Clinical and laboratory assessment of diabetic microvascular complications

Short doctoral thesis

Dr. Gábor Márk Somfai

Semmelweis University
Academic Medical Sciences Programme, 2/10

Tutor:	Professor György Salacz, Ph.D
Co-Tutor:	Dr. Anikó Somogyi, D.Sc.
Official opponents:	Dr. Ágnes Kerényi, Ph.D. Dr. Éva Szökő, D.Sc.
Final exam chair:	Professor János Németh, D.Sc.
Final exam board members:	Professor Péter Kempler, D.Sc. Dr. Tibor Milibák, Ph.D.

Budapest
2008

Introduction

The increasing incidence of diabetes means a growing burden on the societies of developed countries because of the increasing need for disease treatment along with the treatment of complications. In developed countries, among them also in Hungary, the leading cause of legal blindness and visual impairment is diabetes.

There are approximately 200 million people living with diabetes in the world. In about 75% of patients with type 1 diabetes and 50% of those with type 2 diabetes it is expected that diabetic retinopathy (DRP) is present in at least some extent. It is estimated that in 2002 diabetic retinopathy accounted for about 5% of world blindness, representing almost 5 million blind.

The key to the proper prevention and treatment of DRP would be timely and accurate diagnostics, in which fundus imaging techniques are of important help.

Optical coherence tomography (OCT) is an imaging method capable of near-histological resolution scanning of retinal tissue. Its principle of operation is similar to that of ultrasound. The probing light travels through the eye reaching the retina and getting reflected from its various cellular layers with different optical densities. OCT enables the investigator to study the morphological alterations of the retina and objectively measure retinal thickness. A special software enables the operator to measure the thickness of the various cell layers distinguishable on the OCT image.

In diabetic maculopathy fluid rich in fat and cholesterol leaks out of damaged vessels. The condition may develop at any stage of diabetic retinopathy.

There are three different patterns of diabetic maculopathy as observed on OCT, according to Otani et al.:

- Diffuse macula edema (DME), with sponge-like retinal swelling;
- cystoid macula edema (CME), with well defined cysts in the macular area;
- And and serous retinal detachment (SMD), which may accompany any of the above macular edema forms.

The latter is caused by extracellular fluid accumulation which may originate from the disruption and necrosis of Müller cells after long-standing edema.

There are several factors contriubuting to the pathomechanism of diabetic macula edema. Damage to the endothelial cells of retinal vessels causes leakage from capillaries and microaneurysms of increased permeability, which is further enhanced by the damage of the blood-retina barrier. Vitreoretinal traction may also play an important role as it is observed

that patients with diabetes have a tighter vitreous adhesion and a higher prevalence of partial posterior vitreous detachment compared to healthy subjects. The swelling and later the disruption of Müller cells may also be of importance. Leukostasis develops partly because of oxidative stress and partly because of free radicals produced by leukocytes adherent to the endothelium.

The structural changes of the retina are caused by biochemical-physiological alterations, among which an increasing body of evidence is pointing towards the role of an enzyme, called semicarbazide-sensitive amine oxidase (SSAO). All SSAOs catalyze deamination of primary amines with the generation of ammonia and hydrogen peroxide. The catalyzed reaction of aminoacetone and methylamine may lead to the formation of toxic end products, methylglyoxal and formaldehyde, respectively. In both humans and rodents SSAO is found mainly in vascular muscle cells and adipocytes, but besides the above mentioned tissue-bound form there is a soluble isoform with unknown origin circulating in the plasma.

It is known that in humans soluble SSAO activity correlates with the severity of diabetic retinpathy and also the HbA1c levels. Both aldehydes are present in an elevated level in the urine of diabetic patients, while methylamine levels are decreased suggesting an increased activity of the enzyme.

Oxidative stress due to bad glucose metabolism may lead to the formation of reactive oxygen species resulting in tissue damage. The end products of these reactions are often aldehydes (like malondialdehyde) which raises the possibility that the reactions catalyzed by SSAO also leading to aldehyde formation may cause similar oxidative stress to that caused by oxygen or nitrogen radicals.

It has recently been revealed that the sequence of SSAO is identical with an inflammation-inducible adhesion protein, vascular adhesion protein-1 (VAP-1). Membrane-bound VAP-1 is regulating the adhesion and extravasation of leukocytes in inflammatory processes. It is spaeculated that soluble SSA/VAP-1 may play a direct/indirect role int he development of diabetic microangipathy.

Aims

The structural changes in diabetic maculopathy have been studied in depth, however, it is still difficult to detect the first changes in diabetic maculopathy. The value of central foveal thickness represents only one aspect of the macula, but does not reflect any overall changes involving the whole macular region. Analyzing the entire macula is useful for the early detection of subtle thickness changes, ie. the formation of focal edema which can usually not

be seen on angiography at this early stage of the disease. Topographic mapping provided by the built-in software of the OCT device may help qualitative analysis, while total macular volume assessment may be a candidate for the quantitative analysis of macular structural changes.

Serous macular detachments (SMD) occur in some cases of macular edema but the effect of SMD on visual function is unknown.

Thickness information of the various cellular layers of the retina may provide more detailed information about the pathological changes in retinal morphology. The quantification of such pathological changes could permit both a better detection and follow-up of layer injury as well as understanding of the diseased retina. Knowing the limitations of StratusOCT™ thickness measurements it would be crucial to know how imaging artifacts may affect the accuracy of the segmentation algorithm of our own design in order to avoid them in future studies.

