Fluorescein Angiography Parameters in Premature Neonates

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Purpose: To describe fluorescein angiography (FA) parameters observed in premature neonates with retinopathy of prematurity (ROP).

Design: Retrospective case series.

Subjects: Patients with ROP who underwent FA imaging using Retcam at Holtz Children's Hospital from November 2014 to October 2022.

Methods: Fluorescein angiography images of the included patients were analyzed with a focus on the timing of angiography phases, including choroidal flush, retinal, and recirculation phases. Gestational age, birth weight (BW), age at imaging, treatment choice, and any FA complications were documented.

Main Outcome Measures: Dose of fluorescein administered, onset and duration of each angiography phase, and FA findings in ROP-treated patients.

Results: A total of 72 images of 72 eyes were reviewed. Image quality was deemed suitable for inclusion in 64 eyes (88.9%) of 43 patients. The mean gestational age and BW at birth were 24.4 ± 1.9 weeks and 607.8 ± 141.3 g, respectively. The mean postmenstrual age at FA imaging was 50.5 ± 40.8 weeks. All eyes (100%) received treatment with intravitreal injection of anti-VEGF at a mean age of 35.5 ± 2.4 weeks. The onset and duration of angiography phases were relatively variable within the cohort. Choroidal flush occurred at a mean time of 12.2 seconds (range: 6−22 seconds). A subsequent retinal phase was documented at a mean time of 11.96 seconds (range: 3−22 seconds). Recirculation phase was complete at an average time of 2.15 minutes (range: 1−5.45 minutes) postfluorescein injection. None of patients developed allergic reactions to fluorescein injection, such as rash, respiratory distress, tachycardia, fever, or local injection site reactions.

Conclusions: Angiographic phases on FA in preterm infants with ROP are variable and may occur earlier than the established references for adults.

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Fundus fluorescein angiography (FA) has enhanced our ability to diagnose, characterize, and treat retinal disorders. However, most devices and imaging protocols are designed for adult use. Neonates and infants have smaller eyes and unique anatomic considerations.[1] Retinal diseases affecting the pediatric population include several genetic and environmentally influenced conditions that primarily involve the peripheral retina, including retinopathy of prematurity (ROP), familial exudative vitreoretinopathy, and Coats disease.[2]

The increasing survival rate of extremely low birth weight (BW) infants over the last decade has led to an increase in severe forms of ROP.[3−6] The advent of digital fundus imaging made it easier and safer to perform FA in neonatal intensive care units. Instruments such as the RetCam (Clarity) are useful in infants at a high risk of ROP, enabling providers to overcome some limitations of indirect ophthalmoscopy.[7−9] In recent years, many researchers have used FA as a pivotal diagnostic tool to study vascular development and progression in the premature retina.[10]

Despite the well-established protocols for performing FA in adults, there remain a dearth of specific guidelines for children or neonates, particularly concerning the timing of FA phases, optimal dosage, and other nuances of the examination. Thus, this study aims to characterize the parameters of FA and its various phases in premature infants with ROP, serving as an adjunct to conventional examination methods.

Methods

This study was a retrospective case series of patients with ROP who underwent FA imaging at the neonatal intensive care unit at Holtz Children's Hospital (Jackson Memorial Hospital) from November 2014 to October 2022. This study was approved by the University of Miami institutional review board and adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from the patients' parents before FA acquisition.

Screening for ROP adhered to national guidelines, including infants with gestational age <30 weeks and BW <1500 g, as well as larger preterm babies deemed at risk by their pediatricians.[11]

Inclusion criteria comprised patients with ROP who underwent FA either before or after treatment, while exclusion criteria included presence of any media opacity that could compromise image quality in both eyes.

All FA images were acquired by the same photographer (G.O.) using RetCam 3 (Clarity Medical Systems, Inc) equipped with a 130° wide-field contact lens. A new peripheral line was placed in the arm as close to the vein as possible, without using any extension or flexible catheter, such as a peripherally inserted central catheter. After peripheral intravenous cannulation, mydriasis and topical anesthesia were achieved with tropicamide 1%, phenylephrine 2.5%, and proparacaine 0.5%. Eyelids were held open with 2 pediatric Alfonso speculums (Storz). Key steps included inserting a yellow filter into the camera handpiece, switching to blue light for illumination, and activating the software's FA mode.

