science, engineering and health studies

Research Article

Science, Engineering and Health Studies
https://lio1.tci-thaijo.org/index.php/sehs
ISSN (Online): 2630-0087

Vascular and perfusion density in superficial capillary plexus of macula as a biomarker for appearance of diabetic retinopathy

Darina Koseva[1], Elitsa Hristova[2], and Zornitsa Zlatarova[2]*

[1] Specialized Eye Hospital for Active Treatment - Varna, Varna 9002, Bulgaria
[2] Department of Optometry and Occupational Diseases, Faculty of Public Health, Medical University of Varna, Varna 9000, Bulgaria

***Corresponding author**:
Darina Koseva
darikoseva@gmail.com

***Received**: 31 May 2023*
***Revised**: 15 October 2023*
***Accepted**: 15 October 2023*
***Published**: 31 December 2023*

***Citation**:
Koseva, D., Hristova, E., and Zlatarova, Z. (2023). Vascular and perfusion density in superficial capillary plexus of macula as a biomarker for appearance of diabetic retinopathy. Science, Engineering and Health Studies, 17, 23050022.

ABSTRACT

Diabetic alteration of the retinal microenvironment causes damage to the retinal vessels. Biomarkers such as vessel density and perfusion density, measured using optical coherence tomography angiography (OCT-A), may help detect early signs of diabetic retinopathy (DR) and track disease progression. In our study, we categorized 212 individuals into 2 groups: Group I (75 healthy individuals) and Group II (137 people with diabetes). All participants underwent comprehensive eye examinations, including OCT-A with AngioPlex™ system. The measurements taken of the superficial retinal capillary plexus included vessel and perfusion density. Our findings showed that vessel and perfusion densities in the central, inner, and outer ETDRS fields, as well as the full macular area, decreased with age. Notably, vessel and perfusion densities in the inner, outer, and full ETDRS macular areas in diabetic patients with mild DR were significantly lower, compared to those in healthy subjects. In conclusion, this study emphasizes the importance of vessel and perfusion densities as biomarkers for early diabetic retinopathy. However, it is important to note that their reduction does not precede clinical signs of diabetic retinopathy.

Keywords: diabetes; diabetic retinopathy; vessel density; perfusion density; OCT

1. INTRODUCTION

The World Health Organization (WHO) classifies diabetic retinopathy (DR) as a prioritized ocular condition owing to its escalating global prevalence. Among individuals diagnosed with diabetes, approximately one-third develop diabetic retinopathy, with 10% manifesting indicators of sight-threatening retinopathy (Sivaprasad and Pearce, 2019).

The pathogenesis of diabetic complications is intricate, and diabetic retinopathy is no exception. A multitude of factors and diverse pathophysiological mechanisms contribute to the onset of this condition. The American Diabetes Association defines DR as a neurovascular disease that disrupts intercellular communication among various types of retinal cells (Solomon et al., 2017).

When examining the vascular aspect of diabetic retinal damage, it is evident that the retinal microenvironment, affected by diabetes mellitus (DM), plays a pivotal role in the development and advancement of vascular lesions and cell death. Prolonged elevation of glucose levels within the body disrupts the regulation of a myriad of metabolic processes and mediators, including growth factors and vasoactive and inflammatory agents. These alterations ultimately lead to damage to the retinal vasculature.

In accordance with the latest guidelines from the American Academy of Ophthalmology (AAO) and the International Council of Ophthalmology (ICO), the recommended method for diagnosing DR involves the use of slit-lamp biomicroscopy with either a +90D or +78D lens (Flaxel et al., 2019; Wong et al., 2018).

The critical evaluation of vascular retinal abnormalities plays a crucial role in facilitating the diagnosis and treatment of conditions such as DR. This can be effectively achieved through the use of fluorescein angiography, which, although invasive and associated with certain risks, has been a conventional diagnostic method. Alternatively, Optical Coherence Tomography-Angiography (OCT-A) offers a valuable option.

OCT-A leverages a high-performance angiography algorithm and offers several undeniable benefits over traditional fluorescein angiography. One of its primary strengths lies in the low risk of complications since it eliminates the need for intravenous dye injection. Additionally, OCT-A provides three-dimensional visualization of the retinal and choroidal blood supply. This diagnostic method allows for a comprehensive and quantitative assessment of retinal and choroidal vascular abnormalities in vivo.

