ORIGINAL STUDY

Эндотелиальные прогениторные клетки и сосудистый эндотелиальный фактор роста после рентгенэндоваскулярных вмешательств у больных сахарным диабетом 2 типа

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Цель. *Изучить количество эндотелиальных прогениторных клеток (ЭПК) и уровень сосудистого эндотелиального фактора роста (VEGF-A) после рентгенэндоваскулярных вмешательств на коронарных артериях и артериях нижних конечностей у пациентов с сахарным диабетом 2 типа (СД2).*

Материалы и методы. *Обследовано 68 пациентов со стабильной стенокардией напряжения 2–4 функционального класса (ФК) или критической ишемией нижних конечностей (КИНК), поступивших для проведения планового рентгенэндоваскулярного лечения. Пациентам было выполнено чрескожное коронарное вмешательство (ЧКВ) или рентгенэндоваскулярное вмешательство на артериях нижних конечностей. Определение количества ЭПК (CD34+VEGFR2+CD45- и CD34+CD133+CD45-) и уровня VEGF-A проводилось за 1–2 дня до реваскуляризации и на 2–4-й день после вмешательства.*

Результаты. *Установлено, что после рентгенэндоваскулярного вмешательства у пациентов без нарушения углеводного обмена отмечалось статистически значимое повышение количества как CD34+VEGFR2+CD45-клеток ($p<0,0001$), так и CD34+CD133+CD45-клеток ($p=0,041$). У больных СД2 количество ЭПК до и после реваскуляризации статистически не отличалось. Выявлено достоверное повышение фактора мобилизации ЭПК (VEGF-A) после реваскуляризации в обеих группах. Установлена зависимость динамики ЭПК от степени компенсации углеводного обмена и длительности СД2. У пациентов с уровнем $HbA_{1c}<8\%$ и длительностью СД2 менее 10 лет после вмешательства наблюдалось статистически значимое повышение CD34+VEGFR2+CD45-клеток ($p=0,001$) и CD34+CD133+CD45-клеток ($p=0,005$). В то время как у пациентов с уровнем $HbA_{1c}\geq 8\%$ и длительностью СД2 более 10 лет статистически значимого повышения клеток не отмечалось.*

Заключение. *У пациентов с СД2 количество циркулирующих ЭПК не повышалось после рентгенэндоваскулярных вмешательств по сравнению с лицами без СД2. Одними из факторов, влияющих на мобилизацию ЭПК у больных СД2, являлись неудовлетворительный контроль гликемии и длительность заболевания.*

Ключевые слова: *сахарный диабет; эндотелиальные прогениторные клетки; ишемическая болезнь сердца; критическая ишемия нижних конечностей; чрескожные коронарные вмешательства; реваскуляризация нижних конечностей*

Endothelial progenitor cells and vascular endothelial growth factor after endovascular interventions in patients with type 2 diabetes

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Aim. *To study the quantity of endothelial progenitor cells (EPCs) and levels of vascular endothelial growth factor A (VEGF-A) in patients with type 2 diabetes mellitus (T2DM) after endovascular interventions on coronary and peripheral arteries.*

Materials and methods. *We observed 68 patients with stable angina pectoris and critical limb ischaemia, admitted for elective percutaneous coronary intervention and endovascular revascularisation of the lower extremity. The number of CD34+VEGFR2+CD45- and CD34+CD133+CD45- cells and levels of VEGF-A were determined before endovascular intervention and 2–4 days after the surgery.*

Results. *We found that in patients without diabetes, the levels of EPCs increased significantly after endovascular interventions (CD34+VEGFR2+CD45-cells, $p < 0.0001$; CD34+ CD133+CD45-cells $p = 0.041$). The levels of EPCs in the peripheral blood of patients with T2DM before and after endovascular interventions did not significantly differ. The analysis of VEGF-A showed a statistically significant increase after intervention in both groups. In addition, in patients with an HbA1c level of <8% and duration of diabetes of <10 years, the levels of EPCs significantly increased ($p = 0.001$ and 0.005, respectively). In patients*

