Clinical effect of conbercept on improving diabetic macular ischemia by OCT angiography

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Abstract
Purpose Varying degrees of macular ischemia generally occur in diabetic retinopathy (DR). This study aims to evaluate the effect of conbercept with 3+ pro re nata (PRN) on macular perfusion status in patients with diabetic macular edema (DME) and quantitatively assess changes in foveal avascular zone (FAZ) areas and capillary density in macular regions by applying OCT angiography (OCTA).

Methods Fifty patients were divided into ischemic (n=31) and non-ischemic (n=19) groups according to the presence of ischemia on OCTA at baseline. All patients received interval injection of 0.5mg of conbercept with 3+ PRN principle. The FAZ areas and macular vessel density measured using OCTA were evaluated at baseline, 3 months, and 6 months after treatment in both groups.

Results At months 3 and 6, the FAZ area in the ischemic group changed from 0.510±0.171mm² to 0.441±0.158mm² then to 0.427±0.153mm² (p=0.003, p=0.296); in the non-ischemic group, it remained stable (p=0.269, p=0.926). The superficial vessel density changed from 41.1%±4.1% to 42.5%±4.7% then to 42.6%±4.6% (p=0.043, p=0.812), and the deep vessel density changed from 40.7%±4.4% to 42.3%±3.6% then to 42.3%±4.7% (p=0.072, p=0.961) in the ischemic group. In the non-ischemic group, the superficial vessel density changed from 44.8%±3.2% to 46.0%±3.5% then to 45.7%±3.3% (p=0.108, p=0.666), whereas the deep vessel density changed from 43.6%±3.6% to 43.8%±3.2% then to 43.5%±4.5% (p=0.882, p=0.736). Reperfusion in macular nonperfusion areas was observed.

Conclusion Anti–vascular endothelial growth factor treatment may have a positive effect on macular perfusion status, improve macular ischemia, and promote reperfusion appearance in patients with DME. Furthermore, OCTA had advantages in quantifying and calculating blood flow index in the study of macular perfusion status.

Introduction
As extensively reported, the further the diabetic retinopathy (DR) progresses, the severer the condition of diabetic macular ischemia (DMI) in patients with DR [1, 2]. In macular ischemia, the structure of foveal capillary network is damaged. Such damage includes enlargement and irregularity of the foveal avascular zone (FAZ) and appearance of macular nonperfusion (MNP), leading to macular
function disturbance. According to ETDRS Report No. 11, the macular ischemia is defined as a FAZ area enlarging more than 1000 µm in greatest diameter (supposing the FAZ is round or oval), equaling to the size of FAZ more than the area within 500 µm radius circle, and/or broken perifoveal capillary rings at the borders of FAZ with areas of macular capillary nonperfusion within the a1 disk diameter of the foveal center according to fluorescein angiography (FA). In healthy people, the diameter of normal FAZ commonly varies from 500 µm to 600 µm (less than the area within 300 µm radius circle equaling to 0.282mm²) [3]. When a macular region is affected by ischemia, it presents in varying degrees, including disappearance of a part of the macular arch ring capillary network, expansion of the FAZ area, damage in perifoveal capillaries, and appearance of MNP area in the macular region. Different levels of macular ischemia can represent disease severity and progression; for instance, moderate-to-severe macular ischemia affects visual function seriously. Macular ischemia results from the occlusion of foveal capillary network, and vascular endothelial growth factor (VEGF) plays a vital role in the mechanism by which VEGF leads the closure of retinal vascular in patients with diabetic macular edema (DME).

