The assessment of pathological changes in retinal morphology using optical coherence tomography image segmentation

Short doctoral thesis

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1 Introduction

Optical coherence tomography (OCT) has evolved over the past two decades to become one of the most commonly used diagnostic tools in clinical ophthalmology. Its great advantage is that it allows the non-invasive and high-resolution imaging of retinal structure and also the thickness measurement of the retina and peripapillary retinal nerve fiber layer (ppRNFL). The measurement of retinal thickness facilitates diagnostic decisions in many retinal diseases and enables the more precise follow-up of retinal structure, in some cases providing a targeted and more cost-effective treatment.

The image acquisition speed of the latest, 4th generation OCT devices (spectral-domain, SD-OCT) is significantly faster than that of its previous generations. This way, information can be gained from more points of the retina in about the same amount of time which leads to more precise imaging of the examined area. The axial resolution of the 4th generation devices has also improved, being around 3 to 5 µm. Several OCT devices of various manufacturers are commercially available which show only small differences in the resolution and image acquisition speed; however, the size of the examined area, the additional diagnostic procedures and the method of the retinal boundary detection may show a wide variation among the devices. The built-in softwares of the SD-OCT devices define the outer border of the retina at the inner, middle or outer segment of the retinal pigment epithelium (RPE) or the Bruch's membrane and measure the thickness of the retina between this boundary and the vitreoretinal surface. Thus, the retinal thickness measurements of the devices can be significantly different which makes the comparison of results obtained in various studies more difficult.

The images obtained by OCT are giving a representation of the reflectivity of the imaged tissue, with the reflectance displayed on a grayscale or false color-coded. The results of histological studies have shown that the layers which can be seen on the OCT images in different colors due to their different optical densities correspond to the various layers of the retina. Based on the raw data of the A-scans, image processing softwares can differentiate the various layers of the retina, i.e. do a segmentation of retinal borders, using the reflectivity information of the structures. The segmentation of the retinal images enables the improved localization and more precise follow-up of the structural changes of the macula. Several stand-alone image processing applications were developed in the last few years and also the manufacturers of SD-OCT devices are making more and more efforts to provide the segmentation of retinal images to some extent.

It is of great importance that there is a marked difference between the reflectivity of the inner plexiform layer and nuclear layer, thus the boundary of these layers can be easily detected which facilitates the measurement of the thickness of the inner and outer retina. The RTVue OCT (Optovue, Inc., Fremont, CA, USA) uses this method to determine the thickness of the ganglion cell complex (GCC) comprising the ganglion cells and both their proximal and distal nerve fibers. An image processing software called OCT Retinal Image Analysis (OCTRIMA) was used in our studies which was developed by the co-workers of Bascom Palmer Eye Institute, University of Miami. The algorithm processes the data of the A-scans, and following the removal of speckle noise and applying a nonlinear complex diffusion filter it detects the boundaries of the layers by detecting the reflection peaks on the A-scans. Until this step, the procedure is automatic, after this, manual correction of the boundary detection is enabled. The software is able to calculate the thickness of the total retina along with the following intraretinal layers: RNFL, ganglion cell layer and inner plexiform layer complex (GCL+IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL) and the RPE. The layers can also be collapsed, and thus by merging the RNFL and GCL+IPL the thickness of the GCC can also be measured.

Multiple sclerosis (MS) is a chronic inflammatory disorder that affects the central nervous system and is characterized by demyelination and neuronal loss. In the most common, relapsing-remitting subtype of the disease, optic neuritis (ON) is a very common symptom and it is the first symptom of the disorder in 20% of the patients. Although the visual acuity of the patients recovers after the acute phase – usually to the initial value –, the continuous decrease of the thickness of the ppRNFL and GCC was observed in the first 3-6 months following ON. In the eyes previously affected with ON, decreased ppRNFL thickness and macular volume was found.

Interestingly, the above mentioned damage of the nerve fibers was not only observed in the eyes affected with ON but also in the non ON-affected eyes of MS patients; however, the decrease is less pronounced in these eyes than following ON. The precise mechanism of the RNFL loss in the fellow eyes is still unknown, it might be the result of subclinical inflammations or a continuous, slow axonal degeneration independent of inflammation. A strong correlation was observed between the neuronal loss and the EDSS score indicating the physical disability of the patients, thus the OCT-derived thickness values might serve as an objective marker of the disease progression in the future in MS.

