Dynamic versus Static Ultra-widefield Fluorescein Angiography in Eyes with Diabetic Retinopathy: A Pilot Prospective Cross-sectional Study

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Abstract

**Background:** Dynamic angiography can present additional information. However, evidence-based support in clinical application of dynamic modalities remain insufficient. The purpose of this study is to analyze differences in ultra-widefield fluorescein angiography (UWFA) findings between time-lapse dynamic and static imaging in eyes with diabetic retinopathy (DR).

**Methods:** Twenty-eight eyes of 28 DR patients undergoing UWFA were included in the cross-sectional study. A series of UWFA images from each patient were converted into a time-lapse video in a Moving Picture Experts Group 4 movie format as a dynamic image. A single clear arteriovenous phase image was chosen as a static UWFA image. Non-perfusion index (NPI) and its correlation with intraretinal microvascular abnormality and neovascularization in different retinal zones were compared between dynamic and static UWFA imaging in severe nonproliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR).

**Results:** NPI appeared to increase from the center to the far-periphery in both groups. Dynamic NPI appeared to be lower in the total retinal area (0.25 vs 0.28, \(p=0.005\)), far-periphery (0.30 vs 0.34, adjusted \(p=0.040\)) and superior quadrant (0.27 vs 0.34, adjusted \(p=0.045\)) in contrast to static NPI. Far-peripheral NPI was associated with intraretinal microvascular abnormality in posterior area in both groups.

**Conclusion:** Time-lapse dynamic UWFA imaging is a useful method in the differential diagnosis of hypofluorescence in the most peripheral region, which may provide a reliable measurement of NPI and improve the diagnostic accuracy of DR.

**Background**

Diabetic retinopathy (DR) is a leading cause of visual impairment among working-age adults worldwide, occurring in approximately 35% of people with diabetes [1]. Identification of ischemic lesions, such as retinal intraretinal microvascular abnormality (IRMA), neovascularization (NV) and non-perfusion, were important in the diagnosis and the following treatment of DR. Ultra-widefield fluorescein angiography (UWFA) allows for the visualization of up to 200 degrees of the retina, which is largely helpful for the identification of peripheral ischemic lesions [2-6]. Conventionally, retinal results are only recorded using a few typical UWFA images. Due to the spherical aberrations and different focus distances, peripheral blurring may be mistaken for non-perfusion [7]. Moreover, dynamic retinal blood circulation is represented in static pictures, which can lead to impaired identification of vascular lesions, such as subtle IRMA and NV [8]. Therefore, it is important to identify ischemic lesions more precisely.

Time-lapse photography is a cinematography technique which creates dynamic videos from a series of still images. So far, it has been demonstrated to be useful for monitoring neuronal development [9, 10] and clinical practice [11-13]. In ophthalmology, Querques et al [14] re-created an automatic pseudo-movie from five static fluorescein angiography pictures, sharing the visualization of dye leakage in macular degeneration. Gentile et al [15] reported cases of idiopathic macular holes and illustrated the
pathogenesis, progression and surgical closure by morphing serial optical coherence tomography scans into a movie format. We have previously described dynamic angiography through a few typical videos [16]. But no studies have implemented dynamic UWFA to explore the distribution of ischemic lesions in DR eyes. In this study, we hypothesize that a time-lapse dynamic UWFA video presenting the complete angiography process may be of significant value in DR diagnosis and its sequential evaluation of DR progression through treatment. We compared the distribution of non-perfusion index (NPI) in different retinal zones between dynamic videos and static images, and its correlation with vascular abnormalities in DR eyes.

**Methods**

**SUBJECTS**

This pilot prospective, cross-sectional study was conducted at Shanghai General Hospital between January 2017 and December 2019. It conformed to the tenets of the Declaration of Helsinki, and the data collection and analysis were approved by the Medical Ethics Committee of Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine. Written informed consent for participation in research was obtained from each patient before imaging.

The inclusion criteria for patient recruit in the study were: 1) age: 18 years or older with a primary diagnosis of diabetes mellitus, and 2) presence of DR. The exclusion criteria were: 1) ocular comorbidities (such as retinal vein and artery occlusion, ocular tumors or uveitis), 2) lack of clear images due to significant media opacities (such as cataract, vitreous hemorrhage), 3) poor patient cooperation, and 4) allergy to fluorescein.

