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Prognostic biomarkers of non-proliferative diabetic retinopathy progression in type 2 diabetes mellitus

S. Iu. Mogilevskyy [1], A. V. Serdiuk [2], S. V. Ziablitsev [3]

[1] Shupyk National Healthcare University of Ukraine
Kyiv (Ukraine)

[2] Dnipro State Medical University, Ministry of Health of Ukraine
Dnipro (Ukraine)

[3] Bogomolets National Medical University
Kyiv (Ukraine)

Purpose: *To establish prognostic biomarkers of non-prolefarative diabetic retinopathy (NPDR) progression in T2DM on the basis of examination of clinical, ophthalmological and laboratory (carbohydrate and lipid metabolism and coagulation and hemostasis) characteristics.*

Methods: *This study included 358 T2DM2 patients with diabetic retinopathy (DR); for each patient, the eye with more severe DR was included in the study. Cases were dichotomized into three groups based on DR severity: group 1 (NPDR; 189 eyes), group 2 (preproliferative DR or PPDR; 96 eyes), and group 3 (proliferative DR or PDR; 73 eyes). Central retinal thickness (CRT; μm) and central retinal volume (CRV) were assessed by optical coherence tomography (OCT; mm3). Serum samples were taken to assess fasting serum glucose, glycosylated hemoglobin (HbA1c), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), triglycerides, and fibrinogen. Coagulation and hemostasis parameters were assessed. Statistical analyses were performed with EZR version 1.54.*

Results: *Associations of the variables with the risk of NPDR progression were confirmed by a univariate logistic regression analysis. The area under curve (AUC) was largest for HbA1c (AUC = 0.84), followed by CRT and CRV (AUC = 0.79). In addition, the AUC ranged from 0.56 to 0.67 for the rest of variables. Age, gender, diabetes duration, and blood HDL level were not associated with NPDR progression. Our multivatiate logistic regression analysis found six biomarkers (age, HbA1c, LDL, VLDL, prothrombin index and CRT) directly associated with the risk of NPDR progression (AUC = 0.91; $p < 0.001$). The established association was mostly due to the three independent variables, HbA1c, VLDL and CRT (AUC = 0.90; $p < 0.001$).*

Conclusion: *Independent effects on the risk of NPDR progression were confirmed for HbA1c, VLDL and CRT. The built model was found to be of very high predictive quality, which allowed recommending it for confirming high risk for NPDR progression in equivocal clinical cases or as a criterion for conclusive prognosis in the relevant expert systems.*

Keywords:

type 2 diabetes mellitus, diabetic retinopathy, prognosis, modeling, precision medicine, biomarkers, HbA1c, lipoproteins, blood coagulation

Introduction

Diabetes mellitus (DM) is a global endemic. According to the International Diabetes Federation Diabetes Atlas, 9th edition, 9.3% of adults aged 20-79 years – a staggering 463 million people – were living with diabetes in 2019 [1]. Moreover, there was an estimated 374 million people with impaired glucose tolerance. The global diabetes prevalence was estimated to rise to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 [2].

Diabetic retinopathy (DR) is a common microvascular diabetic complication and affects 2 in 3 individuals with diabetes [3]. The incidence of DR is associated with an increased risk of other vascular complications including stroke, cardiac insufficiency, nephropathy and neuropathy [2, 3]. Among individuals with diabetes, global prevalence was 22.27% for DR, 6.17% for vision-threatening DR, and 4.07% for clinically significant macular edema [4].

Although an increasing number of prognostic models have been developed and validated to predict diabetic complications, the concept of precision prognosis is as a component of precision medicine is still in its infancy. Epidemiological and clinical research could inform its further development [5, 6]. The risk of DR progression in T2DM depends on multiple factors including age, male gender, diabetes duration, diabetic nephropathy and neuropathy, fasting blood glucose, total cholesterol, triglyceride, and glycosylated hemoglobin (HbA1c) [7, 8]. In addition, the risk of DR progression in T2DM is associated with high blood pressure [9].

