A Spectrum of Regression Following Intravitreal Bevacizumab in Retinopathy of Prematurity

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• PURPOSE: To describe an improved understanding of the regression patterns following off-label intravitreal bevacizumab (IVB) treatment for retinopathy of prematurity (ROP).

• DESIGN: Retrospective cohort study.

• METHODS: All infants treated with IVB for type 1 ROP at a single institution from June 2013 to March 2018 were retrospectively reviewed and the amount of retinal nonperfusion on fluorescein angiogram was calculated.

• RESULTS: In the 92 eyes of 46 patients analyzed, only 3 eyes (3.3%) reached full vascular maturity. Of the 89 eyes not reaching maturity, 39 eyes (43.8%) had vascular arrest alone (VAA), 34 eyes (38.2%) had vascular arrest with persistent tortuosity (VAT), and 16 eyes (18.0%) had ROP reactivation. Those eyes that reactivated were more likely to be initially classified as having aggressive posterior ROP ($P = .004$) and of Asian ethnicity ($P = .008$). There were greater areas of ischemia in eyes with reactivation as compared to VAT and VAA (112.1 mm^2 vs 72.5 mm^2 vs 56.6 mm^2, respectively, $P = .007$). Younger gestational age at birth was found to be an independent predictor of persistent tortuosity (VAT vs VAA) in a logistic regression model.

• CONCLUSIONS: Incomplete vascularization following IVB is very common and is associated with a younger gestational age at birth, Asian ethnicity, and aggressive posterior ROP. The presence of tortuosity following IVB may be indicative of persistently elevated vascular endothelial growth factor levels and an early indicator of potential reactivation. (Am J Ophthalmol 2019;198:63–69. © 2018 Elsevier Inc. All rights reserved.)

THE TREATMENT OF RETINOPATHY OF PREMATURITY (ROP) has evolved over the past decades from cryotherapy to laser photocoagulation, and now adjunctive or monotherapy injection of an anti–vascular endothelial growth factor (VEGF), typically bevacizumab.[1–5] Though it is very successful in halting progression in type 1 disease, the persistence or reactivation of ROP has been reported following intravitreal bevacizumab (IVB).[6–9] Spontaneous ROP regression has classically followed a stepwise pattern starting with the reversal of plus disease, waning of disease stage, vascular growth beyond the previous avascular demarcation, and, finally, full vascular maturation.[10] The optimal outcome following IVB for type 1 ROP is complete vascular maturation. However, it is common to see vascular arrest with peripheral ischemia or reactivation of ROP despite IVB.[6,7,9]

In an earlier study at the same institution, Toy and associates demonstrated that 30 out of 33 eyes initially treated with bevacizumab (91%) underwent laser treatment on average 14.9 weeks after injection for peripheral nonperfusion, per institutional protocol.[9] The authors noted that there was distinct *scalloped regression* without complete vascular maturation following IVB that they speculated was indicative of low-grade ischemia from the avascular peripheral retina.[9] Interestingly, they also noted that 7 of the 33 eyes treated with IVB also had recurrence of plus disease, which is rarely seen in infants undergoing primary laser therapy.[9,10] Given these previous findings, we postulate that vascular regression after bevacizumab follows a similar stepwise course as spontaneous regression, although it may stop anywhere on a continuum from full vascular maturity, to vascular arrest alone (VAA) with peripheral nonperfusion, to vascular arrest with persistent tortuosity (VAT), to reactivated ROP. VAT seems to be a unique regression pattern following IVB but not laser therapy. We hypothesize that there are demographic, ROP type, and angiographic features associated with the different regression patterns that are related to a quantifiable peripheral ischemic burden. Thus, we conducted a retrospective review of all patients with type 1 Early Treatment for ROP (ETROP) or aggressive posterior ROP (APROP) treated with IVB and evaluated the regression pattern clinically and by fluorescein angiography.

METHODS

• STUDY DESIGN AND PATIENT SELECTION: This was a retrospective study conducted at a single academic center (Stanford University Hospital and Clinics, Palo Alto, California, USA). All babies who received bevacizumab (Genentech, South San Francisco, California, USA)

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administered by a single surgeon (D.M.M.) from June 1, 2013 to March 31, 2018 were included in the study. Per institutional protocol, all babies receiving bevacizumab had to have met criteria for prethreshold or threshold disease as defined by the ETROP study, or APROP as defined by the International Committee for the Classification of Retinopathy of Prematurity (ICROP).[11,12] This study includes eyes (n = 33) previously reported on by Toy and associates[9] at the same institution and was compliant with the Health Insurance Portability and Accountability Act and adhered to the Declaration of Helsinki. Approval was obtained from Stanford University's Institutional Review Board/Ethics Committee.