Several previous large studies regarding the treatment effects of anti-VEGF on DME, including DRCR studies, did not include macular ischemia as an observation indicator. To date, the insight into whether anti-VEGF therapy could aggravate retinal ischemia remains controversial. In the past decade, some clinical studies suggested that blocking VEGF might be harmful to retinal vascular integrity, especially in patients with preexisting retinal ischemia; in these patients, anti-VEGF therapy aggravated retinal ischemia because retinal nonperfusion (RNP) areas enlarged after anti-VEGF therapy [5, 6]. However, increasing studies have shown that retinal ischemia does not worsen after anti-VEFG therapy but has no retinal reperfusion in the preexisted RNP areas [7–9]. Meanwhile, few studies indicated that anti-VEGF therapy could reduce RNP progression. In a multicenter clinical trial, monthly injection of 0.3 or 0.5 mg of ranibizumab can slow retinal vessel closure in DME and can be associated with retinal reperfusion at the anterior nonperfusion area in some patients [10]. Long-term anti-VEGF therapy to patients with DME could improve DR severity and even prevent worsening, especially in mild and severe DR cases. Furthermore, early treatment may prevent visual damage
caused by proliferative DR (PDR) and obtain better therapeutic effects [11]. Thus, anti-VEGF therapy not only improves patients’ vision but also reduces RNP progression, and even enhances blood flow. To date, most of the studies about anti-VEGF effects on DMI were small cross-sectional studies or retrospective studies, and they lack supporting prospective research. Moreover, most studies often use FA to observe macular ischemia changes; however, this tool is invasive and poorly repeatable. Meanwhile, OCT angiography (OCTA) is noninvasive, repeatable, and easily operable, and it is subtler than FA in displaying macular area capillaries. OCTA acquires high-resolution images of macular areas by capturing the signals of moving red blood cells and provides images of the construction of macular capillaries at different plexus of the retina. In addition, OCTA is highly sensitive and consistent compared with FA [12–14]. However, numerous studies only focus on the retinal ischemia status, and more detailed effects on macular ischemia after anti-VEGF therapy have not been studied clearly. Thus, we decided to adopt OCTA as the main evaluation method to observe the blood flow of macular areas and quantify the density of macular vessels.

In this prospective study, we mainly aim to investigate the effects of conbercept with 3 + PRN on macular perfusion status in patients with DME and to quantify FAZ areas and capillary density in the macular region. Conbercept (Langmu; Kanghong, Inc., Sichuan, China) is a new drug comprising a VEGF receptor (VEGFR) fusion protein, with a high binding affinity to VEGF and a long half-life in vitreous, and it has been proven to reduce the chances of intraoperative bleeding in vitrectomy procedures by decreasing VEGF concentrations. Conbercept demonstrates a higher binding activity with VEGF-A, VEGF-B, and placental growth factor (PIGF) than ranibizumab. Clinical trials indicate that intravitreal injections of conbercept can improve visual acuity in patients receiving vitrectomy and seems to reduce the recurrence rate of vitreous hemorrhage in patients with PDR [12–14].

Materials And Methods
The study followed the tenets of the Declaration of Helsinki, all patients gave informed consent before participation in this study and all procedures performed were in accordance with the ethical standards of the Second Xiangya Hospital of Central South University committee. In this study, 50 patients (obtained after informed consent), who consulted in the abovementioned hospital for
diminution of vision caused by diabetes between September 2018 and March 2019, were diagnosed of DR with different levels of DME. All of them were injected with 0.5 mg of conbercept at monthly intervals for the first 3 months and given additional interval injections according to the pro re nata (PRN) principle. All of these patients were evaluated for 6 months, comprising a 3-month treatment period (received monthly interval injection of 0.5 mg of conbercept) and a 3-month observation period (received treatment according to the PRN principle). Before the first intraocular injection, all patients underwent FA, OCT, and OCT angiography (OCTA), which showed whether a patient had apparent macular ischemia (including FAZ area expansion, perifoveal capillary ring damage, and/or MNP appearance). Then, the patients were divided into two groups, namely, the ischemic group and the non-ischemic group. Considering the lack of uniform definition of macular ischemia classification according OCTA, we adopted the basis of grouping at baseline according to macular ischemic grading defined by FA. Inclusion criteria were as follows: patients > 18 years old with type 1 or 2 diabetes; and definite retinal thickening due to DME as the main cause of visual loss (central fovea thickness [CFT] ≥ 250 µm measured on OCT). Meanwhile, the exclusion criteria were the following: visual loss caused by any other retinal disease, excluding DME; retinal treatment or major ocular surgery within the prior 6 months; intraocular pressure ≥ 25 mmHg; and other systemic diseases that needed hospitalization.

