ORIGINAL ARTICLE

Effects of Intravitreal Pegaptanib or Bevacizumab and Laser in Treatment of Threshold Retinopathy of Prematurity in Zone I and Posterior Zone II – Four Year Results

Pašová, P.[1,2], Skorkovská, K.[1], Michálek, J.[3]

[1] Department of Ophthalmology and Optometry, St. Anne's University Hospital, Masaryk University, Brno, Head: doc. MUDr. Svatopluk Synek, CSc.
[2]Eye Centrum Palánek, Vyškov,
[3]Institute of Mathematics, Faculty of Mechanical Engineering, University of Technology, Brno

SUMMARY

Effects of Intravitreal Pegaptanib or Bevacizumab and Laser in Treatment of Threshold Retinopathy of Prematurity in Zone I and Posterior Zone II – Four Year Results

Purpose: To evaluate efficacy and safety of intravitreal injection of pegaptanib or bevacizumab and laser photocoagulation for treatment of threshold stage 3+ retinopathy of prematurity (ROP) affecting zone I and posterior zone II, and to compare the results in terms of regression, development of peripheral retinal vessels with conventional laser photocoagulation or combined with cryotherapy.
Methods: In this prospective comparative study, 174 eyes of 87 premature babies, from January 2008 to December 2011, were included. All infantns were diagnosed with stage 3+ ROP for zone I or posterior II. Patients were randomly assigned to receive intravitreal pegaptanib (0.3 mg) or bevacizumab (0.625 mg/0.025 ml of solution) with conventional diode laser photocoagulation (Group A, 92 eyes of 46 infants) or laser therapy combined with cryotherapy (Group B, 82 eyes of 41 infants), bilaterally. The main evaluated outcomes include time of regression and decrease of plus signs and development of peripheral retinal vessels after treatment, final strucutral-anatomic outcomes compared in the both groups of patients. Risk factors and other characteristics of infnats include birth weight, gestational age, Apgar score, duration of intubation and hospitalizations, postmenstrual age at treatment, sepsis, surgery for necrotizing enterocolitis, intraventricular hemorrhage. Primary outcome of treatment success was defined as absence of recurrence of stage 3+ ROP in one or both eyes (reccurrence rate = 0) by 55 weeks' postmenstrual age. Treatment failure was defined as the recurrence of neovascularization (reccurrence rate = 1 or 2) in one or both eyes requiring retreatment. The mean follow-up after treatment was 23.5 months (range 4 - 45 months) in the Group A, and 25.2 months in the Group B (range 3 - 48 months). Results: Final favorable anatomic outcome and stable regression of ROP at last control examination have 90.2 % of eyes after adjuvant intavitral pagaptanbib or bevacizumab in
the Group A, and 62 % of eyes after only conventional treatment in the Group B (P = 0.0214). Regression of plus disease and peripheral retinal vessels development appeared significantly more rapidly in Group A patients who received intravitreal VEGF inhibitors and laser. An absence of recurrence of neovascularization (stage 3+ ROP) was identified at 87 % of patients in the Group A, and 53 % of patients in the Group B. This difference between the both groups was statistically significant (P = 0.0183). ROP reccured in 7 from 92 eyes (7.6 %) in the Group A, and 23 from 82 eyes (28 %) in the group B (P = 0.0276).
Significantly better treatment effect was found for adjuvant intravitreal pagaptanib or bevacizumab with laser compared with conventional therapy of ROP 3+ in zone I and posterior zone II. Perioperative retinal haemorrhages after laser photocoagulation occured in 8 % of eyes in the Group A, and 11 % of eyes in the group B (P = 0.358), in all eyes with spontaneous resorption. No systemic or significant ocular complications of intravitreal anti-VEGF injections, such as endophthalmitis or retinal detachment were found during follow-up period after operation.
Conclusions: A combination of intravitreal pegaptanib or bevacizumab injection and laser photocoagulation showed to be a safe, well tolerated and effective therapy in patients with stage 3+ ROP in zone I and posterior zone II. Adjuvant intravitreal antiVEGF injection, as compared with conventional laser or cryotherapy, showed significant benefit in terms of better final anatomic outcome, induction of prompt regression, rapid development of peripheral retinal vascularization and decrease

First author:
MUDr. Petra Pašová
Eye Centrum Palánek,
Palánek 1
682 01 Vyškov
e-mail: pasova.petra@gmail.com

of recurrence rate of neovascularization. Results of this study support the administration of pegaptanib and bevacizumab as an alternative usefull therapy in the management of stage 3+ ROP.

Key words: retinopathy of prematurity, stage 3+, zone I, posterior zone II, anti–vascular endothelial growth factor therapy, pegaptanib (Macugen), bevacizumab (Avastin), intravitreal injections, RetCam photography

Čes. a slov. Oftal., 68, 2012, No. 3, p. 28–36

INTRODUCTION

Retinopathy of prematurity (ROP) is a vasoproliferative disorder, which mostly affects prematurely born and immature infants with a birth weight of less than 1500 g, who were born before the 32nd week of gestation, and on whom oxygen therapy is performed due to respiratory and circulatory insufficiency. It is the most frequent cause of congenital blindness in infants in developed countries. Almost 70% of all blind children are patients who have suffered retinopathy of prematurity. ROP occurs in two forms: acute and chronic. The acute form is characterised by impaired vasculogenesis of the immature retina. Chronic late development is characterised by the formation of retinovitreal fibrovascular membranes, which through their traction can cause total or partial retinal detachment, macular ectopy etc. Structural changes on the fundus then determine the degree of functional impairment of sight. In 60% to 80% of cases the disorder has a tendency towards spontaneous regression without more significant permanent anatomical and functional consequences. Approximately 10% to 30% of children with ROP are afflicted with severe cicatrical changes, and total amotion with corresponding loss of sight is not uncommon. The worst course of the disorder is generally in very immature babies with a birth weight of less than 1000 g, born before the 30th week of gestation. Although ROP is a bilateral disorder, the course and affliction of the eyes is frequently asymmetrical. The total incidence of ROP in infants with a birth weight of less than 1000 g is stated within the range of 52% to 88%, in babies above 1000 g it occurs in 34% to 60% of infants. The worldwide prevalence of blindness as a consequence of ROP is 50 000 children (18).

