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RETINAL DISORDERS

Intravitreal bevacizumab monotherapy for type-1 prethreshold, threshold, and aggressive posterior retinopathy of prematurity — 27 month follow-up results from Turkey

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Abstract
Purpose To study the efficacy of intravitreal bevacizumab (IVB) injection as a single treatment for retinopathy of prematurity (ROP).
Methods This was a prospective interventional case series study performed in a clinical practice setting; a total of 122 patients including prethreshold (type 1) (n =79, 152 eyes, six unilateral), threshold (n=12, 24 eyes), and aggressive posterior (APROP) (n=31, 62 eyes); cases were included without any randomization or masking. A total of 253 IVB injections, 238 in the first session, 11 in the second session, and four in the third session were performed, and followed up for a mean of 89.155±4.277 (range 82 to 105) weeks of postmenstrual age (PMA). Regression of ROP, maturation of the retina, and associated complications were evaluated.
Results Total regression was achieved in 227/238 eyes (95.4 %) after the first dose injection. The remaining 11 received a second injection, after which an additional seven (234/238; 98.2 %) regressed; after the third injection, the remaining 4 (238/238; 100 %) regressed. Complete retinal vascular maturation was achieved without any significant complications in all of the cases.
Conclusions IVB injection as monotherapy seems to be a very effective treatment modality for ROP. Based on timely intervention, IVB as a single treatment modality can salvage almost all ROP cases before stage 4.

Keywords Retinopathy of prematurity (ROP) · Anti-VEGF · Bevacizumab · Intravitreal injection

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Introduction

Retinopathy of prematurity (ROP) is a leading cause of childhood blindness in developing and developed countries [1]. CRYO-ROP (Cryotherapy for ROP) and ETROP (Early Treatment of ROP) studies demonstrated various successful outcomes after peripheral thermoablation (cryo/laser) of avascular retina. Cryo-ablation was successful in 75 % of the cases in the CRYO-ROP Study. After the cryo era, even with high success rates of up to 98 % depending upon the severity of the disease, laser was not effective, particularly in zone I and aggressive posterior ROP (APROP) cases [2–11].

Research data demonstrated the role of vascular endothelial growth factor (VEGF) in the pathogenesis of ROP [12–14], and anti-VEGF treatments were developed as another therapeutic option. Several studies demonstrated favorable outcomes after intravitreal anti-VEGF therapy as the first-line

treatment, combined or as a salvage therapy after laser for zone-1 disease or APROP [15–26]. Despite an ongoing debate about the possible systemic adverse effects, dramatically successful results were encouraging, and IVB monotherapy was considered an option for selected cases [27, 28].

ROP is an important health problem in Turkey. Based on the official statistics declared by the Ministry of the Interior General Directorate of Civil Registration and Nationality of Turkey, while there were 1,286,828 live births in 2012, there were 1,283,062 in 2013, representing a decrease of 0.3 % [29]. Worldwide, the preterm birth rate is estimated at approximately 11 percent (range of 5 percent in parts of Europe to 18 percent in parts of Africa), and approximately 15 million children are born preterm each year (range 12 to 18 million) [30]. Based on those figures, approximately 128,300 preterm infants were born in 2013 in Turkey. Although ROP is more frequent in extremely preterm infants, in Turkey all preterm infants of a gestational age (GA) equal to or less than 34 weeks are screened for ROP, and such a high number of preterm cases, together with having very few retina specialists who are experienced in ROP and particularly ROP laser treatment, further exacerbates the problem. Therefore, making the diagnosis and treatment of ROP easier is very important for countries with high numbers of ROP cases, such as Turkey. In this study, we performed a prospective analysis of anti-VEGF monotherapy as a single treatment modality for prethreshold (type-1), threshold and AP-ROP cases in Turkey.

Materials and methods

This is a prospective interventional case series study on the efficacy of intravitreal bevacizumab (IVB) monotherapy as a single treatment modality for ROP, and was performed at Istanbul Yedikule Surp Pirgic Armenian Hospital Ophthalmology Department between January 2012 and April 2014. All patients in need of treatment including type-1 prethreshold (n=79), threshold (n=12) and APROP (n=31) disease, which totaled 122 patients, were included, and patient inclusion was completed between January 2012 and March 2013.