With the assistance of OCT-A, it becomes possible to accurately identify the capillary networks within the retinal or choroidal plexus where diabetic changes may occur (Moir et al., 2021). Three vascular networks are located in the structure of the human retina: the superficial networks in the neurofibrillary and ganglion layers, the intermediate networks in the inner plexiform and nuclear layers, and the deep networks in the inner nuclear and outer plexiform layers (Balaratnasingam et al., 2018; Chan et al., 2012).

In diabetic retinopathy, OCT-A can effectively detect the pathological formation of new blood vessels and visualize ischemic sections (Jia et al., 2015). Furthermore, the literature discusses the concept of "preclinical DR," which incorporates OCT-angiogram changes such as reduced or absent capillary perfusion around the foveolar avascular zone, the disruption of the normal spider-like architecture of capillaries in the perifoveal region, and the presence of microaneurysms that precede any visible abnormalities in the fundus (Cao et al., 2018). This concept highlights the importance of early detection and the potential of OCT-A to reveal subtle vascular changes before they become clinically apparent, offering a fast and non-invasive approach.

In recent years, there has been a growing realization that certain quantitative OCT-A metrics have the potential to assist in detecting the initial signs of DR and monitoring the progression of the disease. Promising biomarkers for DR include vessel density and perfusion density. These metrics can provide valuable insights into the vascular changes associated with DR and offer a more objective and quantitative approach to disease assessment and tracking (Marques et al., 2020; Zhang et al., 2016; Lei et al., 2017). Vascular density in the retina is determined by the number of blood vessels containing erythrocytes, while perfusion density represents the change in the caliber of the vessels (Mendes et al., 2021). Since DR primarily manifests as a microvascular disease, these metrics may be particularly indicative of changes occurring at a capillary level.

In the present study, we investigated whether changes in vessel and perfusion density in the superficial retinal capillary plexus precede the appearance of clinical signs of DR. The primary objective was to assess the significance of vascular and perfusion densities in the superficial capillary macular plexus as biomarkers for the detection of DR.

2. MATERIALS AND METHODS

2.1 Study design

The Ethics Committee of Varna Medical University approved the current study (Approval No. 77/27.09.2018), which was conducted in accordance with the Declaration of Helsinki. Patient recruitment and examinations took place between October 2018 and March 2020 at the specialized hospital for eye diseases in Varna. All patients provided informed consent after receiving a prior explanation of the study's objectives and methods.

Our case-control study included a total of 212 individuals. To achieve the research objectives, two groups of patients were distinguished:

Group I: Control group – This group comprised 75 healthy individuals (148 eyes), including 32 men and 43 women. The distribution by sex and age in this group was comparable to that of individuals with DM.

Group II: Case group – This group consisted of 137 individuals with DM (248 eyes), comprising 60 men and 77 women.

Within Group II, we further divided the patients into two subgroups:

-Subgroup A: 71 diabetics without DR (140 eyes), and

-Subgroup B: 66 diabetics with mild nonproliferative diabetic retinopathy (NPDR; microaneurysms only according to the ICO classification) (108 eyes).

The inclusion criteria for healthy participants encompassed individuals over the age of 18 with the absence of systemic or ocular diseases. In contrast, exclusion criteria for the control group consisted of refractive spherical anomalies exceeding ± 6.0 diopters sphere and/or astigmatism surpassing ± 2.0 diopters, as well as the presence of any eye pathology.

We included type I diabetics with more than five years of disease history, as well as type II diabetics, regardless of diabetes duration. Patients became part of the study provided that they did not have DR or exhibited a mild degree of DR as per the classification of the International Council of Ophthalmology.

Diabetic patients were excluded from the study group if their ocular media transparency hindered obtaining a high-quality OCT image. Additionally, diabetic patients with moderate or severe NPDR, proliferative diabetic retinopathy (PDR), or diabetic macular edema (DME) according to the ICO classification were also excluded from the study.