Morphological changes of the retina are caused by underlying biochemical alterations caused by poor glucose homeostasis in diabetes. Chronic inflammation is observed to accompany diabetes, being an independent risk factor for its late complications. CRP itself exerts direct proinflammatory effects on human endothelial cells, inducing the expression of adhesion molecules. As SSAO is identical with a traditional inflammation-inducible adhesion molecule, VAP-1, it is reasonable to suppose that SSAO activity may be correlated with levels of CRP.

The formation of reactive oxygen species (ROS) leading to oxidative stress is also the consequence of bad metabolic control in diabetes, arising from hyperglycemia. Elevated soluble SSAO activity may be a source of ROS as described above.

Therefore we had the following aims in our work:

- To investigate the role of volumetric macular assessment in comparison with central foveal thickness data obtained by OCT in the early detection of retinal changes in patients with diabetes and preserved visual acuity,
- To show the relationship between visual acuity and retinal thickness/macular volume,
- To assess the relationship between visual acuity (VA), macular morphology, certain risk factors and the occurrence of SMD in patients with diabetic macular edema,
- To investigate how cellular layer thickness measurements extracted with our novel segmentation algorithm OCTRIMA (Optical Coherence Tomography Retinal IMage Analysis) are affected by potential artifacts related to OCT operator errors,

- And to suggest strategies for the recognition and avoidance of these pitfalls,
- To study the correlation between soluble and tissue-bound SSAO activity in streptozotocin-induced diabetic rats,
- To assess how different regimes of insulin treatment influence the activity of soluble and tissue-bound SSAO enzyme in STZ-induced diabetic rats,
- And finally, to investigate the levels of serum total antioxidant status (TAS) and CRP in diabetic rats to reveal the correlation between oxidative stress, subclinical inflammation and SSAO activity.

Patients and Methods

1. Optical coherence tomography examinations in patients with diabetes

In a retrospective observational study we analyzed data of 842 OCT examinations performed in eyes of patients with diabetes at our clinic between March 1. 2002. and October 31. 2003. Exclusion criteria were lack of cooperation, opaque media, any other macular pathology except for diabetic maculopathy, previous pars plana vitrectomy, and retinal photocoagulation within four months prior to the visit. We excluded eyes in which the software did not measure correctly (ie. did not find the vitreoretinal border or the RPE). The Institutional Review Board previously approved the study.

Optical coherence tomography was performed using a commercially available device (OCT Model 2000, Zeiss-Humphrey Instruments, San Leandro, CA, USA). Six radial scans were obtained in both eyes of all patients through a dilated pupil with a length of 6 mm centered on the foveola, using the automated ETDRS-macular map acquisition protocol of the device. Central retinal (foveal thickness (FT) and total macular volume (MV) were assessed by the built-in software of the scanner (Version A-2, Zeiss-Humphrey). Central foveal thickness represents the average thickness measured automatically by the software in the intersection of all six scans belonging to one scan session, while total macular volume represents the data provided by the software for the macular area with a diameter of 6 mm. In cases where FT measurements had an SD of more than 10% we measured foveal thickness manually by the software.

All patients previously underwent a complete ophthalmologic evaluation, including best corrected Snellen visual acuity, indirect ophthalmoscopy, posterior segment biomicroscopy with slit lamp and a fundus lens. The diagnosis of any stage of diabetic retinopathy (DRP) was made and recorded according to the ETDRS classification.

Altogether 389 OCT scan sessions were selected and two study arms were formed: we divided eyes with preserved (1.0) visual acuity (189 OCT scan sessions in 118 patients) from those with visual acuity worse than 1.0 (200 OCT scan sessions in 126 patients).

Retinal thickness data were compared with the normative database of the software and diffuse retinal thickening (DRT) was diagnosed if retinal thickness exceeded the limits of the normative database in any of the ETDRS macular map regions.

Eyes with preserved visual acuity

The following groups were formed according to retinal thickness and the presence of diabetic retinopathy: the DRT group (DRT, no retinopathy, n=17), the DRP group (DRT, retinopathy present, n=32), while those eyes of diabetic patients with normal thickness and no retinopathy were considered normal (DM group, n=140). As OCT normative data are based on a different population as that represented by Hungarian patients, we adjusted an age- and gender-matched Control group consisting of 38 eyes of 25 healthy volunteers who gave their informed consent to participate in the study. Participants in the Control group underwent the same ophthalmologic evaluation as the patients in order to exclude any possible pathology.

Eyes with decreased visual acuity (worse than 1.0)

Snellen visual acuities were transformed into logMAR units. The presence of diffuse and cystoid edema (DME and CME, respectively) and also SMD was recorded. Diabetes duration longer than 15 years, edema type (CME/DME) and accompanying hypertension were entered in a multiple regression analysis model to find risk factors for the presence of SMD.

Differences in FT and MV, diabetes duration, age and sex distribution were compared using the Kruskal-Wallis test one-way analysis of variance. In case of significant result Newman-Keuls post hoc analysis was applied.

In eyes with decreased visual acuity the correlation of FT and MV with logMAR visual acuity was assessed by linear regression analysis. In order to reveal the effect of SMD on visual acuity, the same regression was performed for the eyes with and without SMD and the significance of the regression coefficients was also assessed.

Diabetes duration longer than 15 years, edema type (CME/DME) and accompanying hypertension were analyzed by Fisher exact test in order to reveal risk factors for the presence of SMD. In case of a significant result the odds ration (OR) and relative risk (RR) were computed.

2. Evaluation of potential image acquisition pitfalls during optical coherence tomography and their influence on retinal image segmentation

Eight normal subjects (3 men and 5 women, age 29±5 years) with normal ocular examination and no history of any current ocular or systematic disease were recruited for this study. Informed consent was obtained from each subject after ethics approval was obtained from the Institutional Review Board. All subjects were treated in accordance with the tenets of the Declaration of Helsinki.