All parents signed a detailed informed consent prepared according to the Declaration of Helsinki and approved by the local ethical committee of Cerrahpasa School of Medicine. In a clinical practice setting, without any masking or randomization procedure, all patients requiring treatment as prethreshold, threshold, and APROP were included. For some patients who were referred late, the severity of their disease was approaching the disease threshold defined in the CRYO ROP Study as zone I or II, with 5 contiguous or 8 cumulative clock hours of stage 3 disease and plus disease. Therefore, even though the definition of type-1 prethreshold [as zone I, any stage ROP with plus disease (plus is ≥2 quadrants in the ETROP Study), or zone I, stage 3 ROP with or without plus disease, or zone II, stage 2 or 3 ROP with plus disease] includes the threshold cases, if the patients had stage 3 disease with 5 contiguous or 8 cumulative clock hours with plus, they were grouped and named as threshold, whereas all other forms of type-1 prethresholds were grouped as prethreshold (See supplemental video-S1).

Single or multiple IVB injections, a total of 253 injections, were analyzed prospectively. No other treatment option, i.e., laser or vitrectomy surgery, was administered to any of the patients.

Patients were followed up for 1 week, 2 weeks, or 1 month intervals, with a mean follow-up period of postmenstrual age (PMA) 89.155±4.277 (range 82 to 105) weeks.

A positive response to IVB (regression of the disease) was noted as follows: (1) disappearance of rubeosis iridis with an adequate pupil dilation with a good retinal reflex, (2) decrease in retinal arterial and venous tortuosity and engorgement as regression of plus disease and disappearance of neovascular proliferations and vitreous haze, and (3) the appearance of vessels that continue to vascularize the avascular peripheral retina. Persistence or reappearance of plus disease and neovascularizations were noted as a negative response.

Additional injections were performed if there was an inadequate positive response (regression) at least based on 1-week intervals or if recurrence of the disease occurred.

For statistical analyses, NCSS (Number Cruncher Statistical System) 2007 & PASS (Power Analysis and Sample Size) 2008 Statistical Software (Utah, USA) programs were used, and statistical significance was assessed as $p<0.01$.

Intravitreal bevacizumab injection procedure After the eyes were prepared with 5 % povidone–iodine in a standard fashion, while visualizing the tip of the needle through the dilated pupil under binocular indirect ophthalmoscopic illumination, using a 30-G needle, 0.625 mg (0.025 ml) bevacizumab (Altuzan 100 mg/4 ml flacon, Roche, Turkey) was injected into the vitreous cavity approximately 1 mm behind the limbus via pars plicata under topical anesthesia. An experienced nurse helped to secure the infant during the procedure. All injections were performed by the same surgeon (Dr. H.Y). After the injections, retinal artery patency was checked. Topical antibiotic drugs were administered for 5 days (see supplemental video-S2).

Results

The mean GA (gestational age) and mean BW (birth weight) of the patients was 28.20±1.90 weeks (range 23 to 32) and 1005.46±305.90 grams (range 500 to 1850) respectively. The male to female ratio was 63/59. The mean treatment time was 34.46±1.85 (35.00) weeks of PMA overall and 34.42±1.86

(35.00), 36.00±2.00 (36.00), and 33.97±1.47 (34.00) in the prethreshold, threshold, and APROP groups respectively. All of the baseline characteristics are summarized in Table 1.

During the first injections, there was a zone-I predominance in all 238 eyes: 140/238 (58.8 %) were zone I, and 98/238 (41.2 %) were zone II. The zone-I predominance was present in the prethreshold (57.2 %) and APROP (79 %) groups, but not in the threshold (16.6 %) group.

Overall, the time from birth to the treatment day was a mean of 7 weeks for all groups; it was 7 weeks for the prethreshold group, 8.4 weeks for the threshold group, and 6 weeks for the AP-ROP group.

The mean follow-up period was until a postmenstrual age (PMA) of 89.155±4.277 weeks (range 82 to 105) overall; it was 87.683±3.774 (range 82 to 99) in the prethreshold group, 90.167±3.563 (range 87 to 100) in the threshold group, and 92.516±3.785 (range 86 to 105) in the AP-ROP group (Table 1).

In the first dose of injections, 116 bilateral and six unilateral (all in the prethreshold group), for a total of 238 injections were performed. All eyes in the threshold (n=12) and AP-ROP (n=31) groups were injected bilaterally in the first session; 11/238 (4.6 %) first dose injected eyes required a second dose, including 2/152 (1.32 %) in the prethreshold group, 4/24 (16.7 %) in the threshold group, and 5 /62 (8,1 %) in the AP-ROP group. By the third dose of injections, 4/138 (2, 9 %) first injected eyes, 2/24 (8,3 %) in the threshold group and 2/62 (3.2 %) in the AP-ROP group were treated (Table 1).

