

The diagnostic value of optical coherence tomography angiography in diabetic retinopathy: a systematic review

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Abstract

Purpose Diabetic retinopathy (DR) is one of the leading causes of blindness worldwide. Accurate investigative tools are essential for the early diagnosis and monitoring of the disease. Optical coherence tomography angiography (OCTA) is a recently developed technology that enables visualisation of the retinal microvasculature.

Methods A systematic review of the literature was performed to examine the diagnostic use of OCTA in DR to date. Medline, EMBASE, and Cochrane databases were searched to find relevant studies. Sixty-one original studies were selected for the review.

Results and discussion OCTA has demonstrated the ability to identify microvascular features of DR such as microaneurysms, neovascularisation, and capillary non-perfusion. Furthermore, OCTA is enabling quantitative evaluation of the microvasculature of diabetic eyes. It has demonstrated the ability to detect early microvascular changes, in eyes with or without clinically evident DR. It has also been shown to detect progressive changes in the foveal avascular zone, and

vascular perfusion density, with worsening severity of disease. It provides three-dimensional visualisation of the individual retinal vascular networks and is thereby enhancing our understanding of the role of the deeper vasculature in the pathogenesis of diabetic retinopathy and maculopathy.

Conclusion However, limitations exist with current OCTA technology, in respect to the small field of view, image quality, projection artefact, and inaccuracies in analysis of the deeper vascular layers. While questions remain regarding its practical applicability in its present form, with continuing development and improvement of the technology, the diagnostic value of OCTA in diabetic retinopathy is likely to become evident.

Keywords Optical coherence tomography angiography · OCT angiography · OCTA · OCT-A · Diabetic retinopathy · Diabetic maculopathy · Diabetes

Introduction

Diabetic retinopathy (DR) is one of the leading causes of blindness worldwide [1]. It is the most common cause of blindness in the working population in developed countries [1]. The prevalence of DR is expected to increase due to current diet and exercise

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behaviours [2]. Accurate investigative tools are increasingly important for the diagnosis and monitoring of DR. Fluorescein angiography (FA) is currently the gold standard for the investigation of a number of features of DR including microaneurysms, neovascularisation, capillary non-perfusion, and diabetic macular ischaemia. However, FA is a relatively invasive investigation requiring intravenous injection of fluorescein dye and is known to have a number of risks including nausea, vomiting, itching and urticaria, and even anaphylaxis [3].

Optical coherence tomography angiography (OCTA) is a recently developed technology that enables visualisation of the retinal microvasculature and may offer a noninvasive alternative in the investigation of DR. Several OCTA devices are commercially available currently—the spectral-domain (SD) OCTA device RTVue XR Avanti with AngioVue software (Optovue, Inc., Fremont, California, USA) is the most extensively studied to date. This technology uses the split-spectrum amplitude-decorrelation angiography (SSADA) algorithm to detect blood flow in the retinal tissue [4]. Image areas of 3 mm × 3 mm, or 6 mm × 6 mm, can be acquired. Following acquisition of the retinal OCTA image, it is possible to perform segmentation in order to visualise individual retinal vascular layers separately. The AngioVue software enables segmentation of OCTA images into individual retinal vascular layers—the superficial capillary plexus (SCP) encompasses the vasculature within the ganglion cell layer, and the deep capillary plexus (DCP) encompasses the vasculature bracketing either side of the inner nuclear layer (INL) [4]. The SCP is visualised between an inner boundary at 3 µm below the internal limiting membrane (ILM) and an outer boundary at 15 µm below the inner plexiform layer (IPL) [4]. The DCP is visualised between an inner boundary at 15 µm below the IPL and an outer boundary at 70 µm below the IPL [4]. Additional OCTA devices, as well as alternative segmentation methods, have also been studied and will be discussed throughout the course of this review.

The purpose of this systematic review was to assess the diagnostic value of optical coherence tomography angiography (OCTA) in diabetic retinopathy.

Methods

Literature search

A comprehensive search of multiple databases was performed according to the PRISMA guidelines [5]. Medline, EMBASE, and Cochrane libraries were used to search for the following terms: ‘optical coherence tomography angiography’/‘OCT angiography’/‘OCTA’ and ‘diabetic retinopathy’/‘diabetic maculopathy’/‘diabetic macular ischaemia’/‘diabetic macular oedema’/‘diabetes’, including MeSH terms and synonyms.

Inclusion/exclusion criteria

The search was performed in order to identify all studies in which OCTA imaging had been used in patients with diabetes, diabetic retinopathy, and diabetic maculopathy. OCTA is a relatively recent development in retinal imaging; therefore, studies since 2014 were included. Studies included were limited to those published in English and in peer-reviewed journals. The literature review was limited to original studies and, as such, previous review articles were excluded, as were case reports.

Literature review

Using the search criteria as outlined above, a total of 144 articles were identified. Following an initial review of the abstracts, removal of duplicate studies, and exclusion of papers that did not fit the selection criteria, 61 articles were selected for the full literature review. OCTA has been used to evaluate a variety of microvascular parameters in eyes with DR—namely microaneurysms, neovascularisation, the foveal avascular zone, vessel density/perfusion density, fractal dimensions/vessel spacing/vessel tortuosity, capillary non-perfusion/diabetic macular ischaemia, and diabetic macular oedema. Each of these will be discussed in turn, followed by a discussion on the future directions of OCTA in DR.

Results and discussion

Microaneurysms

Ishibazawa et al. [4] were one of the earliest groups to evaluate OCTA in diabetic retinopathy. It was a prospective pilot study of 47 eyes (of 25 patients) with DR. The RTVue SD-OCTA device captured 3 mm × 3 mm scans centred on the fovea. Microaneurysms (MAs) were identified in each case using FA. OCTA visualised MAs, in both the SCP and DCP, as focally dilated saccular or fusiform capillaries [4]. The findings were promising in that OCTA demonstrated the ability to visualise MAs, in addition to identifying their location within the retinal vasculature—superficial or deep—which is not possible with FA. However, not all MAs present on FA were visualised on OCTA, suggesting that the sensitivity of MA detection may be a limitation of current technology [4].

Couturier et al. [6] also compared OCTA to FA as a method of MA detection, in a retrospective study of 20 eyes (14 patients) with DR. Using the RTVue SD-OCTA device, OCTA identified a significantly lower number of MAs compared to FA ($P = 0.02$) [6]. OCTA detected 62% of MAs that were detected on FA [6]. It is believed that slow blood flow of less than 0.3 mm per second cannot be detected by the SSADA algorithm [7]. There were a significantly higher number of MAs detected in the deep plexus compared to the superficial plexus (Mean {SD} number by eye; 4.4 ± 2.1 vs. 2.9 ± 2.3 , $P = 0.034$), suggesting they primarily originate at this level [6]. It was also noted on OCTA that the majority of MAs occurred at the margin of a capillary non-perfusion area, which appears to support the belief that they are an indicator of retinal ischaemia [6].

Parravano et al. [8] investigated the relationship between the appearance of MAs on structural SD-OCT and their detection with OCTA. One hundred and forty-five MAs, from 30 eyes with diabetic retinopathy, were imaged using the Spectralis SD-OCT and the RTVue SD-OCTA devices [8]. SD-OCT classified MAs based on internal reflectivity—hyporeflexive MAs were significantly less likely to be visualised on OCTA [8]. 66.7% of hyporeflexive MAs were visualised, compared to 88.9% of hyperreflexive and moderately reflexive MAs [8]. The researchers hypothesised that hyporeflexive MAs may be more likely to have a blood flow rate below the threshold as

described by Tokayer et al. [7]. The ability to concurrently acquire both en-face structural OCT and OCTA images has also been explored by Miwa et al. [9], suggesting increased MA detection using these imaging techniques simultaneously.

The distribution of MAs in eyes with diabetic macular oedema (DMO) has also been investigated [10, 11]. Hasegawa et al. [10] published a retrospective study of 33 eyes (27 patients) with DMO who had undergone SD-OCTA (RTVue XR Avanti) and structural SD-OCT imaging. Overall, $77.3 \pm 8.1\%$ of MAs were located in the DCP of eyes with DMO [10]. On focused analysis of the areas of macular oedema, where macular thickness was $> 400 \mu\text{m}$, $91.3 \pm 9.1\%$ of MAs were located in the DCP [10]. The higher proportion of DCP MAs in areas of macular oedema, compared to areas without oedema, was statistically significant ($P < 0.001$) [10].

Salz et al. [12] used a ultrahigh-speed swept-source OCTA (SS-OCTA) prototype device (New England Eye Centre, Boston, Massachusetts) to image the retinal microvasculature of 43 eyes of diabetic patients. FA was performed in 17 of the 43 cases for comparison. In line with previous studies using SD-OCTA, SS-OCTA identified less MAs compared to FA (mean {SD}; $6.4 (4.0)$ vs. $10 (6.9)$) [12]. However, the intraretinal depth of MAs was identified in all cases using SS-OCTA. Compared with FA, detection of MAs with SS-OCTA had a sensitivity of 85% (95% CI, 53–97) and a specificity of 75% (95% CI, 21–98) [12].

Neovascularisation

Ishibazawa et al. [4] also identified neovascularisation at the disc (NVD) using the RTVue SD-OCTA device. Eleven of the 47 eyes in this study had proliferative diabetic retinopathy (PDR) and 3 mm × 3 mm superficial OCTA images of the optic disc head were obtained [4]. In one case, a reduction in blood flow within the neovascularisation at the disc (NVD) was seen in an eye that had been treated with anti-VEGF [4]. It was noted, however, that the reduction in blood flow demonstrated by OCTA does not confirm a structural decrease in the NVD [4].