**IMAGE CAPTURE AND PROCESSING**

Each patient received an intravenous injection of 5-ml of 10% sodium fluorescein and eyes were dilated for a better capture of high-quality image. After intravenous administration of fluorescein dye, UWFA (Optos PLC, Dunfermline, United Kingdom) images were captured by a trained ophthalmology technician. Each patient had one optional eye used for the time-lapse imaging of retinal angiogenesis.

The time-lapse imaging protocols were as follows: images were recorded every 2 seconds in the early phases (within 60 seconds), every 10 seconds in the mid phases (61~300 seconds), and every 15 seconds in the late phases (301~600 seconds). UWFA in the other eye was performed according to the protocol for conventional angiogenesis. The technician was allowed to adjust the recording times without compromising the conventional angiogenesis in the contralateral eyes. The minimum number of shots per study eye was 50.

Images were exported in high quality Joint Photographic Experts Group (jpeg) format and imported into Adobe software (Adobe Photoshop CC, Adobe Photoshop Lightroom and Adobe After Effects, Adobe Systems Inc, San Jose, California, USA). Poor-quality images due to eye movements or blinking were
removed. The retina was divided into three concentric zones (posterior region, mid-periphery and far-periphery) and four quadrants (superior, inferior, temporal, and nasal quadrants) (Figure 1). The ratio of the horizontal and vertical axes of the concentric ellipses was set at 1.2:1 based on the quantification of the Optos 200Tx images evaluated by Oishi et al [17].

MEASUREMENTS

Two independent, trained examiners (H.S and J.W) assessed the presence of the following lesions: non-perfusion area (NPA, areas of significant hypofluorescence flanked by neighboring filled vessels), IRMA (defined as tortuous intraretinal vascular segments), and NV (areas of hyperfluorescence caused by leakage). Disagreements were reviewed by open adjudication with a third examiner (T.N). A single clear arteriovenous phase (between 45-90 seconds) image was chosen for annotation and analysis as the static UWFA image, and was analyzed for the number of IRMAs, NVs, and NPA. The IRMA density was defined as the ratio of the number of IRMAs over the regional area. The NPA was manually outlined and calculated based on the number of pixels according to Adobe Photoshop CC. Since the outline of the NPA on UWFA could be challenging to decipher, images were zoomed in and the brightness was adjusted to optimize the images in Adobe software. The results of two independent examiners were averaged to obtain final values for subsequent analysis.

Serial angiographic images from the same examination were required to generate a Moving Picture Experts Group 4 movie [16], as the time-lapse UWFA image. The examiners obtained the entire dynamic videos and marked areas of retinal non-perfusion on an arteriovenous phase image, designating them as dynamic NPA [see Additional file 1]. To reduce errors, (as the NPA size in pixels could be varied in a series of angiographic frames due to subtle differences in shooting positions) non-perfusion zones on the dynamic and static images of one testing eye were marked on the same original arteriovenous phase image (Figure 2). The global NPI and NPI within each of three concentric zones and four quadrants were calculated and compared between the two imaging modalities, named “dynamic NPI” and “static NPI”. The global NPI was defined as the ratio of the global NPA to the entire UWFA visible retinal area[18, 19]. The NPI of each region was defined as the ratio of the regional NPA to the area of the region.

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS Statistics (version 23.0; IBM Corp, Armonk, New York, USA). The intraclass correlation coefficients (ICC) were used to assess the agreement between examiners, and ICC values greater than 0.80 were considered reflective of good agreement. The normality of the distribution of the continuous variable was assessed using a Shapiro-Wilk test. Multiple groups (>2) were compared using a one-way analysis of variance (ANOVA) or Friedman test (when the ANOVA test was not applicable). Multiple comparisons were done using the false discovery rate. The Wilcoxon signed-rank test was used to compare the correlation between static NPI and dynamic NPI. Bland-Altman plots were used to assess the comparability of two measures. A Spearman's rank correlation was used to examine the correlation between NPI and IRMAs and NVs. We assumed that a coefficient between 0-0.3 indicated
Results

DEMOGRAPHIC FEATURES OF THE STUDY EYES

28 patients with a primary diagnosis of DR were included in the study (Table 1). Four eyes had received panretinal photocoagulation. An average of 62.2 ± 5.1 images were captured in each study eye. After removal of poor-quality images, an average of 60.5 ± 5.2 were included for the analyses.