Furthermore, additional ocular exclusion criteria included the following: intraocular pressure (IOP) exceeding 21 mmHg, the presence of glaucoma, inflammatory diseases affecting the orbit, uvea, or the cornea, macular edema originating from causes other than diabetes, coexistence of other retinal diseases such as hereditary degenerative macular conditions, age-related macular degeneration, retinal detachment, central serous chorioretinopathy, retinal vein or artery occlusion, macular hole, and similar disorders, as well as refractive anomalies exceeding ± 6.0 diopters sphere and/or astigmatism surpassing ± 2.0 diopters. These criteria were

applied to ensure a well-defined and consistent study group.

Twenty-eight eyes were excluded from the study due to reduced ocular media transparency, often caused by initial cataracts, resulting in weak signal strength, low-quality image acquisition, the presence of artifacts, and unreliable recordings.

Following the collection of each patient's medical history, a comprehensive ophthalmological examination was conducted. This examination encompassed multiple key components, including the determination of corrected visual acuity, measurement of intraocular pressure, assessment of the health of the anterior eye segment using a biomicroscope, examination of the retina through stereo ophthalmoscopy, and the performance of OCT-angiography. The OCT-angiography procedure was carried out with the Zeiss Cirrus 5000 HD-OCT device by Carl Zeiss Meditec, Dublin, CA, USA. This rigorous examination protocol aimed to thoroughly assess ocular health and obtain crucial data for the study.

2.2 OCT-A measurements

The AngioPlex metrix system facilitated the measurement of two key parameters within the study area: the total length and the area of the blood-filled vascular network per unit area. These parameters are known as vessel density and perfusion density, respectively (Callan et al., 2022). The scanning protocol used in the study, known as "Angiography 6x6 mm" captures images of a 6-mm by 6-mm area. It detects contrast related to the movement of blood in blood vessels by analyzing differences in individual features across several consecutive B-scans at a single position. For the analysis, the "angiography analysis" protocol was employed, which displays nine pre-set angiographic slices in an en face plane. The inclusion of a structural en–face OCT image in the protocol allowed for accurate analysis of the scanned structures, while excluding potential artifacts.

2.3 Statistical analysis

SPSS 20.0.0., MedCalc 11.6, and Statistica 5.0 were used as software for the statistical processing of the collected data. The selection of statistical methods was based on the nature and volume of the data, as well as the study's objectives. Various techniques, such as empirical distribution, the Kolmogorov-Smirnov test, and hypothesis testing, were applied as appropriate. Results with a *p*-value less than 0.05 were considered statistically significant.

3. RESULTS

Table 1 shows the baseline characteristics of the three groups in the study.

The AngioPlex™ OCT angiography system enabled us to measure vascular density and perfusion density in all macular zones as defined by the Early Treatment Diabetic Retinopathy Study (ETDRS), which includes the central, inner, outer, and entire field. The results of the studied OCT-A parameters in the healthy control group are provided in Table

Table 1. Baseline characteristics of control and case groups

	Sex M / F (number of patients, %)	Mean age ± SD (years)	Diabetes duration (years) ± SD	Type of diabetes (number of patients,%)		Method of treatment (number of patients,%)			
				Type I	Type II	No treatment	Insulin	Peroral	Combined
Group I Control	32(42.6%)/ 43(57.33%)	53±16.45	-	-	-	-	-	-	-
Group II Subgroup A	25(35.2%)/ 46(64.78%)	57.13±14.07	8.78±5.72	11 (15.49%)	60 (84.51%)	3 (4.23%)	48 (67.61%)	15 (21.13%)	5 (7.04%)
Group II Subgroup B	35(46.96%)/ 31(53.03%)	58.97±11.73	12.43±8.0	16 (24.24%)	50 (75.76%)	2 (3.03%)	25 (37.88%)	35 (53.03%)	4 (6.06%)

Table 2. OCT-A parameters measured in group I

Parameter	Mean	Median	Mode	Standard deviation
Vessel Density central, mm^{-1}	9.325	9.2000	8.0000	2.7171
Vessel Density Inner, mm^{-1}	17.0345	17.8750	18.8500	2.2270
Vessel Density Outer, mm^{-1}	17.4534	17.9500	17.8500	1.6529
Vessel Density Full, mm^{-1}	17.1899	17.7750	18.2500	1.6458
Perfusion Density Central, %	20.9939	21.2000	13.6000	6.4416
Perfusion Density Inner, %	40.7608	42.8500	44.2500	5.6951
Perfusion Density Outer, %	43.3932	44.4750	44.5500	4.0687
Perfusion Density Full, %	42.2439	43.7000	43.8000	4.2520

The results indicated that vascular density was the lowest in the central field and the highest in the outer ETDRS field among healthy eyes. Logically, we also observed the lowest percentage of perfusion density in the central field and the highest in the outer ETDRS field.

