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Model for predicting the efficacy of treating diabetic retinopathy in type 2 diabetes on the basis of determination of markers of endothelial dysfunction

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Purpose: *To develop a model for predicting the efficacy of treating DR in type 2 diabetes mellitus (T2DM) on the basis of determination of markers of endothelial dysfunction.*

Material and Methods: *This was a single-center, prospective cohort longitudinal study with a two-year observation period. Totally, 136 patients with T2DM and DR were included. They were divided into three groups: group 1 of 60 eyes with mild non-proliferative DR (NPDR), group 2 of 42 eyes with preproliferative DR (PPDR), and group 3 of 34 eyes with PDR. Enzyme-linked immunosorbent assay (ELISA) was used to determine serum levels of highly sensitive C-reactive protein (hs-CRP), endothelin (ET)-1, endothelial-monocyte-activating polypeptide II (EMAP II), endothelial nitric oxide synthase (eNOS), interleukin (IL)-1β IL-6, and nitric oxide metabolites (NOx). Repeat ophthalmological examination was performed after two years of treatment which included conservative treatment, laser photocoagulation, anti-vascular endothelial growth factor (VEGF) treatment, surgery (vitrectomy), or their combinations.*

Results: *Baseline blood levels of all endothelial dysfunction markers (with the exception of eNOS) among patients with DR were 1.9–16.4 higher compared to controls ($p < 0.001$), and gradually increased with the stage of DR. Baseline blood eNOS levels among patients with DR were 1.5–3.7 times lower compared to controls ($p < 0.001$), and decreased with the stage of DR. Receiver operating characteristic (ROC) curves of the models obtained showed strong associations (area-under-curve (AUC) = 0.77–0.88) of all endothelial dysfunction factors (with the exception of NOx) with the risk of fast DR progression. The univariate models involving EMAP-II, hs-CRP and IL-6 yielded the highest AUC values (> 0.8). Although the specificity of the univariate models involving EMAP-II, hs-CRP, eNOS and IL-6 exceeded 85%, their sensitivity was rather low. The multivariate model for predicting the risk of fast DR progression involved EMAP-II and eNOS, indicating a key role of these markers in determining the efficacy of treatment for DR. The discriminative ability of the model was adequate (AUC = 0.92 (95% CI, 0.86–0.96)), and its sensitivity and specificity were excellent (81.0% and 91.2%, respectively).*

Conclusion: The association of endothelial dysfunction markers with the outcome of treatment for DR was demonstrated. The model predicting the efficacy of treatment of DR in the Ukrainian population and involving the most significant endothelial dysfunction markers (EMAP-II and eNOS) was for the first time developed.

Keywords:

diabetic retinopathy, type 2 diabetes mellitus, endothelial dysfunction, EMAP-II, endothelial NO synthase, prognosis, modeling

Introduction

Strict glycemic control with intensive insulin therapy and individualized selection of antihyperglycemic medications are a mainstay of current treatment for diabetes mellitus (DM) [1]. Diabetes management requires constant monitoring and individualized adjustments in line with the latest achievements in science. The management of diabetic retinopathy (DR) has evolved considerably over the past two decades, with the availability of new technologies and treatment options including intravitreal anti-vascular endothelial growth factor (VEGF) and steroid injections, and laser therapy [2].

As requirements of diabetic patients in particular patient groups may vary, it is important that DR management is personalized in such groups. Glycemic and blood pressure control may slow the progression of early DR, whereas laser treatment and/or may reduce the loss of vision in late DR [3]. The 2017 Diabetic Retinopathy Guidelines from the Ministry of Health of Ukraine [4] regulate the use

of diagnostic techniques, routes of patients and basic methods of treatment including retinal laser photocoagulation and surgical interventions in this country. Additionally, the Adapted Clinical Guidelines on Type 2 Diabetes Mellitus (Ministry of Health Order No. 1118/2022) contain provisions related to ocular lesions [5]. Because these national guidelines do not contain detailed recommendations on the use of anti-VEGF agents or steroid implants, current international guidelines are used in local clinical practice. The 2025 American Academy of Ophthalmology (AAO) Preferred Practice Pattern (PPP) Guidelines for Diabetic Retinopathy recommends anti-VEGF agents (aflibercept, ranibizumab and bevacizumab) as a first-line therapy in center-involved diabetic macular edema (CI-DME) with vision loss [6]. Steroid implants (dexamethasone and fluocinolone) are used in diabetic macular edema (DME) eyes that (1) have a suboptimal response or no response to anti-VEGF therapy or (2) in which anti-VEGF agents are contraindicated. Focal or grid laser photocoagulation (FLP or GLP) surgery is recommended in non-CI-DME. Although panretinal laser photocoagulation (PRP) is still commonly used to manage proliferative DR (PDR), anti-VEGF therapy may be used as an alternative or additional treatment option that requires a more frequent monitoring [6].

