Chronic Vascular Arrest as a Predictor of Bevacizumab Treatment Failure in Retinopathy of Prematurity

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Purpose: To describe a pattern of retinopathy of prematurity (ROP) disease regression and chronic vascular arrest after intravitreal bevacizumab treatment that is not observed after peripheral laser ablation.

Design: Single-institution retrospective cohort study.

Participants: Consecutive sample of 58 eyes in 30 patients treated for type 1 ROP.

Methods: Initial treatment with either a single intravitreal injection of bevacizumab in off-label use (n = 33 eyes) or peripheral laser ablation (n = 25 eyes) as part of standard clinical care. There was bias in recommending off-label bevacizumab for smaller infants with type 1 ROP.

Main Outcome and Measures: Reactivation or persistence of ROP, as determined by clinical examination, fundus photography, and fluorescein angiography.

Results: All eyes treated initially with bevacizumab demonstrated irregular progression of the leading vascular edge in a stereotyped pattern, suggestive of scalloped regression. Recurrence, based on angiographic demonstration of leakage, or chronic vascular arrest, confirmed based on angiographic demonstration of peripheral ischemia, was noted in 30 eyes (91%) in the bevacizumab group, at a median interval of 14.9 weeks after injection (corrected gestational age, 49.3 weeks). Univariate logistic regression indicated that the need for rescue treatment was associated with decreased birth weight (odds ratio [OR], −0.007; $P = 0.04$) and age of initial treatment (OR, −0.35; $P = 0.05$), but not gender, race, or gestational age. Multivariate logistic regression indicated that only decreased birth weight (OR, −0.018; $P = 0.04$) was associated with need for rescue treatment.

Conclusions: Treating ROP with intravitreal bevacizumab results in a characteristic scalloped regression pattern that is highly associated with treatment using biologic anti−vascular endothelial growth factor agents. The presence of this pattern in conjunction with chronic vascular arrest and peripheral retinal ischemia persisting beyond standard screening timelines has significant implications for the management of ROP. Fluorescein angiography is important in assessing vascular maturation in these infants. *Ophthalmology 2016;123:2166-2175 © 2016 by the American Academy of Ophthalmology.*

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Retinopathy of prematurity (ROP) is a disorder of retinal vascular development in premature infants that is characterized by pathologic neovascularization with potentially blinding sequelae, including retinal traction and detachment. Retinopathy of prematurity remains a major cause of childhood visual morbidity, with an estimated worldwide annual incidence of 184 700, resulting in approximately 20 000 new cases of severe visual impairment yearly.[1] The major risk factors for ROP developing are premature birth and low birth weight.[2] Several molecular signaling pathways play a role in the pathogenesis of ROP, including vascular endothelial growth factor (VEGF), erythropoietin, insulin-like growth factor-1, and omega-3 fatty acids.[3]

The pathologic neovascularization characteristic of ROP has 2 phases. Phase 1, occurring during postmenstrual age 22 through 30 weeks, consists of oxygen-induced arrest of normal vascular development, spindle-cell damage and gap junction formation, and obliteration of immature retinal vessels, leading to peripheral retinal avascularity.[3−5] In phase 2, occurring during postmenstrual age 31 through 44 weeks, the retina differentiates and becomes more metabolically active, leading to hypoxia-mediated vasoproliferation in the peripheral areas of ischemia.[3−6] Abnormal levels of VEGF (low in phase 1, high in phase 2) have been implicated in the molecular pathogenesis of ROP.[7]

Intravitreal administration of bevacizumab, a humanized monoclonal antibody against VEGF-A, during phase 2 of ROP, has demonstrated efficacy in causing regression of pathologic neovascularization and promoting progression of putatively normal retinal vascular development through continued canalization of spindle-cell vessel precursors.[8−15] Several studies have reported on the reactivation of ROP

after intravitreal bevacizumab treatment,[8,13,16−18] characterized as either persistence or recurrence of pathologic neovascularization. Recurrence after treatment with anti-VEGF agents tends to occur later than with conventional ablative therapy.[8,13]

In this article, we report on a characteristic pattern of regression of pathologic neovascularization and chronic vascular arrest associated with intravitreal bevacizumab treatment in ROP that is distinct from findings after peripheral laser ablation. We also discuss potential prognostic and management implications with regard to chronic vascular arrest in these infants.

Methods

Patient Inclusion

This was a retrospective, institutional cohort study of 30 patients treated for type 1 ROP disease (including Early Treatment of Retinopathy of Prematurity high-risk prethreshold, threshold, or aggressive posterior retinopathy of prematurity) by a single surgeon (D.M.M.) between January 2013 and June 2015. The protocol was approved by the Stanford University Institutional Review Board, complied with the requirements of the Health Insurance Portability and Accountability Act, and adhered to the tenets of the Declaration of Helsinki.

As part of standard clinical care, premature infants meeting Joint Statement Screening Guidelines criteria[19] were screened routinely for ROP by binocular indirect ophthalmoscopy. Based on confirmation of findings meeting type 1 ROP criteria and the patient's overall health, the patient's caregivers were offered intravitreal bevacizumab in an off-label use versus traditional laser photocoagulation. There was bias in recommending off-label bevacizumab for smaller infants with type 1 ROP.

