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Circulating endothelial progenitor cells as a marker of left ventricular pump function in ischemic chronic heart failure

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The goal of the study was to explore whether the quantity of endothelial progenitor cells (EPCs) associates with ischemic chronic heart failure (CHF) phenotypes.

Materials and methods. Inclusion criteria met 82 patients with ischemic CHF. Sick persons with global left ventricular ejection fraction >40 % were graded as the HFpEF group (n = 39) and others with ≤40 % as the HFrEF group (n = 43). The levels of biomarkers in serum were measured at starting point. The method of flow cytometry was used for predictably distinguishing circulating cell subsets depending on expression of CD45, CD34, CD14, Tie-2, and CD309 antigens.

Results. In multivariate logistic regression model galectin-3 (R^2 = 0.67; P = 0.012), T2DM (R^2 = 0.26; P=0.001), previous MI (R^2 = 0.17; P = 0.012), obesity (R^2 = 0.22; P = 0.001), $CD14^+CD309^+$ cells (R^2 = 0.058; P = 0.001), and $CD14^+CD309^+Tie\text{-}2^+$ cells (R^2 = 0.044; P = 0.028), NT-proBNP (R^2 = 0.11; P = 0.046) were found as autonomous predictors of HFpEF. With help of multivariate Cox-regression analysis we found out, that NT-proBNP (OR 1.08; 95 % CI = 1.03–1.12; P = 0.001) and number of $CD14^+CD309^+$ cells (OR 1.07; 95 % CI = 1.02–1.11; P = 0.05) were independent predictors for HFpEF. The quantity of $CD14^+CD309^+$ cells added to NT-proBNP had more exact predictive value (OR 1.10; 95 % CI = 1.04–1.14; P = 0.001) than these biomarkers unaccompanied.

Conclusion: quantity of NT-proBNP added to $CD14^+CD309^+$ cells, cardiovascular risk influences and clinical information exhibited the best discriminate importance to differentiate HFpEF from HFrEF.

Key words: heart failure, preserved left ventricular function, biomarkers, endothelial progenitor cells.

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Циркулюючі ендотеліальні прогеніторні клітини як маркер насосної функції лівого шлуночка в пацієнтів із хронічною серцевою недостатністю ішемічного генезу

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Мета роботи – дослідити зв'язок числа ендотеліальних прогеніторних клітин (ЕПК) із фенотипами хронічної серцевої недостатності (ХСН).

Матеріали та методи. У дослідженні відповідно до критеріїв включення взяло участь 82 пацієнти з ХСН ішемічного генезу. Пацієнти з фракцією викиду лівого шлуночка >40 % були класифіковані як група хворих із серцевою недостатністю зі збереженою фракцією викиду (СНЗбФВ) (n = 39), а пацієнти з фракцією викиду лівого шлуночка ≤40 % – як група хворих із серцевою недостатністю зі зниженою фракцією викиду (СНЗнФВ) (n = 43). Рівень біомаркерів сироватки вимірювався на початку дослідження. Проточну цитометрію використовували для дослідження відмінностей експресії антигенів CD45, CD34, CD14, Tie-2 та CD309 у пацієнтів, які включені в дослідження.

Результати. У моделі багатовимірної логістичної регресії цукровий діабет 2 типу (R^2 = 0,26, P = 0,001), ожиріння (R^2 = 0,22, P = 0,001), перенесений інфаркт міокарда (R^2 = 0,17, P = 0,012), галектін-3 (R^2 = 0,67; P = 0,012), мозковий натрійуретичний пептид (МНУП) (R^2 = 0,11, P = 0,046), кількість $CD14^+CD309^+$ (R^2 = 0,058, P = 0,001) і $CD14^+CD309^+Tie\text{-}2^+$ (R^2 = 0,044, P = 0,028) визначили як незалежні предиктори СНЗбФВ. За допомогою мультиваріантної моделі регресії Кокса встановлено, що МНУП (OR 1,08, 95 % CI = 1,03–1,12, P = 0,001) і кількість $CD14^+$ $CD309^+$ клітин (OR 1,07, 95 % CI = 1,02–1,11, P = 0,05) були незалежними предикторами для СНЗбФВ. Кількість клітин $CD14^+CD309^+$ спільно з МНУП мало вищу прогностичну цінність (OR 1,10, 95 % CI = 1,04–1,14, P = 0,001), ніж досліджувані біомаркери окремо.