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient Characteristics</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Basic demographics</th>
<th>Mean ± standard deviation (Range) or N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female gender, no. (%)</td>
<td>13 (46.4%)</td>
</tr>
<tr>
<td>Mean age, yrs</td>
<td>63.8 ± 10.1 (39–82)</td>
</tr>
<tr>
<td>Mean diabetes duration, yrs</td>
<td>15.0 ± 5.7 (1–30)</td>
</tr>
<tr>
<td>Diabetes type (type 1/type 2)</td>
<td>0/28</td>
</tr>
<tr>
<td>Study eye (right/left)</td>
<td>11/17</td>
</tr>
<tr>
<td>Total eyes, n = 28</td>
<td></td>
</tr>
<tr>
<td>Mild NPDR</td>
<td>5 (17.9%)</td>
</tr>
<tr>
<td>Moderate NPDR</td>
<td>5 (17.9%)</td>
</tr>
<tr>
<td>Severe NPDR</td>
<td>7 (25.0%)</td>
</tr>
<tr>
<td>PDR</td>
<td>11 (39.2%)</td>
</tr>
<tr>
<td>DR Features</td>
<td></td>
</tr>
<tr>
<td>IRMA</td>
<td>17 (60.7%)</td>
</tr>
<tr>
<td>NV</td>
<td>7 (25.0%)</td>
</tr>
<tr>
<td>NPA</td>
<td>17 (60.7%)</td>
</tr>
</tbody>
</table>

NPDR nonproliferative diabetic retinopathy, PDR proliferative diabetic retinopathy, DR diabetic retinopathy, IRMA intraretinal microvascular abnormalities, NV neovascularization, NPA non-perfusion area

NON-PERFUSION INDEX ON DYNAMIC AND STATIC ULTRA-WIDEFIELD FLUORESCEIN ANGIOGRAPHY

16 of 28 eyes (57.1%) had retinal non-perfusion in severe nonproliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR). Across three concentric regions in these 16 eyes, NPI was
found to be highest in the far peripheral regions and lowest in posterior regions in both groups ($p < 0.05$). Across the four quadrants in these eyes, there was a statistically significant difference in static NPI ($p = 0.006$), but not in dynamic NPI ($p = 0.101$).

Table 2.

Correlation between Dynamic and Static NPI on Ultra-widefield Fluorescein Angiography in severe NPDR and PDR
<table>
<thead>
<tr>
<th>NPI (n = 16)</th>
<th>Dynamic</th>
<th>Static</th>
<th>p value</th>
<th>Adjusted p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>0.25 ± 0.28 (0.15, 0.03-0.38)</td>
<td>0.28 ± 0.30 (0.18, 0.05-0.51)</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Concentric Regions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior</td>
<td>0.06 ± 0.08 (0.02, 0.01-0.09)</td>
<td>0.10 ± 0.14 (0.04, 0.01-0.16)</td>
<td>0.011</td>
<td>0.077</td>
</tr>
<tr>
<td>Mid-periphery</td>
<td>0.24 ± 0.31 (0.07, 0.05-0.34)</td>
<td>0.24 ± 0.30 (0.10, 0.05-0.39)</td>
<td>0.179</td>
<td>0.626</td>
</tr>
<tr>
<td>Far-periphery</td>
<td>0.30 ± 0.33 (0.21, 0.03-0.49)</td>
<td>0.34 ± 0.36 (0.25, 0.03-0.69)</td>
<td>0.017</td>
<td>0.040</td>
</tr>
<tr>
<td>Quadrants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior</td>
<td>0.27 ± 0.33 (0.04, 0.0-0.59)</td>
<td>0.34 ± 0.38 (0.21, 0.01-0.75)</td>
<td>0.026</td>
<td>0.045</td>
</tr>
<tr>
<td>Inferior</td>
<td>0.26 ± 0.34 (0.05, 0.02-0.62)</td>
<td>0.30 ± 0.35 (0.16, 0.02-0.55)</td>
<td>0.535</td>
<td>0.749</td>
</tr>
<tr>
<td>Nasal</td>
<td>0.28 ± 0.28 (0.17, 0.05-0.49)</td>
<td>0.31 ± 0.28 (0.19, 0.07-0.61)</td>
<td>0.063</td>
<td>0.074</td>
</tr>
<tr>
<td>Temporal</td>
<td>0.18 ± 0.31 (0.02, 0-0.19)</td>
<td>0.19 ± 0.30 (0.07, 0-0.18)</td>
<td>0.221</td>
<td>0.221</td>
</tr>
</tbody>
</table>

