Predictive value of circulating apoptotic microparticles in patients with ischemic symptomatic moderate-to-severe chronic heart failure

Aim. To evaluate the prognostic value of circulating CD31+/annexin V+ microparticles (MPs) for cumulative survival in patients with ischemic chronic heart failure (CHF).

Methods. A total of 154 patients with ischemic symptomatic moderate-to-severe CHF were enrolled in the study on discharge from the hospital. Observation period was up to 3 years. Blood samples for biomarkers measurements were collected. Flow cytometry analysis for quantifying the number of CD31+/annexin V+ MPs was used: CD31+/annexin V+ MPs for cumulative survival cases due to CHF were tested. Additionally, all-cause mortality, and CHF-related death were examined.

Results. During a median follow-up of 2.18 years, 21 participants died and 106 subjects were hospitalized repeatedly. Medians of circulating levels of CD31+/annexin V+ MPs in patients who survived and subjects who died were 0.286/mL (95% confidence interval [CI]=0.271–0.309/mL) and 0.673/mL (95% CI=0.65–0.74/mL) (P<0.001). Number of circulating MPs was distributed into Quartiles (Q): Q1 (<0.341/mL), Q2 (0.342–0.514/mL), Q3 (0.521–0.848/mL), and Q4 (>0.850/mL). Receive Operation Curve (ROC) analysis has been shown that cut off point of CD31+/annexin V+ MPs number for cumulative survival function was 0.514/mL. Area under curve was 0.913 (Standard error=0.025; 95% CI=0.863–0.962), sensitivity and specificity were 89.6% and 69.7% respectively. It has been found a significantly divergence of Kaplan–Meier survival curves in patients with high quartile (MPs number >0.514/mL) of MPs numbers when compared with low quartiles. Using a stepwise model selection method for multivariable prediction model we investigated that CD31+/annexin V+ MPs number alone and combination of CD31+/annexin V+ MPs number with NT-pro-brain natriuretic peptide (NT-pro-BNP) remained statistically significant predictors for all-cause mortality, CHF-related death, and CHF-related re-hospitalisations, whereas combination of CD31+/annexin V+ MPs with both NT-pro-BNP and left ventricular ejection fraction did not.

Conclusion. Increased circulating CD31+/annexin V+ MPs associates with increased 3-year CHF-related death, all-cause mortality, and risk for recurrent hospitalization due to CHF.

Key words: apoptotic microparticles, chronic heart failure, prognosis.

Background.
Chronic heart failure (CHF) is considered as a leading cause of morbidity and mortality in worldwide (Roger V.L., 2010). Endothelial dysfunction has been shown to play a critical role in the clinical manifestations of CHF (Matsuzawa Y. et al., 2013). Recent studies suggested that injury of endothelial monolayer due to any reasons leads to dramatic increase of circulating level of endothelial-derived apoptotic microparticles (MPs) (Horstman L.L. et al., 2004). MPs are a heterogeneous population of submicronic vesicles that are released in response to cell activation or apoptosis (Hristov M. et al., 2004). It has been investigated that MPs represent an intercellular communication and delivery mechanism for the efficient and effective transfer of biological information, which selectively packaged as intracellular material included bioactive lipids, integrins, cytokines, enzymes, matrix ribonucleic acid (mRNA) and micro-RNA that lead to reprogramming recipient cells; proatherogenic and prothrombotic effects; as well as modulating inflammatory response (Mallat Z. et al., 2000; Norling L.V., Dali J., 2013). Increase in circulating MPs is detectable in several cardiovascular diseases, such as acute coronary syndrome, atherosclerosis, dyslipidaemia, hypertension, stroke, atrial fibrillation, as well as sepsis, cancer, lupus erythematosus, chronic kidney disease, type two diabetes mellitus, obesity (Pirro M. et al., 2006; Huang P.H. et al., 2010; Jesel L. et al., 2013). While MPs are sensitivity markers of endothelial dysfunction and tissue remodelling, they are also indicator of an imbalance between pro-angiogenic and anti-angiogenic responses, and they could be used to predict value in cardiovascular disease (Sinning J.M. et al., 2011). It has been postulated that CD31+/annexin V+ MPs might be discussed as prognostic factors in CHF, but their predictive value in patients with symptomatic ischemic CHF has not been defined. The aim of this study was to evaluate the potential prognostic value of circulating CD31+/annexin V+ MPs for cumulative survival in patients with ischemic CHF.

