Am J Transl Res 2023;15(1):193-201
www.ajtr.org /ISSN:1943-8141/AJTR0147258

Original Article

Efficacy of laser photocoagulation plus ranibizumab in patients with diabetic retinopathy and their effect on VEGF

Xiaoqiang Zhou, Guoxiao Yu, Li Yao

Department of Ophthalmology, First People's Hospital of Linping District, Hangzhou 311100, Zhejiang, China

Received October 25, 2022; Accepted December 12, 2022; Epub January 15, 2023; Published January 30, 2023

Abstract: Objective: To investigate the efficacy of laser photocoagulation combined with ranibizumab in patients with diabetic retinopathy and their effect on VEGF. Methods: The medical records of 98 patients with diabetic retinopathy treated in the First People's Hospital of Linping District from February 2020 to January 2022 were collected for a retrospective analysis. Among them, 48 patients treated with laser photocoagulation were a control group (CG), and another 50 treated with laser photocoagulation combined with ranibizumab were an observation group (OG). The treatment efficacy, best corrected visual acuity (BCVA), central macular concave thickness (CMT), and change in neovascularization area were compared between the two groups. Also, the changes in serum vascular endothelial growth factor (VEGF), homocysteine (HCY), vitamin B12 and folic acid were compared. The adverse effects that occurred with treatment were assessed. The relationship of pre-treatment BCVA, CMT, neovascularization area and VEGF levels with clinical outcomes were observed, and their predictive values were analyzed by receiver operating characteristic (ROC) curve. Results: The total response rate of patients in the CG was lower than that in the OG ($P<0.05$). The BCVA, CMT, and neovascularization area were dramatically lower, while vitamin B12 and folic acid were markedly higher in the OG than in the CG after treatment ($P<0.05$). There was no statistical difference in adverse reactions between the two groups ($P>0.05$). In patients with effective response, the BCVA, CMT, VEGF and Hcy before treatment were dramatically lower than in those with ineffective response ($P<0.05$), while the neovascularization area, vitamin B12, and folic acid did not differ between them before treatment ($P>0.05$). ROC curve analysis revealed that the areas under the curve of BCVA, CMT, VEGF and Hcy were all greater than 0.7 for predicting patient outcomes, and the area under the curve of the combination of the above indexes was 0.945, with a specificity of 92.30% and sensitivity of 88.23%. Conclusion: Laser photocoagulation combined with ranibizumab may provide good therapeutic efficacy in diabetic retinopathy, by effectively improving neovascularization and reducing VEGF levels to control further progression of the lesions.

Keywords: Laser photocoagulation, ranibizumab, diabetic retinopathy, VEGF

Introduction

Diabetes is a metabolic disease clinically characterized by hyperglycemia, resulting from insufficient insulin secretion and islet dysfunction, presenting as chronic and progressive elevation of blood glucose [1]. There is a clear genetic predisposition, with type 2 diabetes being predominant. Diabetes produces a series of pathologic changes that affect the function of organs, tissues, and blood vessels in the body, leading to a series of complications [2]. In 2015, it was reported [3] that about 415 million people worldwide had diabetes, and this number will probably exceed 640 million in 2040. Diabetic retinopathy is one of the most common and serious microvascular complications of diabetes [4]. Diabetic retinopathy, occurs primarily in the fundus with specific changes, has a prevalence of 20-40% in diabetic patients, and is the leading cause of acquired adult non-traumatic blinding eye disease [5, 6]. It was found that when blood glucose is high, vascular endothelial growth factor (VEGF) expression is upregulated, which activates a series of signal transduction pathways that disrupt the blood-retinal barrier (BRB) and increase the permeability of the vessel wall, leading to retinal edema and hemorrhagic exudation, thus promoting fundus neovascularization [7, 8]. Therefore, the

lesions of diabetic retinopathy can be effectively controlled by regulating VEGF in patients.

Currently, retinal photocoagulation is an effective treatment for diabetic retinopathy by controlling the progression of the disease [9]. However, conventional total retinal photocoagulation has variable results, especially in severe non-proliferative versus proliferative diabetic retinopathy, requiring multiple additional retinal photocoagulations, which in turn may lead to visual impairment and increased risk of blindness [10, 11]. With the development of medical treatment, modified retinal photocoagulation can compensate to some extent for the unstable effect of traditional total retinal photocoagulation [12]. Modified retinal photocoagulation alone has limited success in controlling the progression of proliferative diabetic retinopathy, and there is still a risk of neovascularization [13]. Intravitreal injection of ranibizumab inhibits retinal neovascularization, reduces retinal edema and leakage, and has a positive effect on controlling the progression of diabetic retinopathy [14]. It has been found [15] that retinal angiogenesis in diabetic retinopathy patients can be effectively inhibited by ranibizumab. However, there is a lack of similar studies on whether modified laser photocoagulation plus ranibizumab can improve diabetic retinopathy.