Premature infants meeting Joint Statement Screening Guidelines criteria were screened for ROP by binocular indirect ophthalmoscopy.[13] Based on fundus findings, those meeting type 1 ROP criteria were treated off-label with 0.625 mg bevacizumab in 0.025 mL (half the adult dose) at bedside in affected eyes. The procedure technique has been previously described by Toy and associates.[9] Patients were reexamined at 24-48 hours and then weekly to every other week. Recurrence or reactivation of ROP based on the reoccurrence of stage disease triggered definitive treatment with ablative laser photocoagulation the same week. Patients who had not yet reached vascular maturation by clinical examination at 60 weeks of age underwent an examination under anesthesia (EUA) with photography and fluorescein angiography (FA) using the RetCam3 (Clarity Medical Systems, Pleasanton, California, USA). After indirect ophthalmoscopic examination and real-time review of the angiogram, all eyes with > 2 disc diameters (DD) of peripheral nonperfusion from the ora serrata, regardless of other clinical and angiographic features, underwent ablative laser photocoagulation during the same EUA. The 2 DD cut-off point was adopted by the senior author (D.M.M.) as the designation for vascular arrest and vascular maturity based on the finding by Blair and associates in addition to the Joint Statement Screening Guidelines criteria.[13,14]

• **DATA COLLECTION:** Neonatal characteristics including gestational age (GA), birth weight, sex, race, and delivery type (spontaneous vaginal delivery or cesarean section) were recorded. The dates of 3 visits were recorded, including the date of first ROP screening examination, date of IVB treatment, and date of EUA/FA. ROP characteristics at the time of IVB treatment were recorded, including zone, stage, and the presence of pre-plus or plus disease. Each eye was classified as either APROP or type 1 ROP by ETROP criteria (by D.M.M.).[11,12] During EUA/FA the eye was classified as complete vascular maturity (perfusion to within 2 DD of the ora serrata), VAA (nonperfusion greater than 2 DD from the ora serrata), VAT (nonperfusion greater than 2 DD from the ora serrata and posterior tortuosity), or reactivated ROP (recurrence of stage disease). Digital montages of the FA images were constructed using i2kRetina (DualAlign, Halfmoon, New York, USA). When images could not be automatically aligned by software, they were manually aligned to ensure accurate accounting of the area of nonperfusion. The montage images were analyzed using ImageJ (National Institutes of Health, Bethesda, Maryland, USA) to quantify the area of ischemic retina in square pixels. Using the optic disc as reference scale marker, units of area were converted to mm^2 using the conversion constant provided by the manufacturer (0.0306 mm/pixel) (Figure 1).[15]

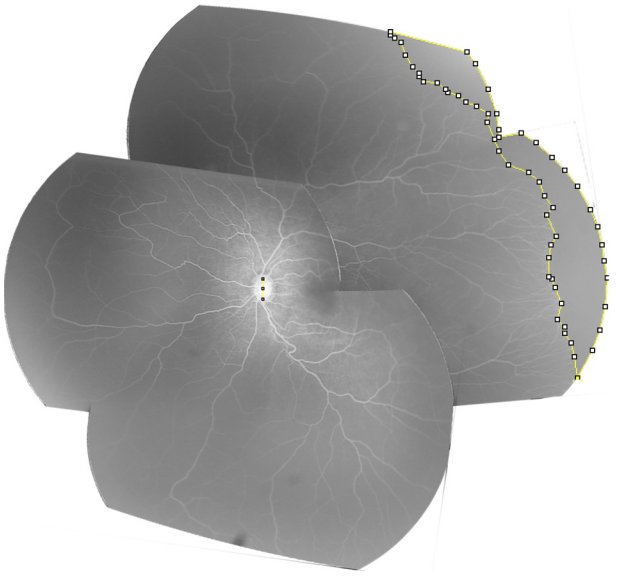

FIGURE 1. Measurement of the optic disc and peripheral nonperfusion using the ImageJ program.