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with an HbA_{1c} level of ≥8% and duration of diabetes of >10 years, the levels of EPCs before and after endovascular interventions did not significantly differ.
***Conclusions**. Patients with diabetes exhibited impaired EPC mobilisation after endovascular interventions. Poor glycaemic control and a long duration of diabetes are among the risk factors of EPC mobilisation.*
***Keywords:** diabetes; endothelial progenitor cells; coronary heart disease; critical limb ischemia; percutaneous coronary intervention; lower extremity revascularization*

Cardiovascular disease is the leading cause of death and disability in patients with type 2 diabetes mellitus (T2DM). Patients with diabetes mellitus (DM) are known to have a faster development and more aggressive course of diseases associated with atherosclerosis. Despite recent advances in the treatment of cardiovascular disease using endovascular techniques, the frequency of cardiovascular events and lower limb amputation in patients with T2DM remains high [1, 2]. It is well known that stent placement damages the endothelium. Timely endothelialisation is needed to restore the integrity of the vascular wall. The endothelial repair process is believed to be mediated by endothelial progenitor cells (EPCs). EPCs are a population of bone marrow-derived cells that have the capacity to differentiate into mature endothelial cells and produce proangiogenic growth factors. Experimental studies have shown that after balloon angioplasty, EPCs migrate to the area of vascular wall injury and promote endothelisation of the damaged endothelial lining [3]. These cells are characterised by the simultaneous expression of CD34, CD133, and vascular endothelial growth factor receptor-2 (VEGFR-2), also known as kinase insert domain receptor (KDR) [4], and these markers are used to identify the cells. The most common EPC phenotype is $CD34^+VEGFR2^+$, and is believed to be specific for endothelial cell lines and have great potential as a biomarker for cardiovascular disease [5]. CD133 antigen, detected on the surface of immature EPCs only, can be used as an additional biomarker. Some data indicate that $CD34^+CD133^+$ cells have greater regenerative and angiogenic potential [6]. Moreover, EPCs are characterised by low-level or absent expression of the leukocyte common antigen CD45 [4]. For the purpose of the study, we have allocated two populations of EPC: $CD34^+VEGFR2^+CD45$- cells and $CD34^+CD133^+CD45-$ cells.

The number of EPCs in the peripheral blood is not high; however, it increases significantly in the case of endothelial damage or tissue ischaemia. Several studies have demonstrated an increase in the number of EPCs after percutaneous coronary intervention (PCI) [7, 8]. Vascular repair in the damaged area is a complex and coordinated multistep process, which includes mobilisation, migration, adhesion, and differentiation of the cells with the involvement of growth factors, chemokines, and adhesion molecules. Vascular endothelial growth factor (VEGF) is one of the most important proteins regulating the arrival of EPCs into the area of injury. Several members of the VEGF family have been described so far: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor. VEGF-A is the earliest identified and the most comprehensively studied protein from the VEGF family. It is considered to be a key mediator of angiogenesis. VEGF was shown to activate cell proliferation and differentiation, as well as inhibiting EPC apoptosis [9]. Its biological effect is mediated through binding to VEGFR-2 tyrosine kinase receptors located on the surface of EPCs and endothelial cells [10].

Patients with DM were shown to have a decreased number and impaired function of EPCs, which can lead to improper re-endothelialisation of the damaged vascular area [4, 11, 12]. Despite a large number of studies devoted to investigating EPCs in T2DM patients, the issue of change in EPC number after endovascular intervention is still controversial.

Aim

The study aimed to assess the number of EPCs and concentration of VEGF-A prior to and after X-ray endovascular interventions on the coronary and lower extremity arteries in patients with T2DM.

Methods

Study design

This was a dynamic prospective study.

Inclusion criteria

Age between 45 and 80 years, patients with stable exertional angina of functional class (FC) II–IV or critical lower limb ischaemia (CLLI) admitted to hospital for balloon angioplasty with stenting of the coronary arteries or lower limb arteries.

