





ARTICLE

Sensitivity and specificity of pseudocolor ultrawide field imaging in comparison to wide field fundus fluorescein angiography in detecting retinal neovascularization in diabetic retinopathy

Swathy Haridas¹, Swati Indurkha¹, Siless Kumar¹, Anantharaman Giridhar¹  and Sobha Sivaprasad² 

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OBJECTIVE: To evaluate the diagnostic accuracy of ultrawide pseudocolor retinal photography (pseudocolor UWF) compared to wide field fundus fluorescein angiography (WFFFA) in the detection of retinal neovascularization (NV) and NV of the disc (NVD) in patients with diabetic retinopathy (DR).

DESIGN: Diagnostic accuracy observational study evaluating pseudocolor UWF as the index test. The reference standard was WFFFA.

SETTING: Single retinal centre in India.

PARTICIPANTS: People with severe non-proliferative DR (sNPDR), early proliferative DR (ePDR) or high-risk proliferative DR (HR PDR).

MAIN OUTCOME MEASURES: Sensitivity and specificity of pseudocolor UWF in the detection of NV.

RESULTS: A total of 176 eyes of 94 subjects with sNPDR, ePDR or HR PDR underwent pseudocolor UWF and WFFFA. The sensitivity and specificity of pseudocolor UWF in detecting NVE were 92.5% (95% CI 86.2–96.5) and 81% (95% CI 64.8–92.0), respectively, with moderate interobserver agreement of 0.722 (*p* value 0.001). The positive predictive value and negative predictive value were 83.0 (71.4–90.5) and 91.5 (84.9–95.3), respectively.

CONCLUSION: Compared to WFFFA as the gold standard, pseudocolor UWF has high sensitivity and specificity in detection of NV in all retinal quadrants and NVD. Therefore, pseudocolor UWF may be used as a non-invasive tool for screening and managing DR.

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INTRODUCTION

The severity of diabetic retinopathy (DR) is determined by mydriatic fundus examination or photography. The severity of DR is broadly classified into non-proliferative DR (NPDR) and proliferative DR (PDR) based on the lesion characteristics on standard ETDRS seven-field fundus photography [1]. As seven-field photography is challenging for both patients and technicians, most DR screening programmes tend to rely on DR severity grades based on 1–3-field fundus photography [2].

Neovascularization (NV) elsewhere of the retina (NVE) and/or NV of the optic disc (NVD) are the hallmark clinical signs of PDR. They develop as a consequence of underlying retinal ischaemia. However, these NVs are quite subtle at times making their identification challenging especially in cases of severe non-proliferative DR (sNPDR) or early proliferative DR (ePDR). Early detection and treatment of these NVs are recommended as they can cause sight-threatening complications such as vitreous haemorrhage (VH) and tractional retinal detachment. Clinicians often tend to intervene even rather than observing the eyes with early PDR in a real-world setting as evidenced by the disease

characteristics of patients included in the recent PDR trials such as Diabetic Retinopathy Clinical Research Network (DRCR.Net) Protocol S [3] and CLARITY [4] trials, although the Diabetic Retinopathy Study (DRS) had shown that panretinal photocoagulation (PRP) can be reserved for high-risk PDR (HR PDR). Hence, most patients undergo confirmatory fundus fluorescein angiography (FFA) to identify NV and coexisting capillary non-perfusion (CNP) areas. Although an established procedure, FFA has disadvantages. It is invasive, requires pupillary dilatation, nurses for intravenous injection of fluorescein dye, trained photographers to capture the images and images should be sequentially captured for up to 10 min. Rarely, patients need to be monitored for allergic reactions.

Pseudocolor ultrawide field retinal imaging (pseudocolor UWF) is captured on Optos (Optos Ltd, Dunfermline, Scotland) through an undilated pupil within a few minutes by non-technical staff. It has revolutionised retinal imaging with the ability to see 200° of retinal surface amounting to three times the area of retina visualised on conventional seven-field retinal photography [5]. Over the last decade, the availability of non-mydriatic ultrawide field (UWF) retinal colour photography has increased our knowledge on DR. It is as

¹Giridhar Eye Institute, Cochin, India. ²NIHR Moorfields Biomedical Research Centre, Moorfields Eye Hospital, 162 City Road, London EC1V2PD, UK. ✉email: giridhareye@gmail.com