Off-Label Bevacizumab Intravitreal Injection Technique and Management

For patients being treated with bevacizumab, a reduced adult dose (0.625 mg in 0.025 ml) of intravitreal bevacizumab was administered at bedside in each eye, using the following technique: sterile gloves, insertion of a lid speculum, instillation of topical povidone−iodine, demarcation 0.5 to 1 mm posterior to the limbus in the inferonasal or inferotemporal quadrants, injection of bevacizumab using a sterile tuberculin syringe with a sterile 30-gauge 0.5-inch needle, removal of the needle with simultaneous occlusion of the sclerotomy site using a sterile cotton-tipped applicator, instillation of topical moxifloxacin, and removal of the speculum. If the other eye was to be treated, all new equipment was used. After injection, patients underwent binocular indirect ophthalmoscopy to assess for lens clarity, retinal breaks, retinal detachment, optic nerve perfusion and spontaneous pulsations, and hemorrhage. Patients were followed up at 24 to 48 hours and then weekly to every other week with dilated ophthalmoscopic examinations until either recurrence or full vascular maturation was noted. Beginning around 50 weeks' postmenstrual age, or when babies were too large to examine safely in the outpatient clinic setting, infants underwent an examination under anesthesia with photography and fluorescein angiography (FA) using the RetCam3 (Clarity Medical Systems, Pleasanton, CA) to assess whether continued evaluation or perhaps treatment were necessary.

We used the following definitions for patterns seen on FA after intravitreal bevacizumab injection: (1) scalloped regression manifesting as an intercalating pattern of vascular loops at the termination of the retinal vasculature, distinct from either immature retinal vasculature, which has no apparent border, and stage 1 ROP, which has a clear, smooth linear demarcation between avascular and vascularized retina; (2) anterior ischemic non-vascularized retina; (3) vascular arrest, defined as failure of the retinal vasculature to grow into zone III, defined as within 2 disc diameters of the ora serrata; and (4) ROP reactivation, manifesting as disease recurrence in stage of disease, plus disease, or neovascularization at the vascular−avascular interface or region of prior extraretinal fibrovascular proliferation.

We performed diode laser photocoagulation (per the protocol below) in the following situations: (1) continued ROP activity (any stage, pre-plus disease, or plus disease), (2) chronic vascular arrest confirmed on FA, and (3) active leakage at the border of the vascular termination or posterior to this area. The rationale for laser photocoagulation was to shut down any continuing VEGF drive. This served 2 purposes: (1) to eliminate the risk of primary retinal detachment from ROP reactivation and (2) to terminate the acute-phase screening of ROP 9 weeks after laser treatment, based on findings by Coats et al.[20]

Traditional Diode Laser Photocoagulation

All caregivers of infants receiving diode laser photocoagulation were informed that the major risks were as follows: decreased peripheral vision, decreased night vision, high myopia, anterior segment ischemia, cataract, phthisis, iris synechiae with irregular pupils, and acute or late angle-closure glaucoma, in addition to the usual risks. All diode laser photocoagulation was performed under general anesthesia in the operating room. Before treatment, binocular indirect ophthalmoscopy with 360° scleral depression was performed to assess and document the disease extent and severity in each eye. This was followed by fundus photography. The laser parameters were as follows: power range, 100 to 300 mW; duration, 150 to 200 ms; and interval, 100 to 200 ms. Laser was applied in a nearly confluent pattern as follows: 1 row anterior to the vascular termination/ridge, 1 row posterior to the ora serrata, and filling in between with 0.25- and 0.5-spot width separation in all locations, except nasal and temporal over the ciliary artery and nerve, where 1- to 1.5-spot width separation was employed to avoid unnecessary damage to the underlying ciliary artery and nerve in an attempt to minimize the possibility of anterior segment ischemia. After laser treatment, binocular indirect ophthalmoscopy with 360° scleral depression was performed to assess and document the absence of skip areas, inadvertent treatment of vascularized retina, new hemorrhage, or retinal break or detachment, or both. This was followed by fundus photography to document these findings. Patients were initiated on postoperative topical prednisolone acetate 1%, atropine 1%, and moxifloxacin 0.5%. Initial assessment was 1 week after diode laser photocoagulation and continued for 9 weeks, when acute-phase screening for ROP was terminated. Patients were scheduled with pediatric ophthalmology 4 to 6 weeks after termination of acute-phase screening for ROP to assess for amblyopia, refractive error, and strabismus.

Data Analysis

Given the small sample size and data that were not normally distributed, continuous variables were summarized as median with range. The Mann−Whitney U test was used to compare continuous data between the 2 groups. Chi-square and Fisher exact tests were used to compare categorical data. Logistic regression analyses were performed to assess the impact of various patient and treatment factors (birth weight, gestational age, age of initial treatment, gender, and race) on ROP disease reactivation requiring rescue

treatment. All statistical analyses were performed using Stata software (StataCorp, College Station, TX) employing an α of 0.05 for statistical significance.

Results

Fifty-eight eyes of 30 patients were included in this study. Thirty-three eyes of 17 patients were treated primarily with intravitreal bevacizumab, and 25 eyes of 13 patients were treated primarily with laser ablation. One patient had a right eye treated initially with laser ablation and a left eye treated initially with intravitreal bevacizumab. To reduce confounding resulting from fellow-eye effects of bevacizumab treatment, the right eye of this patient was excluded. An additional patient who was treated initially with laser in both eyes and received subsequent bevacizumab injection in the left eye for persistent plus disease 1.5 weeks after laser similarly was excluded. One patient in the laser cohort required treatment in 1 eye only.