Sodium fluorescein 10% (Fluorescite; Novartis Pharma) was intravenously administered at a dose of 7.7 mg/kg followed by an immediate saline flush.[12,13] The FA timer was started when the saline flush was completed. Angiographic images were then captured at regular intervals following dye injection, with the camera alternated between eyes to capture relevant phases of dye transit until the late phase was reached. Of note, the faint degrees of fluorescence were much better detected with reduced levels of illumination. The procedure aimed to avoid excessive pressure on the eye to ensure proper dye entry, with a pediatric nurse trained in neonatal life support monitoring vital signs throughout (Fig 1) fluorescein angiography-guided treatment (anti-VEGF, anti-VEGF, and/or laser) was administered on a case-by-case basis.

For each infant, the data collected included gestational age, age at imaging, BW, treatment received, and any FA complications. Retinal images were uploaded to and reviewed on the Optos advance retinal imaging software to identify angiographic phases including choroidal flush (when the dye appears first in the choroid), retinal phase (when the dye reaches the retinal vessels, first sign of retinal circulation), peak phase (when there appears maximal fluorescence), and recirculation phase (when the dye has recirculated and reached the late phase). The duration of the imaging session was determined as the time difference between the first and last captured images, automatically recorded by the system.

Analysis and descriptive statistics were calculated using Microsoft Excel software.

Results

Baseline Characteristics

Out of the 72 identified FA for neonates with ROP, 8 images (11.1%) were excluded because of bad quality imaging (5/8, 62.5%), delayed flush (2/8, 25%), and FA performed without timing (1/8, 12.5%). Consequently, a total of 64 FA examinations (88.9%) of 43 patients were included in this study.

Patient characteristics are summarized in Table 1. The mean gestational age and BW at birth were 24.4 ± 1.9 weeks (range: 22.5−34 weeks) and 607.8 ± 141.3 g (range: 345−1180 g), respectively. The mean postmenstrual age at FA imaging was 50.5 ± 40.8 weeks (range: 32.7−287.4 weeks). Throughout the follow-up period, all eyes (100%) received treatment with intravitreal injection of anti-VEGF at a mean age of 35.5 ± 2.4 weeks. Among the analyzed images, 54.7% (35/64) underwent treatment before FA, while 45.3% (29/64) had follow-up FA after treatment.

FA Findings

Only 3 of eyes (4.6%) had all 4 FA phases imaged, because the photographer was not specifically instructed to capture the choroidal phase. Representative FA images of the 4 phases are shown in Figure 2.

The choroidal flush was observed in only 5 eyes (7.8%), at an average of 12.2 ± 4.0 seconds (range 6−17 seconds). All eyes (100%) had images captured from the first moment of retinal phase, which occurred at a mean value of 11.96 ± 4.3 seconds (range: 3−22 seconds). The peak retinal phase was evident in 61 eyes (95.3%) at an average of 25.2 ± 3.9 seconds (range: 17−37 seconds). The recirculation phase was observed at a mean of 2.15 ± 0.9 minutes (range 1−5.45 minutes) (Table 1) All patients had late phases successfully recorded.
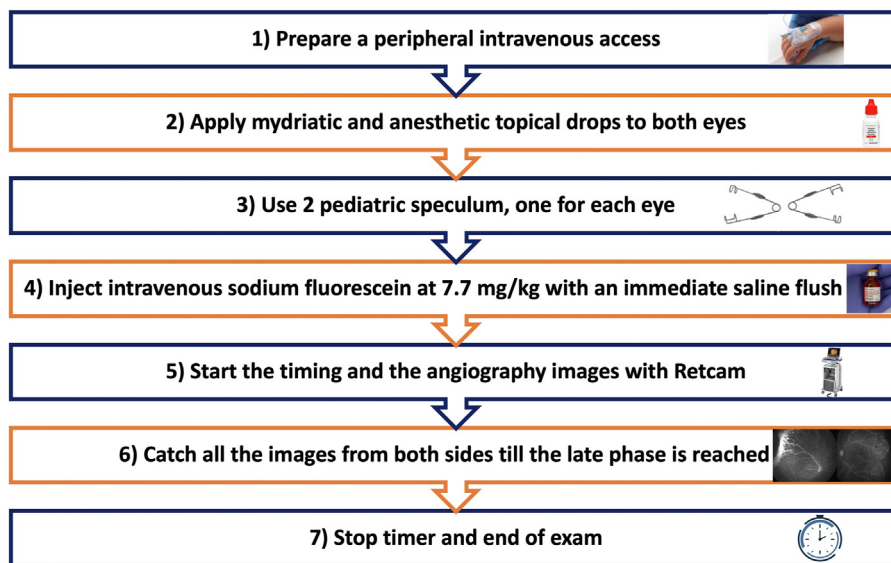