Vascularisation of the retina in prematurely born infants is not complete, and the retinal capillaries end at varying distances before or behind the equator. Vasoconstriction of the retinal arteries and ischemia of the peripheral areas of the retina occurs as a result of oxygen therapy with higher concentrations of oxygen. After the end of oxygen therapy, relative hypoxia is an inducement for dilation of the retinal capillaries. The fusiform cells are stimulated to produce an angiogenic factor, which starts the process of neovascularisation. Arteriovenous losses occur in the area of the interface of the vascularised and avascular retina. The development of newly formed capillaries in characteristic clusters by neovascularisation is accompanied by a stimulation of myofibroblasts for the production of fibrotic tissue. These fibovascular proliferations spread around the posterior surface of the vitreous body and into the vitreous area, and through their pulling force are a cause of tractional retinal detachment.

In each acute stage of ROP a “plus form” may be present, which is characterised by dilation and tortuosity of the retinal capillaries, rubeosis of the iris, and may be a sign of pronounced acceleration of the course of ROP with a more adverse prognosis. The first and second stages of ROP often spontaneously recede. The third stage may regress, but there is a 50% risk of progression with all the attendant serious consequences. In more severe forms of ROP, where there are symptoms of the plus form, in the rush type of ROP and at the beginning of ROP in zone I, the probability of spontaneous regression is negligible. The individual stages of typically ongoing ROP are altered in “atypical” forms. The main clinical units of atypical forms of ROP include: retinopathy of zone I as a posterior variant or rush disease, haemorrhagic forms of ROP, stop form (blocked retinopathy) and late retinopathy (ROP last minute). The majority of atypical forms affect infants with the lowest birth weight and have the most negative prognosis. ROP in zone I and in posterior zone II, especially if the plus form is present, is the most difficult to treat, and repeated procedures are often required as a result of recurrence of neovascularisation (7, 14, 25, 27, 38, 39, 49).

The plus form – or stage ROP 3+ is defined by dilation of veins and tortuosity of arteries from the space from the disc of the optic nerve in 2 or more quadrants of the fundus.

Cryotherapy has been recommended as standard for treatment of stage 3 ROP since 1988 (11).

Since the 1990s laser therapy, or a combination of cryotherapy and transpupillary laser photocoagulation has been applied with ever increasing frequency in the treatment of stage 3+ ROP. Both methods lead to a destruction of cells producing vascular endothelial growth factor (VEGF) in the avascular retina (15, 47).

Since 2003 timely use of confluent ~~laser photocoagulation has been re-~~commended in stage ROP 3+, on the ~~basis of the results of the ET-ROP stu-~~dy (15).

Conventional laser therapy for ROP 3+ in zone I and posterior II is successful on average in 50% of cases. In the case of failure of repeated classical laser therapy, vitrectomy is recommended (40, 53).

VEGF plays a key role in the progression of ROP through the formation of neovascularisations. In recent years, favourable results of intravitreal application of the anti-VEGF preparation bevacizmab have appeared in the literature, stating a reduction of pathological neovascularisation, with a triggering of successful regression of ROP, without ocular and general side effects (19, 22, 26, 28, 35, 52, 54).

VEGF plays a fundamental role in both physiological and pathological neovascularisation. Isoform 165 is responsible for pathological neovascularisation in the eye, isoform 121 is important for the physiological renewal of the vascular system. Of the total quantity of synthesised VEGF, the greatest part is formed by isoform 121 and 165 (24).

Bevacizumab is a non-selective monoclonal antibody, blocking all isoforms of VEGF. Non-selective blockage of all isoforms leads to a restriction of pathological vascularisation, but also of the physiological development of the retinal vascular system (24).

Pegaptanib is a chemically synthesised oligonucleotide – aptamer, which binds with a high affinity to the isoform VEGF 165, which is responsible for pathological neovascularisation, and blocks its receptor on the cell membrane. The physiological isoform VEGF 121 remains free. Systemic resorption of pegaptanib is very low. A concent-

ration corresponding to 0.03-0.15% of the intravitreal concentration was measured in the systemic circulation of tested animals. For the human population the systemic concentration after intravitreal application of 0.3 mg of pegaptanib is beneath the lowest detectability limit (0.008 mg/ml). It has been demonstrated that even after repeated intravitreal applications, no accumulation of pegaptanib was observed (51).
In recent years studies have been published confirming a favourable effect of intravitreal injection of bevacizumab in the treatment of stage ROP 3+ in zones I-II, without ocular and systemic complications (3, 13, 22, 26, 28, 29, 30, 35, 37, 52, 54).
To date, no studies dealing with intravitreal application of a **selective** anti-VEGF preparation – pegaptanib blocking isoform VEGF 165, which is responsible for pathological neovascularisation, in the treatment of ROP, have been published in the professional literature. Our study brings the first long-term results of adjuvant application of pegaptanib or bevacizumab in combination with conventional treatment of stage ROP 3+ in zone I and posterior II, or in the case of the aggressive form of posterior ROP (AP-ROP). The aim of the work is to assess the efficacy and safety of this new treatment in severe forms of ROP, which upon use of the hitherto standard conventional therapy may lead to blindness in up to one half of cases.