Table 1 summarizes the demographic and treatment data for this study cohort, divided by initial treatment arm (intravitreal bevacizumab vs. laser). The median age of initial treatment was significantly different between the 2 groups (34.3 weeks for bevacizumab vs. 37.7 weeks for laser; $P = 0.03$, Mann−Whitney U test). The median age at the time of laser treatment also was significantly different between the 2 groups (49.3 weeks for bevacizumab vs. 37.7 weeks for laser; $P < 0.0001$, Mann−Whitney U test). For the group treated primarily with intravitreal bevacizumab, the median gestational age at birth was 24.7 weeks, and the median birth weight was 645 g. The median corrected gestational age (CGA) of primary intravitreal bevacizumab injection was 34.3 weeks. Recurrence or angiographic demonstration of ischemia, leakage, or both was noted in 30 eyes (91%) at a median interval of 14.9 weeks after injection, or CGA of 49.3 weeks. This was treated with rescue laser ablation. Table 2 details the clinical details and examination findings for each patient in the primary bevacizumab cohort.

For the group treated primarily with laser ablation, the median gestational age was 24.9 weeks, and the median birth weight was 700 g. The median CGA at the time of primary laser treatment was 37.7 weeks. Table 3 details the clinical details and examination findings for each patient in the primary laser cohort.

Initial Bevacizumab Cohort

All 33 eyes (17 patients) treated primarily with intravitreal bevacizumab demonstrated regression of plus disease and irregular progression of the leading vascular edge in a stereotyped pattern, which we call *scalloped regression*. This pattern was noted within 1 week after treatment and has been reported previously to be very nearly pathognomonic of intravitreal bevacizumab injection.[21,22]

Patient 1. Figure 1 illustrates both eyes of a patient treated with intravitreal bevacizumab who demonstrated subsequent vascular arrest without development of leakage on FA. At birth, this female patient was 25 weeks of age and weighed 540 g. At 33 weeks' CGA, she was noted to have bilateral posterior zone II, stage 2 with plus ROP that was treated with bilateral intravitreal injection of bevacizumab. One week later, she was noted to have scalloped regression in both eyes. She was followed up with weekly serial examinations, and at 44 weeks' CGA, she was noted to have reactivation of zone II, stage 2 with plus ROP. She underwent repeat injection of intravitreal bevacizumab bilaterally and was noted again to have scalloped regression in both eyes. She was followed up with weekly serial examinations, and at 49 weeks, she underwent examination under anesthesia with FA that demonstrated vascular arrest without vessel leakage. She subsequently underwent laser photocoagulation and demonstrated complete regression. Montage and single-frame color fundus photographs have been included in Supplemental Figure S1 (available at www.aaojournal.org).

Table 1. Patient Demographics

Patient Demographics	Primary Bevacizumab Treatment	Primary Laser Treatment	P Value
No. of patients (eyes)	17 (33)	13 (25)	
Gender (% female)	59	31	0.13 (Chi-square test)
Gestational age at birth (wks)			0.83 (Mann−Whitney U test)
Median	24.7	24.9	
Range	24.0−28.0	23.6−29.0	
Birth weight (g)			0.10 (Mann−Whitney U test)
Median	645	700	
Range	430−990	560−1160	
Median no. of primary injections	1	—	
CGA of initial treatment (wks)			0.03 (Mann−Whitney U test)
Median	34.3	37.7	
Range	32.6−39.0	33.0−46.1	
No. of eyes undergoing rescue laser (%)	30 (91)	—	
CGA of laser (wks)			<0.0001 (Mann−Whitney U test)
Median	49.3	37.7	
Range	44.3−103.4	33.0−46.1	
Interval between first injection and laser (wks)		—	
Median	14.9		
Range	9.1−68.9		
Race, no. (%)			0.63 (Fisher exact test)
White	4 (24)	2 (15)	
Latino	6 (35)	7 (54)	
Asian	7 (41)	4 (31)	

CGA = corrected gestational age.

Table 2. Initial Bevacizumab Cohort Characteristics: Clinical Characteristics and Examination Findings of Individual Patients in the Group Treated Initially with Intravitreal Bevacizumab Injection

