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Circulating in the blood desquamated endotheliocytes at the cardiovascular diseases. Preliminary communication

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Abstract

Introduction and aim. We previously found that diabetic angiopathy is accompanied by an increase in the level of desquamated endothelial cells (CECs) circulating in the blood, commensurate with its severity. In this study, we set ourselves the aim of analyzing the level of CECs in patients with cardiovascular pathology and their relationship with the level of blood pressure and routine clinical indicators.

Material and methods. The object of clinical observation were patients with stage II hypertension, who were receiving outpatient treatment. Among them, 23 men (62.3±13.2 ys) and 39 women (65.1±10.6 ys). Concomitant ischemic heart disease was diagnosed in 16 men (70±10%) and 28 women (72±7%). The main subject of the study was blood pressure and the level of CECs. In addition, routine general blood analysis and determined metabolic parameters were performed.

Results. Using the method of cluster analysis, the sample was divided into 4 homogeneous groups, different from each other. It was found that in 18 patients of the first cluster, the youngest in the sample, the minimum level of hypertension for the sample occurs against the background of unchanged CECs levels and other registered parameters, instead, it is accompanied by moderate increased creatininemia and a significantly increased rate of erythrocyte sedimentation and a reduced level of hemoglobin and color index. In 18 patients of the second cluster, moderately elevated levels of terminally and markedly changed CECs were found in combination with minimally expressed signs of hyperchromic anemia. In the third cluster (n=16), the levels of terminally and markedly changed CECs are significantly increased in combination with a reduced level of urea and LP index against the background of moderate hyperchromic anemia. Finally, in 10 patients of the fourth cluster, the oldest in the sample, with maximally expressed hypertension, it is accompanied by maximally elevated levels of all three types of CECs against the background of moderate hyperchromic anemia.

Conclusion. Desquamated plasma endothelial cells can be one of the criteria for the severity of hypertonic disease.

Keywords: desquamated plasma endothelial cells, stage II hypertension, ischemic heart disease.

Introduction

We previously found that diabetic angiopathy is accompanied by an increase in the level of desquamated endothelial cells (CECs) circulating in the blood, commensurate with its severity.[1,2,3,4,5,6,7] In this study, we set ourselves the goal of analyzing the level of CECs in patients with cardiovascular pathology and their relationship with the level of blood pressure and routine clinical indicators.

Material and methods

Ethics approval

Tests in patients are conducted in accordance with positions of Helsinki Declaration 1975, revised and complemented in 2002, and directive of National Committee on ethics of scientific researches. During realization of tests from all parent of participants the informed consent is got and used all measures for providing of anonymity of participants. For all authors any conflict of interests is absent.

Participants

The object of clinical observation were patients with stage II hypertension, who were receiving outpatient treatment at the Center for Primary Health Care No.3 (Odessa) in 2019. Among them, 23 men (62.3±13.2 ys) and 39 women (65.1±10.6 ys). Concomitant ischemic heart disease was diagnosed in 16 men (70±10%) and 28 women (72±7%).

Study design and procedure

The main subject of the study was blood pressure and the level of desquamated endothelial cells circulating in the plasma (CECs).

CECs were determined by the method of J. Hladovek.[8] To prepare platelet-rich plasma (PRP), centrifugation (with 1000 g for 10 min) was used. After adding 0.2 ml of Adenosine-Diphosphate to 1 ml of PRP, the mixture was shaken for 10 min using mechanical means. For 10 min, 1000 g of centrifugation were used to remove platelet aggregates. Centrifuged at 3000 g for 15 min, the supernatant's dense sediment was carefully suspended in 0.9% NaC1 in 0.1 M stirring with glass rod. Cells were counted using phase contrast microscopy, which involves filling two platforms in Goryaev's chamber from suspension and using a Micromed XS-3320 binocular microscope with a Plan 10 Ph/0.25 lens (10 fold) and eyepieces WF

16X..[8,9] Within the range of 30-50 m in diameter, the cells represent polygonal objects. The margins are often folded or oiled, indicating an extreme thinness of less than 1 m. The ratio between the number of cells in platforms and the volume of Goriaev's chamber, suspension size (the liquid material), and blood plasma volume is used to determine the number of CECs in 1 ml of blood plasma.

In addition, routine general blood analysis were performed and determined metabolic parameters in serum: triglycerides (by a certain meta-periodate method); total cholesterol (by a direct method after the classic reaction by Zlatkis-Zack) and content of him in composition of α-lipoproteins (HDLP) (by the enzyme method after precipitation of notα-lipoproteins); pre-β-lipoproteins (VLDLP) (expected by the level of triglycerides); β-lipoproteins (LDLP) (expected by a difference between a total cholesterol and cholesterol in composition α-and pre-β-lipoproteins); creatinine (by Jaffe's color reaction by Popper's method); urea (urease method by reaction with phenolhypochlorite); glucose (glucose-oxidase method).