Numeric data are presented as mean ± standard deviation (median, interquartile range)

*p values were calculated using the Wilcoxon signed-rank test and the False Discovery Rate was used to correct for multiple testing.

NPI non-perfusion index, NPDR nonproliferative diabetic retinopathy, PDR proliferative diabetic retinopathy

Regional distributions of dynamic and static NPI from the same eye were compared in Table 2. Dynamic NPI appeared to be lower in the total retinal area (p=0.005), far-periphery (adjusted p=0.040) and superior quadrants (adjusted p=0.046) in contrast to static NPI. Factors influencing the identification of NPI in
static images was listed in Table 3. The leading factor was unrecognizable hypofluorescence in periphery (50.0%), followed by unclear ocular media (18.8%). Dynamic NPI was strongly associated with static NPI in the total retinal area (Spearman's rank coefficient=0.978, \( p<0.001 \)). The mean difference between dynamic and static NPI was 3.58% (range -8.31% to 14.19%, SD ± 5.58%). This relationship is also illustrated by the Bland–Altman plot as shown in figure 3.

Table 3.

Factors Influencing the Identification of Non-perfusion in Static Ultra-widefield Fluorescein Angiography Images
<table>
<thead>
<tr>
<th>Patient#</th>
<th>DR grade</th>
<th>Unclear ocular media</th>
<th>Eye movement</th>
<th>Subretinal hemorrhage</th>
<th>Unrecognizable hypofluorescence in periphery</th>
</tr>
</thead>
<tbody>
<tr>
<td>#2</td>
<td>Severe NPDR</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>#13</td>
<td>Severe NPDR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>#14</td>
<td>Severe NPDR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>#20</td>
<td>Severe NPDR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>#24</td>
<td>Severe NPDR</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>#25</td>
<td>Severe NPDR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>#28</td>
<td>Severe NPDR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>#3</td>
<td>PDR</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>#6</td>
<td>PDR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>#8</td>
<td>PDR</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>#9</td>
<td>PDR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>#11</td>
<td>PDR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>#12</td>
<td>PDR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>#16</td>
<td>PDR</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>#17</td>
<td>PDR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>#22</td>
<td>PDR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No. (%)</td>
<td>3 (18.8)</td>
<td>2 (12.5)</td>
<td>1 (6.3)</td>
<td>8 (50.0)</td>
<td></td>
</tr>
</tbody>
</table>

DR diabetic retinopathy, NPDR nonproliferative diabetic retinopathy, PDR proliferative diabetic retinopathy
+: exist; -: not exist

**CORRELATION OF NON-PERFUSION INDEX AND VASCULAR ABNORMALITIES**

The regional distributions of the IRMAs and NV are presented in Table 4. IRMAs and NVs were most prevalent in the mid-periphery and the nasal quadrant ($p < 0.05$). The density of IRMAs was found to be the highest in the posterior area ($p < 0.001$).
Table 4
Distribution of Intraretinal Microvascular Abnormalities and Neovascularization in Diabetic Retinopathy on Ultra-widefield Fluorescein Angiography

