Adropin in patients with heart failure

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ADROPIN IN PATIENTS WITH HEART FAILURE

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1. INTRODUCTION

1.1 Definition

As the American Heart association (AHA) defines: Heart Failure (HF) is a complex clinical syndrome that results from any structural or functional impairment of ventricular filling or ejection of blood (1).

Heart failure is divided into: HFpEF (heart failure with preserved ejection fraction) with EF ≥ 50% , HFmrEF (heart failure with mid-range ejection fraction) with EF between 40% and 49% , HfrEF (heart failure with reduced ejection fraction) with EF ≤ 40% (11).

More precisely the criteria to diagnose each of the above mentioned types of heart failure are:

- HFpEF
  - LVEF ≥ 50%
  - Elevated levels of natriuretic peptides
  - At least one of the following:
    - Relevant structural heart disease (LVH and/or LAE)
    - Diastolic dysfunction
- HFmrEF
  - LVEF 40-49%
  - Elevated levels of natriuretic peptides
  - At least one of the following:
    - Relevant structural heart disease (LVH and/or LAE)
    - Diastolic dysfunction
- HFrEF
  - LVEF < 40%

1.2 Epidemiology

Worldwide around 26 million of people are affected from Heart Failure (HF). The overall prevalence of HF in the adult population in developed countries is 2%. In 2012 it was responsible for an estimated health expenditure of around $31 billion, equivalent to more than 10 % of the total health expenditure for cardiovascular diseases in the United States (2). Approximately 10% of people above the age of 65 are affected. In Europe and North America, the lifetime risk of developing HF is 20% for a 40 years old. The life expectancy is increasing in all developed countries, moreover better and better treatments are emerging for myocardial infarction, valvular heart disease, arrhythmias and other heart related diseases therefore the overall prevalence of HF is on the rise (2). In general, men are more affected then women. From various epidemiological studies It has emerged that HFpEF patients are more likely to be women and older, obese, with a higher New York Heart Association (NYHA) class and cardiovascular comorbidities (such as hypertension, diabetes and atrial fibrillation) and non-cardiovascular comorbidities (such as anaemia, chronic kidney disease and chronic pulmonary disease); Coronary artery disease is the main determinant of HfrEF and its prevalence is higher in males (2).

1.3 Etiology

Any conditions that can affect the structure or function of the heart can cause heart failure.

Some of the known causes of heart failure are:

- Nonischemic dilated cardiomyopathy
- Arrhythmias
- Chronic lung diseases and Pulmonary vascular disorders (COPD, Pulmonary hypertension)
- Chronic volume overload (Extracardiac/Intracardiac shunting, Regurgitant valvular disease)
- Chronic pressure overload (Hypertension, Obstructive valvular diseases)
- Coronary artery disease

- Toxin/drug induced damage (Metabolic disorders, Viral infections)
- Pathologic hypertrophy (Primary/Secondary hypertrophy)
- Restrictive cardiomyopathy (Infiltrative disorders, Storage diseases, Fibrosis)
- High output states (Thyrotoxicosis, Beri-beri disease)
- Excessive blood flow requirements (Systemic arteriovenous shunting, Chronic anemia)

The great majority of Heart Failure are due to myocardial infarction, chronic obstructive pulmonary disease (COPD), hypertension and rheumatic heart disease (3). Myocardial infarction (MI) happens when the flow through one or more coronary arteries is occluded; this can be due to various causes; from coronary dissection and coronary artery spasm to the widely known and more common atherosclerotic plaque rupture. Risk factors for MI include obesity, smoking and diabetes. COPD is a progressive and chronic disease of the respiratory system; the greatest risk factor for COPD is smoking. Hypertension is a disease whose etiology remains mostly unknown but in a small percentage of cases an underlining etiology can be found for instance renal artery stenosis, coarctation of the aorta, pheochromocytoma, renal parenchymal disease, hyperaldosteronism, obstructive sleep apnea and hormonal disorders.

1.4 Pathophysiology

The Pathophysiology of heart failure is extremely complex and continuously under investigation. The main concept is that, due to a certain event, the cardiac muscle starts losing the capability to pump blood. This event may be directly a lack of systolic function, namely the heart can't pump blood towards the aorta properly, or it can start with a diastolic dysfunction which means that the heart is not able to accommodate enough blood into its chambers; A diastolic dysfunction would eventually transform into a systolic dysfunction if the disease progresses (4).