The success rates were 95.4 % after the first injection, 98.2 % after the second injection and 100 % after the third injection. The following findings are based on the subgroup analyses: the success rates in the prethreshold group were 98.7 % after the first injection and 100 % after the second injection, the success rates in the threshold group were 83.3 % after the first injection, 91.6 % after the second injection, and 100 % after the third injection, and in the AP-ROP group the success rates were 92.0 % after the first injection, 96.8 % after the second injection, and 100 % after the third injection (Table 1). No fluorescein angiography examinations were performed, and these success rates are based on indirect ophthalmoscopic findings.

After a total of 253 intravitreal IVB injections, no serious complications, such as retinal detachment, cataract, endophthalmitis, or intravitreal hemorrhage, were observed. There were only two types of non-serious complications observed throughout the study: (1) subconjunctival hemorrhages limited to the injection site (4.7 %), and (2) very tiny choroidal incarcerations into the needle penetration sites (1.2 %) observed as "black spots", which we referred to as the *"black dot sign"* and which were observed by the parents as well. The black dot sign was consistently observed throughout the study.

Discussion

In this study, we investigated the efficacy of IVB as a monotherapy single-treatment modality for all types of ROP requiring treatment including prethreshold (type-1), threshold, and APROP cases.

By grouping the patients in this manner, we aimed to determine the efficacy of IVB in cases diagnosed in the late stages involving serious intravitreal fibrovascular membranes in zone I or II, with 5 contiguous or 8 cumulative clock hours of stage 3 disease and plus disease. In Turkey, some of the retina specialists are apprehensive of using IVB for cases with intravitreal membranes that are referred in the late stages. Thus, clarifying the risk for such cases is very important for clinical practice.

Table 1 Therapeutic characteristics and results of IVB injections

	Prethreshold (n = 79)	Threshold (n = 12)	APROP (n = 31)	Total (n = 122)
Treatment week (PMA)	34.42 ± 1.86 (35.00)	36.00 ± 2.00 (36.00)	33.97 ± 1.47 (34.00)	34.46 ± 1.85 (35.00)
Timing of first injection (after birthday, in weeks), p**	6. 316 ± 1.629 (4–11) p = 0.614	8.666 ± 1.230 (8–12) p = 0.000	6.322 ± 1.535 (4–10) p = 0.017	6.549 ± 1.710 (4–12)
Timing of additional injections (weeks)				
From *1st to 2nd*	4 ± 0 (n = 2)	1 ± 0 (n = 4)	1.2 ± 0.45 (n = 5)	1.64 ± 1.21 (n = 11)
From *2nd to 3rd*	− (n = 0)	1.5 ± 0.70 (n = 2)	2.5 ± 2.12 (n = 2)	2.0 ± 1.41 (n = 4)
Follow-up period (PMA, weeks)	87.683 ± 3.774 (range 82 to 99)	90.167 ± 3.563 (range 87 to 100)	92.516 ± 3.785 (range 87 to 100) 4	89.155 ± 4.277 (range 82 to 105)
Success rates of injections				
1st dose	150/152 98.7%	20/24 83.3%	57/62 92.0%	227/238 95.4 %
2nd dose	152/152 100%	22/24 91.6%	60/62 96.8%	234/238 98.2%
3rd dose	–	24/24 100%	62/62 100%	238/238 100%

PMA: postmenstrual age, ROP: retinopathy of prematurity, APROP: aggressive posterior retinopathy of prematurity

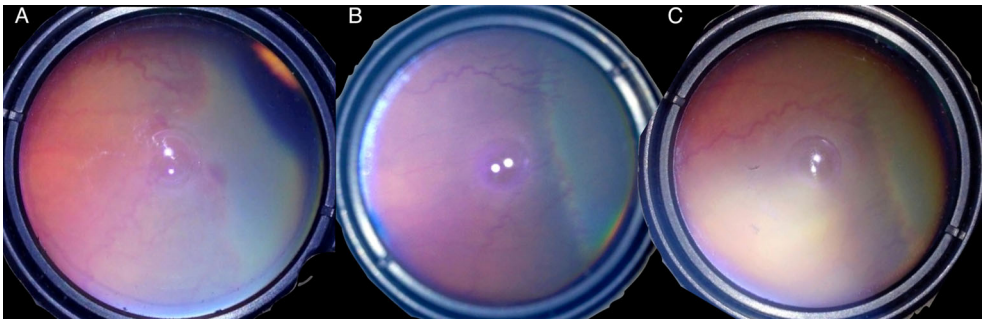

Fig. 1 Funduscopic images of a type-1 prethreshold case before (**a**), 1 week after (**b**) and 1 month after (**c**) a single dose of IVB