Image Acquisition

All OCT and OCT angiography images were acquired from AngioVue OCT system (RTVue XR Avanti, Optovue Inc.) using split-spectrum amplitude decorrelation angiography (SSADA) algorithm to detect blood flow and motion correction technology (MCT) to remove artifacts. The scanning area of OCTA was uniformly obtained in 6 × 6mm² sections consisting of 304 B-scans. Every B-scan was repeated twice, as well as 304 A-scans. In 3 seconds, 209 000 A-scans were obtained. AngioVue provided an automated software algorithm to generate the boundaries of superficial capillary plexus (from 3 µm below ILM to 15 µm below IPL), deep capillary plexus (from 15 µm to 70 µm below IPL) and choriocapillaris (from 30 µm to 60 µm below RPE).

Data Measurement

We assessed BCVA by using a tumbling E chart at an initial testing distance of 5 m, and the result was
displayed in a logMAR unit format. Then, we calculated the CFT as an average value within a circular 1 mm diameter area centered in the fovea measured by OCT. We also obtained vessel density value by calculating the percentage of blood vessel area in a 6 × 6mm² selected area from the OCTA. Furthermore, FAZ area was calculated using an automated software but could be corrected by manual selection.

Statistical Analysis
Main measurements, including BCVA, FAZ area, CFT, and superficial and deep vessel density, in this study were tested by Kolmogorov-Smirnov test (p > 0.05 in all samples). Hence, we adopted t test principally. At months 0, 3, and 6, changes in CFT, FAZ area, and blood flow were evaluated by paired-sample t test. Moreover, p < 0.05 indicated statistical significance, and p values could be adjusted for multiplicity. Statistical analysis was performed using the SPSS 25.0 (SPSS Inc., Chicago, Ill, USA).

Results
Characteristics of Patients with DME at Baseline
This study enrolled 50 patients with DME and divided them into two groups, namely, the ischemic group (n=31) and the non-ischemic group (n=19). The characteristics of patients at baseline are shown in Table 1. These patients comprised 24 males and 26 females, and the mean age was 55.8 years (standard deviation [SD]: 8.4 years [34-80 years]). The BCVA was 0.61±0.34 (mean±SD). The mean duration of diabetes was 9.4 years, and the HbA1c was 9.6%±2.0%. Among these 50 patients, 7 had moderate NPDR (14%), 34 had severe NPDR (68%), and 9 had PDR (18%). Among the 9 patients with DR, 9 had hypertension, 4 had nephropathy, and 1 had thrombocytopenia, showing that DR is associated with many other systemic disorders, especially circulatory diseases and nephropathy. The overall main characteristics of patients with DME were increased central fovea thickness (367±122μm), increased FAZ area (0.455± 0.171mm²), and deceased vessel density (vessel density in superficial plexus [ILM-OPL] and deep plexus (IPL-OPL) was 42.5%±4.2% and 41.8%±4.3%, respectively).
Table 1. Basic characteristics of patients at baseline (n=50)

