The Systemic Blood Pressure and Oxygen Saturation in Retinal Arterioles Predict the Effect of Intravitreal Anti-VEGF Treatment on Diabetic Maculopathy

Toke Bek and Christina Mørup Jørgensen

Department of Ophthalmology, Aarhus University Hospital, Aarhus C, Denmark

PURPOSE. The advent of vascular endothelial VEGF antagonists has increased the therapeutic options for diabetic maculopathy considerably. However, there is a need to identify patients who respond favorably to the treatment from those in whom the treatment is less effective. The purpose of the present study was to test the hypothesis that the oxygen saturation in retinal vessels together with other risk factors can predict the effect of anti-VEGF treatment on diabetic maculopathy.

METHODS. In 73 eyes from 53 patients with center-involving diabetic macular edema, multiple linear regression was used to evaluate the predictive value of oxygen saturation in larger retinal vessels together with age, diabetes duration, diabetes type, hemoglobin A1c (HbA1c), mean arterial blood pressure (MAP), body mass index (BMI), previous retinal photocoagulation, visual acuity (VA), and central retinal thickness (CRT) before treatment as explanatory variables for VA and CRT after three monthly injections of anti-VEGF medication.

RESULTS. Anti-VEGF treatment induced a significant increase in VA and a significant decrease in CRT, but no significant changes in the overall oxygen saturation of larger retinal vessels. Visual acuity and CRT before treatment contributed significantly to predicting the same variable after treatment. Additionally, MAP and the oxygen saturation in retinal arterioles before treatment contributed significantly to predicting VA and CRT after treatment.

CONCLUSIONS. The MAP and oxygen saturation in retinal arterioles might potentially be included as parameters in risk models predicting the effect of anti-VEGF treatment in patients with diabetic maculopathy.

Keywords: diabetic maculopathy, retinal oximetry, anti-VEGF treatment, risk factors

Diabetic maculopathy is a significant vision-threatening complication among patients with diabetes mellitus. The condition can be treated with photocoagulation, which is most effective in the early stages of the disease when retinal edema and exudates are located extrafoveally. Recently, the advent of anti-VEGF therapy has offered new opportunities for treating diabetic maculopathy with center involvement, which is assumed to be related to a reduction in the hyperpermeability of retinal vessels observed in the disease. However, the effect of the treatment shows considerable individual variation and it would be desirable to have access to parameters that allowed the identification of patients who will benefit from treatment from those in whom the treatment is less effective.

The retinal oxygen saturation is a marker of disturbances in metabolism and vascular function in diabetic maculopathy, and can be measured by dual-length oximetry. Using this technique, the oxygen saturation in larger retinal vessels has been shown to increase with increasing diabetic retinopathy grade, but that the beneficial effect of photocoagulation of the disease is not paralleled by a reduction in the oxygen saturation in these vessels. However, the influence of retinal oxygen saturation on intravitreal anti-VEGF therapy in diabetic macular edema is unknown.

Therefore, we tested the hypothesis that the oxygen saturation in larger retinal vessels can predict the effect of anti-VEGF treatment on diabetic maculopathy, considering the contribution of other known risk factors for progression of the disease.

MATERIALS AND METHODS

Study Design

Prospective observational study of the role of oxygen saturation in the larger retinal vessels and other risk factors for the effect of intravitreal injection with ranibizumab in 73 eyes from 53 patients with center-involving diabetic macular edema on visual acuity (VA) and central retinal thickness (CRT).

Patients

Five-hundred-and-sixty-six diabetic patients referred consecutively for specialist evaluation of diabetic retinopathy between November 1, 2011 and March 1, 2015 were studied. The patients were asked about known duration and treatment of diabetes mellitus, followed by measurement of height and weight (Seca Model 708; Vogel & Halke, Hamburg, Germany), which was omitted in 13 patients who declined from the procedure or in whom the examinations were meaningless, for example because of leg amputation. On the basis of this
information, the patients were defined as having type 1 diabetes mellitus (T1D) if the onset of diabetes was before the age of 30 or between 30 and 40 years, if insulin treatment had been initiated within the first year of the disease, and the body mass index (BMI) was below 25. Otherwise, the patient was defined as having type 2 diabetes mellitus (T2D). The arterial blood pressure was scheduled to be measured twice (M4; Omron, Kyoto, Japan), and except for 13 cases where the recordings had been lost, the average of the duplicate measurements was noted. The hemoglobin A1c (HbA1c) value measured closest to but within 3 months from the time of examination was obtained from the central laboratory records of the hospital.