In terms of the number of cases, our study included 122 patients and 238 eyes comparable to both CRYO-ROP [2], with 247 children in 24 centers, and ETROP [3], with 401 patients in 26 centers. The Surp Pırgic Armenian Foundation Hospital is one of the main referral ROP clinics in Turkey, with approximately 2,000 referred ROP cases annually.

In terms of gestational age (mean 28.20±1.90 w, range 23 to 32 weeks) and BW (1,005.46±305.90 grams, 500–1,850 grams), the study patients were 3 weeks older and 300 grams heavier compared to those in the multicenter trials, because all of the patients in those trials weighed less than 1,251 grams, with a mean BW of 703±148 grams and a GA of 25.3± 1.4 weeks in ETROP. This is normal, because ROP screening criteria include babies that are heavier than 1,251 grams. The ETROP study included 54.4 % males, whereas our study has 51.6 %, which shows that both groups were similar.

The timing of the first injection was statistically significantly longer (p=0,000) in the threshold group compared to the prethreshold and APROP cases (Table 1). Therefore, the threshold patients are diagnosed significantly later than the prethreshold and APROP patients.

The number and time intervals of the additional injections, although they were not sufficient for statistical comparisons, demonstrated a more frequent and a shorter time period of re-injections in the threshold and AP-ROP groups compared to the prethreshold group (Table 1). The indication for the second injection in the prethreshold group (2/2; 100 %) was recurrence, but the indication was inadequate regression in both the threshold (4/4; 100 %) and APROP (4/5; 80 %) groups. Indications for third injections were inadequate regression in the threshold (2/2; 100 %) group and recurrence in the APROP (2/2; 100 %) group. Therefore, for threshold cases, disease regression seems to be the primary problem, but for the APROP cases in the early stages of the disease, regression may be easier than threshold but initial regression cannot guarantee full maturation without additional treatment. Furthermore, comparing classical ROP to a more severe form of the disease (prethreshold and threshold versus APROP), APROP treatment resulted in more cases of recurrence that occurred somewhat earlier [in five eyes after only 1.2 weeks (8.1 %,) and two eyes required a third injection within 2.5 weeks (3.2 %)]. This indicates that APROP is associated with a greater percentage of recurrences than the combined classical ROP groups [6/177 eyes (3.4 %) required a third injection in 2/177 eyes (1.1 %)]. This indicates a need to monitor APROP for recurrences more closely.

The overall success rates of the first, second, and third injections were 95.4 %, 98.2 % and 100 % respectively. The first injection success rates were 98.7 % for the prethreshold cases, 92.0 % for the APROP cases and 83.3 % for the threshold cases. After second injections, the success rates increased to 100 % for the prethreshold cases, 91.6 % for the threshold cases and 96.8 % for the AP-ROP cases. No third injection was required for the prethreshold group, but the third injection success rates reached 100 % for the AP-ROP and threshold cases (Figs. 1, 2, and 3). Because the photos are not captured by Ret Cam, they are not wide-field images and depict the 1-month post-injection outcomes.

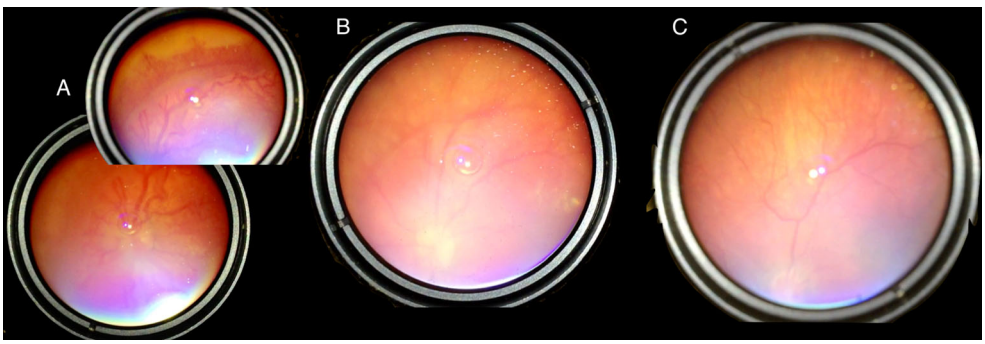

Fig. 2 Funduscopic images of a threshold case before (**a**, composite photo), 1 week after (**b**) and 2 months after (**c**) three doses of IVB