2 Aims

2.1 Reproducibility of thickness measurements using OCTRIMA software

The aim of this study was to determine the reproducibility of the thickness measurements of OCTRIMA software both for the thickness of the intraretinal layers and the total retina. The effect of different observers, graders and visit days on the thickness measurements was also assessed.

2.2 Comparison of retinal thickness measurements by the OCTRIMA software and the RTVue OCT device

It is known, that the time-domain Stratus OCT and SD-OCT devices use different outer boundaries when measuring the thickness of the total retina. Furthermore, the boundary detection of the various SD-OCT devices also differ from each other. OCTRIMA software measures total retinal thickness as the distance between the ILM and the inner border of the OS/RPE junction which is similar to the outer boundary detection of RTVue. Both the built-in software of the RTVue OCT and the OCTRIMA software are able to measure the thickness the GCC. The purpose of this study was to compare the retinal thickness and GCC thickness measurements between RTVue SD-OCT and OCTRIMA segmentation analysis derived from Stratus OCT images.

2.3 Assessment of macular morphology in patients with multiple sclerosis

The purpose of our study was to assess macular morphology in patients with MS with or without ON in previous history and also to determine which OCT parameter has the greatest ability to discriminate the non ON-affected eyes of MS patients from healthy eyes, i.e. to detect neuronal damage in patients with MS.

3 Patients and Methods

3.1 Reproducibility of thickness measurements using OCTRIMA software

OCT examinations were performed on ten eyes of 5 healthy subjects (mean age 29 years, range, 25-34 years) in this study, using the „Macular thickness map" protocol of Stratus OCT. The raw data of the OCT images were processed with the OCTRIMA software. The mean thickness values for the RNFL, GCL+IPL, GCC, INL, OPL, ONL, RPE and the total retina were obtained for each eye.

On the first day of the study, the first observer performed two sets of OCT scans on each eye (assessment of intraobserver repeatability), then a second observer has also performed OCT examination on each eye (assessment of interobserver reproducibility). On the second day, each eye was scanned again by the first operator (assessment of intervisit reproducibility). All these OCT scans were processed by the same grader using OCTRIMA software. For the assessment of intergrader reproducibility, a second grader has also processed the first set of scans and also repeated the processing of the scans one week later for the assessment of intragrader repeatability.

The intraclass correlation coefficient (ICC) and the coefficient of reproducibility (CR) was calculated for each comparison, the latter was also expressed as the ratio of the mean thickness of the layers (CR%). The Wilcoxon test was performed to determine any statistically significant difference between the measurements. The level of significance was set at 5%.

3.2 Comparison of retinal thickness measurements by the OCTRIMA software and the RTVue OCT device

A total of 11 eyes from 11 elderly subjects (six right and 5 left eyes, nine women and two men) were included in this study. The mean patient age was 73 ± 7 years (range, 65-88 years). Taking into consideration that image quality could be affected by media opacities, only eyes which underwent uneventful phacoemulsification surgery with posterior chamber lens implantation 6–12 months prior to enrollment were included. OCT examinations were performed on each eye using the "Macular thickness map" protocol of Stratus OCT and the MM5 and MM6 protocols of RTVue OCT. Stratus OCT raw data were exported and analyzed using OCTRIMA. The mean and regional thickness values of the total retina (measured by

Stratus OCT, RTVue MM5, MM6, OCTRIMA) and the GCC (measured by RTVue MM6 and OCTRIMA) were recorded for each eye.

Total retinal thickness values were compared between the protocols by analysis of variance (ANOVA) followed by Dunnet post hoc test. Because the MM5 protocol uses a scan length of 5 mm, only the foveal and pericentral regional (R1–R5) data were used in the analyses. Paired t-test was performed to compare the thickness of the GCC measured by RTVue MM6 protocol and OCTRIMA. The correlations between the regional thickness values were assessed using Pearson correlation coefficients. Bland-Altman plots were constructed to assess agreement in mean thickness values. The systematic bias between RTVue OCT and OCTRIMA software was analyzed by fitting a linear regression for the difference of the two OCT techniques versus the average of the two thickness values. The level of significance was set at 5%.

3.3 Assessment of macular morphology in patients with multiple sclerosis

Thirty-nine patients with relapsing-remitting multiple sclerosis were consecutively recruited between October 2008 and June 2011 in this cross-sectional case-control study. The mean patient age was 34 ± 8 years, 69,2% of the patients were female. The mean disease duration was 6.5 ± 3.9 years. Five eyes were excluded from the study due to the presence of retinal disease (1 eye), acute ON (1 eye), low signal strength of the OCT image due to media opacity (1 eye) and amblyopia (2 eyes). Thirty-three randomly selected eyes of thirty-three age-and sex-matched controls were enrolled in the control group. The mean age of the control subjects was 34 ± 8 years, 69,7% of the volunteers were female.