METHOD AND SAMPLES

In 2008-2011 a total of 174 eyes were treated in 87 prematurely born infants with severe form of ROP 3+ in zone I and posterior II. The stage and localisations of ROP were classified according to the internationally valid criteria (23).
Plus form – stage ROP 3+ was defined by the presence of dilation and tortuosity of retinal capillaries from the disc of the optic nerve in at least 2 quadrants of the fundus.
The use of adjuvant intravitreal application of anti-VEGF preparations was permitted by the Ethical Commission of the University Hospital in Brno within the framework of the approved grant project **IGA MZ ČR NS/9892-4**. The parents of all the treated infants were informed about the possibility of conventional treatment and the potential expected benefit of intravitreal application of anti-VEGF preparations. Informed consent was obtained from the parents. Conventional treatment by laser photocoagulation was commenced and performed according to the international standards of timely treatment of ROP (ETROP) (15).
Group A consists of 92 eyes (46 infants) treated by means of indirect transpupillary photocoagulation by diode laser (810 nm), with adjuvant intravitreal application of **pegaptanib (60 eyes) or bevacizumab (32 eyes).**
Group B includes 82 eyes (41 infants) with the same form of ROP, who were treated by classic photocoagulation in combination with cryotherapy. In the case of combined treatment in group B, a diode laser was applied in the central parts of the avascular retina, cryotherapy peripherally from photocoagulation upon careful control by indirect ophthalmoscopy in maximum mydriasis.
In group A an intravitreal injection of pegaptanib (0.3 mg) or bevacizumab (0.625 mg/0.025 ml) was performed following photocoagulation under general or local anaesthesia in the pars plana area 2-3 mm from the limbo in the lower nasal or temporal quadrant under aseptic conditions, following rinsing of the conjuctival sac with 5% Betadine solution. Following the procedure, local antibiotics were always applied (Oftaquix gtt or Tobrex gtt) 5x per day for a period of 1 week.
The observed and evaluated parameters cover: degree of ROP before and after surgery, period of achieving regression – clearance of symptoms of "plus disease", completion of normal peripheral vascularisation, birth weight, gestation age at time of procedure and final structural changes on fundus in both groups. From the perspective of the overall condition of health of the prematurely born infants, the Apgar score was used to evaluate the period of applicable intubation, period of hospitalisation in neonatological department, presence of risk factors: sepsis, necrotising enterocolitis, intraventricular haemorrhage.
Successful treatment was defined by the absence of recurrence of stage 3+ ROP in one or both eyes in zone I or posterior zone II up to 55 weeks of gestation age ("recurrence rate" = 0). Unsuccessful treatment was defined as recurrence of neovascularisations in one or both eyes ("recurrence rate = 1 or 2), with the necessity of repeated therapeutic procedure. The fundamental characterising parameters and results of the therapeutic methods in both groups were statistically processed using a t-test, Fischer's exact test and a Mann-Whitney test. The value of $P < 0.05$ was the indicator of statistical significance.
All infants were observed 1-2 months after the procedure 1x per week, afterwards 1x per 14 days, and 1x per month from 4 months after the operation onward.
The average observation period was 23.5 months (range 4-45 months) in group A, 25.2 months (range 3-48 months) in group B. The findings on the fundus before and after surgery were documented using RetCAM.

RESULTS

In both groups of eyes, the occurrence of stage ROP 3+ in zone I and posterior zone II was represented approximately equally. Stage ROP 3+ in zone I at the commencement of treatment was present in 49 eyes in group A and 44 eyes in group B ($P = 0.98$), stage ROP 3+ in posterior zone II was present in 43 eyes in group A and 38 eyes in group B ($P = 0.73$). The average birth weight was 785 g in group A and 813 g in group B ($P = 0.43$). The average gestation age in group A was 24.7 weeks and in group B 25.1 weeks ($P = 0.79$).
Post-conception age at the time of performance of the operation was 33.8 weeks in group A and 34.2 weeks in group B ($P = 0.21$). The parameters evaluating the overall condition of the prematurely born infants (Apgar score, sepsis, intraventricular haemorrhage, enterocolitis, artificial lung ventilation) in both groups are presented in table 1.
In group A a statistically significantly faster clearance of symptoms of plus ROP was recorded, as well as a more rapid achievement of normal peripheral retinal vascularisation than in group B. After the performed treatment there was a clearance of the symptoms of "plus disease" (abnormal dilation of veins and tortuosity of retinal arteries) on average after 1.38 weeks in group A and after 2.61 weeks in group B ($P = 0.0037$).
The average time of conclusion of physiological peripheral retinal vascularisation was 2.35 weeks in group A and 3.48 weeks in group B ($P = 0.0126$).
No recurrence of neovascularisations and no necessity for a repeated treatment procedure (recurrence rate = 0) was recorded for 87% of infants in group A and 53.7% of infants in group B ($P = 0.0183$).
Recurrence of neovascularisations in

one eye was recorded in 10.8% of infants in group A and 36.5% of infants in group B (P = 0.0297).
Favourable anatomical changes on the fundus and stable regression of ROP upon the last check were recorded in 90.2% of all eyes treated with combined therapy with an intravitreal injection of pegaptanib or bevacizumab in group A. In group B favourable structural changes and regression of ROP were recorded in 62% of eyes treated with cryo- and photocoagulation (P = 0.0214) (table 2, graph 1).
Upon an evaluation of the comparison of the effectiveness of intravitreal application of pegaptanib (60 eyes) and bevacizumab (32 eyes) in group A, no statistically significant differences were found between both VEGF blockers, represented in the final favourable anatomical result on the fundus (88.3% versus 87.5%, P = 0.827) at the time of clearance of the symptoms of plus ROP and in the stimulation of normal peripheral retinal vascularisation (table 3, graph 2).
Perioperative complications comprised retinal haemorrhage following photocoagulation in 8% of eyes in group A and 11% of eyes in group B (P = 0.358). In all case there was progressive spontaneous resorption.
No eye or general complications, or side effects of intravitreal injection of pegaptanib or bevacizumab were recorded.
All patients remain under regular observation, and in the next phase the functional results (visual acuity, field of vision, elecrophysiological tests) and the occurrence of refractive errors shall be evaluated.

DISCUSSION

Our study was commenced in 2008 in an endeavour to improve the results of treatment of prematurely born infants with severe form of ROP 3+ beginning in zone I or posterior zone II, thus groups of infants with ROP who are the most at risk of permanent blindness upon the failure of conventional treatment. At this time published results were available of isolated cases of successful adjuvant intravitreal application of non-selective VEGF inhibitors – bevacizumab in the treatment of ROP 3+, in combination with classic laser photocoagulation (19, 22, 26, 28, 52, 54).
In 2008, before the commencement of the grant project, only bevacizumab was available at our clinic for intravitreal application. Thanks to the **support**

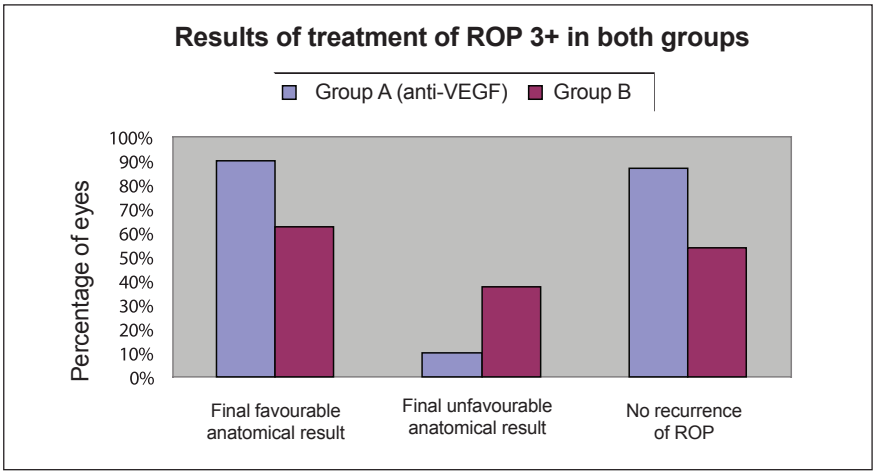
Table 1. Characteristics of 2 groups of infants treated for ROP 3+ in zone I and posterior zone II

