Follow-up to Age 4 Years of Treatment of Type 1 Retinopathy of Prematurity Intravitreal Bevacizumab Injection versus Laser: Fluorescein Angiographic Findings

Domenico Lepore, MD,1 Graham E. Quinn, MD, MSCE,4 Fernando Molle, MD,1 Lorenzo Orazi, MD,1 Antonio Baldascino, MD,1 Marco H. Ji, MD,1 Maria Sammartino, MD,2 Fabio Sbaraglia, MD,2 Daniela Ricci, MD,3 Eugenio Mercuri, MD3

Purpose: To compare structural outcome at age 4 years of eyes treated with intravitreal injection of bevacizumab with fellow eyes treated with conventional laser photoablation in type 1 retinopathy of prematurity (ROP).

Design: Single, randomized, controlled trial.

Participants: All inborn babies with type 1 zone 1 ROP at the Neonatal Intensive Care Unit of the Catholic University, Rome, from September 1, 2009, to March 31, 2012.

Methods: In 21 infants (42 eyes), 1 eye was randomized to receive an intravitreal injection of 0.5 mg bevacizumab; the fellow eye underwent conventional laser photoablation. Digital retinal imaging and fluorescein angiography (FA) were performed at an average of 4 years after treatment in follow-up after these studies performed at treatment and 9 months.

Main Outcome Measures: Fluorescein angiograms were examined by 2 experts to document retinal and choroidal findings.

Results: Among the 20 bevacizumab-treated eyes available at 4 years of age, all showed abnormalities at the periphery (avascular area, vessel leakage, shunts, abnormal vessel branching, and tangles) or the posterior pole (hyperfluorescent lesions, absence of foveal avascular zone). These lesions were not observed in the majority of the lasered eyes. Among the 19 laser-treated eyes, leakage was noted in 1 eye, shunts and tangles were noted in 3 eyes, and macular abnormalities were noted in 3 eyes.

Conclusions: Fluorescein angiography has shown potentially serious and long-term ocular effects that are present more commonly after treatment with bevacizumab for acute-phase ROP than after laser.

Based largely on the results of 2 large randomized clinical trials conducted over the last 30 years, ablation of the peripheral avascular retina is the current standard treatment for retinopathy of prematurity (ROP). However, treatment outcomes from severe retinopathy in zone I need to be improved. For example, zone I stage 3 with or without plus disease eyes treated in the Early Treatment for Retinopathy of Prematurity (ETROP) trial had a 30.4% likelihood of developing unfavorable visual acuity outcome (worse or equal to 20/200) on long-term follow-up at 6 years. As survival of very low—birth-weight infants increased, zone I ROP requiring treatment has become more frequent and other modalities of treatment are being considered. Studies of experimental models for ROP have clearly demonstrated a rationale for the use of anti—vascular endothelial growth factor (VEGF) drugs to prevent progression of serious ROP.

Since 2007, a number of reports have appeared on the use of intravitreal bevacizumab (IVB) in ROP requiring treatment as monotherapy, in combination with laser, or as a rescue treatment in combination or before vitrectomy. The multicenter clinical trial Bevacizumab Eliminates the Angiogenic Threat of Retinopathy of Prematurity study reported a significant reduction of zone I ROP recurrence with a single intravitreal injection of approximately half the adult dose (0.625 mg of bevacizumab in 25 µl of balanced saline solution) compared with conventional laser therapy (6% vs. 42%). After this report, there has been a series with small numbers of subjects or case reports on the use of anti-VEGF drugs, mainly bevacizumab. Wallace et al., on behalf of the Pediatric Eye Disease Investigational Group, recently demonstrated that a dose of bevacizumab as low as 0.031 mg was effective in the treatment of type 1 ROP. Reports of ranibizumab monotherapy also documented successful treatment of severe ROP using doses between 0.2 mg and 0.3 mg. This drug was selected largely on the basis of the finding that the drug has a shorter duration of systemic exposure compared with bevacizumab.

