Scientific Report

December 2019

2nd Phase of execution/2019

I. Study Protocol

- **Patients:**

The inclusion criteria are:
1) age>18 years;
2) HF diagnosis regardless of cause as defined by the Framingham criteria and satisfying the European Society of Cardiology guidelines;
The exclusion criteria are:
1) metallic joint prostheses, cardiac stent or pacemakers, decompensated cirrhosis, pregnancy and limb amputations (due to bioimpedance technique limitations);
2) patients with known persistent pleurisy, pulmonary fibrosis or pneumectomy; (due to lung ultrasonography limitations);
3) end stage renal disease (due to difficulties in the interpretation of overhydration symptoms and signs and also to different biomarker kinetics in this type of patients);
3) absence of congenital heart disease.

- **Methods:**

We collected **blood** from all included patients (for biomarker analysis); all patients had also a complete **clinical evaluation**:
- Personal data (age, sex, height);
- Cardiovascular risk factors (smoking, weight, body mass index);
- Medication;
- The etiology, duration and classification (NYHA class) of heart failure;
- Comorbidities (diabetes, hypertension, stroke, coronary artery disease, chronic kidney disease etc);
- Physical examination (blood pressure, crackles, pedal edema etc);
- Renal function evaluation (serum urea and creatinine, serum electrolytes – K, Na, Cl)
- Nutrition status evaluation: serum protein, cholesterol, triglycerides;
- Inflammation evaluation: CRP;
- Serum glucose and uric acid;
- Hemoleucogram;
- Electrocardiogram.

Bioimpedance spectroscopy analysis

This analysis was performed at baseline using the portable whole-body multifrequency bioimpedance analysis device (BCM®Body Composition Monitor – Fresenius Medical Care D GmbH).

The technique involves attaching electrodes to the patient's forearm and ipsilateral ankle, with the patient in a supine position. The BCM® measures the body resistance and reactance to electrical currents of 50 discrete frequencies, ranging between 5 and 1000 kHz. Based on a fluid model using these resistances, the extracellular water (ECW), the intracellular water (ICW) and the total body water (TBW) are calculated. These volumes are then used to determine the amount of fluid overload. All calculations are automatically performed by the software of the BCM® device. Absolute fluid overload (AFO) is is defined as the difference between the expected patient's ECW under normal physiological conditions and the actual ECW, whereas the relative fluid overload (RFO) is defined as the absolute fluid overload AFO to ECW ratio. In addition, this analysis will provide the LTI and FTI for the included patients.

Echocardiography

Echocardiographic evaluations was performed in each patient at baseline. All echocardiographic measurements were carried out according to the recommendations of the American Society of Echocardiography by an observer unaware of the lung ultrasound and bioimpedance results.

Lung ultrasonography

Examinations were performed in the supine position. Scanning of the anterior and lateral chest was performed on both sides of the chest, from the second to the fourth (on the right side to the fifth) intercostal spaces, at parasternal to mid-axillary lines. B-lines were recorded in each intercostal space and were defined as a hyperechoic, coherent US bundle at narrow basis going from the transducer to the limit of the screen.

B-lines starting from the pleural line can be either localized or scattered to the whole lung and be present as isolated or multiple artifacts. The sum of B-lines produces a score reflecting the extent of lung water accumulation (0 being no detectable B-line).

II. The proposed/achieved objectives

For the second stage of the project the following objectives have been accomplished:

1. We finalized the proposed acquisitions for this stage

- the reagents for biomarkers' assessment

- Galectin-3 Human ELISA kit;
- Leptin Human ELISA;
- Human CT1(Cardiotrophin 1) ELISA Kit;
- Human A-GHRL(Acylated Ghrelin) ELISA Kit;
- HUMAN SOLUBLE CD146 ELISA KIT;
- Soluble IL-1R 4/ST2 (Human) ELISA Kit.

- a laptop for performing an adequate statistical analysis

2. We have finished patient enrollment. We have evaluated 150 patients according to the protocol (clinical assessment, lung ultrasonography, bioimpedance, echocardiography,

blood collection with storage at -82°C for further biomarker analysis); we have also closely monitored their clinical evolution.
4. In all the included patients we have assessed the aforementioned biomarkers; we have completed the database and we are now analyzing the available data.
5. Dissemination
Cardiac Failure – Oral Presentation during the CardioRenal Course – 28 September 2019, Hotel Bavaria Blu, Constanța (https://www.cardioportal.ro/cardiorenal/);
Pulmonary congestion, as assessed by lung ultrasonography, is dependent on renal function and fluid overload in patients with heart failure – Prize winner Poster during the 56th edition of the European Renal Association – European Dialysis and Transplant Association Congress – Budapest 2019
An analysis of the impact of fluid overload and fluid depletion for all-cause and cardiovascular mortality – Manuscript in Nephrology Dialysis Transplantation Journal (IF 4.198)
Prognostic value of lung ultrasonography and bioimpedance spectroscopy in patients with heart failure and reduced ejection fraction – Manuscript (under revision at this moment) in Archives of Medical Sciences Journal (IF 2.380)