	Group A antiVEGF + Laser 92 eyes (46 infants)	**Group B conventional treatment Laser + Cryotherapy 82 eyes (41 infants)**	**P Value**
ROP 3+, zone I (number or eyes)	49	44	0.81
ROP 3+, zone posterior II (number of eyes)	43	38	0.32
Sex – boys (%)	59%	64%	0.67
Birth weight (g, average ± SD)	785 ± 164 g	813 ± 156 g	0.43
Gestation age (weeks, average ± SD)	24.7 ± 1.4	25.1 ± 1.5	0.79
Post-conception age at time of performance of operation (weeks, average ± SD)	33.8 ± 1.7	34.2 ± 1.6	0.21
Apgar score in 1st minute (average ± SD)	4.1 ± 2.3	4.5 ± 1.9	0.35
Apgar score in 5th minute (average ± SD)	6.3 ± 1.8	6.7 ± 1.6	0.47
Sepsis – number of infants (%)	15 (32.6)	14 (34.1)	0.93
Intravetricular haemorrhage – number of infants (%)	11 (23.9)	13 (31.7)	0.69
Enterocolitis – number of infants (%)	13 (28.2)	12 (29.2)	0.97
Intubation – artificial lung ventilation – number	32 (69.5)	27 (65.8)	0.31

Table 2. Comparison of results of treatment of 2 groups of infants treated for ROP 3+ in zones I-II

	Group A antiVEGF + Laser 92 eyes (46 infants)	Group B conventional treatment Laser + Cryotherapy 82 eyes (41 infants)	P Value
Clearance of symptoms of "plus disease" ROP – weeks (average ± SD)	1.38 ± 0.21	2.61 ± 0.73	0.0037
Attainment of peripheral retinal vascularisation after operation – weeks (average ± SD)	2.35 ± 0.54	3.48 ± 0.81	0.0126
Recurrence of neovascularisations ("recurrence rate), number of infants (%)	40 (87%)	22 (53.7%)	0.0183
0 (no recurrence)	5 (10.8%)	15 (36.5%)	0.0297
1 (in one eye)			
2 (in both eyes)	1 (2.2 %)	4 (9.8%)	0.0412
Structural changes relatively favourable (normal posterior pole in zone I, minimal preretinal fibrosis without retinal traction or macular heterotopy) number of eyes (%)	83 (90.2 %)	51 (62.2 %)	0.0214
Structural changes unfavourable – (stage 4A, 4B with affliction of macula), number of eyes (%)	9 (9.8 %)	31 (37.8%)	0.0173

Statistically significant values P < 0.05 (Fischer's exact test, Mann-Whitney U test)

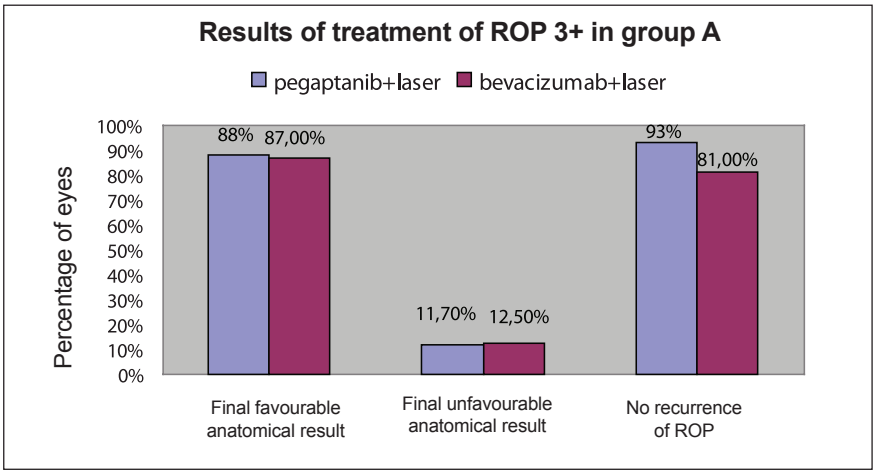


Graph 1. Comparison of results of treatment of 2 groups of infants treated for ROP 3+ in zone I and posterior zone II

Table 3. Comparison of efficacy of pegaptanib and bevacizumab in combination with laser photocoagulation upon treatment of ROP 3+ in zone I and posterior zone II in group A

Evaluated parameters	Pegaptanib + laser 60 eyes (30 infants)	Bevacizumab + laser 32 eyes (16 infants)	P Value
Clearance of symptoms of "plus disease" ROP – weeks (average ± SD)	1.29 ± 0.23	1.43 ± 0.28	0.326
Attainment of peripheral retinal vascularisation after operation – weeks (average ± SD)	2.25 ± 0.56	2.32 ± 0.53	0.651
Recurrence of neovascularisations ("recurrence rate), number of infants (%)	28 (93.3%)	13 (81.2%)	0.134
0 (no recurrence)	2 (6.7%)	2 (12.5%)	
1 (in one eye)	0 (0%)	1 (6.3%)	
2 (in both eyes)			
Structural changes favourable number of eyes (%)	53 (88.3%)	14 (87.5%)	0.827
Structural changes unfavourable – (stage 4A, 4B with affliction of macula), number of eyes (%)	7 (11.7%)	2 (12.5%)	0.731

Statistically significant values P < 0.05 (Fischer's exact test, Mann-Whitney U test)



Graph 2. Comparison of efficacy of pegaptanib and bevacizumab in combination with laser photocoagulation upon treatment of ROP 3+ in zone I and posterior zone II in group A

of the grant IGA MZ ČR it was possible to commence adjuvant treatment of ROP using intravitreal application of the selective VEGF 165 inhibitor – pegaptanib (**Macugen**). The main aim was to improve the results of standard recommended conventional laser or cryotherapy of severe forms of ROP, to evaluate the efficacy and safety of anti-VEGF therapy and to recommend or not recommend the classification of intravitreal application of anti-VEGF preparations within the standard treatment of ROP in the Czech Republic.
No systematic study has been published to date in the Czech or foreign professional literature on the efficacy and safety of intravitreal application of the selective VEGF inhibitor pegaptanib in the treatment of prematurely born infants with ROP.