Methods.
The study evolved 154 patients (86 males) aged 48 to 62 years with ischemic symptomatic moderate-to-severe CHF. CHF was diagnosed according to current clinical guidelines (McMurray J.J.V. et al., 2012). Table 1 shows characteristics of the patients participated in the study. All the patients have given their written informed consent for participation in the study. The following are exclusion criteria: Q-wave and non-Q-wave myocardial infarction within 3 months before study entry; severe kidney and liver diseases that may affect clinical outcomes; malignancy; creatinine plasma level above 440 μmol/L; estimated glomerular filtration rate (GFR) <35 ml/min/m²; brain injury within 3 months before the enrollment; body mass index above 30 kg/m² and less 15 kg/m²; pulmonary edema; tachyarrhythmia; valvular heart disease; thyrotoxicosis; ischemic stroke; intracranial hemorrhage; acute infections; surgery; trauma; all the ischemic events within 3 previous months; inflammations within a previous month; neoplasm; pregnancy; implanted pacemaker, any disorder that may discontinue patient’s participation in the study according to investigators; and patient’s refusal to participate in the study or to give his consent for it. Observation period was up to 3 years. We analyzed cumulative survival related to CHF, and additionally all-cause mortality was examined.

Multispiral computed tomography angiography and/or angiographic study have been carried out to verify the ischemic nature of the disease in patients. Multispiral computed tomography angiography has been carried out for all the patients prior to their inclusion in the study. When atherosclerotic lesions of
the coronary arteries were verified, patients were subjected to conventional angiographic examination provided indications for revascularization were available. Coronary artery disease (CAD) was considered to be diagnosed upon availability of previous angiographic examinations carried out not later than 6 months ago provided no new cardiovascular events occurred for this period. The coronary artery wall structure was measured by means of contrast spiral computed tomographic angiography (Bluemke D.A. et al., 2008) on Somatom Volum Zoom scanner (Siemens, Erlangen, Germany) with two detector rows when holding patients breathe at temperature not higher than –35 °C. Circulating MPs, were isolated from 5 ml of venous citrated blood drawn from the fistula-free arm. Platelet-free plasma was separated from whole blood and then was centrifugated at 20,500 rpm for 30 min. MPs pellets were washed with Dulbecco’s modified Eagle’s medium (DMEM) (supplemented with 10 μg/ml polymyxin B, 100 UI/ml of streptomycin, and 100 μ/ml penicillin) and centrifuged again (20,500 rpm for 30 min). The obtained supernatant was extracted, and pellets were resuspended into the remaining 200 μl of supernatant. Platelet-free plasma, MPs, pellet, and supernatant were diluted five-, 10-, and five-fold in PBS, respectively. Apoptotic MPs were phenotyped by flow cytometry with phycoerythrin (PE)-conjugated monoclonal antibody against CD31 (BD Biosciences, USA) followed by incubation with fluorescent isothiocyanate (FITC)-conjugated annexin V (BD Biosciences, USA) per HD-FACS (High-Definition Fluorescence Activated Cell Sorter) methodology. The samples were then analyzed on a FACs flow cytometer (Beckman Coulter) after 400 μL annexin-V binding buffer was added. For each sample, 500 thousand events have been analyzed.

CD31/annexin V+ MP gate was defined by size, using 0.8 and 1.1 mm beads (Sigma, St Louis, MO, USA). Apoptotic MPs were defined as CD31+/annexin V+ MPs positively labeled for CD31 and annexin V (CD31+/annexin V+) (Lacroix R. et al., 2013).