Therefore, predicting the development and progression of DR on the basis of clinical, ophthalmological and laboratory predictors (biomarkers) is an important task.

The purpose of this study was to establish prognostic **b**iomarkers of non-prolefarative diabetic retinopathy (NPDR) progression in T2DM on the basis of examination of clinical, ophthalmological and laboratory (carbohydrate and lipid metabolism and coagulation hemostasis) characteristics.

Methods

This study included 358 T2DM2 patients with DR; for each patient, the eye with more severe DR was included in the study. Cases were dichotomized into three groups based on DR severity: group 1 (NPDR; 189 eyes), group 2 (preproliferative DR or PPDR; 96 eyes), and group 3 (proliferative DR or PDR; 73 eyes). The median age (interquartile range (IQR)) for groups 1, 2 and 3 was 65 years (59-72), 64.5 years (59-71) and 66 years (61.75-71.25), respectively, but the Kruskal-Wallis difference between groups was not significant ($p = 0.245$). The patient sample consisted of 185 males (51.7%) and 173 (48.3%), and there was no difference between groups in gender ($p = 0.685$).

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 Shupyk National Healthcare University of Ukraine. This was a prospective, randomized cohort study. Informed consent was obtained from all participants of the study.

Patients underwent eye examination including visual acuity assessment with a chart projector (CCP-3100; Huvitz Corp, Gunpo, Korea) and autorefractor/ keretometer (HRK-700, 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 a Goldmann three-mirror lens (Ocular Instruments); and optical coherence tomography with RTVue 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.

Serum samples were taken to assess fasting serum glucose (mMol/l), HbA1c (%), total cholesterol (mMol/l), high-density lipoprotein (HDL; mMol/l), low-density lipoprotein (LDL; mMol/l), very low-density lipoprotein (VLDL; mMol/l), triglyceride (mMol/l), and fibrinogen (mg/dl). Measurements were performed using commercial kits from Roche Diagnostics on a clinical chemistry analyzer (Roche Diagnostics, Mannheim, Germany).

Coagulation and hemostasis parameters (blood activated recalcification time (BART, sec), prothrombin time (sec), prothrombin index (%), Quick prothrombin index (%), international normalized ratio (INR, units), activated partial thrombin time (aPTT, sec), and thrombin time (sec)) were measured by standardized laboratory techniques [10, 11].

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) [12]. Non-normally distributed data were presented as median and IQR. For comparison of more than two groups, Kruskal-Wallis test with Dunn's post-hoc analyses was used. The level of significance $p \leq 0.05$ was assumed. A logistic regression analysis was performed to identify a set of input variables that are predictive of DR progression for a regression model [13]. 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

There was no significant difference between groups in age or diabetes duration (Table 1; $p > 0.2$). There was, however, a small but significant difference between groups in systolic blood pressure (SBP) and diastolic blood pressure (DBP) ($p < 0.02$). No significant difference in SBP was observed between the PPDR and PDR groups ($p > 0.05$), but these groups showed higher SBP values compared to the NPDR group ($p < 0.05$).

Fasting blood glucose level was substantially higher than the norm in all patients, especially those with PDR (Table 1). A significant difference ($p < 0.05$) in HbA1c between the groups was observed, and we found a tendency to a gradual increase in HbA1c with an increase in the severity of DR.

There was a statistically significant difference in lipid metabolism between group 1 and group 2 and between group 1 and group 3 ($p < 0.05$), but not between group 2 and group 3 ($p > 0.05$; Table 1). In groups 2 and 3, total cholesterol, HDL, LDL, VLDL and triglyceride levels were 1.05 to 1.19 times higher than in group 1 ($p < 0.01$).