http://dx.doi.org/10.1016/j.jophtha.2017.08.005
ISSN 0161-6420/17

© 2017 by the American Academy of Ophthalmology
Published by Elsevier Inc.
There are several advantages of the use of anti-VEGF drugs in eyes with high-risk ROP characteristics: easy and quick injection under topical anesthesia, rapid diminution of retinal vascular dilatation and tortuosity, and the possibility of preservation of visual field and lower degrees of myopia in the long run.24 These considerations, combined with some practical considerations, such as laser equipment and well-trained ophthalmologists, may not be readily available in some regions, and the relatively low cost of bevacizumab led to the rapid proliferation of the use of anti-VEGF for ROP treatment in many countries.

Although use of these drugs could represent an important improvement in the therapy of severe ROP, concerns arose about short- and long-term local and systemic adverse effects.25,26 Drug safety, both for adults16 and for premature infants, needs to be a primary concern. For example, a Canadian Neonatal Network report from Morin et al27 on developmental outcomes in a nonrandomized cohort of infants who had received intravitreal injections of bevacizumab compared with a cohort who underwent treatment with laser photocoagulation alone documented a 3.1-times higher risk of severe neurodevelopmental disabilities at 18 months in the 27 IVB infants versus the 98 laser-treated infants. In addition, Lien et al28 recently reported an observational study of 61 infants treated with IVB alone, laser alone, or both. They found that infants treated with bevacizumab and laser had a 5.3-times higher risk of severe psychomotor delays than the laser alone group. Although neither of these studies is a randomized trial, they do add urgency, in agreement with many other investigators, to the need to understand adverse systemic effects of the use of an antiangiogenic in developing neurons, lungs, and other systems of a premature infant.

Thus far, adverse ocular effects have been mainly due to fibrotic reaction after intravitreal injection.31–33 In 2014, we reported a series of fluorescein angiography (FA) studies in a case series of infants who developed type 1, zone I ROP in both eyes. For each infant, 1 eye was randomly assigned to receive bevacizumab in 1 eye and conventional laser photocoagulation in the fellow eye within 24 hours of diagnosis of type 1 ROP. In this case series, IVB-treated eyes had significant residual vascular and macular abnormalities compared with laser-treated eyes on FA 9 months after treatment.34 The purpose of the current report is to present further follow-up results of FA studies,34 along with digital retinal images, of the entire study population (13 from the original report and an additional 8 enrolled from January 1, 2011, to March 31, 2012) 4 years after treatment.

**Methods**

This is a single-center, randomized, controlled trial conducted at the Catholic University in Rome. All inborn infants with type 1 zone I ROP, as defined by ETROP criteria,3 were enrolled in the study after informed consent was obtained from the parent or guardian.

Within a maximum interval of 24 hours after diagnosis of type 1, zone I ROP, all infants underwent general anesthesia for evaluation and treatment.35 For each infant, 1 eye was randomly assigned, using a random number series, to undergo conventional laser photoablation of the peripheral avascular retina while the fellow eye received an intravitreal injection of 0.5 mg bevacizumab in a 0.02-ml balanced salt solution. Before treatment, digital retinal images were obtained using the RetCam imaging system (Clarity Medical Systems, Pleasanton, CA) and then video-digital FA, used as a part of the screening protocol for zone I ROP, was performed using a bolus of 10% fluorescein solution intravenously administered at a dose of 0.1 ml/kg, followed by an isotonic saline flush.33–38 The eye assigned to conventional laser was treated first, and the FA was examined by the treating physician before laser photocoagulation to provide more detailed information about the status of the eye and to indicate areas of the retina that might be treated. The fellow eye was then prepared using 5% povidone/fiode and topical antibiotic, and 0.5 mg (0.02 ml) of bevacizumab was injected intravitreally through the pars plicata. After the injection, intraocular pressure and retinal artery perfusion were checked, and patients received topical tobramycin for 3 days.

After treatment, binocular indirect ophthalmoscopy (BIO) and digital RetCam imaging were performed every 3 days along with FA every 2 weeks until discharge. After discharge from the neonatal intensive care unit, BIO was performed every 2 weeks until 52 weeks postmenstrual age and then monthly until 1 year of age. Infants underwent FA at 4 years of age under general anesthesia.