• **STATISTICAL ANALYSIS:** All data were analyzed with SAS Enterprise Guide 7.1 (SAS Institute, Cary, North Carolina, USA). Baseline characteristics were compared using an analysis of variance test for continuous variables and Fisher exact test or the χ^2 test for categorical variables. Post hoc analyses were conducted for global P values < .05 using the Bonferroni correction ($P < .017$ for 3 pairwise comparisons among the 3 groups) and Tukey test. Measures of association were determined for possible risk factors using crude univariable testing to compare the VAA patients with the VAT patients. Odds ratios (OR) and 95% confidence intervals (CI) were reported for this unadjusted model. Multivariable regression analysis was performed to assess for independent predictors of developing vascular arrest with or without tortuosity (VAT or VAA) and sequentially adjusted for potential confounders.

RESULTS

NINETY-TWO EYES OF 46 PATIENTS MET INCLUSION CRITERIA for this study (including 33 eyes from Toy and associates'

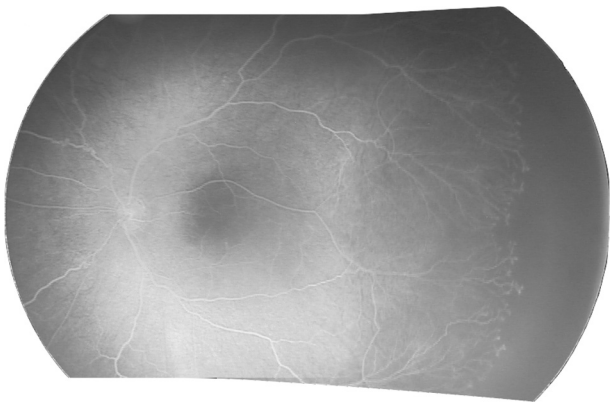

FIGURE 2. Fluorescein angiogram at 60 weeks gestational age demonstrating vascular arrest alone (VAA) after intravitreal bevacizumab.

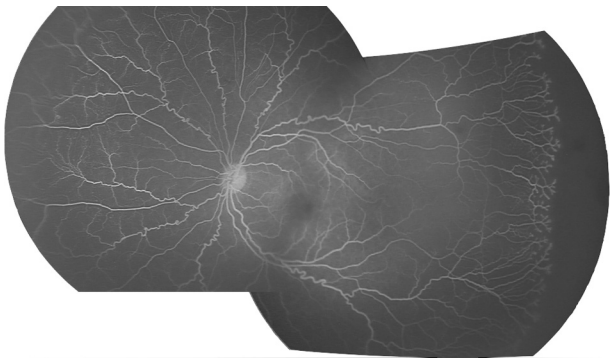

FIGURE 3. Fluorescein angiogram at 60 weeks gestational age demonstrating vascular arrest with tortuosity (VAT) after intravitreal bevacizumab.

study[9]). Only 3 eyes (3.3%) treated with IVB reached complete vascular maturity at the time of EUA/FA and were not included in the final analysis. The remaining eyes (n = 89, 96.7%) had either VAA, VAT, or reactivation prompting the use of ablative laser photocoagulation to the nonperfused retina. Of those eyes not reaching full vascular maturity at the time of FA, 39 eyes (43.8%) had VAA, 34 eyes (38.2%) had VAT, and 16 eyes (18.0%) had reactivated ROP (Figures 2 and 3).

The neonatal characteristics are listed in Table 1. There was a significant difference in GA between the 3 cohorts with those in the reactivation cohort having the earliest GA (24.4 weeks) compared to VAT (24.9 weeks) and VAA (25.4 weeks) (global $P = .005$; post hoc analysis indicated statistical significance between the VAA and reactivation group comparison but not the VAT and reactivation group comparison). There was no difference in birth weight and delivery type. Male patients made up a larger percentage in the VAA group (69%) compared to the VAT group (41%) and the reactivation group (50%, global $P = .049$; post hoc analysis was not significant in pairwise comparison). The racial composition between the 3 cohorts was significantly different (global $P = .007$), especially when comparing Asians with non-Asians among the 3 cohorts (global $P = .008$; post hoc analysis indicated statistical significance between VAA and reactivation group comparison).

The GA at IVB was 35.7 weeks, 35.8 weeks, 34.3 weeks (global $P = .054$) in the VAA, VAT, and reactivated cohorts, respectively. There was a statistical difference between the 3 cohorts with regard to ROP zone, stage, and presence of plus disease at the time of treatment. Those with reactivation were more likely to have zone I, stage 2 plus disease and APROP (Table 2).