The development of retinopathy of prematurity (ROP) takes place in 2 phases: the initial phase of relative hyperoxia with partial vascular obliteration is followed by a proliferation of capillaries as a consequence of relative hypoxia (29). Vascular endothelial growth factor (VEGF) as an angiogenic cytokine plays a key role in the development of both pathogenic phases of ROP. Under physiological conditions, VEGF shares in the normal development of the retina and retinal capillaries from the disc of the optic nerve to the periphery (2, 20). The increased expression of VEGF with an abnormal development of neovascularisations from the retina to the vitreous body is eventually accompanied by a growth of pathological fibrovascular tissue (48). Experimental studies have demonstrated that intravitreal injection of VEGF blockers enables significant inhibition of neovascularisation (1, 41).

Bevacizumab (Avastin; Genetech Inc., South San Francisco, California, USA), a non-selective anti-VEGF antibody, has been approved for the treatment of colorectal carcinoma and is also used in intravitreal application in the treatment of exudative age-related macular degeneration (ARMD) (45) and proliferative diabetic retinopathy (PDR) (5).
A range of clinical studies have demonstrated the beneficial effect of intravitreal injection of bevacizumab in the treatment of severe form of ROP in zones I-II, thus in those cases where conventional treatment by laser photocoagulation and cryotherapy is frequently unsuccessful (8, 12, 15, 25, 38, 39).

Pegaptanib sodium (Magugen®) – a selective RNA aptamer blocking the isoform VEGF-165 is successfully used in the treatment of neovascularisation in the case of ARMD, the ocular and systemic efficacy and safety of which have been demonstrated in a range of studies (4, 33, 46). The selective VEGF-165 isoform inhibitor – pegaptanib in intravitreal application should theoretically be a safer option for the treatment of severe forms of ROP in zones I-II. It blocks only the VEGF isoform responsible for pathological neovascularisation. Other VEGF isoforms which are necessary for the physiological development of nerve tissue and normal vasculogensis remain unaffected.

Retinopathy of prematurity (ROP) and its effects are frequently the cause of infant blindness in developed countries (50). ROP in zone I and posterior zone II occurs in approximately 10-20% of all prematurely born babies who require treatment. Even despite performed conventional treatment by laser photocoagulation or cryotherapy of stage ROP 3+ in zones I and II, the disorder may progress to structurally highly adverse changes – partial or total retinal detachment or formation of fibrotic retrolental tissue.
The CRYO-ROP study states 77.8% of eyes with an adverse anatomical result after cryotherapy and the ET-ROP study demonstrates 55.2% of eyes with an adverse anatomical finding following laser photocoagulation for threshold stage of ROP in zones I-II (12, 15).
Other clinical studies of the treatment of ROP in zones I-II also state a range of 40-77% failure of treatment as a consequence of final pathological changes on the fundus, despite adequately performed laser photocoagulation (25, 27, 38). In these cases the cause of failure of ROP treatment is considered to be aberrant angiogenesis, the mediator of which is VEGF-165 (16). A further source of increased synthesis of VEGF 165 upon ROP is macrophages in the vitreous area (36).

Although the optimum dose of bevacizumab for intravitreal application in the treatment of ROP is not precisely stipulated (range of 0.5 mg to 1.25 mg), the published clinical studies demonstrate an effective and safe dose of 2.5 mg intravitreally without systemic and ocular side effects and complications (17, 31). Experimental studies have demonstrated that doses of 0.5 to 2.5 mg of bevacizumab do not have an adverse effect on the value of visually evoked potentials and electroretinogram, and no toxic effect has been histologically demonstrated on the retina (9, 32, 44). The most frequent dose in clinical studies on the treatment of ROP has been 0.5-0.75 mg administered intravitreally (6, 10, 11, 43). In our sample of infants with ROP, an intravitreal injection of 0.5 to 0.625 mg of bevacizumab or 0.3 mg of pegaptanib was administered in a single dose. These doses were sufficient in order to contribute to a successful regression of severe forms of ROP in zone I – posterior zone II in combination with conventional laser photocoagulation in the majority of our patients.
Our results contribute to the opinion that intravitreal injection of the VEGF inhibitors bevacizumab and pegaptanib in the treatment of stage ROP 3+ need not be repeated.
Upon a systematic analysis of the bibliographical databases, a total of 9 publications were found in the international literature from the period 2007-2009, all of which provide information on the favourable effect of intravitreal application of bevacizumab in the treatment of a total of 48 prematurely born infants (77 eyes) with ROP.
Chung et al. (22) and Lalwani et al. (28) document successful treatment of stage ROP 3+ in zone I by intravitreal application of 0.75 mg and 0.63 mg of bevacizumab in combination with laser photocoagulation. In all eyes there was a regression and stabilisation of the disorder within the course of 3 months of observation following the procedure (22, 28).
The works of Travassos et al. (52) indicate the favourable influence of intravitreal injection of bevacizumab on the regression of neovascularisations in the area of the anterior segment and acceleration of resorption of the tunica vasculosa lentis as a preparation before laser photocoagulation for ROP 3 in zone II. Surprisingly, there was simultaneously a regression of neovascularisations in zone II following the injection of bevacizumab, and subsequent physiological development of retinal capillaries to the periphery, with the result that the finding did not require the originally planned laser photocoagulation (52).
The treatment of stage ROP 4A+ following unsuccessful laser photocoagulation with the help of an intravitreal injection of 0.4 mg of bevacizumab and subsequent persistence of retinal detachment is described by the work of Honda et al. (19). Although there was a marked regression of the neovascular component of the fibrovascular membranes following the injection, the persistent concentration of fibrotic membranes caused retinal detachment in the area of the posterior pole. This amotion was eventually successfully resolved by vitrectomy. No systemic or local complications of the injection of bevacizumab were recorded.
In their retrospective study, Kusaka et al. (26) present the results of 14 prematurely born babies (23 eyes) with ROP 3+ in zones I to II, who were treated by a combination of laser photocoagulation and intravitreal application of bevacizumab. Successful treatment with a favourable anatomical result was recorded in 15 eyes. An unfavourable final result with retinal detachment was recorded in 8 eyes.
A retrospective analysis of the evaluation of intravitreal application of 0.625 mg of bevacizumab as monotherapy without the use of laser photocoagulation on 22 eyes in 11 prematurely born infants with ROP in zones I-II is presented by the work of Mintz-Hittner et al. (35). In all eyes there was a regression of neovascularisations after a single injection, with the establishment of continual physiological vascularisation to the periphery of the retina.
Quiroz-Mercado et al. (54) document the results of a prospective study of 18 eyes in 13 patients with ROP, divided into 3 groups. The first group consisted of eyes with stage 4A or 4B ROP after unsuccessful conventional treatment. The second group comprised eyes in which optical media were not sufficiently clear for conventional treatment by laser photocoagulation. The third group contained eyes with pre-threshold or threshold stage ROP 3. In all cases a single dose of 1.25 mg of bevacizumab was applied intravitreally. In the first group regression to stage I was documented in 2 infants. In another 2 infants it was necessary to perform vitrectomy. In the second and third group all the infants recorded regression to stage I within 4 weeks, and after 12 weeks no signs of ROP were observed. No systemic or ocular complications of intravitreal application were recorded in the entire sample.
In the last 2 years promising results have been published not only of combined laser photocoagulation with intravitreal injection of bevacizumab, but also as anti-VEGF monotherapy.
Lee et al. (30) in their study evaluate combined treatment of 16 eyes (8 infants) with ROP stage 3 by laser photocoagulation with intavitreal injection of

