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Neurodevelopmental Outcomes after Intravitreal Bevacizumab Therapy for Retinopathy of Prematurity

A Prospective Case-Control Study

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Purpose: To evaluate the neurodevelopmental and ocular developmental outcomes in premature children who have undergone intravitreal bevacizumab injection (IVB) for treatment of type 1 retinopathy of prematurity (ROP).

Design: Prospective case-control study.

Participants: We enrolled 3 groups of premature patients: premature children who had no history of ROP (group 0), premature children with history of ROP without treatment (group 1), and premature children with ROP who had received a single IVB (0.625 mg; group 2).

Methods: Ocular developmental assessment, including cycloplegic refractometry, axial length, Cardiff acuity, and neurodevelopmental assessment via the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley III), were performed at 1 to 3 years of age and were compared between groups.

Main Outcome Measures: Ocular developmental outcomes and Bayley III scores.

Results: A total of 148 patients (85 boys and 63 girls) were included. The mean age at assessment was 1.49±0.59 years. Group 0 patients demonstrated significantly higher gestational age (GA), birth weight, and Apgar scores compared with group 1 and 2 patients. There were no significant differences between groups 1 and 2 in demographics or systemic risk factors except for lower GA in group 2. The cylindrical power was significantly larger in groups 1 and 2 compared with group 0. The spherical equivalent was significantly more myopic and the Cardiff acuity was significantly poorer in group 2 than in group 0. There were no significant differences between groups 1 and 2 in refractive status, axial length, or Cardiff acuity. Neurodevelopmental assessment using Bayley III showed no significant difference among the 3 groups in any aspect after adjusting for GA and other systemic risk factors. The risks for poor neurodevelopmental outcomes also were not significantly different.

Conclusions: At the mean age of 1.5 years, children with prior history of IVB (group 2) showed similar refractive and visual outcomes and similar neurodevelopmental outcomes compared with premature patients with ROP without requirement of treatment (group 1), although there is a possibility that a small but clinically significant difference may not have been detected in the current study. *Ophthalmology 2019;126:1567-1577 © 2019 by the American Academy of Ophthalmology*

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See Commentary on page 1578.

Retinopathy of prematurity (ROP) is a retinal disease in preterm infants that is the result of the immaturity of retinal vessels; it is characterized by retinal hypoxia in its early stage and neovascularization in its late stage, which leads to retinal traction and retinal detachment.[1,2] Because ROP is one of the primary causes of childhood blindness,[3] prompt diagnosis and treatment are of paramount importance. The treatment of ROP aims to halt the neovascularization process driven by elevated intraocular vascular endothelial growth factor (VEGF).[4] Laser photoablation of the peripheral avascular retina remains the standard treatment for ROP. Although effective, laser photoablation destroys a sizable portion of the retina and is associated with a narrower anterior chamber angle,[5] the development of myopia,[6] and a reduction in visual field. A new treatment method, intravitreal injection (IVI) of anti-VEGF, has been used increasingly to treat ROP patients after the Bevacizumab Eliminates the Angiogenic Threat of ROP (BEAT-ROP) study showed a significant benefit of intravitreal injection of bevacizumab (IVB) for zone I stage 3+ (i.e., stage 3 with plus disease) ROP compared with conventional laser treatment.[7] The advantages of IVI of

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anti-VEGF include a less time-consuming procedure, fewer risks from general anesthesia in a physically compromised preterm newborn,[8–14] and a potentially lower chance of unfavorable outcomes in zone 1 ROP.[7,15–17] For these reasons, the use of IVI of anti-VEGF for treatment of ROP has gained popularity.

The study of the pharmacokinetics of intraocular anti-VEGF agents has shown potential systemic action of the drug, and concerns of systemic safety have been raised, especially in preterm infants, in whom VEGF is critical for neurodevelopment.[18–20] However, the effect of possible systemic suppression of VEGF after IVB on the neurodevelopment of these patients previously has not been studied widely. A retrospective analysis of neurodevelopmental outcomes in 125 infants with ROP treated with IVB or laser showed that patients treated with IVB had lower Bayley Scales of Infant and Toddler Development, Third Edition (Bayley III), motor composite scores compared with those treated with laser, without a difference in language composite score or cognitive composite score.[21] A higher likelihood of severe neurodevelopmental disabilities in patients treated with IVB compared with laser treatment also was noted.[21] In contrast, our earlier study of 61 patients with ROP reported no difference in neurodevelopment for those who received only IVB versus those who received only laser treatment.[22]

These 2 previous studies were retrospective studies. Some issues related to the study design of the first report have been raised.[14] To provide a better assessment of the neurodevelopmental outcomes, this study was designed prospectively to investigate neurodevelopmental outcomes in ROP patients treated with IVB. The outcomes were compared between ROP patients without requirement of treatment and premature patients without ROP.

Methods

Patients and Grouping

This study was approved by the institutional review board of Chang Gung Memorial Hospital in Taoyuan, Taiwan (contract, IRB104-7500B) and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from each patient's parent for the enrollment of his or her child in the study.

This prospective case-control study was conducted between June 2014 and January 2019 at Chang Gung Memorial Hospital, Taoyuan, Taiwan. Three groups of patients were enrolled in the study: premature children with no ROP (prematurity without ROP group [group 0]), premature children with ROP but who regressed spontaneously without requirement of treatment (ROP without treatment group [group 1]), and premature children with a history of ROP who were treated with a single IVB (ROP with IVB treatment group [group 2]). The indications for treatment were type 1 ROP as defined by the Early Treatment for ROP Study,[23,24] that is, zone I ROP of any stage with plus disease (a degree of dilation and tortuosity of the posterior retinal blood vessels meeting or exceeding that of a standard photograph), zone I stage 3 ROP without plus disease, or zone II stage 2 or 3 ROP with plus disease.[23,24] Prematurity was defined as birth earlier than 37 weeks gestational age (GA). Premature children were enrolled in this study by communicating with their families during admission in the neonatal intensive care unit (NICU; groups 1 and 2) or by invitation during their follow-up in the ophthalmology clinic (groups 0, 1, and 2) or the pediatric psychiatry clinic (groups 0 and 1). The status of the off-label use of IVB for ROP treatment was explained to the parents in detail. Patients with ROP progression to stage 4 or 5 after IVB treatment, other ocular disease, nystagmus, or cerebral palsy or patients who were unable to complete neurologic assessment were excluded. Patients who underwent laser photocoagulation therapy, either before or after IVB, also were excluded from the study (Fig 1).