Exclusion criteria

Non-T2DM, acute coronary syndrome during the last 6 months, endovascular interventions or acute ischaemic stroke during the last 6 months, haemoglobin level lower than 90 g/L, malignant tumours, haemoblastosis, thyrotoxicosis, end-stage renal or liver failure, allergic reactions to iodinated contrast agents.

Conditions and duration of the study

The study was carried out at the Endocrinology Research Centre of the Russian Ministry of Health between 2014 and 2015. All patients underwent standard clinical, laboratory, and instrumental examination. The level of glycated haemoglobin (HbA1c) was measured to evaluate glucose metabolism in patients with T2DM. In patients

without T2DM, both HbA1c and fasting glucose levels were assessed to identify glucose metabolism disorders. Patients with T2DM were examined for the presence of microvascular complications. The sample mostly comprised elderly patients with severe macrovascular complications. According to recommendations, the targeted HbA1C level for these patients was <8% [13].

Description of medical intervention

Peripheral venous blood (used for the assessment of EPC number and VEGF-A level) was collected 1–2 days prior to X-ray endovascular intervention and 2–4 days afterwards.

Main outcome of the study

Within the study, we evaluated the number of EPC and concentration of VEGF-A prior to and after X-ray endovascular intervention.

Methods of outcomes registration

Assessment of circulating EPC: Peripheral venous blood was collected into 4 mL Vacutainer tubes (EDTA K3); staining of the cells was performed not later than 2 h after blood collection in two aliquots. We used panels of monoclonal antibodies conjugated with fluorescent dyes. The first aliquot: FITC-labelled anti-CD34, PC5-labelled anti-CD45, and PE-labelled anti-CD133. The second aliquot: FITC-labelled anti-CD34, PC5-labelled anti-CD45, and PE-labelled anti-hVEGFR2/KDR. Immunostaining of specimens was carried out according to the manufacturer's recommended protocol. Erythrocytes were lysed by incubation with IOTest® 3 Lysing Solution (Beckmancoulter, France) for 10 min at room temperature. Cells suspensions were washed in phosphate saline buffer. Cytofluorometry was performed with the FACSCalibur™ System (Becton Dickinson, USA) using CellQuest Pro Software. For each sample, 300,000–500,000 events (cells) were analysed.

Assessment of VEGF-A level: Blood specimens were centrifuged at 2000 rpm and then frozen at–22°C. VEGF-A level was measured by enzyme-linked immunosorbent assay using an eBiosciense kit.

Subgroup analysis

Patients were divided into two groups: participants with T2DM and participants without glucose metabolism disorders. In order to asses EPC dynamics depending on the quality of glycaemic control (based on the level of glycated haemoglobin), patients with T2DM were further divided into two subgroups: HbA1c < 8% and HbA1c ≥ 8%. To analyse EPC dynamics depending on the disease duration, patients from the first group were divided into two subgroups: patients with T2DM duration less than 10 years and over 10 years.

Ethical review

All patients gave signed informed consent for participation in the study. Study protocol was approved by the Ethic Committee of the Endocrinology Research Centre (protocol No. 11 dated 23 October 2013).

Statistical analysis

Statistical data analysis was performed using SPSS Statistics 22 (SPSS Inc., USA). The data was checked for normality distribution using the Shapiro–Wilk test. The data is presented as median with 25th/75th percentiles. Absolute (n) and relative (%) values were calculated to describe qualitative data. Median comparison between the independent groups was done using the Mann–Whitney test, between the dependant groups using the Wilcoxon test. Pearson 2 test and Fisher's exact test were applied to analyse the correlation between categorical variables. Differences were considered significant for $p < 0.05$.

Results

Study participants

Sixty eight patients were included in the study. Forty of them were males with a mean age of 67 [59; 74] years. The two groups comprised 39 patients with T2DM and 29 patients without glucose metabolism disorders (Fig. 1).

Patients in the groups were matched for age, body mass index (BMI), lipid metabolism parameters, renal function, and cardiovascular events (Table 1). The first group consisted predominantly of females, and the second one of males (60.9% and 78.9% respectively, $p = 0.003$). Smoking was more frequent in patients without glucose metabolism disorders compared with patients with T2DM (69.0% and 28.2% respectively, $p = 0.002$).