Coagulation characteristics tended to increase with DR severity, too (Table 1). In group 3, BART and prothrombin time were larger than in groups 1 and 2. In addition, prothrombin index, Quick prothrombin index, and INR (the prothrombin-time derivatives which characterize the external pathway of plasma hemostasis activation [11]) tended to gradually increase with DR severity. There

Table 1. Characteristics under study in groups of patients with diabetic retinopathy (median (interquartile range))

Characteristic	Groups of patients			p
	Group 1 (NPDR)	Group 2 (PPDR)	Group 3 (PDR)	
Age, years	65 (59–72)	64.5 (59–71)	66 (61.75–71.25)	0.245
T2DM duration, years	14 (9–18)	14 (10.5–18)	15 (12–19)	0.365
SBP, mmHg	145 (135–160)[2,3]	150 (140–167.5)[1]	150 (140–165)[1]	0.004
DBP, mmHg	90 (80–100)	90 (82.5–100)	90 (85–100)	0.018
Glucose, mMol/l	10.25 (7.795–14.1)[3]	10.7 (8.863–14.935)	13.5 (8.915–18.1)[1]	0.004
HbA1c, %	7.9 (7.3–8.8)[2,3]	9,5 (8.4–10.9)[1,3]	11.2 (10.1–11.5)[1,2]	<0.001
Total cholesterol, mMol/l	5.71 (5.18–6.18)[2,3]	6.055 (5.46–6.72)[1]	6.27 (5.49–7.15)[1]	<0.001
HDL, mMol/l	0.54 (0.45–0.61)[3]	0.565 (0.47–0.665)	0.61 (0.505–0.71)[1]	0.006
LDL, mMol/l	4.95 (4.758–5.280)[2,3]	5.22 (4.9–5.755)[1]	5.31 (4.882–5.835)[1]	<0.001
VLDL, mMol/l	0.95 (0.84–1.13)[2,3]	1.11 (0.91–1.24)[1]	1.13 (0.92–1.312)[1]	<0.001
Triglycerides, mMol/l	3.29 (3.16–3.545)[2,3]	3.54 (3.24–3.74)[1]	3.55 (3.218–3.832)[1]	<0.001
BART, sec	65 (61–71)[3]	67 (63–73.5)[3]	71 (62.75–78)[1,2]	<0.001
Prothrombin time, sec	12.5 (11.7–13.2)[3]	12.6 (11.85–14.1)	13.2 (12.2–14.325)[1]	<0.001
Prothrombin index, %	91 (85–97.25)[2,3]	97.5 (89–107)[1]	99 (88–115.25)[1]	<0.001
Quick prothrombin index, %	94 (81–109.5)[2,3]	105 (84–132)[1]	117 (90.5–135)[1]	<0.001
INR, units	1.75 (1.49–1.99)	1.84 (1.572–2.545)	1.81 (1.368–2.347)	0.082
aPTT, sec	29 (27–33)	30.5 (27.5–33)	29 (27.75–32)	0.491
Fibrinogen, mg/div	411 (388.75–432)[2,3]	425.5 (394.5–463)[1]	435 (401–471.5)[1]	<0.001
Thrombin time, sec	21 (17–26)[2,3]	24 (18–31)[1]	27 (19–35)[1]	<0.001
CRT, μm	239 (209–254)[2,3]	309 (252–457.5)[1]	447 (250–589)[1]	<0.001
CRV, mm^3	0.188 (0.165–0.2)[2,3]	0.242 (0.198–0.374)[1]	0.349 (0.2–0.458)[1]	<0.001

Note: Kruskal-Wallis test with Dunn's post-hoc analyses was used for comparison: 1, significant difference from group 1, $p < 0.05$; 2, significant difference from group 2, $p < 0.05$; 3, significant difference from group 3, $p < 0.05$. aPTT, activated partial thrombin time; BART, blood activated recalcification time; CRT, central retinal thickness; CRV, central retinal volume; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin; HDL, high-density lipoproteins; INR, international normalized ratio; LDL, low-density lipoproteins; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus; VLDL, very low-density lipoproteins

was, however, no significant change in aPTT (which characterizes the internal pathway of plasma hemostasis activation) with an increase in DR severity ($p = 0.491$). There was no significant difference in serum fibrinogen between groups 2 and 3, but these groups exhibited higher serum fibrinogen compared to group 1 ($p < 0.001$). Thrombin time characterizes the transformation of fibrinogen into fibrin and its stabilization, and clearly tended to increase with DR severity (Table 1; $p < 0.001$). Therefore, there was a small but statistically significant tendency for coagulation characteristics to increase with DR progression, which could indicate a certain deficiency in blood clotting factors.