A prestudy power analysis was performed to estimate the sample size needed for odds ratios (ORs) of severe neurodevelopmental disability. In a previous study by Morin et al[21] regarding a similar issue, significant differences of ORs between laser-treated and IVB-treated patients were noted in Bayley III motor composite scores of less than 85, neurodevelopmental impairment, and severe neurodevelopmental disability. This was the only available study suitable for our power estimation because other similar studies either used assessment tools other than Bayley III[22] or demonstrated no significant difference in any aspects of neurodevelopmental outcome.[25] Therefore, we conducted a power analysis with the following parameters: OR of 3.1, power $(1 - \beta)$ set at 0.80, $\alpha = 0.05$, 2-tailed. The results showed that a total sample size of 145 would yield a power of more than 0.80 by logistic regression. As a result, we determined our recruitment goal to be a total sample size of 145.

General data, including GA, birth weight (BW), sex, Apgar score at 1 minute and 5 minutes after birth, whether born at our study site (inborn) or at another site (outborn), and use of antenatal corticosteroids were recorded. The data regarding congenital anomalies (patent ductus arteriosus), comorbidities (necrotizing enterocolitis, sepsis, degree of intraventricular hemorrhage, periventricular leukomalacia, respiratory distress syndrome), duration of ventilator use, ROP condition, and the perinatal and therapeutic procedures performed during the infant's NICU stay also were recorded. The 3 study groups of patients were followed up prospectively and compared regarding ocular developmental and neurodevelopmental outcomes at the ages of 1 to 3 years.

Intravitreal Bevacizumab Treatment

The technique of IVB was as described previously.[17,26] The injection was performed under intravenous sedation. After antiseptic preparation using 5% povidone—iodine and topical antibiotics of levofloxacin 0.5% (Cravit ophthalmic solution; Santen Pharmaceutical Co., Osaka, Japan), 0.625 mg (0.025 ml) bevacizumab (Avastin; Genentech, Inc., San Francisco, CA) was injected into the vitreous cavity through the pars plicata using a 30-gauge needle, with a nurse holding the infant during the injection. The needle was directed perpendicularly to the horizontal plane initially, then slightly toward the center of the eyeball after passing the lens equator. Patients were followed up every 1 to 2 weeks after the treatments until vascularization was evident up to less than 2 disc diameters from the oral serrata without active disease or clinically significant tractional elements.

Ocular Examinations

Ocular examinations included cycloplegic refraction, axial length, and visual acuity at the ages of 1 to 3 years. The cycloplegic refraction, including spherical power, cylindrical power, and cylindrical axis, was determined 30 minutes after instilling 2% cyclopentolate and 1% tropicamide (2 instillations with an interval of 10 minutes) using a handheld autorefractometer (FR-5000; Grand Seiko Co., Ltd., Hiroshima, Japan). At least 3 measurements were obtained to determine an average reading. We further categorized astigmatism into 3 types based on the axis: with the rule, cylinder at 0° to 30° or