Figure 1. Standardized protocol for fluorescein angiogram acquisition in premature infants.

1) Prepare a peripheral intravenous access

2) Apply mydriatic and anesthetic topical drops to both eyes

3) Use 2 pediatric speculum, one for each eye

4) Inject intravenous sodium fluorescein at 7.7 mg/kg with an immediate saline flush

5) Start the timing and the angiography images with Retcam

6) Catch all the images from both sides till the late phase is reached

7) Stop timer and end of exam

Table 1. Baseline Characteristics

General Data	Mean	Standard Deviation	Minimum	Maximum
Patients				
Gestational age at birth (weeks)	24.4	1.9	22.5	34
Birth weight (grams)	607.8	141.3	345	1180
Gestational age at treatment (weeks)	35.8	2.5	32.5	45.5
Eyes				
Gestational age at FA (weeks)	50.5	40.8	32.7	287.4
Choroidal phase time (seconds)	12.2	4	6.2	17.1
Retinal phase time (seconds)	11.96	4.3	2.6	22.3
Peak phase time (seconds)	25.2	3.9	17.2	37.2
Recirculation phase time (minutes)	2.2	0.9	0.93	5.45

FA = fluorescein angiography.

Based on these findings, we proposed a new protocol for acquisition of different phases of FA in preterm infants (Table 2). Considering the mean observed time for each phase, we suggest that retinal phase would be best captured between 8 and 16 seconds, the peak phase between 21 and 29 seconds, and the recirculation phase between 1.3 and 3.1 minutes. The choroidal flush was not included in the suggested protocol because of the limited number of patients with choroidal flush data.

FA-Related Complications

Fluorescein angiography was well tolerated by all ROP neonates (100%). None of the patients in the study developed allergic reactions to fluorescein injection, such as rash, respiratory distress, tachycardia, fever, or local injection site reactions.

Discussion

In the current study, we reported the timing of angiographic phases using Retcam in a cohort of neonates with ROP. We were able to obtain images of sufficient quality to evaluate the disease condition in 88.8% of the cases. Our analysis focused on identifying the specific moments when angiographic phases were captured.