In this study, we aimed to analyze the effect and safety of modified laser photocoagulation combined with ranibizumab on diabetic retinopathy to provide a reference for clinical treatment.

Methods and materials

Approval statement

The study was approved by the Medical Ethics Committee of the First People's Hospital of Linping District (ethical approval number 2020-41).

Clinical data

The medical records of 98 patients with diabetic retinopathy treated in the First People's Hospital of Linping District from February 2020 to January 2022 were collected for a retrospective analysis. Among them, 48 patients treated with laser photocoagulation were a control group (CG), and the other 50 treated with laser photocoagulation combined with ranibizumab were an observation group (OG).

Inclusion and exclusion criteria

Inclusion criteria: patients who met the diagnostic criteria for diabetic retinopathy [16]; patients without history of eye surgery; patients who aged <80 years; patients with monocular onset; patients with neovascularization in the retina; patients with clear consciousness and normal communication ability; patients with complete clinical data.

Exclusion criteria: patients with history of anti-VEGF therapy; patients with other non-PDR fundus disease; patients with diseases affecting fundus observation, such as severe cataracts, keratopathy, iritis, glaucoma or history of previous ocular trauma; patients with severe cardiac, hepatic or renal diseases that cannot tolerate the procedure; pregnant women.

Treatment options

In the CG, the pupil was firstly dilated sufficiently with compound tropicamide eye drops (manufacturer: Santen Pharmaceutical Co., Ltd.; registration number: J20110007), and then with proparacaine hydrochloride eye drops (manufacturer: Suzhou Industrial Park Tianlong Pharmacy Co., Ltd.; approval number: SFDA Approval No. H20084062) for surface anesthesia. Laser therapy instrument (multi-wavelength laser therapy instrument produced by Lumenis) was used to perform a "C" shaped photocoagulation in the macula at 500 μm from the central concavity of the macula, with a spot diameter of 100 μm, energy of 150 mW, exposure time of 0.1-0.2 s, producing I-II level spots, with an interval of 1 Spot diameter, around the center of the concave to do 3 rows of circular light spot. The energy setting for the equatorial part, the posterior pole part and the far peripheral light condensation was 180 to 280 mW, and the spot diameter was 300 μm, producing a class II to III spot. For fluorescein fundus angiography showing photocoagulation in areas of non-perfusion and neovascularization, the spot energy was increased to a class III spot response. Photocoagulation was performed in sequence, and patients were treated for a total of 4 weeks, once per week.

In the OG, the control-based treatment regimen was combined with intravitreal injection of ranibizumab injection (manufacturer: Novartis Pharma Schweiz AG; registration number: S20170003). The eyes were disinfected with antibiotic drops for 3 d before surgery, according to ophthalmic surgical disinfection standards. The eye was surface anesthetized, punctured 3.5 mm from the corneoscleral rim in the 11 o'clock direction, entered the vitreous cavity, and slowly injected with 0.05 mL of ranibizumab. The drug was administered once a month for 3 months, followed by modified retinal photocoagulation 1 week later, in the same way as the control group.

Enzyme-linked immunosorbent assay (ELISA)

5 mL of fasting venous blood was collected from patients in the early morning of the day after admission and 5 mL of venous blood in the early morning of the day after treatment. The blood samples were centrifuged at 1509.3*g for 10 min, and the serum was collected for subsequent experiments. ELISA was used to detect VEGF (ml064281), homocysteine (HCY, ml092715), before and after the treatment. The ELISA kits were obtained from Shanghai Enzyme-linked Biotechnology Co., Ltd.

Outcome measures

Main outcome measures: The treatment efficacy, best corrected visual acuity (BCVA), central macular thickness (CMT), and change in neovascularization area were compared between the two groups. The changes in serum VEGF, HCY, vitamin B12 and folic acid in both groups were assessed.

