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LC–MS-based serum fingerprinting reveals significant dysregulation of phospholipids in chronic heart failure

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ABSTRACT

Cardiac and extracardiac lipid metabolism is known to be significantly altered in the course of the heart failure with reduced ejection fraction (HF-REF), however the precise mechanisms are not fully elucidated. The aim of the study was to use of untargeted metabolomics to identify and validate changes in the blood metabolites profile, occurring as a result of HF-REF development. The analyses were performed first in the derivation set (36 chronic HF-REF patients and 19 controls without the disease) and repeated in validation cohort (31 chronic HF-REF patients and 20 controls). Independent analyses of both sets revealed statistically significant decline in intensities of phosphatidylcholine (PC): 34:4 and 36:5, lysophosphatidylcholine (lyso-PC): 14:0, 15:0, 18:0, 18:2, 20:3, lysophosphatidylethanolamine (lyso-PE): 18:1 and 18:2 in chronic HF-REF patients. More symptomatic patients and those with ischaemic etiology of HF-REF presented greater deficit in phospholipids (PLs) intensities. The decrease of identified PLs intensities (as compared to controls) correlated with decreased serum cholesterol level, impaired renal function, reduced exercise capacity, enhanced ventilatory response and metabolic parameters associated with altered fatty acids oxidation. In multiple regression analysis PLs deficit was significantly associated with age, carnitines serum intensity, renal function, uric acid, cholesterol level. In conclusion, HF-REF is associated with significant disturbances in phospholipids metabolism. Greater reduction in serum intensities of particular identified PLs is associated with older age, worse clinical condition, impaired oxidative muscle metabolism and enhanced catabolic status.

1. Introduction

Heart failure with reduced ejection fraction (HF-REF) is a common clinical syndrome which constitutes the final stage of a variety of cardiovascular diseases. Despite significant improvement in the HF-REF treatment (e.g. beta blockers, ACE-inhibitors, aldosterone antagonists – MRA, ivabradine, cardiac resynchronization therapy), prognosis of those patients remains poor. Currently available HF-REF biochemical markers (e.g. natriuretic peptides, troponin, cytokines), in spite of being helpful in the diagnosis and treatment, do not always give a satisfactory answer about the molecular basis of the HF-REF nor the reason of its further progression. To improve our understanding of the molecular mechanisms underlying the heart failure – a multisystemic disease – not only cardiac metabolism perturbations but also metabolic derangements of other organs should be taken into considera-

Abbreviations: ACE-inhibitors, angiotensin-converting-enzyme inhibitors; BNP, B-type natriuretic peptide; BMI, body mass index; CPET, cardiopulmonary exercise test; CRT, cardiac resynchronization therapy; CVD, cardiovascular diseases; FAs, fatty acids; HF, heart failure; HF-REF, heart failure with reduced ejection fraction; IHD, ischaemic heart disease; IQR, interquartile range; LCAC, long-chain acylcarnitine; LC-QTOF-MS, liquid chromatography - quadrupole time-of-flight - mass spectrometry; LC–MS, liquid chromatography mass spectrometry; LC–MS/MS, liquid chromatography tandem mass spectrometry; Lp-PLA2, lipoprotein-associated phospholipase A2; LVEF, left ventricular ejection fraction; lyso-PC, lysophospatidylcholine; lyso-PE, lysophosphatidylethanolamine; MRA, aldosterone antagonists; NYHA class, New York Heart Association class; PC, phosphatidylcholine; PETCO2, end-tidal carbon dioxide pressure; PLs, phospholipids; PVO2, peak oxygen uptake; QC, quality control; SD, standard deviation; 6MWT, six-minute walk test; TG, triglycerides; VE/VCO2 slope, slope of minute ventilation to carbon dioxide output.

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tion. These are reflected in metabolic changes in various body fluids (e.g. urine, serum or plasma) that are available for analysis. Being increasingly used in a wide spectrum of metabolic research [1] metabolomics enables comprehensive characterization of low molecular weight metabolites (e.g. lipids, sugars, organic acids, amino acids) that reflect the complete metabolic phenotype. As a result, it may greatly assist investigator in an interpretation of altered pathways, especially when combined with other high throughput analytical technologies. It has already been shown that in the course of the heart failure development lipid cardiac and extracardiac metabolism [1,2] as well as cardiomyocytes' substrate selection and utilization [1] are significantly altered. Moreover, as the Horwitch et al. [3] previously described, mortality of patients presenting advanced HF-REF is significantly higher in those with low serum total cholesterol level. However, no clear pathway dysregulation has been claimed responsible for worse outcome in these patients. Cholesterols represent only a small subset of a wide variety of lipids (e.g. phospholipids, sphingolipids) which have been proven to be more than major components of cell membranes or energy storage molecules. Phospholipids constitute a heterogeneous group of small amphiphilic molecules that are involved in signaling [4], maintenance of membrane integrity as well as stability [4], cell proliferation and survival [5]. Moreover, recent data have suggested that lysophosphatidylcholines may display proatherogenic properties [6] and dysregulation of their metabolism by gut flora may promote cardiovascular diseases [7]. Despite growing interest of the matter, in the context of heart failure, less is known about specific lipid classes, their metabolism disturbances and potential lipid mechanisms involved in the development and progression of the disease.