|                                | Ischemic Group (n=31) | Non-ischemic Group (n=19) | Total (n=50) |
|--------------------------------|------------------------|---------------------------|-------------|
| Gender, n                      | Male 17 (55%)          | 7 (49%)                   | 24 (48%)    |
|                                | Female 14 (45%)        | 12 (51%)                  | 26 (52%)    |
| Age, years                     | 55.8±8.5               | 55.8±8.4                  | 55.8±8.4    |
| BCVA, logMAR units             | 0.64±0.34              | 0.56±0.34                 | 0.61±0.34   |
| Duration of Diabetes, years    | 10.1±4.5               | 8.2±4.9                   | 9.4±4.7     |
| Stage of DR, n                 | Mild NPDR 0 (0)        | 0 (0)                     | 0 (0)       |
|                                | Moderate NPDR 4 (13%)  | 3 (16%)                   | 7 (14%)     |
|                                | Severe NPDR 21 (68%)   | 13 (68%)                  | 34 (68%)    |
|                                | PDR 6 (19%)            | 3 (16%)                   | 9 (18%)     |
| FGL,%                          | 9.6±2.1                | 9.7±2.0                   | 9.6±2.0     |
| Complication                   | Hypertension 7         | 2                         | 9           |
|                                | Renal Failure 2        | 2                         | 4           |
|                                | Others 0               | 1                         | 1           |
| CFT(1mm), μm                   | 328±109                | 430±118                   | 367±122     |
| FAZ area, mm²                  | 0.510±0.171            | 0.364±0.127               | 0.455±0.171 |
| Vessel Density,%               | Superficial(ILM-IPL) 41.1±4.1 | 44.8±3.2 | 42.5±4.2 |
|                                | Deep(IPL-OPL) 40.7±4.4 | 44.8±3.2                  | 41.8±4.3    |

The composition of sex and age, BCVA, diabetes duration, and FGL between the ischemic group and non-ischemic group had no significant difference (p>0.05, chi-square test and independent-sample t test). Conversely, the CFT, FAZ area, and vessel density between the two groups were significantly different. In the ischemic group, the CFT (328±109μm) was significantly lower than that in the non-ischemic group (p=0.005). The FAZ area in the ischemic group (0.510±0.171mm²) was preoperatively larger than that in the ischemic group (0.364±0.127mm², p=0.001). Additionally, the superficial and deep vessel densities in the ischemic groups were 41.1%±4.1% and 40.7%±4.4%, respectively; both were clearly low than those in the non-ischemic group (44.8%±3.2%, p=0.001 and 44.8%±3.2%, p=0.015). Overall, the patients in the ischemic group showed ischemic changes such as FAZ area expansion, MNP appearance, and blood flow density decrement.

**Anti-VEGF Therapy Decreased the FAZ Area**
|                          | Ischemic Group (n=31) | Non-ischemic Group (n=19) |
|--------------------------|-----------------------|---------------------------|
|                          | Mean±SD               | P value                   | Mean±SD               | P value                   |
| **BCVA, logMAR units**   |                       |                           |                       |                           |
| Month 0                  | 0.64±0.34             |                           | 0.56±0.34             |                           |
| Month 3                  | 0.46±0.26             | 0.004                     | 0.48±0.30             | 0.250                     |
| Month 6                  | 0.38±0.26             | 0.056                     | 0.36±0.26             | 0.108                     |
| **CFT(1mm),μm**          |                       |                           |                       |                           |
| Month 0                  | 329±109               |                           | 430±118               |                           |
| Month 3                  | 298±99                | 0.183                     | 333±120               | 0.005*                   |
| Month 6                  | 299±126               | 0.970                     | 316±113               | 0.255                     |
| **FAZ area, mm²**        |                       |                           |                       |                           |
| Month 0                  | 0.510±0.171           |                           | 0.364±0.1             |                           |
| Month 3                  | 0.441±0.158           | 0.003*                    | 0.379±0.1             | 0.269                     |
| Month 6                  | 0.427±0.153           | 0.296                     | 0.378±0.1             | 0.926                     |
| **Vessel Density(%)**    |                       |                           |                       |                           |
| Superficial (ILM-IPL)    |                       |                           |                       |                           |
| baseline                 | 41.1±4.1              |                           | 44.8±3.2              |                           |
| 3-month                  | 42.5±4.7              | 0.043*                    | 46.0±3.5              | 0.108                     |
| 6-month                  | 42.6±4.6              | 0.812                     | 45.7±3.3              | 0.666                     |
| Deep(IPL-OPL)            |                       |                           |                       |                           |
| baseline                 | 40.7±4.4              |                           | 43.6±3.6              |                           |
| 3-month                  | 42.3±3.6              | 0.072                     | 43.8±3.2              | 0.882                     |
| 6-month                  | 42.3±4.7              | 0.961                     | 43.5±4.5              | 0.736                     |

Table 2. Changes of BCVA, CFT, FAZ area and vessel density after anti-VEGF therapy

All patients were intraocularly injected with 0.5mg of conbercept according to the 3+ PRN principle. The main observation point was set up at months 0, 3, and 6. The changes of BCVA, CFT, FAZ area, and vessel density are listed in Table 2, and the changes in the FAZ area in both groups are depicted in Figure 1.