bevacizumab (0.5 mg) as effective and safe. In all eyes they describe a complete regression within 2 weeks following the procedure and did not record any systemic and ocular complications.

Roohipoor et al. (42) used a combination of intravitreal injection of bevacizumab (0.625 mg) before or after laser photocoagulation in 12 eyes with ROP stage 3 in zones I-II. They describe successful regression of ROP during the course of 1-2 weeks in 10 eyes. In 2 eyes it was necessary to repeat laser coagulation within 1 month following the initial procedure, due to recurrence of neovascularisations. In all eyes intravitreal application of bevacizumab was well tolerated, and established rapid regression without the development of complications such as endophthalmitis or retinal detachment.

In 2011 Mintz-Hittner et al. (34) published results of the largest prospective randomised multicentric study to date, on a total of 150 prematurely born infants with ROP 3+ in zone I or posterior zone II. This study compares the effect of treatment of only intravitreal injection of bevacizumab (0.625 mg) and classic treatment by laser photocoagulation. The main evaluated parameter was the frequency of recurrence of ROP requiring a repeated procedure by the 54th week of post-conception age of the child. The authors demonstrated a significantly higher number of recurrences of ROP in zone I in the group of infants with conventional laser treatment (42%) than in the group of infants with intravitreal application of bevacizumab (6%) (P = 0.003). Upon a comparison of the number of recurrences in infants with ROP 3+ in posterior zone II no significant difference was found between both treatment modalities (laser 12% versus bevacizumab 5%) (P = 0.27). In the entire sample of infants (zone I and posterior zone II) a higher number of recurrences was found after laser therapy (26%) than upon monotherapy with bevacizumab (6%). The study demonstrated a beneficial therapeutic effect of intravitreal application of bevacizumab for regression of ROP 3+ especially in zone I.

Mintz-Hittner et al. (35) in their retrospective study of 22 eyes (11 infants) describe the effective treatment of ROP 3+ in zones I-II by only a single intravitreal injection of bevacizumab, without the necessity of laser therapy. In all the eyes in this small sample the treatment was successful, with establishment of regression of ROP. The average observation period was 48 weeks, and no local or systemic complications of this treatment were recorded.

Dorta et al. (13) provide information on the results of monotherapy by injection of a VEGF blocker in the treatment of 12 eyes in seven prematurely born babies (average birth weight 846 g, average gestation week 25.5) with ROP 3 in zone I (9 eyes) or in zone II (3 eyes). In all cases regression of ROP was achieved and the effectiveness of intravitreal injection of bevacizumab was confirmed. Because this sample of eyes is also very small, the author highlights the need for further, larger studies, which could confirm the efficacy of the promising treatment of ROP by intravitreal application of VEGF inhibitors, either as monotherapy or in combination with conventional laser photocoagulation.

Our presented study confirms that adjuvant intravitreal application of bevacizumab and pegaptanib in combination with conventional treatment of stage ROP 3+ in zones I-II halts the progression of pathological neovascular proliferation more quickly, enables more rapid clearance of the symptoms of “plus disease” (spread and tortuosity of retinal capillaries) and establishes physiological retinal vascularisation to the periphery more rapidly than conventional treatment by laser photocoagulation or cryotherapy alone. Another beneficial effect of intravitreal application of pegaptanib or bevacizumab is the lower occurrence of recurrence of neovascularisations with the attendant necessity of repeated therapeutic procedure. The most important result is the final favourable anatomical – structural finding on the fundus without affliction of the posterior pole in 90% of eyes in our sample. In comparison with 62% of eyes upon the use of conventional treatment, this difference is statistically significant.

Upon a comparison of the efficacy of intravitreal application of pegaptanib (60 eyes) and bevacizumab (32 eyes) in our sample, no statistically significant differences were found between both VEGF blockers in attaining a final favourable anatomical result on the fundus, in the time for clearance of the symptoms of plus ROP, or in the stimulation of normal peripheral retinal vascularisation.

In our study no ocular complications or systemic side effects of intravitreal application of bevacizumab or pegaptanib were recorded during the entire course of the observation period following surgery.

The results of our study demonstrate that intravitreal injection of the anti-VEGF preparations pegaptanib or bevacizumab in combination with photocoagulation by diode laser is effective and safe for the regression of stage ROP 3+ in zone I and posterior II, with the establishment of normal vascularisation of the peripheral retina and a beneficial structural result. Statistically significantly more favourable anatomical results were achieved by means of this combined therapy, with a lower occurrence of undesirable structural changes on the fundus.

In conclusion, it is possible to recommend the classification of intravitreal application of the anti-VEGF preparations pegaptanib or bevacizumab within the standard treatment protocol for prematurely born infants with the development of stage ROP 3+ in zones I-II. In future it is possible to expect a substantially lower percentage of children with severe visual impairment as a consequence of retinopathy of prematurity.

