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Influence of Fluorescein Angiography on the Diagnosis and Management of Retinopathy of Prematurity

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Purpose: To examine the influence of fluorescein angiography (FA) on the diagnosis and management of retinopathy of prematurity (ROP).

Design: Prospective cohort study.

Participants: Nine recognized ROP experts (3 pediatric ophthalmologists and 6 retina specialists) interpreted 32 sets (16 color fundus photographs and 16 color fundus photographs paired with the corresponding FA images) of wide-angle retinal images from infants with ROP.

Methods: All experts independently reviewed the 32 image sets on a secure website and provided a diagnosis and management plan for the case presented, first based on color fundus photographs alone, and then based on color fundus photographs and corresponding FA images.

Main Outcome Measures: Sensitivity and specificity of the ROP diagnosis (zone, stage, plus disease, and category, i.e., no ROP, mild ROP, type 2 ROP, and ROP requiring treatment) were calculated using a consensus reference standard diagnosis, determined from the diagnosis of the color fundus photographs by 3 experienced readers in combination with the clinical diagnosis based on ophthalmoscopic examination. The κ statistic was used to analyze the average intergrader agreement among experts for the diagnosis of zone, stage, plus disease, and category.

Results: Addition of FA to color fundus photography resulted in a significant improvement in sensitivity for diagnosis of stage 3 or worse disease (39.8% vs. 74.1%; $P = 0.008$), type 2 or worse ROP (69.4% vs. 86.8%; $P = 0.013$), and pre-plus or worse disease (50.5 vs. 62.6%; $P = 0.031$). There was a nonsignificant trend toward improved sensitivity for diagnosis of ROP requiring treatment (22.2% vs. 40.3%; $P = 0.063$). Using the κ statistic, addition of FA to color fundus photography significantly improved intergrader agreement for diagnosis of ROP requiring treatment. Addition of FA to color fundus photography did not affect intergrader agreement significantly for the diagnosis of stage, zone, or plus disease.

Conclusions: Compared with color fundus photography alone, FA may improve the sensitivity of diagnosis of ROP by experts, particularly for stage 3 disease. In addition, intergrader agreement for diagnosis of ROP requiring treatment may improve with FA interpretation. *Ophthalmology 2015;122:1601-1608 © 2015 by the American Academy of Ophthalmology.*

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Clinical examination by indirect ophthalmoscopy has long been the standard method for the diagnosis of retinopathy of prematurity (ROP). On the basis of large, well-designed clinical trials, including the Cryotherapy for ROP and Early Treatment for ROP trials,[1,2] a consensus policy statement was established in the United States for the screening and management of ROP.[3] This policy statement recommended that examinations be performed "using binocular indirect ophthalmoscopy."[3] The policy statement also acknowledged a growing role for digital imaging in ROP, but emphasized the need for further studies to parse out the usefulness of these imaging methods in the diagnosis and management of ROP.

Fluorescein angiography (FA) has been shown to be critical for assessing the retinal vasculature in vasoproliferative disorders such as diabetic retinopathy[4] and exudative age-related macular degeneration in adults.[5] Additionally, FA has an important role in the evaluation and management of pediatric vascular disorders, including Coats' disease,[6] choroidal neovascular membranes,[7] sickle cell retinopathy,[8] ocular tumors,[9] and other conditions.[10,11] Fluorescein angiography appears to be safe in children, including neonates with ROP, with no adverse effects reported in several series.[12–15] Cantolino et al,[16] Flynn et al,[17] and O'Grady et al[18] introduced FA as a method to study retrolental fibroplasia in the late 1960s, and other investigators in this era noted the benefit of FA in

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evaluating the peripheral retina in the acute stages of ROP as well as for identifying late complications.[19] These early investigators noted the presence of changes seen on FA that were not visible on clinical examination. Flynn et al[17] used a Zeiss fundus camera (Carl Zeiss Meditec Inc, Dublin, CA) to obtain the angiograms, but because of limitations in obtaining fundus images in neonates with this device, there were limited reports of FA in ROP for many subsequent years.