Angiograms were examined retrospectively by 2 experienced individuals (DL, FM) for the following characteristics as described by Lepore et al.38 Branching abnormalities were considered present if at least 1 quadrant of the eye showed the presence of tangles and shunts. The same criteria were used to assess the capillary loss within the vascularized retina and the posterior pole. Absence of foveal avascular zone and/or the presence of hyperfluorescent lesions and/or pigmented epithelium dystrophy were considered as macular abnormalities. If only large linear choroidal vessels without choriocapillaries were observed in the early FA phases, linear filling pattern was reported.

The Institutional Review Board at the Catholic University of the Sacred Heart of Rome approved the study protocol, and the trial was registered at the EudraCT number 2009-012609-20, protocol number 343/09 April 24, 2009. Group differences were examined using the Fisher exact test.

**Results**

From September 1, 2009, to March 31, 2012, 21 inborn preterm infants who underwent ROP examinations using BIO in the neonatal intensive care unit at the Agostino Gemelli University Hospital developed type 1, zone I ROP in 1 or both eyes and required treatment, according to ETROP criteria. Eight eyes (4 infants; mean birth weight 697 g, range, 380–755 g; mean gestational age 25.3 weeks, range, 22.7–29.3 weeks) were classified as zone I stage 3 with plus disease; 34 eyes (17 infants; mean birth weight 667 g, range, 380–960 g; mean gestational age 25.6 weeks, range, 22.7–29.3 weeks) were classified as zone I stage 3 without plus.

One eye treated with conventional laser progressed to a complete retinal detachment within 4 weeks after treatment. In addition, 1 infant died of pulmonary complications at 3 months of age.

Therefore, FA images of 20 eyes in the bevacizumab-injected group and 19 in the laser-treated group were available for evaluation by 2 different ROP experts. The FA results before treatment and at 9 months post-treatment have been reported, and included in this report are images from the same group of patients at age 4 years (mean post-conceptional age [PCA], 248 weeks; range, 233–275 weeks PCA).
1. Vascular features of the avascular-avascular junction before treatment and at age 4 years: As previously described, the majority of the eyes in this study before treatment showed hyperfluorescence (popcorn abnormalities, focal capillary dilatation, cotton wool, capillary tuft, string of pearls) and hypofluorescent lesions (capillary loss, periarteriolar capillary-free zone).\textsuperscript{34,38} In this case series, all eyes before treatment showed irregular branching (Table 1), starting at various distances from the optic disk. As previously reported, a common feature in both groups before treatment (94.9%, 37/39 eyes) was the presence of arteriolar-venular shunts, frequently running along the avascular-vascular junction, and leakage in the area of junction between avascular and vascular retina (94.9%, 37/39 eyes). Vascular tangles were observed in the majority of the eyes (92.3%, 36/39 eyes).

<table>
<thead>
<tr>
<th></th>
<th>Branching Abnormalities</th>
<th>Tangles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Treatment</td>
<td>9 Mos After</td>
</tr>
<tr>
<td>Avastin</td>
<td>N = 20</td>
<td>20 (100%)</td>
</tr>
<tr>
<td>Laser</td>
<td>N = 19</td>
<td>19 (100%)</td>
</tr>
<tr>
<td></td>
<td>Leakage</td>
<td>Before Treatment</td>
</tr>
<tr>
<td>Avastin</td>
<td>N = 20</td>
<td>19 (95.0%)</td>
</tr>
<tr>
<td>Laser</td>
<td>N = 19</td>
<td>18 (94.7%)</td>
</tr>
<tr>
<td></td>
<td>Shunts</td>
<td>Before Treatment</td>
</tr>
<tr>
<td>Avastin</td>
<td>N = 20</td>
<td>18 (90.0%)</td>
</tr>
<tr>
<td>Laser</td>
<td>N = 19</td>
<td>18 (94.7%)</td>
</tr>
</tbody>
</table>