Studies have shown that OCTA has the ability to quantitatively assess neovascularisation in PDR [13–15]. Savastano et al. [14] conducted a small study of 10 eyes (6 patients) with PDR. It demonstrated that

OCTA (RTVue XR Avanti) can characterise the extent of NVD with greater accuracy compared to FA [14]. This was due to the fact that early leakage of fluorescein during FA obscures parts of the NVD [14]. As OCTA enabled this enhanced delineation of NVD, the area (pixel²) could be manually delineated by observers [14].

A retrospective study by de Carlo et al. [16] evaluated preretinal neovascularisation (NV) in PDR using OCTA. Fifty-two eyes (34 patients) with PDR were imaged using RTVue SD-OCTA [16]. 6 mm × 6 mm scans of the posterior pole were acquired. OCTA segmentation was performed to visualise blood flow above the ILM. Preretinal NV was visualised in the posterior pole of 13 eyes (12 patients) [16]. The retinal vascular layers were assessed in 12 of the 13 cases—1 case was excluded due to poor image quality. The study demonstrated ability of OCTA to not only visualise preretinal NV, but to also identify areas of capillary non-perfusion and IRMA associated with it. In 11 eyes (92%), NV was overlying a capillary non-perfusion area [16]. In 6 eyes (50%), it was noted to be adjacent to an intraretinal microvascular abnormality (IRMA) [16]. However, a considerable drawback of current OCTA technology was also observed in this study—the limited field of view (6 mm × 6 mm scans) of the posterior pole would not have detected more peripheral neovascularisation [16].

A 2016 study by Ishibazawa et al. [17] more extensively described the morphology of neovascularisation that can be visualised on OCTA. SD-OCTA (RTVue XR Avanti) imaging was performed on 40 eyes (33 patients) with PDR. This group consisted of 20 treatment-naïve eyes (14 patients) and 20 eyes (19 patients) previously treated with panretinal photocoagulation (PRP). Patients previously treated with anti-VEGF were not included in the study. Two patterns of NV were noted: ‘exuberant vascular proliferation (EVP)’—defined as an irregular proliferation of fine (small calibre) vessels—and ‘pruned vascular loops’ of filamentous vessels [17]. The EVP pattern was present in 95% of treatment-naïve eyes, compared to 65% of previously treated eyes ($P = 0.043$) [17]. In addition, 36 of the eyes were imaged with FA—all NV sites with EVP (30/30; 100%) demonstrated early phase leakage, while the majority of NV sites without EVP (5/6; 83%) demonstrated only faint leakage [17]. Twelve eyes (eight treatment-naïve, four previously treated) underwent PRP during the study period.

OCTA demonstrated pruning of vessels and a reduction of EVP two-month post-treatment. There was also a significant reduction in the mean flow area of new vessels ($0.70 \pm 0.70 \text{ mm}^2$ to $0.47 \pm 0.43 \text{ mm}^2$, $n = 12$, $P = 0.019$) [17]. These findings suggest that NV in PDR may be categorised morphologically using OCTA as being with, or without, EVP. EVP may also have a role as an indicator of active neovascularisation.

Singh et al. [18] demonstrated that OCTA can differentiate between optic disc venous collaterals (OVCs) and NVD in retinal vascular disease, including DR. In contrast to NVD which arises from the venous circulation and penetrates the ILM, OVCs develop within the existing vascular network and can be visualised on OCTA as thin, loopy vessels in the radial peripapillary capillary layer [18]. Differentiation is important as it is believed that OVCs may offer protection from ischaemia, in contrast to the intervention required in cases of NVD [18]. A limitation of this study is that just 20 eyes were imaged, and only a portion of these eyes (8/20) had DR—other pathologies included branch and central retinal vein occlusions [18]. Further studies are required before conclusions can be drawn on the applicability of this differentiation in DR.

Foveal avascular zone

A number of studies have used OCTA to analyse the foveal avascular zone (FAZ) in diabetic patients [12, 19–34]. Table 1 details the results of FAZ area measurements in the literature to date (Table 1).

Takase et al. [19] demonstrated that eyes of diabetic patients, even without clinical retinopathy (NDR), had significantly larger FAZ areas compared to healthy controls. The OCTA (RTVue XR Avanti) images of 63 eyes were analysed—19 age-matched control eyes, 24 NDR eyes, and 20 eyes with nonproliferative diabetic retinopathy (NPDR) (17 mild NPDR, 3 moderate NPDR) [19]. FAZ area was measured in both the SCP and DCP of each eye, using the ImageJ software—this method involves binarising the images, before manually delineating the FAZ area, which is then measured in mm² [19]. In the SCP, the mean FAZ area was significantly increased in both the NDR ($0.37 \pm 0.07 \text{ mm}^2$) and DR groups ($0.38 \pm 0.11 \text{ mm}^2$), compared to controls ($0.25 \pm 0.06 \text{ mm}^2$) ($P < 0.01$) [19]. In the DCP also,

Table 1 Foveal avascular zone (FAZ) area (mean ± SD) (mm²)

	Total number of eyes	Control eyes	NDR eyes	Mild NPDR	Moderate NPDR	Severe NPDR	PDR	NPDR with DMO	Comments
Takase et al. [19] ^{ad}	63	(n = 19) SCP; 0.25 ± 0.06 DCP; 0.38 ± 0.11	(n = 24) SCP; 0.37 ± 0.07 DCP; 0.54 ± 0.13	(n = 20; 17 mild, 3 moderate) SCP; 0.38 ± 0.11 DCP; 0.56 ± 0.12					NDR versus controls (both SCP and DCP) (<i>P</i> < 0.01) mild–moderate DR vs. controls (both SCP and DCP) (<i>P</i> < 0.01)
Dimitrova et al. [20] ^{ad}	62	(n = 33) SCP; 0.31 ± 0.10 DCP; not reported	(n = 29) SCP; 0.37 ± 0.11 DCP; Not reported						NDR versus controls (SCP) (<i>P</i> = 0.02) NDR versus controls (DCP) (not significant, <i>P</i> value not reported)
Samara et al. [27] ^{ad}	118	(n = 34) SCP; 0.275 ± 0.107 DCP; 0.372 ± 0.113	(n = 32) SCP; 0.365 ± 0.130 DCP; 0.508 ± 0.185	(n = 31) SCP; 0.437 ± 0.203 DCP; 0.625 ± 0.251			(n = 21) SCP; 0.508 ± 0.315 DCP; 0.766 ± 0.113		Each DR group versus controls (in SCP and DCP) (<i>P</i> < 0.05 for each) PDR versus mild NPDR (in DCP) (<i>P</i> = 0.04) NDR versus controls (<i>P</i> = 0.04)
de Carlo et al. [21] ^{ad}	89	(n = 28) Full-thickness; 0.288 ± 0.1364	(n = 61) Full-thickness; 0.348 ± 0.1008						NDR versus controls (<i>P</i> = 0.04)
Di et al. [22] ^{ad}	198	(n = 85) Full-thickness; 0.36 ± 0.11	(n = 53) Full-thickness; 0.40 ± 0.16	(n = 45) Full-thickness; 0.40 ± 0.11			(n = 15) Full-thickness; 0.47 ± 0.18		NDR versus controls (<i>P</i> = 0.04)
Simonett et al. [28] ^{ad}	51	(n = 23) SCP 0.26 ± 0.11 DCP 0.38 ± 0.15	(n = 28; 9 NDR, 19 mild NPDR) SCP 0.26 ± 0.12 DCP 0.40 ± 15						DR group versus controls, in SCP (<i>P</i> = 0.821) DR group versus controls, in DCP (<i>P</i> = 0.510)
Al-Sheikh et al. [23] ^{bd}	68	(n = 40) SCP; 0.339 ± 0.110 DCP; 0.358 ± 0.105	(n = 10) SCP; 0.301 ± 0.401 DCP; 0.608 ± 0.187	(n = 10) SCP; 0.444 ± 0.191 DCP; 0.546 ± 0.250	(n = 2) SCP; 0.681 ± 0.006 DCP; 0.643 ± 0.086		(n = 6) SCP; 0.619 ± 0.119 DCP; 0.734 ± 0.314		Difference between each DR subgroup in DCP (<i>P</i> < 0.05) Difference between severe NPDR group and PDR group in SCP (<i>P</i> < 0.05)
Salz et al. [12] ^{bd}	54	(n = 11) Full-thickness; 0.30 ± 0.11	(n = 13) Full-thickness; 0.41 ± 0.19	(n = 11) Full-thickness; 0.49 ± 0.19			(n = 5) Full-thickness; 0.76 ± 0.16		DR group (NPDR + PDR) versus controls (<i>P</i> < 0.001) PDR group versus NPDR/NDR/Control group (<i>P</i> < 0.05 for each)