Statistical analysis of the results obtained was carried out in SPSS system for Windows, Version 22 (SPSS Inc, Chicago, IL, USA). The data were presented as mean (M) and error of mean (±m) or 95% CI; median (Me) and interquartile range. To compare the main parameters of patients’ groups (subject to the type of distribution of the parameters analyzed), one-tailed Student t-test or Shapiro — Wilk U-test were used. To compare categorical variables between groups, Chi2 test (x²) and Fisher F exact test were used. The circulating CD31+/annexin V+ MP and NT-pro-BNP level in the blood failed to have a normal distribution, while distribution of the TC and cholesterol fractions had a normal character (estimated by means of Kolmogorov — Smirnov test) and was not subjected to any mathematical transformation. The factors, which could be associated potentially with circulating CD31+/annexin V+ MPs, were determined by means of multiple regression analysis. Receiver Operation Curve (ROC) analysis was performed to identify the optimal cutoff points of the CD31+/annexin V+ MPs number with predicted value. Odds ratio (OR) and 95% (CI) were calculated for all the independent predictors of survival of the patients who died in the study (n=21). The factors, which could be associated potentially with circulating CD31+/annexin V+ MPs, were determined by means of multiple regression analysis. Receiver Operation Curve (ROC) analysis was performed to identify the optimal cutoff points of the CD31+/annexin V+ MPs number with predicted value. Odds ratio (OR) and 95% (CI) were calculated for all the independent predictors of survival of the patients who died in the study (n=21).
During a median follow-up of 2.18 years, 21 participants died and CHF-related death was defined in 18 patients. Additionally, 106 subjects were hospitalized repetitively due to advance CHF (17 cases in died cohort and 89 cases in survival cohort). Table 1 shows a general characteristic of the patients included in the study. As one can see from Table 1, no substantial age and gender differences were found among persons who died and survived, as well as differences in body mass index, GFR, glycated hemoglobin (HbA1c), fasting blood glucose level, blood creatinine level, TC, LDL-C and HDL-C, numerous of coronary vessels damaged. No difference was found between the two cohorts in systemic office blood pressure and heart rate. Documented incidence of type 2 diabetes mellitus (T2DM) in patients of the two cohorts was 38.1% and 33.8% (P=0.06). Note that there was not a statistically significant change in peak velocity of early diastolic left ventricular filling to late diastolic myocardial velocity ratio (E/Am) and peak velocity of early diastolic left ventricular filling to early diastolic myocardial velocity ratio (E/Em) between the two cohorts, while decrease in the LVEF value was quite anticipated in the setting in patients who died. At the same time, the level of circulating NT-pro-BNP was statistically significantly higher in patients who died than in persons who survived. When analyzing details of pharmacotherapy, no substantial differences were found between the two cohorts with regard to administration of the majority of drugs.

Medians of circulating levels of CD31+/annexin V+ MPs in cohorts patients who survived and patients who died were 0.286/mL (95% CI=0.271–0.309/mL) and 0.673/mL (95% CI=0.65–0.74/mL) (P<0.001). Number of circulating CD31+/annexin V+ MPs was distributed into Quartiles (Q): Q1 (<0.341/mL), Q2 (0.342–0.514/mL), Q3 (0.521–0.848/mL), and Q4 (>0.850/mL). The data suggested that CD31+/annexin V+ MPs number in plasma was directly related to NewYork Heart Association (NYHA) class of CHF (r=0.514, P<0.001), NT-pro-BNP (r=0.416, P<0.001), T2DM (r=0.402, P=0.003), multi-vessel lesion of coronary arteries (r=0.362, P=0.001), E/A (r=0.360, P=0.001), E/Em (r=0.344, P=0.001), gender (r=0.318, P<0.001 for male), TC (r=0.313, P=0.001), age (r=0.275, P=0.001), smoking (r=0.212, P=0.001) and inversely to LVEF (r=-0.496, P=0.001) and estimated GFR (r=-0.408, P=0.003). No significant association between the levels of circulating CD31+/annexin V+ MPs with fasting plasma glucose, HbA1c, means systolic and diastolic blood pressure, premature CAD in family anamnesis, and medications for both cohorts of the patients was found.

The optimum cut-off point for CD31+/annexin V+ MP number in circulation is determined by the relative importance of the sensitivity and specificity of the test. ROC analysis has been shown that cut-off point of CD31+/annexin V+ MP number for cumulative survival function was 0.514/mL (Fig. 1). Area under curve (AUC) was 0.913 (Standard error=0.025; 95% CI=0.863–0.962), sensitivity and specificity were 89.6 and 69.7%, respectively. Iterations between sensitivity and specificity of CD31+/annexin V+ MPs cut-off point level for other clinical outcomes in study patient population are presented Table 2. For all occasions the model was robust and it has provided a significant results using optimal cut-off point of CD31+/annexin V+ MPs.

Multivariate logistic regression was used to assess whether any combination of assays was able to better discriminate between survivors and died patients. In the logistic regression analysis, the main factors independently related with cumulative mortality and CHF-related re-hospitalisations were MPs, NT-pro-BNP, NYHA class, LVEF, T2DM, and three- and multi-vessel lesion. Circulating MPs number independently predicted all-cause mortality (OR=1.58; 95% CI=1.20–1.98; P=0.001), CHF-related death (OR=1.22; 95% CI=1.12–1.36; P<0.001), and also CHF-related rehospitalisation (OR=1.20; 95% CI=1.11–1.32; P<0.001) within 3 years of observation period (Table 3). NYHA class, NT-pro-BNP and LVEF remained statistically significant for all categories: all-cause mortality, CHF-related death, and CHF-related re-hospitalisations, whereas T2DM and three- and multi-vessel lesion for all variables did not.