Figure 1. Montage of the fluorescein angiography (FA) images showing various features of the junction between vascular and avascular retina in 4-year-old preterm children who received bevacizumab in this eye for type 1 acute-phase retinopathy of prematurity (ROP). A–C, Large areas of avascular retina. A, Mild leakage (white arrows) at the junction between vascular and avascular retina; leakage is more evident in B where an arteriolar-venular shunt at the junction between vascular and vascular retina (black arrows) is present together with hyperfluorescent areas (black dotted circles) and leakage from the vessels inside the vascularized retina (white dotted circles). These areas are evident in C. D, Less-extensive peripheral avascular retina with a dichotomous branching of the peripheral retinal vessels (gray circle).
Figure 2. Montage of fluorescein angiography (FA) images of 6 different 4-year-old preterm children who received bevacizumab in this eye for type 1 acute-phase retinopathy of prematurity (ROP). A–E, Various branching anomalies of the peripheral retinal vessels at various distances from the junction between vascular and avascular retina. The anomalies shown in A, D, and E are finger shaped (grey dotted circles). B, D, and E, Arteriolar-venular shunts between vessel arcades (black arrows). C, Leakage at the junction between vascularized and avascular retina (black dotted circles). F, Hypofluorescent areas at the junction (black circle) and inside the vascularized retina (white circle).

Figure 3. Fluorescein angiography images showing the junction between vascularized and avascular retina in a baby born at 24 weeks gestational age, before treatment, and at 9 months after treatment (A and B, bevacizumab injected; D and E, laser treated), as shown in Figure 1 of our earlier publication.15 Angiograms taken at 4 years have been added (E, bevacizumab-injected eye; F, laser-treated eye). In the eye injected with bevacizumab at 9 months follow-up (B), there was persistence of avascular retina together with hypofluorescent areas (black circles), capillary tufts (white arrow), and peripheral shunt at the junction between vascularized and avascular retina (black arrow). At 4 years of age (C), vascularization is incomplete, there is a large shunt connecting both temporal branches (black arrows), finger-like peripheral branching (gray dotted circle), and some capillary dropout among vessels (black circle). The chorioretinal scar tissue of the conventional laser treatment is similar in E and F with some hypofluorescent areas near the scar (black circle).
Fluorescein angiography at 4 years of age showed that the IVB eyes continue to have extensive areas of non-vascularized peripheral retina (Figs 1A–D and 2A–C, F), whereas the lasered eyes all showed the typical chorioretinal atrophy due to the treatment (Figs 3D, F and 4D, F). In the IVB eyes, the dichotomous branching pattern (Fig 1D) is present in all but 3 eyes at age 4 years (85.0%), and abnormal vascular tangles persist in 15 of 18 eyes that had abnormal tangles before treatment (83.4%) (Table 1; Fig 2A–E). In many cases (65%, 13/20 eyes), some leakage persists in the area of the junction between vascular and avascular retina (Figs 1A–C and 2C). Furthermore, all but 1 of the 18 IVB eyes that had arteriolar-venular shunts at treatment continued to show this abnormality at long-term follow-up: In some cases, shunts cross the median raphe connecting different retinal vessel branches (Figs 3B, C and 5A–C).

2. Abnormalities within the vascularized retina before treatment and at age 4 years: As shown in Table 2, a massive loss of retinal capillary bed at the posterior pole or in the periphery but within the vascularized retina was a common finding (100% of the eyes) in the pretreatment FA (Figs 3A, D and 4A, D). This feature persists at age 4 years in 15 of the 20 IVB eyes (75.0%) (Figs 3C and 6A–D). In contrast, just 2 of the 19 laser-treated eyes (10.5%) showed hypofluorescent lesions within the vascularized retina (Fig 3F).