Secondary outcome measures: The differences in clinical data and the adverse reactions that occurred with treatment were compared between the two groups. The relationship of pre-treatment BCVA, CMT, neovascularization area and VEGF levels with clinical outcomes were observed, and their predictive value was analyzed by receiver operating characteristic (ROC) curve.

Clinical efficacy evaluation

The efficacy of the treatment was evaluated after 6 months of treatment, and the criteria were as follows, markedly effective: the visual acuity level was improved by 2 lines or more on the chart compared with that before treatment; effective: a visual acuity test showed a 1-line improvement on the chart; ineffective: the visual acuity level improved by less than 1 line on the chart, or even worsened. Total number of responses = markedly effective cases + effective cases.

Statistical analysis

SPSS 20.0 (IBM Corp, Armonk, NY, USA) was used for statistical analysis, and GraphPad Prism 7 was used to graph the data. The measured data were expressed as mean ± standard deviation (x ± sd), and t-test was used for comparison between both groups. The counted data were assessed via Chi-square test. The ROC curve was used to evaluate the predictive efficacy of pre-treatment BCVA, CMT, neovascularization area and VEGF on the clinical outcome of diabetic retinopathy patients. The difference was statistically significant when $P<0.05$.

Results

Comparison of clinical data

Comparisons of baseline data between both groups revealed no statistical difference in age, sex, body mass index, course of disease, location of the affected eye, history of hypertension, or history of smoking ($P>0.05$, **Table 1**).

Comparison of clinical outcomes

The treatment effects between both groups revealed that the total number of responses in the CG was dramatically lower than that of the OG ($P<0.05$, **Table 2**).

Changes in BCVA, CMT and neovascularization area in patients

The changes in BCVA, CMT, and neovascularization area before and after 6 months of treatment were compared between the two groups. It was revealed that the BCVA, CMT and neovascularization area were dramatically lower in both groups after treatment compared to before treatment ($P<0.001$), and further comparison demonstrated that the posttreatment BCVA, CMT, and neovascularization area were

Table 1. Baseline data

Factor	Control group (n=48)	Observation group (n=50)	χ^2/t value	*P* value
Age			0.326	0.567
≥60 years	31	5		
<60 years	173	15		
Sex			0.623	0.429
Male	25	30		
Female	23	20		
BMI (kg/m^2)	23.56±2.56	23.23±2.59	0.622	0.535
Course of disease (year)	6.97±2.68	7.28±2.53	0.570	0.569
Location of the affected eye			0.653	0.418
Left side	22	27		
Right side	26	23		
History of hypertension			1.452	0.228
Yes	28	35		
No	20	15		
History of smoking			0.335	0.562
Yes	27	31		
No	21	19		
Disease staging			1.242	0.537
I	18	14		
II	20	26		
III	10	10		

BMI: Body mass index.

Table 2. Clinical efficacy evaluation

Group	Markedly effective	Effective	Ineffective	Total number of responses
Control group (n=48)	20	18	10	38
Observation group (n=50)	33	14	3	57
χ^2 value				4.683
P value				0.030

dramatically lower in the OG than in the CG (P<0.05, **Figure 1**).

Changes in VEGF, Hcy, vitamin B12 and folic acid in patients

Changes in VEGF, Hcy, vitamin B12 and folate were compared between both groups before and after 6 months of treatment. It was found that after treatment, the VEGF and Hcy were dramatically lower while vitamin B12 and folic acid were dramatically higher in both groups compared to those before treatment (P< 0.001). Further comparison revealed that the posttreatment BCVA, CMT, and neovascularization area were lower while vitamin B12 and folic acid were higher in the OG than in the CG (P<0.05, **Figure 2**).

Comparison of patients' adverse reactions

There was no statistical difference in the number of adverse reactions between the two groups (P>0.05, **Table 3**).

Value of clinical indicators for predicting patient outcomes

In this study, we divided the patients into an improvement group (marked effective + effective) and a non-improvement group (ineffective) based on their response to treatments. We then compared the changes in each clinical index between these two groups of patients before treatment. The results found that BCVA, CMT, VEGF, and Hcy were dramatically lower in patients in the improved group than in those in