The analysis carried out according to instructions[10] with the use of analyzers "Reflotron" (BRD) and "Pointe-180" (USA) and corresponding sets of reagents.

Statistical analysis

Statistical processing was performed using a software package “Microsoft Excell” and “Statistica 6.4 StatSoft Inc” (Tulsa, OK, USA).

Results

For the purpose of correct comparison, registered variables (V) expressed as Z-scores calculated by formula:

$Z=(V/N - 1)/Cv$, where

N is Mean of Normal (reference) Variable, Cv is Coefficient its variation.

Reference values of routine variables, taking into account sex and age, are borrowed from the handbook.[11] The norms of CECs are taken from the database of our laboratory.

It turned out (Fig. 1) that most variables fluctuate in a wide range, which indicates the heterogeneity of the sample.

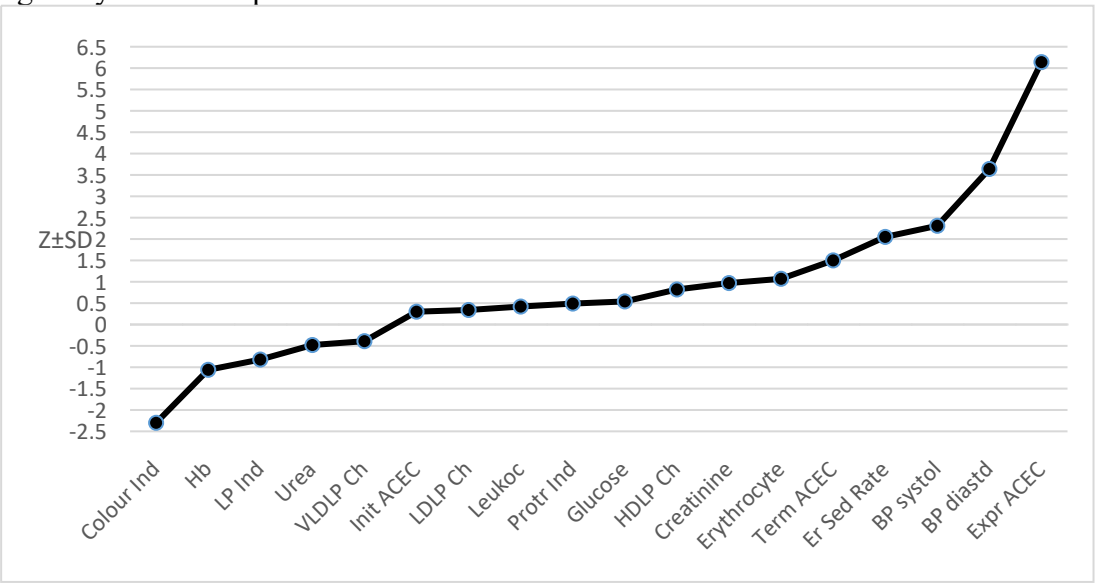

Fig. 1. Profile of registered sample parameters as a whole

Within the range of 30-50 m in diameter, the cells represent polygonal objects. Use of Cluster analysis makes possible the simultaneous consideration of all the signs. Considering the totality of characteristics of persons undertaken in their relationship and conditionality of some of these (derivatives) other (main determinants) allows as to make a natural

classification that reflects the nature of things, their essence. Clustering cohort of persons was realized by iterative k-means method. In this method, the object belongs to the class Euclidean distance to which is minimal. The main principle of the structural approach to the allocation of uniform groups consists in the fact that objects of same class are close but different classes are distant. In other words, a cluster (the image) is an accumulation of points in n-dimensional geometric space in which average distance between points is less than the average distance from the data points to the rest points.[12,13]

We have identified 4 clusters.

Members of Cluster No 1 (Contains 18 cases) and Distances from Respective Cluster Center

C1 C2 C3 C4 C5 C7 C8 C9 C13 C16 C19 C28 C32 C35 C36 C38 C40 C60

Distance

61, 130, 137, 115, 41, 125, 172, 59, 86, 152, 43, 75, 79, 91, 60, 125, 94, 147

Members of Cluster No 2 (Contains 18 cases) and Distances from Respective Cluster Center

C6 C10 C11 C12 C14 C17 C18 C20 C21 C23 C24 C25 C30 C37 C39 C41 C43 C50

Distance

149, 158, 195, 91, 214, 147, 94, 21, 101, 162, 111, 168, 129, 63, 113, 59, 104, 130

Members of Cluster No 3 (Contains 16 cases) and Distances from Respective Cluster Center

C15 C27 C33 C34 C42 C45 C46 C49 C52 C53 C54 C55 C56 C57 C59 C62

Distance

133, 103, 89, 109, 108, 58, 55, 93, 123, 15, 99, 196, 88, 79, 134, 64

Members of Cluster No 4 (Contains 10 cases) and Distances from Respective Cluster Center