The time from IVB to EUA/FA/ablative laser was 26.5 weeks (62.2 weeks GA) in the VAA cohort, 25.3 weeks (61.1 weeks GA) in the VAT cohort, and 13.6 weeks (47.7 weeks GA) in the reactivated cohort (global $P = .002$; post hoc analysis indicated statistical significance between the VAA and reactivation group comparison and between the VAT and reactivation group comparison). The reactivated ROP eyes had the greatest area of ischemia, followed by VAT and VAA eyes (global $P = .007$). Post hoc analysis showed a significant difference between the VAA and reactivation groups (Table 3). When adjusting for gestational age, birth weight, sex, race, age at first examination, age at bevacizumab treatment, ROP type, and area of ischemia on FA, younger age at IVB treatment was predictive for VAT in comparison to VAA (Table 4).

DISCUSSION

WITH THE CONTINUED SUCCESS OF IVB IN HALTING ACTIVE and aggressive ROP, concerns have arisen over the limited number of infants reaching full vascular maturation. Early studies reported a high rate of vascular maturation based on clinical appearance.[2,8] With the aid of FA, the persistence of peripheral nonperfusion and vascular arrest is more detectable, though the implication of the avascular retina is not well known.[16,17] Reactivation and neovascular complications, including retinal detachment, have been reported months to years after IVB and may be a consequence of this peripheral ischemia.[6,7,18,19] This fear may be pushing physicians to perform ablative photocoagulation after 60 weeks (when babies begin to become too big to examine in the clinic).[6,20–22] Nevertheless, the extent of clinically significant peripheral ischemia has not been well defined, especially following IVB. Blair and associates reported that 1.0-1.5 DD of avascular retina posterior to the ora serrata is normal in children, whereas greater than 2 DD of avascular retina posterior to ora serrata is likely a sign of pathologic nonperfusion.[14]

TABLE 1. Characteristics of Infants Treated With Intravitreal Bevacizumab for Type 1 Retinopathy of Prematurity*

	Vascular Arrest Alone (N = 39 Eyes)	Vascular Arrest With Tortuosity (N = 34 Eyes)	Reactivation (N = 16 Eyes)	Global *P* Value
GA, weeks (mean ± SD)	25.4 ± 1.0	24.9 ± 1.3	24.4 ± 0.5	.005[a,*]
BW, g (mean ± SD)	703.7 ± 124.3	686.1 ± 157.1	659.4 ± 113.1	.544
Male sex, n (%)	27 (69.2)	14 (41.2)	8 (50.0)	.049[b,*]
Race, n (%)				.007[c,*]
White	16 (41.0)	8 (23.5)	2 (12.5)	
Hispanic	16 (41.0)	9 (26.5)	6 (37.5)	
Asian	4 (10.3)	8 (23.5)	8 (50.0)	
African American	0 (0.0)	2 (5.9)	0 (0.0)	
Pacific Islander	0 (0.0)	4 (11.8)	0 (0.0)	
More than 1 race	3 (7.7)	1 (2.9)	0 (0.0)	
Other	0 (0.0)	2 (5.9)	0 (0.0)	
Birth delivery, n (%)				.075
SVD	4 (10.3)	6 (17.6)	6 (37.5)	
Cesarean section	35 (89.7)	28 (82.4)	10 (62.5)	

BW = birth weight; GA = gestational age; SVD = spontaneous vaginal delivery.

[a]Post hoc Tukey test indicated significant difference ($P < .05$) between the vascular arrest alone and the reactivation group comparison.

[b]Post hoc pairwise comparison with Bonferroni correction indicated no significant difference between any of the 3 paired groups.

[c]Post hoc pairwise comparison with Bonferroni correction indicated significant difference between (1) the vascular arrest alone and vascular arrest with tortuosity groups, and (2) the vascular arrest alone and reactivation groups.

*$P < .05$ for global comparison between three groups.

