SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

The significance of ocular vascular disorders and the modern management of macular edema due to retinal vein occlusion

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Introduction and review of literature

Vascular disorders of the eye include a number of conditions that can lead to visual impairment or even blindness due to damage of the ocular blood supply. Understanding the pathomechanism of these vascular disorders is crucial for early diagnosis, prevention and treatment. The currently available treatment options are symptomatic therapies, that aim to slow down the progression. Ocular vascular disorders include: retinal artery occlusion (RAO), retinal vein occlusion (RVO), anterior ischemic optic neuropathy (AION), diabetic retinopathy and ocular ischemic syndrome (OIS). In addition to these disorders, pathological vascular processes also play an important role in the development of glaucoma.

RAO is a sudden onset, painless, acute arterial circulatory disorder of the retina, often leading to severe, irreversible visual impairment or loss of vision. It is an emergency condition requiring acute ophthalmic intervention. Depending on the location of the occlusion, it may be a central retinal artery occlusion (CRAO) or a branch retinal artery occlusion (BRAO).

CRAO usually results in severe visual impairment, while BRAO may cause functional impairment in the retinal sector corresponding to the affected area. The fundus abnormalities are of diagnostic value - pale, edematous retina, characteristic "cherry red spot" at the fovea, arterioles are markedly narrow, often with arteriolosclerosis. In some cases, a ruptured blood column and the blocking embolus can also be seen. The characteristic „cherry red spot” in the macular area is due to the anatomical feature of a thinner retina in the fovea, with a choroidal circulation and a pale, edematous surrounding retina.

In BRAO, the retina is pale and edematous in the area supplying the blocked arterioles. When fluorescein angiography (FA) is performed, delayed arteriolar thickening and insufficient perfusion of the supply area of the occluded artery are usually seen. In doubtful cases, optical coherence tomography (OCT) may help in the diagnosis. On OCT images, edema of the inner retinal layers can be seen early on, before the appearance of retinal pallor.

The most common risk factors for RAO are hypertension (HT), type 1 and type 2 diabetes mellitus (T1D; T2D), hyperlipidemia, ischemic heart disease (IHD), atrial fibrillation (AF), smoking, advanced age and male gender.

RVO is one of the most common vascular diseases of the retina. Often a thrombus blocks the venous circulation, but transient venous insufficiency without a thrombus can also result it. It can be subdivided into central retinal vein occlusion (CRVO), branch retinal vein occlusion (BRVO) and hemiretinal vein occlusion (HRVO).

Pathogenetically, we distinguish between ischemic and non-ischemic forms. RVO is a multifactorial disease, often associated with hypertension, atherosclerosis, diabetes mellitus, hyperlipidemia and disorders associated with hypercoagulability. Virchow's triad, which consists of endothelial damage, stasis of blood flow and hypercoagulability, underlies the development of thrombotic events, including RVO. Venous obstruction results in increased venous pressure, leading to fluid leakage, haemorrhage and ischemia. Prolonged ischemia may stimulate the secretion of vascular endothelial growth factor (VEGF) and other inflammatory mediators, promoting neovascularisation and further worsening the clinical picture. It is characterised by a gradual deterioration of vision, with punctate scotomas in the visual field initially, which may later become larger. The veins are fuller and more tortuous. In later stages (after 1-2 weeks), vision deteriorates due to increased circulatory disturbance and macular edema. The fundus picture is characterised by intraretinal haemorrhages along the veins, edematous papilla with a blurred edge. After a few weeks, marked cystoid macular edema and cotton wool spots may develop, corresponding to small infarcted areas of the retinal nerve fiber layer. The arterioles are usually narrower. In the case of transthoracic venous occlusion, haemorrhages develop along all 4 main branches of the veins, and in the case of BRVO, haemorrhages develop only along the affected branch. In HRVO, the lower or upper retinal area is affected.

Macular edema due to RVO is the result of a complex cascade mechanism. The main trigger factor is hypoxia, which causes an increase in vasoactive substances, interleukins and VEGF levels through several pathways, leading to damage of the blood-retinal barrier.