The American Diabetes Association (ADA) Standards of Care in Diabetes-2025 (Section 12, Retinopathy) [7] focus on a multidisciplinary approach to the treatment, with a regular screening strategy (extended fundus examination and optical coherence tomography (OCT)), and strict control of glycated hemoglobin (HbA1c), blood pressure and lipid profile. Additionally, ophthalmologists consider fenofibrate for people with non-proliferative DR and type 2 diabetes to reduce the progression of DR [8]. The National Institute for Health and Care Excellence (NICE) Guideline on Diabetic Retinopathy Management and Monitoring (NG242, 2024) provide the details for monitoring frequency for DR depending on the stage of the condition, and algorithms for the selection of anti-VEGF therapy, laser photocoafulation and steroids [8]. This guideline recommends using current imaging techniques including ultra-wide-field fundus imaging. The 2017 Guidelines for the Management of Diabetic Macular Edema by the European Society of Retina Specialists (EURETINA) [9] confirmed the role of anti-VEGF therapy as the first-line therapy for CI-DME with vision loss and the role of LPC in non-CI-DME. The 2017 Guidelines on Diabetic Eye Care (The International Council of Ophthalmology Recommendations) [10] provide universal standards for managing diabetic eye patients while taking into account the level of national resources related to screening, timely referral for ophthalmologic care and major treatment options.

Therefore, current strategy of treatment for DR is based on a combination of anti-VEGF therapy, laser photocoagulation and surgical interventions depending on a particular case, in addition to an optimal control of systemic risk factors. However, despite wide introduction of current technologies, early and late complications and recurrences may occur after these interventions [11]. Precision medicine in diabetes is believed to help solving these problems, and is based on the idea that diabetic eye care should be tailored to each individual's health profile, which should take into account data of objective ophthalmic examination and comprehensive genetic and metabolic testing, with subsequent development of models predicting DR treatment response and disease progression [12]. The prognostic models available include demographic variables (such as age, sex, and ethnicity), smoking, diabetes duration, and serum HbA1c and lipoprotein levels [12, 13]. Most precision medicine approaches are, however, spuriously precise, overly complex and too narrowly focused on predicting blood glucose levels with a limited set of characteristics of individuals rather than the whole person and their context [14]. The addition of the biomarkers that directly mirror DR mechanisms is seen as an important way for improving the prognostic accuracy of these models.

Endothelial dysfunction, with damage to the vascular wall and reduced nitric oxid (NO) protection, is a major mechanism of the development and progression of DR [15]. Impaired metabolism and accumulation of reactive oxygen species (ROS) hampers endothelial nitric oxide synthase (eNOS) activity, which inhibits endothelium-mediated vasodilation [16]. Another consequence is triggering low-grade metabolic inflammation; markers of this inflammation include highly sensitive C-reactive protein (hs-CRP) and pro-inflammatory cytokines that have a positive correlation with DR severity [17]. Endothelial damage in DR reflects increased production of endothelin-1 (ET-1) in the vascular wall [18]. Another marker of endothelial dysfunction, endothelial-monocyte-activating polypeptide II (EMAP II), has pro-inflammatory and anti-angiogenic activities [19].

The purpose of this study was to develop a model for predicting the efficacy of treating DR in type 2 diabetes mellitus (T2DM) on the basis of determination of markers of endothelial dysfunction.

Material and Methods

This was a single-center, prospective cohort longitudinal study with a two-year observation period.

The procedures followed were in accordance with the ethical standards of the Helsinki declaration (1964, amended most recently in 2008) of the World Medical Association. The study was conducted in compliance with the requirements of the Council of Europe Convention on Human Rights and Biomedicine, and relevant laws of Ukraine. The study was approved by the Bioethics Committee of the Dnipro State Medical University.

Informed consent was obtained from all participants of the study. Inclusion criteria were 50-75-year-old male and female patients with T2DM and DR of any grade which were born and still residing in Ukraine. Exclusion criteria were patients with non-type 2 diabetes; a severe comorbid complication not related to T2DM; acute or chronic dental, maxillary, ear, nose, or throat disease; acute or chronic

infection, uveitis, congenital or acquired glaucoma; or history of ocular injury or surgery.

Totally, 136 patients with T2DM and DR were included in the analysis. Characteristics of the most affected eye were taken into account in ophthalmological studies. Patients were divided into three groups: group 1 of 60 eyes with mild non-proliferative DR (NPDR), group 2 of 42 eyes with preproliferative DR (PPDR), and group 3 of 34 eyes with PDR. Median age (interquartile range) was 65 (59–72) years for group 1, 64.5 (59–71) years for group 2, and 66 (61.75–71.25) years for group 3, with no significant difference between groups (Kruskal-Wallis test $p = 0.245$). The control group was composed of 25 non-diabetics of similar age and sex who were examined and treated for age-related cataract.

Patients were observed and treated with different methods over 2 years [20]. Patients of group 1 received conservative treatment including hypoglycemic medications, restoration of homeostasis and angioprotection. Additionally, they received fibrates and statins and metabolic therapy, if required. Groups 1 and 2 had laser treatment including PRP and, if required, focal FLP. Anti-VEGF therapy was used predominantly in groups 2 and 3 and included an intravitreal anti-VEGF injection once monthly. The treatment course consisted of five injections.