OCT-based CRT and CRV were found to increase almost twice with DR progression from NPDR to PDR, and this increase was statistically significant (Table 1; $p < 0.001$).

Therefore, group 1 (NPDR) showed a significant difference in most parameters compared to groups 2 (PPDR) and 3 (PDR). This allowed analyzing separately associations of studied parameters with NPDR progression, and logistic regression models were built for the analysis [13]. The presence of (PPDR or PDR; Y = 1) or absence of (PPDR or PDR) (i.e., the presence of NPDR; Y = 0) was used as a dependent variable. One hundred and sixty nine patients had PPDR of PDR (Y = 1), and 189 patients had NPDR (Y = 0). Results of univariate analysis are presented in Table 2.

As expected, age and diabetes duration were not associated with NPDR progression ($p = 0.512$ and $p = 0.339$, respectively). Gender, HDL and aPTT were also not associated with NPDR progression ($p = 0.724$, $p = 0.403$, and $p = 0.709$, respectively).

Most other variables were positively associated with NPDR progression, but they differed in their contributions to NPDR progression (Table 2). The AUC was largest for HbA1c (AUC = 0.84), followed by CRT and CRV (AUC = 0.79). In addition, the AUC ranged from 0.56 (INR) to 0.67 (LDL and VLDL) for the rest of variables.

Table 2. Characteristics of univariate logistic regression models for predicting non-proliferative retinopathy (NPDR) progression

Input variable		Model coefficient, b ± m	Significance of the OR difference from 1, p	OR (95% CI) for the model	AUC (95% CI)
Gender	F	Reference value			–
	M	0.07±0.21	0.724	–	
Age, years		0.015 ± 0.012	0.512	–	–
Diabetes duration, years		0.015 ± 0.016	0.339	–	–
SBP, mmHg		0.019 ± 0.006	0.002	1.02 (1.01 – 1.03)	0.60 (0.55–0.65)
DBP, mmHg		0.025 ± 0.010	0.011	1.03 (1.01 – 1.05)	0.58 (0.53–0.64)
Glucose, mMol/l		0.074 ± 0.024	0.002	1.08 (1.03 – 1.13)	0.59 (0.53–0.64)
HbA1c, %		0.96 ± 0.10	<0.001	2.61 (2.15 – 3.16)	0.84 (0.80–0.88)
Total cholesterol, mMol/l		0.7 ± 0.14	<0.001	2.02 (1.53 – 2.6)	0.66 (0.60–0.70)
HDL, mMol/l		0.29±0.34	0.403	–	–
LDL, mMol/l		0.80 ± 0.20	<0.001	2.23 (1.52 – 2.28)	0.67 (0.62–0.72)
VLDL, mMol/l		2.72 ± 0.52	<0.001	15.16 (5.49 – 41.9)	0.67 (0.61–0.71)
Triglycerides, mMol/l		0.56±0.21	0.008	1.75 (1.16 – 2.63)	0.66 (0.60–0.71)
BART, sec		0.062 ± 0.015	<0.001	1.06 (1.03 – 1.10)	0.63 (0.57–0.68)
Prothrombin time, sec		0.47 ± 0.11	<0.001	1.61 (1.29 – 1.99)	0.62 (0.57–0.67)
Prothrombin index, %		0.065 ± 0.011	<0.001	1.07 (1.04 – 1.09)	0.66 (0.61–0.71)
Quick prothrombin index, %		0.028 ± 0.005	<0.001	1.03 (1.02 – 1.04)	0.65 (0.60–0.70)
INR, units		0.55±0.19	0.004	1.73 (1.19 – 2.53)	0.56 (0.50–0.61)
aPTT, sec		0.012 ± 0.033	0.709	–	–
Fibrinogen, mg/div		0.008 ± 0.002	<0.001	1.01 (1.00 – 1.01)	0.64 (0.59–0.69)
Thrombin time, sec		0.094 ± 0.018	<0.001	1.10 (1.09 – 1.14)	0.65 (0.60–0.70)
CRT, μm		0.011 ± 0.001	<0.001	1.012 (1.008–1.015)	0.79 (0.75–0.83)
CRV, mm^3		1.20 ± 0.16	<0.001	3.33 (2.43 – 4.55)	0.79 (0.74–0.83)