All patients underwent an ophthalmic examination including best-corrected Snellen visual acuity, assessment of intraocular pressure, slit lamp biomicroscopy of the anterior segment and binocular ophthalmoscopy with pupil dilation. The same day, OCT examination was performed on each eye using a Stratus OCT device. ‘‘Fast RNFL map protocol’’ was performed and the mean overall and sectoral (superior, nasal, inferior and temporal) ppRNFL thickness values were recorded for each eye. To assess the thickness of the intraretinal layers of the macula, each eye was scanned using the ‘‘Macular thickness map’’ protocol; the raw data of the OCT images were processed using OCTRIMA software. The mean thickness values for the RNFL, GCL+IPL, GCC, INL, OPL, ONL, RPE and the total retina were obtained for each eye. The eyes of the MS patients were divided into two study groups for further analyses. The first group (ON+) was composed of 39 eyes which had ON at least 6 months prior to enrollment. The second group (ON-) was composed of 34 eyes which had no

history of ON. The diagnosis of optic neuritis was based on the patient's medical history and defined by clinical symptoms such as decreased visual acuity developing in few days, pain on eye movement, abnormal response on visual evoked potential examination confirming prechiasmal lesion and decrease in the critical flicker frequency. The control group consisted of 33 healthy eyes.

The correlation between the disease duration, age, EDSS score and the ppRNFL and macular intraretinal thickness parameters was calculated by linear correlation. All measured thickness values were compared among the groups using mixed model ANOVA. Receiver operating characteristic (ROC) curves were constructed to describe the ability of each parameter to discriminate between the eyes of MS patients not affected with ON and the eyes of the control group. The level of significance was set at 5%.

4 Results

4.1 Reproducibility of thickness measurements using OCTRIMA software

The coefficient of reproducibility was less than 5.3 µm for each comparison for the intraretinal layers and less than 12.8 µm for the total retina.

The CR in/as the ratio of the thickness of the layer (CR%) was less than 6.2% for each comparison for the layers except for the RPE, where it was between 6.7% and 30.7%. The CR% value for the total retina was less than 4.6% for each comparison.

The ICC values were between 0.25 and 1.00 for the comparisons. The highest ICC values were observed for the assessment of intragrader repeatability (0.81-1.00) and intergrader reproducibility (0.67-1.00). The lowest ICC values were observed for most of the intraretinal layers and the total retina for the interobserver reproducibility (0.25-0.96). The ICC values for the RNFL, GCL+IPL, ONL and the total retina were above 0.78. The ICC values calculated for the INL and OPL layers were ranging between 0.37 and 0.98. The lowest ICC values were observed for the RPE, ranging between 0.25 and 0.90.

4.2 Comparison of retinal thickness measurements by the OCTRIMA software and the RTVue OCT device

A strong correlation was observed for the regional retinal thickness values when comparing OCTRIMA to RTVue MM5 and MM6 protocols (Pearson correlation coefficients

range: 0.93–0.97 and 0.82–0.94, respectively). A slightly weaker correlation was obtained for the regional GCC measurements when comparing OCTRIMA to RTVue MM6 protocol (Pearson correlation coefficients range: 0.73–0.88).

ANOVA followed by Dunnett post hoc test showed no significant differences in regional thickness measurements between OCTRIMA and RTVue MM6 protocol except for ETDRS regions R6 and R7; while there was no significant difference between the thickness values measured by OCTRIMA and RTVue MM5 protocol in the ETDRS regions R1-R5. The mean difference in retinal thickness measurements between OCTRIMA, MM6 and MM5 protocols was <7 μm in each ETDRS region except for R1, R6, and R7. In the ETDRS region R1, retinal thickness was higher measured by the RTVue MM6 and MM5 protocols compared to OCTRIMA by 12 and 14 μm, respectively; however, these differences were not significant. OCTRIMA produced significantly thicker measurements for R6 and R7 compared to RTVue MM6, with 25 and 18 μm, respectively.

In the case of the GCC thickness measurements, the mean difference range was from 6 to 12 μm in the ETDRS regions. GCC measurements were significantly thicker for the MM6 protocol, except for R6 and R7 where OCTRIMA produced thicker results.