A routine ophthalmologic examination was performed, including measurement of best corrected VA using Early Treatment of Diabetic Retinopathy Study (ETDRS) charts, followed by induction of mydriasis by phenylephrine 10% (SAD, Copenhagen, Denmark) and tropicamide 1% (Alcon, Copenhagen, Denmark) eye drops. Subsequently, the patients were subjected to slit-lamp examination, binocular inspection of the fundus using a 90-diopter (D) lens, and fundus photography (CF 60Z; Canon Amstelveen, Holland) with two images obtained, one centered on the fovea, two centered on the temporal vascular arcades in the center of the image. On the basis of measurements at two different fields on each eye, one centered on the fovea and another nasally displaced field centered on the optic disk. Optical coherence tomography (OCT) scanning was performed using the Heidelberg Spectralis version 1.7.0.0 (Heidelberg Engineering, Heidelberg, Germany) using the IR & OCT 30° ART protocol, which includes a 30° infrared fundus image centered on the fovea displaying the position of an array of 19 horizontal OCT scans with a length of 20° and spaced with a vertical interval of 0.8°.

**Oximetry**

Retinal oximetry was performed using the Oxymap model T1 oximeter (Oxymap, Reykjavik, Iceland) and the procedures described previously. The patient was positioned in front of the oximeter and five 50° fundus photographs were obtained, one centered on the fovea, two centered on the optic disk, and two images displaced 1 disk diameter temporally, respectively, upwards and downwards with the larger temporal vascular arcades in the center of the image. The image with the optic disk in the center, which was considered to have the best quality, was used for the analysis (see Fig.). The oximetry images from nine eyes from nine patients were discarded, in one patient the flash in the camera had been set to a wrong intensity, in two patients the oximetry images had been lost, and in the remaining patients the images were too blurred for the oxygen saturation to be obtained. This left examinations from 64 eyes from 44 patients (26 males and 18 females, 6 with T1D, and 38 with T2D) to contribute with oxygen saturation values. Three patients had previously received panretinal photocoagulation, 11 patients had received macular photocoagulation, and two of these also had intravitreal injection with anti-VEGF medication. Additionally, 16 of the studied eyes from 12 patients had received cataract surgery, all more than 3 months before the examination.

The clinical data of the patients before treatment are shown in Table 1. It appears that the patients with T1D were significantly younger, had a significantly longer diabetes duration, and had a significantly lower BMI than the T2D patients. There was no significant difference between logVA and CRT on the first treated eye of patients with T1D and T2D, \( (P = 0.49 \) for both comparisons).

**Treatment**

The patients entered a treatment protocol consisting of three intravitreal injections of ranibizumab 0.3 mg (Novartis, Copenhagen, Denmark) with 1-month intervals on the affected eye(s). The compound was injected in a volume of 0.05 ml using a 30-gauge needle (Becton Dickinson, Albertslund, Denmark) after disinfection with povidone iodide 5% and tetracaine 1% (Skanderborg Pharmacy, Skanderborg, Denmark). The patients were scheduled for a follow-up examination similar to the examination before treatment approximately 3 months after the last injection, but the time was adjusted according to the patients’ preferences, which resulted in a follow-up time from the first injection of (mean ± SEM, range): 200.97 ± 17.61 days, 97 to 858 days, \( n = 73 \) for the treated eyes. At the follow-up examination after treatment measurement of best corrected VA and OCT scanning was repeated in all treated eyes from all patients except one, whereas oximetry was performed in 55 eyes from 34 patients.

**Data Analysis**

**VA, CRT, and Blood Pressure.** The VA recorded in decimal values were log transformed (logVA) for the further analysis. The OCT examination was displayed to present the “thickness map” option on the Heidelberg instrument, which shows the average retinal thickness within a central circle with a diameter of 1 mm and two concentric circles with diameters of 5 and 6 mm, respectively, each divided into four quadrants by oblique meridians. The thickness value from the central circle was noted as the CRT. The mean arterial pressure (MAP) was calculated as \( BP_d + (BP_s - BP_d)/3 \), where \( BP_d \) is the diastolic and \( BP_s \) the systolic blood pressure.