Following a comparison between the age of healthy individuals and the studied parameters, we identified several correlations, primarily demonstrating an inverse relationship with age. Specifically, we observed statistically significant negative age correlations with ETDRS vessel and perfusion density in the central, outer, and full ETDRS fields, as well as ETDRS vessel density in the inner field. While perfusion density in the inner field also displayed a negative age correlation, it did not reach statistical

significance. Consequently, it can be concluded that vascular density in the central, inner, and outer ETDRS fields, along with the entire macular area, decreased with age. Additionally, the percentage of perfusion density across the entire macular area, as well as in the central and outer ETDRS zones of healthy eyes, significantly declined with age (Table 3).

The results from OCT-A measurements in Group II are presented in Table 4 (subgroup II A) and Table 5 (subgroup II B). In both Group II A and Group II B, we observed that vascular and perfusion densities were the lowest in the central ETDRS field and the highest in the outer ETDRS macular field.

Table 3. Correlation of age with the measured OCT-A parameters in group I

Parameter	Correlation coefficient	*p*-value
Vessel density central	-0.231	0.047*
Vessel density inner	-0.259	0.026*
Vessel density outer	-0.332	0.004*
Vessel density full	-0.303	0.009*
Perfusion density central	-0.239	0.040*
Perfusion density inner	-0.225	0.054
Perfusion density outer	-0.284	0.014*
Perfusion density full	-0.277	0.017*

Note: statistical significance is presented - p; correlation coefficient - Pearson correlation

Table 4. Macular microstructural features observed with OCT-A in subgroup II A – DM patients without DR

Parameter	Mean	Median	Mode	Standard deviation
Vessel density central, mm^{-1}	8.8986	9.4500	10.3500	3.3905
Vessel density inner, mm^{-1}	16.3845	17.3000	18.6000	2.7757
Vessel density outer, mm^{-1}	16.8810	17.2500	17.7000	2.0879
Vessel density full, mm^{-1}	16.4901	17.1500	16.5500	2.2468
Perfusion density central, %	20.1472	21.5000	21.9000	7.9638
Perfusion density inner, %	39.7007	42.0500	44.4000	6.9407
Perfusion density outer, %	41.9514	43.1000	42.4500	5.4868
Perfusion density full, %	40.8366	42.3000	37.2000	5.6840

Table 5. Macular microstructural features observed with OCT-A in subgroup II B – DM patients with mild DR

Parameter	Mean	Median	Mode	Standard deviation
Vessel density central, mm^{-1}	8.5278	8.5500	8.7000	3.1273
Vessel density inner, mm^{-1}	16.1341	16.6500	17.2000	2.0318
Vessel density outer, mm^{-1}	16.2484	16.4000	18.2000	1.9243
Vessel density full, mm^{-1}	16.0032	16.2000	17.2000	1.9172
Perfusion density central, %	19.1929	19.0500	17.2000	7.9214
Perfusion density inner, %	39.2698	40.0000	43.3000	5.2905
Perfusion density outer, %	40.6214	40.9500	40.9500	4.9962
Perfusion density full, %	39.7246	40.5000	43.7000	4.9539

3. DISCUSSION

The retinal microvasculature and blood flow play a potential role in the pathophysiology of various retinal diseases including diabetic retinopathy. Therefore, in our study, we measured vessel density and perfusion density in the superficial vascular retinal plexus. In a study examining vascular density in the superficial capillary retinal plexus of healthy individuals, the authors highlighted the repeatability of the obtained values in automated measurements. Using OCT-A, they reported a results of 19.43±3.10 mm^{-1} in the first session and 19.72±3.78 mm^{-1} in the second session, respectively (Al-Sheikh et al., 2017). In our measurements within Group I, we specifically concentrated on the mean perfusion density in the macula, resulting in a value of 42.2439% for perfusion density in the superficial plexus. It's noteworthy that this value aligns with a study conducted by Durbin et al., where the authors also reported a perfusion density of 42% in this vascular network. These congruent findings align our study with previous research in this area (Durbin et al., 2017).