Table 1 continued

	Total number of eyes	Control eyes	NDR eyes	Mild NPDR	Moderate NPDR	Severe NPDR	PDR	NPDR with DMO	Comments
Hwang et al. [29] ^{ae}	24	(n = 12) Full-thickness; 0.17 ± 0.05		(n = 12; 2 mild NPDR, 1 moderate NPDR, 9 PDR) Full-thickness; 0.33 ± 0.19					DR group versus controls (P = 0.024)
Sambhav et al. [30] ^{ae}	162	(n = 60) SCP; 0.334 ± 0.11 DCP; 0.363 ± 0.1	(n = 21) SCP; 0.390 ± 0.14 DCP; 0.418 ± 0.13	(n = 36) SCP; 0.377 ± 0.17 DCP; 0.415 ± 0.15	(n = 13) SCP; 0.399 ± 0.15 DCP; 0.431 ± 0.17		[32] SCP; 0.538 ± 0.19 DCP; 0.579 ± 0.13		DCP increase versus SCP increase (P < 0.05) NPDR with DMO group versus controls/mild/moderate/severe (P < 0.05)
Durbin et al. [31] ^{cd}	100	(n = 50) SRL; 0.25 ± 0.1	(n = 50; 10 early/NDR, 27 mild NPDR, 11 moderate–severe NPDR) SRL; 0.26 ± 0.1						Whole DR group versus controls (P = 0.28)
Camevali et al. [32] ^{cd}	50	(n = 25) SCP; 0.251 ± 0.104 DCP; 0.762 ± 0.231	(n = 25) SCP; 0.223 ± 0.1 DCP; 0.747 ± 0.199						NDR versus controls, in SCP (P = 0.341) NDR versus controls, in DCP (P = 0.808)
Bhanushali et al. [33] ^{ad}	269	(n = 60) SCP; 0.38 ± 0.01	(n = 35) SCP; 0.46 ± 0.03	(n = 95) SCP; 0.45 ± 0.01	(n = 57) SCP; 0.46 ± 0.02		(n = 22) SCP; 0.47 ± 0.02		Control group versus DR grades, (P < 0.001) Among DR grades (P = 0.82)
Lee et al. [34] ^{cd}	151	(n = 30) SRL; 0.325 ± 0.08 DRL; 0.797 ± 0.21	(n = 31) SRL; 0.354 ± 0.14 DRL; 0.822 ± 0.25	(n = 26) SRL; 0.350 ± 0.11 DRL; 0.902 ± 0.30	(n = 31) SRL; 0.431 ± 0.66 DRL; 1.174 ± 0.69		(n = 33) SRL; 0.492 ± 0.21 DRL; 1.24 ± 0.77		PDR versus controls, in DRL (P = 0.029) Other groups versus controls (not significant)

Full-thickness; segmentation of retinal vasculature into individual layers not performed
 SCP superficial capillary plexus (RTVue XR Avanti segmentation software); DCP deep capillary plexus (RTVue XR Avanti segmentation software); SRL superficial retinal layer (Cirrus SD-OCTA segmentation software); DRL deep retinal layer (Cirrus SD-OCTA segmentation software)

^aRTVue XR Avanti SD-OCTA (with AngioVue) device

^bPrototype SS-OCTA device

^cCirrus SD-OCTA (with Angioplex) (Carl Zeiss Meditec, Inc.)

^d3 mm × 3 mm scans

^e6 mm × 6 mm scans

the mean FAZ area was significantly increased in both the NDR ($0.54 \pm 0.13 \text{ mm}^2$) and DR groups ($0.56 \pm 0.12 \text{ mm}^2$), compared to controls ($0.38 \pm 0.11 \text{ mm}^2$) ($P < 0.01$) [19]. There was no significant difference between the NDR and DR groups, possibly due to the mild–moderate NPDR classification of the DR group [19]. These findings suggest that OCTA may be useful for the detection of early microvascular changes, before retinopathy is present on clinical examination.

Dimitrova et al. [20] found the FAZ area to be significantly increased in NDR patients compared to controls in the SCP, but not the DCP. OCTA (RTVue XR Avanti) imaging was performed on 33 control eyes, and 29 NDR eyes. In the SCP, the mean FAZ area was increased in NDR eyes compared to controls ($0.37 \pm 0.11 \text{ mm}^2$ vs. $0.31 \pm 0.10 \text{ mm}^2$, $P = 0.02$) [20]. In the DCP, the mean FAZ area was also increased in NDR eyes; however, this difference was not significant [20].

A prospective study by de Carlo et al. [21] similarly found that OCTA (RTVue XR Avanti) can identify early microvascular alterations in eyes of diabetic patients, before DR is clinically evident. Sixty-one NDR eyes (39 subjects) and 28 control eyes (22 age-matched healthy subjects) were imaged [21]. The mean FAZ area was significantly increased in NDR eyes compared to controls ($0.348 \pm 0.1008 \text{ mm}^2$ vs. $0.288 \pm 0.1364 \text{ mm}^2$, $P = 0.04$) [21]. However, the findings are not directly comparable to Takase et al., as full-thickness images of the retinal vasculature were obtained—i.e. SCP/DCP segmentation was not performed.

A larger cross-sectional study by Di et al. [22] also demonstrated increased FAZ area in NDR eyes compared to healthy controls. This study consisted of 85 control eyes (62 healthy subjects) and 113 eyes of diabetic patients (65 patients)—classified using the modified ETDRS system as; 53 NDR eyes, 45 NPDR eyes, and 15 PDR eyes. As with de Carlo et al., full-thickness retinal vasculature images were acquired using OCTA (RTVue XR Avanti). The mean FAZ area was significantly increased in the NDR group compared to controls ($0.40 \pm 0.16 \text{ mm}^2$ vs. $0.36 \pm 0.11 \text{ mm}^2$, $P = 0.04$) [22].

Al-Sheikh et al. [23] used a prototype swept-source OCT device (DRI OCT Triton; Topcon) to acquire OCTA images of eyes with DR and controls. Forty control eyes (20 healthy subjects) and 28 DR eyes (18

patients) were imaged—the DR group consisted of ten mild NPDR eyes, ten moderate NPDR eyes, two severe NPDR eyes, and six PDR eyes [23]. The mean FAZ area was significantly increased in the DR eyes (whole group) compared to controls, in both the SCP and DCP ($P = 0.003$, and $P < 0.001$, respectively) [23]. On subgroup analysis within the DR group, there was a significant difference in FAZ area between all subgroups at the level of the DCP, and between the severe NPDR and PDR subgroups at the level of the SCP [23] (Table 1).

Additional FAZ parameters have also been investigated using OCTA—including FAZ remodelling measurements, FAZ acircularity and axis ratio, horizontal and vertical FAZ radius, horizontal FAZ diameter, and angle of maximum FAZ diameter.

Krawitz et al. [35] determined that the OCTA parameters of ‘acircularity index’ (AI) and ‘axis ratio’ (AR) of FAZ can differentiate between control or NDR eyes, and NPDR or PDR eyes. OCTA (RTVue XR Avanti) images were acquired for 27 age-matched controls and 73 diabetic eyes (16 NDR eyes, 11 mild NPDR eyes, 10 moderate NPDR, 8 severe NPDR eyes, and 28 PDR eyes—graded using the ETDRS system) [35]. FAZ AI was defined as the ratio of the perimeter of the FAZ to the perimeter of a circle of equal area—i.e. a perfectly circular FAZ area would have an AI of 1. FAZ AR was defined as the ratio of the major axis to the minor axis of an ellipse generated in the FAZ—i.e. a perfectly circular FAZ would have an axis ratio of 1. There was a significant increase in both AI and AR in the NPDR and PDR groups compared to the control and NDR groups ($P < 0.01$ for each) [35]. On subgroup analysis, a significant difference was also noted in AI between controls and NPDR eyes ($P < 0.05$), and in AR between NDR and mild NPDR eyes ($P < 0.01$) [35].

Di et al. [22] found that the horizontal radius (HR) and vertical radius (VR) of the FAZ were significantly increased in eyes with DR (NPDR and PDR eyes) compared to controls, but no significant difference was observed between NDR eyes and control eyes with these parameters. Freiberg et al. [36] demonstrated that OCTA can be used to distinguish DR eyes from healthy controls based on horizontal FAZ diameter and the angle of maximum FAZ diameter. Twenty-nine DR eyes and 25 control eyes were imaged using the RTVue XR Avanti OCTA device. The mean horizontal FAZ diameter was significantly larger in

DR eyes compared to controls in both the SCP ($753 \mu\text{m} \pm 272 \mu\text{m}$ vs. $573 \mu\text{m} \pm 177 \mu\text{m}$, $P = 0.029$) and DCP ($1009 \mu\text{m} \pm 342 \mu\text{m}$ vs. $659 \mu\text{m} \pm 194 \mu\text{m}$, $P = 0.001$) [36].

Vessel density/perfusion density

Several OCTA-based methods have been described for measuring the density of vascular perfusion at the macula in eyes with DR—these include capillary perfusion density (CPD), vessel density (VD), flow index (FI), skeleton density (SD), vessel area density (VAD), vessel length density (VLD), and capillary density index (CDI) (Table 2).

A retrospective review by Agemy et al. [37] used OCTA to investigate perfusion density in diabetic eyes. The RTVue OCTA device was used to generate retinal vascular perfusion maps by identifying blood flow [37]. Capillary perfusion density (CPD) was then measured in the SCP, DCP, and choriocapillaris. $3 \text{ mm} \times 3 \text{ mm}$ and $6 \text{ mm} \times 6 \text{ mm}$ scans were obtained in each case. The study included 21 control eyes (12 age-matched subjects), and 56 DR eyes (34 eyes)—the DR group consisted of 11 mild NPDR, 9 moderate NPDR, 9 severe NPDR, and 26 PDR eyes (ETDRS grading) [37]. In all three layers, CPD was found to be significantly reduced in the diabetic group as a whole, compared to controls ($P < 0.05$ for each) [37]. A trend of reducing CPD was seen along the spectrum of disease from controls to NPDR to PDR. On subgroup analysis of the $3 \text{ mm} \times 3 \text{ mm}$ scans, mild NPDR patients had significantly lower CPD in both the SCP and DCP compared to controls ($P < 0.05$ for each) [37].