With the introduction of newer digital wide-angle and ultrawide-field imaging systems, including those designed for pediatric use (e.g., RetCam; Clarity Medical Systems, Inc, Pleasanton, CA),[10,11,20] it is now becoming more common to perform bedside fundus imaging[21,22] and FA[12,23] in the pediatric population. Given that bedside FA is now more accessible and may provide useful information regarding developing retinal vasculature, there has been renewed interest in using this diagnostic method in the evaluation of ROP. Moreover, the shortage of trained ROP experts worldwide has prompted an interest in the role of telemedicine for this disease.[24,25] In turn, there has been particular interest in the usefulness of digital imaging for ROP.

The role of FA in the diagnosis of ROP by digital imaging remains unclear. Fluorescein angiography recently was implemented to evaluate retinal vascular morphologic features in eyes receiving intravitreal anti−vascular endothelial growth factor (VEGF) therapy[26−28]; however, current studies of FA in ROP are predominately descriptive, limiting their clinical impact and conclusions.[12,14,15,26,27,29] As such, a gap exists in understanding the usefulness of FA related to the accuracy of diagnosis and management of ROP by pediatric ophthalmologists and retina specialists. The purpose of this study was to evaluate the influence of FA on the diagnosis and management of ROP by ROP experts using wide-angle fundus images.

Methods

This study was approved as a prospective study by the institutional review board at Weill Cornell Medical College. Informed consent was obtained from all study participants before participation, and waiver of consent was obtained for use of de-identified retinal images. This study was conducted in accordance with Health Insurance Portability and Accountability Act guidelines and adhered to the tenets of the Declaration of Helsinki.

Image Acquisition

Wide-angle images of the posterior retina and corresponding FA images were captured bilaterally from 8 infants with ROP (16 eyes) using the RetCam II (Clarity Medical Systems, Inc.). Images were obtained from infants between 33 and 44 weeks postmenstrual age. For acquisition of FA images, 4 of 8 infants (50%) were imaged in the neonatal intensive care unit without intubation or sedation, whereas the remaining 4 of 8 infants (50%) were imaged in the operating room under sedation.

Consensus Reference Standard Diagnosis

For each image set, a consensus reference standard ROP diagnosis was established. This was accomplished by combining the clinical diagnosis as determined by indirect ophthalmoscopy with the image-based diagnosis from multiple experienced readers, as described previously.[30] This consensus reference standard then was used for the current study.

Study Experts

Eligible participants for this study were defined as board-certified practicing pediatric ophthalmologists or retina specialists who routinely evaluate infants for ROP and met at least 1 of the following criteria: was a principal investigator or certified investigator for the Cryotherapy for ROP study or Early Treatment for ROP study or published at least 2 peer-reviewed articles about ROP. These participants are referred to herein as *experts*.

Study Design

Study experts were directed to a secure website developed by the authors (M.A.K., S.N.P., M.F.C., R.V.P.C.). Initial baseline demographic data were collected from each expert, including what fellowship training had been completed (pediatric ophthalmology, medical retina, surgical retina), years since completion of fellowship, and level of comfort with reading color fundus photographs and FA images in ROP (not comfortable, somewhat comfortable, comfortable). Experts also were asked for the percentage of patients in their clinical practice with type 2 or worse ROP from whom they obtained FA imaging (0%, 1%−25%, 26%−50%, 51%−75%, 75%−99%, 100%), and, finally, whether they believe FA is safe in infants and neonates (yes or no). Type 2 ROP was defined as (1) zone I, stage 1 or 2 without plus disease, or (2) zone II, stage 3 without plus disease. Retinopathy of prematurity requiring treatment was defined as (1) zone I, any stage with plus disease; (2) zone I, stage 3 with or without plus disease; or (3) zone II, stage 2 or 3 with plus disease.

Experts were presented with a series of 8 ROP cases. Each case consisted of baseline demographic information (birth weight, gestational age, and postmenstrual age at the time of imaging) and an image set of color fundus photographs (Fig 1, available at www.aaojournal.org). Color fundus photographs were displayed as a set of 3 retinal images of each eye (temporal, posterior, nasal). For each image set, experts were asked to choose the zone (I, II, II posterior, III); stage (1, 2, 3, 4, 5); plus (no, preplus, plus); category (mild, type 2 ROP, ROP requiring treatment); management (observation, laser only, anti-VEGF only, laser with anti-VEGF, surgery); presence of aggressive posterior ROP (yes or no); and recommended clinical follow-up (less than 1 week, 1 week, 2 weeks, more than 2 weeks). Experts were asked their level of confidence (confident, somewhat confident, not confident) in determining the clinical diagnosis based on color fundus photographs provided and whether they would obtain FA imaging based on the color fundus photographs (yes or no). This was performed in sequential order for each of the 8 cases.