C22 C26 C29 C31 C44 C47 C48 C51 C58 C61

Distance

184, 173, 178, 121, 131, 113, 180, 129, 102, 28

Cluster Number	Euclidean Distances between Clusters (End_BP.STA) Distances below diagonal Squared distances above diagonal			
	No. 1	No. 2	No. 3	No. 4
No. 1	0	36020	131120	274792
No. 2	190	0	30955	113213
No. 3	362	176	0	27761
No. 4	524	336	167	0

In the next stage carried Analysis of Variance and ranking variables for coefficient η^2:

$\eta^2=Sb^2/(Sb^2+Sw^2)$,

$R=\eta$,

$F=[Sb^2(n-k)]/[Sw^2(k-1)]$, where

Sb^2 is Between Variance; Sw^2 is Within Variance; n is number of persons (62); k is number of groups-clusters (4).

It was found (Table 1) that the most characteristic feature of clusters is the level of Markedly altered circulating endotheliocytes (ACEC). Terminally ACEC and systolic BP

make much smaller but significant contributions to the distribution of the sample into clusters. Instead, the contribution of Initially ACEC is meager and diastolic BP is insignificant.

Table 1. Analysis of Variance

Variables	Between SS	Within SS	η^2	R	F	signif. p
Markedly ACEC	24633148	1817820	0,931	0,965	262	10^{-6}
Terminally ACEC	814331	1536153	0,346	0,589	10,2	10^{-4}
BP systolic	2748	9916	0,217	0,466	5,36	,003
Initially ACEC	206824	1155111	0,152	0,390	3,46	,022
BP diastolic	135	11712	0,011	0,107	0,222	,881

Clustering, by definition, minimized the variability of variables. It was found that in the patients of the first cluster, the youngest in the sample, hypertension occurs against the background of unchanged CECs levels and other registered parameters, instead, it is accompanied by moderate increased creatininemia and a significantly increased rate of erythrocyte sedimentation and a reduced level of hemoglobin and color index. In the patients of the second cluster, moderately elevated levels of terminally and markedly changed CECs were found in combination with minimally expressed signs of hyperchromic anemia. In the third cluster, the levels of terminally and markedly changed CECs are significantly increased in combination with a reduced level of urea and LP index against the background of moderate hyperchromic anemia. Finally, in patients of the fourth cluster, the oldest in the sample, with maximally expressed hypertension, it is accompanied by maximally elevated levels of all three types of CECs against the background of moderate hyperchromic anemia (Fig. 2).

Further, the registered variables were condensed into 8 patterns (Fig. 3).

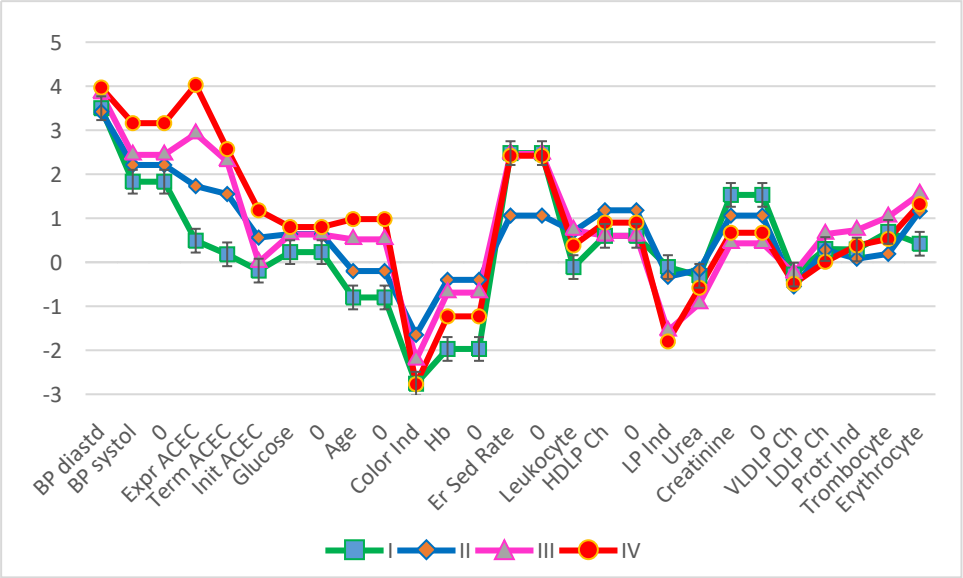

Fig. 2. Profiles of registered parameters of CEC&BP cluster members. Y axis: Z±SE