Note: aPTT, activated partial thrombin time; BART, AUC, area under the receiver operating characteristic curve; blood activated recalcification time; CI, confidence interval; CRT, central retinal thickness; CRV, central retinal volume; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin; HDL, high-density lipoproteins; INR, international normalized ratio; LDL, low-density lipoproteins; OR, odds ratio; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus; VLDL, very low-density lipoproteins

These results confirmed the high value of most of the examined variables as possible predictors (biomarkers) of NPDR progression. It was, however, necessary to determine the most significant contributors to NPDR progression, taking into account interactions of all possible predictors. Multivariate regression models were built to identify a set of independent variables associated with NPDR progression, taking into account contributions from other factors [13]. A stepwise forward and backward selection was conducted for inclusion and exclusion of the potential risk factor (a value of $p < 0.05$ was used for inclusion and a value of $p > 0.1$, for exclusion).

Six independent variables (age, HbA1c, LDL, VLDL, prothrombin index and CRT) were selected by multivariate regression. A logistic regression model was built based on selected variables, and the model fit was adequate ($\chi^2 = 225.3$ for 6 degrees of freedom; $p < 0.001$). The results of the multivariate regression model are presented in Table 3.

All independent variables were positively associated with the risk of NPDR progression. Figure 1 shows the ROC curve for the model.

The six-variate regression model for predicting the risk of NPDR progression yielded an AUC of 0.91 (95% CI, 0.88–0.94), indicating a strong association with selected predictors. For the optimal cut-off point associated with the Youden Index (Youden Index Criterion > 0.3723), the model sensitivity was 87.0% (95% CI, 81.0%–91.7%), and specificity, 83.1% (95% CI, 76.9%–88.1%). Therefore, the built model was found to be of very high predictive quality, which allowed recommending it for confirming high risk for NPDR progression in equivocal clinical cases or as a criterion for conclusive prognosis in the relevant expert systems.

Table 3. Characteristics of multivariate logistic regression model for predicting non-proliferative retinopathy (NPDR) progression

Independent variable	Model coefficient, b ± m	Significance of the OR difference from 1, p	OR (95% CI) for the model
Age, years	0.041 ± 0.019	0.031	1.04 (1.00 – 1.08)
HbA1c, %	0.90 ± 0.12	< 0.001	2.46 (1.94 – 3.11)
LDL, mMol/l	0.55 ± 0.26	0.033	1.73 (1.04 – 2.85)
vLDL, mMol/l	2.04 ± 0.74	0.006	7.72 (1.80 – 33.2)
Prothrombin index, %	0.043 ± 0.018	0.016	1.04 (1.01 – 1.08)
CRT, mm	0.010 ± 0.002	< 0.001	1.010 (1.00–1.014)

Note: CRT, central retinal thickness; HbA1c, glycosylated hemoglobin; LDL, low-density lipoproteins; OR, odds ratio; VLDL, very low-density lipoproteins