<table>
<thead>
<tr>
<th></th>
<th>IRMA (n = 17)</th>
<th>IRMA density (10^{-5} per pixel)</th>
<th>NV (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>14.50 ± 12.61</td>
<td>1.12 ± 0.94</td>
<td>4.20 ± 3.78</td>
</tr>
<tr>
<td>Concentric Regions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior</td>
<td>4.03 ± 3.22</td>
<td>2.57 ± 2.01</td>
<td>0.81 ± 0.96</td>
</tr>
<tr>
<td>Mid-periphery</td>
<td>8.06 ± 6.80</td>
<td>1.68 ± 1.35</td>
<td>2.86 ± 2.87</td>
</tr>
<tr>
<td>Far-periphery</td>
<td>2.41 ± 4.34</td>
<td>0.37 ± 0.64</td>
<td>0.42 ± 0.84</td>
</tr>
<tr>
<td>p value</td>
<td>0.004</td>
<td>&lt; 0.001</td>
<td>0.03</td>
</tr>
<tr>
<td>Quadrants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior</td>
<td>2.12 ± 2.09</td>
<td>0.79 ± 0.73</td>
<td>0.81 ± 1.41</td>
</tr>
<tr>
<td>Inferior</td>
<td>2.41 ± 2.54</td>
<td>1.01 ± 1.09</td>
<td>0.57 ± 1.13</td>
</tr>
<tr>
<td>Nasal</td>
<td>7.18 ± 7.03</td>
<td>1.91 ± 1.80</td>
<td>1.90 ± 2.78</td>
</tr>
<tr>
<td>Temporal</td>
<td>2.79 ± 3.26</td>
<td>0.70 ± 0.81</td>
<td>0.81 ± 0.84</td>
</tr>
<tr>
<td>p value</td>
<td>&lt; 0.001</td>
<td>0.005</td>
<td>0.478</td>
</tr>
</tbody>
</table>

Numeric data are presented as mean ± standard deviation

p values (2-tailed) < 0.05 were considered statistically significant and corrected by False Discovery Rate method

IRMA intraretinal microvascular abnormalities, NV neovascularization, NPA non-perfusion area

In dynamic group, far-peripheral NPI is significantly associated with posterior segment IRMA density (R = 0.517, p = 0.04), however, not substantially associated with posterior segment IRMA quantity (R = 0.471, p = 0.066). In static group, far-periphery NPI is significantly associated with posterior segment IRMA density (R = 0.583, p = 0.018) and quantity (R = 0.541, p = 0.03). There was no correlation between far-periphery NPI and mid-peripheral IRMA, posterior segment NV or posterior segment NV in both groups (p > 0.05).

The ICCs between examiners were 0.936 for IRMA, 0.964 for NV and 0.977 for NPI.

**Discussion**

With advances in imaging technology, the development of fundus angiography from static to dynamic is an inevitable trend. However, one of remaining difficulties is that the entire record required a huge amount of memory. For instance, a traditional angiography device is adequate for recording short, one-minute videos. The size of a one-minute 1080P30 (1920*1080 image with progressive scan at 30 frames per second) high definition UWFA video could approach 100 GB. Time-lapse photography is a technique that
meets the need for recording the complete angiography process. With the use of a time-lapse UWFA video, hundreds of GB of imaging data could be reduced to dozens of MB. In previous studies, it has been proven to be useful in visualizing abnormal vasculature leakage [14] and illustrating the progression of macular hole [15]. The present study used time-lapse technique to visualize the complete UWFA process in DR patients. Compared to static NPI, dynamic NPI generated using a time-lapse technique was significantly lower in the total retina (0.25 vs 0.28, \( p = 0.005 \)), far-periphery (0.30 vs 0.34, adjusted \( p = 0.040 \)) and superior quadrant (0.27 vs 0.34, adjusted \( p = 0.045 \)) in eyes with severe NPDR and PDR. The far-periphery NPI was associated with posterior segment IRMA density in both groups, and posterior segment IRMA quantity in the static group.

NPI appeared to increase from the center to the periphery in both groups. The lower perfusion pressure at further distances from the posterior pole may cause a higher NPI in the peripheral retina [20]. We noticed that NPI could be quite variable. The NPI in Son et al’s study [21] was found to be highest in the temporal quadrant and lowest in the superior quadrant, with a global NPI of 0.59. In Silva et al’s [19] study, the NPI was observed to be the highest in the modified superotemporal quadrant. On one hand, variable NPI values may be caused by the different grading protocols and study characteristics. On the other hand, areas of hypofluorescence is a nonspecific hallmark on static images, which can be related to non-perfusion, subretinal hemorrhage, or vitreous opacity.

