Ganglion cell complex. Part II. GCC depending on the intraocular pressure in hypertensive glaucoma.

Jan Lestak[1,2,3]* , Elena Nutterova[1], Libuse Bartosova[1] and Pavel Rozsival[3]

1.JL Clinic, V Hurkach 1296/10, Prague, Czech Republic

2.Czech Technical University in Prague (Faculty of Biomedical Engineering), Czech Republic

3.Charles University (Faculty of Medicine in Hradec Kralove), Czech Republic

Abstract

Objective: The aim of the study was to determine whether there is a correlation between changes in visual fields, thickness of retinal ganglion cells and their axons in hypertensive and normotensive glaucoma and whether these findings differ in both diagnostic groups.

Patients and methods of examination: A total of 100 eyes of 50 people, of whom 30 were women (25-82 years) and 20 men (21-81 years) were included in the study. The first group included 26 patients with hypertensive glaucoma (HTG) (14 women and 12 men). The second group included 24 patients with normotensive glaucoma (NTG) (16 women and 8 men). All patients were subject to the assessment of average thickness of the ganglion cell complex (GCC) by SD-OCT RTvue -100, retinal nerve fibre layer thickness by GDx (parameter NFI) and the visual field by the fast threshold program of Medmont M 700 device.

Results: Correlation between studied parameters (GCC, NFI and visual field) was evaluated by correlation coefficients. For the HTG group, there was moderately strong correlation between visual field changes (pattern defect -PD) and GCC (r = -0.5). There was also moderately strong correlation between GCC and NFI (r = -0.64) and between NFI and PD (r = 0.67). Very weak correlation was found between GCC and the overall defect (OD) (r = 0.1). The above correlations were significant at the p = 0.05 significance level. For the NTG group, there was moderately strong correlation between visual field changes (PD) and GCC (r = -0.42). There was also moderately strong correlation between GCC and NFI (r = -0.45) and between NFI and PD (r = 0.36). Very

weak correlation was found between GCC and OD (r = -0.24). The above correlations were significant at the p = 0.05 significance level.

Conclusion: Examination of GCC and NFI showed moderately strong correlation for both NTG and HTG patients. As we expected, the correlation was not identical between the two diagnostic groups.

Keywords: GCC, NFI, visual field, hyper-tension glaucoma, normal-tension glaucoma

***Corresponding author:** Jan Lestak, MD, PhD, FEBO, MBA, LLA, DBA, FAOG
JL Clinic, V Hurkach 1296/10, 158 00 Prague 5, Czech Republic, E-mail: lestak@seznam.cz

Introduction

Glaucoma is currently defined as a disease with progressive loss of retinal ganglion cells and their axons, which leads to changes in the visual field with atrophy and excavation of the optic nerve. [12] This definition, which emphasises the damage of retinal ganglion cells before the damage of their axons, is not complete because it does not take into account the damage of ganglion cells in the subcortical and cortical centres of the brain. The current definition also does not distinguish between hyper-tension (HTG) and normal-tension (NTG) glaucoma.

Based on this definition, correlation close to 1 of changes in the visual field (they are the function of the entire visual pathway) and thickness of retinal ganglion cells and their axons cannot be expected. The correlation in NTG patients, where the changes occur mainly in the visual pathway, should be even smaller. [5]

The aim of the study was therefore to determine whether there is any correlation between changes in visual fields, thickness of retinal ganglion cells and their axons in hypertensive and normotensive glaucoma patients and whether these findings differ in both diagnostic groups.

Material and methods

The study was performed on two groups of patients. The HTG group consisted of 14 women (30-72 years) with the average age of 57 years and 12 men (25-75 years) with the average age of 57 years. The second – NTG – group consisted of 16 women (21-71 years) with the average age of 57 years and 8 men (32-65 years) with the average age of 55 years. All patients were subject to the assessment of average thickness of the ganglion cell complex (GCC) by SD-OCT RTvue -100 (Optovue Inc, CA USA), retinal nerve fibre layer thickness by GDx Carl Zeiss Meditec, Inc. (parameter NFI) and visual field by the fast threshold program of Medmont M 700 device (Medmont International Pty Ltd Australia). Tables 1 and 2.