**Oximetry.** The calculation of the oxygen saturation assumes that the absorbance of light through the retinal blood vessels obeys Lambert-Beers law and depends on the concentrations of the two light-absorbing pigments hemoglobin and oxyhemoglobin. The absorbance is calculated from the intensity of light reflected from the vessel and the perivascular retina. On the basis of measurements at two different wavelengths the relative concentration of the two hemoglobin types, and thereby the oxygen saturation, can be calculated.\(^9\)
**TABLE 2.** The Studied Risk Factors Before and After Treatment in the First (or Only) Treated Eye, and \( P \) Values From the Analysis Stratified According to Whether One or Both Eyes Were Treated

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SEM) Before</th>
<th>( N )</th>
<th>Mean (SEM) After</th>
<th>( N )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>logVA</td>
<td>–0.45 (0.04)</td>
<td>53</td>
<td>0.04 (0.04)</td>
<td>52</td>
<td>0.007*</td>
</tr>
<tr>
<td>CRT, ( \mu )m</td>
<td>495.7 (17.3)</td>
<td>52</td>
<td>241.4 (52.0)</td>
<td>52</td>
<td>0.001*</td>
</tr>
<tr>
<td>Sat-A, %</td>
<td>96.7 (0.86)</td>
<td>44</td>
<td>1.09 (34)</td>
<td>34</td>
<td>0.60</td>
</tr>
<tr>
<td>Dia-A, pixels</td>
<td>12.6 (0.22)</td>
<td>44</td>
<td>1.24 (34)</td>
<td>34</td>
<td>0.36</td>
</tr>
<tr>
<td>Sat-V, %</td>
<td>65.1 (1.15)</td>
<td>44</td>
<td>1.24 (34)</td>
<td>34</td>
<td>0.95</td>
</tr>
<tr>
<td>Dia-V, pixels</td>
<td>16.3 (0.25)</td>
<td>44</td>
<td>1.29 (34)</td>
<td>34</td>
<td>0.65</td>
</tr>
<tr>
<td>AV-Sat, %</td>
<td>31.6 (1.05)</td>
<td>44</td>
<td>0.31 (34)</td>
<td>34</td>
<td>0.73</td>
</tr>
</tbody>
</table>

Asterisks indicate significant \( P \) values (\( P < 0.05 \)).

On the disk-centered fundus image recorded by the oximeter, a circle with the best possible fit to the disk margin was defined manually using the marking tool in the oximeter software, and subsequently two larger circles concentric with the disk were defined, an inner circle with a 15-pixel larger radius and an outer circle with a one disk diameter larger radius. Subsequently, the saturation and diameter were collected from the longest segment with uninterrupted recording of oxygen saturation in eight vessels within these two circles, that is, from the major arterioles and venules from the four quadrants. Saturation and diameter values from the segments on each of the four arterioles and venules were averaged to provide an overall value for saturation and diameter for each of the two vessel types in the eye.

**Ethical Approval**

The patients gave their informed consent for the procedures, which were approved by the regional committee for scientific ethics and adhered to the tenets of the Declaration of Helsinki.

**Statistical Analysis**

The background data at the time of treatment of the first eye were compared between the patients with T1D and T2D using the Mann-Whitney \( U \) test.

For all eyes where logVA, CRT, saturation, and diameters of retinal vessels were available, the change from before to after treatment was calculated. Additionally, the patients were allocated to one of two strata according to whether data were available for both eyes or only one eye. A weighted mean across the strata was calculated using strata-specific weights equal to the inverse of the squared standard error, which was used to test whether the differences differed significantly from zero.14

Finally, multiple linear regression was used to test if logVA and CRT after treatment could be explained by the available epidemiologic, anthropometric, and clinical parameters in the first (or only) treated eye from each patient before treatment. Diabetes type (T1D or T2D) and previous retinal photocoagulation (performed or not) were included in the model as categorical variables, and the remaining parameters: age, diabetes duration (Dur), HbA1c, BMI, MAP, logVA, CRT, arterial and venous saturations (Sat-A and Sat-V) and diameters (Dia-A and Dia-V) as continuous variables. To ensure that all available data were included in the multiple regression, single missing values of a parameter were replaced with the average of that parameter among the remaining patients.

**RESULTS**

Table 2 shows that anti-VEGF treatment induced a significant increase in logVA and a significant decrease in CRT, whereas neither oxygen saturations nor diameters of the larger retinal vessels changed significantly.

The results of the multiple regression are shown with logVA and CRT (Table 4) after treatment as dependent variables. It appears that logVA and CRT before treatment contributed significantly to predicting these same variables after treatment. Additionally, increased MAP and Sat-A before treatment contributed significantly to predicting a lower logVA and a higher CRT after treatment. The adjusted \( R \) square values showed that the included parameters predicted 53% of the variation in logVA and 45% of the variation in CRT after treatment (not shown).