For imaging purposes the commercially available StratusOCT^{TM} unit (software version 4.0; Carl Zeiss Meditec, Inc., Dublin, CA) was used. A single operator collected all scans per subject in one session. Consequently, a total of 8 and 32 horizontal B-scans (7-mm long, horizontal line scan protocol) were obtained under optimal scan acquisition and specific error's operator related artifacts, respectively). The error's operator related artifacts included: 1) defocusing, 2) depolarization, 3) decentration, and 4) a combination of defocusing and depolarization. First, an optimal scan was acquired with fine adjustment of the focus and automatic optimization of polarization by the StratusOCT^{TM} software. The manufacturer-provided image assessment parameter (SS) was collected from the OCT data. Decentration was modeled by manual movement of the fixation point upwards on the StratusOCT^{TM} interface, resulting in a downward gaze. Thus, the macula would get approximately 2 optic disk diameters from its original position as seen on the CCD camera image of the device. The scan line was then manually adjusted to run through the center of the macula. Afterwards, macular fixation was repeated and the scan line readjusted to intersect the foveal center. Defocusing was achieved by turning the focus knob -4.0 Diopters. As a next step, image focusing was readjusted and depolarization was achieved by enhancing polarization by clicking ten times on the increasing button on the StratusOCT^{TM} interface. For the effect of both artifacts, the focus was then simultaneously turned -4.0 Diopters to achieve defocusing and depolarization.

The OCT raw data was exported to a compatible PC and analyzed using an automated computer algorithm of our own design (OCTRIMA) capable of segmenting the various cellular layers of the retina, as described above. A total of 7 layers [retinal nerve fiber layer (RNFL), ganglion cell layer (GCL) along with the inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), photoreceptor inner/outer segment junction (IS/OS); and the section including the retinal pigment epithelium (RPE) along with the choriocapillaries (ChCap) and choroid layer] were automatically extracted using the algorithm and their average thickness was calculated.

An accuracy measure ("segmentation accuracy measure", SAM) was introduced to evaluate the performance of the new segmentation algorithm.

For the statistical analyses of signal strength (SS) and average thickness, Friedman analysis of variance was used. In the case of a significant result, Dunnett post hoc analysis was performed in order to reveal difference from the optimal scan test results. If there was more than one significant difference from the optimal scan test, Newman-Keuls post-hoc analysis was also performed.

3. Assessment of SSAO activity in diabetic rats.

Five-week-old male Wistar rats (n=35, body weight 140-160g) were used in our studies. In 26 animals diabetes was induced by a single intraperitoneal (ip.) injection of STZ (60mg/kg) under pentobarbital anesthesia. Control group was injected with ip. saline (n=9). All animals were kept under the same conditions and followed for 3 weeks, which time period was determined as "short-term" for diabetes. Only STZ-treated animals with polyuria, polydipsia and plasma glucose concentrations above 15 mmol/l were considered diabetic and involved in the study. Treatment consisted of 2-4 IU subcutaneous insulin (Humulin U) administered once (at 8:00 AM) (DM 1×I, n=8) or twice (at 8:00 AM and 17:00 PM) daily (DM 2×I, n=9), depending on blood glucose levels obtained from the tail of the animals. A group of STZ-treated rats was left without treatment (DM, n=9). At the end of the study blood from the animals was collected in Vacutainer tubes without anticoagulant and centrifuged at 2500 *g* for 10 min. Aortas of all rats were prepared and stored separately from the sera at -80 °C pending assay.

In order to determine serum SSAO activity a radiometric procedure was adapted [23]. The radioenzymatic assay is based on the extraction of ^{14}C-benzaldehyde formed by the enzyme from ^{14}C-benzylamine. Benzylamine, chlorgyline and semicarbazide were purchased from Sigma-Aldrich (Germany). ^{14}C-benzylamine was obtained from Amersham International (United Kingdom; specific activity: 2.04 GBq/mmol). All other chemicals were of analytical grade.

The sera were preincubated in phosphate buffer ($5x10^{-2}$ mol/L; pH=7.4) with chlorgyline (10^{-4} mol/L) at room temperature for 20 min to ensure that any MAO activity, if present, was completely inactivated. The enzyme was then incubated in the presence of ^{14}C-benzylamine (5×10^{-4} mol/L. 2×10^{5} dpm, 0.1 μCi) in a final volume of 200 μl at 37°C for 40 min. The enzyme reaction was stopped by adding 200 μl of 2 mol/L citric acid. The oxidized products were extracted into 1 ml toluene/ethyl acetate (1:1 v/v) mixture, of which 600 μl was

transferred to a counting vials containing 5 ml Aquasafe fluid. Radioactivity was determined using a liquid scintillation counter (Beckman LS5000TA).

Rat aortas were carefully cleaned of all adjunct tissues in order to determine tissue-bound SSAO activity more exactly on protein base. Tissue samples were then homogenized and centrifuged at 800 rpm for 10 min. The supernatants were then centrifuged (14000 rpm for 40 min.) and the membrane fractions were incubated for 20 min in phosphate buffer ($5x10^{-2}$ mol/L; pH=7.4), in the presence of chlorgyline (10^{-4} mol/L). The enzyme was then incubated in the presence of ^{14}C-benzylamine (5×10^{-4} mol/L, 5×10^{4} dpm, 0.025 μCi) in a final volume of 220 μl at 37°C for 40 min. All other procedures were carried out as in the case of plasma.

Protein content of the samples was determined according to Lowry, using bovine serum albumin as standard. Serum SSAO activity was expressed in the following unit: pmol substrate oxidized /mg protein/h. Aorta SSAO activity was expressed in nmol substrate oxidized /mg protein/h.

