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CLINICAL STUDY

Blood flow velocity measured using the Retinal Function Imager predicts successful ranibizumab treatment in neovascular age-related macular degeneration: early prospective cohort study

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Abstract

Purpose **Anti-VEGF treatment has a potent vasoconstrictive effect. Early changes of retinal blood flow velocity (RBFV) measured using the Retinal Function Imager (RFI) combined with indicators of vascular status may help in predicting the visual outcome 1 month post injection in patients with neovascular age-related macular degeneration (nvAMD) under ranibizumab treatment. To develop a simple prediction model based on the change in RBFV 3 days post injection and indicators of a patient's vascular status to assess the probability of a successful visual outcome 1 month post injection.**

Methods **RBFV measured using RFI were prospectively collected pre-injection and 3 days post injection in 18 eyes of 15 patients. Indicators of vascular status (history of hypertension, diabetes mellitus without retinal affection, and smoking) were assessed by medical history. By univariate analyses, parameters associated with visual outcome were weighted (−1 to 6 points). A multivariate logistic regression model with the categorized visual outcome parameter (≥0 letters gained after 1 month) as the dependent variate and the sum score as the independent variate (continuous scale) was used to estimate the score value-specific probabilities of letters gained ≥0 1 month post injection.**

Results **The indicators of vascular status negatively influenced the likelihood of a letter gain ≥0 whereas an increase in the arterial RBFV strongly increased it. The area under the receiver operating characteristics curve for these parameters investigated was 0.71 (95% CI: 0.43–1.00).**

Conclusion **Changes in the arterial RBFV following 3 days after ranibizumab injection combined with three indicators of the vascular status identified nvAMD patients with favorable visual outcome accurately.**

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Introduction

Early changes in retinal blood flow velocity (RBFV) measured with the Retinal Function Imager (RFI) may help in predicting the visual outcome of patients under ranibizumab treatment for neovascular age-related macular degeneration (nvAMD), but the evidence is sparse.[1,2] It has been demonstrated that intravitreal ranibizumab injection (IVRI) in patients with nvAMD causes a significant vasoconstriction in retinal arterioles.[3,4] This effect can be explained by the inhibition of vascular endothelial growth factor (VEGF), which functions as a vasodilator by activating endothelial nitric oxide synthase, resulting in the production of the potent vasodilator nitric oxide.[5] In order to maintain the retinal blood flow in case of vasoconstriction following IVRI,

RBFV is expected to increase (flow = cross-section area × RBFV). Assuming that this is the case, the effect of a ranibizumab therapy would be expressed by a gain in arterial RBFV.

Retinal function imaging is a new, noninvasive diagnostic concept that allows to perform a quantitative mapping of RBFV.[6–8] The RBFV is measured by direct observation of erythrocyte movement. The RFI has proven to be a valuable tool in investigating various retinal conditions[9–16] as well as in evaluating the medical treatment.[17] We hypothesize that the extent of RBFV gain early after injection could be used as a proxy for an anti-VEGF treatment effect. Therefore, in this study we quantified the early changes in RBFV measured by RFI following ranibizumab injection and examined the association with visual outcomes after 1 month of follow-up.

Materials and methods

This study received the approval of the ethics committee of Canton Lucerne (#13103) and was performed according to the standards of good clinical practice.

Setting and recruitment

In this prospective and longitudinal study, patient records of individuals with nvAMD admitted to the Lucerne Eye Clinic for monthly check-up in the *pro re nata* regimen were screened. If patients met the inclusion criteria, they were informed about the study and included if interested. Written informed consent was obtained by all participating patients.

Inclusion and exclusion criteria

A total of 18 eyes of 15 patients were included. We included patients with nvAMD who have been treated with ≥ 3 intravitreal injections of only ranibizumab in the study eye. Indication for and conduction of retreatment on the actual visit were mandatory. If both eyes of one individual qualified for inclusion, both eyes were investigated.

All patients with history of epileptic or any other type of seizure ($n = 0$) were excluded as the RFI works with a stroboscopic flash light. To guarantee an optimal image quality, patients with optical media opacity ($n = 2$), poorly dilating pupil ($n = 1$), or high refractive error ($\geq +10$dpt or -6dpt) ($n = 0$) were excluded. Factors possibly altering RBFV that accounted for exclusion were eye surgery or trauma within ≤ 3 months ($n = 1$), diabetic retinopathy ($n = 0$), and intraocular pressure (IOP) > 21 mm Hg ($n = 1$).