Based on the Bland-Altman analysis, RTVue MM6 protocol provides lower mean retinal thickness values than OCTRIMA software, while RTVue MM5 protocol measures higher retinal thickness than OCTRIMA. The difference is at the level of the axial resolution of SD-OCT in both cases and corresponds to <2% of mean retinal thickness, which is not clinically significant. There was no statistical difference for the mean thickness results obtained for the GCC. However, it should be noted that the slope computation gave a significant result for the Bland-Altman plot of the GCC measurements. This particular result indicates that the RTVue algorithm overestimates low thickness values and underestimates high GCC thickness values compared to the OCTRIMA algorithm. The average GCC thickness difference between the two methods was 3.74 μm (95% CI –1.98, +9.48) when the thickness was <100 micrometer and –2.66 μm (95% CI –6.47, +1.12) when the thickness was >100 micrometer.

4.3 Assessment of macular morphology in patients with multiple sclerosis

All eyes had a best-corrected Snellen visual acuity of 1.0. The strongest correlation was observed between the EDSS and the thickness of the GCL+IPL, GCC and mean overall ppRNFL (p= 0.007, p = 0.007 and p = 0.008, respectively; r =20.43 for all variables) while the correlations with the inferior ppRNFL, superior ppRNFL and the thickness of the RNFL

in the macula was weaker (p= 0.02, p= 0.05 and p = 0.05, respectively; r =20.38, r =20.33 and r =20.32, respectively). The remaining intraretinal layers and ppRNFL parameters showed no correlation with the EDSS. There was no correlation between any of the thickness values measured and either disease duration or age.

The mean overall ppRNFL thickness and the ppRNFL thickness in the superior, nasal, inferior and temporal quadrants was significantly decreased in the eyes of MS patients previously affected with ON compared to the non-affected eyes of MS patients. Each ppRNFL thickness parameter was significantly lower in the ON-affected eyes compared to controls except for the ppRNFL thickness in the nasal quadrant. However, the eyes not affected with ON showed significantly lower ppRNFL thickness values compared to the control group only in the temporal quadrant. The ppRNFL was thinner in the superior, nasal, inferior and temporal quadrants by 16%, 11%, 16% and 27%, respectively in the ON-affected eyes compared to controls and by 6%, 4%, 4% and 17%, respectively in the eyes without ON in medical history compared to controls.

The mean thickness of the total retina, RNFL, GCL+IPL and GCC showed a significant decrease in both the ON-affected and non-affected eyes of MS patients compared to the control group. Furthermore, the eyes previously affected with ON had significantly lower thickness values in these layers than the eyes not affected with ON. There was no statistically significant difference in the thickness of the outer retinal layers among the groups.

The strongest correlation was observed between the mean overall ppRNFL thickness and the thickness of the GCL+IPL and GCC in the macula (r = 0.76 and r = 0.75, respectively) while the correlation was weaker in the case of the total retina in the macula (r = 0.68). An average 10 mm loss of the average ppRNFL thickness was associated with 7.5 mm reduction in the total retinal thickness and also a 7.5 mm reduction in the thickness of the GCC, the latter resulting from a 5.3 mm reduction of the GCL+IPL and a 2.2 mm reduction of the RNFL.

The largest area under the curve (AUC) value for the discrimination between the non-affected eyes of MS patients and the eyes of the control group was obtained for the mean thickness of the GCC (0.892). The AUC values for the thickness data obtained by the built-in software of the Stratus OCT – namely the ppRNFL thickness in the temporal quadrant and the thickness of the total retina – were below that of the GCC, 0.745 and 0.709, respectively.

5 Conclusion

1. A high repeatability and reproducibility of the thickness measurements of OCTRIMA software was found in healthy eyes. The different observers, graders or the different days of the examinations did not have any effect on the thickness values.

2. A good correlation was found between the mean total retinal and GCC thickness measurements of RTVue SD-OCT and the OCTRIMA software. Our results have shown that both RTVue MM6 protocol and OCTRIMA software measured significantly higher total retinal thickness than Stratus OCT; the comparable difference between the measurements are due to the similar boundary detections of the softwares. The regional GCC values measured by RTVue MM6 and OCTRIMA software were different, thus care should be taken when comparing the regional thickness values.

3. A similar degree of correlation was observed between the mean overall peripapillary RNFL thickness and the thickness of the total retina in the macular area as it was described in previous studies. We were the first to observe that the correlation between the mean overall ppRNFL thickness and the thickness of the GCL+IPL and GCC in the macula is even stronger than the correlation with total retinal thickness.