Results

VA	Refraction	GCC		VF PD RE/LE	VF OD RE/LE	NFI RE/LE	Therapy
		RE	LE				
1	0	73.28	86.53	3.5/1.8	0.8/4.8	53/23	xalatan
1	-1	103.68	101.87	1.8/1.8	2.8/2.7	9.0/3.0	xalatan
1	-1.25	63.95	90.32	8.2/2.0	0.2/2.7	77/28	lumigan
1	0	86.32	85.1	2/2.2	2.8/2.8	26/23	xalacom
1	-1	89.94	91.49	1.9/1.1	1.0/1.21	16.0/10.0	monopost
1	0	96.04	85.52	2.9/2.7	0.1/-0.5	22/25	ganfort
1	0	81.56	101.9	8.8/8.4	1.0/4.1	98/10	luxfen
1	1	95.84	96.93	1.8/2.3	1.4/2.6	38/33	timolol
1	1	71.88	74.47	3.1/4.3	3.9/3.7	41/49	xalatan
1	-2	92.56	97.04	3.3/2.2	6.1/6.1	33/15	monopost
1	2	90.55	89.61	4.4/1.9	0.3/0	25/20	xalatan
1	0	71.88	74.47	3/4.3	3.9/3.7	41/49	xalatan
1	0	102	99.3	2.1/1.7	3.2/2.9	28/29	xalatan
1	-2.5	101	119	3.5/3	0/0	28/20	xalacom
1	0	91.25	87.36	2.1/1.9	4.8/3.7	14.0/11.0	xalatan
1	0	94.79	97.61	1.8/1.8	3.3/26	22/17	xalatan
1	2	95.12	91.01	1.8/2.6	3.9/3.3	15/2.0	monopost
1	2	94.6	89.8	3/2.1	4.2/3.8	24/34	monopost
1	0	99.45	100.1	2.4/1.7	2.9/3.3	19/7.0	xalatan

1	-2.5	81.92	81.88	1.4/1.7	3.5/3.4	17.0/12.0	xalatan
1	-1.75	90.4	92.03	2.2/2.9	2.8/3.7	29/28	xalatan
1	0.75	96.97	92.87	1.8/2.4	3.7/4.7	53.0/29.0	luxfen
1	0	65.18	70.48	13.9/20.2	0/0	71/76	xalatan+oftidor
1	-1	102.84	97.19	2.1/2	3.6/2.7	24/26	monopost
1	0	102	98.56	2.3/2.4	3.1/2.9	11.0/10.0	monopost
1	0	101	106.1	1/1.6	2.7/3.2	19/21	ganfort

Table 1. Summary table for patients with hypertensive glaucoma. VA-visual acuity. GGC-ganglion cells complex (um). VF PD-pattern defect of the visual field. VF OD-overall defect of the visual field. RE-right eye. LE-left eye.

Sex/Age	VA	Refraction	GCC		VF PD RE/LE	VF OD RE/LE	NFI RE/LE
			RE	LE			
F/59	1	0	93.75	91.12	1.4/1.6	1.8/1.8	31/32
F/21	1	-1.5	90.45	90.15	2.4/2.1	1.7/1.4	41/28
F/54	1	1.5	102.13	101.99	1.7/2.5	3.6/3.1	16/22
F/51	1	-1	91.88	87.17	1.3/1.4	4.4/4	15/16
F/58	1	1	95.05	97.81	1.4/2.3	2/1.5	21.0/29.0
F/66	1	1	88.61	84.05	2.3/3	4/2.8	24/14
F/56	1	0	90.51	90.69	1.7/1.9	2.1/0.3	26/29
F/47	1	-1	72	76	9.6/2.6	3.4/3.9	68/24
F/71	1	0.5	75	63.7	3.6/8.3	4.1/3.7	40/29
F/62	1	-0.75	99.6	100.84	2.4/4.1	3.9/3.8	34/20
F/61	1	-5.00	82.24	91.192	1.5/1.6	4.6/4.6	36/36
F/59	1	1	96.3	101.1	2/2.8	1.8/2.0	15/2
F/68	1	3	89.56	98.14	2.2/2.3	3.6/3.1	37/14
F/64	1	2	94.37	91.4	1.9/1.9	3.6/3.7	20/20
F/49	1	0	103	101.16	1.9/2.0	3.5/3.5	21.0/10.0
F/66	1	-3	84.64	78.43	11.5/21.8	2.2/2.1	23/38