Upon admission, most patients received dual antiplatelet therapy and lipid-lowering therapy (Table 2). Among patients with T2DM, 16 (41%) received oral hypoglycaemic therapy, and 16 (41%) received intensified insulin therapy. Seven patients (17%) received combination therapy with insulin and oral hypoglycaemic agents (OHAs).

The severity of coronary lesions was comparable in both groups (Table 3). However, the frequency of multi-vessel lesions in the coronary arteries was higher in patients with T2DM, although the difference was not statistically significant due to the small number of patients.

Coronary arterial stenting was carried out for 18 patients from the T2DM group (46%) and 18 patients from the second group (62%) (Table 4). We mainly performed anterior interventricular artery stenting. The number of stents per patient was 1–2 in both groups. All patients with T2DM were implanted with drug-eluting stents (DESs). In the non-T2DM group, 15 participants were implanted with DESs and three participants were implanted with halometasone stents. Immediate angiographic success was achieved in all patients.

X-ray endovascular interventions on the lower extremity arteries was carried out for 21 patients in the first group (53%) and 11 patients in the second group (37%). The severity of lesions in the lower extremities arteries was comparable in both groups. In most of the cases

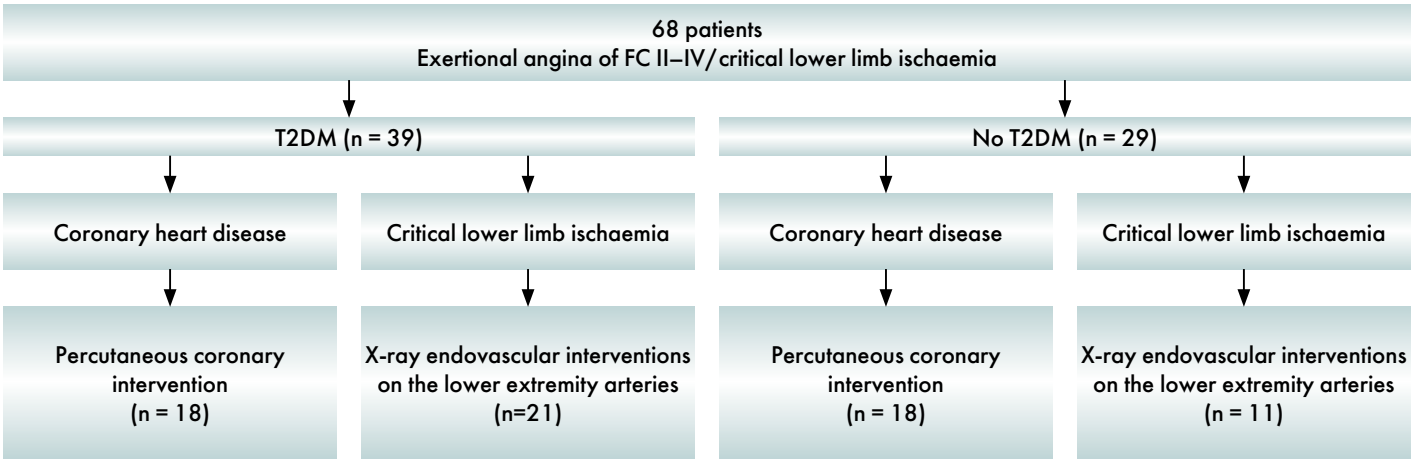

Fig. 1. Study participants

Table 1

Clinical characteristics of the patients

Parameter	T2DM (n = 39)	No T2DM (n = 29)	p
Age, years	67 [48;78]	67 [45;79]	0,862
Males, n (%)	17 (43)	23 (79)	**0,003**
Smoking, n (%)	11 (28)	20 (69)	**0,002**
Body mass index, kg/m2*	28,5 [27,3;33,3]	28,3 [27,1;31,0]	0,564
Glycated haemoglobin, %	8,5 [7,0;9,3]	5,9 [5,7;6,0]	**0,0001**
Total cholesterol, mmol/L*	4,3 [3,6;4,9]	4,07 [3,4;4,4]	0,217
LDL cholesterol, mmol/L*	2,4 [2,0;3,3]	2,27 [1,86;2,53]	0,192
Creatinine μmol/L	71,7 [64,9;87,2]	72,6 [63,4;86,8]	0,980
Glomerular filtration rate (calculated using CKD-EPI formula) ml/min/1.73 m2*	87 [67,0;93,0]	87 [79,0;96,0]	0,309
Left ventricular ejection fraction, %	55 [51;58]	56 [52;59]	0,571
Hypertension, %	38 (97)	27 (93)	0,453
MI in anamnesis, n (%)	13 (33)	11 (37)	0,334
Myocardial revascularisation in anamnesis, n (%)	8 (20)	3 (10)	0,263