Patients began receiving PRP one month after the first aflibercept injection. Up to five PRP sessions were performed, with a subsequent PRP session administered only in the presence of ischemic areas. A 532-nm laser was used. Laser settings utilized pulse durations from 100 to 200 ms, pulse-to-pulse periods of 100 to 150 ms, and powers from 100 to 200 mW, to obtain medium-intensity grade 2 or 3 coagulation burns.

In group 3, surgical treatment included a 25-G three-port subtotal pars plana vitrectomy (PPV) plus PRP session, epiretinal membrane removal and endotamponade with 18% C3F8 or silicone oil 5700 centistokes, depending on the stage of the process.

Patients in groups 2 and 3 were administered a combination of intravitreal anti-VEGF medications, PRP and a 25-G three-port subtotal PPV.

Patients underwent eye examination including visual acuity assessment with a chart projector (CCP-3100; Huvitz Corp, Gunpo, Korea) and phoropter (HDR 7000, Huvitz Corp); static Humphrey perimetry (Humphrey Field Analyzer model 740i, Carl Zeiss Meditec Inc, Dublin, CA); refractometry with a autorefractor/ keretometer (HRK-700, Huvitz Corp); tonometry with a non-contact air-puff tonometer (HNT-7000, Huvitz Corp); corneal pachymetry with a biometer (Pentacam AXL, Oculus, Werzlar, Germany); slit-lamp biomicroscopy (SLM-2ER, Chongqing Kanghua Ruiming Science Technology Co, Ltd, Chongqing, China), gonioscopy with a Goldmann three-mirror lens (Ocular Instruments, Bellevue, WA, USA); ophthalmoscopy with Volk digital wide-field lens (Volk Optical, Mentor, OH) and Goldmann three-mirror lens (Ocular Instruments); and optical coherence tomography with RT-Vue RT-100 apparatus (Optovue Inc., Fremont, CA). Fundus photohraphy (TRS-NW7SF; TOPCON, Tokyo, Japan) and fluorescein angiography were performed, if indicated.

Central retinal thickness (CRT, μm) and central retinal volume (CRV, mm^3) were assessed.

No DR progression was defined as the stability of ophthalmological characteristics at two years. Slow DR progression was defined as the worsening of some ophthalmological characteristics at two years. Fast DR progression was defined as worsening from NPDR to PPDR, worsening from PPDR to PDR, or the worsening of the most ophthalmological characteristics at two years. Treatment effect was defined as no DR progression or slow DR progression at 2 years.

General clinical characteristics, carbohydrate and lipid metabolism characteristics and homeostasis characteristics were also assessed. We have reported previously on the analysis of the results obtained [21, 22].

In the current study, enzyme-linked immunosorbent assay (ELISA) was performed using enzyme immunoassays for hs-CRP kit (Monobind Inc., Lake Forest, CA, USA), Endothelin (ET)-1 kit (Peninsula Laboratories, San Carlos, CA), Endothelial-Monocyte Activating Polypeptide II protein (EMAP-II) kit (BioSource International, Invitrogen Corporation, Carlsbad, CA), endothelial nitric oxide synthase (eNOS) kit (BCM Diagnostics, USA), and IL-1β and IL-6 kits (Bender Medsystems, Vienna, Austria). Photometric measurements were performed on an ELISA plate reader (Stat Fax 303 Plus, Awareness Technology Inc, Palm City, FL). Blood nitric oxide levels were determined biochemically via nitric oxide metabolism end products, nitrites (NO2-) and nitrates (NO3-), using the acidic Griess reaction [23].

Statistical analyses were performed with EZR version 1.54 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical interface for R (The R Foundation for Statistical Computing, version 4.03, R Foundation for Statistical Computing, Vienna, Austria) [24].

A logistic regression analysis was performed to identify a set of input variables that are predictive of DR progression for a regression model [25]. Model classifications were developed using Statistica Neural Networks v. 4.0C (StatSoft Inc., Tulsa, OK). A method of linear neural network model classifications was employed for the analysis of the impact of input variables associated with the risk of DR progression [26]. A genetic algorithm was used in probabilistic classification models to select significant risk factors [27]. The area under curve (AUC) was calculated for each curve with 95% confidence intervals (CIs) and its associated sensitivity and specificity were calculated. The model was considered adequate if the AUC was significantly different from 0.5. Odds ratios (ORs) and their 95% CIs were calculated to assess the relative contribution of each independent variable.

Results

In this study, we analyzed the results of treating patients with DR based on baseline serum levels of endothelial dysfunction markers (Fig. 1). Baseline serum levels of all endothelial dysfunction markers (with the exception of eNOS) among patients with DR were 1.9–16.4 higher compared to controls ($p < 0.001$), and increased with the stage of DR. Baseline serum eNOS levels among patients with DR were 1.5–3.7 times lower compared to controls ($p < 0.001$), and decreased with the stage of DR. Baseline serum NOx levels among patients with DR were 1.3–3.14 higher compared to controls ($p < 0.001$), with no significant difference between stages of DR.