M/65	1	0	83.95	77.75	2/2.6	3.7/3.3	25/26
M/48	1	1.5	88.69	87.02	3.1/3.1	3.8/3.3	30/30
M/47	1	0	97.95	99.19	1.7/1.3	3.3/3	22/26
M/32	1	-2	96.15	95	2.5/2.8	3.1/2.2	19/19
M/44	1	0	89.6	90.18	1.9/2.1	2.4/2.3	5.0/13
M/57	1	1	77.05	76.51	2.6/2.6	3.7/4.4	21/24
M/51	1	1	95.06	95.02	1.5/2.3	3.1/1.4	20/21
M/55	1	-3	83.94	85.16	1.6/1.6	3.2/3.4	25/19

Table 2. Summary table for patients with normotensive glaucoma. GGC-ganglion cells complex (um). VF PD-pattern defect of the visual field. VF OD-overall defect of the visual field. RE-right eye. LE-left eye.

Correlation between the studied parameters (GCC, NFI, PD and OD of the visual field) was assessed using correlation coefficients arranged in the correlation matrix. According to the value of the correlation coefficient, the following categories could be distinguished: weak ($|r| < 0.3$), moderate ($0.3 < |r| < 0.8$) and strong ($|r| > 0.8$) linear relationship (correlation).

For the HTG group, there was moderately strong correlation between visual field changes (PD) and GCC ($r = -0.5$). There was also moderately strong correlation between NFI and GCC ($r = -0.64$) and between NFI and PD ($r = 0.67$). Very weak correlation was found between GCC and OD ($r = 0.1$). The above correlations were significant at the $p = 0.05$ significance level. Figures 1 and 2.

For the NTG group, there was moderately strong correlation between visual field changes (PD) and GCC ($r = -0.42$). There was also moderately strong correlation between NFI and GCC ($r = -0.45$) and between NFI and PD ($r = 0.36$). Very weak correlation was found between GCC and OD ($r = -0.24$). The above correlations were significant at the $p = 0.05$ significance level. Figures 3 and 4.

We also studied partial correlation coefficients, which showed that when the role of age is compensated for, simple and partial correlation coefficients show no differences.
The problem of apparent correlation due to the impact of age was therefore not present in the data.

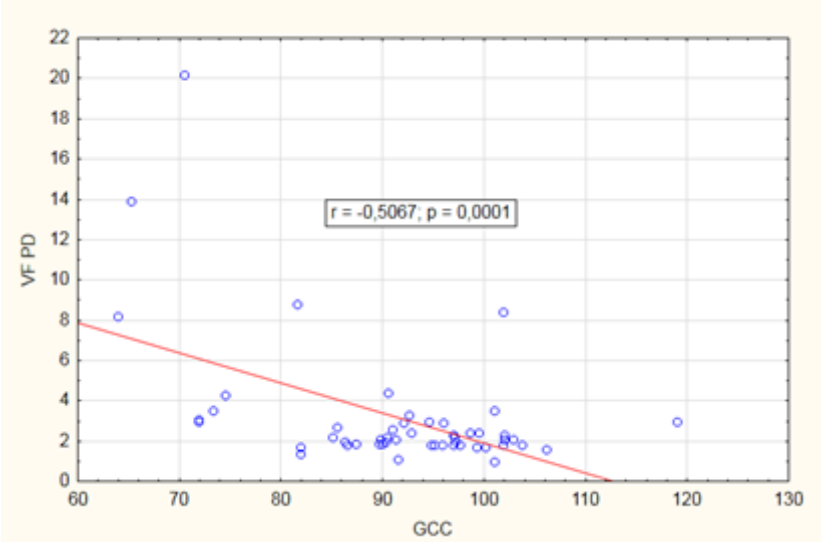

Figure 1. Scatter graph showing correlation between VF PD and GCC thickness in HTG patients