Hwang et al. [29] evaluated the use of automated quantification of vessel density (VD) on OCTA in eyes with DR. This retrospective study used $6 \text{ mm} \times 6 \text{ mm}$ scans acquired with the RTVue OCTA device and consisted of 12 controls eyes and 12 DR eyes (DRSS grading—two mild NPDR, one moderate NPDR, nine PDR). Segmentation of the individual vascular layers was not performed. The automated quantification technique for VD was obtained by calculating the mean decorrelation signal in eyes of controls—a threshold was defined as 2.3SDs above this mean [29]. The percentage of pixels with flow signal greater than this threshold was used to determine VD. Parafoveal and perifoveal VD were measured. There was a 12.6% reduction in parafoveal VD (95% CI, 7.7–17.5%;

$P < 0.001$) and a 10.4% reduction in perifoveal VD (95% CI, 6.8–14.1%; $P < 0.001$) in DR eyes compared to controls [29]. The findings suggest that this automated technique may be able to differentiate DR eyes from controls.

Sambhav et al. [30] expanded on these findings in a prospective study of 102 newly diagnosed NPDR eyes—classified using the ETDRS system as 36 mild NPDR eyes, 21 moderate NPDR eyes, 13 severe NPDR eyes, and 32 NPDR eyes with DMO. $6 \text{ mm} \times 6 \text{ mm}$ scans were obtained using OCTA (RTVue XR Avanti). Sixty control eyes were also imaged for comparison. In contrast to Hwang et al., segmentation of the individual vascular layers was performed using the built-in segmentation software. Vessel density (VD) was defined as the percentage of area occupied by vessels [30]. Flow index (FI) was defined as the average decorrelation value in the segmented area [30]. Analysis was performed on the parafoveal and perifoveal area; however, there were slight differences as to how these areas were defined compared to Hwang et al.—the parafoveal area was defined as a central circular area with diameter 3 mm, excluding the FAZ, while the perifoveal area was defined as a ring area from 3 to 6 mm from centre of FAZ [30]. In both the SCP and DCP, perfusion indices were found to reduce with increasing severity of DR [30]. On subgroup analysis, each NPDR subgroup significantly differed from control eyes for all parameters—i.e. parafoveal VD and FI, and perifoveal VD and FI, in both the SCP and DCP ($P < 0.001$) [30]. However, the reduction in perfusion indices was significantly more pronounced in the DCP than the SCP ($P < 0.05$) and in the perifoveal region compared to the parafoveal region ($P < 0.05$) [30].

Simonett et al. [28] similarly demonstrated that DCP parafoveal (VD) was significantly reduced in T1DM patients (NDR or mild NPDR) compared to controls ($P < 0.001$); however, the difference was not significant in the SCP. On subgroup analysis, mild NPDR eyes had a significantly reduced DCP parafoveal VD compared to controls ($P < 0.001$) (Table 2) [28]. DCP parafoveal VD was also reduced in NDR eyes compared to controls, but this was not statistically significant ($P = 0.052$) (Table 2) [28]. Direct comparisons with the aforementioned studies may not be appropriate as the definition of the parafoveal area in this study also differed—it was delineated by an annulus between a circle of 1 mm diameter (centred

Table 2 Perfusion indices (vessel density unless otherwise stated)

	Total number of eyes	Control eyes	NDR eyes	Mild NPDR	Moderate NPDR	Severe NPDR	PDR	NPDR with DMO	Comments
Hwang et al. [29] ^{af}	24	(n = 12) Parafoveal; (full-thickness) 89.6% ± 4.2 Perifoveal; (full-thickness) 91.2% ± 3.3		(n = 12; 2 mild NPDR, 1 moderate NPDR, 9 PDR) Parafoveal; (full-thickness) 77.0% ± 7.0 Perifoveal; (full-thickness) 80.7% ± 5.1					Parafoveal; DR group versus controls (<i>P</i> < 0.001) Perifoveal; DR group versus controls (<i>P</i> < 0.001)
Samara et al. [27] ^{pe}	118	(n = 34) (VAD) SCP; 55.09% ± 2.6 DCP; 61.32% ± 1.7		(n = 32) (VAD) SCP; 49.69% ± 4.0 DCP; 58.09% ± 2.7	(n = 31) (VAD) SCP; 49.09% ± 4.0 DCP; 55.83% ± 3.0	(n = 21) (VAD) SCP; 49.55% ± 3.7 DCP; 55.68% ± 1.8			Each DR group versus control group (SCP and DCP) (<i>P</i> < 0.001) Moderate–severe NPDR group (in DCP) (<i>P</i> = 0.002)
Sambhav et al. [30] ^{af}	162	(n = 60) Parafoveal; SCP; 46.8% ± 9.0 DCP; 36.93% ± 8.1 Perifoveal; SCP; 52.0% ± 9.5 DCP; 44.2% ± 9.0		(n = 36) Parafoveal; SCP; 35.9% ± 7.6 DCP; 25.2% ± 6.1 Perifoveal; SCP; 42.8% ± 8.6 DCP; 32.0% ± 9.1	(n = 21) Parafoveal; SCP; 29.8% ± 7.1 DCP; 20.2% ± 6.2 Perifoveal; SCP; 35.2% ± 8.7 DCP; 24.9% ± 9.1	(n = 13) Parafoveal; SCP; 20.3% ± 6.8 DCP; 11.2% ± 4.2 Perifoveal; SCP; 26.1% ± 7.9 DCP; 16.1% ± 6.1	(n = 32) Parafoveal; SCP; 30.9% ± 7.9 DCP; 17.9% ± 4.4 Perifoveal; SCP; 36.0% ± 9.4 DCP; 23.0% ± 6.6		Each DR group versus control (all indices) (<i>P</i> < 0.001) Reductions in DCP versus reductions in SCP (<i>P</i> < 0.05) Reductions in perifoveal versus reductions in parafoveal region (<i>P</i> < 0.05)
Dimitrova et al. [20] ^{pe}	62	(n = 33) Parafoveal; SCP; 51.39% ± 13.05 DCP; 41.53% ± 14.08	(n = 29) Parafoveal; SCP; 44.35% ± 13.31 DCP; 31.03% ± 16.33						NDR versus controls in SCP (<i>P</i> = 0.04) NDR versus controls in DCP (<i>P</i> < 0.01)

Table 2 continued

	Total number of eyes	Control eyes	NDR eyes	Mild NPDR	Moderate NPDR	Severe NPDR	PDR	NPDR with DMO	Comments
Al-Sheikh et al. [23] ^{bc}	68	(n = 40) VD (ratio) SCP; 0.709 ± 0.038 DCP; 0.714 ± 0.049	NDR eyes	(n = 10) VD (ratio) SCP; 0.591 ± 0.097 DCP; 0.699 ± 0.076	(n = 10) VD (ratio) SCP; 0.566 ± 0.114 DCP; 0.649 ± 0.118	(n = 2) VD (ratio) SCP; 0.589 ± 0.043 DCP; 0.704 ± 0.079	(n = 6) VD (ratio) SCP; 0.519 ± 0.081 DCP; 0.636 ± 0.017	NPDR with DMO	Whole DR group versus control ($P < 0.001$ in SCP) ($P = 0.028$ in DCP) Mild NPDR versus controls, in SCP ($P = 0.004$) Control group versus mild NPDR, in SCP ($P = 0.005$) Control group versus severe NPDR, in DCP ($P = 0.085$)
Kim et al. [39] ^c	98	(n = 14) VD (ratio) SRL; 0.426 ± 0.019 DRL; 0.433 ± 0.009	NDR eyes	(n = 32; mild–moderate) VD (ratio) SRL; 0.382 ± 0.039 DRL; 0.425 ± 0.015	(n = 16; moderate–severe) VD (ratio) SRL; 0.349 ± 0.056 DRL; 0.406 ± 0.051	(n = 22) (CDI) SCP; 0.342 ± 0.019 DCP; 0.351 ± 0.025	(n = 21) (CDI) SCP; 0.346 ± 0.01 DCP; 0.352 ± 0.35	(n = 21) (CDI) SCP; 0.338 ± 0.012 DCP; 0.345 ± 0.020	Mild NPDR versus controls, in DCP ($P < 0.001$) NDR versus controls, in DCP ($P = 0.052$) NDR versus controls, in SCP ($P = 0.805$) NDR versus controls, in DCP ($P = 0.005$)
Ting et al. [41] ^d	100	(n = 19) (CDI) SCP; 0.358 ± 0.017 DCP; 0.361 ± 0.019	(n = 9) Parafoveal; SCP; not reported DCP; 58.4% ± 2.9	(n = 17) (CDI) SCP; 0.354 ± 0.09 DCP; 0.353 ± 0.02	(n = 19) Parafoveal; SCP; not reported DCP; 56.3% ± 3.3	(n = 23) Parafoveal; SCP; 51.5% ± 4.0 DCP; 60.7% ± 2.4	(n = 25) SCP; 0.430 ± 0.020 DCP; 0.477 ± 0.014	(n = 25) SCP; 0.432 ± 0.023 DCP; 0.464 ± 0.016	PDR group versus NDR, in SCP ($P < 0.001$) PDR group versus NDR, in DCP ($P = 0.04$) Mild NPDR versus controls, in DCP ($P < 0.001$) NDR versus controls, in DCP ($P = 0.052$) NDR versus controls, in SCP ($P = 0.805$) NDR versus controls, in DCP ($P = 0.005$)
Simonett et al. [28] ^{ac}	51	(n = 23) Parafoveal; SCP; 51.5% ± 4.0 DCP; 60.7% ± 2.4	(n = 9) Parafoveal; SCP; not reported DCP; 58.4% ± 2.9	(n = 19) Parafoveal; SCP; not reported DCP; 56.3% ± 3.3	(n = 25) SCP; 0.430 ± 0.020 DCP; 0.477 ± 0.014	(n = 25) SCP; 0.432 ± 0.023 DCP; 0.464 ± 0.016	(n = 25) SCP; 0.430 ± 0.020 DCP; 0.477 ± 0.014	(n = 25) SCP; 0.432 ± 0.023 DCP; 0.464 ± 0.016	PDR group versus NDR, in SCP ($P < 0.001$) PDR group versus NDR, in DCP ($P = 0.04$) Mild NPDR versus controls, in DCP ($P < 0.001$) NDR versus controls, in DCP ($P = 0.052$) NDR versus controls, in SCP ($P = 0.805$) NDR versus controls, in DCP ($P = 0.005$)
Camevali et al. [32] ^{ce}	50	(n = 25) SCP; 0.430 ± 0.020 DCP; 0.477 ± 0.014	(n = 9) Parafoveal; SCP; not reported DCP; 58.4% ± 2.9	(n = 19) Parafoveal; SCP; not reported DCP; 56.3% ± 3.3	(n = 25) SCP; 0.432 ± 0.023 DCP; 0.464 ± 0.016	(n = 25) SCP; 0.430 ± 0.020 DCP; 0.477 ± 0.014	(n = 25) SCP; 0.432 ± 0.023 DCP; 0.464 ± 0.016	(n = 25) SCP; 0.432 ± 0.023 DCP; 0.464 ± 0.016	PDR group versus NDR, in SCP ($P < 0.001$) PDR group versus NDR, in DCP ($P = 0.04$) Mild NPDR versus controls, in DCP ($P < 0.001$) NDR versus controls, in DCP ($P = 0.052$) NDR versus controls, in SCP ($P = 0.805$) NDR versus controls, in DCP ($P = 0.005$)