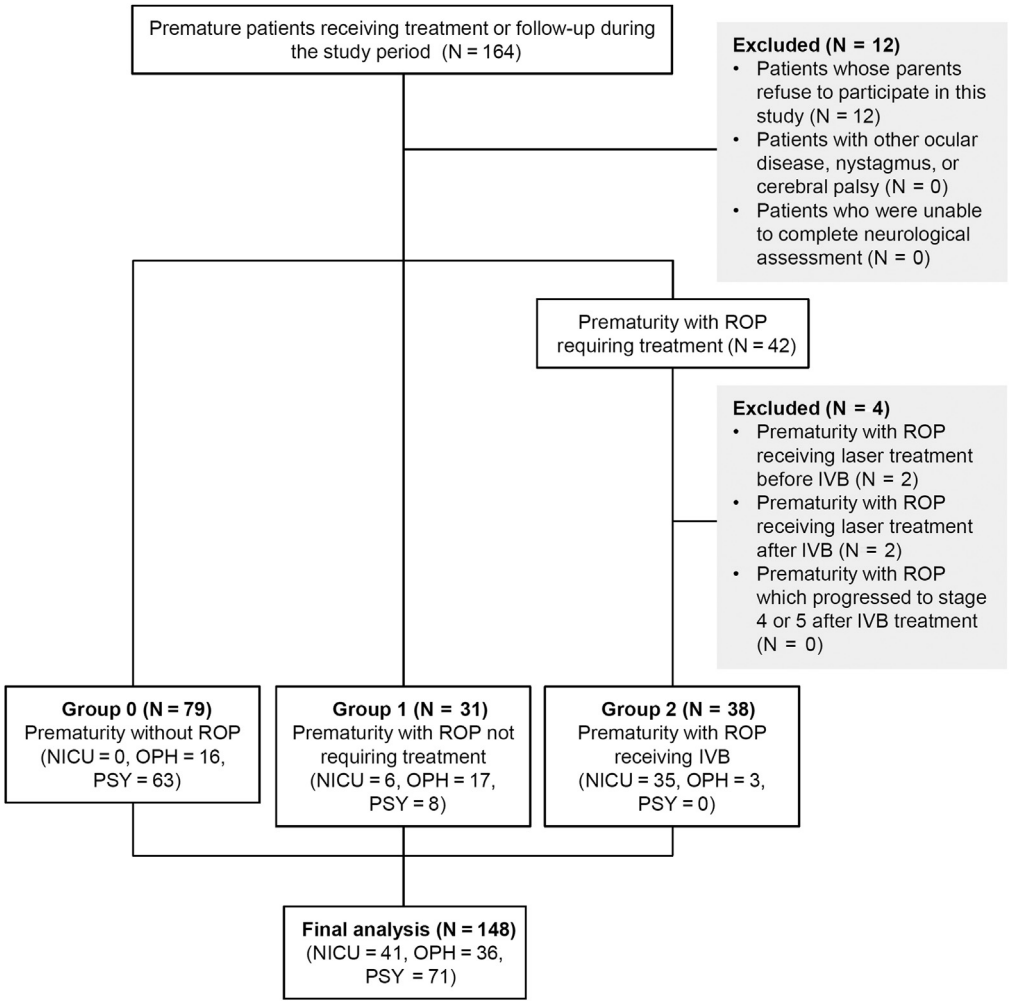

Figure 1. Flowchart showing the inclusion and exclusion of patients during the study period. IVB = intravitreal injection of bevacizumab; NICU = neonatal intensive care unit; OPH = ophthalmology clinic; PSY = pediatric psychiatry clinic; ROP = retinopathy of prematurity.

150° to 180°; against the rule, cylinder at 60° to 120°; and oblique type, cylinder at 31° to 59° or 121° to 149°.[27] Measurements of the axial length were obtained with an optical coherence biometer (IOL Master; Carl Zeiss, Jena, Germany). Visual acuity measurements were obtained using the Cardiff acuity test.[28,29]

Neurodevelopmental Outcomes Assessment

Neurodevelopmental outcomes were assessed using the Bayley III[30] at the ages of 1 to 3 years. The Bayley III is an individually administered instrument that consists of 5 distinct scales, namely, the cognitive scale (91 items), the receptive language scale (48 items), the expressive language scale (49 items), the fine motor scale (72 items), and the gross motor scale (66 items), and is designed to measure the developmental functioning of infants and toddlers 1 to 42 months of age. When scoring, each of the 5 scales was given a raw score based on the number of test items the child successfully completed. Higher scores indicated more mature development. Raw scores then were converted to 5 scaled scores, namely, cognitive, receptive language, expressive language, fine motor, and gross motor scaled scores. These scores were converted further into 3 composite scores (cognitive, language, and motor composite scores) and to 3 percentile ranks (cognitive, language, and motor percentile ranks). In the current study, a certified pediatric psychiatrist (Y.-S.H.), experienced with this assessment and masked to the patient's prior ophthalmic history and treatment, performed the evaluation and scoring for all patients.

The rates of poor neurodevelopmental outcomes, including Bayley III composite scores of less than 85 and severe neurodevelopmental disability, also were recorded and analyzed. Because patients with cerebral palsy were excluded from our study, the definition of severe neurodevelopmental disability was adapted from the study by Morin et al[21] to the presence of any one or more of the following: hearing impairment (hearing loss with use of hearing aids or cochlear implants), visual acuity poorer than 0.5 logarithm of the minimum angle of resolution, or any Bayley III composite scores less than 70. We also analyzed the rates of severe neurodevelopmental disability excluding visual impairment, which was defined as the presence of hearing impairment or any Bayley III composite scores less than 70.

Statistical Analysis

Qualitative data were expressed as number and percentage, whereas continuous values were expressed as mean and standard deviation or median and range when appropriate. Demographics and systemic risk factors were compared among the study groups

using the analysis of variance (ANOVA), Kruskal-Wallis test, chi-square test, or Fisher exact test when appropriate. Retinopathy of prematurity conditions were compared between groups 1 and 2 using a chi-square test. The Scheffe post hoc test was used for post hoc pairwise comparisons to identify significantly different groups after the ANOVA. For the comparison of ocular developmental outcomes among the study groups, the Kruskal-Wallis test was used for comparison of refractive powers and visual acuity, ANOVA for comparison of axial length, and Fisher exact test for comparison of astigmatism types. The Dunn's post hoc test was used for post hoc pairwise comparisons after the Kruskal-Wallis test. For the comparison of neurodevelopmental outcomes among the study groups, the analysis of covariance (controlled covariates: GA, Apgar score at 5 minutes, sepsis, and grade 3 or 4 intraventricular hemorrhage) was used. The partial eta squared (η_p^2) and its 95% confidence interval also were calculated. The effect size magnitude was defined as follows: η_p^2 0.01 − 0.06 = small effect, η_p^2 0.06 − 0.14 = medium effect, $\eta_p^2 > 0.14$ = large effect. Odds ratios of poor neurodevelopmental outcomes were calculated by logistic regressions and were adjusted for GA, Apgar score at 5 minutes, sepsis, and grade 3 or 4 intraventricular hemorrhage. All statistical analyses were performed using SAS software version 9.4 (SAS Institute, Inc, Cary, NC). A P value less than 0.05 was considered statistically significant.

Results

Study Participants

One hundred forty-eight patients (85 boys and 63 girls) were included in the final analysis. A flowchart showing how patients were enrolled and excluded from the study is presented in Figure 1. The mean age of patients at outcome measurement was 1.49±0.59 years. Among these patients, 79 were premature without development of ROP (prematurity without ROP group [group 0]), 31 demonstrated ROP but did not require treatment (ROP without treatment group [group 1]), and 38 demonstrated ROP and received IVB (ROP with IVB treatment group [group 2]). The average postmenstrual age of group 2 patients at first IVB treatment was 36.78±3.85 weeks. Table 1 shows the demographic data, systemic risk factors, and ROP conditions of the 3 groups. There were no significant differences in the mean age at outcome measurement ($P = 0.24$), sex ($P = 0.14$), grade 3 or 4 intraventricular hemorrhage ($P = 0.45$), or periventricular leukomalacia ($P = 0.06$) among the 3 groups.