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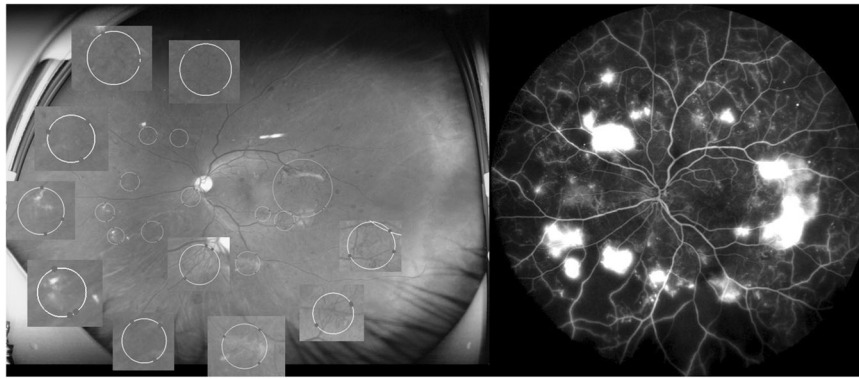


Fig. 1 Pseudocolor UWF image of an asymptomatic eye with high-risk PDR showing multiple NVs. All the smaller NV lesions have been encircled and separately magnified in the inset, except for an obvious large neovascular frond temporal to the macula. Active NV lesions are seen more in superior and temporal quadrant, whereas fibrovascular proliferations are seen in inferior quadrant. The WFFFA of the same eye done on Spectralis, Heidelberg, demonstrates all the NV lesions as hyperfluorescent areas. Peripheral capillary non-perfusion areas are seen more in the inferior quadrant where more fibrovascular proliferations are noted than the active NVs. Note that the NVD is better appreciated in the pseudocolor UWF image than in the WFFFA.

good as clinical screening [6] and more effective than seven-field photography in DR screening [7]. Eyes with predominantly peripheral lesions such as microaneurysms, hard exudates, intraretinal microvascular abnormalities (IRMA), venous beading and NVE detected outside the standard seven-field fundus photography showed four times more progression (25%) to PDR in comparison to eyes without predominantly peripheral lesions (6%) before adjusting for the baseline DR severity, glycosylated haemoglobin (HbA1c) levels, duration and type of diabetes. However, correlation with each lesion separately was not evaluated in particular [8]. In addition, as more area of the retina is visible on pseudocolor UWF, it enables visualisation of more numbers and areas of NV.

We hypothesised that pseudocolor UWF retinal imaging is as accurate as standard FFA to robustly detect PDR in people with diabetes. The aim of our study was to assess the diagnostic accuracy of pseudocolor UWF compared to standard FFA to detect NVE and NVD in people with diabetes.

MATERIALS AND METHODS

This is a diagnostic accuracy study based on retrospective case notes review. The study was approved by the Institutional ethics committee. Informed consent was obtained from all the study participants. The study followed the tenets of Declaration of Helsinki. The sample size calculation was not done since it was done as a pilot study.

Identification of participants

Consecutive eligible patients were identified from the retinal imaging database. Eligibility criteria included participants with sNPDR or early PDR or HR PDR who had been imaged on both pseudocolour UWF and had FFA in the same clinic visit from January 2019 to January 2020. All the subjects underwent a complete ophthalmological examination including best corrected visual acuity in logMAR, intraocular pressure examination with Goldmann applanation tonometry, slit-lamp biomicroscopy and fundus examination using 78D as well as indirect ophthalmoscopy. Other baseline data included age, gender, duration of diabetes, fasting blood sugar (FBS), post-prandial blood sugar (PPBS) and HbA1c. Eyes with ungradable images due to media opacities or already ablated with PRP were excluded.

Index test was defined as pseudocolor UWF imaging for mydriatic fundus photography on Optos Daytona plus (Optos Ltd, Dunfermline, Scotland) without any eye steering.

Reference test was wide field FFA (WFFFA) on Spectralis, Heidelberg (Heidelberg Engineering, Germany), obtained after administration of 3 mL 20% fluorescein sodium dye intravenously and then capturing early and late frames up to 8 min.

The images from pseudocolor and WFFFA were divided into superotemporal, superonasal, inferotemporal and inferonasal quadrants with fovea as the centre according to the recommendations from the International Widefield Imaging Study Group [9].

Table 1. DR severity level of the study population.