Treatment with anti-VEGF (e.g. bevacizumab, ranibizumab, aflibercept) in the form of intravitreal injections, is considered the first-line treatment for macular edema due to RVO, reducing edema and improving visual acuity. Intravitreal corticosteroids (e.g. triamcinolone acetonide, dexamethasone implants) may be

an alternative, especially for patients who do not respond adequately to anti-VEGF therapy. Especially in BRVO, if macular edema or neovascularisation persists, macular grid or focal laser photocoagulation may be performed in selected cases. This typically does not eliminate the macular edema but may be useful in limiting its extent. In CRVO, panretinal photocoagulation may be helpful in preventing or treating neovascular complications. These different treatment methods of RVO aim to reduce the risk factors involved in its pathogenesis, improve retinal blood flow and prevent or if necessary, treat complications, in particular macular edema and neovascularisation.

1. Objectives

Assessment of cardio- and cerebrovascular risk factors for RAO, acute ischaemic stroke and ST-elevation myocardial infarction

We intended to:

- analyse the risk of developing acute ischemic stroke (AIS) and ST-elevation myocardial infarction (STEMI) among RAO patients.
- investigate the role of age and gender in the development of RAO, AIS and STEMI in the eastern region of Hungary.
- investigate the role of cardio- cerebrovascular risk factors such as HT, T1D, T2D, IHD, AF and hyperlipidemia among RAO patients.
- analyze the combined effect of several risk factors in the development of RAO, AIS and STEMI in Hungarian patients.
- investigate whether the reduction of risk factors and the appropriate management of concomitant diseases can prevent or reduce AIS/STEMI and mortality in RAO patients.

Evaluation of the efficacy of anti-VEGF treatment in RVO-induced cystoid macular edema

We intended to:

- analyse the clinical efficacy of intravitreal bevacizumab (IVB) treatment for macular edema caused by BRVO and CRVO.
- investigate and determine the extent of visual improvement and reduction in macular edema using OCT.

- assess the effectiveness of treatment by the examination of 4 main parameters (corrected visual acuity measured at the end of the study period (final corrected visual acuity), improvement in corrected visual acuity, the thickness of the central 1 mm diameter macular area measured by OCT at the end of the study period (final CST), and the change (decrease) in CST during the study period).
- determine a correlation between the 4 main parameters examined and the number of intravitreal injections received during treatment.
- analyse a correlation between the number of injections and the age of the patients.

2. Patients and Methods

All the subjects were recruited at the Department of Ophthalmology, Faculty of Medicine, University of Debrecen, Hungary. The results were analysed after anonymisation of the patient data, which were stored in a database accessible only to the participants.

The methodology of our studies is in accordance with the international literature, and the research design was drawn up in accordance with the legislation in force and the Declaration of Helsinki. Patients were included after informed consent and with the approval of the Regional and Institutional Research Ethics Committee of the University of Debrecen.

The relationship between RAO, AIS and STEMI and analysis of cardio- and cerebrovascular risk factors in Hungarian patients

We analysed data from 181 RAO patients (114 CRAO and 67 BRAO) who presented to our clinic between 2009 and 2019. The data were collected retrospectively from our electronic database (e-MedSolution 6.0) and the National eHealth Infrastructure (EESZT – Hungarian acronym). We selected patients who presented for examination within 1 week of RAO onset. We considered symptoms of diagnostic value as sudden visual deterioration, visual loss, visual field defects. Inclusion criteria were characteristic fundus abnormalities, such as pale, edematous retina, "cherry red spot" in the foveolar area, disconnected blood column in the arterioles, and a visible embolus blocking the circulation.

We excluded all patients with any other pathological fundus abnormality from our study. We also excluded RAO patients whose electronic documentation was not available due to digital self-determination or who did not have adequate documentation of comorbidities and medications taken regularly. A total of 12 patients were excluded from the study, making a total of 169 RAO patients (106 CRAO and 63 BRAO) eligible for inclusion.

We recorded the age, sex, onset time of RAO, type of obstruction (CRAO/BRAO) and documented deaths from cardiovascular or cerebrovascular causes. In both groups, we recorded the presence of AIS and STEMI and their time of onset. We also examined the presence of concomitant diseases such as HT, T1D, T2D, IHD, AF and hyperlipidemia and the time of their onset. We randomly selected 169 age and sex homogeneous control patients from our database. The control group consisted of cataract surgery patients with no previous history of ocular vascular disease. All RAO patients and control members underwent a complete ophthalmic examination, including best corrected visual acuity testing, slit-lamp examination, Goldmann applanation intraocular pressure measurement, and fundus examination with dilated pupils.