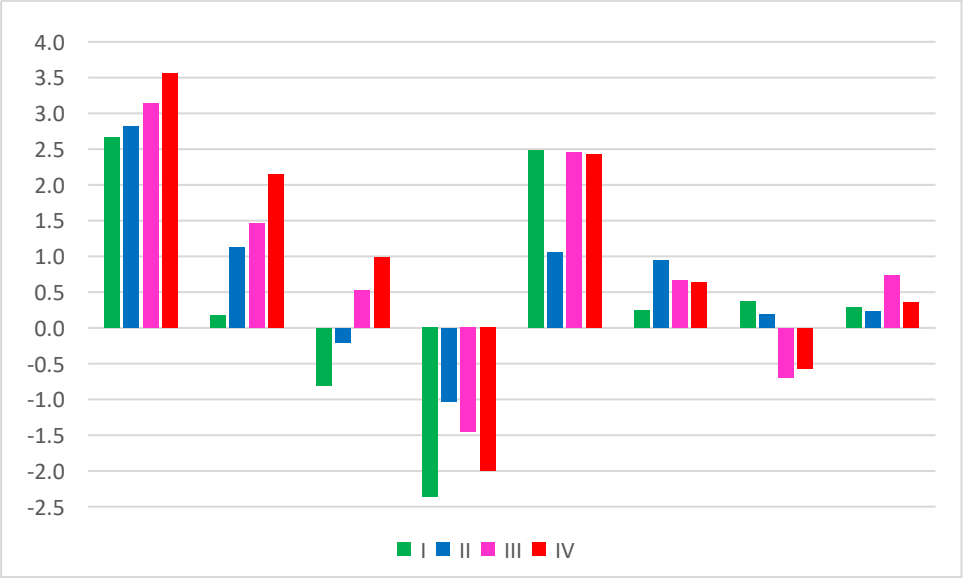

Fig. 3. Patterns of registered parameters of CEC&BP cluster members. Y axis: Z±SE

In order to identify among the registered parameters, those for which the ACEC&BP clusters differ from each other, a discriminant analysis was performed.[14] The program forward stepwise included in the discriminant model 8 parameters. In addition to CECs and systolic BP by default, the following variables were identified as characteristic: HDLP Cholesterol, Hemoglobin, Erythrocytes Sedimentation Rate, and Trombocytes level. The rest of the variables were left out of the model, but some of them still carry identifying information (Tables 2 and 3).

Table 2. Discriminant Function Analysis Summary for Variables, their actual levels (Mean±SE) for Clusters of Endotheliocytes & Blood Pressure as well as Reference levels and Coefficients of Variability

Variables currently in the model	**Clusters of ETC&BP (n)**				**Parameters of Wilk's Statistics**						
	I (18)	**II (18)**	**III (16)**	**IV (10)**	Wilks Λ	Partial Λ	F-remove	p-level	Tolerancy	Reference (62)	Cv
Markedly altered endotheliocytes, c/mL	**956 46**	**1583 18**	**2181 26**	**2740 52**	,0351	,121	123	10^{-6}	,988	711 21	,236
Terminally altered endotheliocytes, c/mL	**217 26**	**389 16**	**481 47**	**510 59**	,0051	,840	3,2	,029	,753	194 16	,647
Blood Pressure Systolic, mmHg	**152 3.0**	**158 1.4**	**162 2.8**	**173 4.2**	,0046	,916	1,6	,212	,905	124.5 1.9	,122
Initially altered endotheliocytes, c/mL	**178 24**	**267 15**	200 27	**340 62**	,0046	,919	1,5	,224	,906	200 15	,594
HDLP Cholesterol, mM/L	1.60 0.08	**1.79 0.07**	1.53 0.06	1.62 0.09	,0050	,848	3,0	,037	,780	1.32 0.05	,297
Hemoglobin, g/L	120.7 2.3	**133.8 3.2**	132.3 4.2	125.7 2.8	,0050	,860	2,8	,050	,946	136.7 1.0	,057
Erythrocytes Sedimentation Rate, mm/h	14.6 2.0	**10.2 0.4**	13.8 2.3	14.2 0.8	,0056	,758	5,4	,003	,715	7.4 0.4	,392
Trombocytes, 10^9/L	274 9	**257 3**	286 10	269 14	,0050	,855	2,9	,044	,845	250 4	,140
Variables currently not in model	**I (18)**	**II (18)**	**III (16)**	**IV (10)**	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy	Reference (62)	Cv
Age, years	**54.7 2.3**	**61.7 2.5**	**70.1 1.8**	**75.3 1.8**	,0042	,977	,39	,761	,799	64.0 1.5	,181
Glucose, mM/L	**5.41 0.22**	**5.79 0.23**	5.78 0.17	**5.94 0.19**	,0041	,964	,63	,601	,887	5.20 0,12	,177
LP Index	**0.89 0.03**	**0.87 0.01**	**0.74 0.03**	**0.72 0.04**	,0041	,962	,67	,576	,928	0.90 0.01	,111
Colour Index	0.82 0.02	**0.88 0.01**	0.85 0.04	0.82 0.04	,0041	,952	,85	,470	,780	0.955 0.006	,050
Urea, mM/L	5.18 0.39	**5.58 0.50**	4.81 0.23	4.95 0.29	,0041	,954	,81	,494	,827	5.71 0.13	,184
Leukocytes, 10^9/L	6.28 0.31	**7.20 0.13**	7.20 0.46	6.82 0.35	,0042	,978	,37	,775	,871	6.40 0.14	,172
Blood Pressure Diastolic, mmHg	102.8 3.3	**102.2 1.3**	105.0 3.2	106.0 5.5	,0042	,984	,28	,841	,633	79.0 0.7	,086
LDLP Cholesterol, mM/L	3.86 0.25	4.01 0.26	**4.29 0.20**	3.85 0.28	,0041	,971	,50	,687	,856	3.80 0.09	,193
VLDLP Cholesterol, mM/L	0.47 0.03	0.41 0.01	**0.52 0.05**	0.44 0.06	,0277	1,00	,00	1,00		0.59 0.04	,577
Protrombine Index, %	99.3 1.9	98.0 0.7	**104.5 3.4**	100.9 3.1	,0041	,951	,85	,473	,789	97.5 0.8	,064
Erythrocytes, 10^{12}/L	4.44 0.10	4.64 0.14	**4.76 0.17**	4.65 0.18	,0041	,952	,83	,482	,915	4.35 0.03	,057
Sex Index (M=1; F=2)	1.67 0.11	1.61 0.12	**1.56 0.13**	1.70 0.18	,0043	,999	,02	,996	,945	1.63 0.06	,299
Creatinine, μM/L	103 4	101 1	**92 5**	94 7	,0043	,999	,02	,995	,750	83.7 2.0	,188
Entropy CECG	0.701 0.023	0.696 0.009	**0.607 0.024**	0.622 0.028						0.819	
Entropy LPG	0,746 0.018	0.739 0.006	**0.731 0.017**	0.745 0.014						0.768	