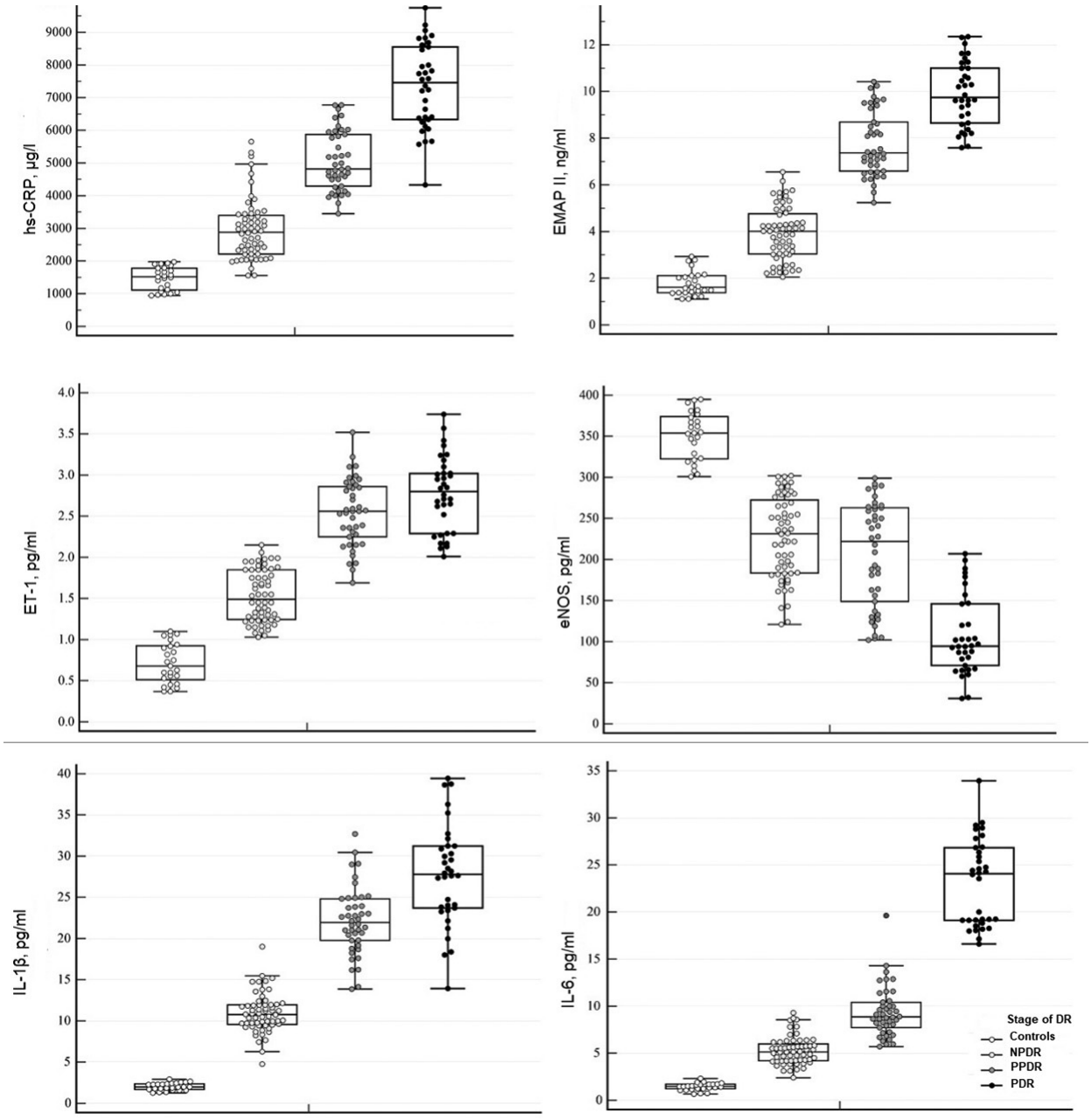


Fig. 1. Serum levels of endothelial dysfunction markers in patients with non-proliferative, pre-proiferative and proliferative diabetic retinopathy and controls. Box plots denote median (center line), first and third quartiles (boxes), and full range (whiskers). Circles denote levels of a particular marker in patients of the groups. All differences between groups were statistically significant ($p < 0.05$). DR, diabetic retinopathy; EMAP II, endothelial-monocyte-activating polypeptide II; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; IL-1β, interleukin 1β; hs-CRP, highly sensitive C-reactive protein; IL-6, interleukin 6; NPDR, non-proliferative diabetic retinopathy (group 1); PDR, proliferative diabetic retinopathy (group 3); PPDR, pre-proliferative diabetic retinopathy (group 2).