Lower extremity revascularisation in anamnesis, n (%)	5 (13)	5 (17)	0,731
Acute stroke in anamnesis, n (%)	5 (13)	5 (17)	0,446

Note. LDL – low-density lipoprotein, MI – myocardial infarction, * median Me [Q25; Q75]

Table 2

Pharmacotherapy

Therapy	T2DM (n = 39)	No T2DM (n = 29)	p
Statins, n (%)	33 (85)	27 (93)	0,451
Aspirin, n (%)	37 (95)	27 (93)	0,789
Clopidogrel, n (%)	30 (77)	22 (76)	0,919
ACE inhibitors/ ARBs, n (%)	31 (79)	18 (62)	0,113
β-blockers, n (%)	31 (79)	23 (79)	0,986
Calcium antagonists, n (%)	10 (26)	8 (28)	0,857
OHA, n (%)	16 (41)	-	-
Insulin and OHA, n (%)	7 (18)	-	-
Insulin therapy, n (%)	16 (41)	-	-

Note. ACE – angiotensin-converting enzyme, ARBs – angiotensin receptor blockers, OHA – oral hypoglycaemic agent.

we performed stenting of the superficial femoral artery. Majority of the participants also underwent angioplasty of the crural arteries during the intervention (Table 5). The number of stents per patient was 1–2 in both groups. Satisfactory angiographic results were achieved in all cases.

Main results of the study

The number of endothelial progenitor cells and level of VEGF in the two groups

The analysis showed that there was a significant increase in EPC number in patients without T2DM after endovascular intervention, while patients with T2DM

Table 3

Characteristics of coronary lesions

Parameter	T2DM (n = 18)	No T2DM (n = 18)	p
1-vessel lesion, n (%)	2 (11)	7 (39)	0,268
2-vessels lesion, n (%)	4 (22)	3 (17)	
3-vessels lesion, n (%)	12 (67)	8 (44)	
Lesions in the left coronary artery trunk, n (%)	4 (22)	6 (33)	

Table 4

Results of the coronary arterial stenting

Parameter	T2DM (n = 18)	No T2DM (n = 18)	p
Stenting of the left coronary artery trunk, n (%)	2 (11)	4 (22)	0,371
Stenting of the anterior interventricular artery, n (%)	11 (61)	10 (56)	0,725
Stenting of the circumflex artery, n (%)	5 (28)	4 (22)	0,989
Stenting of the right coronary artery, n (%)	5 (28)	5 (28)	0,979

Table 5

Results of stenting and balloon angioplasty of the lower extremities arteries

Parameter	T2DM (n = 21)	No T2DM (n = 11)	p
Stenting of the iliac arteries, n (%)	2 (10)	1 (9,0)	0,967
Stenting of the lower extremity arteries and angioplasty of the crural arteries, n (%)	19 (90)	10 (91)	0,180

had no change in the number of cells after the surgery. A significant increase in the level of VEGF-A was observed in both groups (Table 6).