Step 10, N of vars in model: 8; Grouping: 4 grs; Wilks' Λ: 0,00426; approx. $F_{(24)}$=34,5; $p<10^{-6}$

Table 3. Summary of Stepwise Analysis for Variables, ranked by criterion Lambda

Variables currently in the model	F to enter	p-level	**Λ**	F-value	p-value
Markedly altered endotheliocytes, c/mL	262	10^{-6}	,069	262	10^{-6}
Hemoglobin, g/L	3,3	,028	,059	59	10^{-6}
HDLP Cholesterol, mM/L	2,1	,116	,053	36	10^{-6}
Erythrocytes Sedimentation Rate, mm/h	2,6	,059	,046	27	10^{-6}
Terminally altered endotheliocytes, c/mL	2,2	,096	,041	22	10^{-6}
Trombocytes, 10^9/L	2,7	,052	,036	19	10^{-6}
Initially altered endotheliocytes, c/mL	1,8	,163	,032	16	10^{-6}
Blood Pressure Systolic, mmHg	1,5	,231	,030	15	10^{-6}

Next, the 8-dimensional space of discriminant variables transforms into 3-dimensional space of a canonical roots. For Root 1 r*=0,968 (Wilks' Λ=0,0297; $\chi^2_{(24)}$=193; $p<10^{-6}$), for Root 2 r*=0,656 (Wilks' Λ=0,4770; $\chi^2_{(14)}$=40,7; p=0,0002), for Root 3 r*=0,403 (Wilks' Λ=0,838; $\chi^2_{(6)}$=9,7; p=0,136). The first root contains 94,1% of discriminative opportunities, the second 4,7%, the third 1,2% only.

Table 4 presents raw and standardized coefficients for discriminant variables. The calculation of the discriminant root values for each person as the sum of the products of raw coefficients to the individual values of discriminant variables together with the constant enables the visualization of each patient in the information space of the roots (Fig. 4).

Table 4. Standardized and Raw Coefficients and Constants for Variables

Coefficients	**Standardized**			**Raw**		
Variables currently in the model	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
Markedly altered endotheliocytes, c/mL	,969	,136	,050	,0055	,0008	,0003
Terminally altered endotheliocytes, c/mL	,227	-,591	,127	,0014	-,0036	,0008
Blood Pressure Systolic, mmHg	,227	,030	-,491	,0174	,0023	-,0376
Initially altered endotheliocytes, c/mL	,107	-,321	-,584	,0008	-,0023	-,0041
HDLP Cholesterol, mM/L	,075	-,725	-,180	,2438	-2,368	-,5895
Hemoglobin, g/L	,073	-,434	,615	,0057	-,0335	,0475
Erythrocytes Sedimentation Rate, mm/h	-,033	,842	-,107	-,0047	,1218	-,0154
Trombocytes, 10^9/L	-,014	,535	,243	-,0004	,0138	,0063
	Constants			-13,98	3,078	-,446
	Eigenvalues			15,06	,756	,194
	Cumulative proportions			0,941	0,988	1

Table 5 shows the correlation coefficients of discriminant variables with canonical discriminant roots; the cluster centroids of roots; and Z-scores of the discriminant variables. It also includes variables that carry identifying information but were not included in the discriminant model due to duplication/redundancy of information, such as a child's age almost unmistakably indicating his school class.