In our study, vessel density in the macula, as measured in the group of healthy eyes, displayed the lowest values in the central ETDRS field and the highest values in the outer ETDRS field. Logically, we also observed that the lowest percentage of perfusion density in the macula was in the central ETDRS field, while the highest percentage was in the outer ETDRS field. This pattern can be attributed to the retinal anatomy, where the distribution of vessels and perfusion in the macular area follows a logical pattern based on the organization of the retinal vasculature.

Our statistical analysis provided clear evidence that vascular density in the central, inner, and outer ETDRS fields of the macula, as well as throughout its entire area, decreases with advancing age. Additionally, the percentage of perfusion density across the entire macular area, as well as in the central and outer ETDRS fields of healthy eyes, significantly decreases with increasing age. The only parameter that did not reach statistical significance in

relation to the age of healthy controls was perfusion density in the inner field. These results emphasize the impact of age on macular vascular and perfusion characteristics in healthy individuals. In a manner similar to our study, Shahlaee et al. (2016) and Iafe et al. (2016) investigated the influence of age on retinal vessel density. The authors of both studies observed a tendency of decreased vessels in the superficial retinal plexus among healthy subjects as they age.

In the comparison among the three study groups using the two quantitative indices, our results revealed distinct patterns. The results are as follows: vascular density in the internal and external ETDRS fields of the macula as well as throughout its entire area is statistically significantly lower in diabetics with DR, when compared to the healthy group. Regarding ETDRS perfusion density (inner, outer, and full), the percent perfusion density in the internal and external ETDRS fields of the macula as well as throughout its entire area is also statistically significantly lower in diabetics with mild DR, compared to the control group. Notably, the values of vessel density and perfusion density in the central field exhibited no significant differences across the three studied groups.

There is a rather limited number of OCT-A analyses in the literature comparing vascular and perfusion density in SCP in the macula in healthy and diabetic eyes. *In vitro* tests offer insights into retinal tissue damage caused by prolonged high blood glucose levels in diabetic eyes, which can lead to disruptions in both normal tissue architecture and retinal vascular and perfusion density (Cheung and Wong, 2008). In a study by El-Din (2019), a comparison was made between healthy eyes and eyes of diabetics without DR. The mean value of vessel density in the control group was 20.40±1.56 mm^{-1}, while in the diabetic group, the mean value of this parameter was statistically significantly lower at 17.65±2.87 mm^{-1}. In terms of perfusion density, the authors reported a statistically significant difference between the two groups, with a value of 36% in healthy patients and 33% in the group of patients with DM (El-Din, 2019). In a study of foveal and perifoveal values of both parameters, Kim et al. (2018) observed significantly lower values in the eyes of individuals with DM, regardless of the presence of DR, in comparison to the eyes of healthy individuals. Agemy et al. (2015) reported a statistically significant decrease in the density of perfusion in both the superficial and deep capillary plexus of the retina across all stages of diabetic retinopathy (mild, moderate, severe NPDR, and PDR) in comparison to controls. Their study showed that as the disease progresses, both vascular and perfusion densities in both superficial and deep retinal capillary networks exhibit a significant downward trend. This pattern is evident in the general linear model analysis conducted in their study, emphasizing the impact of disease progression on these retinal parameters. Similarly found a strong correlation between vessel density in the macula and the severity of retinopathy. However, their study reported a significant difference in vascular density in the deep vascular plexus, but not in the superficial vascular plexus, when comparing diabetics without DR to a healthy control group. This indicates that alterations in vascular density may be more pronounced in the deeper retinal capillary networks in individuals with DM. In another study by Nesper et al. (2017), neither the superficial nor the deep retinal vascular plexus in DM patients without DR and healthy subjects showed a statistically significant difference in vascular density.

