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Research Article

Variation of Retinal Vascularisation Post Bevacizumab for Treatment of Type 1 Retinopathy of Prematurity: A Dose Comparison Based on Retinal Fluorescein Angiography

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Abstract

Retinopathy of prematurity (ROP) is increasingly treated with intravitreal bevacizumab (IVB) but consensus on the optimal dose remains to be determined. Peripheral avascular retina (PAR) and fluorescein leakage are recognised sequelae following anti-vascular endothelial growth factor (VEGF) therapy. The impact of IVB dose on the frequency of these outcomes requires further investigation. This study aimed to compare the prevalence of these two findings on fundus fluorescein angiography (FFA) in infants post treatment of ROP with either 0.625 mg or 0.3125 mg IVB. This was a retrospective study of 74 eyes to compare the prevalence of PAR, fluorescein leak and length of temporal retinal vascularisation (LTRV) after receiving IVB as primary therapy for severe ROP. PAR was observed in almost all eyes treated with IVB (P=1). The number of eyes demonstrating fluorescein leak was double in those receiving 0.3125 mg (6 out of 34) compared to 0.625 mg (3 out of 40) though this difference was not statistically significant (OR 2.61, 95% CI 0.50-17.53, P=0.286). Eyes treated with 0.625 mg IVB had a non-significantly greater mean LTRV by 0.37 disc diameters (P=0.573). Despite lacking statistical significance, the doubled fluorescein leakage rate and trend towards lower LTRV in patients receiving 0.3125 mg may warrant caution in high-risk cases.

Keywords

Retinopathy of Prematurity, Bevacizumab, Dose Comparison Peripheral Avascular Retina

1. Introduction

Retinopathy of prematurity (ROP) is a sight threatening neovascular disorder affecting preterm infants with low gestational age or birth weight. [1] Despite advances in neonatal and ophthalmic care, it remains a leading cause of preventable childhood blindness. [1] The efficacy of anti-vascular endothelial growth factor (VEGF) treatment has been established by the BEATROP and RAINBOW studies. [1, 2]. Compared with laser photocoagulation, anti-VEGF monotherapy produces both improved anatomical and refractive outcomes in posterior ROP disease. [1] Since the landmark BEATROP

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study used 0.625 mg bevacizumab, comparable efficacy has been reportedly achieved with lower doses in terms of acute retreatment rates and disease regression. [3-9] However, there is no consensus among ophthalmologists regarding the ideal dose of intravitreal bevacizumab (IVB) for retinopathy of prematurity (ROP) treatment. The competing clinical priorities include minimising potential systemic side-effects while maximising visual outcomes. [3-9].

It is well known that peripheral avascular retina (PAR) occurs frequently in eyes treated with anti-VEGF therapy, [10] but there is a paucity of research into whether IVB dose influences the frequency and extent of PAR or vascular leakage that may require further treatment. Our study attempted to answer these questions by utilising fundus fluorescein angiography (FFA) to assess the peripheral retinal vasculature following treatment with 0.625 mg versus a reduced dose of 0.3125 mg IVB for ROP.

2. Methods

This retrospective cohort study included all infants who underwent FFA imaging post primary IVB therapy for severe ROP (Type 1 ROP and aggressive ROP) between October 2018 and January 2023 at a tertiary paediatric referral centre. Ethics approval was obtained from our institutional Human Research Ethics Committee (EX/2023/QCHQ/94315). Patients were split into Group 1 or 2 and received 0.625 mg or 0.3125 mg IVB respectively. Pre-treatment RetCam images were de-identified and re-graded to confirm treatment requiring ROP by two paediatric ophthalmologists experienced in ROP management. A third paediatric ophthalmologist blinded to treatment groups reviewed the follow-up angiograms for fluorescein leakage and the presence of PAR (Figures 1 and 2). Fluorescein leakage was defined as intense early vascular hyperfluorescence with increased late fluorescence.

The length of temporal retinal vascularisation (LTRV) was used as a surrogate to gauge retinal vascularisation with a high LTRV indicating less PAR. The LTRV was measured from the optic disc centre through the fovea to the vascular-avascular border using xScope (xScope, version 4.6, The Iconfactory; 2022) as shown in Figure 3. Optic disc diameter (DD) and LTRV were recorded in pixels. To account for differences between sizes of eyes LTRV was compared in DD instead of pixels.

Statistical analyses were performed in RStudio (RStudio, version 2023.03.0+386, R Foundation for Statistical Computing, Vienna, Austria; 2022). Categorical variables were assessed using Fisher's exact or chi-squared test based on cell counts. The Shapiro-Wilk test for normality was conducted on numerical variables. A Mann-Whitney U test was performed for those with normal distribution and a student's T-test for the remainder. To compare the difference in LTRV a linear mixed model analysis accounting for inter-eye correlation in bilateral cases was used.