Figure 4. Fluorescein angiography images of the posterior pole retina in a baby born at 25 weeks gestational age, before treatment, and 9 months after treatment (A and B, bevacizumab injected; D and E, laser treated) as shown in Figure 2 of our earlier publication. Angiograms taken at 4 years have been added (C, bevacizumab-injected eye; F, laser-treated eye). Both eyes at the time of treatment (A and C) show absence of foveal avascular zone and hypofluorescent areas, persisting after injection (B, white circle). Anomalies of vessel branching with finger-like anastomoses (grey dotted circles) are observed in the bevacizumab-injected eye (C), presumably starting from the point the junction between vascular and avascular retina. Similar scarring from photocoagulation is observed at 9 months and 4 years (E and F).

Figure 5. Montage of the features of shunts (A–C; black arrows) observed in various eyes treated with a single injection of bevacizumab at 4 years. B, Finger-like anastomosis seems to start from the shunt (grey dotted circle).
Table 2. Lesions within Vascularized Retina in Bevacizumab versus Laser Groups: Number and Percentage of Eyes with Abnormalities

<table>
<thead>
<tr>
<th>Macular Abnormalities (Hyperfluorescent Lesions)</th>
<th>Capillary Bed Loss (Central or Peripheral)</th>
<th>Linear Choroidal Filling Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Treatment</td>
<td>9 Mos After</td>
<td>4 Yrs After</td>
</tr>
<tr>
<td>Avastin N=20</td>
<td>18 (90.0%)</td>
<td>13 (65.0%)</td>
</tr>
<tr>
<td>Laser N=19</td>
<td>19 (100%)</td>
<td>4 (21.1%)</td>
</tr>
</tbody>
</table>

Macular abnormalities (hyperfluorescent lesions) were observed in both groups with FA at the posterior pole before treatment (37/39 eyes, 94.87%). At age 4 years, these lesions persist in 55% (10/18 eyes) of the IVB eyes compared with 16.0% (3/19 eyes) of the laser-treated eyes (Figs 7A–C and 8D).

3. Choroidal filling pattern before treatment and at age 4 years: Data on choroidal filling pattern are provided for the 2 treatment groups in the last 2 columns of Table 2. As previously noted,34,38 the presence of a linear choroidal pattern is associated with an immature choroid and retina vascular system. In this series, the majority of eyes (36/39 eyes, 93.9%) showed a linear choroidal filling pattern, confirming the observation that filling of the choriocapillaries with more physiologic lobular structure was largely absent in both groups before treatment. This immature pattern persists in 11 of 20 eyes injected with bevacizumab at 4 years of age (Figs 6C, D and 8D); at the same time point, the more mature lobular (Fig 3F) pattern is found in 18 of 19 laser-treated eyes (94.7%).

Comments

The purpose of this report is to determine whether the findings we previously reported at age 9 months in eyes treated with IVB or laser persisted into childhood. The major findings at an average age of 4 years of eyes of infants with type 1 ROP treated with IVB are the persistence of large areas of avascular retina and persistence of shunts in the periphery, suggesting continued abnormalities of circulation. Further, vessel leakage is still noted in 65% of the 20 IVB eyes. Both abnormal vessel branching and vascular tangles persist in more than 80% of the 20 eyes. When

Figure 6. Montage of the features observed in several eyes treated with a single injection of bevacizumab at 4 years after treatment; large area of retinal capillary obliteration (white circle) in the vascularized retina at 13 seconds from injection (A); lobular filling pattern of the choroid with diffuse hypo-fluorescent areas (white circles) at 16 seconds from injection (B); linear choroidal filling pattern with hypo-fluorescent areas (white circles), possibly related to abnormal choriocapillaries perfusion, at 11 seconds after injection (C); diffuse capillary obliteration outside vascular arcades (white circles) at 11 seconds after injection (D).
considering findings in the posterior retina, macular abnormalities persist in half of the IVB eyes with a similar reduction in choroidal blood flow abnormalities.

Considering the laser-treated fellow eyes of these children at age 4 years, there is evidence of peripheral retinal ablation with vessel leakage noted in 1 of 19 eyes on FA, abnormal shunts in 2 eyes, and vascular tangles found in 1 eye. There continued to be macular abnormalities, such as hyperfluorescent lesions, noted in 3 of the 19 laser-treated eyes. There was persistent linear choroidal filling in 1 eye.