In previous study, patients with severe NPDR and PDR were reported to have a significantly greater vision-related functional burden than those without DR, which correlated well with degree of retinopathy [22]. It is important to precisely identify ischemic lesions in these DR patients. The present study showed significant differences between dynamic and static NPI among 16 eyes with severe NPDR and PDR in the far-periphery and in the superior quadrant. It demonstrated that low image contrast and eyelash artifact in the most peripheral region in UWFA imaging can contribute to a higher NPI. Some non-perfusion in peripheral retinal zones may actually be perfused. We considered that a 3.58% NPI may be an overestimation when calculated on a single UWFA image. In comparison with static pictures, videos are helpful for the differential diagnosis of hypofluorescence and for providing more information with regard to the peripheral retina.

We observed that the highest NPI was in the far-periphery, while IRMAs and NV occurred more frequently in the mid-periphery and posterior area. Analysis by Spearman’s rank correlation showed the severity of non-perfusion in the peripheral zone was associated with the prevalence of IRMAs in the posterior zone. Lange et al [23] found that far-peripheral NPI was significantly associated with mid-peripheral NV index (linear regression: \( Y = 0.103*X + 0.841, \ p = 0.007 \)). These results indicate that the ischemia-induced vascular abnormalities usually occurs at the border between the perfusion and non-perfusion areas, in concordance with previous study [24]. Of note, static far-peripheral NPI was associated with posterior segment IRMA quantity (\( R = 0.517, \ p = 0.04 \)). No association was found of dynamic far-peripheral NPI and posterior segment IRMA quantity (\( R = 0.471, \ p = 0.066 \)). The results imply that the correlation between NPI and vascular abnormalities tend to be magnified in a single UWFA image. Moreover, the presence of vascular abnormalities might be impacted by multiple factors, besides ischemia. Posterior vitreous
detachment has been a common occurrence in the vitreous, though this has not been mentioned in either previous or current studies. It has been shown that vitreoretinal attachment plays an important role in retinal vascular proliferation [25, 26]. Other risk factors of retinal vascular abnormalities include the long duration of diabetes, male gender, insulin use, hypertension, and PDR in the contralateral eye [27, 28].

The present study demonstrated that dynamic UWFA imaging allows for the accurate measurement of retinal non-perfusion. As a result, the precise application of panretinal photocoagulation and targeted retinal photocoagulation based on accurate non-perfusion delineation may be possible. An additional advantage is that a time-lapse video formed by a sequence of UWFA images is more likely helpful in differentiating retinal vascular lesions [see Additional file 2]. While leakage on fluorescein angiography conventionally helps to evaluate NV activity [8], a single image may be insufficient to distinguish NV from other lesions causing leakage, such as IRMA or other vascular abnormalities (Fig. 4). Therefore, with regard to clinical and scholarly presentations, active classic neovascularization on disc and neovascularization elsewhere could be easily detected in the videos (corresponding to the arrows in [Additional file 3]). Vascular leakage, non-perfusion, and granular background fluorescence at the far periphery, with increasing hyperfluorescence in the late phase, were clearly detected (corresponding to the arrows in [Additional file 4]). In addition, the UWFA time-lapse imaging allowed the posterior vitreous detachment with a Weiss ring as hypofluorescence at the posterior pole (corresponding to the arrow in [Additional file 5]).

The strength of this study lies in the fact that we assessed the value of dynamic UWFA imaging in precise identification of peripheral retinal non-perfusion and vascular abnormalities in DR. In addition, images were analyzed and retinas graded by two independent examiners. It should be noted that greater peripheral DR lesions in UWFA have been associated with more severe DR [29–31]. However, whether UWFA will attain a key position as a clinically relevant and irreplaceable tool remains unknown. Future studies, including the ongoing Intravitreal Aflibercept as Indicated by Real-Time Objective Imaging to Achieve Diabetic Retinopathy Improvement (registered at http://www.clinicaltrials.gov, with a registration number of NCT03531294) and Peripheral Diabetic Retinopathy Lesions on Ultrawide-field Fundus Images and Risk of DR Worsening over Time (DRCR.net, Protocol AA) [32], will help shed light on the potential role of UWFA findings in the evaluation and clinical management of DR eyes.