Next, a second image set comprising the same color fundus photographs accompanied by their corresponding FA images was presented for each of the 8 cases in the same sequential order. For each image set, the expert again was asked to determine zone, stage, plus, category, management, presence of aggressive posterior ROP, and recommended clinical follow-up. Experts also were asked to gauge their level of confidence in determining the diagnosis based on color fundus photographs and FA images (confident, somewhat confident, not confident) and whether they believed that the FA images had provided clinically useful information for management purposes (yes or no).

Data Analysis

All data were analyzed using statistical software (Stata/SE version 12.0; StataCorp LP, College Station, TX). A Wilcoxon

signed-ranked test was run to determine whether there were differences in image quality or confidence in identifying zone, stage, and category of disease using the 2 imaging methods.

Using the consensus reference standard diagnosis, the performance of individual experts was evaluated for each method (color fundus photographs and color fundus photographs plus FA) by comparing areas under the receiver operating characteristic curves. These results were averaged among all experts to determine the sensitivity and specificity of each method for detecting stage 1 or worse disease, stage 2 or worse disease, or stage 3 or worse disease; disease in zone I or disease in either zone I or zone II; preplus or worse disease or plus or worse disease; and mild or worse ROP, type 2 ROP or worse, or ROP requiring treatment. The nonparametric sign test was performed to determine whether the mean difference in sensitivity and specificity between paired color fundus photographs and FA images was significantly different from 0.[31]

To evaluate intergrader reliability, each International Classification of ROP criterion was dichotomized, forming a 2-level categorization: stage 3 or worse disease or not, zone I disease or not, plus disease or not, type 2 or worse ROP or not, and ROP requiring treatment or not. The unweighted κ statistic was calculated to measure chance-adjusted agreement for each head-to-head paring of readers. These results were averaged to determine the mean unweighted κ for each reader in each category. A widely accepted scale was used to interpret the results: 0 to 0.20 indicated slight agreement, 0.21 to 0.40 indicated fair agreement, 0.41 to 0.60 indicated moderate agreement, 0.61 to 0.80 indicated substantial agreement, and 0.81 to 1.00 indicated almost perfect agreement.[22]

Results

Study Experts

Nine ROP experts (based on the study definition) consented to participate. Among these experts, 6 of 9 (67%) were retina specialists and 3 of 9 (33%) were pediatric ophthalmologists. The experts had been practicing ophthalmology for an average of 18.9 years (range, 10−33 years).

Each expert evaluated 32 image sets (16 color fundus photographs and 16 color fundus photographs plus FA images) from 16 eyes, for a total of 288 readings. Eight of 9 experts (89%) reported that they were comfortable with reading color fundus photographs, whereas 1 of 9 experts (11%) was somewhat comfortable. When experts were asked about comfort with reading FA images, 6 of 9 (67%) were comfortable, 2 of 9 (22%) were somewhat comfortable, and 1 of 9 (11%) was not comfortable.

Distribution of Expert Responses Based on Imaging Method

Figure 2 shows the diagnostic responses of the 9 experts when the consensus reference standard diagnosis was type 2 ROP (Fig 2A), ROP requiring treatment (Fig 2B), stage 3 disease (Fig 2C), stage 2 disease (Fig 2D), and zone I ROP (Fig 2E).

Notably, among the 12 eyes with the consensus reference standard diagnosis of stage 3 disease, experts diagnosed stage 3 disease or worse in 43 of 108 responses (40%) using color fundus photographs alone versus 80 of 108 responses (74%) using color fundus photographs and FA images (Fig 2C). Among the 4 eyes with the consensus reference standard diagnosis of stage 2, experts diagnosed stage 2 in 19 of 36 responses (53%) using color fundus photographs alone versus 15 of 36 responses (42%) using color fundus photographs and FA images (Fig 2D). Furthermore, among these 4 eyes with a consensus reference standard diagnosis of stage 2, experts diagnosed stage 3 or worse in 13 of 36 responses (36%) using color fundus photographs alone versus 21 of 36 responses (58%) using color fundus photographs and FA images.