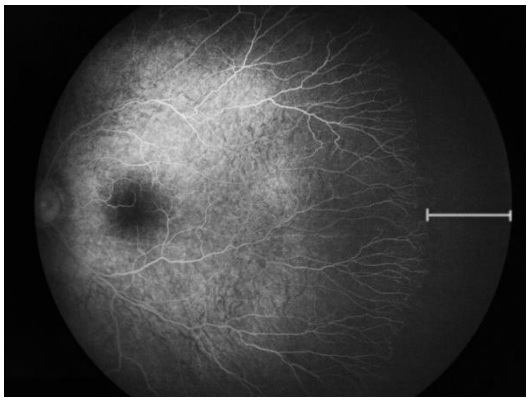

Figure 1. *Peripheral avascular retina post 0.3125 mg bevacizumab treatment for retinopathy of prematurity. (GA 25 weeks, BW 800 g and PMA 92 weeks at time of FFA).*

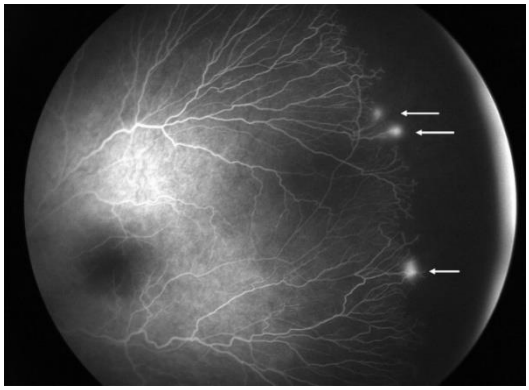

Figure 2. *Fluorescein leak post 0.3125 mg bevacizumab treatment for retinopathy of prematurity. (GA 23 weeks, BW 526 g and PMA 84 weeks at time of FFA).*

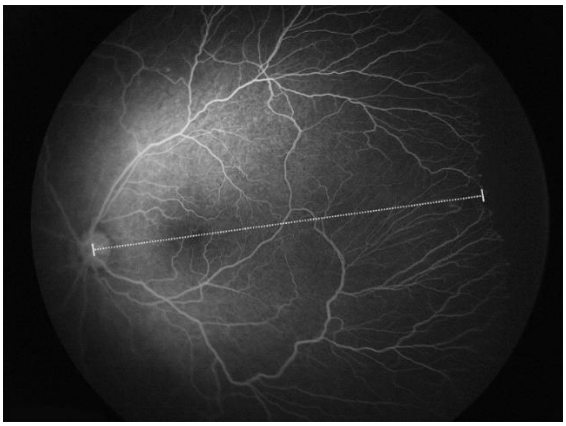

Figure 3. *Measurement of temporal vascularisation from optic disc through fovea to avascular-vascular border.*

3. Results

Eighty-six eyes of 44 infants received IVB during the study period. Seventy-four had FFAs available for review with 40 (54%) having received 0.625 mg bevacizumab. Four from each treatment group were lost to follow-up and four Group 2

eyes did not receive FFA imaging post IVB as they were deemed low risk on indirect ophthalmoscopy. The mean gestational ages (GA) at birth for Group 1 and 2 respectively were 24.3 (range, 23-28) and 24.7 weeks (range, 23-27). Mean birth weights (BW) for Group 1 and 2 were 687.8 (range, 429-1300) and 743.4 g (range, 420-1331).

In Group 1, the mean postmenstrual age (PMA) at time of IVB was 34.9 (range, 32-40) while Group 2 doses were delivered at 35.4 weeks (range, 33-39). Two infants in Group 2 required retreatment one week post initial IVB due to insufficient disease regression. FFAs were performed significantly later in Group 2 at a mean PMA of 94 weeks (range, 42-176) while Group 1 underwent imaging at 74.3 weeks (range, 46-125). Table 1 outlines the baseline characteristics for the two cohorts.

Table 1. *Characteristics of Patients Treated with Intravitreal Bevacizumab for Retinopathy of Prematurity.*

Characteristic	0.625 mg (40 eyes)	0.3125 mg (34 eyes)	P Value
Mean GA	24.3	24.7	P=0.2921
Mean BW	687.8	743.4	P=0.5937
Sex			
M	28 (73.3%)	22 (76.5%)	P=1
F	12	12	
AROP	10 (25.0%)	8 (23.5%)	
Zone 1 plus	6 (15.0%)	10 (29.4%)	P=0.6122
Zone 2 plus	24 (60.0%)	16 (47.1%)	
Mean PMA at Treatment	34.9	35.4	P=0.3514
Mean PMA at FFA	74.3	94.0	P=0.049
Retreatment	0	2 (5.8%)	P=0.4595

PAR was observed in almost all treated eyes with a higher number amongst Group 2 patients (P=1). Group 1 demonstrated a lower rate of fluorescein leak, but this was not statistically significant (OR 2.61, 95% CI 0.50-17.53, P=0.286). Two of three eyes in Group 1 demonstrating fluorescein leak had aggressive ROP and the other belonged to a patient with zone 1 plus disease. Among the six Group 2 eyes, two had zone 1 disease with plus and the remainder were zone 2.