Elevated blood glucose levels in patients with DM lead to occlusion of blood vessels supplying the retina. This occlusion is due to changes in the capillary lumen, damage to the basal membrane, endothelial cells and capillary pericytes (Usman, 2018). The progressive loss of capillaries in the superficial macular plexus of diabetic patients with DR may provide an explanation for the reduced vascular and perfusion density in the macula of these patients, compared to healthy individuals and diabetics without DR. This capillary loss is a key feature of DR and can significantly impact the retinal vasculature, leading to changes in vascular and perfusion density measurements. While our research identifies vessel and perfusion density as significant biomarkers for DR development in DM patients, our findings suggest that reductions in these densities do not precede clinical DR signs, particularly in the superficial capillary plexus of the retina. Existing literature, however, hints that the deep capillary network in the macula may be more susceptible to ischemic damage, potentially causing decreased vascular and perfusion density at an earlier DM stage, even before clinical DR signs emerge. Nonetheless, the limitation of our study is the software's constraint to the superficial vascular plexus, which should be acknowledged. Although vessel and perfusion density hold promise for predicting retinopathy progression, further studies encompassing a broader patient spectrum are required for confirmation, especially as our study solely involved patients with mild DR.

4. CONCLUSION

The vascular and perfusion density in the superficial capillary macular plexus were found to be significantly lower in eyes with mild NPDR, compared to healthy eyes. While these measurements serve as important biomarkers for the onset of DR, our findings indicate that their reduction does not occur before the clinical signs of DR become apparent.

ACKNOWLEDGMENT

Special thanks to Assistant Professor Mariana Kaneva, Varna University of Economics, for her invaluable advice and assistance with the statistical data processing.

REFERENCES

Agemy, S. A., Scripsema, N. K., Shah, C. M., Chui, T., Garcia, P. M., Lee, J. G., Gentile, R. C., Hsiao, Y.-S., Zhou, Q., Ko, T., and Rosen, R. B. (2015). Retinal vascular perfusion density mapping using optical coherence tomography angiography in normals and diabetic retinopathy patients. *Retina*, 35(11), 2353–2363.

Al-Sheikh, M., Tepelus, T. C., Nazikyan, T., and Sadda, S. R. (2017). Repeatability of automated vessel density measurements using optical coherence tomography angiography. *British Journal of Ophthalmology*, 101(4), 449–452.

Balaratnasingam, C., An, D., Sakurada, Y., Lee, C. S., Lee, A. Y., Mcallister, I. L., Freund, K. B., Sarunic, M., and Yu, D.-

Y. (2018). Comparisons between histology and optical coherence tomography angiography of the periarterial capillary-free zone. *American Journal of Ophthalmology*, 189, 55–64.

Callan, T., de Sisternes, L., Lewis, W., Bonnin, S., Santos, T., Cunha-Vaz, J. G., and Kubach, S. (2022). Comparison of vessel density and vessel perfusion measurements in SD-OCT and SS-OCT devices. *Investigative Ophthalmology & Visual Science*, 63(7), 2949–F0102.

Cao, D., Yang, D., Huang, Z., Zeng, Y., Wang, J., Hu, Y., and Zhang, L. (2018). Optical coherence tomography angiography discerns preclinical diabetic retinopathy in eyes of patients with type 2 diabetes without clinical diabetic retinopathy. *Acta Diabetologica*, 55(5), 469–477.

Chan, G., Balaratnasingam, C., Yu, P. K., Morgan, W. H., McAllister, I. L., Cringle, S. J., and Yu, D.-Y. (2012). Quantitative morphometry of perifoveal capillary networks in the human retina. *Investigative Opthalmology & Visual Science*, 53(9), 5502–5514.

Cheung, N., and Wong, Y. T. (2008). Diabetic retinopathy and systemic vascular complications. *Progress in Retinal and Eye Research*, 27(2), 161–176.

Durbin, M. K., An, L., Shemonski, N. D., Soares, M., Santos, T., Lopes, M., Neves, C., and Cunha-Vaz, J. (2017). Quantification of retinal microvascular density in optical coherence tomographic angiography images in diabetic retinopathy. *JAMA Ophthalmology*, 135(4), 370–376.

El-Din, A. E.-M. M. T. (2019). Comparative study between patients with subclinical diabetic retinopathy and healthy individuals in the retinal microvascular changes using optical coherence tomography angiography. *Delta Journal of Ophthalmology*, 20(3), 132–137.

Flaxel, C. J., Adelman, R. A., Bailey, S. T., Fawzi, A., Lim, J. I., Vemulakonda, G. A., Ying, G.-S. (2019). Diabetic retinopathy preferred practice pattern. *Ophthalmology*, 127(1), 66-145.