Clinical assessments

The data evaluated in this study derived from three visits per patient.

On the first (T_0) and third (T_{1m}) visit, approximately 1 month after T_0, patients underwent testing of best-corrected visual acuity (BCVA) by Early Treatment of Diabetic Retinopathy Scale (ETDRS) and a Spectralis-Optical Coherence Tomography (OCT) (Heidelberg Engineering GmbH, Heidelberg, Germany) in follow-up mode was conducted by the optometrist to obtain central macular thickness (CFT) and total macular volume (TMV) (version 1.7.1.0., 2012, Heidelberg Engineering GmbH, Germany).

Indication for retreatment was determined by a resident physician using the retreatment criteria based on the PrONTO-Studies.[18,19] In addition any intra- and subretinal fluid assessed by OCT was treated.

Before IVRI (T_0) and 3 days after IVRI (T_3) images were taken with the RFI after dilatation (tropicamide 0.5% gtt) to analyze the changes in the arterial, venous, and mean RBFV.

Indicators of vascular status and physiological parameters

On the first visit (T_0), we assessed the history of hypertension, diabetes mellitus without retinal affection, and smoking. Moreover, systolic and diastolic blood pressure, mean arterial pressure (MAP), pulse frequency, IOP, and mean ocular perfusion pressure (MOPP) were assessed.

RFI measurements

Details of RFI measurements have been described elsewhere.[6] In brief, the device calculates the cross-correlation of moving patterns of erythrocytes and single erythrocytes over eight consecutive pictures and quantifies the RBFV of venules and arterioles.

The first RBFV measurement (T_0) was performed minutes before the injection, the second measurement was conducted 3 days after IVRI (T_3). The field of vision was set to 35 degrees. The three series of best image quality, each with at least four consecutive images, were included for the RBFV analysis. For quality assurance, segments with coefficients of variance > 0.45 were excluded. In each hemisphere three arterioles and three venules were measured using the automated integrated software, resulting in a total of 12 vessel segment measurements per patient, 6 venules and 6 arterioles (Supplementary Figure 1).

Statistical analysis

Dichotomous variates were described with percentages and continuous variates with means and standard

deviations (SD). We measured the changes in arterial, venous, and mean RBFV between T_0 and T_3 with a paired *t*-test. In the same manner, the outcomes (change in BCVA, CMT, and TMV) were assessed. To quantify the association between history of hypertension, diabetes mellitus, any smoking and the three RFI parameters (arterial, venous, and mean RBFV difference between T_0 and T_3), we performed univariate analyses.

Multivariate analysis

For each patient we calculated the vascular score as the sum of positive vascular risk factors. Values of this score could range from 0 (no history of hypertension, diabetes, and smoking) up to a value of 3 (all risk factors present). In a multivariate analysis (using a random intercept model with subject as the random factor to adjust for the fact that three patients provided data on both eyes), we assessed the association of the two independent variates, arterial RBFV and vascular score, with the visual outcome parameter (≥0 letters gained after 1 month) as the dependent variate.

Prediction score

The arterial blood flow velocity was categorized into two groups (RFI $>=1$ *vs* else) and a score value was attributed to each category as follows: Each of the three vascular parameters (hypertension, diabetes mellitus without retinal affection, and smoking history) received 1 minus point and the velocity parameter received 6 points. Thus, the sum score could theoretically reach values between −3 and 6 points and was calculated for each patient. A univariate logistic regression model with the categorized visual outcome parameter (≥0 letters gained after 1 month) as the dependent variate and the prediction score as the independent variate (continuous scale) was used to estimate score value-specific probabilities of letters gained ≥0 using the following formula:

$$P = \text{probabilityofgoodvisualoutcome} = e(\alpha + \beta\text{score})/1 + e(\alpha + \beta\text{score}),$$

where α = intercept and β = regression coefficient for score variate.

The corresponding estimated probabilities are shown in Table 2. The area under the receiver operator curve (aROC) was estimated. A *P*-value of <0.05 was considered statistically significant. Analyses were performed using the Stata 11.2 statistics software package (StataCorp LP, College Station, TX, USA).

Results

Patients' characteristics

Patients' characteristics are listed in Table 1a. Mean age was 80.4 years (SD ± 4.8), 67% of eyes were female, and 44% was right eyes. On average, patients have received 13.7 injections (SD ± 9.0) before inclusion into the study. Overall, 39% of patients were suffering from a treated hypertension and 17% were suffering from a diabetes mellitus without retinal affection, whereof 67% were insulin dependent and 33% treated with oral anti-diabetics. Also, 56% of eyes came from former or current smokers, with an average pack years of 12.8 (SD ± 18.9).