In 67 eyes with FFAs of sufficient quality for measurement, a greater mean LTRV of 0.37 DD was measured in patients who received 0.625 mg bevacizumab however this was not significant (P=0.573). Table 2 compares peripheral retinal findings between the two treatment groups.

Table 2. *Fluorescein Angiography Findings Following Different Doses of Intravitreal Bevacizumab for Retinopathy of Prematurity.*

Finding	0.625 mg (40 eyes)	0.3125 mg (34 eyes)	P value
Eyes with fluorescein leak	3 (7.5%)	6 (17.6%)	P=0.286
Eyes with peripheral avascular retina	38 (95%)	34 (100%)	P=1
Mean amount of temporal retinal vascularisation in disc diameters (n=67)	12.13 DD	11.76 DD	P=0.573

4. Discussion

Study findings

Our retrospective study compares FFA findings following 0.625 mg versus 0.3125 mg bevacizumab for treatment of ROP. No significant statistical difference in fluorescein leak, PAR and LTRV was found between groups. Notably despite this, double the number of infants had fluorescein leakage in Group 2. Given the small sample size, it is possible the study had insufficient power to detect a significant difference between the two groups.

A higher LTRV was noted in Group 1 infants that received 0.625 mg although the difference was not significant. Due to clinician preference, all Group 1 infants proceeded directly to FFA post IVB without clinic review and were imaged significantly earlier by almost 20 weeks on average. It is possible if Group 1 FFAs were performed later, further vascularisation could have occurred and a statistically difference in retinal vascularisation may be detected. In contrast to our findings, a comparable study by Bayramoglu *et al.* of 84 eyes also utilising FFA imaging found a lower LTRV in their 0.625 mg group. [11] Although this result was also statistically non-significant, the authors of that paper acknowledged selection bias may have occurred as their 0.3125 mg group had lower plus disease severity and higher GA. [11].

Clinician use of FFA post anti-VEGF for ROP varies and there is currently no standard practice internationally including Australia. [12] FFA post IVB treatment can help identify ROP sequelae not visualised otherwise. [13-15] Where the vascular-avascular junction can be unclear on examination or colour fundus photography, areas of PAR are well-demarcated [13, 15] Mansukhani *et al.* noted fluorescein leak was present without abnormal vessels on indirect ophthalmoscopy in 40% of patients. [14] To improve diagnostic reliability, FFA has good interoperator concordance in evaluation of leakage and ischaemic retina amongst ophthalmologists with varied expertise. [13].

Almost all patients in the study demonstrated PAR on FFA irrespective of IVB dose. The management of PAR follow-

ing anti-VEGF therapy remains without consensus. [13, 16] Some clinicians opt to laser all PAR observed on indirect ophthalmoscopy post IVB treatment, others may use FFA as an ancillary tool to aid decision making and ablate eyes with residual PAR or fluorescein leakage. [13, 16] At our centre, all fluorescein leak post anti-VEGF therapy for ROP is treated using laser. It is debatable if all PAR post anti-VEGF therapy should receive prophylactic laser photocoagulation considering the destructive modality and increased risk for myopia. [15, 17] A prospective study to determine the two-year outcomes of PAR and fluorescein leakage with or without laser retreatment finished recruitment in January 2022. [17] Although ranibizumab was chosen as the anti-VEGF agent, the results when available, could help determine ideal management of these findings. [17] Alternatively, as earlier recipients of IVB begin to have long-term follow-up, the results of differing practice preferences will also become available. Notwithstanding this, FFA is an important modality for evaluation of the peripheral retina post IVB, and we would recommend routine use in follow-up.

Limitations

Study limitations in addition to clinician preferences and patient number discussed previously include the retrospective design and small sample size. A larger multi-centre collaboration may help to increase power in future studies examining treatment outcomes in ROP. The effect of attrition bias is likely limited as both treatment groups lost patients to follow-up at a similar rate. As dose of IVB was not chosen based on severity of disease but rather clinician preference, there is low risk of confounding by indication as a result.

In conclusion, our study shows eyes treated with 0.625 mg IVB had increased retinal vascularisation and decreased fluorescein leak however this was not statistically significant. Routine FFA post IVB treatment is recommended for clear visualisation of ROP sequelae and can be useful to guide any retreatment decision.

Abbreviations

BW	Birth Weight
DD	Disc Diameter
FFA	Undus Fluorescein Angiography
GA	Gestational Age
IVB	Intravitreal Bevacizumab
LTRV	Length of Temporal Retinal Vascularisation
PAR	Peripheral Avascular Retina
PMA	Postmenstrual Age
ROP	Retinopathy of Prematurity
VEGF	Vascular Endothelial Growth Factor

Declaration of Funding Sources

None.

Conflicts of Interest

The authors declare no conflicts of interest.

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