Serum total antioxidant status was measured by a Randox HA3830 standard kit (Randox Laboratories, Ardmore, UK). The level of serum hsCRP was assessed by an immunoturbidimetric method (Erix Daytona Chemical Analyser, Randox Laboratories, Ardmore, UK).

Serum glucose concentrations were measured with an automatic analyzer (Hitachi 917, Boehringer-Mannheim Diagnostic Systems, Diagnostic kit). Serum fructosamine concentrations were measured using a reagent kit from Roche Diagnostics GmbH by Hitachi 907. All other chemicals were obtained from Sigma.

Results were assessed using ANOVA followed by Newman-Keuls multiple comparisons test. Linear correlation was performed and the Pearson correlation coefficient (r) was calculated for the pairwise relationship between se/aoSSAO, fructose amine, TAS and hsCRP levels as measured in all rats participating in the study.

For the analyses Statistica 6.0 for Windows software was used (Statsoft Inc, Tulsa OK, USA), significance was accepted at $p<0.05$ level.

Results

1. Optical coherence tomography examinations in patients with diabetes

In the eyes with preserved visual acuity central foveal thickness (FT) values were markedly higher in the DRP group than in all other groups. In the case of MV both DRT and

DRP groups had significantly higher values than the other two groups, and there was significant difference between thee DRT and the DRP groups, as well ($p<0.001$ in all cases).

In eyes with decreased visual acuity both FT and MV correlated with logMAR VA ($p<0.001$ both cases, $r=0.65$ for FT and $r=0.69$ for MV). Serous macular detachment was found in 28 eyes (14%). Linear regression was also significant separately in eyes with SMD as well as in eyes without SMD ($p<0.001$, all cases). Linear regression showed that eyes with SMD had significantly higher FT and MV values compared to eyes without SMD.

The presence of cystoid macular edema and diabetes duration longer than 15 years proved to be significant risk factors for SMD ($p=0.030$, OR=3.15, RR=2.69 and $p=0.047$, OR=2.66, RR=2.32), while the presence of hypertension had no effect.

2. Evaluation of potential image acquisition pitfalls during optical coherence tomography and their influence on retinal image segmentation

The SS score showed significant difference compared to the optimal acquisition protocol in all groups ($p<0.001$ in all cases), and all groups showed a significant difference compared to each other (data not shown) except for the comparison of decentration with defocus and depolarization ($p=0.19$ and $p=0.13$, respectively).

The SAM values were below 1.0 for the outer boundaries of the IPL, INL and OPL in the cases of all image acquisition pitfalls, the worst results were observed in the case of the depolarization-defocus artifact, which were 0.64, 0.56, 0.51, respectively. In all other layers the SAM was 1.0, inner and outer retina misidentification artifacts were not observed under any error's operator related artifact

A statistically significant difference in average thickness was found for depolarization-defocus in the case of GCL+IPL complex and ONL layer segmentation (71.61±13.40 vs. 51.43±22.68μm and 84.28±5.80 vs. 118.56±33.82μm, respectively, $p<0.05$ both cases). This particular result is in agreement with the lowest SAM values obtained for the outer boundaries of the IPL, INL and OPL.

3. The correlation between soluble and tissue-bound SSAO activity, oxidative stress and subclinical chronic inflammation in diabetic rats.

Serum fructose amine levels were markedly higher in the DM and DM 1×I groups ($p<0.001$ in both cases), while animals with semi-intensive insulin therapy (DM 2x1) showed only modest significant difference compared to Control ($p=0.04$). Nevertheless, Newman-Keuls post hoc analysis showed that both DM and DM 1×I groups had serum fructose amine levels significantly higher than the DM 2×I group ($p<0.01$ in both cases).

Serum SSAO activity had increased five-fold in the DM group and to a lesser degree, in the DM 1×I group, while there was no statistically significant difference between the DM 2×I and the control group. A reversed response in tissue-bound SSAO activity was assessed regarding metabolic state: under poor glycemic control a marked decrease in activity was seen in aorta, while semi-intensive treatment returned aortic enzyme activity closer to, though still significantly lower than control. Serum SSAO activities were in negative correlation with tissue-bound SSAO activities.

Serum TAS significantly decreased in the DM and DM 1×I groups compared to both the control and the DM 2×I groups, while the control group did not differ significantly from the DM 2×I group. High sensitivity C-reactive protein levels increased both in the DM and the DM 1×I groups, while semi-intensive insulin treatment reduced its concentration back to the level of the control group. Plasma TAS levels showed significant negative correlation with hsCRP and seSSAO, while hsCRP and seSSAO were in positive correlation.

A very strong positive correlation was found between serum fructose amine and seSSAO, and a strong negative correlation between serum TAS and hsCRP levels. Also strong correlation was revealed in the relationship between hsCRP and seSSAO. Tissue-bound SSAO had no significant relationship with serum TAS, and only weakly correlated with hsCRP. All other parameters have shown significant, though not strong correlation with each other.

Conclusion

The clinical and laboratory assessment of diabetic microvascular complications is essential not only in the early diagnosis and possible treatment of the condition, but also in the understanding of the disease process.

In our work we aimed to understand the morphological alterations of the macula in diabetic patients with and without visual acuity loss by the help of optical coherence tomography. We investigated what morphological data may give the earliest signs of edema formation and also aimed to describe the role of observed macular edema patterns in visual function.

Future studies will try to elucidate the role played by the various cellular layers of the retina in disease-related thickness changes, therefore we investigated the sensitivity of optical coherence tomography image segmentation for operator-related imaging errors.