Using a stepwise model selection method for multivariable prediction model we investigated the summary effect of any combinations of CD31+/annexin V+ MPs, NT-pro-BNP, LVEF on all-cause mortality, CHF-related death, and CHF-related re-hospitalisations. We found that CD31+/annexin V+ number alone (Model 1) and combination of CD31+/annexin V+ MPs number with NT-pro-BNP reached be able significance in 60 weeks after study entry.

It has been found a significantly divergence of Kaplan — Meier survival curves in patients with high quartile (>0.514/mL) of CD31+/annexin V+ MPs numbers when compared with low quartiles (Q1–Q3) (Fig. 2). The curves divergence of events accumulation reached a statistical significance in 50 weeks of observation period (P<0.001 for all cases). No statistically significance differences between survival in patient cohorts with Q1 and Q2, as well as Q2 and Q3 in numbers of CD31+/annexin V+ MPs were found. The divergence between two cohorts with CD31+/annexin V+ MPs numbers in Q1 and Q3 was
(Model 2) remained statistically significant predictors for all-cause mortality (B-coefficient=5.38, p=0.001, and B-coefficient=6.32, p=0.001, respectively), CHF-related death (B-coefficient=4.34, p=0.001, and B-coefficient=5.11, p=0.001, respectively), and CHF-related re-hospitalisations (B-coefficient=3.82, p=0.001, and B-coefficient=3.26, p=0.001, respectively), whereas combination of CD31/annexin V MPs with both NT-pro-BNP and LVEF (Model 3) did not (B-coefficient=0.16, p=0.72, and B-coefficient=0.22, p=0.58, and B-coefficient=-0.021, p=0.52, respectively). A stepwise model selection method demonstrated that NTHA class, LVEF, T2DM and three- and multi-venol diseased of coronary arteries added to combination of CD31/annexin V MPs and NT-pro-BNP do not offer any additional information to discriminate between survived and died patients with symptomatic ischemic CHF (B-coefficient of 0.14; 0.018; 0.086 and 0.016, respectively; p-values of 0.86; 0.65; 0.58; and 0.56, respectively).

Discussion

Circulating CD31/annexin V MPs play a pro-inflammatory and procoagulant detrimental role in the vascular dysfunction that is a key mechanism in the development and progression of a wide range of cardiovascular diseases (Camussi G. et al., 2010). Recent studies revealed that CD31/annexin V MPs may trigger endothelial dysfunction by disrupting production of nitric oxide release from vascular endothelial cells and subsequently modifying vascular tone (Horstman L.L. et al., 2004, Jesel L. et al., 2013; Louren F., Verma S., 2014). Circulating CD31/annexin V MPs affect both pro-inflammatory and pro-atherosclerotic processes, promote coagulation and inflammation, and also modulate angiogenesis and apoptosis in endothelial cells (Louren F., Verma S., 2013; Montoro-Garcia S. et al., 2013; Ohtsuka M. et al., 2013). Because endothelium is one of the primary targets of circulating microparticles, CD31/annexin V MPs have been considered as biomarkers of vascular injury, inflammation and stages of progression of cardiovascular diseases. Recent study has revealed an association between circulating apoptotic MPs labelled as CD31/annexin V cells with cardiovascular outcomes (Sinning J.M. et al., 2011). However, no previous study has mentioned the possible predicted role of circulating CD31/ annexin V MPs levels in the CHF. In our investigation we found a significantly increase of CD31/annexin V MPs level in circulation in ischemic CHF patients who died when compared with those who survived. Quartile distribution of CD31/annexin V MPs with further cumulative survival analysis with Kaplan — Meier has been shown a significant divergence between curves in Q4 CD31/ annexin V MPs and other quartiles. Therefore, cut-off point for survived and died patients with different plasma level of CD31/annexin V MPs was 0.514/mL, and it was equal normal cell numbers that divided Q4 and Q3 in CD31/ annexin V MPs. Using this data we found that increased CD31/annexin V MPs number more 0.514/mL independently predicted all-cause mortality, CHF-related death, and also CHF-related rehospitalisation (P<0.001 for all cases) within observation period. Multivariable prediction model has been shown a high decremented potential of CD31/annexin V MPs alone and in combination with NT-pro-BNP in CHF patients during 3 years after baseline. Therefore, the suggestion that increased CD31/annexin V MPs number might improve the predictive value of contemporary model in CHF based on clinical performances and NT-pro-BNP measurements. Although the cellular mechanism of action of CD31/ annexin V MPs largely remains unclear, we believe that increased CD31/annexin V MPs in CHF may reflect a reduced vascular repair capacity and severity of endothelial dysfunction that is, probably, considered as staging disease. In this study, levels of CD31/ annexin V MPs and NT-pro-BNP were sufficient to predict long-term changes significant as independent factors in cumulative survival, re-hospitalisation due to CHF, and CHF-related death. It should emphasizes that while CD31/annexin V MPs have large diagnostic potential as biomarker for cardiovascular diseases and cancer; however, due to current technological limitations in purification of CD31/annexin V MPs and an absence of standardized methods of detection, the role of CD31/annexin V MPs became controversial (Buda M. et al., 2012; Barteneva N.S. et al., 2013; Muller F. et al., 2013). There are data elucidated that a large pool of nanoparticles is produced after blood sampling due to fragmentation of blood cells (SuStar V. et al., 2011). Indeed, such a possibility is not excluded, that in our opinion should be taken into account when interpreting the data. New studies with more statistical powerful are required. Knowledge of the functional properties of CD31/annexin V MPs will contribute to a better understanding of the pathological mechanisms of communication between cells and CHF progression, because CD31/ annexin V MPs may be an attractive prognostic biomarker for CHF.