Subsequent regression analysis with separate inclusion of each of the significant explanatory variables showed that logVA, MAP, and Sat-A before treatment predicted 36.1%, 2.5%, and 9.2%, respectively, of the variation in logVA after treatment, whereas CRT, MAP, and Sat-A before treatment predicted 20.4%, 11.6%, and 16.4%, respectively, of the variation in CRT after treatment.
The findings of the present study confirmed previous reports that the oxygen saturation in larger retinal venules is higher in patients with diabetic maculopathy than in normal persons, whereas the saturation in retinal arterioles is similar in the two groups. The oximetry examination was standardized as described previously and the resulting reproducibility of 1.5% or better is comparable to other sources of variation, such as differences in fundus pigmentation, lens opacities, and age. The oxygen saturation calculated on the basis of dual-length oximetry depends on the diameter of the vessels, which was corrected automatically by the oximeter using an algorithm identical for arterioles and venules. This correction can be considered to have been valid since the contributions of venuel diameters to explaining VA and CRT after treatment were not significant. Although the conclusions of the study are robust, it cannot be excluded that the number of patients has been too low to identify other of the included parameters as being minimally predictive for the effect of anti-VEGF therapy on diabetic macular edema.

The lack of reduction of the pathologically increased venous oxygen saturation after intravitreal injection with anti-VEGF compound is similar to findings after retinal photoagulation, and supports that the beneficial effects of treatment for diabetic retinopathy are unrelated to changes in the oxygen saturation in the larger retinal venules. However, this does not exclude that the oxygen saturation in the retinal tissue had been affected by the treatment. Thus, substantial parts of the retinal blood flow could have been shunted to bypass the capillary exchange, or the site of oxygen exchange might have been affected, potentially facilitated by regional differences in the regulation of retinal blood flow. Therefore, the oxygen saturation in the larger retinal vessels might be less relevant as an indicator of retinal metabolism, and the increased oxygen saturation in larger retinal venules in patients with diabetic maculopathy could be an epiphenomenon rather than a causal factor in the underlying disease process.

It is notable that parameters related to retinal and systemic metabolism before treatment, such as previous retinal photoagulation, BMI, and HbAlc, had no significant predictive value for the outcome of anti-VEGF treatment. A separate analysis including sex as an explanatory variable showed no significant contribution to the treatment effect from this parameter, but sex was excluded from the regression model, since this parameter has been found previously to covary with metabolic regulation in the studied population. The significant contribution of the MAP and the Sat-A before anti-VEGF treatment to the outcome after treatment suggests that the predictive effect was related to changes in the hemodynamic conditions in the retinal vessels. The arterial blood leaving the heart is nearly fully saturated with oxygen, and the lower arterial oxygen saturation in the retina is assumed to be due to countercurrent exchange in the optic nerve, where the central retinal artery and vein are adjacent located over a distance of several centimeters. The amount of oxygen and other compounds exchanged between the central retinal artery and vein in the optic nerve will depend on the amount of blood per time unit exposed to the diffusion area between the vessels, and an increased arterial oxygen saturation in retinal vessels, therefore, can be interpreted as a consequence of increased flow rate in the central retinal vessels. A higher retinal blood flow combined with increased arterial blood pressure and impaired retinal pressure autoregulation may increase the hydrostatic pressure in the capillaries and the stress on the walls may counteract the tightening effect of anti-VEGF medication on the blood-retina barrier. This may explain how increased systemic blood pressure and oxygen saturation in retinal arterioles can contribute to increasing CRT with a consequent reduction in visual acuity. The lack of predictive value of age on the therapeutic outcome may be due to an increased stiffening of the retinal vascular walls with age that hinders dilatation when the blood pressure increases.

The findings of the present study are limited by several factors. Thus, the measurements of oxygen saturation and diameters in the larger retinal vessels were not performed repeatedly, and, therefore, an influence of dynamic properties of vascular function to the observed effects may have been overlooked. Additionally, the relationship between increasing CRT and decreasing VA after anti-VEGF treatment can be explained only partially by the findings. Thus, a positive predictive value of VA and CRT before treatment for the same parameters after treatment can be interpreted that the more deranged these parameters were before treatment, the more likely it was that the parameters also were deranged after treatment. A recalculation of the multiple regression model with the changes in logVA and CRT instead of the outcome values of these parameters as effect variables showed that a higher CRT before treatment resulted in a larger improvement of this parameter after treatment. The lack of a similar finding for VA is opposite to experiences from other studies, which may be due to a lower VA before treatment than in the present study. However, it is notable that neither logVA nor CRT before...
Intravitreal Anti-VEGF in Diabetic Maculopathy


Jeppesen P, Gregersen PA, Bek T. Age-dependent decrease in the myogenic response of retinal arterioles as studied with the...