There were statistically significant differences among the 3 groups in GA ($P < 0.001$), BW ($P < 0.001$), Apgar score at 1 minute ($P < 0.001$) and 5 minutes ($P < 0.001$) after birth, inborn rate ($P = 0.04$), rate of use of antenatal steroids ($P = 0.02$), patent ductus arteriosus ($P < 0.001$), necrotizing enterocolitis ($P = 0.04$), sepsis ($P < 0.001$), grade 1 or 2 intraventricular hemorrhage ($P = 0.04$), respiratory distress syndrome ($P < 0.001$), and number of days of ventilator use ($P < 0.001$). Based on the results of the post hoc tests, BW and Apgar scores at 1 minute and 5 minutes after birth were significantly lower in groups 1 and 2 compared with group 0 but were not significantly different between groups 1 and 2. In contrast, GA was significantly lower in groups 1 and 2 compared with group 0 and also was significantly lower in group 2 compared with group 1 (group 0: mean GA, 31.96±2.78 weeks; BW, 1660.53±571.20 g; group 1: mean GA, 28.24±2.60 weeks; BW, 1046.94±346.23 g; group 2: mean GA, 26.35±2.09 weeks; BW, 833.29±199.98 g). The number of days of ventilator use was significantly higher in groups 1 and 2 compared with group 0 but was not significantly different between groups 1 and 2. Overall, there were no significant differences in the demographics of patients or associated systemic risk factors between groups 1 and 2, except for lower GA in group 2 compared with group 1; however, there were higher rates in some systemic risk factors in groups 1 and 2 compared with group 0 (Table 1). Regarding ROP condition, patients in group 2 were significantly more likely to show stage 3, zone 1 ROP and were more likely to show plus disease compared with group 1 (Table 1).

Ocular Refractive and Visual Outcomes

Table 2 summarizes the results of ocular refractive and visual outcomes, including spherical power, cylindrical power, spherical equivalent, astigmatism type, axial length, and Cardiff acuity in the 3 groups. There was no significant difference among the 3 groups in spherical power ($P = 0.12$). Significant differences among the 3 groups were noted in cylindrical power ($P = 0.0001$) and spherical equivalent ($P = 0.006$) by Kruskal-Wallis test. After further analysis by Dunn's post hoc test, cylindrical power was significantly higher in groups 1 and 2 compared with group 0, but it was not significantly different between groups 1 and 2. The spherical equivalent was significantly more myopic in group 2 compared with group 0 but was not significantly different between groups 1 and 2 (Table 2). We further categorized astigmatism into with-the-rule, against-the-rule, and oblique types based on the axis. In all 3 groups, most patients demonstrated with-the-rule astigmatism, and there were no significant differences in the rates of the 3 astigmatism types among the 3 groups ($P = 0.20$; Table 2). There was also no significant difference among the 3 groups in their axial length measurement by ANOVA ($P = 0.22$). In brief, groups 1 and 2 showed no significant differences in any aspects of refractive status (Table 2).

A significant difference among the 3 groups was noted in Cardiff acuity by Kruskal-Wallis test ($P = 0.0001$). After further analysis with Dunn's post hoc test, Cardiff acuity was significantly poorer in group 2 than in group 0, but there was no significant difference in Cardiff acuity between groups 1 and 2 (Table 2).

Neurodevelopmental Outcomes

Table 3 shows the neurodevelopmental outcomes in the 3 study groups based on Bayley III assessment. When comparing the Bayley III scores by analysis of covariance with controlled covariates of GA, Apgar scores at 5 minutes, sepsis, and grade 3 or 4 intraventricular hemorrhage, no significant differences were found among the 3 groups in any of the Bayley III scores, including cognitive scaled score ($P = 0.08$; $\eta_p^2 = 0.004$), receptive language scaled score ($P = 0.13$; $\eta_p^2 = 0.03$), expressive language scaled score ($P = 0.24$; $\eta_p^2 = 0.02$), fine motor scaled score ($P = 0.12$; $\eta_p^2 = 0.03$), gross motor scaled

Table 1. Demographics, Systemic Risk Factors, and Retinopathy of Prematurity Conditions of the 3 Study Groups

	Prematurity without Retinopathy of Prematurity (Group 0; n = 79)	Retinopathy of Prematurity without Treatment (Group 1; n = 31)	Retinopathy of Prematurity with Intravitreal Injection of Bevacizumab Treatment (Group 2; n = 38)	*P* Value
Age (yrs), mean ± SD	1.57±0.55	1.42±0.67	1.39±0.59	0.24*
Sex (male/female), no. (%)	40 (50.6)/39 (49.4)	22 (71.0)/9 (29.0)	23 (60.5)/15 (39.5)	0.14†
GA (wks), mean ± SD	31.96‡,§±2.78	28.24‡,‖±2.60	26.35§,‖±2.09	<0.001*
BW (g), mean ± SD	1660.53‡,§±571.20	1046.94‡±346.23	833.29§±199.98	<0.001*
Apgar score, median (range)				
1 minute	7‡,§ (1–9)	6‡ (1–8)	6§ (1–8)	<0.001¶
5 minutes	9‡,§ (5–10)	8‡ (3–9)	8§ (2–9)	<0.001¶
Inborn, no. (%)	74 (93.7)	24 (77.4)	32 (84.2)	0.04#
Use of antenatal steroids, no. (%)	44 (57.1)	21 (67.7)	30 (83.3)	0.02†
Patent ductus arteriosus, no. (%)	15 (19.0)	16 (51.6)	32 (84.2)	<0.001†
Necrotizing enterocolitis, no. (%)	2 (2.5)	4 (12.9)	5 (13.2)	0.04#
Sepsis, no. (%)	13 (16.7)	13 (41.9)	19 (51.4)	<0.001†
IVH, no. (%)				
Grade 1 or 2	10 (12.8)	5 (16.1)	12 (32.4)	0.04 by chi-square test
Grade 3 or 4	3 (3.9)	3 (9.7)	1 (2.7)	0.45#
PVL, no. (%)	0 (0)	2 (6.5)	1 (2.7)	0.06#
RDS, no. (%)	50§ (63.3)	27 (87.1)	38§ (100.0)	<0.001#
No. of days of ventilator use, median (range)	14.50‡,§ (0–82)	71.50‡ (0–126)	83.00§ (11–217)	<0.001¶
ROP stage, no. (%)				
No ROP	79 (100.0)	0 (0)	0 (0)	<0.001†,**
1	0 (0)	19 (61.3)	0 (0)	
2	0 (0)	11 (35.5)	3 (7.9)	
3	0 (0)	1 (3.2)	35 (92.1)	
ROP zone, no. (%)				
I	0 (0)	1 (3.2)	4 (10.5)	0.03#,**
II	0 (0)	26 (83.9)	34 (89.5)	
III	0 (0)	4 (12.9)	0 (0)	
Plus disease, no. (%)				
Yes	0 (0)	3 (9.7)	35 (92.1)	<0.001†,**