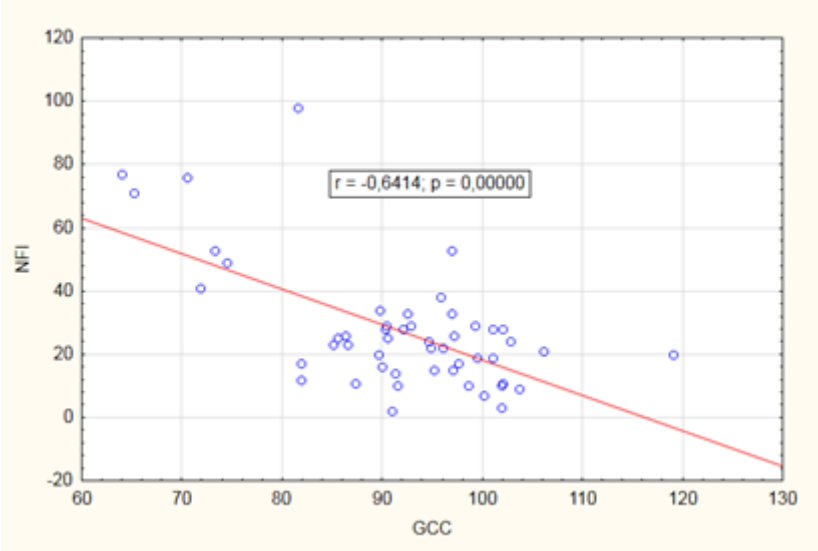

Figure 2. Scatter graph showing correlation between NFI and GCC thickness in HTG patients

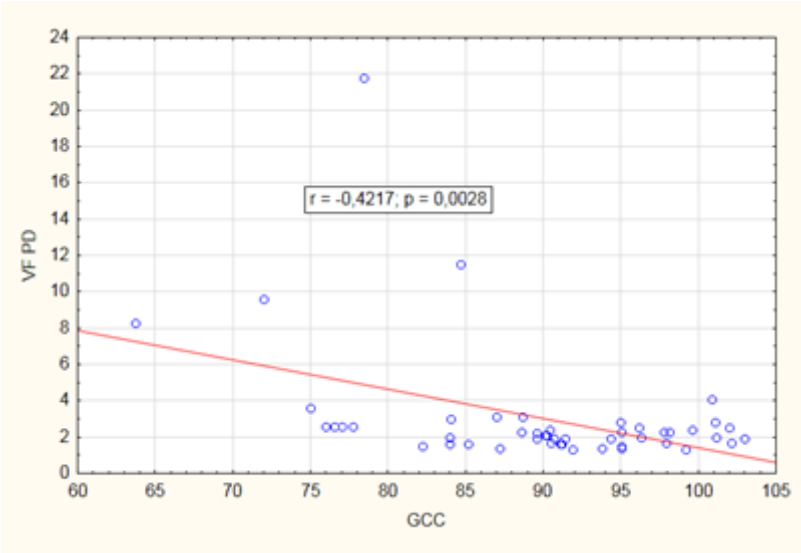

Figure 3. Scatter graph showing correlation between PD VF and GCC thickness in NTG patients

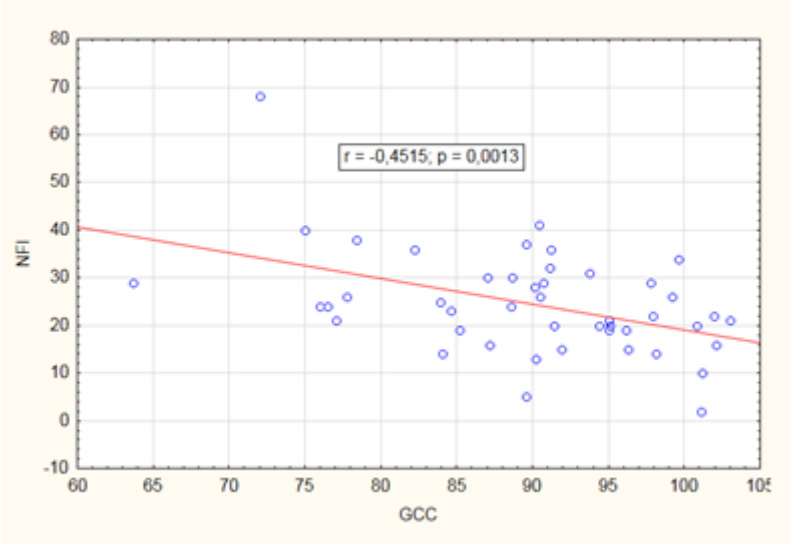

Figure 3. Scatter graph showing correlation between NFI and GCC thickness in NTG patients