Fluorescein angiography has been extensively studied in ROP,[12,14,15] offering valuable insights, such as clearly delineating the junction between the vascular and
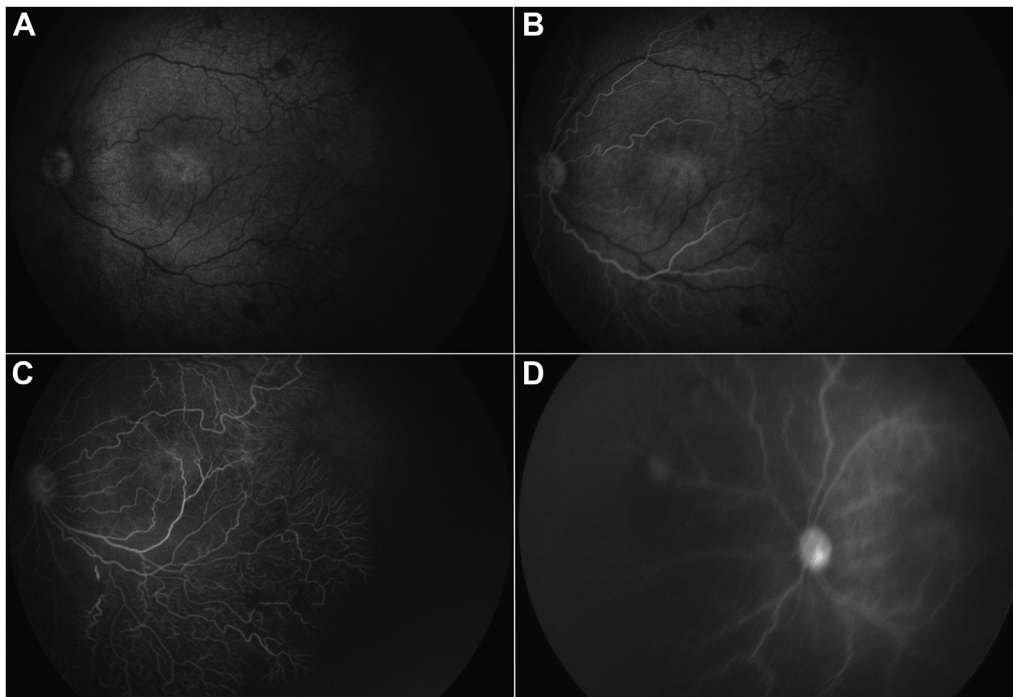

Figure 2. Representative fluorescein angiograms of a premature neonate showing the 4 phases: (A) choroidal flush, (B) retinal phase, (C) peak phase, and (D) recirculation phase.

Table 2. Suggested Protocol for Acquisition of Different Phases of Fluorescein Angiography in Preterm Infants

Angiogram Phase	Time After Injection	Findings
Retinal phase	8–16 seconds	Dye reaches the retina vessels
Peak phase	21–29 seconds	Concentration of fluorescein is maximum
Recirculation phase	1.3–3.1 minutes	Dye has recirculated and reached the late phase

avascular retina, identifying flat neovascularization not easily discernible to the naked eye, predicting the early progression of severe ROP, and assessing treatment response.[10,16–18] However, our study represents the first description of FA timing specifically performed in preterm newborns at a single institution.

Conventionally, the timing of angiographic phases in adults serves as a reference point. For instance, the choroidal phase typically begins 10 to 15 seconds after the dye is injected into the arm, followed by the retinal circulation phase 1 to 2 seconds later. A peak phase of the angiogram, characterized by a well-defined perifoveal capillary network, occurs around 30 seconds, with recirculation starting around 10 minutes after injection.[19]

Herein, we observed a highly variability in the circulation rate of the fluorescein dye from the arm to the infant's eye. Choroidal flush and retinal phase could manifest anywhere from 6 to 17 seconds and 4 to 22 seconds, respectively, with an average retinal phase onset at 11.95 seconds. Lepore et al reported a similar variability of fluorescein circulation.[12] Of note, the average time for choroidal flush (12.2 seconds) is longer than the average time to the retinal phase (11.96 seconds). This is likely attributed to the small number of patients with choroidal flush data. The recirculation phase was complete within 5.45 minutes after the fluorescein injection, quicker than previously reported in infants.[20,21] It is noteworthy that this variability of fluorescein circulation was observed despite following a preestablished standardized protocol, executed by a sole provider responsible for administering the dye and saline flush in the upper extremity, and a single technician in charge of initiating the timer.

These findings suggest differences in angiographic phases among premature infants with severe ROP. We propose several factors contributing to the difference between neonates with ROP and adult patients, including the higher heart rate of newborns, which is often double compared to adults,[22] shorter arm-to-retina circulation time, smaller size of the eyes, and the presence of tunica vasculosa lentis.