TABLE 2. Retinopathy of Prematurity Disease Level and Type at Time of Bevacizumab Treatment

	Vascular Arrest Alone (N = 39 Eyes)	Vascular Arrest With Tortuosity (N = 34 Eyes)	Reactivation (N = 16 Eyes)	Global *P* Value
ROP disease level, n (%)				.002[a,*]
Zone I				
Stage 1, plus	3 (7.7)	3 (8.8)	0	
Stage 2, plus	4 (10.3)	2 (5.9)	8 (50.0)	
Stage 3, plus	5 (12.8)	3 (8.8)	2 (12.5)	
Zone II				
Stage 1, plus	0	0	0	
Stage 2, plus	5 (12.8)	1 (2.9)	4 (25.0)	
Stage 3, plus	21 (53.8)	18 (52.9)	2 (12.5)	
Stage 3, pre-plus	1 (2.6)	4 (11.8)	0	
Stage 3, no plus	0	3 (8.8)	0	
ETROP type I	27 (69.2)	24 (70.6)	4 (25.0)	.004[a,*]
APROP	12 (30.8)	10 (29.4)	12 (75.0)	

APROP = aggressive posterior ROP; ETROP type I = early treatment for ROP type I; ROP = retinopathy of prematurity.

[a]Post hoc pairwise comparison with Bonferroni correction indicated significant difference between (1) vascular arrest alone and reactivation groups, and (2) vascular arrest with tortuosity and reactivation groups.

In this review of infants with type I ROP or APROP treated with IVB, 89 of 92 eyes (96.7%) failed to achieve complete vascular maturity to within 2 DD of the ora serrata by 60 weeks GA. Additionally, of the 89 eyes with prior plus or pre-plus disease, only 42 eyes (47.2%, including 3 eyes with full maturity) had resolution of posterior tortuosity at the time of FA. Both the rate of complete vascular maturity and resolution of plus disease were lower than prior reports.[16,17,23,24] Lorenz and associates, using half-dose bevacizumab (0.312 mg), treated 27 eyes with IVB and found that 11 eyes (41%) did not achieve vascular outgrowth to within 2 DD of the ora serrata.[17] Tahija and associates treated 20 eyes with IVB and found that 9 eyes (45%) failed to achieve complete vascularization.[23] Henaine-Berra and associates reported the FA findings on 47 eyes following IVB and demonstrated that 45 eyes (96%) had resolution of vascular tortuosity, though 39 eyes (83%) demonstrated persistent perivascular leakage.[24] Moreover, in addition to vascular

TABLE 3. Area of Ischemia in Eyes Treated with Intravitreal Bevacizumab for Type 1 Retinopathy of Prematurity That Did Not Reach Maturity

	Vascular Arrest Alone (N = 39 Eyes)	Vascular Arrest With Tortuosity (N = 34 Eyes)	Reactivation (N = 16 Eyes)	Global *P* Value
Area of ischemia (mm^2)	56.6	72.5	112.1	.007[a,*]

[a]Post hoc Tukey test indicated significant difference ($P < .05$) between the vascular maturity and reactivation groups.

arrest and persistent tortuosity, multiple other FA abnormalities can be detected post IVB.[15,24] Lepore and associates reported on 12 eyes receiving IVB and demonstrated abnormalities on FA, including large peripheral avascular areas, abnormal vascular branching and shunts, and absence of the foveal avascular zone.[16]

The amount of peripheral ischemia on FA between the 3 cohorts (based on mm^2) was statistically different, with reactivated eyes having more ischemia than VAT, followed by VAA (Table 3). However, with pairwise testing we were only able to demonstrate a difference between reactivated eyes and VAA eyes. The trend would indicate that eyes with VAT have an increased amount of ischemia and potentially elevated VEGF levels. Previous studies have demonstrated that tortuosity is directly related to the VEGF burden, which supports the notion that VAT eyes have a higher VEGF burden than VAA eyes.[10,25,26] Further supporting a higher VEGF burden in the VAT compared to the VAA cohort is the fact that patients undergoing definitive laser therapy rarely if ever have reoccurrence of plus disease.[10]

In this study, infants with younger GA were more likely to have VAT or reactivation on FA (Table 1). Multiple studies have demonstrated that younger GA and lower birth weight are risk factors for APROP and more severe ROP.[27,28] With an adjusted regression model, we were able to demonstrate that younger gestational age was predictive of VAT in comparison to VAA. Those of Asian ethnicities were more likely to reactivate in comparison to other races. Previous observational studies have demonstrated that Asian infants were at increased risk of threshold ROP, which was possibly related to improved survival and/or lower birth weight in Asian versus white infants.[29,30] Additionally, in a rat model it was found that rats with increased pigmentation were at an increased risk of complications related to retinal ischemia, which may partially explain the more severe ROP and worse response to IVB in Asian infants.[31] Nevertheless, pigmentation is likely not the only explanation for Asian infants, as Hispanic and African-American infants did not demonstrate the same rate of incomplete response to IVB as Asians. Additional research is required to elucidate a potential physiologic or genetic factor(s) involved in Asian infants' response to IVB for ROP.