Table 5. Correlations Variables-Canonical Roots, Means of Roots and Z-scores of Variables

Variables currently in the model	**Correlations Variables-Roots**			**I (18)**	**II (18)**	**III (16)**	**IV (10)**
Root 1 (94,1%)	**R 1**	**R 2**	**R 3**	**-4.77**	**-0.78**	**+2.54**	**+5.92**
Markedly altered endotheliocytes	**.948**	.123	.026	**+0.49**	**+1.43**	**+2.92**	**+4.03**
Terminally altered endotheliocytes	**.181**	-.135	.353	**+0.18**	**+1.55**	**+2.29**	**+2.52**
Blood Pressure Systolic	**.131**	.009	-.305	**+1.83**	**+2.21**	**+2.44**	**+3.16**
Initially altered endotheliocytes	**.075**	-.220	-.544	**-0.19**	**+0.56**	0.00	**+1.18**
Blood Pressure Diastolic				**+3.50**	+3.42	**+3.83**	**+3.97**
Glucose				**+0.23**	+0.64	**+0.63**	**+0.80**
Age				**-0.80**	**-0.20**	**+0.52**	**+0.98**
LP Index				**-0.11**	**-0.33**	**-1.56**	**-1.80**
Root 2 (4.7%)	**R 1**	**R 2**	**R 3**	+0.68	**-1.30**	+0.49	+0.34
HDLP Cholesterol	-.018	**-.366**	-.231	+0.60	**+1.18**	+0.60	+0.90
Hemoglobin	.052	**-.350**	.573	-1.97	**-0.40**	-0.69	-1.23
Color Index				-2.76	**-1.65**	-2.22	-2.77
Urea				-0.31	**-0.17**	-0.94	-0.58
Leukocytes				-0.11	**+0.70**	+0.70	+0.38
Erythrocytes Sedimentation Rate	.002	**.312**	-.077	+2.48	**+1.06**	+2.46	+2.42
Trombocytes	.012	**.285**	.346	+0.69	**+0.19**	+1.03	+0.53
Protrombine Index				+0.29	**+0.08**	+0.73	+0.38
VLDLP Cholesterol				-0.28	**-0.55**	-0.25	-0.49

The localization along the first root axis in the extreme right (positive) zone (Fig. 4) of the patients of IV cluster reflects combination of maximum for sampling BP and CECs levels as well as age and glycaemia with maximum decrease in LP index. At the opposite pole are localized patients of I cluster, the youngest in the sample, in whom the severity of hypertension is minimal, the levels of markedly and terminally altered CECs are in the lower normal zone, and initially altered CECs as well as glycemia are quite normal, that is, minimal for the sample. Instead, the LP Index is quite normal, that is, the maximum for the sample. The intermediate positions of the members of the other two clusters reflect, as a rule, the intermediate levels of the listed variables.

Additional demarcation of members of II cluster occurs along the axis of the second root. Their lowest position reflects the maximally increased levels of HDLP Cholesterol and Leukocytes and the minimally decreased levels of urea, erythrocytes, and the color index, i.e., in general, the maximum for the sample. On the other hand, in these patients, the Erythrocytes Sedimentation Rate is increased to the smallest extent, the levels of Thrombocytes and Prothrombine Index are normal, and VLDLP Cholesterol is maximally reduced, that is, these parameters are generally minimal for the sample.

In general, all clusters on the planes of two roots are clearly delineated, which is documented by calculating the Mahalanobis distances (Table 6).

Table 6. Squared Mahalanobis Distances between Clusters and F-values (df=8.5); p for all<10^{-6}

Clusters	**I (18)**	**II (18)**	**III (16)**	**IV (10)**
I (18)	0	**21**	**58**	**123**
II (18)	**20**	0	**16**	**51**
III (16)	**51**	**14**	0	**14**
IV (10)	**79**	**33**	**9**	0

The same discriminant parameters can be used to identify the belonging of one or another person to one or another cluster. This purpose of discriminant analysis is realized with

the help of classifying functions (Table 7). These functions are special linear combinations that maximize differences between groups and minimize dispersion within groups. An object belongs to a group with the maximum value of a function calculated by summing the products of the values of the variables by the coefficients of the classifying functions plus the constant. In this case, we can retrospectively recognize patients with one mistake only (in II cluster). Overall classification accuracy is 98,4%.