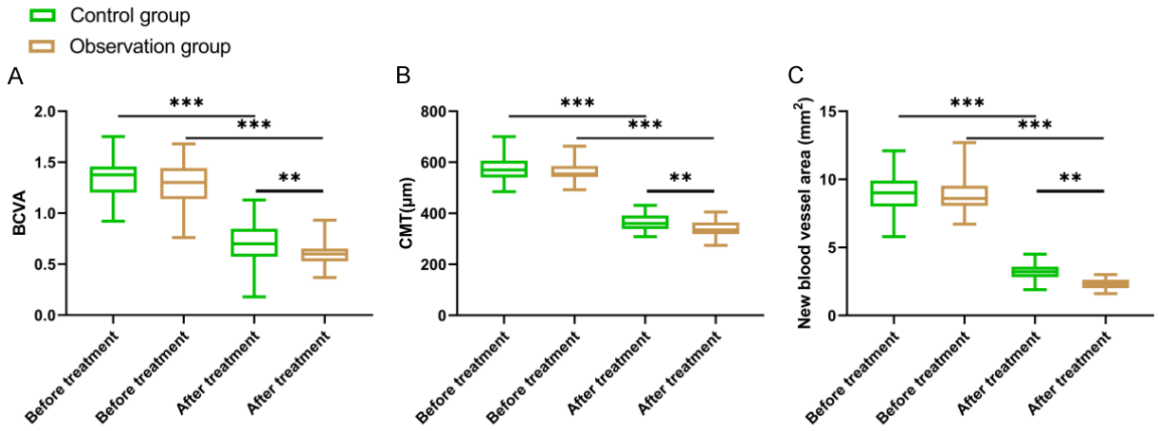

Figure 1. Comparison of changes in BCVA, CMT, and neovascularization area in patients before and after treatment. A. Comparison of BCVA changes in patients before and after treatment; B. Comparison of changes in CMT before and after treatment in patients; C. Comparison of changes in neovascularization area in patients before and after treatment. Note: ** indicates P<0.01, *** indicates P<0.001, best corrected visual acuity (BCVA), central macular thickness (CMT).

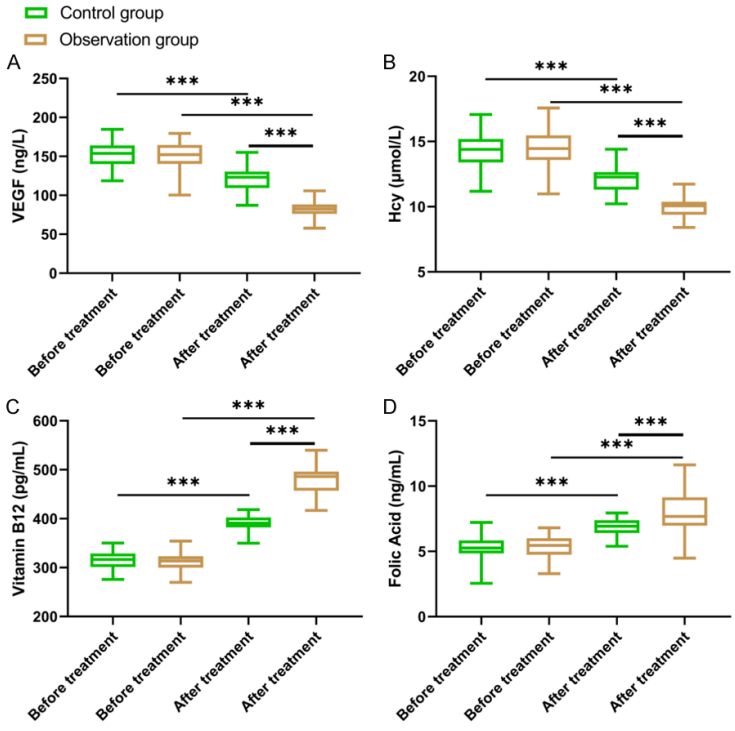

Figure 2. Comparison of changes in VEGF, Hcy, vitamin B12, and folic acid in patients before and after treatment. A. Comparison of VEGF changes in patients before and after treatment; B. Comparison of Hcy changes in patients before and after treatment; C. Comparison of vitamin B12 changes in patients before and after treatment; D. Comparison of folic acid changes in patients before and after treatment. Note: ** indicates P<0.01, *** indicates P<0.001, vascular endothelial growth factor (VEGF), homocysteine (Hcy).

the non-improved group (P<0.05, **Figure 3**), while there were no differences in neovascular area, vitamin B12 and folic acid between the two groups before treatment (P>0.05, **Figure 3**). Subsequently, ROC curve revealed that the areas under the curve for BCVA, CMT, VEGF and Hcy were all greater than 0.7 in predicting patient outcome. In addition, the area under the curve of the combination of the above indexes was 0.945, with a specificity of 92.30% and sensitivity of 88.23% (**Figure 4**).