Therefore the aim of the study was to use an untargeted metabolomic analysis to identify and validate changes in the blood metabolites profile occurring as a result of heart failure development. Among analytical platforms applied for untargeted metabolomics analyses LC–QTOF–MS allows for detection and reliable identification of lipids from different classes [8], therefore this platform was used in this study.

2. Material and methods

2.1. Study population

We decided to carry out the study in two sets of patients: derivation (36 chronic HF-REF patients and 19 age-matched controls without the disease) and validation (31 chronic HF-REF patients and 20 age-, gender- and IHD-matched controls without the disease). The enrolment of patients (HF-REF and control group) to the study was conducted between 2012 and 2015 at Cardiology Department of the University Hospital in Bialystok, Poland. The investigation conforms with the principles outlined in the Declaration of Helsinki. HF-REF study participants were ambulatory optimally treated patients with stable moderate chronic HF-REF (left ventricular ejection fraction – LVEF $\leq$ 35%) who provided written informed consent. All of the HF-REF patients had a minimum six-month history of the disease and did not have an episode of decompensation within the last month. HF-REF was clinically, biochemically (natriuretic peptide – BNP) and echocardiographically (LVEF $\geq$ 50%) excluded from the control group which consisted of ambulatory treated patients with arterial hypertension, atrial fibrillation, ischemic heart disease and/or hypercholesterolemia. The inclusion/exclusion criteria were the same for respective groups in both sets. Patients with acute and chronic inflammatory diseases (rheumatoid arthritis and asthma), severe chronic obstructive pulmonary disease (forced expiratory volume in one second – FEV1 less than 50% of a predicted value), severe renal dysfunction (estimated glomerular filtration rate – eGFR <30 mL/min/1.73 m^2), diabetes mellitus, thyroid dysfunction requiring pharmacotherapy, a history of implantation of the cardiac resynchronization therapy device (CRT) or those with a diagnosis of cancer in the past five years were excluded from the study. Information about concomitant diseases, current treatment and prior hospitalizations were gathered from the medical documentation. All of the enrolled patients were assessed clinically (body mass index – BMI, systolic and diastolic blood pressure – SBP/DBP, heart rate – HR, New York Heart Association – NYHA class), biochemically (complete blood count, iron level, parameters of renal function, urea, uric acid, fasting lipid profile, natriuretic peptide – BNP, C reactive protein – CRP), echocardiographically (left ventricular ejection fraction – LVEF, left ventricular end-diastolic diameter – LVEDd) and as far as possible also functionally (six-minute walk test – 6MWT, cardiopulmonary exercise test – CPET with prior rest spirometry).

2.2. Clinical parameters

The BMI and mean arterial pressure (MAP) were calculated in the usual way: weight [kg]/height [m]^2, DBP + 1/3(SBP – DBP); respectively. The estimated glomerular filtration rate (eGFR) was computed using the modification of diet in renal disease (MDRD) formula GFR calculator. The incidence of the ischaemic heart disease (IHD) was calculated taking into account confirmed cases of IHD as a cause of chronic HF-REF and as invasively confirmed or pharmacologically treated cases in the control group. Cardiopulmonary exercise test (CPET) was carried out in 44 (66%) patients with chronic heart failure. Due to the lack of feasibility of exercise test in all HF-REF patients CPET result was not taken into initial model of backward stepwise multiple regression test. The decision about whether the patient is able to do CPET was taken after the 6MWT. Main reasons of not performing CPET were: difficulties with walking due to non-cardiac causes (e.g. problems with lumbar spine, peripheral occlusive arterial disease), significant fatigue in the 6MWT and fear of exercise on a treadmill.

Peak oxygen uptake (PVO2) is the rate of an oxygen consumption (the difference between inspired and expired volume of oxygen) measured at peak exercise on a treadmill and expressed as milliliters of O_2 per kg per minute (mL/kg/min). It depends on cardiac output reserve and the arteriovenous O_2 difference. Oxygen and carbon dioxide ventilatory equivalents (EQO2, EQCO2) indicate the volume of minute ventilation needed to provide one liter of oxygen or to eliminate one liter of carbon dioxide, respectively. Oxygen pulse (O_2 pulse) is expressed as a rate of an oxygen uptake per heartbeat (mL/beat) and is an indirect indicator of stroke volume and arteriovenous O_2 difference. The slope of a minute ventilation to carbon dioxide output (VE/VCO2 slope) indicates the effectiveness of ventilation and its increase in HF-REF patients is known as an indicator of poor outcome. The basic clinical characteristics of the study population is shown in Table 1.

2.3. Chemicals

Purified water was obtained using the Milli-Q Integral 3 system (Millipore SAS, Molsheim, France). LC–MS grade methanol, acetonitrile, formic acid and LC grade ethanol as well as carnitine, acetylcarnitine and stearoylcarnitine standards were purchased from Sigma-Aldrich Chemie GmbH, (Steinheim, Germany). The API-TOF reference mass solution kit (G1969-850001) and tuning solutions, ESI-L low concentration tuning mix (G1969-85000), and ESI-TOF Biopolymer Analysis reference masses (G1969-850003) were purchased from Agilent Technologies (Santa Clara, California, USA).