Clinical grading of DR severity	Number of eyes, N (%)
Severe NPDR	63 (35.79)
Early PDR	53 (30.11)
High-risk PDR	60 (34.19)

Both the investigations were performed on the same day and hence the images were captured after mydriasis using tropicamide 0.8% and phenylephrine hydrochloride 5% eye drops. Two independent masked investigators (SI and SH) evaluated the pseudocolor UWF images and WFFFA images, respectively, and any discrepancies were settled by principal investigator (AG). The images from both the modalities were recruited by SH who among the investigators alone had access to the patient identification number (PIN) and the electronic medical record (EMR) system which carries the clinical diagnosis made by the consultant physicians who have seen the patient in the clinic. No patients who were initially seen by SI or SH in the clinic were included in the study. The pseudocolor UWF images and the WFFFA images were assessed by SI and SH, respectively, and were masked to each other as well as the clinical diagnosis due to their inaccessibility to the EMR system. The pseudocolor UWF and the Heidelberg databases need the PIN alone to access the patient image. This obviated the need for accessing the patient details in any step of grading, thus making it entirely based on their interpretation of the images alone.

Definition of positive test

In the pseudocolor UWF images, NVs were identified as active NVs appearing as reticular red coloured lesions arising from the retinal vessels assuming a sea-fan pattern or as fibrovascular proliferation NVs in the form of pale yellow tissue with lesser vascular component. Similar lesions when present over the optic disc or within 1 disc diameter were considered to be positive test for NVD. The images were resized and contrast adjusted accordingly to identify the NVE and NVD.

The NVs in WFFFA were defined as the arborising network-like areas which appear in early frames of FFA with increase in fuzziness and leakage towards the late phase.

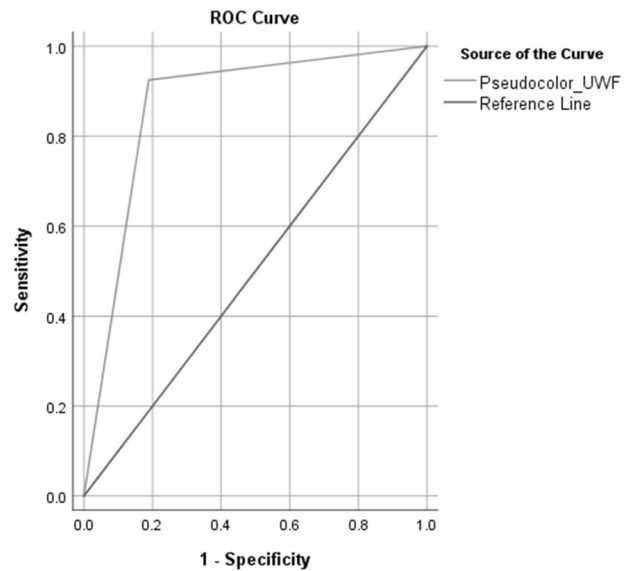
The number of NVs in each quadrant was recorded separately for the two modalities by the masked investigators. The sample image of pseudocolor UWF imaging and WFFFA of a same eye is shown in Fig. 1.

After assessing the number of NVE/NVD in each quadrant, the eyes were classified into sNPDR, ePDR or HR PDR based on the following classification system for each imaging modality. In grading the pseudocolor UWF images, sNPDR was defined as presence of retinal haemorrhages in all four quadrants, venous beading in at least two quadrants or presence of IRMA in at least one quadrant, ePDR as presence of NVE without VH/preretinal haemorrhage and HR PDR as NVD > 1/3–1/4 DD or NVD if < 1/3–1/4 DD with VH/preretinal haemorrhage or NVE with VH/preretinal haemorrhage.

Table 2. Diagnostic accuracy of pseudocolor UWF in detecting NVE (whole image and each retinal quadrant separately) and NVD.