Discussion

Diabetic retinopathy is the most common fundus disease and is the leading cause of blindness in diabetic patients [17]. The development of diabetic retinopathy is influenced by systemic glucose blood pressure and local ocular factors. Long-term chronic elevated blood glucose, among these factors, is the main cause of diabetic retinopathy progression, and patients with poorly controlled long-term blood glucose levels have more severe diabetic retinopathy and higher rates of blindness than patients with stable glycemic control [18]. Once diabetic retinopathy develops, it is usually irreversible, but the initial phase of diabetic

Table 3. Incidence of adverse reactions in patients

Group	Macular edema	Vitreous hemorrhage	Optic disc vascular leakage	Total adverse reactions
Control group (n=48)	3	3	2	8
Observation group (n=50)	3	4	4	11
χ^2 value				0.051
P value				0.821

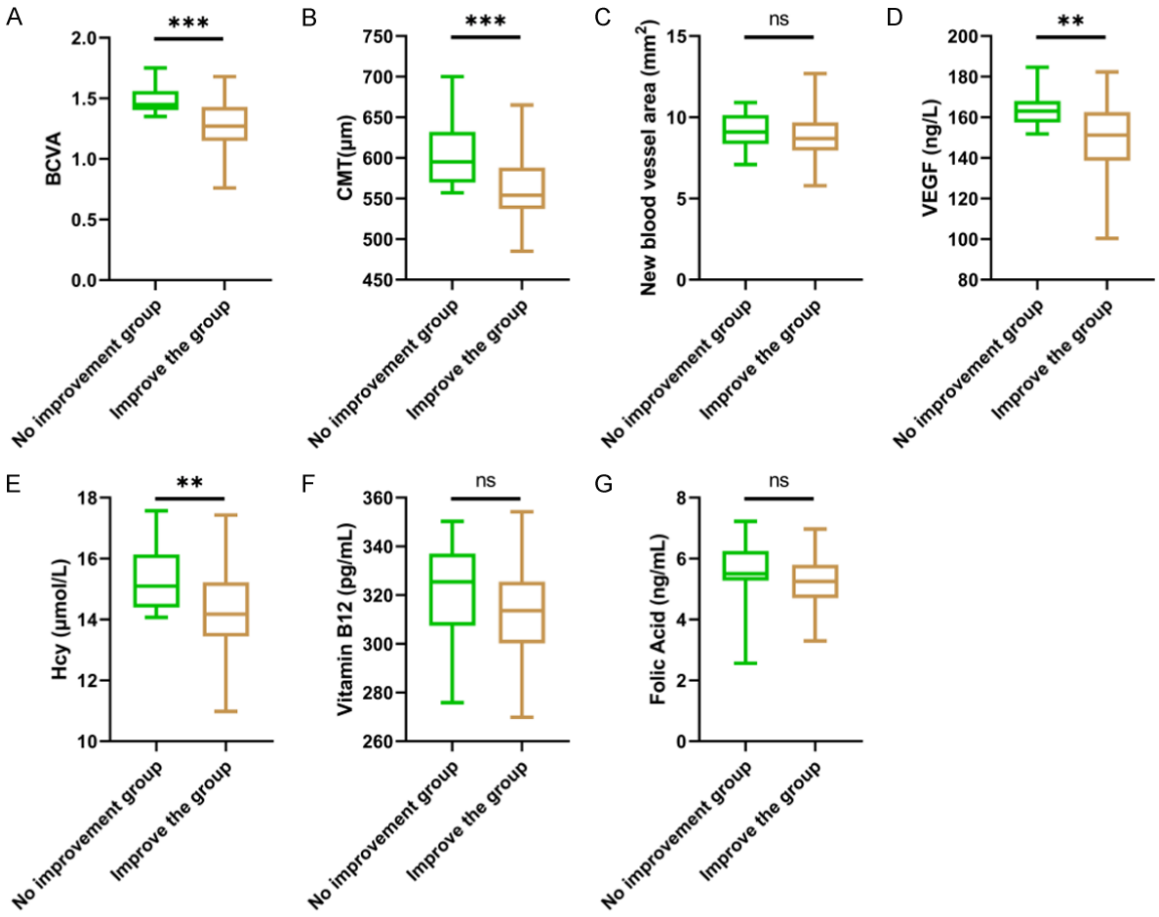

Figure 3. Pre-treatment expressions of clinical indicators in patients with different outcomes. A. Comparison of BCVA between the improved and unimproved groups; B. Comparison of CMT between the improved and unimproved groups; C. Comparison of neovascularization area between the improved and unimproved groups; D. Comparison of VEGF between the improved and unimproved groups; E. Comparison of Hcy between the improved and unimproved groups; F. Comparison of vitamin B12 