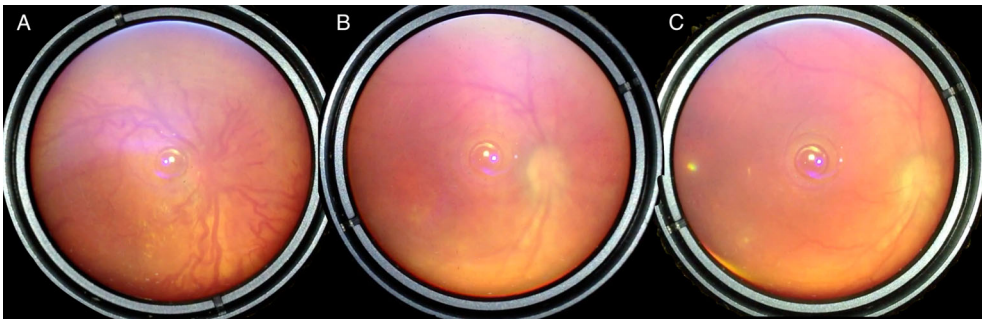

Fig. 3 Funduscopic images of an APROP case before (**a**), 1 week after (**b**) and 1 month after (**c**) a single dose of IVB

One of the most important contributions of this study to the literature is re-injections. Two main studies on IVB monotherapy for ROP in the literature, the BEAT ROP (Bevacizumab Eliminates the Angiogenic Threat of Retinopathy of Prematurity) Study and a multicenter Taiwanese Study, both investigated the results of a single dose of IVB without any additional injections but additional LPC when needed [15, 20]. Based on the use of IVB as the single treatment modality and the use of re-injections, our study is unique in the literature to date.

On the other hand, in terms of the follow-up period with a mean post-injection follow up of 51.683±3.570 (range 46 to 63) weeks until 87.683±3.774 (range 82 to 99) weeks of PMA, our study has a relatively long follow-up period after IVB injection therapy for ROP (Table 1).

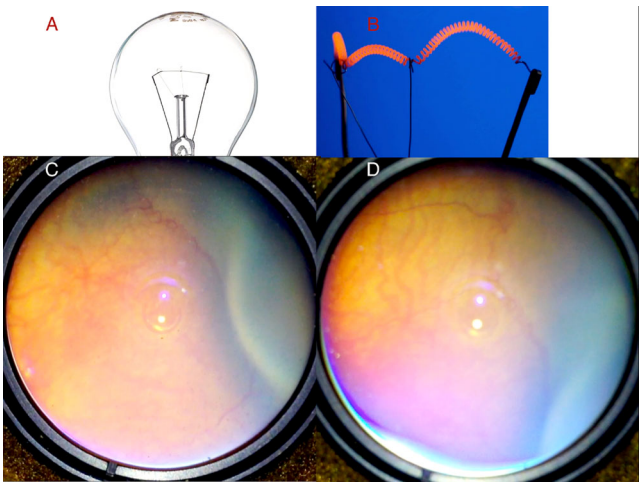

Fig. 4 In this picture, the tungsten filament sign is observed. At least 1 week after IVB, in most of the cases, the anterior segment becomes very silent, pupils can be fully dilated easily, the vitreous becomes crystal clear, ILM shines brightly under ophthalmoscopic light, and the demarcation line together with proliferations are replaced by a corkscrew-like communicating shunt-vessel between the main branching retinal vessels (**c** and **d**), like the tungsten filament in tungsten bulbs (**a** and **b**), which we named the *"tungsten filament sign"*; we consider this sign almost a pathognomonic sign of an anti-VEGF injected ROP in the presence of all above-mentioned anterior segment and posterior segments findings. This condition might be misdiagnosed as persistent plus disease or inadequate regression, but it is a sign of inactive disease and does not require any treatment

Consistent with the observations in the literature, IVB monotherapy provided a remarkable effect on the management of Zone I and AP-ROP in our study as well.