Analysis of intravitreal bevacizumab treatment of macular edema due to RVO

A total of 66 patients who developed macular edema due to RVO between May 2014 and February 2019, were prospectively examined at our retinal vascular specialty clinic. At the first visit, best corrected visual acuity testing, slit-lamp examination, intraocular pressure measurement using a Goldmann applanation tonometer, fundus examination with dilated pupils, and OCT scan were performed. If necessary, FA examination was also performed. CST was determined using Stratus TD-OCT (Carl Zeiss Meditec Inc, Dublin, California). The above tests were repeated on all control visits.

Our study included patients with macular edema due to RVO less than 9 months from the development, with a CST greater than 300 μm. Patients with STEMI, AIS, or pulmonary embolism within 1 year, as well as patients with severe liver and kidney disease, coagulopathy, and poor cooperative skills were not included in our study. We also excluded patients with other ocular fundus abnormalities. Seven patients were excluded due to the exclusion criteria, and a total of 59

patients with macular edema due to RVO (34 CRVO and 25 BRVO), who received IVB treatment, were included in the final analysis.

According to the treatment protocol, members of the study group received 1.25 mg of bevacizumab intravitreally once every month for three months in a volume of 0.05 µl. After three months, a further treatment plan (pro re nata) was determined on an individual basis, considering changes in anatomy and visual acuity. Patients in whom macular edema had resolved were no longer treated, but regular follow-up visits were ordered. Initially monthly, then every 3 to 6 months. If deemed necessary based on best corrected visual acuity or CST values (inadequate functional and anatomical outcome), patients continued to be treated after the third month. In cases where IVB treatment proved ineffective, we discontinued it after the third month.

At the start of our study, there was a relatively long time interval between the onset of RVO symptoms and IVB treatment (up to 9 months), as this treatment option only became available in our country in 2014. Later, this time was reduced to 6 weeks on average.

As a control group, we retrospectively collected data from 80 RVO patients from our electronic database (e-MedSolution 6.0) and the National eHealth Infrasructure (EESZT – hungarian acronym), who presented between July 1998 and January 2005. We selected patients who had not received any invasive therapy (intravitreal anti-VEGF treatment, intraocular argon laser treatment, subtenon steroid treatment). During this period, intravitreal anti-VEGF treatment was not yet available in our country.

3. Results

Results of RAO, AIS and STEMI association and analysis of cardio- and cerebrovascular risk factors

We compared data from 169 RAO patients (106 CRAO and 63 BRAO) with data from 169 control patients who had no previous history of retinal vascular disease. Our data showed a non-normal distribution according to the Shapiro-Wilk test ($p < 0.05$). There were significantly more male patients than female patients in the RAO patient group ($p = 0.007$). RAO and control patients were homogeneous with respect to age and gender by Mann-Whitney U test ($p =$

0.575). The mean age in the RAO group was 64.18 ± 10.00 years, while in the control group it was 63.88 ± 10.43 years.

During our study period, RAO became bilateral in two of our patients, one developing CRAO and the other BRAO. Among our 169 RAO patients, 40 developed AIS and 32 developed STEMI. The difference was found to be significant by Pearson's chi-square test for the same disease in the control group ($p1 < 0.001$ and $p2 < 0.001$, respectively).

In terms of cardio- and cerebrovascular disease, HT and hyperlipidemia were significantly more frequent in the study group compared to the control group according to Pearson's chi-square test ($p1 = 0.005$, $p2 < 0.001$, respectively). In contrast, no significant differences were found between the two groups for T1D, T2D, AF and IHD ($p1 = 0.346$, $p2 = 0.096$, $p3 = 0.309$ and $p4 = 0.641$, respectively).

We used a two-sample Z test to examine how the number of AIS and STEMI cases varies with age in 10-year intervals. We also analysed whether advancing age plays a role as a pathogenetic/risk factor in the development of AIS and STEMI. We found, that the prevalence of both AIS and STEMI increases substantially above the age of 50 years. In the 60-70 age group, AIS and STEMI continue to develop more frequently, but no further increase in risk is observed. There was a significant difference in the incidence of AIS and STEMI between the two age groups (under 50 and over 50 years; $p1 < 0.001$ and $p2 < 0.001$, respectively), but no such difference between the RAO group and the control group.