After the patients were intraocularly injected with 0.5mg of conbercept thrice, their BCVA improved and CFT decreased in both groups. The FAZ area of all patients decreased significantly at month 3 (p=0.017). Especially in the ischemic group, the FAZ area decreased from 0.510mm² to 0.441mm² (p=0.003), continually decreasing to 0.427mm² at the end point (p=0.296). In contrast, the FAZ area did not expand significantly in the non-ischemic group. A representative case of DMI showed an apparent decrease in FAZ area, as illustrated in Figure 2.

**Anti-VEFG Therapy Improved the Vessel Density in the Macular Region**
In the ischemic group, the superficial vessel density increased from 41.1%±4.1% to 42.5%±4.7% then to 42.6%±4.6% at the end point, and the deep vessel density changed from 40.7%±4.4% to 42.3%±3.6% then to 42.3%±4.7% at month 6. The capillary density increased in both superficial plexus ($p_{\text{month3}}=0.043$) and deep plexus ($p_{\text{month3}}=0.072$) in the first 3 months after anti-VEGF therapy, especially in the superficial plexus. Moreover, the vessel density remained stable during the observation period ($p_{\text{month6}}=0.812$, $p_{\text{month6}}=0.961$). In the non-ischemic group, the superficial vessel density changed from 44.8%±3.2% to 46.0%±3.5% then to 45.7%±3.3% ($p_{\text{month3}}=0.108$, $p_{\text{month6}}=0.666$), whereas the deep vessel density changed from 43.6%±3.6% to 43.8%±3.2% then to 43.5%±4.5% at the end point ($p_{\text{month3}}=0.882$, $p_{\text{month6}}=0.736$). Some patients had increased vessel density and reperfusion in previous nonperfusion area in the macular region (Figure 4).

Discussion

In our study, varying degrees of macular ischemia gained different therapeutic effects after anti-VEGF therapy in both the ischemic and non-ischemic groups. Furthermore, the macular perfusion status improved after the anti-VEGF therapy, especially in the FAZ area and superficial vessel density. The superficial vessel density is associated with DME development, whereas the vessel density in deep plexus corresponds to macular photoreceptors and is important to the oxygen requirements of photoreceptors and outer retina in patients with DMI. Some patients with DMI obtained reperfusion at previous nonperfusion areas. As observed in Fig. 4, the OCTA detected that the FAZ area of a 59-year-old patient with DME, the structure of arch ring capillaries already disrupted, clearly decreased after receiving the anti-VEGF therapy and indicating rebuilding especially in the inferior-nasal macular fovea. Therefore, the anti-VEGF therapy improved macular ischemia and blood supply even the occurrence of macular reperfusion in some patients with DME.

Macular ischemia is a contraindication of anti-VEFG therapy, but some patients developed capillary nonperfusion after such therapy. Severe macular ischemia may be a limitation of visual acuity outcomes in patients with DME after receiving anti-VEGF therapy [16]. However, trials in DME suggested that anti-VEGF therapy did not induce retinal ischemia at least in healthy retina. In addition, repeated anti-VEGF therapy on macular perfusion in patients with DME did not cause treatment-related significant changes in FAZ sizes and capillary loss around the fovea. Patients with DME who had severe ischemia still achieved favorable changes in BCVA and central macular thickness
after long-term anti-VEGF therapy [7, 17]; thus, anti-VEGF therapy may be an alternative for such patients. Therefore, patients with severe macular ischemia receiving anti-VEFG therapy should be individualized, and they need a comprehensive assessment of possible risks and closer follow-up to prevent the worsening of ischemia. Overall, patients with DMI in our study experienced improved eyesight and macular ischemia after anti-VEGF therapy.