4. In agreement with the results of previous studies, the most pronounced reduction in the thickness of the ppRNFL was found in the temporal quadrant in the eyes of patients with multiple sclerosis. This finding confirm that the fibers of the papillomacular bundle are the most susceptible to damage in multiple sclerosis.

5. By applying OCT image segmentation we were among the first groups to show that the thickness of the RNFL, GCL+IPL and consequently the GCC in the macula is decreased both in the ON-affected and non ON-affected eyes of patients with multiple sclerosis. The decrease correlates with the physical disability of the patients also in the non ON-affected eyes which is probably due to an axonal degeneration independent of inflammation.

6. We were the first the show that the OCT-derived value most capable of discriminating the non ON-affected eyes of multiple sclerosis patients from healthy eyes was the mean thickness of the GCC in the macula.

6 Publications

6.1 Papers of the author in the scope of the present work

1. Cabrera DeBuc D, Somfai GM, Ranganathan S, **Tátrai E**, Ferencz M, Puliafito CA. (2009) Reliability and reproducibility of macular segmentation using a custom-built OCT retinal image analysis software. J Biomed Opt, 14: 064023. **IF: 2.501**

2. Cabrera Debuc D, Salinas HM, Ranganathan S, **Tátrai E**, Gao W, Shen M, Wang J, Somfai GM, Puliafito CA. (2010) Improving image segmentation performance and quantitative analysis via a computer-aided grading methodology for OCT retinal image analysis. J Biomed Opt, 15: 046015. **IF: 3.188**

3. Somfai GM, **Tátrai E**, Ferencz M, DeBuc DC, Ranganathan S, Németh J. (2011) In vivo quantitative assessment of the macula by OCT image segmentation: the study of reproducibility and age-related macular changes. (Hungarian) Ophthalmologia Hungarica, 148: 11-16.

4. **Tátrai E**, Ranganathan S, Ferencz M, DeBuc DC, Somfai GM. (2011) Comparison of retinal thickness by Fourier-domain optical coherence tomography and OCT retinal image analysis software segmentation analysis derived from Stratus optical coherence tomography images. J Biomed Opt, 16: 056004. **IF: 3.188**

5. **Tátrai E**, Simó M, Iljicsov A, Németh J, Debuc DC, Somfai GM. (2012) In vivo evaluation of retinal neurodegeneration in patients with multiple sclerosis. PLoS One, 7: e30922. **IF: 4.411**

6. Tátrai E, Simó M, Iljicsov A, Németh J, DeBuc DC, Somfai GM. (2011) Assessment of macular morphology in patients with multiple sclerosis. (Hungarian) Ophthalmologia Hungarica, 148: 134-139.

6.2 Other papers outside the scope of the present work

1. Somfai GM, **Tátrai E**, Ferencz M, Puliafito CA, Cabrera DeBuc D. (2010) Retinal layer thickness changes in eyes with preserved visual acuity and diffuse diabetic macular edema on optical coherence tomography. Ophthalmic Surg Lasers Imaging, 41: 593-597. **IF: 0.715**
2. Iljicsov A, Barsi P, Várallyay Gy, **Tátrai E**, Somfai GM, Bereczki D, Rudas G, Simó M. (2010) Devic syndrome – case report, diagnostic and therapeutic principles. (Hungarian) Ideggyogy Sz, 63: 320–326. **IF: 0.236**
3. Somfai GM, **Tátrai E**, Simó M. The role of opical coherence tomography in the diagnosis of ocular neurodegenerative disorders. (Hungarian) In: Somlai J, Kovács T (ed.) Neuroophthalmologia. ISBN: 978-963-08-1357-0 Private publishing, Budapest, 2011: 93-99.
4. Vámos R[*], **Tátrai E**[*], Németh J, Holder GE, DeBuc DC, Somfai GM. (2011) The structure and function of the macula in patients with advanced retinitis pigmentosa. Invest Ophthalmol Vis Sci, 52: 8425-8432. **IF: 3.466**
5. Gao W, **Tátrai E**, Ölvedy V, Varga B, Laurik L, Somogyi A, Somfai GM, DeBuc DC. (2011) Investigation of changes in thickness and reflectivity from layered retinal structures of healthy and diabetic eyes with optical coherence tomography. J Biomed Sci Eng, 4: 657-665.
6. Nagy ZZ, Ecsedy M, Kovács I, Takács Á, **Tátrai E**, Somfai GM, DeBuc DC. (2012) Macular morphology assessed by OCT image segmentation after femtosecond laser assisted and standard cataract surgery. J Cataract Refract Surg, 38: 941-946. **IF: 2.942**