The elevated heart rate in newborns facilitates faster arrival and departure of the fluorescein dye because of increased cardiac output to meet metabolic demands during the first several months of life.[23] Additionally, the arm-to-retina distance is shorter in neonates compared to adults, primarily because of their smaller size at birth. Consequently, blood flow containing fluorescein can reach the retina more rapidly from the systemic circulation, especially when the dye is administered through a proximal intravenous access point. This dynamic leads to quicker timing of the FA.

Our hypothesis of faster fluorescein circulation in neonates compared to adults finds support in the understanding that various factors, more common in the adult population, can potentially cause delays in the arm-to-retina circulation. These factors include myocardial and pulmonary disease, alterations in blood viscosity, advancing age, hypertension, and glaucoma, all of which might contribute to delayed FA timing.[24] Furthermore, the shorter axial lengths of preterm infants' eyes can facilitate faster arrival and recirculation of the dye because of the reduced area for circulation.[25] Finally, preterm infants' eyes may have a tunica vasculosa lentis, potentially resulting in leakage of the dye into the anterior chamber. Consequently, this leakage may decrease the fluorescein concentration in the retina, contributing to a faster washout of the dye from the retina (Fig 3).

Although FA is not optimal for assessing choroidal circulation, previous series of FA examinations have provided some insights. Existing literature shows extreme variability in the pattern of choroidal filling, even across serial exams of the same eye.[10,16] For instance, fluorescein angiograms to evaluate the choroid in neonatal rhesus monkeys revealed that fluorescein first entered the large choroidal vessels at 4.4 seconds after injection. Subsequently, at 4.8 seconds, the retinal arterial phase appeared, followed by the arteriovenous phase at 5.6 seconds, and the late venous phase of the retinal circulation at 6.4 seconds, after which all choroidal fluorescence dissipated.[26] Notably, the faster timing of choroidal vessels filling observed compared to the adults' protocol was similar to our findings.

Regarding the dosage of fluorescein sodium, consensus for children or newborns remains elusive. Dosages may vary, ranging from a 10% or 20% fluorescein solution at a
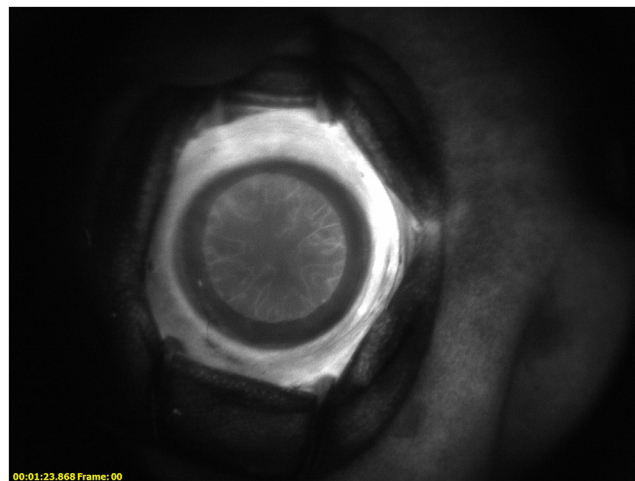

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Figure 3. Representative fluorescein angiogram of a premature neonate showing the leakage from a tunica vasculosa lentis.

dose of 0.04 to 0.1 ml/kg. Also, FA may or may not be followed by a small isotonic saline flush, considering the small quantity of the dye.[1,16,20,21,27,28] In our study, the dose of fluorescein of 7.7 mg/kg, previously reported to be safe in the pediatric population,[12,13] and the method of the examination were administered safely, with no reported complications.

This study was limited because of its retrospective nature. The angiograms were taken in patients with ROP with standardized protocol designed for adults and adapted for pediatric use. Another limitation is that video FA acquisitions were not available. Despite these limitations, it was evident that FA timing in preterm infants can be variable and occur earlier than adults.

In conclusion, this paper established reference ranges for different phases in pediatric FA. The timing for each phase was notably shorter than adults, likely secondary to physiologic and anatomic differences. These findings can provide a benchmark for future clinical and research investigations.

Footnotes and Disclosures

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