When viewing the corresponding color fundus photographs and FA images, experts altered their choice of category (none, mild, type 2 ROP, ROP requiring treatment) in 66 of 144 responses (46%). Pediatric ophthalmologists had 19 of 48 changes in category (40%), whereas retina specialists had 47 of 96 changes in category (49%) after viewing the corresponding FA. Of the 66 changes in category after viewing the color fundus photographs and FA, 31 of 66 (47%) were changes to a diagnosis consistent with the consensus reference standard diagnosis, 15 of 66 (23%) were changes from one consistent with the consensus reference standard diagnosis to a diagnosis inconsistent with the consensus reference standard diagnosis, and 20 of 66 (30%) were changes from a diagnosis inconsistent with the consensus reference standard diagnosis to another diagnosis also inconsistent with the consensus reference standard diagnosis.

After viewing the FA images corresponding to the color fundus photographs, experts altered their choice of management in 37 of 144 responses (26%). Pediatric ophthalmologists made 11 of 48 changes (23%) in management after viewing the corresponding FA; specifically, 2 of 11 responses (18%) changed from observation to laser treatment, whereas 5 of 11 responses (45%) changed from observation to anti-VEGF therapy. Retina specialists made 26 of 96 changes (27%) in management after viewing the corresponding FA; specifically, 13 of 26 responses (50%) changed from observation to laser treatment, whereas 3 of 26 (12%) changed from observation to anti-VEGF therapy.

Sensitivity and Specificity of Retinopathy of Prematurity Diagnosis

Table 1 shows the mean (95% confidence interval) sensitivity and specificity of stage, zone, plus disease, and category for study experts compared with the consensus reference standard diagnosis. With the supplementation of FA images to color fundus photographs, there were statistically significant improvements in the sensitivity for diagnosing stage 2 or worse disease ($P = 0.016$), stage 3 or worse disease ($P = 0.008$), preplus or worse disease ($P = 0.031$), and type 2 ROP or worse ($P = 0.013$; Table 1). No significant changes in specificity were seen with the supplementation of FA images to color fundus photographs.

Intergrader Agreement of Zone, Stage, Plus, and Category of Disease

Figure 3 displays a strip plot for the mean intergrader agreement for each expert compared with all other experts using an unweighted κ value. When stage was dichotomized into stage 3 ROP or not, experts had a mean κ value of 0.06 (range, −0.05 to 0.20) with color fundus photographs versus 0.09 (range, −0.06 to 0.21) with color fundus photographs and FA images ($P = 1.00$). When zone was dichotomized into zone I or not, experts had a mean κ value of 0.34 (range, 0−0.47) with color fundus photographs versus 0.48 (range, 0−0.64) with color fundus photographs and FA images ($P = 0.07$). When plus was dichotomized into plus disease or not, experts had a mean κ value of 0.07 (range, −0.05 to 0.23) with color fundus photographs versus 0.04 (range, 0−0.13) with color fundus photographs and FA images ($P = 0.38$). When category was dichotomized into whether there was type 2 ROP or worse, experts had a mean κ value of 0.11 (range, −0.02 to 0.22) with