BW = birth weight; GA = gestational age; IVH = intraventricular hemorrhage; PVL = periventricular leukomalacia; RDS = respiratory distress syndrome; ROP = retinopathy of prematurity; SD = standard deviation.
$P < 0.05$ was considered to be statistically significant.
**P* values calculated by analysis of variance and post hoc tests performed by the Scheffe test.
†*P* values calculated by chi-square test.
‡Significant difference between groups 0 and 1.
§Significant difference between groups 0 and 2.
‖Significant difference between groups 1 and 2.
¶*P* values calculated by Kruskal-Wallis test and post hoc tests performed by the Dunn test.
#*P* values calculated by Fisher exact test.
**Group 0 was excluded.

score ($P = 0.73$; $\eta_p^2 = 0.004$), cognitive composite score ($P = 0.10$; $\eta_p^2 = 0.03$), language composite score ($P = 0.39$; $\eta_p^2 = 0.01$), motor composite score ($P = 0.33$; $\eta_p^2 = 0.02$), cognitive percentile ranks ($P = 0.13$; $\eta_p^2 = 0.03$), language percentile ranks ($P = 0.58$; $\eta_p^2 = 0.008$), and motor percentile ranks ($P = 0.42$; $\eta_p^2 = 0.01$; Table 3).

Table 4 shows the ORs of Bayley III composite scores of less than 85, severe neurodevelopmental disability, and severe neurodevelopmental disability excluding visual impairment. Although group 2 was most likely to show severe outcomes, there were no significantly higher or lower risks for Bayley III composite scores of less than 85, severe neurodevelopmental disability, or severe neurodevelopmental disability except for visual impairment in either group 2 or 0 compared with group 1 after adjustment for GA, Apgar score at 5 minutes, sepsis, and grade 3 or 4 intraventricular hemorrhage (Table 4).

Discussion

In this study, we found that the neurodevelopmental outcomes at the mean age of 1.49±0.59 years were similar between ROP patients who did not require treatment and ROP patients with IVB treatment after adjusting for GA, Apgar score at 5 minutes, sepsis, and grade 3 or 4 intraventricular hemorrhage. We also found that the risks for poor neurodevelopmental outcomes were similar between ROP patients who did not require treatment and ROP patients with IVB treatment after adjustment for GA and other

Table 2. Refractive and Visual Outcomes among the 3 Study Groups

	Prematurity without Retinopathy of Prematurity (Group 0; n = 79)	Retinopathy of Prematurity without Treatment (Group 1; n = 31)	Retinopathy of Prematurity with Intravitreal Injection of Bevacizumab Treatment (Group 2; n = 38)	P Value
Spherical power (D), median (minimum −maximum)	1.50 (−3.75 to 7.75)	1.00 (−1.50 to 5.00)	1.00 (−15.75 to 3.50)	0.12*
Cylindrical power (D), median (minimum −maximum)	−0.75†,‡ (−5.25 to −0.25)	−1.25† (−5.50 to −0.25)	−1.75‡ (−4.50 to −0.25)	0.0001*
Spherical equivalent (D), median (minimum −maximum)	1.00‡ (−3.88 to 7.25)	0.50 (−2.25 to 3.38)	0.13‡ (−17.5 to 3.00)	0.006*
Astigmatism type, no. (%)				
WTR	41 (65.1)	18 (78.3)	21 (61.8)	0.20§
ATR	12 (19.0)	5 (21.7)	6 (17.7)	
Oblique	10 (15.9)	0 (0.0)	7 (20.6)	
Axial length (mm), mean ± SD	21.21±0.96	21.82±1.47	21.07±0.99	0.22‖
logMAR Cardiff acuity	0.10‡ (0.00–0.70)	0.30 (0.00–0.50)	0.40‡ (0.00–1.00)	0.0001*
Snellen acuity, median (minimum −maximum)	0.79 (0.20–1.00)	0.50 (0.32–1.00)	0.40 (0.10–1.00)	

ATR = against-the-rule astigmatism; D = diopter; logMAR = logarithm of the minimum angle of resolution; SD = standard deviation; WTR = with-the-rule astigmatism.
$P < 0.05$ was considered to be statistically significant.
*P values were calculated by Kruskal-Wallis test and post hoc tests were performed by Dunn's test.
†Significant difference between groups 0 and 1.
‡Significant difference between groups 0 and 2.
§P values calculated by Fisher exact test.
‖P values calculated by analysis of variance.

risk factors. However, because our sample size was small, we could not rule out the possibility that small but clinically significant differences may not have been detected in the current study.