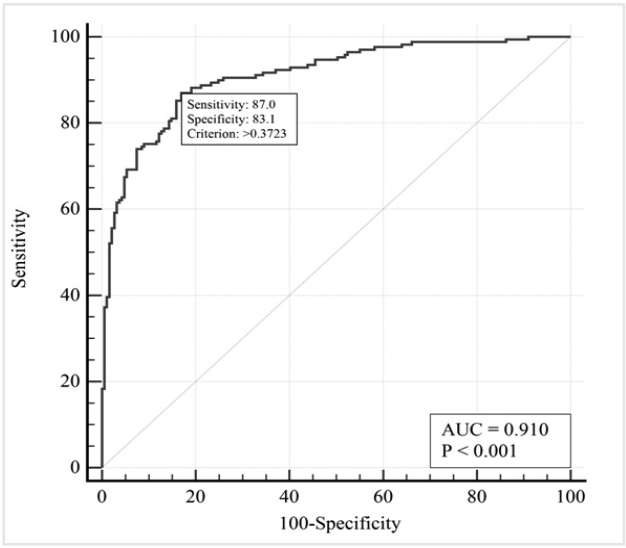

Fig. 1. Receiver operating characteristic (ROC) curve of the six-variate model for predicting the risk of NPDR progression. Note: AUC, area under the receiver operating characteristic curve; Criterion, calculated Y criterion of the model; p, significant difference

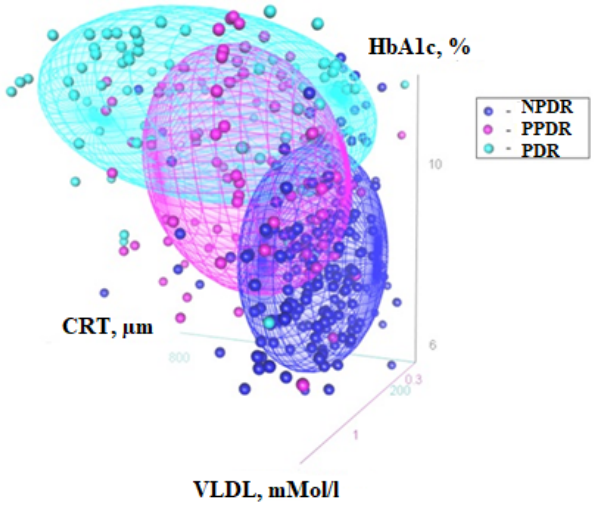

Fig. 2. Distribution of patients with different stages of DR in the three-dimensional (HbA1c (%), VLDL (mmol/l), CRT (µm)) system. Note: CRT, central retinal thickness; HbA1c, glycosylated hemoglobin; VLDL, very low-density lipoproteins

It should be noted that the established association was mostly due to HbA1c, VLDL and CRT. The model built on these predictors yielded an AUC of 0.90 (95% CI, 0.87–0.93), with predictive quality comparable to that of the six-variate regression model. Figure 2 shows the scatter field for patients with different DR stages in a three-dimensional system (HbA1c (%); VLDL (mMol/l); CRT (mm)).

Of note that NPDR patients mostly had small HbA1c and CRT values, whereas PDR patients mostly had large HbA1c and CRT values. PPDR patients had an intermediate position in the three-dimensional system.

Therefore, the current study allowed identifying relationships of biomarkers (like blood pressure, carbohydrate and lipid metabolism, and blood clotting characteristics) with the risk of NPDR progression. Six independent variables (age, HbA1c, LDL, VLDL, prothrombin index and CRT) were found to have independent effects on the risk of NPDR progression in a multivariate regression model, and the established association was mostly due to HbA1c, VLDL and CRT.

Discussion

The concept of precision medicine has been substantially expanded by options determining biological variations of humans through the assessment of genetic and metabolic heterogeneity, assessment of physical examination findings, and building prognostic models that take into account detailed data on the way of life and environmental effects [14].

Most prognostic models for DR include demographic characteristics (age, gender, and ethnicity), smoking, HbA1c and diabetes duration [5, 14]. Increasing the age of participants, longer duration of diabetes and co-morbid hypertension were independent predictors of microvascular complications in T2DM [15]. Our univariate regression analysis found no significant differences in age, gender or

diabetes duration between T2DM patients with different stages of DR. This finding was expected given the absence of significant difference in these characteristics between patients of different groups ($p > 0.1$). Age, nevertheless, was a predictor included in our six-variate logistic regression model for predicting NPDR progression; therefore, it is likely that age is mildly, but positively associated with NPDR progression.