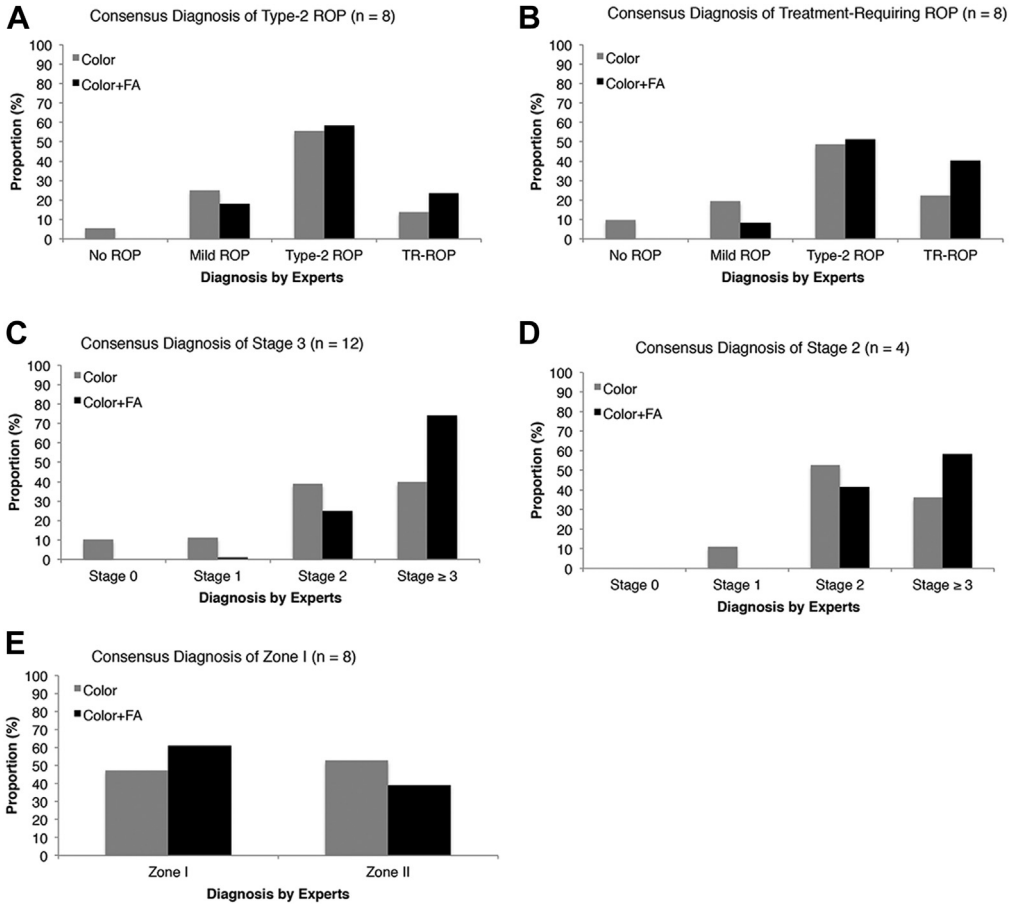

Figure 2. Bar graphs showing the mean distribution of retinopathy of prematurity (ROP) diagnoses by 9 ROP experts using color fundus photographs only versus color fundus photographs with corresponding fluorescein angiography (FA) images. Gray bars indicate diagnosis using color fundus photographs only and black bars indicate diagnosis using color fundus photographs and corresponding FA images. **A**, Distribution of responses for the 8 eyes with a consensus diagnosis of type 2 ROP. **B**, Distribution of responses for the 8 eyes with a consensus diagnosis of ROP requiring treatment (TR-ROP). **C**, Distribution of responses for the 12 eyes with a consensus diagnosis of stage 3 ROP. **D**, Distribution of responses for the 4 eyes with a consensus diagnosis of stage 2 ROP. **E**, Distribution of responses for the 8 eyes with a consensus diagnosis of zone I ROP.

color fundus photographs versus 0.01 (range, −0.11 to 0.08) with color fundus photographs and FA images ($P = 0.29$). When category was dichotomized into ROP requiring treatment or not, experts had a mean κ value of 0.003 (range, −0.06 to 0.05) with color fundus photographs versus 0.089 (range, 0–0.18) with color fundus photographs and FA images ($P = 0.02$).

Confidence of Clinical Diagnosis and Image Quality

For the 144 color fundus photographs, image quality was scored as adequate in 85 of 144 responses (59%), somewhat adequate in 37 of 144 responses (26%), and not adequate in 22 of 144 responses (15%). For the 144 FA images, image quality was scored as adequate in 72 of 144 responses (50%), somewhat adequate in 58 of 144 responses (40%), and not adequate in 14 of 144 responses (10%). Overall, there was no difference in image quality scoring between color fundus photographs and FA images ($P = 0.517$).