Area of NVE/NVD	AUC	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR- (95% CI)
All quadrants together	0.868 (0.804–0.916)	92.5 (86.2–96.5)	81.0 (64.8–92.0)	83.0 (71.4–90.5)	91.5 (84.9–95.3)	4.89 (2.50–9.55)	0.09 (0.05–0.18)
Supertemporal quadrant	0.741 (0.659–0.812)	71.4 (57.8–82.7)	76.8 (66.2–85.4)	75.5 (66.7–82.5)	72.8 (63.6–80.5)	3.08 (2.01–4.73)	0.37 (0.24–0.57)
Superonasal quadrant	0.716 (0.633–0.789)	62.9 (49.6–74.8)	80.2 (69.5–88.5)	76.1 (66.1–83.9)	68.4 (60.6–75.3)	3.19 (1.95–5.21)	0.46 (0.33–0.65)
Inferotemporal quadrant	0.744 (0.663–0.814)	63.4 (47.8–77.6)	85.1 (76.2–91.6)	81.1 (71.5–87.9)	70.1 (61.1–77.7)	4.27 (2.51–7.28)	0.43 (0.29–0.64)
Inferonasal quadrant	0.728 (0.646–0.801)	56.0 (41.3–70.0)	89.6 (81.3–95.2)	84.4 (73.5–91.3)	67.1 (59.7–73.7)	5.41 (2.78–10.53)	0.49 (0.36–0.68)
Neovascularization of the disc	0.83 (0.756–0.889)	67.9 (47.7–84.1)	98.1 (93.4–99.8)	97.3 (89.9–99.3)	75.3 (64.0–83.9)	36.3 (8.98–146.69)	0.33 (0.19–0.56)

AUC area under receiver operating characteristic curve, PPV positive predictive value, NPV negative predictive value, LR+ positive likelihood ratio, LR- negative likelihood ratio.

**Fig. 2** Receiver-operating characteristic curve (ROC) for detection of NVE in diabetic retinopathy for UWF Optos and WFFFA. The figure below depicts the area under ROC (AUC) for pseudocolor UWF for NVE detection in comparison to WFFFA as the reference line.

While grading WFFFA, sNPDR was defined as presence of microaneurysms alone with CNP areas in all quadrants, but no NVE/NVD, ePDR as any NVE without blocked fluorescence of VH/preretinal haemorrhage and HR PDR as NVE or NVD with blocked fluorescence due to preretinal haemorrhage or VH.

Statistical analysis. The data were entered in Microsoft Excel spreadsheet. The primary outcome of sensitivity and specificity with 95% confidence intervals were calculated for pseudocolor UWF in detecting NVE and NVD using the WFFFA as a reference standard considering each pseudocolor UWF image as whole and also separately for each quadrant. *p* value of <0.05 was considered statistically significant for the data. Other outcomes included positive and negative predictive values (PPV and NPV) and positive and negative likelihood ratios (LR+ and LR-) and the area under the receiver operating characteristics (ROC) curve, with 95% confidence intervals. The interobserver agreement using Kappa statistics was also calculated. The kappa value between 0.6 and 0.79 was considered moderate agreement, 0.8 and 0.9 suggested strong agreement and more than 0.9 was perfect agreement.

The statistical analyses were carried out in SPSS 26.0 Version (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp).

RESULTS

A total of 176 eyes of 94 patients with sNPDR and PDR were recruited for the study. The study population comprised of 62 males and 32 females. The mean duration of diabetes was 15.27 months. The mean FBS, PPBS and HbA1c were 143.56 mg/dL, 225.82 mg/dL and 10.25%, respectively. The distribution of various severity grades of DR in the study population is shown in Table 1.

Of these, 10 eyes were excluded from NV assessment in WFFFA and 12 eyes from the pseudocolour UWF due to media haze from VH, nuclear or cortical cataract. Seven eyes were indeterminate due to confluence of NVE and NVD.

The sensitivity and specificity of pseudocolor UWF in detecting NV in comparison to gold standard WFFFA were 92.5% (95% CI 86.2–96.5) and 81% (95% CI 64.8–92.0), respectively. Table 2 shows the PPV and NPV, AUC of pseudocolour UWF considering the image as a whole and per quadrant. AUC for NV in pseudocolor UWF was 0.868 as demonstrated in Fig. 2. When we evaluated the sensitivity and specificity of NVD detection, pseudocolor UWF

Table 3. Diagnostic accuracy outcomes of pseudocolor UWF in detecting NVE in subgroup analysis of eyes with sNPDR and early PDR.

DR severity	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
sNPDR	82.14 (63.11–93.94)	86.67 (69.28–96.24)	86.03 (70.88–93.97)	82.92 (68.62–91.58)
ePDR	93.02 (80.94–98.54)	75.00 (19.41–99.37)	78.82 (40.48–95.32)	91.49 (75.87–97.35)
sNPDR and ePDR	88.73 (79.00–95.01)	85.29 (68.94–95.05)	85.78 (72.78–93.16)	88.33 (79.52–93.65)

sNPDR severe NPDR, ePDR early PDR, PPV positive predictive value, NPV negative predictive value.

imaging was more specific than sensitive with very high positive likelihood ratio.