Study Limitations

There are several limitations in this study. These include the within-subject randomization that, because IVB has been shown to leak from the eye into the systemic circulation, may have decreased the likelihood of finding differences between fellow eyes. The prevalence of abnormalities also may be related to the dose of bevacizumab used in this study, although it was lower than that used in the Bevacizumab Eliminates the Angiogenic Threat of Retinopathy...
of Prematurity study. Masking was not possible when examining peripheral abnormalities after treatment in these eyes. In addition, a more detailed description of the macular abnormalities using visual acuity measures and optical coherence tomography would improve our understanding of the implications of the structural abnormalities noted on FA.

One of the criticisms of this work might be that the number of eyes treated without plus disease is more than would be expected in eyes with type 1 disease. However, with the use of FA, extraretinal neovascularization is more easily noted and thus stage 3 ROP can be diagnosed earlier in such eyes. This could help explain the number of treated eyes with zone I ROP without plus disease. In addition, use of FA to determine more precisely those retinal areas in need of laser treatment may have led to fewer structural sequelae after laser treatment.

The use of additional modalities to describe abnormalities seen in eyes with ROP will likely affect the traditional classification of ROP that is based on the appearance of the fundus using the indirect ophthalmoscope or digital fundus photographs. Including techniques such as FA and OCT in describing ROP changes may eventually influence the timing and types of eyes that need treatment. It is certainly well understood that some eyes with type 1 ROP would have regressed without treatment, and it may well be that using additional methods to describe the abnormalities will allow identification of those eyes that would have regressed without treatment.

**Conclusions**

The use of FA in this study has allowed the description of potentially serious and long-term ocular effects from treatment of acute-phase ROP that are more frequent with IVB than with laser photoablation. Many of the abnormalities noted would not have been detected without the use of this additional modality, and many of these findings are worrisome. It is incumbent on the medical community caring for the premature infant to determine the optimum treatment regimen for serious ROP, not only from an ocular perspective but also from a systemic perspective.

**References**

24. Geloneck M, Chuang A, Clark L, et al. Refractive outcomes following bevacizumab monotherapy compared with


Footnotes and Financial Disclosures

Originally received: April 16, 2017.
Final revision: July 19, 2017.
Accepted: August 2, 2017.
Available online: ■■■. Manuscript no. 2017-897.

1 Department of Ophthalmology, Catholic University of Sacred Heart, Rome, Italy.
2 Department of Anesthesiology, Catholic University of Sacred Heart, Rome, Italy.
3 Department of Pediatric Neurology, Catholic University of Sacred Heart, Rome, Italy.
4 Division of Pediatric Ophthalmology, The Children’s Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania.

Financial Disclosure(s):
The author(s) have made the following disclosure(s): Since 2015 (3 years after the end of this study recruitment), D.L. is a paid member of the Steering Committee of the Rainbow Study (Clinical Trial Protocol CRFB002H2301E1) sponsored by Novartis.

Author Contributions:
Conception and design: Lepore, Quinn, Molle, Sammartino, Sbaraglia, Ricci, Mercuri
Data collection: Lepore, Molle, Orazi, Baldascino, Ji, Sammartino, Sbaraglia, Ricci, Mercuri
Analysis and interpretation: Lepore, Quinn, Molle, Orazi, Ji, Ricci, Mercuri
Obtained funding: Not applicable
Overall responsibility: Lepore, Quinn

Abbreviations and Acronyms:

**BIO** = binocular indirect ophthalmoscopy; **ETROP** = Early Treatment for Retinopathy of Prematurity; **FA** = fluorescein angiography; **IVB** = intravitreal bevacizumab; **ROP** = retinopathy of prematurity; **VEGF** = vascular endothelial growth factor.

Correspondence:
Domenico Lepore, MD, Department of Ophthalmology, Catholic University of Sacred Heart, Largo Francesco Vito 8, 00168 Rome, Italy. E-mail: domenico.lepore@unicatt.it.