Table 2 continued

Total number of eyes	Control eyes	NDR eyes	Mild NPDR	Moderate NPDR	Severe NPDR	PDR	NPDR with DMO	Comments
Zahid et al. [42] ^{ac}	(n = 56) SCP; 55.56% ± 1.69 DCP; 60.58% ± 1.5	(n = 13; 5 mild NPDR, 8 PDR) SCP; 48.22% ± 4.59 DCP; 53.57% ± 3.21						DR group versus controls, in SCP (<i>P</i> < 0.001) DR group versus controls, in DCP (<i>P</i> < 0.001)
Bhanushali et al. [33] ^{ae}	(n = 60) SCP; 49.7% ± 0.55 DCP; 53.1% ± 0.73	(n = 35) SCP; 39.2% ± 1.21 DCP; 39.7% ± 1.57	(n = 95) SCP; 40.1% ± 0.58 DCP; 40.2% ± 0.53	(n = 57) SCP; 38.5% ± 0.76 DCP; 39.4% ± 0.68	(n = 22) SCP; 38.9% ± 1.38 DCP; 39.2% ± 0.94			Controls versus DR grades, in SCP and DCP (<i>P</i> < 0.001) Among DR groups, in SCP (<i>P</i> = 0.11)

Full-thickness; segmentation of retinal vasculature into individual layers not performed

VAD vessel area density; CDI capillary density index; SCP superficial capillary plexus (RTVue XR Avanti segmentation software); DCP deep capillary plexus (RTVue XR Avanti segmentation software); SRL superficial retinal layer (Cirrus SD-OCTA segmentation software); DRL deep retinal layer (Cirrus SD-OCTA segmentation software)

^aRTVue XR Avanti SD-OCTA (with AngioVue) device

^bPrototype SS-OCTA device

^cCirrus SD-OCTA (with Angioplex) (Carl Zeiss Meditec, Inc.)

^dSS-OCTA (Topcon Corp) device

^e3 mm × 3 mm scans

^f6 mm × 6 mm scans

on the fovea) and a circle of 2.5 mm diameter (also centred on the fovea) [28].

A number of studies have discussed the difficulty in accurately assessing the DCP vasculature using current OCTA technology [20, 23]. Projection artefact, whereby the vascular flow of more superficial vessels can alter the interpretation of deeper vessels, may account for this difference [38]. This may account for some of the inconsistencies observed in relation to DCP perfusion density data, in addition to the previously discussed DCP FAZ measurements.

For example, Dimitrova et al. [20] found that DCP VD was lower than the SCP VD in both control eyes and NDR eyes. SCP VD was significantly reduced in NDR eyes compared to controls ($44.35\% \pm 13.31$ vs. $51.39\% \pm 13.05$, $P = 0.04$) [20]. DCP VD was also significantly reduced in NDR eyes compared to controls ($31.03\% \pm 16.33$ vs. $41.53\% \pm 14.08$, $P < 0.01$) [20]. Al-Sheikh et al. [23] found that mean VD was also significantly lower in both the SCP and DCP in eyes with diabetic retinopathy compared to normal subjects. However in this study, using a prototype SS-OCTA device, a more severe VD reduction was seen in the SCP (Table 2) [23]. Mild NPDR eyes could be differentiated from control eyes in the SCP ($P = 0.004$), but not the DCP ($P = 0.544$) [23].

Kim et al. [39] used the Cirrus SD-OCT with Angioplex (Carl Zeiss Meditec, Dublin, CA, USA) to quantify skeleton density (SD) (a measurement of the length of vessels based on a skeletonised OCTA image), and vessel density (VD) (the proportion of image containing OCTA signal compared to total area imaged). An automated segmentation algorithm was used to produce three slabs—a superficial retinal layer (SRL); ILM to 110 μm above the retinal pigment epithelium (RPE) (inner 60% of retina), a deep retinal layer (DRL); 110 μm above RPE to RPE (outer 40%), and an outer retinal layer; 110 μm above RPE to external limiting membrane [39]. The study included 14 control eyes and 84 DR eyes—graded using the ETDRS as; 32 mild–moderate NPDR, 16 moderate–severe NPDR, and 36 PDR eyes. On full-thickness slabs (i.e. non-segmented), there were statistically significant differences in the parameters when comparing healthy eyes with moderate–severe NPDR or PDR eyes and when comparing mild NPDR eyes with moderate–severe or PDR eyes (Table 2) [39]. However, a statistically significant difference between

healthy and mild–moderate NPDR was noted only in the SRL (Table 2) [39]. A notable limitation of this study was the number of eyes excluded from the final analysis due to insufficient image quality—116 eyes had been imaged during the defined study period, and 14 of these were excluded for this reason.

Samara et al. [27] also used the RTVue OCTA device to quantitatively analyse VD in eyes with DR. Two methods of VD measurement were used—vessel area density (VAD) and vessel length density (VLD) [27]. In contrast to Hwang et al., 3 mm \times 3 mm scans were used for analysis—and VD measurements were obtained for the entire scan area (unlike para/perifoveal areas) [27, 29]. Similar to Hwang et al., images were binarised using an automated thresholding algorithm, and blood vessels were identified by pixels that had decorrelation values above a threshold [27, 29]. The FAZ area was excluded. VAD was calculated as the area where vasculature was present—(pixels)/(Total area – FAZ area) (pixels) [27]. The image was then skeletonised for measurement of VLD and calculated as—the length of skeletonised vasculature (mm)/(Total area – FAZ area) (mm^2) [27]. Measurements were obtained for the SCP and DCP. The study consisted of 34 control eyes and 84 DR eyes—the DR group included 32 mild NPDR, 31 moderate–severe NPDR, and 21 PDR eyes [27]. VAD and VLD were significantly reduced in all stages of DR compared to controls in both the SCP and DCP ($P < 0.001$ for all) (Table 2) [27]. In the SCP, VAD and VLD did not differ significantly between DR stages [27]. In the DCP, VAD was significantly reduced in the moderate–severe NPDR group compared to the mild NPDR group (55.83% vs. 58.09% , $P = 0.002$) and was also significantly reduced in the PDR group compared to the mild NPDR group (55.68% vs. 58.09% , $P = 0.004$) [27]. In the DCP, VLD was significantly reduced in the moderate–severe NPDR group compared to the mild NPDR group (20.81 mm^{-1} vs. 22.12 mm^{-1} , $P = 0.015$) and was also significantly reduced in PDR compared to mild NPDR (20.34 mm^{-1} vs. 22.12 mm^{-1} , $P = 0.002$) [27]. These findings suggest that the VD parameters, VAD and VLD, may be useful in evaluating the severity of DR.