Finally, as the observed retinal alterations in diabetes are caused by underlying pathophysiological changes, we focused on soluble and tissue-bound SSAO activity in an

experimental rat model. It has been previously described that elevated soluble levels of SSAO are correlated with diabetic retinopathy, therefore we investigated its relationship to insulin treatment and other biochemical markers of disease.

In summary, we have shown the following:

- We have shown the usefulness of high resolution retinal imaging by OCT to detect the earliest macular changes in diabetic retinopathy. Macular volume seems to be a more sensitive indicator of pathological changes than central foveal thickness.
- Visual acuity and retinal thickness/macular volume are strongly correlated in diabetic macular edema.
- Eyes with serous macular detachment have better visual acuity compared to eyes with similar central foveal thickness and macular volume but without SMD. The risk of developing SMD may be higher in the presence of cystoid macular edema and long diabetes duration (longer than 15 years).
- OCT image segmentation by OCTRIMA is sensitive to image artifacts related to OCT operator errors, significantly in the combined cases of decentration and depolarization.
- Careful fine-tuning of imaging settings is important in obtaining a best-possible scan, the value of SS might be of guidance with a target value of ≥ 6
- Soluble and aorta SSAO activities are inversely influenced by hyperglycema in diabetes: serum activity increases while that of the tissue-bound form decreases.
- Oxidative stress caused by diabetes triggers inflammatory processes, as there was strong correlation both between oxidative stress related to diabetes, soluble SSAO activity and the increase in the level of hsCRP. As an adhesion molecule, SSAO takes part in these processes, and may facilitate future atherosclerosis.
- Insulin treatment normalizes the above pathobiochemical changes. We suppose that our results give indirect evidence that soluble and tissue-bound SSAO activity are in negative interrelationship in diabetes. Also, our results are pointing at the importance of tight glucose control in patients with diabetes.

Papers of the author in the scope of the present work

1. **Somfai GM**, Somogyi A, Nemes J: The relationship between macular morphology and macular function in diabetic patients assessed by optical coherence tomography. (Hungarian) *Ophthalmologia Hungarica* 2004;141(1):45-54.

2. **Somfai GM**, Salacz Gy: Optical biopsy of the retina in vivo: on optical coherence tomography and its clinical use in ophthalmology. (Hungarian) *Orv Hetil* 2005;146(21):1157-63.

3. **Somfai GM,** Knippel B, Ruzicska E, Stadler K, Tóth M, Salacz G, Magyar K, Somogyi A: Soluble semicarbazide-sensitive amine oxidase (SSAO) activity is related to oxidative stress and subchronic inflammation in streptozotocin-induced diabetic rats. *Neurochem Int* 2006 Jun;48(8):746-52. **(IF: 3,159)**

4. **Somfai GM**, Knippel B, Ruzicska É, Stadler K, Tóth M, Salacz Gy, Magyar K, Somogyi A: Soluble semicarbazide-sensitive amine oxidase (SSAO) activity and its role in streptozotocin-induced diabetic rats treated with insulin. (Hungarian) *Metabolizmus,* 2006. Szept(3);4:187-192.

5. **Somfai GM**, Salinas HM, PuliafitoCA, Fernández DC: Evaluation of Potential Image Acquisition Pitfalls during Optical Coherence Tomography and their Influence on Retinal Image Segmentation. *J Biomed Opt.* 2007 July/August, 12: 041209. **(IF 2,870)**

Other papers

1. Nemes J, **Somfai GM**, Hargitai J: Structural changes studied by optical coherence tomography after photocoagulation for diabetic macular edema. (Hungarian) *Ophthalmologia Hungarica* 2004;141(1):41-44.
2. Ferencz M, **Somfai GM**, Récsán Zs: The effect of vitrectomy with peeling of the ILM for treatment of macular edema in diabetic retinopathy. (Hungarian) *Ophthalmologia Hungarica* 2004; 141(1):15-19.
3. Hargitai J, **Somfai GM**, Vámos R, Farkas A, Allikmets R: Foveolar thickness and macular volume changes in Stargardt macular dystrophy. (Hungarian) *Ophthalmologia Hungarica* 2004;141(1):35-39.
4. Ecsedy M, Récsán Zs, Nemes J, **Somfai GM**, Papp M: The role of optical coherence tomography and fluorescein angiography in age-related macular degeneration. (Hungarian) *Ophthalmologia Hungarica* 2004;141(1):55-60.
5. **Somfai GM**, Miháltz K, Tulassay E, Slavei K, Rigó J: The diagnosis of serous macular detachments in patients with severe preeclampsia with the use of optical coherence tomography. (Hungarian) *Magyar Nőorvosok Lapja* 2005;68:5-10.
6. Somogyi A, Herold M, Kocsis I, Nagy G, **Somfai GM**, Studinger P: Effect of vitamin E supplementation on the vitamin content of lipoprotein in young men and women. (Hungarian) *Orv Hetil* 2005;146(35):1813-1818.
7. Tönköl T, **Somfai GM**, Vámosi P: Cogenital pigmentepithelial hypertrophy. (Hungarian) *Ophthalmologia Hungarica* 2005;142(1):45-49.
8. Lengyel M, Debreczeni R, **Somfai GM**, Szigeti Zs, Rigó J Jr.: Post-partum eclampsia complicated by cortical blindness. (Hungarian) *Magyar Nőorvosok Lapja* 2005;68:341-344.