For the color fundus photographs, confidence in diagnosis was scored as confident in 20 of 72 responses (28%), somewhat confident in 36 of 72 responses (50%), and not confident in 16 of 72 responses (22%). For the combined color fundus photograph and FA image set, degree of confidence in diagnosis was scored as confident in 46 of 72 responses (64%), somewhat confident in 24 of 72 responses (33%), and not confident in 2 of 72 responses (3%). There was a statistically significant increase in confidence with the diagnosis when experts used color fundus photographs and FA images compared with the color fundus photographs only ($P < 0.001$).

After viewing the color fundus photographs for each case, 9 of 72 responses (13%) indicated that they would obtain FA images. After viewing the color fundus photographs and FA for each case, 58 of 72 responses (81%) stated they believed the FA provided clinically useful information for management purposes.

Discussion

The key findings of this study are as follows. First, the addition of FA images to color fundus photographs resulted in a statistically significant increase in sensitivity for diagnosis of stage 2 or worse, stage 3 or worse, pre-plus or worse, and type 2 ROP or worse compared with a consensus reference standard diagnosis. Second, the addition of FA images to color fundus photographs resulted in a statistically significant improvement in intergrader agreement for diagnosis of ROP requiring treatment.

The first key finding is that compared with color fundus photographs alone, the addition of FA resulted in an increased sensitivity of diagnosis of stage 2 or worse, stage

Table 1. Sensitivity and Specificity of Retinopathy of Prematurity Diagnosis by Retinopathy of Prematurity Experts Based on a Consensus Reference Standard Diagnosis

	Sensitivity (95% Confidence Interval)			Specificity (95% Confidence Interval)		
	Color Fundus Only	*Color Fundus + Fluorescein Angiography*	**P Value***	*Color Fundus Only*	*Color Fundus + Fluorescein Angiography*	**P Value***
Stage						
1 or worse	92.4 (86.7–96.1)	100.0 (97.5–100)	0.063	—	—	—
2 or worse	81.3 (73.9–87.3)	99.3 (96.2–99.9)	0.016	—	—	—
3 or worse	39.8 (30.5–49.7)	74.1 (64.8–82.0)	0.008	63.9 (46.2–79.2)	41.7 (25.5–59.2)	0.453
Zone						
I	47.2 (35.3–59.3)	61.1 (48.9–72.4)	0.219	100 (95–100)	100 (95–100)	**1.000**
II	97.2 (93–99.2)	97.2 (93–99.2)	**1.000**	—	—	—
Plus						
Pre-plus or worse	50.5 (40.3–60.7)	62.6 (52.3–72.1)	0.031	73.3 (58.1–85.4)	55.6 (40–70.4)	0.125
Plus	13.3 (5.1–26.8)	20.0 (9.6–34.6)	**1.000**	87.9 (79.8–93.6)	84.8 (76.2–91.3)	**1.000**
Category						
Mild or worse	91.7 (85.9–95.6)	100 (97.5–100)	0.0625	—	—	—
Type 2 or worse	69.4 (61.2–76.8)	86.8 (80.2–91.9)	0.013	—	—	—
Requiring treatment	22.2 (13.3–33.6)	40.3 (28.9–52.5)	0.063	86.1 (75.9–93.1)	76.4 (64.9–85.6)	0.375

— = Specificity is undefined as there were no condition negative eyes.
Boldface values indicate statistically significant *P* values.
*Nonparametric sign test.

3 or worse, pre-plus or worse, and type 2 ROP or worse. Perhaps the most notable effect of FA was on the sensitivity of diagnosis of stage 3 or worse. In eyes with a consensus reference standard diagnosis of stage 3, the sensitivity for diagnosis of stage 3 or worse by experts increased from 43 of 108 (40%) using color photographs alone to 80 of 108 (74%) with color fundus photographs and FA images. In turn, there was an increase in the sensitivity of diagnosis of type 2 ROP or worse ($P = 0.013$) and a trend toward an increased sensitivity for diagnosis of ROP requiring treatment ($P = 0.063$). Concomitant with the improvement in sensitivity of stage 3 or worse diagnosis was a trend toward reduction in specificity, which did not reach significance ($P = 0.375$). Indeed, even in patients with a consensus reference standard diagnosis of stage 2, FA resulted in a shift toward fewer stage 2 and more stage 3 diagnoses (Fig 2D). By contrast, this study also demonstrated that FA may offer less of an advantage over color fundus photographs alone in the diagnosis of zone or plus disease.