You et al. [40] suggested that there is good intravisit and intervisit reproducibility of OCTA vessel density measurements in both healthy and diseased eyes. Fifteen control eyes and 22 eyes with retinopathy—16

of whom had DR without DMO—were imaged using the RTVue OCTA device. Vessel density in the SCP was calculated using the AngioVue software, defined as the proportion of the area occupied by vessels—each patient attended two separate scanning sessions, with two scans at each session. The intravisit coefficient of variation varied from 2.1 to 4.9% for healthy eyes and 3.4 to 6.8% for eyes with retinopathy [40]. The intervisit coefficient of variation varied from 2.9 to 5.1% for healthy eyes and 4.0 to 6.8% for eyes with retinopathy [40]. However, as DR was not the only retinopathy investigated and eyes with DMO were not included, these findings may not apply to all DR eyes.

Ting et al. [41] investigated capillary density index (CDI) as a parameter for vascular perfusion in DR. The SS-OCTA (Topcon Corp) device was used to image 100 diabetic eyes attending a DR screening clinic at the Singapore National Eye Centre—this consisted of 19 NDR, 17 mild NPDR, 21 moderate NPDR, 22 severe NPDR, and 21 PDR eyes. CDI was calculated using ImageJ software, and a technique in which OCTA images were binarised and the luminal area was measured [41]. CDI analysis was performed in a circle with a radius of 1.5 mm, centred on the fovea. There was a trend of reducing CDI from NDR eyes through to PDR eyes in both the SCP and DCP (Table 2) [41]. There were statistically significant differences between the PDR group and NDR group in both the SCP ($P < 0.001$), and DCP ($P = 0.04$) [41]. The findings demonstrate the potential of CDI as another perfusion index for evaluating the severity of DR; however, larger studies are required to investigate its ability to distinguish less severe disease.

Fractal dimension/vessel spacing/vessel tortuosity

OCTA has also been used to investigate retinal microvasculature in terms of branching complexity, vessel spacing, and vessel tortuosity.

It is hypothesised that a loss of small branching vessels in diseases such as DR, results in a reduced branching complexity of the retinal vasculature—fractal dimensional (FD) analysis can be used as a measurement of branching complexity [39, 42]. A retrospective study by Zahid et al. [42] analysed the OCTA (RTVue XR Avanti) images of 56 control eyes and 13 DR eyes (five mild NPDR and eight PDR eyes). FD was calculated using ImageJ software and a ‘box-counting’ method on binarised OCTA images [42].

Mean FD was significantly reduced in DR eyes compared to controls in both the SCP (1.56 ± 0.08 vs. 1.64 ± 0.05 , $P = 0.005$) and DCP (1.60 ± 0.11 vs. 1.72 ± 0.04 , $P = 0.003$) [42]. The aforementioned study by Kim et al. [39] similarly demonstrated that there is a progressive reduction in FD with increasing severity of DR. Using the Cirrus SD-OCTA device, 14 control eyes and 84 DR eyes (32 mild NPDR, 16 severe NPDR, 36 PDR) were imaged. In full-thickness, non-segmented slabs, FD was significantly reduced in severe NPDR or PDR eyes compared to controls or mild NPDR eyes [39]. Following analysis of the segmented slabs, FD in the SRL was significantly reduced in mild NPDR eyes compared to controls (1.695 ± 0.018 vs. 1.717 ± 0.006 , $P = 0.026$).

Bhanushali et al. [33] investigated the potential value of large vessel spacing as a marker of DR progression. This was a prospective study of 60 controls and 209 DR eyes—graded using the ETDRS system as 35 mild NPDR, 95 moderate NPDR, 57 severe NPDR, and 22 PDR eyes. The RTVue OCTA device was used to measure the spacing between large and small vessels, in the SCP and DCP. Large vessel spacing in the SCP was significantly higher in the severe NPDR and PDR groups compared to mild NPDR and moderate NPDR ($P = 0.04$) [33]. However, small vessel spacing in the SCP was significantly higher in mild NPDR compared to other DR grades ($P < 0.001$) [33].

Lee et al. [34] quantitatively analysed vessel tortuosity (VT) on the Cirrus SD-OCTA images of diabetic eyes. The study consisted of 30 control eyes and 121 diabetic eyes—graded as per the modified Airlie House Classification system as: 31 NDR, 26 mild NPDR, 31 moderate–severe NPDR, and 33 PDR eyes. 3 mm × 3 mm scans of the SRL and DRL were acquired, within which 3 mm or 1.5 mm diameter circular areas were analysed. VT was calculated as the sum of actual branch lengths divided by sum of straight lengths between branch connections, using skeletonised OCTA images [34]. In the SRL, VT trended upwards with progressing DR severity until moderate–severe NPDR [34]. There was no significant increase in VT between healthy eyes and NDR eyes. However, in the SRL 1.5 mm area scans, VT was significantly increased in mild NPDR compared to healthy eyes and NDR eyes ($P < 0.05$) [34]. In both the 3 mm and 1.5 mm area scans, moderate to severe

NPDR eyes demonstrated significantly higher VT compared to healthy eyes and NDR eyes [34]. In the DRL, similar trends were observed; however, the only statistically significant increase in VT was noted between moderate–severe NPDR and healthy/NDR eyes [34].

Capillary non-perfusion/diabetic macular ischaemia

Ishabazawa et al. [4] demonstrated that areas of retinal non-perfusion can be visualised in eyes with DR using OCTA. Furthermore, these areas can be delineated in both the SCP and DCP and measured in mm^2 [4]. However, a limitation of retinal non-perfusion analysis with current OCTA technology was noted in that the small field of view imaged, $3 \text{ mm} \times 3 \text{ mm}$, does not allow for evaluation of more peripheral perfusion abnormalities [4].

Couturier et al. [6] compared OCTA to FA in terms of its ability to identify macular capillary non-perfusion in 20 eyes with DR. $3 \text{ mm} \times 3 \text{ mm}$ OCTA (RTVue XR Avanti) images were compared to magnified $3 \text{ mm} \times 3 \text{ mm}$ early phase FA squares. OCTA identified areas of non-perfusion in the SCP of all eyes, but in the DCP of just 35% of cases, indicating a difference in the pattern of microvascular change in each plexus [6]. OCTA more accurately delineated areas of capillary non-perfusion outside the FAZ, compared to FA [6]. This was due to the fact that there was superposition of the deep and superficial capillary plexus, as well as some obscuring vessel leakage, with FA imaging.

The ability of OCTA to detect eyes with DR based on capillary non-perfusion was investigated by Hwang et al. [29]. The term, total avascular area (TAA), was used to describe the sum of the avascular (non-perfused) areas in a $6 \text{ mm} \times 6 \text{ mm}$ OCTA (RTVue XR Avanti) image. An automated quantification technique was used to calculate TAA—the mean decorrelation signal in controls was calculated, and areas with flow signal less than 1.2SDs above this mean were identified as being avascular [29]. TAA was significantly increased in DR eyes compared to controls ($1.00 \pm 0.27 \text{ mm}^2$ vs. $0.18 \pm 0.07 \text{ mm}^2$, $P < 0.001$) [29]. The findings suggest that TAA may have a diagnostic role in eyes with DR; however, the small sample size (12 controls, 12 DR eyes) is a limitation of this study. Furthermore, nine of the DR

group were graded as PDR. Larger studies with increased numbers of NPDR eyes are required.

OCTA has enabled a greater understanding of the relationship between capillary non-perfusion and diabetic macular ischaemia (DMI). Minnella et al. [43] demonstrated that eyes with DMI had significantly increased perifoveal ‘no flow’ areas compared to controls, in both the SCP and DCP. A 2016 study by Scarinci et al. found that areas of DCP non-perfusion in eyes with DMI, corresponded precisely with areas of outer retinal disruption on structural OCT imaging [44]. By contrast, eyes with normal DCP perfusion did not exhibit outer retinal structural abnormalities [44].

A number of studies have investigated the ability of OCTA to grade DMI [45–48]. Currently, DMI is graded with FA images using the ETDRS protocols as; absent (no FAZ disruption), questionable (FAZ not smooth/oval, but no clear pathology), mild (< half FAZ circumference destroyed), moderate (> half FAZ circumference destroyed), severe (FAZ outline completely destroyed), or ungradable [49].

A 2016 study by Bradley et al. [45] compared OCTA to FA in the grading of DMI. The RTVue OCTA images—SCP, DCP, and choriocapillaris—of 24 DR eyes were retrospectively analysed. The SCP OCTA images were graded using the ETDRS protocols [49] and compared to FA images. The DCP and choriocapillaris OCTA images were graded with a newly devised system [45]. In the DCP, this grading was absent (no disruption of FAZ), questionable (FAZ not smooth/oval, but no clear pathology), mild/moderate (FAZ disrupted in ≤ 2 quadrants), severe (FAZ disrupted in ≥ 3 quadrants), or ungradable (poor image quality, artefact) [45]. In the choriocapillaris, it was graded as ischaemia present (loss of speckled hyperreflectance, or dark defects), ischaemia absent, or ungradable [45]. Two masked graders independently assessed the images. There was moderate agreement between DMI grading with the FA and OCTA images of the SCP (intragrader weighted κ values; 0.53 for grader 1, 0.41 for grader 2)—there was no difference in DMI grade in 60.4% of eyes, 33.3% had a difference of one grade, 2.1% had a difference of two grades, and 4.2% were ungradable [45]. In terms of reproducibility of this OCTA-based grading, there was substantial intergrader agreement in terms of the DMI grade acquired using OCTA for the SCP, DCP, and choriocapillaris (weighted κ values; 0.65, 0.61, and 0.65, respectively) [45]. While larger, prospective

trials are required to validate these findings, they suggest that OCTA may provide an alternative to FA in the investigation of DMI.