9. Hargitai J, Zernant J, **Somfai GM**, Vamos R, Farkas A, Salacz G, Allikmets R: Correlation of clinical and genetic findings in Stargardt disease patients from Hungary. *Invest Ophthalmol Vis Sci* 2005 Dec;46(12):4402-8. **(IF: 3,643)**
10. **Somfai GM**, Mihăltz K, Tulassay E, Slavei K, Rigó J Jr: Diagnosis of serous neuroretinal detachments of the macula in severe preeclamptic patients with optical coherence tomography. *Hypertens Pregnancy* 2006 Mar;25(1):11-20. **(IF: 0,871)**
11. Lesch B., Szabó V, Salacz Gy, Varsányi B, **Somfai GM**, Farkas Á: Up-to date and complex examination of X-linked juvenile retinoschisis. *Ophthalmologia Hungarica*, 2006; 143(3):165-174.
12. Ferencz M, **Somfai GM**, Farkas Á, Kovács I, Lesch B, Récsán Zs, Nemes J, Salacz Gy: Functional assessment of the possible toxicity of indocyanine green dye in macular hole surgery. *Am J Ophthalmol.* 2006 Nov;142:765-70. **(IF 2,468)**
13. Kovács I, Ferencz M, Nemes J, **Somfai GM**, Salacz Gy, Récsán Zs: Intraocular lens power calculations for combined cataract surgery, vitrectomy and peeling of epiretinal membranes for macular edema. *Acta Ophthalmol Scand, 2007 Feb;85(1):88-91*. **(IF: 1,458)**
14. Varsanyi B, **Somfai GM**, Lesch B, Vamos R, Farkas A.: Optical coherence tomography of the macula in congenital achromatopsia. *Invest Ophthalmol Vis Sci,* 2007 May;48(5):2249-53. **(IF: 3,766)**
15. **Somfai GM**, Ferencz M, Fiedler O, Varga T, Somogyi A, Németh J: Diabetic retinopathy at the beginning of the 21st century: prevention, diagnostics and therapy. (Hungarian) *Magyar Belorvosi Archivum (Journal of the Hungarian Society of Internal Medicine)* 2007;60(2):123-127

Presentations within the scope of the present work:

1. Nemes J, **Somfai GM**, Salacz Gy: A diabeteses maculopathia vizsgálata optikai koherens tomográffal (OCT). MSzT Éves Kongresszus, Miskolc, 2002.
2. Nemes J, **Somfai GM**, Somogyi A, Salacz Gy: A maculopathia diabetica vizsgálata optikai koherencia tomográfiás készülék segítségével. Fiatal Diabetológusok VI. Országos Találkozója. Gyula, 2003.
3. **Somfai GM**, Knippel B, Ruzicska É, Salacz Gy, Magyar K, Somogyi A: A szemikarbazid-szenzitív aminooxidáz enzim aktivitása cukorbeteg és hypertóniás patkányok aortagyökében. Fiatal Diabetológusok VI. Országos Találkozója. Gyula, 2003.
4. **Somfai GM**, Knippel B, Ruzsicska É, Salacz Gy, Magyar K, Somogyi A: A szemikarbazid-szenzitív aminooxidáz enzim aktivitása cukorbeteg és hypertóniás patkányok aortagyökében. Semmelweis Egyetem PhD Tudományos Napok. Budapest, 2003.
5. Nemes J, **Somfai GM**, Salacz Gy: Diabeteses maculopathia miatt végzett laser photocoagulatio után kialakult strukturális változások vizsgálata optikai koherencia tomográffal. MSzT Éves Kongresszus, Budapest, 2003.
6. **Somfai GM**, Knippel B, Ruzicska É, Magyar K, Salacz Gy, Somogyi A: A szérum és az aorta szemikarbazid-szenzitív aminooxidáz (SSAO) enzim aktivitása kontroll és streptozotocin diabeteses patkányokban. Magyar Szabadgyökkutató Társaság Kongresszusa, Sopron, 2003..
7. **Somfai GM**: Vajon a megnövekedett macula térfogat előfutára-e a korai macula elváltozásoknak diabetesben? Fiatal Diabetológusok VII. Országos Találkozója és Továbbképzése, Siófok, 2005.
8. **Somfai GM,** Nemes J, Somogyi A, Salacz Gy: Serosus macula leválás vizsgálata diabeteses maculopathiában optikai koherencia tomográfia segítségével. MSzT Éves Kongresszus, Szeged, 2005.

Posters, citable abstracts within the scope of the present work

1. **Somfai GM**, Kovácsvölgyi S, Somogyi A, Nemes J, Salacz Gy: Optikai Koherencia Tomográfiás vizsgálatok diabetes mellitusban. Semmelweis Egyetem PhD Tudományos Napok, Budapest, 2002. (*különdíj*)
2. **Somfai GM**, Salacz Gy: Az Optikai Koherens Tomográf szemészeti alkalmazásáról. MSzT Éves Kongresszus, Miskolc, 2002.
3. Nemes J, **Somfai GM**, Salacz Gy: Structural and functional changes studied by optical coherence tomography in diabetic maculopathy after laser photocoagulation for clinically significant macular edema. 3rd Euretina Congress, Hamburg, Germany, 2003. May 15-17.
4. Nemes J, **Somfai G**, Salacz G: Examination of diabetic macular edema with optical coherence tomography. 14th Congress of the European Society of Ophthalmology, Madrid, Spain, 2003. June 7-12.
5. Vámos R, Farkas Á, **Somfai G**, Hargitai J: Multifocal electroretinography and Optical Coherence Tomography in cases with Stargardt's macular dystrophy. 14th Congress of the European Society of Ophthalmology, Madrid, Spain, 2003. June 7-12.
6. **Somfai GM**, Nemes J, Somogyi A, Salacz G: Optical coherence tomography is a new and reliable method for the assessment of diabetic maculopathy. 18th International Diabetes Federation Congress, 24 - 29 August 2003, Paris, France (Diabetologia (2003) 46: [Suppl. 2.]: A393, 1145)
7. **Somfai GM**, Nemes J, Salacz Gy, Somogyi A: Cukorbetegek retinavastagsága és látásélessége közötti összefüggés vizsgálata optikai koherencia tomográfiás berendezéssel. Az MDT XVII. Kongresszusa, 2004. April 22-25. Tihany, Hungary.
8. **Somfai GM**, Nemes J, Ferencz M, Hargitai J, Somogyi A, Salacz G: Is increased macular volume the first sign of minor macular changes in diabetes? Ninth Annual Vision Research Conference – Neuroimaging the retina. April 29-30., 2005, Fort Lauderdale, USA.
9. **Somfai GM**, Nemes J, Ferencz M, Somogyi A., Salacz G: Volumetric measurements obtained by optical coherence tomography may provide a useful clinical tool for the early detection of macular changes in diabetes. 41st EASD Meeting, 12-15 September 2005, Athens, Greece (Diabetologia (2005) 48: [Suppl. 1.]: 989)
10. **Somfai GM**, Nemes J, Hargitai J, Somogyi A, Salacz G: Increased macular volume measured by optical coherence tomography may be the first sign of minor macular changes in diabetes. 15th SOE and 103rd DOG Joint Congress, 25-29 September 2005, Berlin, Germany.
11. **Somfai GM,** Denis CE, Salinas HM, Nagy ZZ, Németh J, Puliafito CA, Cabrera FD: Evaluation of Potential Pitfalls Related to Operator Errors During OCT Image Acquisition. ARVO 2006, April 30-May 4, 2006, Fort Lauderdale, USA., *Invest. Ophthalmol. Vis. Sci.* 2006;47(5):2631-.