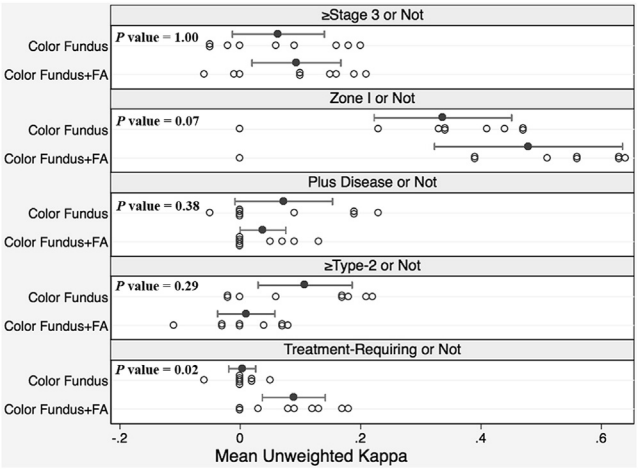

Figure 3. Graphs showing intergrader agreement of retinopathy of prematurity (ROP) diagnosis by 9 ROP experts using color fundus photographs only versus color fundus photographs with corresponding fluorescein angiography (FA) images. Intergrader agreement is measured as the unweighted κ statistic for each expert compared with all other experts. Each white circle represents the mean unweighted κ for a single expert. Each black circle is the value for the unweighted κ averaged across all experts, and the whiskers off the black circles denote the 95% confidence interval for that mean. Significance is calculated using a nonparametric sign test that analyzes mean κ differences using color fundus photographs versus color fundus photographs with corresponding FA images.

The impact of FA on stage 3 diagnosis likely reflects this imaging method's ability to highlight retinal vessels, and in turn, stage 3 (extraretinal fibrovascular proliferation) may be more easily identified on FA. The ability of FA to demonstrate retinal neovascularization not apparent on clinical examination has long and extensively been demonstrated in other vascular retinal disorders.[32] The ability of FA to highlight retinal vascular structures also may underlie the increased diagnosis of stage 3 by the ROP experts in this study after incorporation of FA in cases with a consensus reference standard diagnosis of stage 2. The characteristic clinical finding in stage 2 is the presence of a ridge without extraretinal fibrovascular proliferation. This ridge comprises endothelial cells that may form vascular channels and shunts.[17] As previously demonstrated by Flynn et al,[17] these vascular channels and shunts in stage 2 may exhibit leakage on FA. Misinterpretation of this leakage as neovascularization may have contributed to the shift in diagnosis from stage 2 to stage 3 with the addition of FA in cases with a consensus reference standard diagnosis of stage 2.

Our findings therefore emphasize the potential need to develop standardized criteria for FA findings for each ROP stage. Because there are currently no standardized criteria for

classifying FA findings in stage 2 versus stage 3 disease, it is unclear what metrics were implemented by the study experts in the evaluation of the FA images. Although ROP classification using color fundus photographs is standardized according to the criteria outlined by International Classification of ROP,[33] there is no defined role or consensus classification of FA images in ROP diagnosis. If more physicians begin to incorporate FA into their management of ROP, devising a standardized classification system, as has occurred with other ophthalmic disorders using FA, such as diabetic retinopathy and age-related macular degeneration, would be useful.[4,5] Nevertheless, if FA and other imaging methods, such as optical coherence tomography, are incorporated in routine evaluation of ROP, there is an inherent cost that should be considered. Previous reports have suggested that FA may provide a more objective assessment of zone in part because it provides high-contrast images of the peripheral retina.[12,15] However, our study did not find a statistically significant improvement in sensitivity or specificity of a zone I or II diagnosis when color fundus photographs were supplemented with FA images (Table 1). We did observe that a greater proportion of experts did trend toward selection of zone I disease when presented with FA images (Fig 2E) and that for the diagnosis of zone I or not, intergrader agreement trended from fair to moderate ($P = 0.07$; Fig 3).