Table 1. Characteristics of univariate logistic regression models for predicting the risk of fast diabetic retinopathy progression

Independent variable	Model coefficient, b ± m	Significance of the odds ratio difference from 1, p	Odds ratio (95% confidence interval) for the model	Area-under-curve (95% confidence interval) for the model
hs-CRP, per 1000 μg/l	0.86 ± 0.15	<0.001	2.33 (1.74 – 3.14)	0.84 (0.77 – 0.90)
EMAP-II, per 1 ng/ml	0.69 ± 0.11	<0.001	2.00 (1.60 – 2.49)	0.88 (0.81 – 0.93)
ET-1, per 1 pg/ml	1.64 ± 0.33	<0.001	5.18 (2.69 – 9.98)	0.77 (0.69 – 0.84)
NOx, per 1 μMol/l	0.52 ± 0.28	0.061	1.68 (0.98 – 2.89)	0.59 (0.50 – 0.67)
eNOS, per 10 pg/ml	-1.83 ± 0.85	<0.001	0.16 (0.08 – 0.31)	0.79 (0.71 – 0.85)
IL-1β, per 10 pg/ml	1.44 ± 0.29	<0.001	4.22 (2.39 – 7.47)	0.78 (0.70 – 0.85)
IL-6, per 10 pg/ml	2.07 ± 0.47	<0.001	7.95 (3.16 – 20.0)	0.83 (0.76 – 0.89)

Note: AUC, area under the receiver operating characteristic curve; b ± m, model coefficient ± standard error; CI, confidence interval; OR, odds ratio; P, P-value of the odds difference from 1; *EMAP II*, endothelial-monocyte-activating polypeptide II; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; IL-1β, interleukin 1β; IL-6, interleukin 6

We have previously reported on the efficacy of treatment for different stages of DR in a cohort of 358 patients with DR and T2DM [20]. In the current study, we analyzed the relationships of endothelial dysfunction markers with the results of treatment for DR. A logistic regression analysis was performed to identify a set of input variables that are predictive of DR progression for a regression model [25]. Fast DR progression (Y = 1) or no fast DR progression (Y = 0) was used as a dependent variable. After treatment, 57 patients showed no DR progression or slow progression of DR (Y = 0), and 79 patients showed fast DR progression (Y = 1). Results of univariate analysis are presented in Table 1.

All independent variables (with the exception of NOx) were strongly associated with the dependent variable. Receiver operating characteristic (ROC) curves of the models obtained showed their strong associations (AUC=0.77–0.88) with the dependent variable Y (Fig. 2). This indicated the importance of the characteristics studied for the onset and progression of DR and established their capability of being used as biomarkers of DR.

The models involving EMAP-II, hs-CRP and IL-6 yielded the highest area-under-curve (AUC) values (> 0.8). The specificity of the models involving hs-CRP, EMAP-II, eNOS and IL-6 exceeded 85%, but the sensitivity of the models obtained was low, varying from 51.9% for eNOS to 81.0% for ET-1. This indicated the possibility for their use in practical studies was limited.

Building multivariate regression models seemed to be more promising for obtaining improved prediction accuracy. Multivariate regression models were built to identify a set of independent variables associated with fast DR progression, taking into account contributions from other risk factors [26]. A stepwise forward and backward selection was conducted for inclusion and exclusion of the potential risk factor (a value of $p < 0.1$ was used for inclusion and a value of $p > 0.2$, for exclusion).

In the current study, the analysis was conducted using the database of DR patients with available clinical, laboratory and eye examination data, which has been previously described by us [21, 22]. Thirty independent variables were employed in calculations: age, sex, diabetes duration, DR stage, presence of maculopathy and DME, type of hypoglycemic therapy, and serum levels of fasting glucose, HbA1c, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), triglycerides, fibrinogen, prothrombin and other coagulation factors, and endothelial dysfunction markers. Three independent variables (DR stage and serum EMAP II and eNOS levels) were selected by multivariate regression. A logistic regression model was built based on variables selected, and the discriminative ability of the model was adequate ($\chi^2 = 93.2$ for 4 degrees of freedom; $p < 0.001$). The characteristics of the multivariate regression model are presented in Table 2.

Therefore, while taking into account DR stage, only two of the parameters examined (high serum EMAP II level and low serum eNOS level) were associated with the outcome of treatment at two years. Figure 3 shows the ROC curve for the model.

The trivariate regression model for predicting the risk of fast DR progression yielded an AUC of 0.92 (95% CI, 0.86–0.96), indicating a well-fit model. For the optimal cut-off point associated with the Youden Index (Youden Index Criterion > 0.5921), the model sensitivity was 81.0% (95% CI, 70.6%–89.0%); specificity, 91.2% (95% CI, 80.7%–97.1%); prognostic significance of a positive outcome, 92.8% (95% CI, 84.6%–96.7%), and prognostic significance of a negative outcome, 77.6% (95% CI, 68.6%–84.6%).

After stratification of patients by treatment method, it was found that the model performed well in predicting fast DR progression for any treatment method (Fig. 4).