Discussion

In 1987, we previously had doubts about damage affecting only the axons of retinal ganglion cells in HTG when we simultaneously measured the Pattern Electroretinogram (PERG) and the Pattern Visual Evoked Potential (PVEP). We examined the healthy eyes of a 20-year-old man with an Intraocular Pressure (IOP) of 15 mmHg. Subsequently, we increased the IOP to 40 mmHg and repeated the examination. To our surprise, neurotransmission at the level of ganglion cells became blocked, while the PVEP changed only slightly. Based on our findings, we concluded that initial changes will be at the level of the ganglion cells and not their axons and that not only the retinal ganglion cell layer but the entire visual pathway will be included in the process of elevated IOP. [6]

Similar results at the retinal level were also obtained by Crowston *et al*., who described a model of acute intraocular pressure (IOP) elevation in the mouse eye that induced reversible loss of inner retinal function associated with oxidative stress, glial cell activation and minimal loss of retinal ganglion cell (RGC) number. Young healthy mouse eyes recovered inner retinal function within 7 days, but more persistent functional loss was seen in older mice. They believe that systematic investigation into the characteristics and determinants of RGC recovery following an IOP challenge will shed light on the processes that govern RGC vulnerability in the early stages of glaucoma. [1]

The morphological basis of these findings, in terms of changes of ganglion cells and their axons, can be obtained from the works of Hyashi *et al.,* Weber *et al*., Pavlidis *et al.,* Shou *et al*., Kim *et al*. and others. [2,3,7,9]

Hayashi *et al*. hypothesised that a reduction of dendrite projections and the dendrite tree itself is not sufficient to induce apoptosis of retinal ganglion cells. Dendrite projections are extremely variable elements, providing structural mechanism for synaptic plasticity. [2]

According to Weber *et al*., changes observed within the ganglion cells are mutually linked and dendritic changes are steps to ensure cell survival. The authors assume that the first change necessary for the survival of retinal ganglion cells is to avoid excessive

use of the most distal dendrites to conserve energy and maintain homeostasis at the level of the cell body. [11]

Shou *et al*. found in retinal ganglion cells that cell density, cell body size, dendrite radius, the length of the protrusions and the number of dendrite bifurcations decrease significantly in glaucoma eyes compared to controls. Dendrite structural changes and corresponding physiological deficiency of retinal ganglion cells appeared before cell death. [9]

Similar conclusions were reached by Kim *et al*., who, after cauterisation of episcleral veins and prior retrograde labelling of retinal ganglion cells by Fluoro-Gold (Fluorochrome, Denver, USA), investigated the number of cells that died of apoptosis using the TUNEL method. [3]

Soto *et al*., in their work on DBA/2 mice models, found that degeneration of retinal ganglion cells in glaucoma comprises two separate stages. The first stage involves atrophy of ganglion cells and the second stage involves the damage of their axons. Retrolaminar degeneration of axons occurs before the degeneration of intraretinal parts of cells. [10]

Similar conclusions in human glaucoma were reached by Roddick *et al*., who demonstrated a reduction in the number of dendritic bifurcations in both major classes of retinal ganglion cells — parvocellular and magnocellular. [8]

A histological study of the final stages of glaucoma in humans was also presented by Pavlidis *et al*. In the advanced stages of the disease, there was almost no retinal ganglion cell layer, which degenerated and could not even be visualised by staining. The remaining ganglion cells were considered glaucoma resistant. These cells showed drastic morphological alterations, abnormal axonal beading, cell bodies were smaller and their dendrite branches were also shorter. [7]

The above overview was provided intentionally, because it points to the shrinkage of ganglion cells, reduction of their dendritic tree and dendrite length before cell death. This is important information because, after the increase of intraocular pressure, the thickness of the retinal ganglion cell layer is reduced, while the layer of cell axons should remain intact at least temporarily. Therefore, in HTG patients, high correlation between the parameters under consideration cannot be expected. In our group, the correlation between GCC and NFI (r = -0.64) was moderately strong. In NTG patients,

the correlation between NFI and GCC was also moderately strong, but lower when compared to HTG (r = -0.45).
Similar conclusions were reached by Kim *et al*., who found that GCC loss in the NTG group was more localised compared to the diffuse GCC loss in the HTG group. [4]