Compared to the laterality for the first injection, only six of 158 (3.79 %) eyes were treated unilaterally, and they were all in the prethreshold group (6/79; 7.59 %). Because the contralateral eyes were type-2 prethreshold, ROP disease required bilateral treatment in 96.21 % of the cases.

Another very important regression sign is the clarity of the vitreous. After injection, if adequate regression occurs, the vitreous becomes crystal clear, and the mature internal limiting membrane regains its normal bright shiny appearance. If this silent clinical picture can be achieved, any remaining isolated vascular tortuosity, twisting, or even twirling vasculature, without engorgement and hazy vitreous, should not be accepted as a sign of active disease. Therefore, persistent or reappearance of vascular engorgement with only persistent or reappearance of vitreous haze should be accepted as recurrence or inadequate regression, and an additional injection should be applied. Otherwise, even persistent stage 3 disease with intravitreal extensions of retinal vascularization, if not associated with vitreous haze and/or vascular engorgement, should not be accepted as a need for additional treatment. Those isolated persistent stage 3 areas will be observed to be separated from the retinal plane, and will resolve spontaneously during the follow-up period.

After IVB, for most of the cases, the demarcation line disappears, and a corkscrew-like communicating shunt-

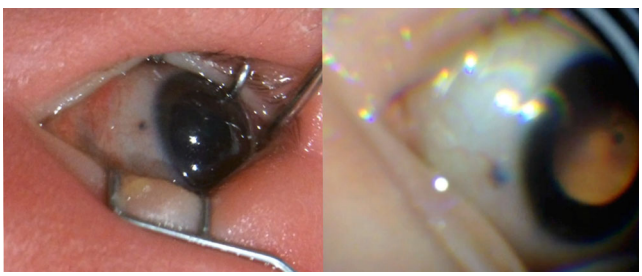

Fig. 5 In this picture, there seems to be a *black dot* 1 mm posterior to the inferonasal limbus that indicates a very tiny choroidal incarceration into the injection puncture site (**a** and **b**). In our cases, these remain stable, and have not yet disappeared

vessel becomes obvious between the main branching retinal vessels, like tungsten coil in tungsten bulbs; we named this sign the "*tungsten filament sign*" and consider this sign almost a pathognomonic sign of an anti-VEGF injected ROP (Fig. 4). Based on our experience with the referred cases, this clinical situation is misdiagnosed as incomplete regression or re-activation, and further treatment is offered. However, this is a sign of regression, and after a mean of 4.5–5 months of follow-up, the retina matures without any further treatment.

Anterior segment signs of the active ROP disease rapidly disappear after the injection. An easily and fully dilated pupil is another regression and successful treatment sign.

Another important observation of this study was that IVB injections did not induce and/or increase the contraction of ROP-associated vitreoretinal proliferations and membranes. Based on our experience as well as literature data, it is well-known that LPC for ROP can induce/increase contraction of ROP membranes, and IVB can have this effect in other proliferative diseases of the retina, i.e., diabetic retinopathy, BRVOs, and CRVOs. However, this was not the case for ROP. Typically, having no neovascular glaucoma or other associated neovascular complications that are characteristic of ischemic–neovascular retinal vascular diseases even in the latest stages of the disease, ROP is not a classical ischemic–neovascular disease of the retina. In contrast, intravitreal injection itself might have a liquefaction or softening effect on the compact vitreous gel of premature infants. Thus when injecting under indirect ophthalmoscopic visualization, it was noted that during the first injection, the drug spilled forcefully into the compact vitreous gel, during additional injections the drug easily spilled into a syneresis-like cavity.

The complication rate after injection was 5.9 %, with 4.7 % due to subconjunctival hemorrhage and 1.2 % due to pinpoint choroidal incarceration to the injection site, which we referred to as "black dot sign" (Fig. 5). For most of the cases, the parents became aware of this black dot and asked the doctor about it; it is likely that the use of "smaller" gauge (27 to 30 gauge) needles may decrease the rate of this complication. Therefore, we started to inform parents of this type of complication before the injections. In our experience, all of these cases remained as a black spot throughout the follow-up period. We think that these spots may disappear over the years as the sclera thickens.