In comparison with previous studies using Bayley III for neurodevelopmental assessment,[21,25] the Bayley III composite scores of patients receiving IVB in the present study (group 2) seemed to be higher. However, compared with

Table 3. Neurodevelopmental Outcomes among the 3 Study Groups

	Prematurity without Retinopathy of Prematurity (Group 0; n = 79)	Retinopathy of Prematurity without Treatment (Group 1; n = 31)	Retinopathy of Prematurity with Intravitreal Injection of Bevacizumab Treatment (Group 2; n = 38)	P Value	Partial Eta Squared (η_p^2)	95% Confidence Interval
Bayley III scaled scores, mean ± SD						
Cognitive	10.42±2.31	9.87±2.46	8.95±3.25	0.08	0.04	0.00–0.11
Receptive language	9.83±2.63	9.68±2.64	8.79±2.58	0.13	0.03	0.00–0.10
Expressive language	9.49±2.31	8.84±2.28	9.21±2.53	0.24	0.02	0.00–0.08
Fine motor	10.35±2.46	9.81±2.95	9.16±3.48	0.12	0.03	0.00–0.10
Gross motor	9.37±2.78	8.45±2.84	7.50±2.72	0.73	0.004	0.00–0.04
Bayley III composite scores, mean ± SD						
Cognitive	102.28±11.40	99.35±12.30	95.29±16.50	0.10	0.03	0.00–0.10
Language	98.28±12.29	95.84±12.76	95.05±12.42	0.39	0.01	0.00–0.06
Motor	97.78±15.82	94.97±14.77	90.82±17.48	0.33	0.02	0.00–0.07
Bayley III percentile ranks, mean ± SD						
Cognitive	54.47±24.04	49.23±25.74	43.20±28.42	0.13	0.03	0.00–0.10
Language	46.32±25.77	41.39±27.08	41.27±23.29	0.58	0.008	0.00–0.05
Motor	49.02±26.28	41.43±25.74	35.51±28.03	0.42	0.01	0.00–0.06

Bayley III = Bayley Scales of Infant and Toddler Development, Third Edition; SD = standard deviation; η_p^2 = partial eta squared.
P values were calculated by analysis of covariance test, adjusted for gestational age, Apgar score (at 5 minutes), sepsis, and grade 3 or 4 intraventricular hemorrhage. $P < 0.05$ was considered to be statistically significant. The effect size magnitude was defined as follows: η_p^2 0.01 − 0.06 = small effect, η_p^2 0.06 − 0.14 = medium effect, $\eta_p^2 > 0.14$ = large effect.

Table 4. Comparison of Poor Neurodevelopmental Outcomes among the 3 Study Groups

	Prematurity without Retinopathy of Prematurity (Group 0; n = 79)	Retinopathy of Prematurity without Treatment (Group 1; n = 31)	Retinopathy of Prematurity with Intravitreal Injection of Bevacizumab Treatment (Group 2; n = 38)	Unadjusted Odds Ratio (95% Confidence Interval)	Adjusted Odds Ratio (95% Confidence Interval)*
Bayley III composite scores <85, no. (%)					
Cognitive	8 (10.1)	4 (12.9)	9 (23.7)	OR_1, 2.1 (0.6–7.6) OR_2, 0.8 (0.2–2.7)	OR_1, 4.6 (0.8–27.9) OR_2, 1.5 (0.2–9.9)
Language	12 (15.2)	7 (22.6)	4 (10.5)	OR_1, 0.4 (0.1–1.5) OR_2, 0.6 (0.2–1.7)	OR_1, 0.4 (0.1–1.7) OR_2, 0.5 (0.1–2.1)
Motor	12 (15.2)	6 (19.4)	9 (23.7)	OR_1, 1.3 (0.4–4.1) OR_2, 0.7 (0.3–2.2)	OR_1, 1.4 (0.4–5.2) OR_2, 0.7 (0.2–2.7)
Severe neurodevelopmental disability, no. (%)	1 (1.3)	2 (6.45)	11 (29.0)	OR_1, 5.9† (1.2–29.1) OR_2, 0.2 (0.02–2.1)	OR_1, 4.6 (0.8–25.9) OR_2, 0.4 (0.03–5.4)
Severe neurodevelopmental disability excluding visual impairment, no. (%)	1 (1.3)	2 (6.45)	5 (13.2)	OR_1, 2.2 (0.4–12.2) OR_2, 0.2 (0.02–2.1)	OR_1, 1.3 (0.2–9.6) OR_2, 0.4 (0.03–6.9)

Bayley III = Bayley Scales of Infant and Toddler Development, Third Edition; OR_1 = odds ratio of group 2; OR_2 = odds ratio of group 0.
Group 1 is the reference group. Severe neurodevelopmental disability: hearing impairment, visual acuity poorer than 0.5 logarithm of the minimum angle of resolution, or any Bayley III composite scores <70. Severe neurodevelopmental disability excluding visual impairment: hearing impairment or any Bayley III composite scores <70.
*Adjusted for gestational age, Apgar score (at 5 minutes), sepsis, and grade 3 or 4 intraventricular hemorrhage.
†$P < 0.05$.

those previous studies, the patients receiving IVB in the present study also showed higher GA and BW, which may be related to the better neurodevelopmental performance of those patients.

To date, the BEAT-ROP study is among the few prospective randomized studies that have included patients with IVB treatment for ROP.[7] Although the BEAT-ROP study demonstrated the effectiveness of IVB for ROP, it did not address the issue of safety because of its small sample size and the relatively low rates of adverse events recorded in that study (i.e., corneal opacity requiring corneal transplant, lens opacity requiring cataract removal, and death). As mentioned by Micieli et al,[31] a large patient population of 2800 infants, which is difficult to achieve clinically, would be needed to determine whether IVB is associated with a significantly higher mortality rate compared with laser treatment.[7,31] Therefore, although no increased number of adverse events have ever been reported in patients with ROP receiving IVB,[7,17] concerns about the safety of using IVB in newborns nevertheless have been raised.[21,32,33] There are currently no definite conclusions in how to use bevacizumab properly for ROP patients.