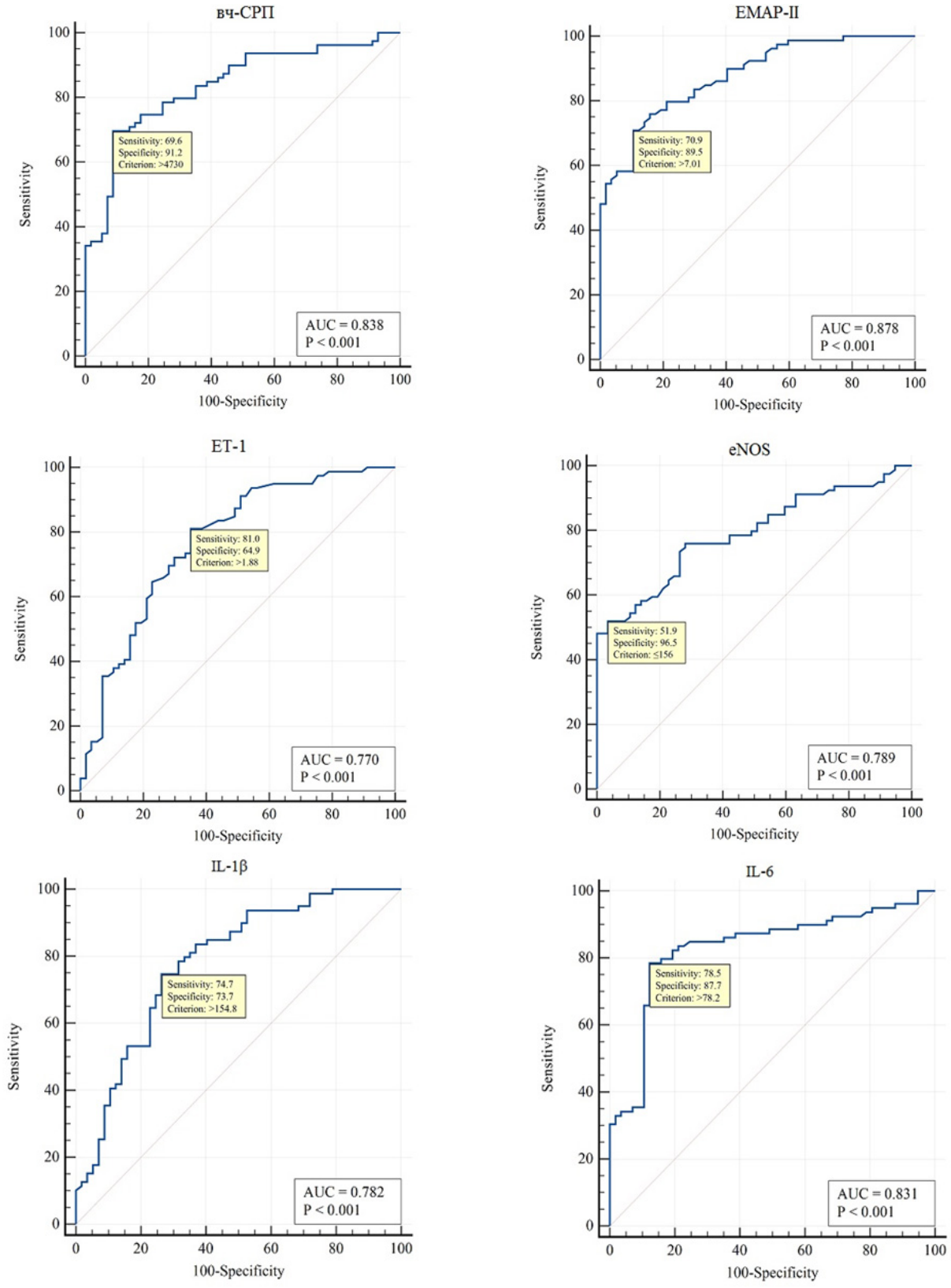


Fig. 2. Receiver operating characteristic (ROC) curve of the models for predicting the risk of fast diabetic retinopathy progression based on serum levels of markers of endothelium dysfunction. Note: AUC, area under the receiver operating characteristic curve; Criterion, calculated Y criterion of the model; p, P-value; DR, diabetic retinopathy; EMAP II, endothelial-monocyte-activating polypeptide II; eNOS, endothelial nitric oxide synthase; hs-CRP, highly sensitive C-reactive protein; ET-1, endothelin-1; IL-1β, interleukin 1β; IL-6, interleukin 6; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; PPDR, pre-proliferative diabetic retinopathy

Table 2. Characteristics of trivariate logistic regression model for predicting the risk of fast diabetic retinopathy progression

Independent variable		Model coefficient, b ± m	Odds ratio difference from 1, p	Odds ratio (95% confidence interval) for the model	Area-under-curve (95% confidence interval) for the model
Diabetic retino-pathy stage	NPDR	Reference			0.92 (0.86 – 0.96)
	PPDR	-1.56 ± 0.99	0.119	–	
	PDR	-6.02 ± 1.63	<0.001	0.002 (0.001 – 0.059)	
EMAP-II, per 1 ng/ml		1.41 ± 0.33	<0.001	4.10 (2.13 – 7.89)	
eNOS, per 10 pg/ml		-1.82 ± 0.62	0.003	0.16 (0.05 – 0.55)	