Other posters, citable abstracts outside the scope of the present work:

1. Vamos R, Farkas A, Entz B, **Somfai G**, Salacz G: Functional and morphological evaluation of central areolar choroidal atrophy by multifocal electroretinography and ocular coherent tomography. EVER 2002, Alicante, Spain, 2002. October 2-5.
2. Vamos R, Hargitai J, **Somfai G**, Farkas A, Lesch B, Salacz G: Multifocal electroretinography and optical coherence tomography in cases with Stargardt's macular dystrophy. XXXXI Symposium of the International Society for Clinical Electrophysiology of Vision, Nagoya, Japan, 2003. April 1-5.

3. Hargitai J, **Somfai G**, Vámos R, Farkas Á, Ekesten B, Allikmets R, Gouras P, Salacz G: Foveal thickness and macular volume changes in Stargardt macular dystrophy. ARVO 2003, Fort Lauderdale, Florida, USA, 2003. May 4-9., *Invest. Ophthalmol. Vis. Sci.* 2003;44(5):544-.
4. **Somfai G**, Hargitai J, Vámos R, Salacz G: Optical Coherence Tomography in the study of Stargardt macular dystrophy. 14th Congress of the European Society of Ophthalmology, Madrid, Spain, 2003. June 7-12.
5. **Somfai GM**, Salacz G: Traumatic macular hole with a pseudooperculum in the fellow eye assessed by optical coherence tomography. 6th Meeting of the International Society on Ocular Trauma, September 13-16, 2003, Sopron, Hungary.
6. Gombos K, Kusnyerik Á, Sebestyén M, **Somfai GM**, Salacz G: Volume changes of the macula measured by OCT after phacoemulsification and PCL implantation. EVER, October 8-11, 2003, Alicante, Spain.
7. Vámos R, Hargitai J, **Somfai GM**, Farkas A: Functional and morphologic alterations in Stargardt's macular dystrophy. EVER, October 8-11, 2003, Alicante, Spain.
8. Hargitai J, Zernant J, **Somfai GM**, Vamos R, Farkas A, Nemes J, Salacz G, Allikmets R: Correlation of clinical and genetic findings in Stargardt disease patients from Hungary. ARVO 2004, April 24-29, 2004, Fort Lauderdale, USA, *Invest. Ophthalmol. Vis. Sci.* 2004;45(5):1763-.
9. Barabasi Z, **Somfai GM**, Farkas A, Nemes J, Ferencz M, Salacz G: Bilateral Optical Coherence Tomography abnormalities in patients with central serous chorioretinopathy. ARVO 2004, April 24-29, 2004, Fort Lauderdale, USA, *Invest. Ophthalmol. Vis. Sci.* 2004;45(5):2381-.
10. Varsanyi B, **Somfai GM**, Farkas A: Optical coherence tomography in patients with achromatopsia. EVER 2004, September 24-27, 2004, Algarve, Portugal.
11. **Somfai GM**, Mihăltz K, Tulassay E, Slavei K, Rigó J Jr.: Serous neuroretinal detachments of the macula diagnosed by optical coherence tomography in patients with severe preeclampsia. Conference of the International Society for the Study of Hypertension in Pregnancy, November 14-17, 2004, Vienna, Austria. (Young Investigator Award.)
12. **Somfai GM**, Kovács I, Mihátz K, Rigó J, Salacz G: Serous neuroretinal detachments of the macula diagnosed by optical coherence tomography in patients with severe preeclampsia. Ninth Annual Vision Research Conference – Neuroimaging the retina. April 29-30., 2005., Fort Lauderdale, USA.
13. **Somfai GM**, Barabási Z, Nemes J, Somogyi A., Salacz G: The Influence of Serous Macular Detachment on Visual Acuity in Diabetic Macular Edema. ARVO 2005, May 1-5, 2005, Fort Lauderdale, USA., *Invest. Ophthalmol. Vis. Sci.* 2005;46(5):394-.
14. Hargitai J, Zernant J, **Somfai GM**, Vámos R, Farkas A, Nemes J, Salacz G, Allikmets R: ABCR Mutations and Clinical Phenotype in Hungarian Patients With Stargardt Disease. ARVO 2005, May 1-5, 2005, Fort Lauderdale, USA., *Invest. Ophthalmol. Vis. Sci.* 2005;46(5):1638-.
15. Ferencz M, Kovács I, Lesch B,.Farkas A, **Somfai GM**, Barabási Z, Récsán Z, Nemes J, Fiedler O, Salacz G: Assessment of Possible Toxic Effect of Indocianine Dye Applied in Macular Hole Surgery. ARVO 2005, May 1-5, 2005, Fort Lauderdale, USA., *Invest. Ophthalmol. Vis. Sci.* 2005;46(5):4563-.
16. Lesch B, Szabó V, Vámos R, **Somfai GM**, Hargitai J, Varsányi B, Farkas Á: Optical coherence tomography and electroretinography findings in the cases of 4 Hungarian X-linked juvenile retinoschisis families. 15th SOE and 103rd DOG Joint Congress, 25-29 September 2005, Berlin, Germany.
17. Hargitai J, Zernant J, **Somfai GM**, Vámos R, Farkas Á, Salacz G, Allikmets R: Corelation of clinical and genetic findings in Stargardt disease patients from Hungary. 15th SOE and 103rd DOG Joint Congress, 25-29 September 2005, Berlin, Germany.