Висновки. Спільне визначення кількості $CD14^+$ $CD309^+$ клітин спільно з МНУП, клінічні дані та серцево-судинні фактори ризику показали найкращу передбачувальну цінність для диференціації СНЗбФВ від СНЗнФВ.

Ключові слова: серцева недостатність, функція лівого шлуночка, біомаркери, ендотеліальні прогеніторні клітини.

Актуальні питання фармацевтичної і медичної науки та практики. – 2017. – Т. 10, № 3(25). – С. 302–305

Циркулирующие эндотелиальные прогениторные клетки как маркер насосной функции левого желудочка у пациентов с хронической сердечной недостаточностью ишемического генеза

А. А. Кремзер

Цель работы – исследовать связь числа эндотелиальных прогениторных клеток (ЭПК) с фенотипами хронической сердечной недостаточности (ХСН).

Материалы и методы. В исследовании в соответствии с критериями включения участвовало 82 пациента с ХСН ишемического генеза. Пациенты с фракцией выброса левого желудочка >40 % были классифицированы как группа больных с сердечной недостаточностью с сохраненной фракцией выброса (СНСоФВ) (n = 39), а пациенты с фракцией выброса левого желудочка ≤40 % – как группа больных с сердечной недостаточностью со сниженной фракцией выброса (СНСнФВ) (n = 43). Уровень биомаркеров сыворотки измерялся в начале исследования. Проточная цитометрия использовалась для исследования различий экспрессии антигенов CD45, CD34, CD14, Tie-2 и CD309 у пациентов, включённых в исследование.

Результаты. В модели многомерной логистической регрессии сахарный диабет 2 типа (R^2 = 0,26, P = 0,001), ожирение (R^2 = 0,22, P = 0,001), перенесенный инфаркт миокарда (R^2 = 0,17, P = 0,012), галектин-3 (R^2 = 0,67; P = 0,012), мозговой натрийуретический пептид (МНУП) (R^2 = 0,11, P = 0,046), количество $CD14^+CD309^+$ (R^2 = 0,058, P = 0,001) и $CD14^+CD309^+Tie\text{-}2^+$ (R^2 = 0,044, P = 0,028) определены как независимые предикторы СНСоФВ. С помощью мультивариантной модели регрессии Кокса установлено, что МНУП (OR 1,08, 95 % CI = 1,03–1,12, P = 0,001) и количество $CD14^+CD309^+$ клеток (OR 1,07, 95 % CI = 1,02–1,11, P = 0,05) были независимыми предикторами для СНСоФВ. Количество клеток $CD14^+$ $CD309^+$ совместно с МНУП имело более высокую прогностическую ценность (OR 1,10, 95 % CI = 1,04–1,14, P = 0,001), чем исследуемые биомаркеры в отдельности.

Выводы. Совместное определение количества $CD14^+CD309^+$ клеток совместно с МНУП клинические данные и сердечно-сосудистые факторы риска показали наилучшую предсказательную ценность для дифференциации СНСоФВ от СНСнФВ.

Ключевые слова: сердечная недостаточность, функция левого желудочка, биомаркеры, эндотелиальные прогениторные клетки.

Актуальные вопросы фармацевтической и медицинской науки и практики. – 2017. – Т. 10, № 3(25). – С. 302–305

Chronic heart failure (HF) is a foremost cause of cardiovascular (CV) mortality and morbidity worldwide [1]. HF with reduced ejection fraction (HFrEF) shows sufficiently difference in hospital fees and mortality rate compared to HF with preserved ejection fraction (HFpEF) [2]. In this background, the routine use of biomarkers of HFrEF and HFpEF can be helpful to stratify the patients at higher risk of death and medical consequences [3].