In adult patients, the systemic half-life of bevacizumab has been shown to be 20 days after intravitreal injection.[34] The median plasma level of VEGF has been noted to be reduced by 42% in patients with age-related macular degeneration 28 days after receiving the third monthly IVI of bevacizumab.[35] In a study regarding the treatment of age-related macular degeneration, IVB was shown to suppress VEGF for at least 1 year after monthly or as-needed treatments.[36]

However, the pharmacokinetics of IVB may be different between newborns and adults.[32,37] Sato et al[37] found that systemic VEGF levels were depressed for at least 2 weeks after the administration of either 0.25 mg or 0.5 mg IVB in patients with stages 3, 4, and 5 ROP. Kong et al[18] demonstrated that serum-free VEGF levels decreased 2 days after treatment of either 0.25 mg or 0.625 mg IVB or laser and that the reductions were more significant in both IVB-treated groups. They also found that clearance of bevacizumab from the bloodstream in premature infants takes at least 2 months after IVB.[18] Our previous studies have shown further that VEGF levels in type 1 ROP infants were decreased significantly up to 12 weeks after administration of 0.625 mg IVB.[38–40]

Previous laboratory studies have shown that VEGF plays an important role in neurogenesis in embryos and newborns. Breier et al[20] found that VEGF transcript levels were abundant in the ventricular neuroectoderm of embryonic and neonatal murine brains when endothelial cells proliferate rapidly but were reduced in adults when endothelial cell proliferation has ceased.[20] Bagnard et al[41] found that VEGF164 stimulates the migration survival of a neuroectodermal progenitor cell line.[41] Jin et al[42] found that intracerebroventricular VEGF administration to adult rat brains stimulates neurogenesis, astrocyte production, and endothelial cell growth in the hippocampus and the lateral subventricular zone.[42] Zhang et al[43] found that neuronal progenitors derived from the newborn rat rostral subventricular zone expressed VEGF receptor after

fibroblast growth factor 2 stimulation, and VEGF guided the directed migration of the undifferentiated neural progenitors.[43] Malik et al[19] found that rabbit pups in utero exhibit higher levels of VEGF, that preterm delivery and room air exposure reduces VEGF expression, and that treatment with hypoxia mimetics significantly enhances VEGF expression, thus restoring neurogenesis in these preterm pups.[19] These findings were proposed to explain partially the neurodevelopmental delay and reduced growth of the cerebral cortex in preterm infants.[19] Furthermore, they also found that significant neurogenesis continued in human preterm infants born at a GA less than or equal to 28 weeks;[19] therefore, deprivation of serum VEGF in preterm infants may have effects on the neurodevelopment and development of other organ systems.

Because VEGF plays a key role in neurogenesis in embryos and preterm newborns, investigation of the impact of anti-VEGF treatment for ROP on neurodevelopment is needed for better understanding of its safety in such infants. The neurodevelopmental outcomes after IVB for ROP have been addressed in a few studies. In a prospective noncomparative case series by Martínez-Castellanos et al,[44] 13 infants who received IVB for ROP were evaluated annually by the pediatricians using the standardized Denver Developmental Screening Test II, and most patients showed normal neurodevelopmental scores 5 years after the use of IVB, with the exception of 1 patient who experienced developmental delay.[44] However, the study included a relatively small number of patients, and the study design was noncomparative.

Morin et al[21] retrospectively compared the neurodevelopmental outcomes of 125 preterm infants treated with IVB (n = 27) and laser (n = 98) at 18 months' corrected age using the Bayley III for assessment. The only difference between the 2 groups was noted in the motor composite score, with lower motor composite score in patients receiving IVB compared with laser treatment (median, 81 vs. 88; $P = 0.02$). The study also showed a higher risk of motor composite score of less than 85, neurodevelopmental impairment, and severe neurodevelopmental disabilities in patients receiving IVB compared with laser ablation.[21] However, the neurodevelopmental disabilities in that study may not be a reliable indicator of neurodevelopmental outcomes, because some of the items included were questionable. First, cerebral palsy is a movement disorder that is present from birth rather than a postnatal developmental disorder; therefore, infants born with cerebral palsy should be excluded rather than included in the study. Second, visual or hearing impairment was determined through parental or medical record review, or both, in the study. In addition to the inaccuracy of subjective assessment, visual impairment itself can be highly related to the consequence of ROP, rather than being a neurodevelopmental disability related to the treatments. Blair and Shapiro[14] also commented that the IVB group in the study included patients with more severe systemic illnesses and more severe ROP statuses compared with the laser group and that more patients were excluded from the laser group than from the bevacizumab group because of inability to undergo Bayley III assessment, which may be related to poorer neurodevelopment in the laser group.[14]

An earlier study by Lien et al[22] retrospectively compared the neurodevelopmental outcomes of 61 preterm infants treated with IVB only (n = 12), laser only (n = 33), and a combination of laser and IVB treatment (n = 16), with neurodevelopmental assessments performed at the corrected ages of 6, 12, 18, and 24 months using the Bayley II (i.e., the second edition) for assessment. The results showed that patients treated with laser alone and IVB alone did not differ significantly in mental or psychomotor development at up to 2 years of follow-up, whereas the worst neurodevelopmental outcomes were noted in the combined IVB and laser group. Patients in the combined IVB and laser group showed a significantly lower mental developmental index ($P = 0.028$) and psychomotor developmental index ($P = 0.002$) as well as higher risk of severe psychomotor impairment at 24 months ($P = 0.042$) compared with the patients in the laser group.[22] However, as stated by Blair and Shapiro,[14] it is worth noting that patients in the combined IVB and laser group showed lower GA and BW and were significantly more likely to have zone I disease, which may have affected the neurodevelopmental outcomes.[14]

Recently, Kennedy and Mintz-Hittner[25] analyzed the medical and neurodevelopmental outcomes of a subgroup of 18 inborn infants at 1 study site of the BEAT-ROP study. In the study, patients were randomized to receive either IVB or laser treatment, as in the BEAT-ROP study, and routine neurodevelopmental assessments were performed at the corrected age of 18 to 22 months using the Bayley III. The results showed that there were no significant differences between the IVB group and laser group in all 3 composite scores (cognitive, language, and motor), but there seemed to be a trend toward higher scores in patients with IVB treatment. However, the study included a relatively small number of patients (7 in the IVB group and 9 in the laser group) and therefore might not be powered sufficiently to identify small but important differences.