Note: AUC, area under the receiver operating characteristic curve; b ± m, model coefficient ± standard error; CI, confidence interval; OR, odds ratio; P, P-value of the odds difference from 1; *EMAP II*, endothelial-monocyte-activating polypeptide II; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; IL-1β, interleukin 1β; IL-6, interleukin 6; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; PPDR, pre-proliferative diabetic retinopathy

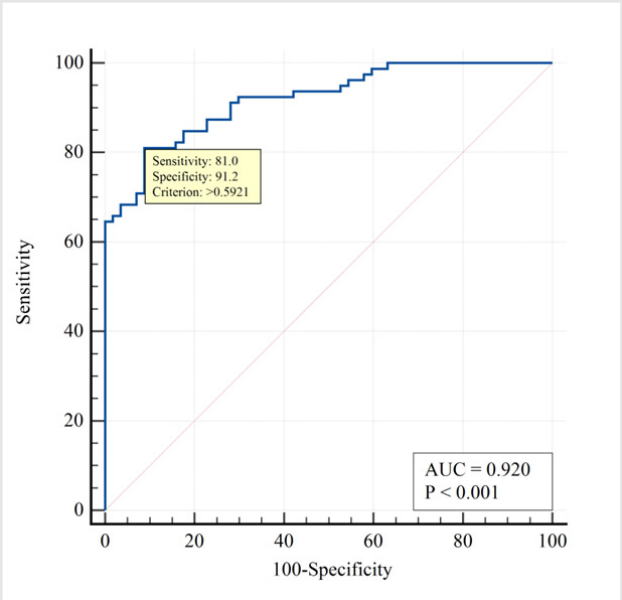
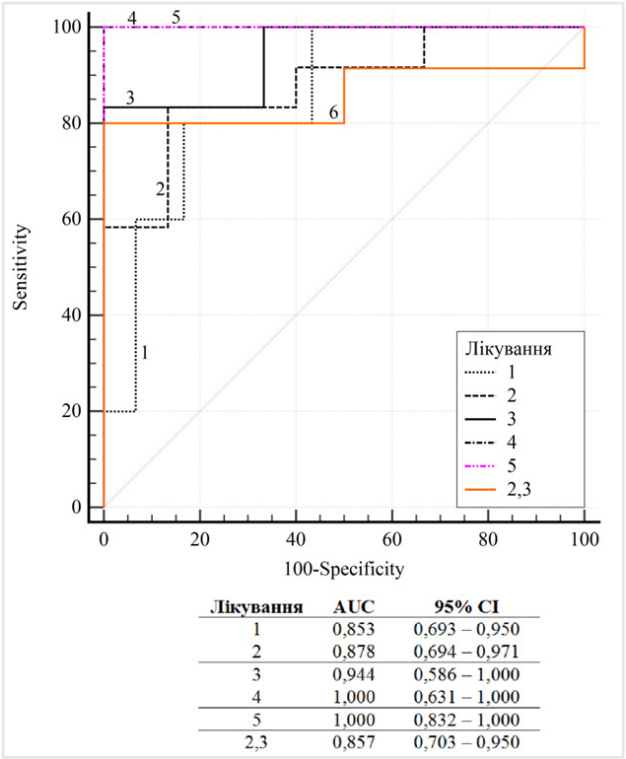


Fig. 3. Receiver operating characteristic (ROC) curve of the trivariate model for predicting the risk of fast diabetic retinopathy progression. Note: AUC, area under the receiver operating characteristic curve; Criterion, calculated Y criterion of the model; p, P-value.



Лікування	AUC	95% CI
1	0,853	0,693 – 0,950
2	0,878	0,694 – 0,971
3	0,944	0,586 – 1,000
4	1,000	0,631 – 1,000
5	1,000	0,832 – 1,000
2,3	0,857	0,703 – 0,950

Fig. 4. Receiver operating characteristic (ROC) curve of the trivariate model for predicting the risk of fast diabetic retinopathy progression in patients treated with various treatment options. Note: AUC, area under the receiver operating characteristic curve; Criterion, calculated Y criterion of the model; p, P-value; methods of treatment: 1, conservative treatment; 2, laser treatment; 3, anti-vascular endothelial growth factor (VEGF) therapy; 4, surgery; 5, anti-VEGF therapy combined with laser treatment and surgery; 2,3, anti-VEGF therapy combined with laser treatment.

The 3-variate models for predicting fast DR progression yielded an AUC of 0.85 to 1.0, indicating that the fit of the models developed was excellent. This was evidence that the predictors found (high serum EMAP II level and low serum eNOS level) are biomarkers of a high risk of fast DR progression at two years of treatment, i.e., actually, the predictors of an unfavorable treatment outcome.

Discussion

The close relationship between endothelial dysfunction characteristics, DR progression and the outcome of DR treatment at two years demonstrated they are capable of being used as biomarkers. Thus, we found a progressive increase in the serum hs-CRP level with an increase in DR stage, which is consistent with the findings of a large meta-analysis [28]. It has been also found that hs-CRP predicts microvascular complications of T2DM and is directly associated with pro-inflammatory and angiogenic factors [29, 30].