Birth Weight (g)	Gestational Age (wks)	Age at Bevacizumab Treatment (wks)	Age at Laser Treatment (wks)	Examination Notes
540	24.00	35.71	44.86	II1+ both eyes → bevacizumab both eyes → vascular arrest with recurrent plus both eyes → laser both eyes
650	24.00	32.57	47.43	APROP both eyes → laser right eye, bevacizumab left eye → vascular arrest (II1) left eye → laser left eye
540	24.00	35.00	47.29	I2+ both eyes → bevacizumab both eyes → vascular arrest (II1) with peripheral ischemia, minimal leakage both eyes → laser both eyes
540	24.57	33.29, 44.29	49.14	IIposterior2+ both eyes → bevacizumab both eyes No. 1 → vascular arrest with recurrent plus both eyes → bevacizumab both eyes No. 2 → scalloped regression with peripheral ischemia → laser both eyes
645	24.71	35.14	48.86	II2+ right eye, II3+ left eye → bevacizumab both eyes → scalloped regression with peripheral ischemia both eyes → laser both eyes
740	24.71	35.14	48.86	II2+ right eye, II3+ left eye → bevacizumab both eyes → scalloped regression with peripheral ischemia both eyes → laser both eyes
640	25.00	34.14	44.29	I2+ → bevacizumab both eyes → vascular arrest (IIposterior1pre+) with minimal leakage both eyes → laser both eyes
750	25.29	33.71	50.71	II2+ both eyes → bevacizumab both eyes → scalloped regression with ischemia both eyes, extensive temporal leakage left eye → laser both eyes
780	25.29	34.86	50.71	I3 both eyes → bevacizumab both eyes → II1 both eyes → peripheral ischemia both eyes → laser both eyes
990	28.00	33.14		APROP both eyes → bevacizumab both eyes → vascular arrest (posterior II1) both eyes → immature vessels (III0, within 2 disc diameters of ora) without leakage
630	24.00	33.00	50.00	I2+ both eyes → bevacizumab both eyes → temporal reactivation with leakage both eyes → laser both eyes
700	24.00	33.86	52.43	II2+ both eyes → bevacizumab both eyes → scalloped regression with leakage and recurrent plus both eyes → laser both eyes
645	24.00	34.00	48.86	APROP both eyes → bevacizumab both eyes → peripheral ischemia both eyes → laser both eyes
780	25.29	34.57	103.43	APROP both eyes → bevacizumab both eyes → peripheral temporal ischemia both eyes → laser both eyes
500	25.00	37.57	63.14	II2+ both eyes → bevacizumab both eyes → temporal and inferior scalloped regression with retinal ischemia left eye → laser left eye
430	24.00	34.29	50.14	II3 both eyes → bevacizumab both eyes → II1 peripheral ischemia with leakage both eyes → laser both eyes
510	26.86	39.00	49.43	II3+ both eyes → bevacizumab both eyes → scalloped regression with recurrent plus both eyes → laser both eyes
Overall, median (range) 645 (430–990)	24.71 (24.0–28.0)	34.29 (32.6–39.0)	49.3 (44.3–103.43)	

APROP = aggressive posterior ROP; arabic numeral = stage of ROP disease; roman numeral = zone of ROP disease; ROP = retinopathy of prematurity; + = plus disease present.

Patient 2. Figure 2 illustrates both eyes of a patient treated with intravitreal bevacizumab that demonstrated vascular arrest with persistent peripheral avascularity and ischemia. At birth, this female patient was 25 weeks of age and weighed 780 g. At 35 weeks' CGA, she was noted to have bilateral zone II, stage 3 ROP that was treated with bilateral intravitreal bevacizumab injection. One week later, she was noted to have scalloped regression. She was followed up with weekly serial examinations, and at 51 weeks' CGA, she underwent examination under anesthesia with FA that demonstrated

persistent peripheral avascularity in both eyes, which were treated with peripheral laser ablation, resulting in complete regression of disease. Montage and single-frame color fundus photographs have been included in Supplemental Figure S2 (available at www.aaojournal.org).

Patient 3. Figure 3 illustrates both eyes of a patient treated with intravitreal bevacizumab that demonstrated subsequent vascular arrest with the development of minimal leakage at the advancing vascular edge. At birth, this female patient was 25 weeks of age and weighed 640 g. At 33 weeks' CGA, she was noted to have

Table 3. Initial Laser Cohort Characteristics: Clinical Characteristics and Examination Findings of Individual Patients in the Group Treated Initially with Peripheral Laser Ablation

Birth Weight (g)	Gestational Age (wks)	Age at the Time of Laser Treatment (wks)	Examination Notes
1160	27.29	42.43	III3+ left eye → laser left eye
580	23.86	34.71	II3+ both eyes → laser both eyes
660	25.86	38.14	II3+ both eyes → laser both eyes
1080	29.00	38.14	II3+ left eye, II1 right eye → laser both eyes
810	25.00	42.71	III3+ right eye, III1+ left eye → laser both eyes
990	28.00	46.14	Persistent III3 with vitreous condensation and hemorrhage both eyes → laser both eyes
620	25.14	34.43	APROP both eyes → laser both eyes
602	23.57	33.00	I2+ both eyes → laser both eyes
560	23.86	38.00	II3+ both eyes → laser both eyes
706	24.29	33.14	I2+ both eyes → laser both eyes
770	24.86	36.14	Posterior II3+ both eyes → laser both eyes
700	24.00	34.86	APROP both eyes → laser both eyes
650	24.57	37.71	II3 pre-plus both eyes → laser both eyes
Overall, median (range)			
700 (560–1160)	24.86 (23.6–29.0)	37.7 (33.0–46.1)	

APROP = aggressive posterior ROP; arabic numeral = stage of ROP disease; roman numeral = zone of ROP disease; ROP = retinopathy of prematurity; + = plus disease present.

bilateral zone I, stage 2 with plus ROP that was treated with bilateral intravitreal injection of bevacizumab. One week later, she was noted to have scalloped regression. She was followed up with weekly serial examinations, and at 44 weeks' CGA, she underwent examination under anesthesia with FA that demonstrated posterior zone II, stage 1 pre-plus ROP with minimal leakage at the vascular–avascular junction in both eyes, which were treated with peripheral laser ablation, resulting in complete regression. Montage and single-frame color fundus photographs have been included in Supplemental Figure S3 (available at www.aaojournal.org).