The kappa statistics demonstrating the interobserver agreement in pseudocolor UWF and WFFFA in NVE and NVD detection was found to be 0.722 (p value: 0.001) and 0.727 (p value: 0.001), respectively, and both were statistically significant.

In the subgroup analysis of eyes with sNPDR and early PDR, there was increased sensitivity in the early PDR in comparison to the sNPDR eyes when analysed separately, although the sensitivity and specificity increased when they were analysed together as evidenced by the results given in Table 3.

DISCUSSION

Pseudocolor UWF imaging utilises red (633 nm) and green (532 nm) lasers in scanning laser ophthalmoscope via an ellipsoid mirror to produce UWF pseudocolor image of retinal surface. The utility of pseudocolor UWF in detecting peripheral retinal diseases such as retinal tears, retinal detachment [10], adult onset Coats' disease [11], proliferative retinopathy in sickle cell disease [12] as well as posterior lesions like neovascular age related macular degeneration are well established [13]. It has also been utilised in deep learning to detect NV in sickle cell retinopathy with sensitivity of 97.4% (95% CI, 86.5–99.9%) and specificity of 97.0% (95% CI, 93.5–98.9%) in comparison to clinical examination [14]. Similar attempt in the setting of DR has been successfully done using different fundus cameras such as Centervue DRS, Optovue ICam, Canon CR1/DGi/CR2, and Topcon TRC NW6 non-mydiatic camera but in a restricted field of only up to 45° [15]. Furthermore, all of these studies were done in comparison to clinical grading and not to the gold standard modality of FFA to detect early NV.

Pseudocolor UWF imaging is as good as the standard ETDRS seven-field photography in comparison to clinical assessment in DR and diabetic macular oedema grading with an agreement of 95.8% and 90.8%, respectively [7]. However, the utility of pseudocolor UWF imaging in detecting DR changes in comparison to the gold standard FFA in detecting NV that warrant active intervention has not yet been evaluated. In our study, we have found pseudocolor UWF imaging to have good sensitivity and reasonable specificity in NV detection with good interobserver agreement which proves its utility as a good screening tool in finding the eyes that need treatment or referral to higher centre. NV lesions develop as a result of retinal hypoxia evidenced as CNP areas, both of which are identified by FFA. Presence of CNP areas in the absence of NV are managed conservatively until NVs are seen clinically. Active intervention in the form of PRP is mandatory, once the NV has been identified. Patients are often lost to follow-up and waiting for HR PDR to ensue is not recommended in real life [16] unlike what was proven in DRS [17]. So, the presence of NV can be a surrogate marker to detect the eyes that need treatment.

Although there is a generalised increased sensitivity, a drop in sensitivity is noted in the inferior quadrants, probably due to the eyelash artefacts in pseudocolor UWF imaging as stated in another study [10]. However, our study shows numerous strengths for pseudocolor UWF. It has the advantages of decreasing patient exposure to light and imaging, decreasing the waiting time and it can be obtained even in non-mydiatic eyes, although imaging in our cohort was done in mydiatic setting because we performed

both the procedures on the same day. Pseudocolor UWF imaging also has the advantage of shorter image capture time and repeatability in comparison to gold standard FFA. Our study has proven that NV can be detected using pseudocolor UWF imaging with excellent sensitivity and specificity even in comparison to the gold standard test.

CONCLUSION

The purpose of any DR screening programme is primarily to identify eyes with vision threatening DR. Pseudocolor UWF imaging is an effective tool in detecting NV which is a surrogate marker of retinal ischaemia and has excellent sensitivity and reasonable specificity when compared to WFFFA. The increased sensitivity in the subset of eyes diagnosed as sNPDR or ePDR adds to its clinical relevance.

Summary

What was known before

- Fundus fluorescein angiography is mandatory for the diagnosis of treatable retinopathy.

What this study adds

- Pseudocolor UWF imaging using Optos will aid in identifying eyes that need treatment.

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AUTHOR CONTRIBUTIONS

SH was responsible for recruiting the patients for the study. SI graded the pseudocolor UWF images and SH graded the WFFFA images apart from extracting and analysing the data and result interpretation. SS and AG overviewed writing the report.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Anantharaman Giridhar.

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