Increasing prematurity and the severity of ROP and laser ablative therapy have been shown to be associated with the development of myopia.[45–49] Our study showed that ocular developmental outcomes at the age of 1.49±0.59 years after birth were similar between ROP patients who did not require treatment and ROP patients with IVB treatment. In the current study, the spherical equivalent of ROP patients receiving IVB was 0.13 diopter (D; range, −17.5 to 3.00 D), which was similar to the previously reported spherical equivalent of ROP patients receiving IVB treatment alone (range, −1.59 to 0.64 D)[44,50–53] and seemed less myopic than the previously reported spherical equivalent of patients receiving cryotherapy (range, −6.5 to −3.51 D) and laser ablative treatment or combined IVB and laser treatment (range, −4.71 to −1.50 D).[45,47,50–52,54] Our findings suggest that IVB treatment may have less influence on refractive outcomes compared with laser ablative treatment.

Furthermore, we found that the visual acuity measured by the Cardiff acuity test at the age of 1.49±0.59 years was similar between ROP patients who did not require treatment and ROP patients who underwent IVB treatment. Few

studies have been conducted regarding the visual outcome after treatment of ROP. The Cryotherapy for ROP Cooperative Group[55] has demonstrated that patients with threshold ROP treated with cryotherapy developed poorer visual outcomes 5.5 years after treatment compared with patients with threshold ROP without cryotherapy treatment.[55] Saint-Geniez et al[56] demonstrated that VEGF neutralization leads to neuroretinal cell apoptosis and loss of retinal function in mice, which results in significant reduction of both a- and b-wave amplitude in electroretinography, indicating a considerable loss of photoreceptor function.[56] These results show that endogenous VEGF is required for visual function, indicating that anti-VEGF therapies should be administered with caution, particularly in developing eyes in infants and children. In the present study, our findings suggest that a single IVB treatment may have less influence on visual outcomes compared with cryotherapy.

This study has several limitations, including the limited numbers of patients enrolled, the nonrandomized design, and differences in the source of enrollment among the 3 groups. Although our institution is the largest medical center in Taiwan and one of the major referring centers for severe ROP, enrolling infants into a prospective study remains difficult. The small sample size, although satisfying the prestudy power analysis and larger than most of the previous studies,[22,25,44] still may have lacked sufficient power to identify small but clinically significant differences.

Some also may be concerned that the difference in the source of enrollment between groups (Fig 1) may result in bias because of enrolling seemingly sicker patients from outpatient clinics (because they may be likely to come back for follow-up), or the opposite, enrolling seemingly healthier ones (because the sicker ones may be likely to drop out of standard care). We checked the data of the premature infants hospitalized in the NICU during the enrollment period and analyzed their demographics, systemic risk factors, and ROP conditions. The results showed no significant difference in those variables between the enrolled and nonenrolled patients. These findings are summarized in Table S1 (available at www.aaojournal.org). As a result, although we were not able to approach and enroll all the patients admitted to the NICU during the enrollment period, the patients we enrolled showed demographics similar to those of the patients not included in the study, and thus our study did not bias toward enrolling healthier or sicker patients in any of the 3 study groups. Nevertheless, the higher percentage of enrollment in the so-called sicker group suggested that sicker patients tended to be enrolled. Although this phenomenon is common in prospective studies, the potential bias from this, which may be difficult to avoid, is still a limitation and could serve as a potential bias in the interpretation of the presented data.

The early outcome measurement at the ages of 1 to 3 years also may be a limitation, because some fluctuations in neurodevelopment were noted as a result of systemic development being incomplete during this period. Further studies assessing long-term neurodevelopmental and ocular developmental outcomes are needed to evaluate better the long-term efficacy and safety of IVB on ROP patients. Furthermore, the 3 groups were not comparable in several aspects, including GA, BW, and comorbidities. Although a post hoc test revealed no significant differences in these aspects between the ROP without treatment group (group 1) and ROP with IVB treatment group (group 2) except for GA, there seemed to be a trend for group 2 patients to show lower BW and higher rates of comorbidities compared with group 1 patients. Those differences were related to the natural history of ROP, as demonstrated in our previous study.[57]

Notwithstanding the aforementioned limitations, the present study had certain strengths. In this study, we enrolled patients and evaluated their outcomes prospectively. We also used clear definitions of patient enrollment, exclusion criteria, treatment indication, and designated tests performed for patients.

In conclusion, we did not detect a significant difference in neurodevelopmental outcomes or refractive or visual outcomes between the ROP patients treated with IVB and the ROP patients without treatment. Because of the limitations mentioned previously, further prospective, larger-scaled, randomized trials are needed to verify whether anti-VEGF treatments for ROP patients had a small but clinically significant impact on neurodevelopmental and ocular developmental outcomes.

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Abbreviations and Acronyms:
ANOVA = analysis of variance; **BEAT-ROP** = Bevacizumab Eliminates the Angiogenic Threat of Retinopathy of Prematurity; **Bayley III** = Bayley Scales of Infant and Toddler Development, Third Edition; **BW** = birth weight; **D** = diopter; **GA** = gestational age; **IVB** = intravitreal injection of bevacizumab; **IVI** = intravitreal injection; **NICU** = neonatal intensive care unit; **OR** = odds ratio; **ROP** = retinopathy of prematurity; **VEGF** = vascular endothelial growth factor; η_p^2 = partial eta squared.

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