Diabetes duration, however, showed no association with NPDR progression in our sample of patients with T2DM. Patients included in this study had a rather long diabetes duration ranging between 9–19 years. It is likely that the pathological changes determining NPDR progression have accumulated in the retina over such a long period. We found that these changes included those in several laboratory characteristics associated with abnormal carbohydrate and lipid metabolism and abnormal hemostasis.

DM is diagnosed in the presence of hyperglycemia which contributes to the development of microvascular complications in the organs and tissues of the body [2]. Hyperglycemia, in turn, results from numerous pathophysiological processes developing over many years and involving almost all body systems. It is however, not blood glucose, but a more stable characteristic, HbA1c, that is recommended for making a precision diagnosis of DM [14]. The level of glycosylated hemoglobin depends on the factors affecting hemoglobin stability and erythrocytes, and the level of mean glycemia which may vary among patients with DM [16].

Most available prognostic models confirmed a positive association of an elevated HbA1c level with the onset and progression of DR (e.g., NPDR) [17, 18]. Our findings in a rather large sample of T2DM patients with different stages of DR also confirmed this tendency. It is interesting that it is HbA1c level that was found by our univariate analysis of associations of the studied parameters with NPDR progression to have the largest AUC.

A review by Liu and colleagues [19] included DR-related studies involving intensive glucose control with large sample size and long follow-up time. The found that individuals who have DR lesions that are equivalent or less severe that moderate NPDR achieve benefits for the retina by intensive glycemic control. In addition, if the severity of DR lesions is worse than moderate NPDR, intensive glycemic control may not bring benefits. This in agreement with our finding that elevated blood HbA1c level is a major biomarker of the risk for NPDR progression.

Hypertension and hyperlipidemia are believed to be other traditional risk factors, in addition to diabetes duration and elevated blood HbA1c level, for NPDR progression [20]. We found the risk for NPDR progression to be positively associated with SBP (OR = 1.02; 95% CI, 1.01-1.03) and DBP (OR = 1.03; 95% CI, 1.01-1.05), which is in agreement with the findings of others [8, 9].

Srinivasan and colleagues [21] aimed to elucidate the influence of serum lipid control on the incidence a progression of DR in subjects with T2DM. Poor control of total cholesterol was associated with the incidence of sight-threatening DR (OR = 7.2, $p = 0.012$), poor control of triglycerides was associated with progression to PDR (OR = 3.2; $p = 0.048$). The authors [21] concluded that poor lipid control is a risk factor for incidence of and progression to late stages of DR. In a study by Alattas and colleagues [22], triglyceride levels, SBP, LDL and presence of macular edema were significantly associated with DR progression ($p < 0.05$). The following idea seems quite reasonable in this regard [23]: higher risk of polyneuropathy in T2DM is associated with a certain cluster of interrelated factors (including obesity, hypertension, reduced HDL levels, inflammation, and insulin resistance), which affects fatty acid metabolism and underpins the onset and progression of DR, diabetic kidney disease and neuropathy. The current study demonstrated that the risk of DR progression increased with increased blood levels of all investigated lipid metabolism characteristics with the exception of HDL. In addition, VLDL and LDL were included in our six-variate regression model for predicting the risk of NPDR progression. We believe that it was associated with a pathological (anti-inflammatory) nature of systemic and retinal dyslipidemia in T2DM. Systemic lipoprotein abnormalities in T2DM include plasma hypertriglyceridemia and low plasma HDL due to insulin resistance [24]. Using post-mortem human retina cells, Elm and colleagues obtained data suggesting alterations in lipid metabolism in retinas from donors with DR, consistent with the reductions observed in a diabetic mouse model [25].

Zhou and colleagues [26] performed a meta-analysis to evaluate the relationship between triglycerides (TG), serum total cholesterol (TC), HDL, LDL and DR. They did not find obvious differences in TG, TC and HDL levels between patients with DR and without DR. However, slightly higher LDL levels were observed in the DR cases. This association could be caused by the LDL oxidation-induced oxidative stress, which may mediate neuropathy, as was demonstrated in a diabetic mouse model [27].