Patient 4. Figure 4 illustrates both eyes of a patient treated with intravitreal bevacizumab that demonstrated subsequent vascular arrest with the development of leakage at the vascular–avascular junction, consistent with disease reactivation. At birth, this female patient was 24 weeks of age and weighed 630 g. At 33 weeks' CGA, she was noted to have bilateral zone I, stage 2 ROP that was treated with bilateral intravitreal bevacizumab. One week later, she was noted to have scalloped

regression. She was followed up with weekly serial examinations, and at 50 weeks' CGA, she underwent examination under anesthesia with FA that demonstrated temporal reactivation with leakage at the region of prior extraretinal fibrovascular proliferation in both eyes, which were treated with peripheral laser ablation, resulting in complete regression of disease. Montage and single-frame color fundus photographs have been included in Supplemental Figure S4 (available at www.aaojournal.org).

Patient 5. Figure 5 illustrates both eyes of a patient treated with intravitreal bevacizumab that demonstrated reactivation of plus disease. At birth, this female patient was 24 weeks of age and weighed 700 g. At 34 weeks' CGA, she was noted to have bilateral zone II, stage 2 with plus ROP that was treated with bilateral intravitreal bevacizumab. One week later, she was noted to have scalloped regression. She was followed up with weekly serial examinations, and at 52 weeks' CGA, she underwent examination under anesthesia with FA that demonstrated reactivation of plus disease, which was treated with peripheral
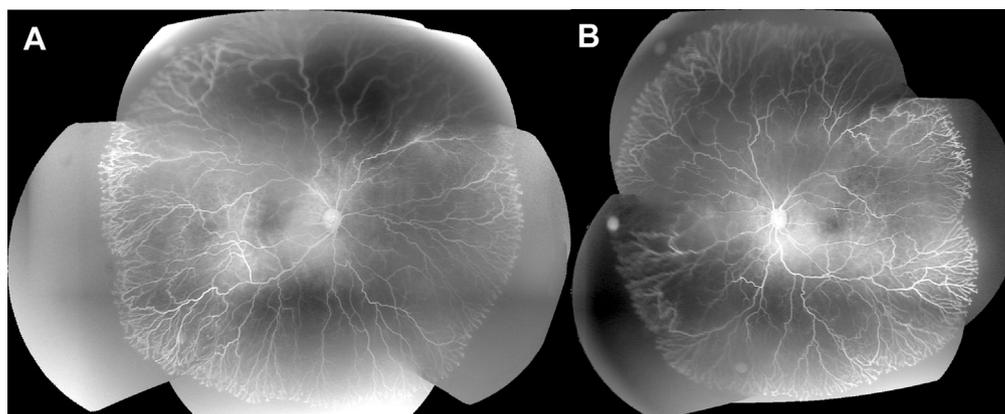

Figure 1. Montage fluorescein angiograms of the (**A**) right and (**B**) left eyes of patient 1 demonstrating vascular arrest without leakage at 49 weeks' corrected gestational age (CGA), after treatment with bilateral intravitreal bevacizumab injections at 33 and 44 weeks' CGA.
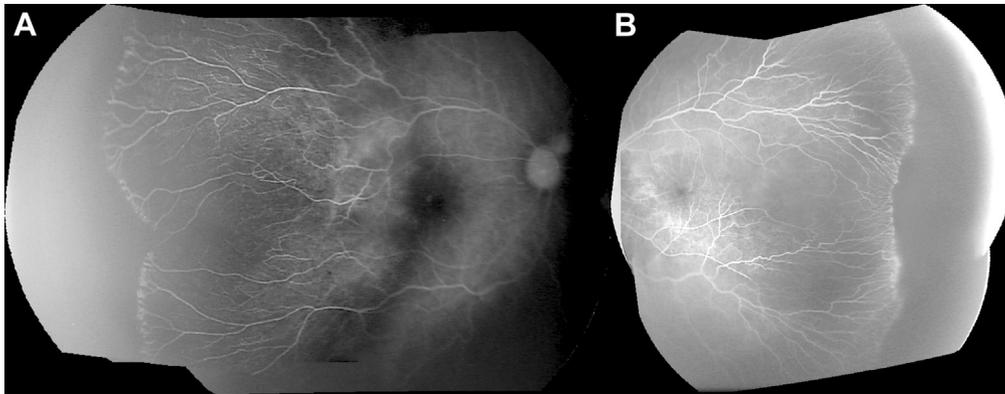
Figure 2. Montage fluorescein angiograms of the (**A**) right and (**B**) left eyes of patient 2 demonstrating vascular arrest with persistent peripheral avascularity and ischemia at 51 weeks' corrected gestational age (CGA), after treatment with bilateral intravitreal bevacizumab at 35 weeks' CGA.

laser ablation, resulting in complete regression of disease. Montage and single-frame color fundus photographs have been included in Supplemental Figure S5 (available at www.aaojournal.org).