Conclusion

Among patients with symptoms of CHF, increased circulating CD31/annexin V MPs number associates with increased 3-year CHF-related death, all-cause mortality, and risk for recurrent hospitalization due to CHF.

Conflict of interests

Not declared

Ethical principles

The study was carried out in conformity with the Declaration of Helsinki.

Study Restrictions

This study has some restrictions. The author believes that a greater cohort of patients with more incenences detected is able to improve the power of the study. It is necessary to note that large pool of nanopar- ticles might be produced after blood sampling. I believe that these risks are systemic, and to minimize them, author refused to freeze the blood samples before measurement of MPs. The authors suppose that these restrictions might have no significant impact on the study data interpretation.

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Обстежено 154 пацієнти з ішемічною ХСН.

Прогностична цінність циркулюючих апоптотичних мікрочастинок у хворих з ішемічною маніфестною хронічною серцевою недостатністю

О. О. Кремзер

Резюме. Мета. Оцінити прогностичне значення циркулюючих CD31/аннексин V-мікрочастинок для кумулятивної виживаемості у хворих з ішемічною хронічною серцевою недостатністю (XCH). Методи. Обстежено 154 пацієнти з ішемічною XCH протягом 3 років після госпіталізації внаслідок зміни лабірінту. Зразки крові для наступного визначення рівня біомаркерів були зібрані одноразово на початку дослідження. Фенотипування популяції мікрочастинок здійснювали методом проточного цитофлуориметриї з допомогою моноклональних антител, мішених флуороскопіями FITC (флуоресценція ізотіоцианат) або подвійною міткою FITC/PE (фікоеритрин) для CD31- та аннексин V-антігенів. Результати. За період дослідження (медіана стосунок показаний становила 2,18 року) було зареєстровано 21 смерчийний випадок та 106 повторних госпіталізацій внаслідок XCH. Медіана циркулюючого рівня CD31/аннексин V-мікрочастинок у пацієнтів з XCH, що вижили або померли, були 0,286/мл (95% довірчий інтервал [ДІ]=0,271–0,309/мл) та 0,673/мл (95% ДІ=0,65–0,74/мл) (p<0,001). Кількість циркулюючих мікрочастинок була розподілена на квартили (Q): Q1 (0,341/мл), Q2 (0,342–0,514/мл), Q3 (0,521–0,848/мл) і Q4 (>0,850/мл). Аналіз отриманих даних показав значне розходження кривих виживання Каплана — Мейера у пацієнтів з високим квартилем вмісту мікрочастинок з фенотипом CD31/аннексин V (>0,514/мл) порівняно з більш низькими квартилями. При цьому кількість CD31/аннексин V-мікрочастинок та її поєднання з концентрацією N-термінального фрагмента проміжкового мікрочастина вплинула статистично значущим відмінність в середньому 3-річній виживаності пацієнтів з XCH, а також госпіталізацій, пов’язаних із прогресуванням XCH. Висновок. Підвищення циркулюючого фітоцитоциту CD31/аннексин V асоціюється з погіршенням 3-річної виживаності пацієнтів з XCH.

Ключові слова: апоптотичні мікрочастинки, хронічна серцева недостатність, прогноз.

Прогностична ценность цирулярующих апоптотических микрочастиц у больных с ишемической хронической сердечной недостаточностью

А. А. Кремзер

Резюме. Цель. Оценить прогностическое значение циркулирующих CD31/аннексин V-микрочастиц и их сочетание с концентрацией N-терминального фрагмента промежуточного мембранного белка для оценки эффективности терапии у пациентов с хронической сердечной недостаточностью.

Ключевые слова: апоптотические микрочастицы, хроническая сердечная недостаточность, прогноз.

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