18. Ferencz M, Kovács I, Lesch B, Farkas Á, **Somfai GM**, Barabási Z, Récsán Z, Nemes J, Fiedler O, Salacz G: Assessment of possible toxic effect of indocyanine dye applied in macular hole surgery. 15th SOE and 103rd DOG Joint Congress, 25-29 September 2005, Berlin, Germany.
19. Varsányi B, **Somfai GM**, Lesch B, Farkas A: Comparison of OCT2 and OCT3 measurements in patients with achromatopsia. EVER 2005, 5-8 September, Vilamoura, Portugal
20. Cabrera FD, Salinas HM, **Somfai GM**, Puliafito CA: Potential use of combining the diffusuion equation with the free Schrödinger equation to improve Optical Coherence Tomography image analysis. APS Meeting, 14-18. March 2006, Baltimore, MD, USA
21. Vámos R, Lesch B, **Somfai GM**, Farkas A: Follow-up of young patients with retinitis pigmentosa by multifocal ERG: 382. Acta Ophthalmologica Scandinavica. 84 Supplement 239:49, October 2006.
22. Lesch B, Szabo V, Pámer Zs, Kenézy K, Salacz G, Papp A, **Somfai GM**, Varsanyi B, Vamos R, Hargitai J, Farkas A: 1 X-linked juvenile retinoschisis - age related macular changes by optical coherence tomography: 323. Acta Ophthalmologica Scandinavica. 84 Supplement 239:34, October 2006.
23. Varsányi B, Lesch B; **Somfai GM**, Farkas A: Age dependence of macular changes in congenital achromatopsia. Acta Ophthalmologica Scandinavica. 84 Supplement 239: October 2006.
24. **Somfai GM**, Fischer E, Ferencz M, Salacz Gy, Nagy Z: Differences in Retinal Thickness Measurements Obtained With the Fast and Slow Scanning Protocols of Stratus OCT. AAO Annual Meeting 2006, November 10-14, Las Vegas, USA
25. Ferencz M, Szepessy Z, **Somfai GM**, Kovács I, Entz B, Récsán Z, Salacz G: Posterior Capsule Opacification and Anatomical Success in Combined Operations for Macular Holes. ARVO 2007, May 6-10, 2007, Fort Lauderdale, USA., *Invest. Ophthalmol. Vis. Sci.* 2007;48(5):4127-.
26. Szepessy Z, **Somfai GM**, Fischer E, Ferencz M, Salacz G: The Assessment of Macular Volume and Thickness Symmetry in Healthy Eyes by Optical Coherence Tomography. ARVO 2007, May 6-10, 2007, Fort Lauderdale, USA., *Invest. Ophthalmol. Vis. Sci. 2007;48(5):156-.*
27. **Somfai GM**, Tatrai E, Ferencz M, Puliafito CA, Cabrera D: Quantifying Retinal Layer Thickness Changes in Eyes With Diabetic Diffuse Macular Edema Using Optical Coherence Tomography. ARVO 2007, May 6-10, 2007, Fort Lauderdale, USA., *Invest. Ophthalmol. Vis. Sci.* 2007;48(5):1426-
28. Ferencz M, Bokrétás G, **Somfai GM**, Kovács I, Fiedler O, Farkas A: The role of multifocal electroretinography in the prognosis of macular hole development. 7th EURETINA Congress, May 17-20, 2007, Monte Carlo
29. Tátrai E, **Somfai GM**, Ferencz M, Puliafito CA, Cabrera D: The assessment of retinal layer thickness and volume subsegmentation reproducibility of optical coherence tomography images. Annual meeting of the Hungarian Society of Ophthalmology, Debrecen, Hungary 2007. June 21-23.
30. Tátrai E, **Somfai GM**, Puliafito CA, Cabrera FD: The assessment of retinal layer thickness and volume segmentation reproducibility of optical coherence tomography images. Acta Ophthalmologica Scandinavica 85 (supplement 240): 3410. doi:10.1111/j.1600-0420.2007.01062_3410.x (EVER 2007, October 4-7, Portoroz, Slovenia)
31. **Somfai GM**, Tátrai E, Ferencz M, Puliafito CA, Cabrera FD: Retinal layer thickness changes in eyes with diffuse diabetic macular edema. Acta Ophthalmologica Scandinavica 85 (supplement 240): 3381. doi:10.1111/j.1600-0420.2007.01062_3381.x (EVER 2007, October 4-7, Portoroz, Slovenia)