Additional results of the study

Dynamics of EPC and the quality of glycaemic control (level of HbA1c)

Patients with T2DM were divided into two subgroups: patients with HbA1c < 8% (17 individuals) and patients with HbA1c ≥ 8% (22 individuals). Patients in both groups were matched for gender, age, and renal function (Table 7). Patients with HbA1c ≥ 8% were more often diagnosed with diabetic retinopathy and had longer duration of the disease.

After endovascular intervention, we observed a statistically significant increase in the number of both $CD34^+VEGFR2^+CD45-$ cells and $CD34^+CD133^+CD45-$ cells among T2DM patients with HbA1c < 8%, whereas the patients with HbA1c ≥ 8% had no significant changes in the number of cells (Table 8).

Dynamics of the EPC number and T2DM duration

Patients with T2DM were divided into two subgroups depending on disease duration: the first consisted of 15 patients with T2DM duration less than 10 years, the second included 24 patients with T2DM duration over 10 years. Patients with T2DM for more than 10 years demonstrated no changes in the number of cells in response to intervention. Patients from the first subgroup were found to have a significant increase in the number of EPC after surgery (Table 9).

Discussion

In the current study, we demonstrated that patients with T2DM had no increase in the number of circulating EPC after X-ray endovascular intervention. The

Table 6

The number of EPC and level of VEGF-A prior to and after endovascular intervention in the two groups

EPC, % of leukocytes	T2DM (n = 39)	No T2DM (n = 29)
CD34+VEGFR2+CD45– prior to endovascular intervention	0,012 [0,009;0,016]	0,010 [0,007;0,016]
CD34+VEGFR2+CD45– after endovascular intervention	0,014 [0,009;0,019]	0,016 [0,012;0,020]
	p=0,092	p<0,0001
CD34+CD133+CD45– prior to endovascular intervention	0,018 [0,013;0,023]	0,014 [0,011;0,020]
CD34+CD133+CD45– after endovascular intervention	0,021 [0,018;0,022]	0,022 [0,016;0,027]
	p=0,068	p=0,041
VEGF-A, pg/mL		
VEGF-A prior to endovascular intervention	410,2 [287,4;500,3]	409,7 [300,1;540,3]
VEGF-A after endovascular intervention	569,7 [330,4;723,5]	502,7 [314,5;660,1]
	p=0,037	p=0,043

Table 7

Characteristics of T2DM patients			
Parameter	HbA_{1c}<8% Me [Q25; Q75]	HbA_{1c}≥8% Me [Q25; Q75]	p
Number, n	17	22	
Female, n (%)	9 (52,9)	13 (59)	0,751
T2DM duration, years	6 [2,5;12]	14,5[12;20]	**0,0001**
HbA1c, mmol/L	7 [6,35;7,35]	9,25 [8,7;10,1]	**0,0001**
Glomerular filtration rate (calculated using CKD-EPI formula) ml/min/1,73 m2	87 [71;93]	83,5 [64,5;90,7]	0,312
Urine albumin in a single portion, mg/L	16,5 [10,5;26,7]	22 [12,7;34,5]	0,454
Diabetic retinopathy, n (%)	3 (17,7)	13 (59)	**0,022**

Table 8

The number of EPC depending on the quality of glycaemic control (level of HbA1c)		
EPC, % of leukocytes	HbA_{1c}<8% (n=17)	HbA_{1c}≥8% (n=22)
CD34+VEGFR2+CD45– prior to endovascular intervention	0,009 [0,008;0,013]	0,013 [0,008;0,013]
CD34+VEGFR2+CD45– after endovascular intervention	0,014 [0,011;0,018]	0,015 [0,011;0,018]
	p=0,001	p=0,498
CD34+CD133+CD45– prior to endovascular intervention	0,016 [0,014;0,021]	0,021 [0,012;0,024]
CD34+CD133+CD45– after endovascular intervention	0,021 [0,019;0,024]	0,022 [0,014;0,026]
	p=0,005	p=0,289
VEGF-A, пг/мл		
VEGF-A до эндоваскулярного вмешательства	410,2 [287,4;500,3]	409,7 [300,1;540,3]
VEGF-A после эндоваскулярного вмешательства	569,7 [330,4;723,5]	502,7 [314,5;660,1]
	p=0,037	p=0,043