Changes in clinical parameters

RFI Before anti-VEGF injection, the average RBFV of all patients studied was 3.68 mm/s for arterial, 3.08 mm/s for venous, and 3.38 mm/s for mean arterio-venous RBFV. Three days after the injection, the RBFV measurements increased to 4.27 mm/s (+16%) for the arterial RBFV, 3.24 mm/s (+5%) for the venous RBFV, and 3.85 mm/s (+14%) the mean arterial-venous RBFV. Although the arterial and mean RBFV increased significantly 3 days following the injection ($P=0.023$, $P=0.042$), venous RBFV only increased to a non-significant level ($P=0.464$).

In the subgroup of patients with stable gaining of vision, the RBFV values increased from 3.63 (SD 1.14) to 4.79 (SD 1.41) mm/s for the arterial RBFV, from 3.38 (SD 1.16) to 3.28 (SD 0.38) mm/s for the venous RBFV, and from 3.50 (SD 1.10) to 4.03 (SD 0.83) mm/s for the mean arterial-venous RBFV before and 3 days after the treatment, respectively. In contrast, in the subgroup of patients losing vision the RFFV readings changed from 3.72 (SD 0.74) to 3.94 (SD 1.21) mm/s for the arterial RBFV, from 2.89 (SD 0.81) to 3.22 (SD 0.97) mm/s for the venous RBFV and from 3.30 (SD 0.69) to 3.74 (SD 1.04) mm/s for the mean arterial-venous RBFV before and 3 days after treatment. The difference in changes of arterial RBFV was statistically significant ($P=0.048$), whereas those of the venous ($P=0.344$) and mean RBFV ($P=0.486$) were not.

Visual acuity and morphologic parameters Overall, BCVA decreased slightly, but non-significantly, from 75.6 letters (SD ± 7.8) to 74.9 letters (SD ± 7.3) ($P=0.517$) after 1 month. Letter gain 1 month post injection was significantly influenced by the arterial RBFV (coef. 1.23, 95% CI: 0.23–2.23, $P=0.016$). At the same time, a significant decrease in the morphologic parameters CFT (−17.72 (SD 23.73); $P=0.006$) and TMV (−0.08 μm^3 (SD 0.16); $P=0.044$) was observed; however, it was not influenced by the arterial RBFV (coef. −1.21,

95% CI: −13.81 to 11.39, $P=0.841$; and −0.004, 95% CI: −0.088 to 0.081, $P=0.931$). The eyes of patients with diabetes without retinal affection were associated with a lower decrease in CFT (coef. −27.27, 95% CI: −56.71 to 2.17). The changes in clinical parameters are summarized in Table 1b.

Physiological parameters All physiological parameters (systolic and diastolic blood pressure, pulse frequency, MAP, and MOPP) except for IOP ($P=0.039$) were comparable at T_0 and T_3.

The changes in all parameters are summarized in Table 1b.

Prediction model

Full model The regression coefficients of the full model were: $\beta_0=-0.76$ (95% CI: −2.56 to 1.05); $\beta_{\text{vasc.status}}=-0.42$ (95% CI: −1.59 to 0.75); and $\beta_{\text{aRBFV}}=1.23$ (95% CI: 0.00–2.46). The corresponding aROC of this model was 0.82 (95% CI: 0.56–1.00). The estimated probabilities ranged from 10.8 to 81.8%. Goodness-of-fit and the comparison between observed and estimated probabilities are shown in Supplementary Figures 2 and 3.

Score An increase in arterial RBFV of ≥1 mm/s strongly increased the likelihood for a letter gain ≥0 one month post injection, whereas the history of hypertension, diabetes mellitus without retinal affection, and smoking decreased this likelihood. ROC plot is depicted in Figure 1 with the aROC amounting to 0.71 (95% CI: 0.43–1.00). A comparison between observed probabilities and corresponding estimated probabilities generated by the simple prediction score are shown in Table 2.