The second key finding in this study is that supplementing FA to color fundus photographs resulted in an improvement in intergrader agreement among experts for the diagnosis of ROP requiring treatment, with a mean κ improvement from 0.003 to 0.089 (Fig 3). Although this is a statistically significant improvement, the interpretation of the mean κ remains in slight agreement with the addition of FA images based on a widely accepted scale for κ interpretation.[22] One previous study demonstrated that agreement between expert and nonexpert interpretations of retinal photomontages was greater for FA images than for color fundus photographs.[34] Furthermore, prior studies have revealed that variability exists in intergrader agreement for diagnosis of ROP: the Cryotherapy for ROP trial noted disagreement between 2 unmasked certified examiners as to whether threshold disease was present in 12% of eyes.[1] Disagreement in the diagnosis of severe ROP also has been noted among ROP experts even when examining the exact same image sets.[22,35] Given prior reports of poor intergrader agreement in ROP diagnosis using color fundus photographs only, our findings in this study of improved intergrader agreement for the diagnosis of ROP requiring treatment suggest that FA may be a useful adjunct in improving diagnostic agreement among ROP examiners.

Several previous studies investigated expert versus nonexpert diagnosis of ROP with wide-angle color fundus photographs only,[36,37] and one other introduced FA images.[34] This area deserves special attention given the increasing need for digital or telemedicine systems to undertake ROP screening with a limited supply of expert ophthalmologists available to perform screenings.[38] Previous studies of ROP screening among trainees have noted that variability exists among pediatric ophthalmology and retinal fellows in the diagnostic accuracy of clinically significant ROP.[39,40] If FA is able to improve the ability of imaging to detect disease requiring treatment among nonexperts,[34] and potentially among experts, this could have important implications for ROP screening protocols. Indeed, we found poor sensitivity for diagnosis with color fundus photographs, especially for more advanced ROP (i.e., zone I, stage 3, plus, and ROP requiring treatment). The addition of FA improved the sensitivity of stage 3 diagnosis significantly, resulting in a statistically significant increase in the sensitivity of type 2 ROP or worse diagnosis and a trend toward increased sensitivity for the diagnosis of ROP requiring treatment. This study may demonstrate the potential for FA to offset some of the shortcomings of using color fundus photographs alone for ROP diagnosis. Also, because our consensus reference standard diagnosis strongly incorporated the clinical diagnosis using indirect ophthalmoscopy, our findings suggest that FA may add a dimension to digital image analysis that is more consistent with examination findings from indirect ophthalmoscopy.

Limitations of this study include first the lack of multiple early, middle, and late FA images. Some examinations were performed under anesthesia, and the need to limit anesthesia time prompted the acquisition of only a finite number of FA images. Second, limitations also exist with color fundus photographs, which may have peripheral distortion, glare, and compromised contrast, depending on the skill of the user obtaining the photographs and other optical factors at the time of image acquisition.[41] Indeed, the 8 clinical cases for the study were selected to be of excellent quality and represent a range of ROP severity. Third, this study did not assess the impact of FA on ROP diagnosis in the clinical setting as other similar studies have done for FA in other retinal conditions,[42,43] but rather the impact of FA on ROP diagnosis based on digital imaging alone. However, we integrated the clinical diagnosis based on color fundus photographs into the consensus reference standard diagnosis to account for the findings noted on clinical examination. Fourth, this study did not incorporate other imaging methods such as optical coherence tomography, nor did we perform a cost analysis if FA was incorporated into ROP telemedicine screening programs. Finally, there may be variability among experts in their comfort with reading color fundus photographs and FA images that may be a function of years of experience, current practice patterns, and other factors.

Overall, this study contributes to the body of ROP knowledge by showing that, compared with color fundus photographs alone, FA may improve the sensitivity of diagnosis by ROP experts, particularly for stage 3 disease, and in turn may improve accuracy of diagnosis of type 2 ROP or worse and ROP requiring treatment. Fluorescein angiography continues to have an evolving role in the screening, diagnosis, and management of ROP in the era of multimodal imaging and telemedicine. Larger studies using standardized reading criteria for FA images in ROP are needed to elucidate further the optimal use of FA in ROP.

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Abbreviations and Acronyms:
FA = fluorescein angiography; **ROP** = retinopathy of prematurity; **VEGF** = vascular endothelial growth factor.

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