Recent investigations showed that endothelium injury is common for HF onset and development beyond etiology [4]. Endothelial dysfunction closely associates with activation and/or apoptosis of endothelial cells. Therefore, lack of endothelial progenitor cells (EPCs) $CD14^+CD309^+$(VEGFR2) and $CD14^+CD309^+$(VEGFR2) $Tie\text{-}2^+$ cells was found as indicator of endothelial dysfunction and compensation ability [5].

The aim of the study

The aim of the study was to explore whether the number of endothelial progenitor cells with angiogenic capacity would associate with HF phenotypes.

Materials and methods

A total of 82 subjects with CHF were added in this study after reviewing discharge reports. Chronic HF was defined according modern criteria published by actual scientific recommendation [6]. Criteria for including patients with CHF were: LVEF <59 %, increased level of NT-proBNP >220 pg/mL and clinical signs of CHF. Excluding conditions were: malignancies; severe kidney and liver diseases; estimated GFR index <35 mL/min/м^2; brain damage within 3 months before the admission; valvular disease of heart; acute infections; thyrotoxicosis; ischemic stroke; surgery; intracranial hemorrhage; pregnancy; trauma; implanted cardioverter. Patients with overall left ventricular ejection fraction ≤40 % were categorized as the HFrEF group (n = 43) and those with >40 % as patients with HFpEF group (n = 39).

Before the study beginning, the local Ethics Committee Review Board of State Medical University of Zaporizhzhia (Ukraine) approved the study procedure. The study meet the terms with the Declaration of Helsinki and informed written agreement was obtained from all patients involved in this study.

End-diastolic and end-systolic left ventricular volumes and LVEF were measured by modified Simpson's method [7].

Calculation of glomerular filtration rate (GFR) was intended by CKD-EPI formula [8]. Fast blood samples were drawn in the morning (at 7–8 a. m.) into chilled silicone test pipes where in 2 mL of 5 % Trilon B solution were added; at that point they were directly centrifuged upon permanent chilling at 6.000 rpm for 10 minutes. Then, plasma was chilled immediately and stored at a temperature -70 °C. All laboratory examinations were performed using standard procedures to measure the serum fasting plasma glucose, fasting phospholipid profiles and other biomarkers.

We used flow cytometric method (FCM) for predictably distinguishing circulating cell subgroups, that depend on manifestation of CD45, CD34, CD14, Tie-2, and CD309 (VEGFR2), using High-definition Fluorescence Activated Cell Sorter (HD-FACS) procedure [8].

Determination of Circulating EPCs was defined as CD34/CD309 (VEGFR2) positive cells with deficiency of CD45 expression. From every pipe, 500.000 measures were analyzed. Using quadrant analysis co-expression with $Tie\text{-}2^+$ and/or $VEGFR\text{-}2^+$ for $CD14^+$ populations was determined. Standardized cell amounts were offered as a percentage of the entire white blood cell count, identified as the total number of all $CD45^+$ cells. The FITC-labeled isotype control was examined with the same gate and window sets. Pro-angiogenic phenotype for EPCs was resolute as $CD14^+CD309^+$ (VEGFR2) $Tie\text{-}2^+$ antigen appearance. The reproducibility of EPC amounts using the standard protocol was 3.5 %. Information was analyzed using SPSS 20.0 (SPSS, IBM Corporation, NY, USA). Numerical variables were expressed as mean (M) and standard deviation (± SD), median and interquartile range (IQR), estimated marginal mean (95 % confidence interval [CI]) or number (percentage).

The potential influences that may be associated with HFpEF were acknowledged first with the univariate examination (ANOVA), and then the independent prognosticators of HFpEF were searched with the multivariate one-step backward logistic regression analysis, firstly including variables for which a P value <0.1 was reached from the univariate analysis. For all regression models, we calculated R^2. The odds ratio (OR) and 95 % CI were calculated for influences independently predicted phenotype of HF in Cox regression model. A difference of $P < 0.05$ was considered significant.