Our findings of high serum ET-1 levels in T2DM and their association with vascular complications are also in agreement with findings of other studies [31, 32]. Niranjan and colleagues [33] found plasma ET-1level to be a prognostic marker of PDR. Low-grade inflammation plays a critical role in the pathogenesis of DR as multiple inflammatory factors, such as IL-1β, IL-6, IL-8 and tumor necrosis factor-α, are increased in the vitreous and retina of DR patients [34]. IL-1β accumulation results in pericyte and capillary endothelial damage, leading to retinal capillary degeneration [35]. In a clinical study on patients with T2DM and with or without DR, Feng and colleagues [36] concluded that the aqueous levels of IL-1β, IL-6, and IL-8 may be associated with the pathogenesis, severity, and prognosis of DR. This is in agreement with our findings. Meta-analyses of prospective studies indicated that elevated circulating IL-6 and IL-1β had predictive value for diabetes and the overall Hazard Ratio (HR) of T2DM was 1.28 (95 % CI: 1.17, 1.40; P<0.001) per 1 log pg/ml increment in IL-6 levels [37]. Additionally, the increased level of IL-6 was significantly associated with several diabetic complications [37].

In the current study, the risk of fast DR progression was four times increased in patients with a high serum IL-1β level and eight times increased in those with a high serum IL-6 level (Table 1), thus confirming the value of IL-1β and IL-6 in the pathogenesis of DR. Additionally, of all the endothelial dysfunction markers examined, only EMAP-II and eNOS were found to be biomarkers of a high risk of fast DR progression. There is a direct relationship between EMAP-II and the parameters of carbohydrate and lipid metabolism in diabetic patients [22], and elevated serum EMAP-II levels were found to influence microvascular complications [38]. Singling out this parameter by building a trivariate regression model in the current study indicated (1) an independent effect of EMAP-II on fast DR progression and (2) its capability of being used as a biomarker of DR progression.

Our findings confirmed the importance of the depletion of eNOS that is expressed in endothelial cells, keeps blood vessels dilated, controls blood pressure, and has numerous other vasoprotective and anti-atherosclerotic effects [39]. Under chronic hyperglycemic conditions, an excess of NO formed by inducible NOS from the effect of inflammatory mediators interacts with ROS, producing highly toxic peroxynitrite that mediates retinal cell apoptosis [16, 41]. Impaired eNOS activity results in reduced NO bioavailability, increased oxidative stress and endothelial dysfunction, which has been considered a mechanism for the development of microvascular lesions in DR [41].

Studies noted the role of eNOS in the pathogenesis of DR, although did not identify it as a well established clinical biomarker of DR [42]. In the current study, the regression models built confirmed the role of eNOS depletion and its use as a prognostic biomarker of the late outcome of treatment of DR.

Therefore, we demonstrated that most of the predictors of endothelial dysfunction studied were associated with treatment results, which indicated the important role of these predictors in the mechanisms of pathogenesis of DR. However, low sensitivity of the univariate models obtained has limited their use in clinical practice. Multivariate regression found that serum levels of EMAP-II and eNOS remained significant independent risks factors for unfavorable late outcome of any method of treatment we used for DR. Therefore, based on our clinical studies, the model predicting the efficacy of treatment of DR in the Ukrainian population and involving endothelial dysfunction markers was for the first time developed.

Conclusion

First, baseline serum levels of all endothelial dysfunction markers (with the exception of eNOS) among patients with both T2DM and DR were 1.9–16.4 higher compared to controls ($p < 0.001$), and increased with the stage of DR. Baseline serum eNOS levels among patients with DR were 1.5–3.7 times lower compared to controls ($p < 0.001$), and decreased with the stage of DR.

Second, ROC curves of the models obtained showed strong associations (AUC=0.77–0.88) of all endothelial dysfunction factors (with the exception of NOx) with the risk of fast DR progression at two years. The models involving EMAP-II, hs-CRP and IL-6 yielded the highest AUC values (> 0.8).

Third, the multivariate model for predicting the risk of fast DR progression involved EMAP-II and eNOS, indicating a key role of these markers in determining the efficacy of treatment for DR. The discriminative ability, sensitivity and specificity of the model were excellent (AUC = 0.92 (95% CI, 0.86–0.96), 81.0% and 91.2%, respectively). The model performed well when predicting the risk of fast DR progression for any method of treatment, with the AUC ranging from 0.85 to 1.0.

Finally, the model predicting the efficacy of treatment of DR in the Ukrainian population and involving endothelial dysfunction markers was for the first time developed.

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Abbreviations: *AUC, area under curve; CI, confidence interval; DME, diabetic macular edema; DM, diabetic mellitus; DR, diabetic retinopathy; EMAP II, endothelial-monocyte-activating polypeptide II; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; HbA1c, glycated hemoglobin; hs-CRP, highly sensitive C-reactive protein; IL-1β, interleukin 1β; IL-6, interleukin 6; NO, nitric oxide; NOx, nitric oxide metabolites; NPDR, non-proliferative diabetic retinopathy; OCT, optical coherence tomography; PDR, proliferative diabetic retinopathy; PPDR, preproliferative diabetic retinopathy; PRP, panretinal laser photocoagulation*