between the improved and unimproved groups; G. Comparison of folic acid between the improved and unimproved groups. Note: ns indicates P>0.05, ** indicates P<0.01, *** indicates P<0.001, best corrected visual acuity (BCVA), central macular thickness (CMT), vascular endothelial growth factor (VEGF), and homocysteine (Hcy).

retinopathy is usually asymptomatic [19]. When diabetic retinopathy enters the proliferative phase, the progression of the disease is accelerated. Therefore, the earlier the treatment, the better the prognosis.

Retinal photocoagulation is currently the clinical choice for proliferative diabetic retinopathy. Its treatment mechanism is the absorption of laser light through the refractive interstitium of the eye by the retinal and choroidal pigment tissues and hemoglobin, and the conversion of light energy into heat energy to destroy local retinal REP cells, thereby reducing oxygen consumption and outer layer metabolism [20]. A study showed that when treated with photoco-

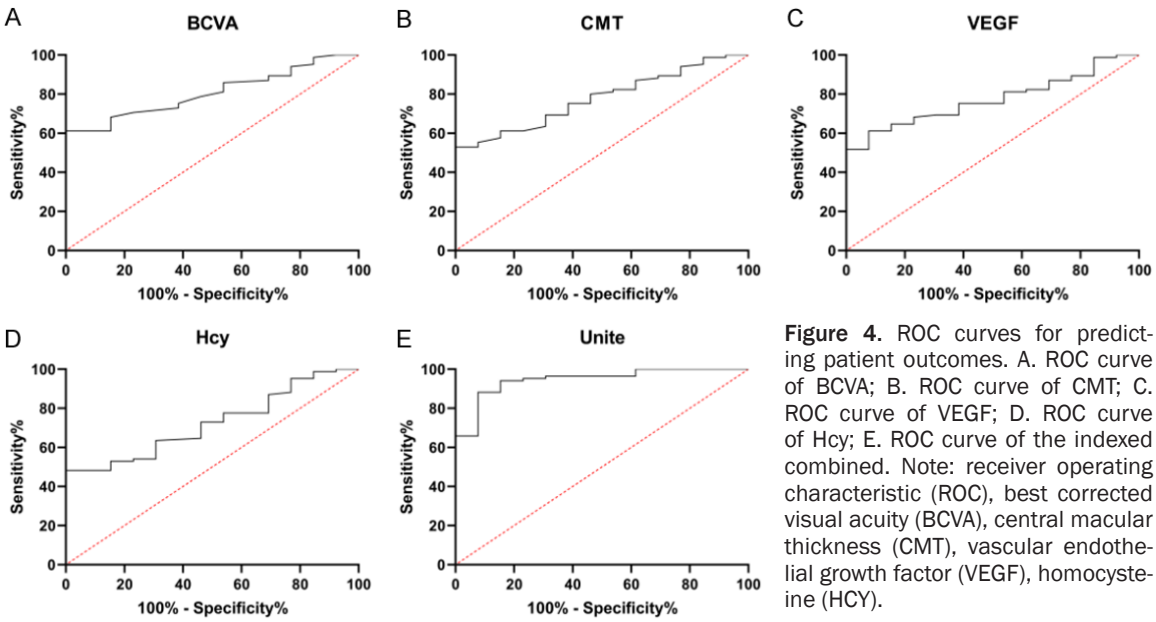

Figure 4. ROC curves for predicting patient outcomes. A. ROC curve of BCVA; B. ROC curve of CMT; C. ROC curve of VEGF; D. ROC curve of Hcy; E. ROC curve of the indexed combined. Note: receiver operating characteristic (ROC), best corrected visual acuity (BCVA), central macular thickness (CMT), vascular endothelial growth factor (VEGF), homocysteine (HCY).