Iafe, N. A., Phasukkijwatana, N., Chen, X., and Sarraf, D. (2016). Retinal capillary density and foveal avascular zone area are age-dependent: Quantitative analysis using optical coherence tomography angiography. *Investigative Ophthalmology & Visual Science*, 57(13), 5780–5787.

Jia, Y., Bailey, S. T., Hwang, T. S., McClintic, S. M., Gao, S. S., Pennesi, M. E., Flaxel, C. J., Lauer, A. K., Wilson, D. J., Hornegger, J., Fujimoto, J. G., and Huang, D. (2015). Quantitative optical coherence tomography angiography of vascular abnormalities in the living human eye. *Proceedings of the National Academy of Sciences of the United States of America*, 112(18), E2395–E2402.

Kim, K., Kim, E. S., and Yu, S.-Y. (2018). Optical coherence tomography angiography analysis of foveal microvascular changes and inner retinal layer thinning in patients with diabetes. *British Journal of Ophthalmology*, 102(9), 1226–1231.

Lei, J., Durbin, M. K., Shi, Y., Uji, A., Balasubramanian, S., Baghdasaryan, E., Al-Sheikh, M., and Sadda, S. R. (2017). Repeatability and reproducibility of superficial macular retinal vessel density measurements using optical coherence tomography angiography en face images. *JAMA Ophthalmology*, 135(10), 1092–1098.

Marques, I. P., Alves, D., Santos, T., Mendes, L., Lobo, C., Santos, A. R., Durbin, M., and Cunha-Vaz, J. (2020). Characterization of disease progression in the initial stages of retinopathy in type 2 diabetes: A 2-year longitudinal study. *Investigative Opthalmology & Visual Science*, 61(3), 20.

Mendes, L., Marques, I. P., and Cunha-Vaz, J. (2021). Comparison of different metrics for the identification of vascular changes in diabetic retinopathy using OCTA. *Frontiers in Neuroscience*, 15, 755730.

Moir, J., Khanna, S., and Skondra, D. (2021). Review of OCT angiography findings in diabetic retinopathy: Insights and perspectives. *International Journal of Translational Medicine*, 1(3), 286–305.

Nesper, P. L., Roberts, P. K., Onishi, A. C., Chai, H., Liu, L., Jampol, L. M., and Fawzi, A. A. (2017). Quantifying microvascular abnormalities with increasing severity of diabetic retinopathy using optical coherence tomography angiography. *Investigative Ophthalmology & Visual Science*, 58(6), BIO307–BIO315.

Shahlaee, A., Samara, W. A., Hsu, J., Say, E. A. T., Khan, M. A., Sridhar, J., Hong, B. K., Shields, C. L., and Ho, A. C. (2016). In vivo assessment of macular vascular density in healthy human eyes using optical coherence tomography angiography. *American Journal of Ophthalmology*, 165, 39–46.

Sivaprasad, S., and Pearce, E. (2019). The unmet need for better risk stratification of non-proliferative diabetic retinopathy. *Diabetic Medicine*, 36(4), 424–433.

Solomon, S. D., Chew, E., Duh, E. J., Sobrin, L., Sun, J. K., VanderBeek, B. L., Wykoff, C. C., and Gardner, T. W. (2017). Diabetic retinopathy: A position statement by the American diabetes association. *Diabetes Care*, 40(3), 412–418.

Usman, M. (2018). An overview of our current understanding of diabetic macular ischemia (DMI). *Cureus*, 10(7), e3064.

Wong, T. Y., Sun, J., Kawasaki, R., Ruamviboonsuk, P., Gupta, N., Lansingh, V. C., Maia, M., Mathenge, W., Moreker, S., Muqit, M. M. K., Resnikoff, S., Verdaguer, J., Zhao, P., Ferris, F., Aiello, L. P., and Taylor, H. R. (2018). Guidelines on diabetic eye care: The international council of ophthalmology recommendations for screening, follow-up, referral, and treatment based on resource settings. *Ophthalmology*, 125(10), 1608–1622.

Zhang, M., Hwang, T. S., Dongye, C., Wilson, D. J., Huang, D., and Jia, Y. (2016). Automated quantification of nonperfusion in three retinal plexuses using projection-resolved optical coherence tomography angiography in diabetic retinopathy. *Investigative Opthalmology & Visual Science*, 57(13), 5101–5106.