Table 9

Changes in the number of EPC depending on the duration of T2DM		
EPC, % of leukocytes	Длительность СД2 <10 лет (n=15)	Длительность СД2 ≥10 лет (n=24)
CD34+VEGFR2+CD45– prior to endovascular intervention	0,010 [0,007;0,013]	0,014 [0,014;0,021]
CD34+VEGFR2+CD45– after endovascular intervention	0,016 [0,011;0,018]	0,015 [0,014;0,021]
	p=0,001	p=0,811
CD34+CD133+CD45– prior to endovascular intervention	0,016 [0,014;0,021]	0,021 [0,012;0,018]
CD34+CD133+CD45– after endovascular intervention	0,021 [0,019;0,024]	0,022 [0,014;0,030]
	p=0,008	p=0,432

pathophysiological processes initiated by persistent hyperglycaemia have a negative impact on the EPC at all stages of their life cycle. Oxidative stress, reduced nitric oxide bioavailability, and inflammation disrupt the mobilisation and targeted migration of EPC to the area of damage and ischaemia. Furthermore, hyperglycaemia inhibits the proliferation, differentiation, and adhesion of EPCs [14, 15]. Moreover, chronic hyperglycaemia has a negative impact on bone marrow, which is the main source of EPCs. Furthermore, we cannot ignore the fact that the development of microangiopathy and bone marrow autonomic neuropathy causes impaired EPC mobilisation [16]. These mechanisms explain inadequate mobilisation of EPCs from the bone marrow in T2DM patients with high level of VEGF. In an earlier Russian study [11], the number of circulating EPCs was assessed in coronary artery disease patients with impaired glucose metabolism. The most significant decline in the number of progenitor cells was observed in participants with stable angina and concomitant glucose metabolism disorders; a negative correlation between the number of EPCs and the blood glucose level was revealed.

Among the currently available Russian publications, there are several manuscripts devoted to the investigation of the impact of the X-ray endovascular interventions on the number of circulating progenitor cells. Vyborov et al. [17] demonstrated that the number of $CD34^+$ cells in the peripheral blood of patients with stable angina declined during the first day after coronary angioplasty with stenting but was restored 3–5 days post-surgery. However, DM was an exclusion criterion in this study. On the contrary, another study devoted to the assessment of EPC ($CD34^+133^+$ cells and $CD34^+KDR^+$ cells) number before and after myocardial revascularisation or lower limb revascularisation showed an increase in the number EPC 6 months post-surgery. Coronary angioplasty was associated with doubling of EPC number, whereas the lower limb revascularisation induced the raise of EPC number by 35%–36% [18]. This work did not imply the assessment of EPC dynamics in T2DM patients as well. So far, several studies have explored the

mobilisation of EPC in T2DM patients. Lin Ling et al. [19] evaluated the dynamics of both circulating EPC and the factors stimulating EPC mobilisation (VEGF and stromal cell-derived factor-1 alpha (SDF-1)) in patients with acute myocardial infarction (AMI). Decreased mobilisation and reduced peak concentration of EPC was detected in individuals with T2DM compared with those without the disease, while VEGF and SDF-1 levels in plasma were higher in T2DM patients. In the group of patients without diabetes, the number of circulating EPCs was high within the first 24 h after AMI with a subsequent peak on day 5 followed by a decrease. T2DM patients demonstrated a similar pattern of changes, but the peak occurred on day 7 and was lower than that in patients without diabetes. Another study revealed a decrease in the initial number of cells and a delayed peak in patients with T2DM compared with individuals without glucose metabolism disorders [20]. These studies aimed to assess EPC dynamics in patients with acute myocardial infarction, whereas our study included patients with stable angina or CLLI, and patients with acute coronary syndrome were excluded. In one studies, the authors explored EPC dynamics in the case of AMI depending on the intraoperative glycaemic control during PCI. The authors concluded that intensive glycaemic control (compared with conventional glycaemic control) during the PCI and within the first 24 h after it increases the number of EPCs and their ability to differentiate [21]. The results obtained in our earlier work also suggest the violation of EPC mobilisation after endovascular treatment [22].