Similar morphological studies are not available for NTG patients. However, our electrophysiological work shows that in NTG patients, the damage affects mainly visual pathways and the retinal ganglion cells remain relatively intact. [5]

There is currently a search for diagnostic methods to allow HTG diagnosis in the earliest stages. One of these is GCC layer thickness measurement. It should be emphasised that, before the ganglion cell dies, it "collapses". If this condition persists long enough to deplete the energy supply that protects the ganglion cells, the cells die of apoptosis. Collapsed cells may then also result in the change of the thickness of GCC. This should be taken into account because, depending on the IOP, the thickness of this layer can also change. This is evidenced by some of our other works.

Conclusion

Examination of GCC and NFI showed moderate correlation in both NTG and HTG patients. As we expected, the correlation was not identical in both diagnostic groups.

The study protocol was approved by the local Ethics Committee and the study was performed in accordance with Good Clinical Practice and the Declaration of Helsinki.

Acknowledgements
Grant support, research funding and proprietary interest is none, and presentation at meeting is also none.

Conflict of interest statement
The authors state that there are no conflicts of interest regarding the publication of this article.

References

1.Crowston JG, Kong YX, Trounce IA, Dang TM, Fahy ET, Bui BV, Morrison JC, Chrysostomou V. An acute intraocular pressure challenge to assess retinal ganglion cell injury and recovery in the mouse. Exp Eye Res 2015; Mar 7. pii: S0014-4835(15)00088-3. doi: 10.1016/j.exer.2015.03.006

2.Hayashi, K., Ishikawa, R., Ye, L.H, a jiní:. Modulatory role of drebrin on the cytoskeleton within dendritic spines in the rat cerebral cortex. J Neurosci 1996;6:7161–7170

3.Kim, D.H., Kim, H.S., Ahn, M.D., Chun, M.H.: Ganglion Cell Death in Rat Retina by Persistent Intraocular Pressure Elevation. Korean J Ophthalmol 2004;18:15-22

4.Kim NR, Hong S, Kim JH, Rho SS, Seong GJ, Kim CY. Comparison of macular ganglion cell complex thickness by Fourier-doman OCT in normal tension glaucoma and primary open-angle glaucoma. J Glaucoma 2013;22:133-139

5.Lešták, J., Nutterová, E., Pitrová, Š., Krejčová, H., Bartošová, L., Forgáčová, V.: High tension versus normal tension glaucoma. A comparison of structural and functional examinations. J Clinic Exp Ophthalmol 2012, S:5, http://dx.doi.org/10.4172/2155-9570.S5-006. ISSN: 2155-9570

6.Lešták J, Tintěra J, Kynčl M, Svatá Z. Rozsíval P: High Tension Glaucoma and Normal Tension Glaucoma in Brain MRI. J Clin Exp Ophthalmol 2013, 4:4 http://dx.doi.org/10.4172/2155-9570.1000291

7.Pavlidis M, Stupp T, Naskar R. a jiní: Retinal Ganglion Cells Resistant to Advanced Glaucoma: A Postmortem Study of Human Retinas with the Carbocyanine Dye DiI. Inves Ophthalmol Vis Sci 2003;44:5196-5205

8.Rodieck, R.W., Binmoeller, K.F., Dineen, J.: Parasol and midget ganglion cells of the human retina. J.Comp.Neurol. 1985; 233:115–132

9.Shou, T., Liu, J., Wang, W. a jiní: Differential Dendritic Shrinkage of α and ß Retinal Ganglion Cells in Cats with Chronic Glaucoma. Invest Ophthalmol Vis Sci 2003; 44:3005-3010

10.Soto, I., Oglesby, E., Buckingham, B.P. a jiní: Retinal Ganglion Cells Downregulate Gene Expression and Lose Their Axons within the Optic Nerve Head in a Mouse Glaucoma Model. J Neurosci 2008;28:548-561

11.Weber, A.J., Kaufman, P.L., Hubbard, W.C.: Morphology of single ganglion cells in the glaucomatous primate retina. Invest Ophthalmol Vis Sci 1998;39:2304–2320

12.Weinreb RN, Khaw PT. Primary open-angle glaucoma. Lancet 2004;365:1711-1720