Highly vitreous concentration of VEGF can lead to serious retina ischemia and hypoxia. The retinal arterioles would contract immediately, and once ischemia occurs, vascular occlusion and RNP areas progression take place, resulting in retinal microvascular abnormality and neovascularization elsewhere, especially in patients with severe NPDR and PDR [4]. VEGF is the strongest angiogenic factor. The VEGF family in mammals mainly includes five species and PIGF. In DR, VEGF and PIGF disrupt the blood–retinal barrier, leading to DMI progression. Anti-VEFG therapy improves retinal hypoxia and ischemia by reducing the VEGF content. Conbercept is a new VEGFR fusion protein that reduces the concentration of VEGF and PIGF by specifically binding to VEGF-A, VEGF-B, and PIGF [18]. However, the molecular mechanism on how anti-VEGF therapy improves ischemia and realizes reperfusion remains unclear. A study using retinal ischemia animal models proved that anti-VEGF therapy could reduce autophagy and apoptosis rate and activate ischemia-damaged microglia to protect the retinal ganglion cells and bipolar cells [19]. In another study on tumor, VEGF inhibition can normalize peripheral cells, stabilize the basement membrane, remodel the immature vessels to a more mature version by destroying the vessels that lack peripheral cells, and provide frameworks for new vessels to grow in again by stabilizing the basement membrane. Surmising that the mechanism is the same in the retina [20, 21].

Fig 5. Simultaneous fundus fluorescein angiography and OCT angiography images in a left eye of a 60-year-old male with severe NPDR. The red arrows indicate the damage in the macular arch ring capillary network; the yellow arrows indicate a large area of nonperfusion at the posterior pole. The FAZ and nonperfusion areas presented in two images are highly similar.

(A) FFA demonstrates the presence of FAZ area expansion and macular nonperfusion. (B) FA image was cropped to $6 \times 6 \text{mm}^2$, same as the OCT angiography image. (C) OCT angiography shows the
disruption occurring along the outline of the FAZ, increasing in FAZ area and nonperfusion area around macular. (D) Superficial capillary plexus. (E) Deep capillary plexus. (F) Choriocapillaris.

In this study, we adopted OCTA as the main evaluation method to observe the blood flow of the macular region and to quantify the macular vessel density. Despite acknowledging FA as a gold standard in the diagnosis of DR and classification of DMI, by comparing the FA and OCTA, we found that both tests were consistent in showing the vessels in the macular area, but OCTA is unacted on the leakage of fluorescein. (Fig. 5: the FAZ and non-perfusion areas presented in fundus FA and OCTA are highly similar) [12–14]. OCTA not only evaluated macular ischemia and DR severity but also predicted the peripheral nonperfusion by observing the change of FAZ size. However, OCTA still has several defects. Fluid may induce segmentation artifacts, and OCTA quantitative metrics lacks consensus and normative database. Other limitations in our study mainly include the small sample size and the short study period. The effects of anti-VEGF therapy on DMI need a larger sample size and a longer observation period to determine how anti-VEGF plays a role in improving macular ischemia and retinal ischemia and realizing reperfusion in nonperfusion areas.

In conclusion, anti-VEGF treatment improved the FAZ size, increased the vascular density in the macular region of patients with DME after receiving intraocular injection of conbercept according to the 3 + PRN principle. Furthermore, conbercept had a positive impact on macular perfusion status and promoted reperfusion in macular nonperfusion areas. Lastly, OCTA may be used as an important imaging modality to evaluate retinal vasculature and to quantify the perfusion status in DR.

Declarations

**Ethics approval and consent to participate**

The study followed the tenets of the Declaration of Helsinki, all patients gave informed consent before participation in this study and all procedures performed were in accordance with the ethical standards of the Second Xiangya Hospital of Central South University committee. Subjects were provided a written informed consent in accordance with the guidelines of the Second Xiangya Hospital of Central South University.