Triglyceride-enriched VLDL is secreted by the liver into circulation; excessive nutrition and a high-fat diet lead to higher VLDL secretion [28]. The pathological role of VLDL has been traditionally associated with the development of metabolic syndrome, insulin resistance and proatherogenic effects [29]. Li and colleagues [30] conducted a meta-analysis of thirteen cohort studies (7459 participants). They found that higher levels of total cholesterol, triglycerides and LDL were observed in T2DM patients with later onset of DR, but no significant difference in the HDL level was observed between patients with DR and without DR. They concluded that their results suggest that baseline triglyceride and cholesterol levels are significantly associated with the occurrence of DR in patients with DM. Their findings are in agreement with ours and confirm the value of diabetogenic dyslipidemia in NPDR progression.

The implications of altered coagulation-fibrinolytic system in the pathophysiology of stroke and myocardial infarction have been well researched upon and established. However, its role in DR progression has not been explored much [31]. Hyperglycemia is associated with the hypercoagulation state, which has been found to be an independent risk factor for diabetic cardiovascular complications and DR [32]. In the current study, coagulation characteristics showed a mild but significant tendency to increase with the progression of DR, which could indicate a certain deficiency in blood clotting factors. In addition, the plasma fibrinogen level in patients with PPDR or PDR was higher than in patients with NPDR, which is in agreement with findings of others [33, 34]. Moreover, almost all investigated coagulation parameters showed a positive relationship with NDPR progression, but their prognostic value as assessed by AUC was lower than the value of other potential predictors of NDPR progression. It should be, however, noted that, our six-variate model for predicting the risk of NPDR progression included prothrombin index as a significant predictor ($OR = 1.04$; $p = 0.016$). Therefore, coagulation abnormalities, in addition to other factors, were important for NPDR progression.

OCT is sensitive in early detection of small changes in macular thickness; in diabetic patients there was an initial decrease in central macular thickness which gradually increased with increasing duration of diabetes [35]. CRT and CRV, major indicators of the state of the retina, are well established biomarkers for detecting retinal changes and determining the severity of DR [36]. In the current study, the stage of DR clearly depended from CRT and CRV, and our univariate analysis found these two parameters to be strongly correlated with NPDR progression ($AUC = 0.79$; $p < 0.001$ for both parameters). In addition, CRT was included in our six-variate model for predicting NPDR progression, and, along with HbA1c and LDLC, was important for building a model of very high predictive quality ($AUC = 0.90$; $p < 0.001$).

Therefore, the current study not only demonstrated the importance of such NPDR progression mechanisms as carbohydrate and lipid metabolism abnormalities and coagulation and hemostasis abnormalities, but also identified major biomarkers of NPDR progression (blood HbA1c, LDLC and CRT). The built models were found to be of very high predictive quality, which allowed recommending them for confirming high risk for NPDR progression in equivocal clinical cases or as a criterion for conclusive prognosis in the relevant expert systems.

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Corresponding author: *A. V. Serdiuk, PhD, Assistant; Dnipro State Medical University, Ministry of Health of Ukraine, Dnipro, Ukraine. E-mail: anton777serdiyk@gmail.com*

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Abbreviations: *aPTT, activated partial thrombin time; AUC, area under the ROC curve; BART, blood activated recalcification time; BMI, body mass index; CI, confidence interval; CRT, central retinal thickness; CRV, central retinal volume; DBP, diastolic blood pressure; DKD, diabetic kidney disease; DM, diabetes mellitus; DME, diabetic macular edema; DR, diabetic retinopathy; HbA1c, glycosylated hemoglobin; HDL, high-density lipoproteins; INR, international normalized ratio; LDL, low-density lipoproteins; NDPR, non-proliferative diabetic retinopathy; OR, odds ratio; PDR, proliferative diabetic retinopathy; PPDR, preproliferative diabetic retinopathy; SBP, systolic blood pressure; 2DM, type 2 diabetes mellitus; VLDL, very low-density lipoproteins*