Diabetic macular oedema

OCTA has also been used to investigate the microvascular abnormalities associated with diabetic macular oedema (DMO) [22, 39, 41, 50].

The previously discussed study by Kim et al. which evaluated a number of perfusion indices in eyes with NPDR, performed additional analysis eyes with DMO [39]. Of the 32 mild NPDR eyes imaged using the Cirrus SD-OCTA device, 8 had co-existing DMO. This subgroup had significantly lower vessel density, skeleton density, and fractal dimension in both the SRL and DRL compared to those without DMO [39]. Further longitudinal studies will be required to investigate these associations with DMO—if these microvascular changes are a risk factor for DMO, or if they occur as a result of DMO. The authors hypothesise that intraretinal fluid may physically displace the vasculature or attenuate the decorrelation signal, or, alternatively, occluded vessels may lead to the development of DMO [39].

Ting et al. [41] demonstrated similar findings using SS-OCTA (Topcon Corp). Of the 100 diabetic eyes imaged, 16 had DMO. Capillary density index was reduced in eyes with DMO compared to those without DMO in both the SCP and DCP; however, in contrast to the indices evaluated by Kim et al., these reductions were not statistically significant (SCP; 0.344 vs. 0.347, $P = 0.16$, and DCP; 0.349 vs. 0.357, $P = 0.12$) [41]. Di et al. [22] analysed the RTVue OCTA images of 113 diabetic eyes, of which 30 had DMO. This group demonstrated that eyes with DMO exhibited increased FAZ areas (non-segmented) compared to those without DMO and that this increase was statistically significant ($0.46 \pm 0.12 \text{ mm}^2$ vs. $0.40 \pm 0.13 \text{ mm}^2$, $P = 0.017$) [22].

Lee et al. [51] investigated the OCTA microvascular features of eyes with DMO, undergoing anti-VEGF therapy. SD-OCT (Spectralis) was used to evaluate the response of DMO eyes to anti-VEGF. A good response was defined as a reduction of $> 50 \mu\text{m}$ after three consecutive injections. RTVue OCTA imaging was performed on 103 eyes—51 DMO eyes with a poor response to anti-VEGF, 32 DMO eyes with a good response to anti-VEGF, and 20 control eyes (fellow

eyes without a history of DMO) [51]. FAZ area and the number of MAs were increased to a greater extent in DCP compared to SCP in both responders and non-responders [51]. Vascular flow density was reduced to a greater extent in the DCP compared to SCP in both groups also [51]. DMO eyes with a good response to anti-VEGF were compared to DMO eyes with a poor response. In the DCP, poor responders exhibited larger FAZ areas, higher numbers of MAs, and reduced vascular flow density ($P < 0.001$ for each parameter) [51]. There was no significant difference in these parameters in the SCP. In addition, DMO eyes were compared to non-DMO (control) eyes. In the DCP, DMO eyes exhibited larger FAZ areas, more MAs, and reduced vascular flow density ($P < 0.001$ for each parameter) [51]. The findings suggest that DCP microvascular integrity may be associated with anti-VEGF treatment response, and disease pathogenesis, in eyes with DMO.

De Carlo et al. [52] demonstrated that OCTA has the ability to differentiate between DMO and capillary non-perfusion. The RTVue OCTA images of 17 eyes with DMO were retrospectively analysed. In all cases, OCTA was able to demonstrate cystoid spaces of DMO as areas of complete flow void, appearing as completely black—they demonstrated rounded edges and could therefore be differentiated from the more irregularly delineated capillary non-perfusion areas [52]. Nine eyes from the study were treated with anti-VEGF during the period of the study. OCTA demonstrated a corresponding reduction in DMO in all nine cases [52]. The findings are promising in that they suggest OCTA may have a role in the diagnosis of DMO, as well as monitoring the treatment response to anti-VEGF therapy. However, similar to Lee et al., the reliability of current OCTA technology in eyes with DMO was questioned as the presence of DMO resulted in greater difficulty in the segmentation of SCP and DCP [51, 52].

Mane et al. [53] used OCTA to investigate the relationship between capillary non-perfusion and the location of cystoid oedema in DMO. Retrospective analysis was performed on the OCTA (RTVue XR Avanti) images of 20 controls and 24 eyes with chronic diabetic cystoid macular oedema (DCMO). The findings suggest that cystoid spaces are associated with sites of capillary non-perfusion, particularly in the DCP—in the DCMO eyes, cystoid spaces were surrounded by non-perfusion areas in the SCP in 71%

of cases, and in the DCP in 96% of cases [53]. Compared to controls, the mean capillary density was significantly reduced in DCMO eyes, in both plexuses (SCP; $58.43 \pm 2.28\%$ vs. $44.98 \pm 3.32\%$, and DCP; $58.84 \pm 2.43\%$ vs. $51.55 \pm 3.41\%$ $P < 0.001$ for both comparisons) [53]. In contrast to Lee et al., this study noted a greater reduction in perfusion the SCP compared to DCP [51, 53]. However, the authors eluded to the fact that an overestimation on DCP capillary density was likely to have occurred—due to a falsely detected correlation signal from the walls of cystoid spaces and projection artefacts from the SCP [53]. In addition, this study of chronic DCMO cases may not translate to all cases of DCMO.

Alterations in OCTA-based microvascular parameters in response to anti-VEGF have not been extensively researched to date. De Carlo et al. [52] suggested that reappearance of retinal microvasculature may occur following anti-VEGF therapy; however, this has not been definitively demonstrated. In the study by Mane et al. [53], DCMO resolution had occurred in 11 of the cases studied, either spontaneously or after anti-VEGF therapy—and capillary density, in the SCP or DCP, did not significantly change after DCMO resolution. Similarly, Ghasemi et al. [54] found no significant difference in vessel density or FAZ area in eyes with macular oedema before and after anti-VEGF injections. However, limitations of these studies are noted in terms of sample size, and further research is warranted in this area.

Future directions of OCTA in diabetic retinopathy

A number of potential future directions of OCTA technology in DR have been investigated by recent publications. These include additional segmentation analysis [55–57], reduction of projection artefacts [56–58], and swept-source OCTA technology [23, 59–61].

Additional segmentation of retinal vascular layers was investigated in diabetic eyes by Park et al. [55]. Standard OCTA software, as previously described, separates retinal vasculature at the middle of the INL into the SCP and DCP. The middle capillary plexus (MCP) is located at the inner aspect of the INL, therefore it is incorporated into both these plexuses, mainly the SCP, using this method [55]. This group used the RTVue OCTA device; however, instead of

using the built-in segmentation software to visualise the SCP and DCP, they presented a novel method of manual segmentation to evaluate the SCP (3 μm below ILM to the outer IPL), MCP (a 30 μm slab from outer IPL boundary), and DCP (a 15 μm slab from outer INL, set at 45–60 μm below IPL) [55]. Ten control eyes and 12 DR eyes were imaged. Qualitative analysis was performed. In healthy eyes, the MCP appeared to have the most clearly delineated FAZ and a smaller FAZ diameter compared to the SCP and DCP [55]. In diabetic eyes, FAZ disruption and parafoveal non-perfusion occurred to varying extents in the three different plexuses—there was marked disruption of the FAZ outline in the MCP, compared to healthy controls [55]. Further quantitative studies are required in this area.

The reduction of projection artefacts, thereby improving the accuracy of DCP analysis has also been investigated. Hwang et al. used projection-resolved OCTA (PR-OCTA) in eyes with DR and healthy controls [56]. The RTVue OCTA device was used to image 29 control eyes and 47 DR eyes—graded as 8 mild/moderate NPDR, 8 severe NPDR, and 13 PDR. A projection-resolution (PR) algorithm, as described by Zhang et al. [62], was used to correct for projection artefacts. Following this, a semi-automated algorithm was used to segment the images into three distinct vascular plexuses—superficial layer; the inner 80% of ganglion cell layer, intermediate layer; the outer 20% of the ganglion cell layer and inner 20% of INL, and deep layer; half the depth of the INL to outer border of OPL [56]. Three-layer PR-OCTA identified capillary non-perfusion with greater sensitivity than standard two-layer (SCP, DCP) OCTA, 100% (95% CI, 90.8–100%) vs. 78.7% (95% CI, 63.9–88.8%) [56]. It also differentiated severe DR (PDR and severe NPDR) from mild DR (mild/moderate NPDR) with greater sensitivity, 72.2% (95% CI, 54.6–85.2%) vs. 25.0% (95% CI, 12.7–42.5%) [56]. PR-OCTA, and subsequent visualisation of three individual vascular plexuses with reduced projection artefacts in the intermediate and deep layers, may enable improved identification of microvascular abnormalities compared to two-layer OCTA.

This technology was further evaluated by Zhang et al. [57]. PR-OCTA images were obtained for the superficial, intermediate, and deep layers as described by Hwang et al. [56]. This study, however, focused on eyes with mild NPDR—13 control eyes and 13 mild

NPDR eyes [57]. Non-perfusion was measured in each of the three vascular layers using a novel automated algorithm—the total avascular area (TAA) was calculated in each 3 mm × 3 mm scan, and the extrafoveal avascular area (EAA) was defined as the TAA excluding a 1 mm central circle [57]. The EAA from each plexus was summated. The sum of the EAA's from the three plexuses was significantly larger in mild NPDR eyes compared to controls ($P < 0.001$) and had a sensitivity for DR detection of 94% (AROC = 0.99) [57]. The results indicate that quantification of avascular area in these three vascular layers using PR-OCTA may be useful in the early detection of DR.