**Consent for publication**
Written informed consent was obtained from the patients for publication and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

**Availability of data and materials**

All data is included in Excel sheet, and medical records and picture information is included in this published article and supplementary files.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors’ contributions**

Ziyi Z, Youling L and Jing L were responsible for the conception and design of this review. Ziyi Z, Bin Y, Kejun L and Yiwei Z acquired the data. Ziyi Z and Zhishang M analyzed and interpreted the data. Ziyi Z wrote the draft. Youling L and Jing L revised the manuscript critically. All authors have read and approved the final manuscript.

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**References**

[1]. Arend, O., et al., Retinal microcirculation in patients with diabetes mellitus: dynamic and morphological analysis of perifoveal capillary network. Br J Ophthalmol, 1991. 75(9): p. 514-8.

[2]. Yalcin, N.G. and S. Ozdek, The Relationship Between Macular Cyst Formation and Ischemia in Diabetic Macular Edema. Turk J Ophthalmol, 2019. 49(4): p. 194-200.

[3]. Classification of diabetic retinopathy from fluorescein angiograms. ETDRS report number 11. Early Treatment Diabetic Retinopathy Study Research Group. Ophthalmology, 1991. 98(5 Suppl): p. 807-22.

[4]. El, D.Y., et al., Reduced baseline diameter and contraction of peripheral retinal arterioles immediately after remote ischemia in diabetic patients. Graefes Arch Clin Exp Ophthalmol, 2019.

[5]. Li, J.K., et al., Changes in vitreous VEGF, bFGF and fibrosis in proliferative diabetic
retinopathy after intravitreal bevacizumab. Int J Ophthalmol, 2015. 8(6): p. 1202-6.

[6]. Bonini-Filho, M., et al., Intravitreal bevacizumab for diabetic macular edema associated with severe capillary loss: one-year results of a pilot study. Am J Ophthalmol, 2009. 147(6): p. 1022-30, 1030.e1-5.

[7]. Karst, S.G., et al., Association of Changes in Macular Perfusion With Ranibizumab Treatment for Diabetic Macular Edema: A Subanalysis of the RESTORE (Extension) Study. JAMA Ophthalmol, 2018. 136(4): p. 315-321.

[8]. Bonnin, S., et al., ANTI-VASCULAR ENDOTHELIAL GROWTH FACTOR THERAPY CAN IMPROVE DIABETIC RETINOPATHY SCORE WITHOUT CHANGE IN RETINAL PERFUSION. Retina, 2019. 39(3): p. 426-434.

[9]. Couturier, A., et al., Widefield OCT-Angiography and Fluorescein Angiography Assessments of Nonperfusion in Diabetic Retinopathy and Edema Treated with Anti-Vascular Endothelial Growth Factor. Ophthalmology, 2019.

[10]. Peter A, C., et al., Neutralization of vascular endothelial growth factor slows progression of retinal nonperfusion in patients with diabetic macular edema. Ophthalmology, 2014. 121(9).

[11]. Ip, M.S., et al., Long-term effects of therapy with ranibizumab on diabetic retinopathy severity and baseline risk factors for worsening retinopathy. Ophthalmology, 2015. 122(2): p. 367-74.

[12]. Garcia, J.M., et al., Diabetic Macular Ischemia Diagnosis: Comparison between Optical Coherence Tomography Angiography and Fluorescein Angiography. J Ophthalmol, 2016. 2016: p. 3989310.

[13]. Jia, Y., et al., Split-spectrum amplitude-decorrelation angiography with optical coherence tomography. Opt Express, 2012. 20(4): p. 4710-25.

[14]. Cole, E.D., et al., Contemporary retinal imaging techniques in diabetic retinopathy: a review. Clin Exp Ophthalmol, 2016. 44(4): p. 289-99.