Univariate logistic regression analysis was used between RAO patients and controls for each risk factor/co-morbidity. We examined the effect of each factor on the incidence of developing AIS and STEMI. We found that RAO increased the risk of developing AIS by 8.18-fold (odds ratio - OR: 8.18, $p < 0.001$, 95% CI: 3.09-21.64).

Our results suggest that RAO is associated with a 3.10-fold increase in risk of developing STEMI (OR: 3.10, $p = 0.007$, 95% CI: 1.36-7.08). By having T1D the same risk is elevated by 3.51-fold (OR: 3.51, $p = 0.013$, 95% CI: 1.31-9.42).

AF increases risk of STEMI by 2.35-fold (OR: 2.35, p = 0.030, 95% CI: 1.08-5.07), hyperlipidemia by 2.30-fold (OR: 2.30, p = 0.023, 95% CI: 1.12-4.72), and ISZB increased the odds of developing STEMI by 2.13-fold (OR: 2.13, p = 0.044, 95% CI: 1.02-4.46). Differences between RAO patients and controls were also significant for the same risk factors.

Multivariate logistic regression analysis was used to investigate how the co-occurrence of different risk factors/cardiovascular diseases influences the development of AIS and STEMI in RAO patients.

For RAO patients, the odds ratio for developing AIS increased by 8.18-fold (OR: 8.18, p < 0.001, 95% CI: 3.09-21.64). If RAO patients had HT, this increased the odds ratio for developing AIS by a larger factor of 11.65-fold (OR: 11.65, p < 0.001, 95% CI: 4.44-30.55). However, the odds ratio did not increase further in cases where PF was present along with RAO (OR: 4.36, p = 0.001, 95% CI: 1.83-10.34), and when hyperlipidemia was associated with RAO, in which case the odds ratio was increased to 2.66-fold (OR: 2.66, p = 0.009, 95% CI: 1.28-5.53) for the development of AIS. In RAO patients, who had HT, and IHD (OR: 10.04, p < 0.001, 95% CI: 3.26-30.94), T1D (OR: 25.11, p < 0.001, 95% CI: 4.86-129.71), or T2D (OR: 15.38, p < 0.001, 95% CI: 4.80-49.24) was present as a third factor, the risk of AIS increased further. The presence of hyperlipidaemia (OR: 2.99, p = 0.004, 95% CI: 1.43-6.25) in addition to RAO and HT did not further increase the risk ratio.

The risk of developing STEMI is increased 3.10-fold in RAO patients (OR: 3.10, p = 0.007, 95% CI: 1.36-7.08). In RAO patients presence of HT increased risk 4.93-fold (OR: 4.93, p < 0.001, 95% CI: 2.24-10.85), PF to 4.92-fold (OR: 4.92, p < 0.001, 95% CI: 2.09-11.56), and those who had hyperlipidemia had a 3.34-fold (OR: 3.34, p = 0.002, 95% CI: 1.56-7.15) increased risk of developing STEMI. In RAO patients with concomitant HT, if T1D (OR: 4.22, p = 0.013, 95% CI: 1.36-13.15), T2D (OR: 4.79, p = 0.005, 95% CI: 1.60-14.37) or hyperlipidaemia (OR: 3.62, p = 0.001, 95% CI: 1.69-7.78) were also present as a third factor, the risk of STEMI was further increased. In RAO patients, the coexistence of HT and IHD increased the risk of STEMI even more markedly than the previous factors (OR: 5.52, p = 0.001, 95% CI: 2.10-14.53).

During the study period, 23 RAO patients died of cardio- or cerebrovascular complications. There was no significant difference in the number of deaths

known to be related to AIS or STEMI (3 and 2 patients, respectively). 13 patients developed AIS before RAO and 12 after RAO. 19 patients developed STEMI before RAO and 6 after RAO.

Clinical results of intravitreal bevacizumab treatment for macular edema due to RVO

The mean age of the patients was 58.83±12.30 years, the youngest participant was 23 years and the oldest was 85 years old. There were 28 males and 31 females in the IVB treated group.

The mean age of the control group was 59.97±14.15 years, the youngest patient was 21, and the oldest patient was 88 years old. The control group consisted of 36 men and 44 women. The control group members did not undergo any further ophthalmic follow-up examination at the time of data collection. The initial and final best corrected visual acuity values were processed using data from our electronic database. For comparability, the same follow-up time was defined for the control and IVB groups. The final best corrected visual acuity and best corrected visual acuity change values were used in our calculations. The average follow-up time in both groups was 9 months.