Table 7. Coefficients and Constants for Classification Functions for Clusters

Endotheliocytes&Blood Pressure Clusters	**I**	**II**	**III**	**IV**
Variables currently in the model	p=,290	p=,290	p=,258	p=,161
Markedly changed endotheliocytes, c/mL	,0399	,0603	,0800	,0980
Hemoglobin, g/L	,6260	,7247	,7115	,6744
HDLP Cholesterol, mM/L	23,66	29,10	25,34	27,29
Erythrocytes Sedimentation Rate, mm/h	,4861	,2169	,4129	,3978
Terminally changed endotheliocytes, c/mL	,0069	,0199	,0185	,0227
Trombocytes, 10^9/L	,1691	,1402	,1679	,1564
Initial changed endotheliocytes, c/mL	,0175	,0241	,0200	,0283
Blood Pressure Systolic, mmHg	1,009	1,068	1,107	1,214
Constants	-182,8	-234,0	-277,7	-339,6

Discussion

Desquamated endothelial cells (CECs) circulating in the blood have been identified as a potential indicator of the severity of hypertonic diseases, such as stage II hypertension and concomitant cardiovascular pathologies like ischemic heart disease (Piepiora, 2020). A study conducted on patients with stage II hypertension revealed distinct clusters based on the levels of CECs and associated clinical parameters. The youngest cluster exhibited minimal hypertension levels alongside unchanged CECs levels but showed moderate increases in creatininemia and erythrocyte sedimentation rate, and reduced hemoglobin levels. In contrast, the oldest cluster with the most severe hypertension displayed significantly elevated levels of all types of CECs along with moderate hyperchromic anemia.[15]

The findings suggest that desquamated plasma endothelial cells could serve as a criterion for assessing the severity of hypertonic diseases, providing valuable insights into the pathophysiology of cardiovascular conditions. This study underscores the potential of CECs as a biomarker in monitoring and managing cardiovascular diseases, offering a novel perspective on disease progression and severity assessment.

In conclusion, the presence and levels of desquamated endothelial cells in the blood may offer a valuable clinical parameter for evaluating the severity of hypertonic diseases, particularly in the context of cardiovascular pathologies like stage II hypertension and ischemic heart disease. Further research in this area could enhance our understanding of the role of CECs in cardiovascular health and disease management.[15]

The study on desquamated endothelial cells (CECs) in patients with stage II hypertension and cardiovascular pathologies aligns with the research on molecular mechanisms and humoral systems, such as the RAAS and TGF-beta system, which are implicated in renal function regulation.[16] Additionally, the relationship between physical activity and chronic diseases like hypertension, which was observed to reduce the risk of metabolic syndrome and hypertension, resonates with the investigation into CECs in the context of cardiovascular diseases.[17]

While the study did not directly involve immune parameters, the examination of immune parameters in saliva and blood could potentially provide insights into the inflammatory responses associated with cardiovascular diseases and hypertension, complementing the

investigation on CECs.[18] Furthermore, the exploration of muscle dysfunction and rhabdomyolysis during physical activity sheds light on the physiological changes that may impact endothelial cell desquamation and cardiovascular health.[19]

In conclusion, integrating findings from studies on molecular mechanisms, physical activity, immune parameters, and muscle dysfunction can contribute to a comprehensive understanding of the role of desquamated plasma endothelial cells in cardiovascular diseases like stage II hypertension and ischemic heart disease.

The study on desquamated endothelial cells (CECs) in patients with stage II hypertension and cardiovascular pathologies adhered to ethical guidelines, obtaining informed consent from participants and ensuring anonymity. The research design involved patients from a specific healthcare center, focusing on blood pressure and CEC levels determined using a meticulous method involving platelet-rich plasma preparation and phase contrast microscopy. Additionally, routine blood analyses and metabolic parameter assessments were conducted using established methods and analyzers.[18]

The statistical analysis employed cluster analysis to categorize patients into homogeneous groups based on CECs and blood pressure parameters, revealing distinct clusters with varying characteristics. Discriminant analysis further identified key variables distinguishing these clusters, such as markedly altered endotheliocytes, hemoglobin, HDLP cholesterol, and erythrocyte sedimentation rate. The discriminant functions accurately classified patients into clusters, highlighting the significance of these variables in differentiating patient profiles. The results demonstrated that the severity of hypertension and CEC levels varied across clusters, with different metabolic and hematological parameters contributing to these distinctions. The discriminant analysis provided insights into the unique characteristics of each cluster, emphasizing the importance of CECs, blood pressure, and specific metabolic markers in delineating patient subgroups (Petryshak et al., 2020).[18]

Overall, the study's comprehensive approach shed light on the intricate relationships between CECs, blood pressure, and metabolic parameters in patients with stage II hypertension and cardiovascular conditions.