The study was conducted thanks to support of the grant project IGA MZ ČR (NS/9892-4)

LITERATURE

1. Aiello, L.P., Pierce, E.A., Foley, E.D., et al.: Suppression of retinal neovascularization in vivo by inhibition of vascular endothelial growth factor (VEGF) using soluble VEGF-receptor chimeric proteins. Proc Natl Acad Sci U S A 1995; 92: 10457–10461.
2. Alon, T., Hemo, I., Itin, A., Peter, J., Stone, J., Keshet, E.: Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. Nat Med 1995; 1: 1024–1028.
3. Altinsoy, H.I., Mutlu, F.M., Günör, R., Sarici, S.U.: Combination of laser photocoagulation and intravitreal bevacizumab in aggressive posterior retinopathy of prematurity. Ophthalmic Surg Lasers Imaging 2010; 27: 345–349.
4. Apte, R.S., Modi, M., Masonson, H., et al.: Pegaptanib 1-year systemic safety results from a safety--pharmacokinetic trial in patients with neovascular agerelated macular degeneration. Ophthalmology 2007; 114: 1702–12.
5. Arevalo, J.F., Garcia-Amaris, R.A.: Intravitreal bevacizumab for diabetic retinopathy. Curr Diabetes Rev 2009; 5: 39–46.

6. Avery, R.L., Pearlman, J., Pieramici, D.J. et al.: Intravitreal bevacizumab (Avastin) in the treatment of proliferative diabetic retinopathy. Ophthalmology, 2006; 113: 1695–1705.
7. Axer-Siegel, R., Snir, M., Cotlear, D., et al.: Diode laser treatment of posterior retinopathyof prematurity. Br J Ophthalmol 2000; 84: 1383–6.
8. Axer-Siegel, R., Snir, M., Cotlear, D, et al.: Diode laser treatment of posterior retinopathy of prematurity. Br J Ophthalmol 2000; 84: 1383–1386.
9. Bakri, S.J., Cameron, J.D., McCannel, C.A., Pulido, J.S., Marler, R.J.: Absence of histologic retinal toxicity of intravitreal bevacizumab in a rabbit model. Am J Ophthalmol, 2006; 142: 162–164.
10. Beer, P.M.,Wong, S.J., Hammad, A.M., Falk, N.S., O'Malley, M.R., Khan, S.: Vitreous levels of unbound bevacizumab and unbound vascular endothelial growth factor in two patients. Retina 2006, 26: 871–876.
11. Cryotherapy for Retinopathy of Prematurity Cooperative Group. Multicenter trial of cryotherapy for retinopathy of prematurity: preliminary results. Arch Ophthalmol 1988; 106: 471–9.
12. Cryotherapy for Retinopathy of Prematurity Cooperative Group. Multicenter trial of cryotherapy for retinopathy of prematurity. Three--month outcome. Arch Ophthalmol 1990; 108:195–204.
13. Dorta, P., Kychenthal, A. Treatment of type 1 retinopathy of prematurity with intravitreal bevacizumab (Avastin). Retina 2010; 30: Suppl: S24–S31.
14. Drenser, K.A., Tresem M.T., Capone, A. Jr.: Aggressive posterior retinopathy of prematurity. Retina 2010; 30: Suppl: S37–S40.
15. Early Treatment for Retinopathy of Prematurity Cooperative Group Revised indications for the treatment of retinopathy of prematurity: results of the Early Treatment for Retinopathy of Prematurity randomized trial. Arch Ophthalmol 2003; 121: 1684–94.
16. Flynn, J.T., Chan-Ling,T.: Retinopathy of prematurity: Two distinct mechanisms that underlie zone 1 and zone 2 disease. Am J Ophthalmol, 2006, 142: 46–59.
17. Fung, A.E., Rosenfeld, P.J., Reichel, E.: The International Intravitreal Bevacizumab Safety Survey: using the internet to assess drug safety worldwide. Br J Ophthalmol,2006, 90:1344–1349.
18. Gilbert, C.: Retinopathy of prematurity: a global perspective of the epidemics, population of babies at risk and implication for control. Early Hum Dev 2008; 84: 77–82.
19. Honda, S., Hirabayashi, H., Tsukahara, Y., Negi, A.: Acute contraction of the proliferative membrane after intravitreal injection of bevacizumab for advanced retinopathy of prematurity. Graefes Arch Clin Exp Ophthalmol 2008; 246: 1061–3.
20. Chan-Ling, T., Gock, B., Stone, J.: The effect of oxygen on vasoformative cell division. Evidence that 'physiological hypoxia' is the stimulus for normal retinal vasculogenesis. Invest Ophthalmol Vis Sci 1995; 36: 1201–1214.
21. Chen, J., Smith, L.E.: Retinopathy of prematurity. Angiogenesis 2007; 10: 133–140.
22. Chung, E.J., Kim, J.H., Ahn, H.S., Koh, H.J.: Combination of laser photocoagulation and intravitreal bevacizumab (Avastin) for aggressive zone I retinopathy of prematurity. Graefes Arch Clin Exp Ophthalmol 2007; 245: 1727–30.
23. International Committee for the Classification of Retinopathy of Prematurity. The International Classification of Retinopathy of Prematurity revisited. Arch Ophthalmol 2005; 123: 991–9. (Erratum, Arch Ophthalmol 2006; 124: 1669–70.)
24. Ishida, S.,, Usui, T., Yamashiro, K., et al.: VEGF 164-mediated inflammation is required for pathological, but not physiological, ishcemia-reduced retinal neovascularization. J Exp Med 2003, 198: 483–489.
25. Katz, X., Kychenthal, A., Dorta, P.: Zone Iretinopathy of prematurity. J AAPOS 2000; 4: 373–6.
26. Kusaka, S., Shima, C.,Wada, K., et al.: Efficacy of intravitreal injection of bevacizumab for severe retinopathy of prematurity: a pilot study. Br J Ophthalmol 2008; 92: 1450–5.
27. Kychenthal, A., Dorta, P., Katz, X.: Zone I retinopathy of prematurity: clinical characteristics and treatment outcomes. Retina 2006; 26: Suppl: S11–S15.
28. Lalwani, G.A., Berrocal, A.M., Murray, T.G., et al.: Off-label use of intravitreal bevacizumab (Avastin) for salvage treatment in progressive threshold retinopathy of prematurity. Retina 2008; 28: Suppl: S13–S18. (Erratum, Retina 2009; 29: 127.)
29. Law, J.C., Recchia, F.M., Morrison, D.G., Donahue, S.P., Estes, R.L.: Intravitreal bevacizumab as adjunctive treatment for retinopathy of prematurity. J AAPOS 2010;14: 6-10.
30. Lee, J.Y., Chae, J.B., Yang, S.J, Yoon, Y.H., Kim, J.G.: Effects of intravitreal bevacizumab and laser in retinopathy of prematurity therapy on the development of peripheral retinal vessels. Graefes Arch Clin Exp Ophthalmol 2010; 248: 1257–62.
31. Lynch, S.S., Cheng, C.M.: Bevacizumab for neovascular ocular diseases. Ann Pharmacother 2007, 41: 614–625.
32. Manzano, R.P., Peyman, G.A., Khan, P., Kivilcim, M.: Testing intravitreal toxicity of bevacizumab (Avastin). Retina, 2006, 26: 257–261.
33. Marcus, D.M.: VEGF Inhibition Study in Ocular Neovascularization Clinical Trial Group. Four-year safety of pegaptanib sodium in neovascular age-related macular degeneration (AMD): results of the V.I.S.I.O.N. trial. Invest Ophthalmol Vis Sci 2008; 49: E--abstract 5069.
34. Mintz-Hittner, H.A., Kennedy, K.A., and Chuang, A.Z.: Efficacy of Intravitreal Bevacizumab for Stage 3+ Retinopathy of Prematurity N Engl J Med 2011, 364;7: 603–615.
35. Mintz-Hittner, H.A., Kuffel, R.R. Jr.: Intravitreal injection of bevacizumab (Avastin) for treatment of stage 3 retinopathy of prematurity in zone I or posterior zone II. Retina 2008; 28: 831–8. (Erratum, Retina 2008; 28: 1374.)
36. Naug, H., Browning, J., Gole, G., Gobe, G.: Vitreal macrophages express VEGF165 in oxygen-induced retinopathy. Clin Exp Optom, 2000, 28: 48–52.
37. Nazari, H., Modarres, M., Parvaresh, M.M., Falavarjani, K.G.: Intravitreal bevacizumab in combination with laser therapy for the treatment of severe retinopathy of prematurity (ROP) associated with vitreous or retinal hemorrhage. Graefes Arch Clin Exp Ophthalmol 2010; 248: 1713–8.