Additionally, in terms of possible systemic IVB side-effects, with the neonatologists we observed a positive systemic course after IVB injections as the systemic parameters improve after IVB, i.e., decrease in oxygen dependency, more rapid advancement to oral nutrition, and weight-gain. We are still evaluating this very interesting clinical observation in conjunction with a possible pathogenic correlation between ROP, bronchopulmonary dysplasia (BPD), and necrotizing enterocolitis (NEC), to determine whether they are commonly driven by a VEGF-related abnormal (aberrant) vasculogenesis processes simultaneously at the retinal, pulmonary, and intestinal levels. Nevertheless, those are all our clinical observations, and it is impossible to completely exclude systemic side-effects because no systemic VEGF and bevacizumab levels were tested. On the other hand, the risk of the development of systemic adverse events may be higher with bevacizumab compared to other anti-VEGF drugs such as ranibizumab. Because ranibizumab has a higher affinity for VEGF than does bevacizumab, and as an antibody-binding fragment, it lacks the domain necessary to activate complement-mediated cytotoxicity or to interact with Fc receptors on immune cells. Therefore, bevacizumab is more likely to induce immunologic activation than ranibizumab. Thus, bevacizumab administration may lead to a higher risk of systemic adverse events.

In conclusion, in this study, IVB monotherapy as a single treatment modality for ROP experience in a clinical practice setting in Turkey showed promising successful results with ophthalmoscopically normal-appearing mature retina without ablation in any of the cases. IVB monotherapy as a single treatment modality with re-injections when needed seems to be safe and effective. However, it should be kept in mind that the results of this study depend on the indirect ophthalmoscopic examinations performed using 360-degree scleral indentation and do not include fluorescein angiography during a follow-up period that was limited to a mean postmenstrual age (PMA) of 89.155±4.277 weeks. Therefore, peripheral avascular zones cannot be totally excluded based on long-term follow-up. Those are all important weak points of the study. Although ROP management is based on indirect ophthalmoscopic examination in most cases worldwide, longer-term follow-up examinations are needed for IVB-treated cases. Longer-term follow-up will clarify whether those peripheral retinal areas remain normal or whether they will develop other unpredicted complications, require further treatment, or lead to peripheral retinal degenerations, holes, or tears.

However, those encouraging results indicate a potential shift in the main aim of ROP management from preserving vision to both preserving vision and retinal tissue; achieving this goal requires more support from more comprehensive studies with longer follow-up periods. On the other hand, there is no need for complex equipment, endotracheal anesthesia, and availability of bedside performance in a neonatal intensive care unit or in an office environment; this may make IVB superior to laser treatment in the management of ROP, particularly in countries with a high number of ROP cases, such as Turkey.

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Conflict of Interest There is no conflict of interest for any author. Authors have no proprietary interest in the material described in the article