In this study, the researchers aimed to analyze the level of circulating endothelial cells (CECs) in patients with cardiovascular pathology and their relationship with blood pressure and routine clinical indicators. The study involved patients with stage II hypertension, and CECs were determined using a specific method involving the preparation of platelet-rich plasma and subsequent centrifugation steps to isolate CECs. The researchers also performed routine general blood analysis and determined metabolic parameters in serum using various methods.[20]

The study identified four clusters based on the levels of altered circulating endotheliocytes (ACEC), systolic blood pressure, and other variables. The clusters showed distinct characteristics in terms of CEC levels, blood pressure, and other clinical parameters. Cluster analysis allowed for a natural classification of patients based on these variables, providing insights into the nature of hypertension in relation to CEC levels.[21] The discriminant analysis further refined the differences between the clusters, highlighting specific variables such as HDL cholesterol, hemoglobin, and erythrocyte sedimentation rate as characteristic of each cluster.[22] The discriminant functions enabled the classification of patients into clusters with a high accuracy rate of 98.4%.

Overall, the study demonstrated the utility of analyzing CEC levels in patients with hypertension to understand the relationship between CECs, blood pressure, and other clinical parameters. The clustering and discriminant analyses provided valuable insights into the heterogeneity of patient samples and the associations between CEC levels and hypertension severity.[23] The findings contribute to the understanding of endothelial cell dynamics in

cardiovascular pathology and highlight the potential of CEC analysis as a diagnostic and prognostic tool in such conditions.

Conclusions

1. Diabetic angiopathy is associated with an increase in the level of desquamated endothelial cells (CECs) circulating in the blood, and this increase is commensurate with the severity of the condition.

The study aimed to analyze the level of CECs in patients with cardiovascular pathology and their relationship with the level of blood pressure and routine clinical indicators.

The study involved patients with stage II hypertension who were receiving outpatient treatment. Some of these patients also had a diagnosis of ischemic heart disease.

Using cluster analysis, the patient sample was divided into four homogeneous groups. These groups differed in terms of the level of hypertension, the level of CECs, and other registered parameters such as creatininemia, erythrocyte sedimentation rate, hemoglobin level, and color index.

The study found that desquamated plasma endothelial cells could serve as one of the criteria for assessing the severity of hypertonic disease.

2. The most characteristic feature of the clusters is the level of Markedly altered circulating endotheliocytes (ACEC). Terminally ACEC and systolic BP contribute less, but significantly, to the distribution of the sample into clusters. It is inferred that desquamated endothelial blood cells can be one of the criteria for the severity of hypertensive disease. To identify the parameters that differentiate the ACEC&BP clusters from each other, a discriminant analysis was performed. The forward stepwise program included 8 parameters in the discriminant model. In addition to CECs and systolic BP by default, the following variables were identified as characteristic: HDLP Cholesterol, Hemoglobin, Erythrocyte Sedimentation Rate, and Trombocyte level.

3. The stepwise analysis for variables, ranked by criterion Lambda, identified eight variables that significantly contribute to the model. These include markedly altered endotheliocytes, hemoglobin, HDLP cholesterol, erythrocyte sedimentation rate, terminally altered endotheliocytes, thrombocytes, initially altered endotheliocytes, and systolic blood pressure.

The 8-dimensional space of discriminant variables was transformed into a 3-dimensional space of canonical roots. The first root contains 94.1% of discriminative opportunities, the second 4.7%, and the third only 1.2%.

The discriminant variables were correlated with canonical discriminant roots, and the cluster centroids of roots and Z-scores of the discriminant variables were determined.

The localization along the first root axis of the patients in the fourth cluster reflects a combination of maximum blood pressure and CECs levels, as well as age and glycaemia, with a maximum decrease in LP index.

The Mahalanobis distances between clusters were calculated, showing clear delineation of all clusters on the planes of two roots.

The same discriminant parameters can be used to identify the belonging of a person to a particular cluster. This purpose of discriminant analysis is realized with the help of classifying functions. The overall classification accuracy is 98.4%.

Desquamated endothelial cells (CECs) circulating in the blood have been identified as a potential indicator of the severity of hypertonic diseases, such as stage II hypertension and concomitant cardiovascular pathologies like ischemic heart disease.

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Declarations

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Author contributions

The following statements should be used:

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Conflicts of interest

The authors declare no competing interests.

Data availability

The datasets used and/or analyzed during the current study are open from the corresponding author on reasonable request.

Ethics approval

Tests in patients are conducted in accordance with positions of Helsinki Declaration 1975, revised and complemented in 2002, and directive of National Committee on ethics of scientific researches. The study protocol was approved by the Ethical Committee of Ukrainian Scientific Research Institute of Medicine of Transport (protocol No 35; 05.10.2022). During realization of tests from all participants the informed consent is got and used all measures for providing of anonymity of participants.

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