Limitations of this study were that we did not correct the peripheral warp present in UWFA. Since the most peripheral part of a UWFA image is magnified, we evaluated retinal non-perfusion using NPI instead of evaluating absolute areas of non-perfusion. Tan et al [33] used stereographic projection software to calculate precise NPA (in mm²) and compared corrected NPI to original NPI. They found that corrected NPI correlated with original NPI (Spearman correlation $R = 0.978$, $p < 0.001$), with no significant difference between the two NPI values (Wilcoxon signed-rank test, $p = 0.239$). Therefore, we believe that the discrepancy in NPI values may not significantly alter our conclusions. Besides, the significant differences between dynamic and static NPI in the far-periphery (adjusted $p = 0.040$) and superior quadrant (adjusted $p = 0.045$) are near minimal. The small sample size of this study increased the possibility of biased statistical significance (with a higher type 2 error). In addition, this technique requires patients to maintain
good fixation long enough to attain high-quality images, which can be modified with the development of imaging technology.

**Conclusions**

In summary, we conclude that dynamic UWFA video can provide a precise evaluation of NPI. It helps to improve the diagnostic accuracy of DR. Further studies with a large sample are warranted to reveal the potential value of treatment and follow-up of DR patients.

**Declarations**

**Ethics approval and consent to participate**

This study conformed to the tenets of the Declaration of Helsinki. All patients signed a written informed consent for participation in research, which was approved by the Medical Ethics Committee of Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine.

**Consent for publication**

Obtained.

**Availability of data and materials**

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors’ contributions**

H.S performed the experiments, and was a major contributor in writing the manuscript. J.W and T.N analyzed and interpreted the patient data. J.C and X.X edited and revised manuscript. All authors read and approved the final manuscript.
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Abbreviations

DR: diabetic retinopathy

ICC: intraclass correlation coefficients

IRMA: intraretinal microvascular abnormality

NPA: non-perfusion area

NPDR: nonproliferative diabetic retinopathy

NPI: non-perfusion index

NV: neovascularization

PDR: proliferative diabetic retinopathy

UWFA: ultra-widefield fluorescein angiography

References


33. Tan CS, Chew MC, van Hemert J, Singer MA, Bell D, Sadda SR. Measuring the precise area of peripheral retinal non-perfusion using ultra-widefield imaging and its correlation with the ischaemic
Figures

Figure 1

Ultra-widefield fluorescein angiography imaging of a right eye. The retina was divided into compartments. The posterior pole zone (P) and mid-peripheral zone (M) were demarcated by a concentric grey ellipse (1.2:1) centered on the fovea, and the far-peripheral zone's (F) boundaries were demarcated by the outer grey ellipse and the edge of the image (shown as the grey dotted borders). Four quadrants, namely the superior (S), inferior (I), temporal (T), and nasal (N) quadrants, were demarcated with red lines.
Delineation of the non-perfusion area in static and dynamic ultra-widefield fluorescein angiography imaging. The non-perfusion area was outlined in yellow as regional hypofluorescence relative to the background, flanked by neighboring filled vessels. (A) The non-perfusion area was outlined based on a single image. Hypofluorescence in the most peripheral region of the retina due to low image contrast and
eyelash artifact was related to a larger non-perfusion region. (B) The non-perfusion area was outlined based on a time-lapse video. Videos are helpful for the differential diagnosis of hypofluorescence.

Figure 3

Bland–Altman plot of the static and dynamic non-perfusion index.

Figure 4

Intraretinal microvascular abnormalities and neovascularization in ultra-widefield fluorescein angiography. (A) Intraretinal microvascular abnormalities (starred polygon) and neovascularization (arrow) was outlined on the total visible retina. (B) A region of the imaging. A single image may be
insufficient to distinguish neovascularization from Intraretinal microvascular abnormalities as both of lesions can cause leakage.

Supplementary Files

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- Additionalfile1.mp4
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