Initial Laser Cohort

Twenty-five eyes of 13 patients were included in the initial laser treatment cohort. All demonstrated complete regression of ROP after treatment by ablation of the peripheral retina (Table 3) in a manner consistent with prior published studies.[23] Of note, the patients in the initial laser cohort were approximately 13 weeks younger at the time of laser ablation (median age, 37.7 weeks for laser vs. 49.3 weeks for bevacizumab), and this difference was statistically significant ($P < 0.0001$, Mann−Whitney U test). The laser cohort was 3 weeks older (median age, 37.7 weeks for laser vs. 34.3 weeks for bevacizumab) at the time of initial treatment, and this difference was statistically significant ($P = 0.03$, Mann−Whitney U test).

Univariate logistic regression indicated that the need for rescue treatment was associated with decreased birth weight (odds ratio [OR], −0.007; $P = 0.04$) and age at initial treatment (OR, −0.35; $P = 0.05$), but not with gender (female OR, 0.84; $P = 0.26$), race,

gestational age (OR, −0.50; $P = 0.12$), or age at initial treatment (OR, −0.02; $P = 0.72$). Multivariate logistic regression indicated that only decreased birth weight (OR, −0.018; $P = 0.04$) was associated with the need for rescue treatment.

Discussion

Over the past 10 years, there has been a shift toward increasing use of intravitreal bevacizumab for the treatment of prethreshold disease in ROP. Clinical experience with bevacizumab has shown a dramatic effect on inducing regression of active retinopathy, contributing to a significant increase in its use; however, there is concern that treatment with intravitreal bevacizumab, especially in very premature infants, may lead to chronic vascular arrest beyond standard screening guidelines of 54 weeks, posing a risk of late reactivation months to years later.[24]

In designing our treatment protocol, we were guided by the only randomized controlled trial of bevacizumab for ROP (Bevacizumab Elimiates the Angiogenic Threat for
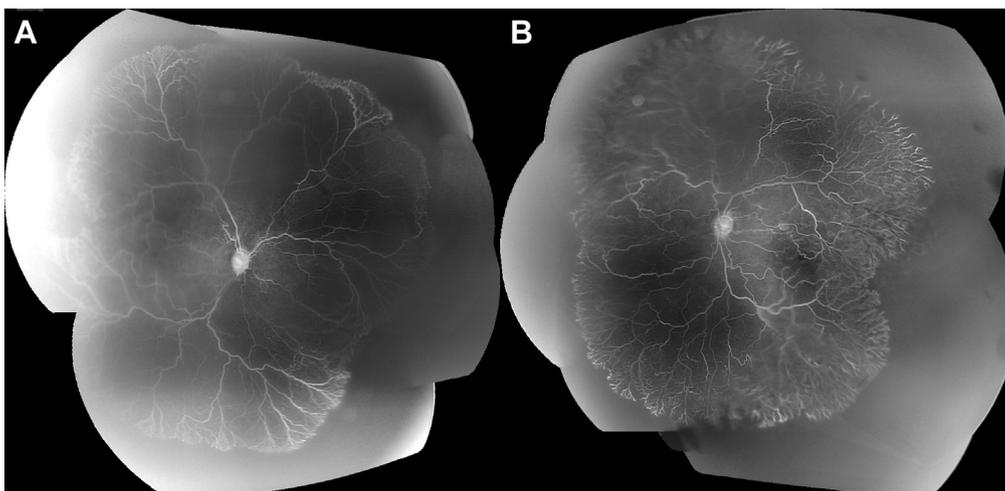

Figure 3. Montage fluorescein angiograms of the (**A**) right and (**B**) left eyes of patient 3 demonstrating vascular arrest with the development of leakage at the advancing vascular edge at 44 weeks' corrected gestational age (CGA), after treatment with bilateral intravitreal injection of bevacizumab at 33 weeks' CGA.
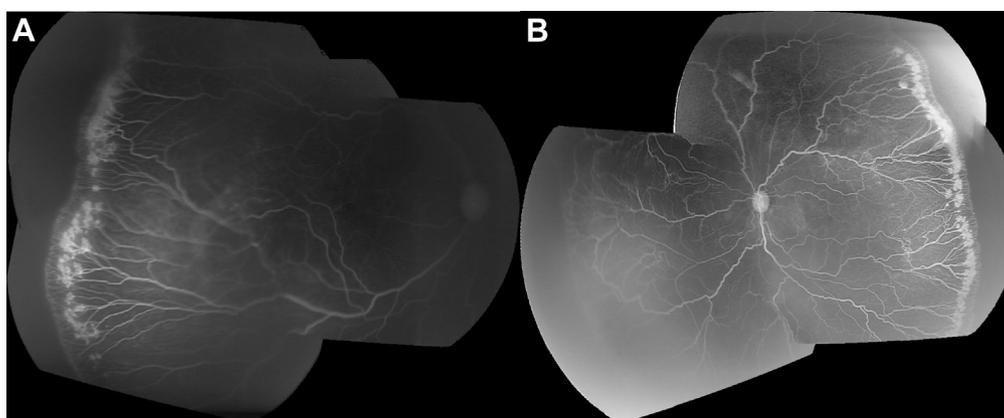
Figure 4. Montage fluorescein angiograms of the (**A**) right and (**B**) left eyes of patient 4 demonstrating vascular arrest and the development of leakage at the vascular—avascular junction, consistent with disease reactivation, at 50 weeks' corrected gestational age (CGA), after bilateral intravitreal bevacizumab injection at 33 weeks' CGA.