Patients with reactivated ROP were much more likely to have been treated for zone 1 disease and APROP (Table 2).

TABLE 4. Predictors of Vascular Arrest With Persistent Tortuosity vs Vascular Arrest Alone (Excluding Reactivation Eyes)

	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Gestational age	0.68 (0.44, 1.04)	0.47 (0.25, 0.88)*
Birth weight	1.00 (1.00, 1.00)	1.00 (1.00, 1.01)
Male sex	0.31 (0.12, 0.82)*	0.28 (0.07, 1.21)
Gestational age at first examination	0.61 (0.37, 1.01)	0.97 (0.50, 1.89)
Gestational age at bevacizumab treatment	1.02 (0.83, 1.25)	1.30 (0.92, 1.85)
APROP	0.94 (0.34, 2.56)	0.76 (0.15, 3.82)
Area of ischemia on FA	1.01 (1.00, 1.02)	1.01 (1.00, 1.02)

APROP = aggressive posterior retinopathy of prematurity; CI = confidence interval; FA = fluorescein angiogram; OR = odds ratio.

Final regression model adjusted for gestational age, birth weight, delivery type, sex, Asian race, gestational age at first examination, gestational age at bevacizumab treatment, ROP type, and area of ischemia on FA.

Asterisk (*) indicates statistical significance, $P < .05$.

Ahn and associates demonstrated that those with APROP required more laser spots to photocoagulate the avascular retina in comparison to non-APROP, which corresponds with our finding of an increased area of avascular retina in those with reactivation (of which 75% were APROP).[27] This larger area of ischemia may result in an increased VEGF burden and reactivation of ROP. Those with reactivated ROP also underwent laser therapy at an earlier age than those with VAA or VAT and this may in part explain the larger areas of peripheral nonperfusion between reactivated ROP and VAA or VAT. As it is this institution's standard to perform EUA/FA at 60 weeks GA, it is unclear if those in the VAA or VAT groups would be more likely to reactivate, as we were unable to demonstrate a statistically significant difference in the area of ischemia between VAA and VAT.

Of the 92 eyes treated with IVB in this study, 89 underwent prophylactic laser treatment for reactivation or peripheral nonperfusion. Though this study did not aim to address the efficacy of prophylactic laser, the combination of IVB and prophylactic laser may minimize the risk of early

and late retinal detachment, particularly in comparison to laser therapy or IVB alone.[32,33]

Given the retrospective nature of this study, there are inherent limitations. This study only included eyes treated with IVB, without a control group treated with primary laser. Additionally, 89 of the 92 eyes were treated with laser for persistent peripheral nonperfusion at 60 weeks. There was not a control group of infants with peripheral nonperfusion at 60 weeks who did not receive laser. The decision to treat peripheral nonperfusion was also at the discretion of the surgeon and is based on the concern that residual peripheral nonperfusion at 60 weeks carries a lifelong risk of reactivation or redetachment.[9] This treatment strategy does not reflect universal practice. Lastly, the distinction between VAA and VAT was a subjectively determined based on masked photographs.

IVB is a very powerful tool in the treatment of ROP. Nevertheless, ROP is a chronic disease and infants treated with IVB need close follow-up with a skilled ROP specialist. An accurate understanding of the physiologic response to IVB is paramount to preventing late reactivation and retinal detachments. We propose a new sequence of ROP regression following IVB, with full vascular maturity as the optimal outcome, followed by VAA, VAT, reactivated ROP, and finally retinal detachment. VAT may represent a higher risk pattern than VAA, given the trend toward larger areas of ischemia seen on fluorescein angiography. We argue that any sign of persistent vascular tortuosity should alert the physician to continuing VEGF production. Severely preterm infants, Asian infants, and infants with APROP were at a statistically significant higher risk of failed vessel maturation and reactivation of ROP. One inference from our results is that all children undergoing IVB may benefit from fluorescein angiography prior to discharge from acute-phase screening of ROP.

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