Table 1a Patients' characteristics

Patient characteristics	*Mean*	*SD*
Age (years)	80.4	4.8
No. of pack years	12.8	18.9
No. of injections	13.7	9.0
Ranibizumab treatment duration (days)	918.2	834.6
	Frequency	*Percentage*
Females	12	67
Right eye	8	44
Pseudophakia	14	78
History of hypertension	7	39
History of diabetes	3	17
Current or previous smokers	10	56

Discussion

Main findings

A simple score including changes in arterial RBFV following 3 days after ranibizumab injection combined with three indicators of vascular status (history of hypertension, diabetes mellitus, and smoking) predicted nvAMD patients with a favorable visual outcome after 1 month accurately. Arterial RBFV increased 3 days after ranibizumab injection, while most physiological parameters remained stable, rendering chances of confounding of RBFV changes low. The finding of a major increase of RBFV occurring in arterioles supports the theory of vasoconstriction being the major driver of increase in RBFV.[20,21] In this set of patients, we found only a weak association between changes in IOP and blood flow velocity (data not shown). We therefore conclude that changes in RBFV were not substantially affected by changes in IOP. As treatment success was strongly positively influenced by increase in RBFV, thus

Table 1b Patients' characteristics

Characteristics	*Baseline*				*Follow-up*[a]				P-*values*
	Mean	*SD*	*Min*	*Max*	*Mean*	*SD*	*Min*	*Max*	
Letters	75.6	7.8	59	89	74.9	7.3	59.0	86.0	0.517
CFT (μm)	326.3	94.0	223	583	308.6	79.8	231.0	509.0	0.006
TMV (mm^3)	2.6	0.6	1.9	3.9	2.5	0.6	2.0	3.9	0.044
IOP (mm Hg)	13.6	2.3	11	18	14.7	2.4	11	20	0.039
Systolic blood pressure (mm Hg)	146.1	22.0	106	180	146.3	20.3	111	180	0.969
Diastolic blood pressure (mm Hg)	83.7	8.9	65	100	84.0	10.6	64	104	0.880
Pulse frequency (b.p.m.)	74.7	15.3	52	100	73.8	14.0	56	98	0.715
MAP (mm Hg)	104.5	12.2	83.3	121.3	104.7	12.0	81.3	124.0	0.919
MOPP (mm Hg)	56.0	7.4	42.9	66.9	55.1	9.0	40.4	68.2	0.610
Venous RBFV (mm/s)	3.08	0.96	1.62	4.97	3.24	0.78	1.76	4.85	0.464
Arterial RBFV (mm/s)	3.68	0.88	2.61	5.44	4.27	1.32	2.37	6.70	0.023
Mean RBFV (mm/s)	3.38	0.85	2.15	5.21	3.85	0.95	2.06	5.29	0.042

[a] 3 days except for letters, CFT, and TMV (1 month).

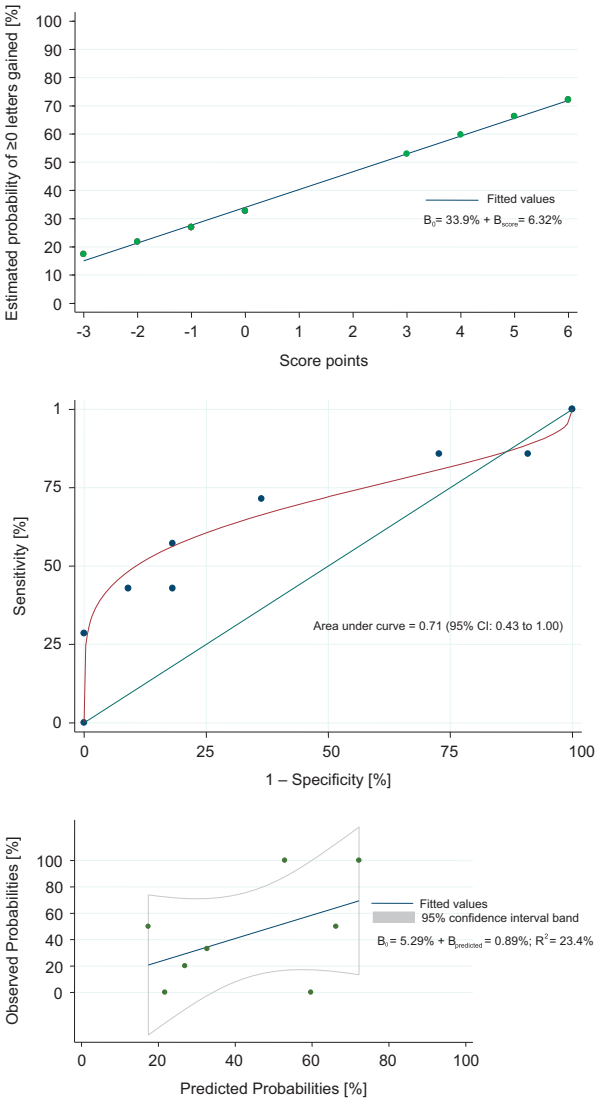

Figure 1 Discriminative capacity of a score combining the arterial RBFV with history of hypertension, history of diabetes mellitus without retinal affection, and history of smoking.