Results

The study population consisted of 82 HF subjects with mean age 52.13 ± 7.80 years. The reference point data of eligible persons listed in *Table 1*. As one can see, the majority of the patients included in the study was male (53.6 %) with II

(32.9 %) and III (40.2 %) functional class (FC) of NYHA and previously determined myocardial infarction (MI) (68.2 %). At least 63 % of the patients were hypertensive and 24 % of individuals had diabetes mellitus. There was not a significant difference between cohorts of the patients in age, sex, frequency of hypertension, whereas individuals with HFrEH were frequently diagnosed II and III FC of NYHA, MI.

The hemodynamics and biochemical parameters reported in *Table 2*. There is strong evidence that patients with HFrEF had significantly higher creatinine, NT-proBNP, galectin-3, numerous of $CD14^+CD309^+$ and $CD14^+CD309^+Tie\text{-}2^+$ to HFpEF. In contrast, individuals with HFrEF had lower GFR when compared to HFpEF.

In HFrEF cohort serum galectin-3 associated positively with NYHA class of CHF (r = 0.27, P = 0.001), and negatively with $CD14^+CD309^+$ cells (r = -0.28; P = 0.003), $CD14^+CD309^+Tie\text{-}2^+$ cells (r = -0.23; P = 0.001), GFR (r = -0.23; P = 0.001). Therefore, NT-pro-BNP positively associated with NYHA class of CHF (r = 0.43, P = 0.001), and negatively with LVEF (r = -0.43, P = 0.001), GFR (r = -0.28; P = 0.001), obesity (r = -0.25; P = 0.001).

In HFpEF galectin-3 positively associated with type two diabetes mellitus (r = 0.26; P = 0.001), and negatively with $CD14^+CD309^+$ cells (r = -0.32; P = 0.001), $CD14^+CD309^+$ $Tie\text{-}2^+$ cells (r = -0.29; P = 0.001). NT-pro-BNP positively associated with NYHA class of CHF (r = 0.36, P = 0.002), and negatively with LVEF (r = -0.28, P = 0.001), $CD14^+CD309^+$ cells (r = -0.26, P = 0.003), obesity (r = -0.24, P = 0.001).

In multivariate logistic regression model type 2 diabetes mellitus (R^2 = 0.26; P = 0.001), obesity (R^2 = 0.22; P = 0.001), previous MI (R^2 = 0.17; P = 0.012), galectin-3 (R^2 = 0.67; P = 0.012), NT-proBNP (R^2 = 0.11; P = 0.046), $CD14^+CD309^+$ cells (R^2 = 0.058; P = 0.001), and $CD14^+CD309^+Tie\text{-}2^+$ cells (R^2 = 0.044; P = 0.028) were found as independent predictors of HFpEF.

Using multivariate Cox-regression analysis adjusted etiology (previous myocardial infarction), cardiovascular risk influences (obesity, type 2 diabetes mellitus) we found that NT-proBNP (OR 1.08; 95 % CI = 1.03–1.12; P = 0.001) and $CD14^+CD309^+$ cells (OR 1.07; 95 % CI = 1.02–1.11; P = 0.02) remained independent predictors for HFpEF. Moreover, number of $CD14^+CD309^+$ cells added to NT-proBNP had more accurate

Table 1. The basic characteristics of participants in the study

Variables	Entire patient group (n = 82)	Subjects with HFrEF (n = 43)	Subjects with HFpEF (n = 39)	P value between HF cohorts
Age, years	56.13 ± 7.80	57.50 ± 6.70	54.79 ± 6.62	0.78
Male	44 (53.6 %)	25 (58.1 %)	19 (48.7 %)	0.24
II NYHA class	27 (32.9 %)	18 (41.8 %)	9 (23.1 %)	0.026
III NYHA class	33 (40.2 %)	15 (34.8 %)	18 (46.2 %)	0.048
IV NYHA class	22 (26.8 %)	10 (23.3 %)	12 (30.7 %)	0.12
Previous MI	56 (68.2 %)	32 (74.1 %)	24 (61.5 %)	0.01
Hypertension	52 (63.4 %)	25 (58.1 %)	26 (66.7 %)	0.28
T2DM	20 (24.3 %)	8 (18.6 %)	12 (30.8 %)	0.01