[15]. Ren, X., et al., Safety and efficacy of intravitreal conbercept injection after vitrectomy for the treatment of proliferative diabetic retinopathy. Eye (Lond), 2019. 33(7): p. 1177-1183.

[16]. Douvali, M., et al., Effect of macular ischemia on intravitreal ranibizumab treatment for
diabetic macular edema. Ophthalmologica, 2014. 232(3): p. 136-43.

[17.] Bonini-Filho, M., et al., Intravitreal bevacizumab for diabetic macular edema associated with severe capillary loss: one-year results of a pilot study. Am J Ophthalmol, 2009. 147(6): p. 1022-30, 1030.e1-5.

[18.] Zhou, J., et al., Concentrations of VEGF and PIGF Decrease in Eyes After Intravitreal Conbercept Injection. Diabetes Ther, 2018. 9(6): p. 2393-2398.

[19.] Palmhof, M., et al., Fewer Functional Deficits and Reduced Cell Death after Ranibizumab Treatment in a Retinal Ischemia Model. International Journal of Molecular Sciences, 2018. 19(6): p. 1636.

[20.] Inai, T., et al., Inhibition of vascular endothelial growth factor (VEGF) signaling in cancer causes loss of endothelial fenestrations, regression of tumor vessels, and appearance of basement membrane ghosts. Am J Pathol, 2004. 165(1): p. 35-52.

[21.] Dickson, P.V., et al., Bevacizumab-induced transient remodeling of the vasculature in neuroblastoma xenografts results in improved delivery and efficacy of systemically administered chemotherapy. Clinical Cancer Research An Official Journal of the American Association for Cancer Research, 2007. 13(13): p. 3942-50.

Figures
Figure 1

Changes in FAZ area after anti-VEFG therapy
Figure 2

FAZ area clearly decreasing in the eye of a 59-year-old male, 6 months after receiving conbercept treatment. Notes: OCT angiography images (A–E) depict macular ischemia and apparent FAZ expansion captured in 6×6mm² sections in the macular area at month 0. (A)

FAZ area with 0.604mm², showing severe FAZ area expansion and arch ring capillary network breakage; (B) Superficial capillary plexus (ILM-IPL), vessel density of 39.2%; (C)

Deep capillary plexus (IPL-OPL), vessel density of 43.5%; (D) Choriocapillaris (BRM-BRM+30μm). At month 3, OCT angiography images (A’–E’) showed FAZ area decrease. (A’)

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FAZ area with 0.464mm²; (B’) Superficial capillary plexus vessel density of 40.4%; (C’) Deep capillary plexus vessel density of 43.1%; (D’) Choriocapillaris.

Figure 3

Changes of vessel density in both superficial plexus and deep plexus in all patients
Reperfusion occurred in the left eye of a 52-year-old male, 6 months after receiving conbercept treatment. Red arrows indicate growth of new capillaries and reperfusion in the macular nonperfusion areas. OCT angiography images (A–C) at baseline illustrate the macular nonperfusion area. (A) Superficial capillary plexus; (B) Deep capillary plexus; (C) Angio overlay. OCT angiography images (A’–C’) at month 6 indicate reperfusion in previous macular nonperfusion areas. (A’) Superficial capillary plexus; (B’) Deep capillary plexus; (C’) Angio overlay.
Simultaneous fundus fluorescein angiography and OCT angiography images in a left eye of a 60-year-old male with severe NPDR. The red arrows indicate the damage in the macular arch ring capillary network; the yellow arrows indicate a large area of nonperfusion at the posterior pole. The FAZ and nonperfusion areas presented in two images are highly similar. (A) FFA demonstrates the presence of FAZ area expansion and macular nonperfusion. (B) FA image was cropped to 6×6mm², same as the OCT angiography image. (C) OCT angiography shows the disruption occurring along the outline of the FAZ, increasing in FAZ area and nonperfusion area around macular. (D) Superficial capillary plexus. (E) Deep capillary plexus. (F) Choriocapillaris.

Supplementary Files
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Data.xlsx