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References

1. Gilbert C, Rahi J, Eckstein M, O'Sullivan J, Foster A (1997) Retinopathy of prematurity in middle-income countries. Lancet 350(9070):12–14
2. Cryotherapy for Retinopathy of Prematurity Cooperative Group (1988) Multicenter trial of cryotherapy for retinopathy of prematurity: preliminary results. Arch Ophthalmol 106(4):471–479
3. Early Treatment for Retinopathy of Prematurity Cooperative Group (2003) Revised indications for the treatment of retinopathy of prematurity:results of the early treatment for retinopathy of prematurity randomized trial. Arch Ophthalmol 121(12):1684–1694
4. International Committee for the Classification of Retinopathy of Prematurity (2005) The International Classification of Retinopathy of Prematurity revisited. Arch Ophthalmol 123(7):991–999
5. Morizane H (1976) Initial sign and clinical course of the most severe form of acute proliferative retrolental fibroplasias (type 1) [in Japanese]. Nippon Ganka Gakkai Zasshi 80(1):54–61
6. O'Keefe M, Lanigan B, Long VW (2003) Outcome of zone I retinopathy of prematurity. Acta Ophthalmol Scand 81(6):614–616
7. Onofrey CB, Feuer WJ, Flynn JT (2001) The outcome of retinopathy of prematurity: screening for retinopathy of prematurity using an outcome predictive program. Ophthalmology 108(1):27–35
8. Hatsukawa Y, Ueki N, Yamagishi T, Kiytajima H (2005) The effect of early treatment at the initial stage 3 moderate phase of retinopathy of prematurity on severe retinal changes. Jpn J Ophthalmol 49(5):391–396
9. Jalali S, Kesarwani S, Hussain A (2011) Outcomes of a protocol-based management of zone 1 retinopathy of prematurity: the Indian Twin Cities ROP Screening Program Report Number 2. Am J Ophthalmol 151(4):719–724
10. Yuka S, Fujino T, Hatsukawa Y (2008) Progression and timing of treatment of zone 1 retinopathy of prematurity. Am J Ophthalmol 146(3):369–374
11. Kychenthal A, Dorta P, Katz X (2006) Zone 1 retinopathy of prematurity. Retina 26(7):11–15
12. González Viejo I, Ferrer Novella C, Pueyo RV (2011) Use of anti-VEGF (anti-vascular endothelial growth factor) in retinopathy of prematurity (ROP). Arch Soc Esp Oftalmol 86(7):207–208
13. Romagnoli C (2009) Risk factors and growth factors in ROP. Early Hum Dev 85(10):79–82
14. Alon T, Hemo I, Itin A et al (1995) Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. Nat Med 1:1024–1028
15. Wu WC, Yeh PT, Chen SN, Yang CM, Lai CC, Kuo HK (2011) Effects and complications of bevacizumab use in patients with retinopathy of prematurity: a multicenter study in Taiwan. Ophthalmology 118(1):176–183
16. Chung EJ, Kim JH, Ahn HS, Koh HJ (2007) Combination of laser photocoagulation and intravitreal bevacizumab (Avastin) for aggressive zone 1 retinopathy of prematurity. Graefes Arch Clin Exp Ophthalmol 245:1727–1730
17. Mintz-Hittner HA, Kuffel RR (2008) Intravitreal injection of bevacizumab (Avastin) for treatment of stage 3 retinopathy of prematurity in zone 1 or posterior zone 2. Retina 28(6):831–838
18. Altınsoy HI, Mutlu FM, Gungor R, Sarici SU (2010) Combination of laser photocoagulation and intravitreal bevacizumab in aggressive posterior retinopathy of prematurity. Ophthalmic Surg Lasers Imaging 9:1–5
19. Law JC, Recchia FM, Morrison DG, Donahue SP, Estes RL (2010) Intravitreal bevacizumab as adjunctive treatment for retinopathy of prematurity. J AAPOS 14(1):6–10
20. Mintz-Hittner HA, Kennedy KA, Chuang AZ; BEAT-ROP Cooperative Group (2011) Efficacy of intravitreal bevacizumab (Avastin) for stage 3+ retinopathy of prematurity. N Engl J Med 364(7):603–615
21. Lee JY, Chae JB, Yang SJ, Yoon YH, Kim JG (2010) Effects of intravitreal bevacizumab and laser in retinopathy of prematurity therapy on the development of peripheral retinal vessels. Graefes Arch Clin Exp Ophthalmol 248(9):1257–1262
22. Mintz-Hittner HA (2012) Treatment of retinopathy of prematurity with vascular endothelial growth factor inhibitors. Early Hum Dev 88(12):937–941
23. Harder BC, Schlichtenbrede FC, von Baltz S, Jendritza W, Jendritza B, Jonas JB (2013) Intravitreal bevacizumab for retinopathy of prematurity: refractive error results. Am J Ophthalmol 155(6):1119–1124
24. Spandau U, Tomic Z, Ewald U et al (2013) Time to consider a new treatment protocol for aggressive posterior retinopathy of prematurity? Acta Ophthalmol 91(2):170–175
25. Nazari H, Modarres M, Parvaresh MM et al (2010) 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 248(12):1713–1718
26. Lalwani GA, Berrocal AM, Murray TG et al (2008) Off-label use of intravitreal bevacizumab (Avastin) for salvage treatment in progressive threshold retinopathy of prematurity. Retina 28(3):13–18
27. Fierson WM, American Academy of Pediatrics Section on Ophthalmology, American Academy of Ophthalmology, American Association for Pediatric Ophthalmology and Strabismus, American Association of Certified Orthoptists (2013) Screening examination of premature infants for retinopathy of prematurity. Pediatrics 131(1):189–195, originally published online December 31, 2012. DOI: 10.1542/peds.2012-2996
28. Kashani AH, Drenser KA, Capone Jr A (2014) Retinopathy of prematurity. In: Yanoff M, Duker JS (eds) Ophthalmology, 4th Edition. Elsevier Saunders, pp 535–540
29. http://www.turkstat.gov.tr/PreHaberBultenleri.do;jsessionid=PJhDTnPKfGBrjQQGqRstp6NpTt9GpvCTMBFGL1mQJqnr2QWJp2FJ!-568420742?id=16048 Accessed 20 Aug 2014
30. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, Adler A, Vera Garcia C, Rohde S, Say L, Lawn JE (2012) National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet 379(9832):2162–2172