the macula is necessary before a GCC parameter classified as "borderline" or "outside normal limits" by the instrument's software is considered a sign of glaucoma in the elderly.

6. We have shown that the change of RNFLT and macular thickness during the course of acute optic neuritis in multiple sclerosis strongly depends on the method used for the measurement. Inner macula thickness, measured with RTVue-100 OCT, was especially useful for the follow-up, since it was not influenced by initial disc edema and had consistently high image quality.

## LIST OF PUBLICATIONS

### *Peer-reviewed publications*

1. **Garas A**, Vargha P, Holló G. Reproducibility of retinal nerve fiber layer and macular thickness measurement with the RTVue-100 optical coherence tomograph. **Ophthalmology** 2010;117:738-746. **IF: 5.491**
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6. Holló G, Kóthy P, **Garas A**, Géczy Anna, Vargha P. Non-population-based glaucoma screening exercise in an osteoporosis patient organization: can elderly persons with advanced health awareness identify their risk for glaucoma? **J Glaucoma** 2011; early online publication, DOI: 10.1097/IJG.0b013e3182208a48. **IF: 1.744**

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### ***Book chapter***

1. Holló G, Tóth M, **Garas A**. A retinalis idegrostréteg vizsgálata. In: Németh J (szerk.), Szemészeti diagnosztikus képalkotó eljárások. Semmelweis Kiadó, Budapest, **2011**:137-145.

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1. **Garas A**, Papp A, Holló G. Influence of age-related macular degeneration on glaucoma measurements made with Fourier-domain optical coherence tomography. **Invest Ophthalmol Vis Sci** **2011**;52:E-abstract No:258.
2. Holló G, Kóthy P, **Garas A**, Géczy A. Non-population-based glaucoma screening exercise in an osteoporosis patient organization: Can elderly persons with advanced health awareness identify their risk for glaucoma? **Invest Ophthalmol Vis Sci** **2011**;52:E-abstract No:5046.
3. **Garas A**, Vargha P, Holló G. Agreement and repeatability of different disc-definition methods for optic nerve head and retinal nerve fiber layer measurements with the RTVue-100 optical coherence tomograph. **Invest Ophthalmol Vis Sci** **2010**; 51:E-abstract No:2754.
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5. Kim PY, Iftekharuddin KM, Gunvant P, Tóth M, **Garas A**, Holló G, Essock EA. Feature-based glaucomatous progression prediction using scanning laser polarimetry (SLP) data. In: **Medical Imaging 2011**; Computer-Aided Diagnosis, edited by Ronald M. Summers, Bram van Ginneken, Proceedings of SPIE Vol. 7963.
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10. **Garas A**, Papp A, Holló G. Fourier-domain optical coherence tomography in glaucoma: Influence of age-related macular degeneration on macular thickness measurements. **Ophthalmologia Hungarica 2011**;148:54.
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12. **Garas A**, Tóth M, Vargha P, Holló G. Reproducibility of retinal nerve fiber layer and macular thickness measurement with the RTVue-100 optical coherence tomography. **Ophthalmologia Hungarica 2010**;147:134.
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15. **Garas A**, Holló G. Evaluation of the repeatability of measurements of Fourier-domain optical coherence tomography for glaucoma diagnostics. **Ophthalmologia Hungarica 2009**;146:126-127.

## ***Presentations and posters***

1. **Garas A**, Papp A, Holló G. Influence of age-related macular degeneration on glaucoma measurements made with Fourier-domain optical coherence tomography (poster). **Annual Meeting of Association for Research in Vision and Ophthalmology (ARVO) 2011**, May 1-5; Fort Lauderdale, USA.
2. Holló G, Kóthy P, **Garas A**, Géczy A. Non-population-based glaucoma screening exercise in an osteoporosis patient organization: Can elderly persons with advanced health awareness identify their risk for glaucoma? (poster) **Annual Meeting of Association for Research in Vision and Ophthalmology (ARVO) 2011**, May 1-5; Fort Lauderdale, USA.
3. **Garas A**, Vargha P, Holló G. Agreement and repeatability of different disc-definition methods for optic nerve head and retinal nerve fiber layer measurements with the RTVue-100 optical coherence tomograph (poster). **Annual Meeting of Association for Research in Vision and Ophthalmology (ARVO) 2010**, May 2-6; Fort Lauderdale, USA.
4. **Garas A**, Tóth M, Vargha P, Holló G. Repeatability and influence of pupil dilation on RNFL measurement with the Optovue OCT in different severity of glaucoma (poster). **Annual Meeting of Association for Research in Vision and Ophthalmology (ARVO) 2009**, May 3-7; Fort Lauderdale, USA.
5. Holló G, **Garas A**, Vargha P. Repeatability and influence of pupil dilation on RNFL measurement with the Optovue OCT in different severity of glaucoma (poster). **World Glaucoma Congress (WGC) 2009**, July 8-11; Boston, USA.
6. **Garas A**, Kóthy P, Holló G. RTVue Fourier-domain OCT: reproducibility of RNFLT and macular thickness measurements. **Annual Meeting of European Association for Vision and Eye Research (EVER) 2009**, Sept. 30 - Oct. 3; Portoroz, Slovenia.

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8. Holló G, **Garas A**. Reproducibility of Measurement with the RTVue Fourier-Domain OCT in Glaucoma. **8th International Symposium on Ocular Pharmacology and Therapeutics (ISOPT) 2009**, Dec. 3-6; Rome, Italy.
9. **Garas A**, Papp A, Holló G. Influence of age-related macular degeneration on glaucoma measurements made with the RTVue Fourier-domain OCT. **Congress of the Austrian Ophthalmic Society (ÖOG) 2011**, June 2-4; Mayrhofen, Austria.
10. **Garas A**, Holló G. Influence of pupil dilation and patient-related factors on the repeatability of the RTVue-100 optical coherence tomography. **International Congress of Alpe Adria Association (AAA) 2009**, Nov. 13-14; Graz, Austria.
11. Kim PY, Iftekharuddin KM, Gunvant P, Tóth M, **Garas A**, Holló G, Essock EA. Feature-based glaucomatous progression prediction using scanning laser polarimetry data. **Society of Photographic Instrumentation Engineers (SPIE) 2011**, Feb. 12-17; Orlando, USA.
12. **Garas A**, Holló G. Evaluation of the repeatability of measurements of Fourier-domain optical coherence tomography for glaucoma diagnostics. **6th Congress of South-East European Ophthalmological Society (SEEOS) 2009**, June 25-27; Budapest, Hungary.
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19. **Garas A**. Fourier-domain optical coherence tomography and scanning laser polarimetry in glaucoma diagnostics: evaluation of repeatability. **PhD Scientific Days**, Semmelweis University **2009**, March 30-31; Budapest, Hungary.
20. **Garas A**, Tóth M, Vargha P, Holló G. Repeatability and influence of pupil dilation on RNFL measurement with the Optovue OCT in different severity of glaucoma (poster). **Night of Researchers 2009**, Sept. 25; Budapest, Hungary.