38. O'Keefe, M., Lanigan, B., Long, VW.: Outcomes of zone I retinopathy of prematurity.Acta Ophthalmol Scand 2003; 81: 614–6.
39. Récsán, Z., Vámos, R., Salacz, G.: Laser treatment of zone I prethreshold andstage 3 threshold retinopathy of prematurity. J Pediatr Ophthalmol Strabismus 2003;40: 204–7.
40. Repka, M.X., Tung, B., Good, W.V., et al.: Outcome of eyes developing retinal detachment during the Early Treatment for Retinopathy of Prematurity Study (ETROP). Arch Ophthalmol 2006; 124: 24–30.
41. Robinson, G.S., Pierce, E.A., Rook, S.L., Foley, E., Webb, R., Smith, L.E.: Oligodeoxynucleotides inhibit retinal neovascularization in a murine model of proliferative retinopathy. Proc Natl Acad Sci U S A 1996; 93: 4851–4856.
42. Roohipoor, R., Ghassemi, H., Ghassemi, F., Karkhanech, R. et al.: Intravitreal bevacizumab in retinopathy of prematurity: an intervetional case series Graefes Arch Clin Exp Ophthalmol 2011; 2489: 1295–1301
43. Shah, P.K., Narendran,V.,Tawansy,K.A., Raghuram, A., Narendran, K.: Intravitreal bevacizumab (Avastin) for post laser anterior segment ischemia in aggressive posterior retinopathy of prematurity. Indian J Ophthalmol, 2007, 55: 75–76.
44. Shahar, J., Avery, R.L., Heilweil, G. et al.: Electrophysiologic and retinal penetration studies following intravitreal injection of bevacizumab (Avastin). Retina, 2006, 26: 262–269.
45. Schouten, J.S., La Heij, E.C., Webers, C.A., Lundqvist, I.J., Hendrikse, F.: A systematic review on the effect of bevacizumab in exudative age-related macular degeneration. Graefes Arch Clin Exp Ophthalmol 2009; 247: 1–11.
46. Singerman, L.J., Masonson, H., Patel, M., et al.: Pegaptanib sodium for neovascularage-related macular degeneration: third-year safety results of the VEGF Inhibition Study in Ocular Neovascularisation (VISION) trial. Br J Ophthalmol 2008; 92: 1606–11.
47. Smith, L.E.H.: Through the eyes of achild: understanding retinopathy throughROP: the Friedenwald lecture. Invest Ophthalmol Vis Sci 2008; 49: 5177–82.
48. Smith, L.E.,Wesolowski, E., McLellan, A., et al.: Oxygen induced retinopathy in the mouse. Invest Ophthalmol Vis Sci 1994; 35: 101–111.
49. Spencer, R.: Long-term visual outcomes in extremely low-birth-weight children (an American Ophthalmological Society thesis). Trans Am Ophthalmol Soc 2006;104: 493-516.
50. Stenkuller, P.G., Du, L., Gilbert, C et al.: Childhood blindness. J AAPOS 1999, 3: 26–32.
51. Storkebaum, E., Carmeliet, P.: VEGF: critical player in neurodegeneration. J.CLin Invest., 2004, 113: p. 14-18.
52. Travassos, A., Teixeir, S., Ferreira, P., et al.: Intravitreal bevacizumab in aggressive posterior retinopathy of prematurity. Ophthalmic Surg Lasers Imaging 2007; 38: 233–7.
53. Quinn, G.E., Dobson, V., Barr, C.C., et al.: Visual acuity of eyes after vitrectomy forretinopathy of prematurity: follow-up at5 1/2 years. Ophthalmology 1996; 103: 595–600.
54. Quiroz-Mercado, H., Martinez--Castellanos, M.A., Hernandez--Rojas, M.L, Salazar- Teran, N, Chan, R.V.: Antiangiogenic therapy with intravitreal bevacizumab for retinopathy of prematurity. Retina 2008; 28: Suppl: S19–S25.