Retinopathy of Prematurity [BEAT-ROP]),[8] which evaluated a 0.625-mg dose. It has been published and anecdotally demonstrated that half of that dose may be effective,[25] but no randomized controlled trial data presently exist to support universal efficacy or to guide case selection for the reduced dose.

There is evidence that late recurrence after bevacizumab is not merely a theoretical concern. Hu et al[16] reported on 17 eyes that had undergone bevacizumab injection for prethreshold disease and then demonstrated recurrence of a new demarcation line, ridge, extraretinal fibrovascular proliferation, or leakage on FA. Five of these eyes progressed to retinal detachment at a median age of 55 weeks' CGA (range, 49–69 weeks' CGA). Wu et al[26] reported on 3 of 162 eyes treated with bevacizumab injection for prethreshold ROP that progressed to retinal detachment at 35, 41, and 69 weeks' CGA. There also have been single case reports of patients with subsequent retinal detachment at 54 weeks',[27] 81 weeks',[28] and 2.5 years'[24] CGA after treatment with initial bevacizumab injection. Although there were methodologic concerns related to follow-up in some of these studies, it does highlight the potential for catastrophic late recurrence after intravitreal bevacizumab for type 1 ROP.[29]

Most literature about intravitreal bevacizumab or ranibizumab, for ROP maintains that full vascular maturation occurs as a consequence of treatment.[8,18,25,26,30,31] These studies relied mainly on funduscopic examination with or without fundus photography. When angiography was applied uniformly, full vascularization was not observed consistently (although these studies focused largely on the diversity of angiographic findings).[13,21,22] In this study, full retinal maturation was the exception, occurring in only 9% of bevacizumab-treated eyes by 54 weeks' CGA. It is possible that with longer follow-up, these infants eventually would have shown vascularization; however, continued examinations past 60 weeks of age become logistically difficult: caregivers are burdened with repeated patient visits, the child becomes too big to examine safely and reliably, and practitioners have an unsustainable increase in patient volume.

Our report contributes to the growing evidence that a significant subset of infants treated with intravitreal bevacizumab demonstrates chronic vascular arrest that extends beyond standard screening guidelines devised in the era before bevacizumab treatment. In our case series, 17 patients receiving bevacizumab for type 1 ROP had persistent vascular arrest at CGAs ranging from 60 to 108 weeks. Furthermore, 11 of 33 eyes showed peripheral leakage on FA at the
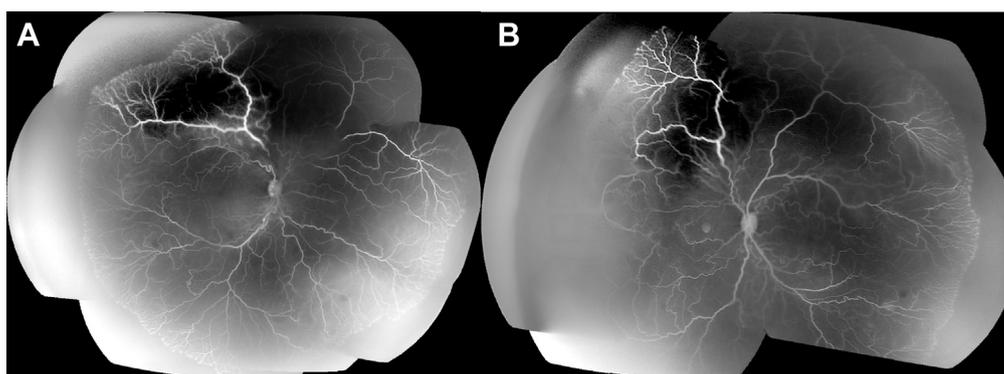

Figure 5. Montage fluorescein angiograms of the (**A**) right and (**B**) left eyes of patient 5 demonstrating reactivation of plus disease at 52 weeks' corrected gestational age (CGA), after bilateral intravitreal bevacizumab at 34 weeks' CGA.

vascular−avascular junction or region of prior extraretinal fibrovascular proliferation that is thought to indicate persistent or recurrent hypoxia-mediated neovascular drive.[3] Other studies have suggested, and we agree, that definitive ablative treatment to these eyes can curb the risk of late exudative and tractional consequences developing from this persistent area of avascular retina.[17,32,33]

In addition to chronic vascular arrest, we also have observed a characteristic appearance of fine vessel arborization at the junction of vascular−avascular retina that occurs after bevacizumab treatment. This vessel pattern does not occur after laser-induced regression of ROP. It is reminiscent of the acute capillary budding phase of spontaneously regressing ROP, but in contrast, it is present after many weeks and does not have the associated retinal atrophy and scarring noted in involution with partial vascularization.[34,35] In our practice, we call this regression pattern *scalloped regression* and find that it can distinguish reliably between infants who have and have not previously received bevacizumab. The significance of this distinct regression pattern is not known. It may represent the terminal extent of spindle-cell migration, beyond which normal vasculogenesis by spindle-cell canalization cannot occur. We believe its presence is tied to the higher rate of vascular arrest and greater area of peripheral avascular retina noted in infants previously treated with bevacizumab, which perhaps facilitates persistence of a chronic low-grade ischemia that gradually recrudesces.