Moreover, we have confirmed the hypothesis related to the impact of persistent hyperglycaemia (duration of the disease) and poor glycaemic control on the mobilisation of EPCs. One study demonstrated a close relationship between the glycaemic control and circulating EPCs: poor glycaemic control was associated with a low number of circulating EPCs, while satisfactory glycaemic control ensured higher number of EPC [23]. Churdchomjan observed a decrease in the number of circulating EPCs among patients with T2DM and poor glycaemic control (HbA1C > 7.0%) compared with patients with satisfactory glycaemic control (HbA1C ≤ 7.0%) [24]. In two subsequent studies the researchers showed that intensive glycaemic control increases the number and activity of EPCs within 3 months [25, 26]. One factor which probably affected the mobilisation of EPCs is duration of the disease. Within our study we discovered an association between EPC dynamics and duration of T2DM. Patients with disease duration of more than 10 years were found to have impaired EPC mobilisation in response to intervention. An earlier study revealed that patients suffering from T2DM for more than 20 years have the lowest number of EPCs compared with patients with disease duration of 0–19 years [27]. Thus, we can assume that the mobilisation of progenitor cells is affected by the presence of T2DM, quality of glycaemic control, and disease duration.

The following classical cardiovascular risk factors may also affect the number of EPC: dyslipidaemia, smoking, and obesity [29]. We have already mentioned that patients in our groups were matched for BMI and lipid profile parameters, but the number of smokers was higher in patients without T2DM. No significant differences in the number of EPCs were found between the smokers and non-smokers. Some authors suggest that the number of EPCs may depend on gender. For example, the number and the activity of EPCs were shown to be higher in women than men [30]. Other studies demonstrated no differences in the number of EPCs between men and women [31, 32]. We should mention that our study included postmenopausal women. We also analysed the effect of gender on the EPC dynamics but found no significant difference.

We assessed the impact of the different types of hypoglycaemic therapy on the number of EPCs. In one study, adding insulin glargine or neutral protamine Hagedorn insulin to a standard oral hypoglycaemic therapy induced an increase in the number of EPCs [33]. Another study aimed at assessing the impact of metformin and a sulfonylurea (gliclazide) on the number of EPCs in patients with newly diagnosed T2DM. Metformin monotherapy ensured an increase in numbers of EPCs, but combination therapy with metformin with gliclazide was more effective [34]. Another study showed that the use of sitagliptin (a dipeptidyl peptidase-4 inhibitor) doubled the number of EPC compared with patients receiving glimepiride, although satisfactory glycaemic control was achieved in both groups [35]. Similar data was obtained for patients receiving linagliptin [36]. In our study, the type of hypoglycaemic therapy (insulin therapy or OHA) had no impact on the number of EPCs prior to and after endovascular intervention.

Limitations of the study

The sample size used in our study is not representative enough; therefore, the results obtained cannot be extrapolated to the entire population with T2DM. Within this study, we did not evaluate the functional characteristics of EPCs. The absence of a standard method for EPC counting may have affected the results.

Conclusion

Patients with T2DM had no increase in number of circulating EPCs after endovascular interventions compared with patients without T2DM. Poor glycaemic control and duration of the disease were found to influence EPC mobilisation in patients with T2DM.

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Conflict of interest

The authors declare no conflict of interest related to current manuscript.

Authors contribution

I.I. Dedov, V.Yu. Kalashnikov, O.M. Smirnova–development of a research concept and study design, editing and final approval of the manuscript; O.N. Ivanova, S.M. Stepanova, A.V. Ilyin–laboratory testing, verification and editing of the manuscript; S.A. Terekhin–X-ray endovascular interventions, verification and editing of the manuscript; M.S. Michurova–collection of the clinical material, statistical data analysis, results interpretation, drafting the manuscript.

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