Table 2. The hemodynamics and biochemical parameters of participants in the study

Variables	Entire patient group (n = 82)	Subjects with HFrEF (n = 43)	Subjects with HFpEF (n = 39)	P value between HF cohorts
Systolic BP, mm Hg	132 ± 9	130 ± 7	133 ± 6	0.88
Diastolic BP, mm Hg	77 ± 6	76 ± 5	78 ± 5	0.88
Heart rate, beat per min	72.35 ± 6.95	76.20 ± 5.11	66.70 ± 5.24	0.12
LVEF, %	45.5 (30.4–55.3)	36.50 (30.7–39.1)	55.1 (42.7–58.4)	0.001
GFR, mL/ min/1.73 m^2	82.3 (68.7–102.6)	79.6 (63.1–92.3)	88.2 (77.1–102.1)	0.046
Fasting glucose, mmol/L	5.17 (3.5–9.6)	4.98 (3.8–8.1)	5.27(3.6–9.3)	0.28
Creatinine, μmol/L	72.3 (58.7–92.6)	82.1 (64.9–90.5)	67.7 (59.1–84.1)	0.01
Total cholesterol, mmol/L	5.1 (3.9–6.1)	5.3 (4.6–6.0)	5.0 (3.5 –5.9)	0.02
NT-pro-BNP, pg/mL	2336.2 (988.5–3552.8)	2774.5 (1520.4–3870.2)	2130.8 (954.5–3056.2)	0.02
Galectin-3, μg/L	18.92 (14.25–23.15)	19.03 (15.80–23.96)	16.99 (13.77–19.20)	0.022
$CD14^+CD309^+$, cells/μL	0.296 (0.225–0.351)	0.236 (0.202–0.325)	0.325 (0.233–0.407)	0.001
$CD14^+CD309^+$ $Tie\text{-}2^+$, cells/μL	0.032 (0.025–0.410)	0.030 (0.021–0.403)	0.036 (0.019–0.465)	0.26

The values correspond to medians and IQR of 25–75 %. Statistical comparisons are made using Mann–Whitney test with significance levels of <0.05 (for 2-tailed).

Abbreviations: **GFR** – glomerular filtration rate; **BMP** – brain natriuretic peptide; **BP** – blood pressure; **LVEF** – left ventricular ejection fraction.

predictive value (OR 1.10; 95 % CI = 1.04–1.14; P = 0.001) than the only one of these biomarkers.

The results of the study confirm the hypothesis regarding the potential deficiency of pro-angiogenic EPCs labeled $CD14^+CD309^+$ and $CD14^+CD309^+$ Tie-2^+ that could be a marker of severity of HF. It turns out that transformation of HFpEF as temporary stage of HF to HFrEF may associate with development of EPC dysfunction. Moreover, there is suggested that EPC dysfunction is not only result in etiology effect and co-morbidity influence, but it is independent predictor of HF advance and probably HF-related outcomes. Indeed, previously reported results of other studies provided by wide circle of investigators have shown that the EPCs can appear prior to asymptomatic HF and cooperate with numerous CV risk factors [9,10]. Therefore, EPC dysfunction reflects lowered ability of EPCs in survival, migration and differentiation and thereby it is confirmation a pivotal role of endogenous endothelial repair system in shaping maladaptive cardiac and vascular remodeling and HF development [11,12]. Nevertheless, EPC dysfunction as a marker of endothelial dysfunction and worsening vascular repair may be the best biomarker for personified risk stratification among HF individuals.

Conclusions

Quantity of NT-proBNP added to $CD14^+CD309^+$ cells, cardiovascular risk aspects and clinical data showed the best discriminate value and higher reliability to forecast HFpEF compared with NT-proBNP and medical data / cardiovascular risk influences alone.

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