How does this differ from traditional regression patterns? The hallmark of de novo regression in ROP is the resolution of plus disease, disappearance of stage disease, and retinal vascular growth beyond the previous ridge with full vascular maturation.[36] In cases of only partial vascularization, the peripheral avascular retina becomes atrophic and scarred.[34] After retinoablative therapy with either cryotherapy or laser photocoagulation, stage and plus disease involute in most successful cases within 3 weeks, with retinal detachment rarely noted in the 7 weeks after treatment.[20] This regression pattern is characterized by the development of a hyperpigmented and hypopigmented retinopathy in the previously avascular retina that had received ablative therapy. Vascular growth may continue to the ora serrata, but the overlying retina is atrophic. The Early Treatment of Retinopathy of Prematurity (ETROP) and CRYO-ROP studies demonstrated the efficacy of peripheral retinal ablation treatment in high-risk (type 1) prethreshold and threshold ROP, respectively.[23,37] Various longitudinal studies have characterized the course of ROP involution after ablative treatments to the peripheral retina and have demonstrated similar results.[20,38−42] In this study, all 25 eyes treated initially with laser ablation demonstrated complete regression of ROP, without reactivation or progression of ROP after treatment.

We hold to a strict policy of monitoring until vascularization to within 2 disc diameters of the ora serrata. When children become too big to examine safely in the clinic or their caregivers are unable to maintain appropriate follow-up, patients undergo examination under anesthesia with fluorescein angiography. Our findings highlight that after bevacizumab injection, a subset of patients have incomplete peripheral vascularization.

We hypothesize that treatment for ROP can be considered successful only with complete vascularization to the ora serrata (or within 2 disc diameters) and no active disease. It is our hypothesis that it is not possible to determine whether peripheral avascular retina will or will not progress to recurrence of ROP and possible subsequent detachment. The natural history of ROP is that for the vast majority of patients, it involutes with full vascularization extending to the ora serrata. A subset of prethreshold and even threshold disease will regress and not progress to stage 4 or 5.[38,43] In those eyes with more severe ROP, the timeline for involution is protracted, extending beyond 55 weeks' CGA.

The underlying pathophysiologic concern is that there is a mismatch between the extent of retinal vasculature and neurosensory retinal tissue, with the potential for ischemia, subsequent neovascularization, and the attendant risk of late retinal detachment. There is evidence that peripheral avascular retina may be associated with retinal hole formation in children with spontaneously regressed ROP[10] and retinal detachment in adults.[44] Preslan and Butler[38] hypothesized that the choroid may be capable of supplying some of the retinal oxygen needs in the far periphery, and thus in the Cryotherapy for Retinopathy of Prematurity (CRYO-ROP) study, one of the criteria for involution of disease was retinal vascularization reaching into zone III, defined as, "if the vessels in the 2 nasal sectors reached within 1 disc diameter of the ora serrata and there was no ROP in the 2 nasal sectors." We used the criterion of vessels extending to within 2 disc diameters of the ora serrata.

The goal is to prevent stage 4 or 5 disease in all infants. The amount of avascular retina beyond 2 disc diameters that may be safe has not been defined clearly, but with multiple case reports of late retinal detachment after anti-VEGF treatment for ROP, we hypothesize that one factor may be persistent avascular retina elaborating VEGF and other angiogenic factors that promote neovascularization with risk of subsequent traction and detachment. Accordingly, because there is still uncertainty regarding the natural history of avascular zones larger than 2 disc diameters, we have adopted the approach of applying laser photocoagulation to peripheral avascular regions identified on FA in infants older than 60 weeks' CGA with chronic vascular arrest. This approach eliminates the chance of late reactivation.

Admittedly, this conservative approach increases the number of infants exposed to the side effects of peripheral laser ablation. We believe that the alternative (assuming that peripheral avascular retina cannot cause reactivation under any circumstance) risks infants returning with late reactivation or retinal detachment. Although only small numbers have been observed thus far, as bevacizumab use increases, there is concern about an increasing prevalence of reactivation in infants presumed to have completed vascularization.

Ideally, patients would be followed up prospectively and receive FA at fixed intervals to classify better the effects of bevacizumab on the peripheral retina. Unfortunately, repeated anesthesia poses an unnecessary risk to the developing brain.[45,46] Despite its retrospective nature, our study highlights that avascular retina does exist in infants treated with bevacizumab for up to 108 weeks, and likely longer. Although this peripheral avascular retina eventually

may perfuse, in-clinic examinations are no longer feasible for the reasons mentioned above. Because of the potential for catastrophic late retinal detachments, we use FA to identify areas of avascular retina and the degree of leakage in infants without complete vessel maturation. Although national guidelines require a more complete dataset (to determine specific factors predisposing patients to stage 4 or 5 disease, factors affecting completion of vascularization, etc.), we emphasize the need not to presume that peripheral vascularization occurs, but instead either to confirm complete vascularization with direct clinical examination or through an examination under anesthesia with FA.

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Footnotes and Financial Disclosures

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Abbreviations and Acronyms:

BEAT-ROP = Bevacizumab Elimiates the Angiogenic Threat for Retinopathy of Prematurity; **CRYO-ROP** = Cryotherapy for Retinopathy of Prematurity; **CGA** = corrected gestational age; **ETROP** = The Early Treatment of Retinopathy of Prematurity; **FA** = fluorescein angiography; **OR** = odds ratio; **ROP** = retinopathy of prematurity; **VEGF** = vascular endothelial growth factor.

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