Swept-source OCTA technology may also offer enhanced investigation of microvascular abnormalities in DR. Studies have been performed in diabetic eyes using the SS-OCTA (Carl Zeiss Meditec) device [59] and the SS-OCTA (DRI OCT Triton; Topcon) device [23, 41, 61].

A qualitative by Matsunaga et al. [59] used the SS-OCTA (Carl Zeiss Meditec) device to image 47 eyes with DR. Phase-contrast and intensity-contrast algorithms used to construct three retinal slabs; inner retina (ILM to superficial IPL), middle retina (deep IPL to superficial ONL), and deep retina (deep ONL to ELM) [59]. Like SD-OCTA, SS-OCTA demonstrated the ability to identify previously described microvascular abnormalities such as microaneurysms, capillary non-perfusion and NVD, but also identified additional abnormalities such as IRMA and cotton wool spots [59]. In many cases, SS-OCTA enabled enhanced delineation of the non-perfusion areas, particularly in the inner and middle retina layers [59].

Choi et al. [60] imaged diabetic eyes, with and without retinopathy, using a prototype ultrahigh-speed SS-OCTA device with a light source wavelength of 1050 nm and 400 kHz scan rate. This prospective study consisted of 63 normal eyes, 51 NDR eyes, 29 NPDR eyes, and 9 PDR eyes [60]. In eyes with DR, reduced capillary density, capillary dropout, and FAZ irregularities were observed [60]. Choriocapillaris flow impairment was noted in 7 PDR eyes and 15 NPDR eyes [60]. In addition to visualisation of the retinal vascular layers, ultrahigh-speed SS-OCTA technology enables deeper tissue penetration, allowing more accurate analysis of the choriocapillaris [60]. With faster image acquisition speeds, the technology may also permit wider field imaging [60].

An improved method for detection of blood flow has recently been described. The SSADA algorithm, as used in conventional SD-OCTA technology, characterises vasculature by detecting the presence of blood flow [4]. In addition to visualising the microvasculature, a novel variable interscan time analysis (VISTA) algorithm has demonstrated the ability to evaluate the speed of the detected blood flow [63, 64]. The technology has been implemented in combination with a vertical cavity surface emitting laser (VCSEL) swept light source OCTA prototype, with promising results [63, 64]. It is hoped that this technology will improve the quantitative interpretation of OCTA.

Conclusion

OCTA can identify microaneurysms (MAs) in eyes with DR, allowing enhanced analysis compared to FA, in that their intraretinal location can be identified—that is SCP or DCP [4, 6]. A significantly higher number of MAs occur in the DCP, compared to the SCP [6, 11]. DCP MAs also appear to be significantly associated with areas of DMO [10]. However, the sensitivity for MA detection is a limitation of current OCTA technology [6]. It is hypothesised that blood flow in MAs below a threshold of 0.3 mm per second is not detected by the SSADA algorithm [7]. With advancing technology, this may improve—ultrahigh-speed SS-OCTA has been shown to have a sensitivity of 85% and a specificity of 75% for MA detection compared to FA [12].

By adjusting the segmentation boundary to detect blood flow above the internal limiting membrane (ILM), OCTA can visualise preretinal neovascularisation in eyes with proliferative DR [4]. OCTA can characterise the extent of NVD with greater accuracy than FA—due to the absence of obscuration of its outline with fluorescein dye leakage [14]. Enhanced delineation of NVD can potentially enable quantitative assessment of neovascularisation, whereby its area can be precisely measured [14]. OCTA can also identify areas of capillary non-perfusion associated with the neovascularisation [16]. Furthermore, it may enable a morphological classification on NV, based on the presence or absence of exuberant vascular proliferation (EVP)—a potential indicator of disease activity for monitoring of treatment [17]. However, in its present form, the restricted field of view is a significant

drawback of OCTA imaging, in that peripheral neovascularisation cannot be evaluated [16].

The foveal avascular zone has been one of the most extensively investigated areas in DR using OCTA. Eyes of diabetic patients, even without retinopathy (NDR), have been shown to have significantly enlarged FAZ areas compared to controls—in both the SCP and DCP [19]. However, inconsistencies exist in the literature. Dimitrova et al. found that the FAZ area was significantly increased in NDR eyes compared to controls in the SCP, but the increase was not significant in the DCP [20]. OCTA has also demonstrated the ability to differentiate between different subgroups of DR severity—Al-Sheikh et al. found significant differences in FAZ area between all DR subgroups at the level of the DCP, but just between the severe NPDR and PDR subgroups at the level of the SCP [23]. A number of OCTA devices and segmentation methods have been used to date (Table 1). In addition, the aforementioned inaccuracies in interpretation of the deeper vascular networks due to projection artefact [38] mean it is difficult to draw conclusions on the diagnostic value of FAZ area in DR at present. Further FAZ parameters of acircularity index, axis ratio, FAZ perimeter, radius, and diameter have also shown promise as markers of DR on OCTA imaging [22, 35, 36, 65].

OCTA-based measurements have been used as perfusion indices in eyes with DR (Table 2). Several OCTA devices have been used, as well as different segmentation methods (Table 2). The region in which perfusion is evaluated has also varied between studies—some analysing the entire 3 mm × 3 mm or 6 mm × 6 mm scan area, others analysing a parafoveal or perifoveal area. Furthermore, the definition of these para-/perifoveal areas has differed between studies [20, 28–30]. This makes overall interpretation of this data more difficult. Nonetheless, there is significant evidence that the density of vascular perfusion reduces with increasing severity of DR and that OCTA parameters can be used to identify these changes (Table 2). It can also be used to detect early DR. Capillary perfusion density (CPD) has been shown to be significantly lower in eyes with mild NPDR compared to controls, in both the SCP and DCP [37]. It has also been demonstrated that vessel density is significantly reduced in NDR eyes compared to controls, in both the SCP and DCP [20]. However, as with FAZ measurements, there have been

inconsistencies noted in the literature, particularly in relation to deep plexus analysis [20, 23, 28].

OCTA enables evaluation of the branching complexity of vessels in DR—in the form of fractal dimension (FD) measurements. FD has been shown to be significantly reduced in DR eyes compared to controls [42]. It can be used to differentiate mild NPDR eyes from controls, based on SCP analysis [39]. Measurement of vessel spacing and vessel tortuosity using OCTA has also been proposed as methods of analysing DR severity [33, 34].

Compared to FA, OCTA has demonstrated moderate agreement for grading of DMI [45]. Using OCTA, areas of macular non-perfusion can be accurately delineated and measured in both the SCP and DCP [4]. As a result of this, and the absence of fluorescein dye leakage, OCTA may enable enhanced analysis of non-perfusion [6]. However, the small field of view obtained is a limitation with current technology, meaning that peripheral retinal non-perfusion cannot be seen [4]. Nevertheless, OCTA is showing promising results for the evaluation capillary non-perfusion and particularly automated methods of analysis [29].

OCTA has also been used to investigate microvascular abnormalities associated with diabetic macular oedema (DMO). Kim et al. [39] demonstrated that DR eyes with DMO have significantly reduced vessel density, skeleton density, and fractal dimension, compared to DR eyes without DMO. OCTA is improving our understanding of the role of the DCP in the pathogenesis of DMO. In eyes with DMO, FAZ area and the number of MAs appear to be increased to a greater extent in the DCP compared to the SCP [51]. In addition, flow density appears to be reduced to a greater extent in the DCP [51]. The ability to simultaneously obtain structural OCT and OCTA images may be of benefit for monitoring of treatment response in DMO. It has been demonstrated that poor responders to anti-VEGF have significantly larger FAZ areas, more MAs, and reduced flow density in the DCP, compared to good-responders [51].

OCTA technology is evolving, and the future directions of OCTA are becoming apparent. Additional segmentation analysis, and evaluation of the middle capillary plexus, may further enhance the ability of OCTA to detect early microvascular changes in DR [55–57]. Projection-resolution algorithms have been developed to reduce projection artefact and thereby improve the accuracy of analysis of the middle

and deep vascular layers [62]. Ultrahigh-speed swept-source OCTA technology may also offer improved microvasculature analysis, enhanced depth penetration and choriocapillaris visualisation, and wide-field OCTA imaging [59, 60].

In conclusion, OCTA has demonstrated the ability to accurately visualise retinal microvasculature in eyes with diabetic retinopathy. It has the potential to quantitatively analyse neovascularisation, capillary non-perfusion, and diabetic macular ischaemia, in addition to novel vascular parameters such as fractal dimension, vessel tortuosity, and skeleton density. It can detect early microvascular changes, in eyes with or without clinically evident DR. It has been shown to detect progressive changes in the foveal avascular zone, and vascular perfusion density, with worsening severity of disease. By enabling three-dimensional visualisation of the individual retinal vascular networks, OCTA is enhancing our understanding of the role of the deeper vasculature in the pathogenesis of diabetic retinopathy and maculopathy. However, limitations exist with current OCTA technology, in respect to the small field of view, projection artefact, and inaccuracies in analysis of the deeper vascular layers. While questions remain regarding its practical applicability in its current form, with continuing development and improvement of the technology, the diagnostic value of OCTA in diabetic retinopathy is likely to become evident.

Compliance with ethical standards

Conflict of interest All authors certify that they have no affiliations with or involvement in any organisation or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge, or beliefs) in the subject matter or materials discussed in this manuscript.

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