vasoconstriction, and negatively influenced by suboptimal vascular conditions (history of hypertension, diabetes mellitus without retinal affection, and smoking) probably only allowing restricted response by vasoconstriction, one can conclude that treatment success as visual outcome is directly influenced by vessel response. Morphologic findings in OCT did not correspond to our indicators, which is why the prediction model was based on visual development solely.

Results in context with the existing literature

Finger *et al*[22] in a review classified possible predictors of anti-VEGF treatment outcome as genetic, clinical, and behavioral, where they found that clinical parameters are likely to majorly contribute to treatment outcome. Although the levels of pretreatment visual acuity and several morphologic parameters in OCT have been assessed in detail as possible predictors for treatment outcome,[23] the role of factors of retinal hemodynamics indicating at a treatment response are ill understood. We are aware of only one study by Barak *et al*[17] that looked into the short-term effects of bevacizumab (Avastin) on RBFV. Stimulated by their findings, we focused on the vascular response to ranibizumab. Our findings contribute to a better understanding the extent to which RBFV may be useful in the prediction of a ranibizumab treatment success.

Table 2 Comparison of observed and estimated probabilities when using the clinical score

Score value	#	*Not improved*	*Improved*	*Observed probability (%)*	*Mean estimated probability (%)*
6	2	0	2	100	72.2
5	2	1	1	50	66.2
4	1	1	0	0	59.7
3	1	0	1	100	52.9
2	0	—	—	—	—
1	0	—	—	—	—
0	3	2	1	33	32.7
−1	5	4	1	20	26.9
−2	2	2	0	0	21.7
−3	2	1	1	50	17.4

Strengths and limitations

The strengths of this study lie in its prospective design presenting a uniform and typical cohort of nvAMD patients in terms of mean age (80.4 years, SD ± 4.8), female predominance[24] (67%), and ranibizumab history (13.7 injections, SD ± 9.0). The simple score that was derived is easy to use and has an acceptable goodness-of-fit particularly in higher probability regions. Where are the limitations? This prediction model does only take into account the outcome of functional success, ie, visual acuity. Anatomic features, such as CMT and TMV, that do contribute to a fair degree to definition of treatment success and are commonly used for indication for retreatment[18,19] were not included. Also, the sample size was rather small and further exploration of its usefulness is certainly warranted.

Implications for research and practice Further studies should validate our score and our findings in their settings and with their patients. It might be necessary to calibrate the parameters for specific clinical circumstances. The prediction model could contribute as a puzzle piece to the ongoing quest of early definition of therapy response to anti-VEGF treatment.[22,25,26]

Randomized trials comparing a therapeutic strategy based on the prediction model with standard care are necessary to assess the clinical impact of the score in the clinical management. If, by the score, treatment is expected to be successful, the treatment interval until the next check-up could possibly be stretched out, combined with means of patient empowerment and self-monitoring of their disease in the meantime.[27]

Conclusions

To our knowledge, the developed prediction score is the first tool allowing an estimation of visual treatment success as early as 3 days post injection in nvAMD therapy with ranibizumab. In regard to the current research to predict responders and nonresponders of anti-VEGF therapy, we believe that our findings make a valuable contribution in creating the full picture of early detection of treatment success. If confirmed in cross validations this simple score based on RBFV and clinical information could assist ophthalmologists optimizing nvAMD management of individual patients.

Summary

What was known before

- Anti-VEGF treatment has a potent vasoconstrictive effect.
- Early changes of rRBFV measured with the RFI may help in predicting the visual outcome 1 month post injection in patients with nvAMD under ranibizumab treatment.

What this study adds

- A simple score including the changes of arterial RBFV following 3 days after ranibizumab injection combined with three indicators of vascular status (history of hypertension, diabetes mellitus, and smoking) accurately predicted nvAMD patients with a favorable visual outcome after 1 month.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on Eye website (http://www.nature.com/eye)