In the IVB treated group, 20 patients had HT, 1 patient had T1D, 6 patients had T2D, and 2 patients had hyperlipidemia. In the control group, 39 patients had HT, 4 patients had T1D, 9 patients had T2D, and 33 patients had hyperlipidemia.

Based on a two-sample t-test, the change in best corrected visual acuity showed a significant improvement within the treated group of patients (p1: CRVO = 3.25×10-4; BRVO = 5.52×10-4) and compared to the control group (p2: CRVO = 3.46×10-4; BRVO = 0.003). The magnitude of CST reduction in the treated group was also significant (p3: CRVO = 6.94×10-9; BRVO = 1.67×10-4). In the control group, the change in best corrected visual acuity was negative in the CRVO group, and a slight improvement was observed in the BRVO group, but the change was not significant ($p = 0.357$).

In terms of final best corrected visual acuity, only the BRVO subgroup showed a significant improvement ($p = 0.016$) compared to the control group. The CRVO subgroup did not show significantly better final best corrected visual acuity than the control ($p = 0.204$).

Pearson's correlation demonstrated a positive correlation between the number of injections administered during IVB treatment and all four parameters we examined. In the CRVO subgroup, there was a significant positive correlation between the final best corrected visual acuity ($p = 0.011$, $r = 0.431$) and best corrected visual acuity improvement ($p = 0.015$, $r = 0.414$) (Table 9). No similar correlation was confirmed in the BRVO subgroup.

ANOVA test was performed to investigate the effect of age on the four parameters we investigated (final best corrected visual acuity, best corrected visual acuity change, final CST, CST change) in the IVB treated group. At younger ages, the magnitude of CST reduction was significantly greater ($p = 0.018$, $R2 = 0.094$). No significant association was found between age and number of IVB treatments ($p = 0.265$, $R2 = 0.022$).

4. Main findings and conclusions

Increased cardio- and cerebrovascular risk in patients with retinal artery occlusion

- RAO is associated with an increased risk of AIS and STEMI, especially in patients with multiple cardiovascular risk factors.
- Early detection, appropriate medical evaluation and management of concomitant diseases in collaboration with ophthalmologists, neurologists and cardiologists can potentially prevent RAO, AIS and STEMI in cardiovascularly burdened patients.
- It is essential to recognise the increased risk and refer patients promptly for appropriate investigations and secondary prevention measures. The use of antithrombotic treatment may be beneficial in the secondary prevention of RAO development in the fellow eye, AIS/STEMI development, provided there are no contraindications.

Results of anti-VEGF treatment in patients with retinal vein occlusion

- Based on our data, the separation of anti-VEGF treatment protocols for CRVO and BRVO may be useful. The degree of ischaemic damage and the degree of macular edema are effective predictors of the length and intensity of treatment required to achieve the expected outcome.

- Our results can also help patients who have suffered thrombosis to know what intensity of IVB treatment to expect and what degree of visual acuity improvement to expect. This information can help them decide whether or not to undergo IVB treatment. This should be carefully considered, especially in older, polymorbid patients.

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List of publications related to the dissertation

1. **Balla, S.**, Vajas, A., Pásztor, O., Rentka, A., Lukucz, B., Kasza, M., Nagy, A. C., Fodor, M., Nagy, V.: Analysis of the Association between Retinal Artery Occlusion and Acute Ischaemic Stroke/ST-Elevation Myocardial Infarction and Risk Factors in Hungarian Patients. *Medicina. 59* (9), 1-11, 2023.
   DOI: http://dx.doi.org/10.3390/medicina59091680
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2. **Balla, S.**, Zöld, E., Potor, L., Lukucz, B., Vajas, A., Ujhelyi, B., Nagy, V.: Analysis of intravitreal bevacizumab treatment for macular oedema due to retinal vein occlusion. *Eur. J. Ophthalmol. 31* (5), 2528-2534, 2021.
   DOI: http://dx.doi.org/10.1177/1120672120962051
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List of other publications

3. Tóth, N., Silver, D. M., **Balla, S.**, Káplár, M., Csutak, A.: In vivo corneal confocal microscopy and optical coherence tomography on eyes of participants with type 2 diabetes mellitus and obese participants without diabetes.
Graefes Arch. Clin. Exp. Ophthalmol. 259 (11), 3339-3350, 2021.
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