agulation alone, the retina around the serrated edge is in a state of ischemia because it does not receive photocoagulation, which can lead to an increase in the vascular supply burden at the junction of the large branch retinal vessels and the laser spot, as well as an increase in neovascularization-inducing factors in the ischemic area, which does not completely control the progression of the disease and carries some risk [21]. The mechanism of action of ranibizumab, a type of anti-VEGF drug, was analyzed by inhibiting the binding of VEGF to its receptor, which results in a cascade reaction that effectively inhibits vascular leakage [22]. In the present study, we found that the efficacy in diabetic retinopathy patients treated with laser photocoagulation combined with ranibizumab was dramatically higher than that of those treated with laser photocoagulation alone, and the combination reduced the BCVA, CMT, and neovascularization areas. This suggests that combination therapy can enhance the clinical outcome of patients. Cao et al. [23] claimed that intravitreal ranibizumab combined with total retinal photocoagulation was found to dramatically improve the clinical outcome of patients, with significant postoperative improvements in BCVA, CMT, and neovascularization area, which is consistent with the results of our study. We believe that this is due to the ability to promote the closure of the retinal plexiform layer of aggregated fluid through photocoagulation, thereby inhibiting the production of VEGF, preventing the presentation of neovascularization, and effectively promoting the regression of neovascularization. In contrast, intravitreal injection of ranibizumab can be effective in promoting neovascular regression, reducing retinal neovascular leakage, decreasing retinal thickness in the central macular sulcus, and improving visual acuity.

In the current study, we also compared the changes in VEGF, Hcy, vitamin B12, and folic acid before and after treatment. The results showed that patients had a significant decrease in VEGF and Hcy but an increase in vitamin B12 and folic acid after treatment. This is because ranibizumab, a humanized recombinant anti-VEGF monoclonal antibody fragment Fab part, inhibited neovascularization in the fundus, improved macular edema, and promoted neovascular atrophy. Research has confirmed [24] that VEGF is involved in ocular neovascularization and is a major factor in the development and progression of diabetic retinopathy. Impaired microcirculation in the eye can lead to a large release of VEGF into the vitreous cavity, and high VEGF concentrations can stimulate retinal neovascularization, thereby increasing the risk of neovascularization and macular edema. Hcy is an intermediate product of methionine metabolism that increases free radical activity in the body and causes vascular endothelial damage, which accelerates platelet-mediated vascular endothelial smooth mus-

cle proliferation [25]. Vitamin B12 and folic acid are important antioxidants in the body and are involved in a variety of metabolic activities. Reduced levels of vitamin B12 and folic acid [26] can affect the metabolism of Hcy in the body. The above results show that laser photocoagulation combined with ranibizumab can effectively improve the treatment outcome of diabetic retinopathy.

Earlier studies found [27] that clinical outcomes of patients with type 2 diabetes mellitus treated with troglitazone could be predicted by observing leptin concentrations before treatment. In the present study, we grouped patients according to their response and analyzed the relationship of clinical indicators to patient outcomes. We found that pre-treatment BCVA, CMT, VEGF, and Hcy were dramatically lower in the improved group than in the unimproved group. This suggests that BCVA, CMT, VEGF, and Hcy may be related to patient outcome. To further analyze their clinical value, we plotted ROC curves and found that BCVA, CMT, VEGF, and Hcy all had areas under the curve >0.7 in predicting patient outcome, and the area under the combined prediction curve was 0.945, with a specificity of 92.30% and sensitivity of 88.23%. This denotes that BCVA, CMT, VEGF, and Hcy have some clinical value in predicting the outcome of diabetic retinopathy patients, and the value for predicting the outcome by combining the indexes is more obvious.

In the present study, we first analyzed the indicators that could predict the outcome of patients with diabetic retinopathy. Nevertheless, the current study still has some limitations. First of all, the current study was conducted retrospectively, so the sample collected may have been biased. Moreover, patients were not followed up in this study, and the long-term efficacy of the two treatment regimens remains unclear. Therefore, we hope to conduct a prospective follow-up study to refine our findings.

In summary, laser photocoagulation combined with ranibizumab can provide good therapeutic efficacy in diabetic retinopathy, by effectively improving neovascular regression and reducing VEGF levels to control further progression of the lesions.

Disclosure of conflict of interest

None.

Address correspondence to: Xiaoqiang Zhou, Department of Ophthalmology, First People's Hospital of Linping District, Hangzhou 311100, Zhejiang, China. E-mail: zxq15372002316@163.com

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