The event that starts the pathophysiologic cascade of HF can be either acute such as MI or chronic such as aortic stenosis. The patient can present acutely symptomatic and, depending on the cause, have chest pain, dyspnea, leg edema, palpitation etc. or he/she can be completely asymptomatic and only develop signs and symptoms of heart failure when the disease is more advanced (5). The reason of the latter is the presence of multiple compensatory mechanisms such as:

- Activation of the RAA (renin-angiotensin-aldosterone) system
- Increase in myocardial contractility through activation of the sympathetic nervous system
- Production of ANP (atrial natriuretic peptide), BNP (brain natriuretic peptide), PGE2 (prostaglandin E2), PGI2 (prostacyclin), NO (nitric oxide)

The RAA system increases the absorption of NaCl and H_20 from the kidney increasing the blood volume and venous return. Moreover, it causes a peripheral vasoconstriction that increases TPR (total peripheral resistance).

The increase in production of norepinephrine and epinephrine from the nerve endings and adrenal gland increases the capability of the heart to pump the blood forward (contractility).

The production of vasodilatative substances such as ANP, BNP, NO allows for a counterbalance in the rise in TPR thus keeping the afterload in check (5). The event that accompanies the pathophysiologic cascade of HF is cardiac remodeling. The final events of heart failure are systolic ventricular dysfunction, arrhythmias and death (6). Cardiac remodeling is characterized by cellular and interstitial changes that leads to changes in size, mass, geometry and function of the heart after injury (6). In various studies it was seen that cardiac remodeling happens mostly during the transition from asymptomatic to symptomatic HF. Cardiac remodeling is characterized by hypertrophic, contractile and metabolic changes; apoptosis and necrosis of the myocytes; desensitization to catecholamines; reorganization of the extracellular matrix with dissolution of the organized structural collagen surrounding myocytes and subsequent replacement by an interstitial collagen matrix that does not provide structural support to the myocytes. These changes are stimulated by mechanical stretch of the myocytes, increase in production of norepinephrine, angiotensin II, inflammatory cytokines, peptides such as endothelin, growth factors and ROS (reactive oxygen species). Essentially the mechanical

and neurohormonal changes that “are the cause of “ or “ follow “ heart failure and that are, in part, compensating for the HF, are also the cause of cardiac remodeling thus setting up a vicious circle. Clinicians fight against cardiac remodeling with drugs such as ACE inhibitors, B-blockers, ARBs, MR antagonists (4). Due to the underlying pathology that started HF and the structural changes above described, the patient ends up with a cardiomyopathy that can be restrictive, dilatative or hypertrophic in nature or, of course, a combination of the above. The end stage is usually a dilatated heart that is unable to pump blood (low ejection fraction) and prone to various arrhythmias such as VT and Vfib; all leading up to death.

1.5 Clinical presentation

Cardinal symptoms related to heart failure are: fatigue, shortness of breath while upright, orthopnea and paroxysmal nocturnal dyspnea

Fatigue is a generalized sense of tiredness with multiple mechanisms and a complex underlying pathophysiology; shortness of breath while standing, orthopnea and paroxysmal nocturnal dyspnea happen due to fluid collection in the lungs. When laying down the symptoms of dyspnea worsen due to the fluid affecting a wider area of the lungs. Other symptoms of HF may be GI disturbances such as indigestion and bloating (due to congestion of GI mucosa), right upper quadrant pain (due to liver congestion and enlargement) and encephalopathy in cases of advanced HF with decreased cerebral perfusion (4). Encephalopathy happens in the end stage of the disease because the brain has a privileged and well regulated blood flow (8).

Clinical examination of the patient may reveal:

- Hepatojugular reflux
- Jugular vein distension
- Cyanosis
- Pleural effusion
- Pulmonary edema
- S3 heart sound
- S4 heart sound
- Valve related murmurs
- Hepatomegaly
- Peripheral edema
- Cachexia

Depending on the cause of heart failure the right side of the heart might not be able to accommodate the blood coming from the inferior vena cava. Pressing on the liver and increasing the venous return will pool the blood up into the jugular veins (hepatojugular reflux). For the same reason the Jugular veins may be distended even without pressure on the liver (jugular vein distension) (9). Decreased oxygenation of the blood and/or decreased delivery of oxygen will cause cyanosis. Since in heart failure the heart can't accommodate the blood properly in its chambers or can't push it forward, the blood will accumulate proximal to the chambers. In the case of the left ventricle, this happens in the lung interstitium and/or alveoli (pulmonary edema). The lung vasculature can withstand an increase in hydrostatic pressure of about 30 mmHg before extravasation of the blood; this is due to the capability of the lung vessels to dilate and accommodate more blood (5). However, this defence mechanism is overcome in heart failure patients. S_3 heart sound happens when the blood coming from the atria, come into contact with a compliant ventricle whereas S_4 happens when the atrium contracts pushing the blood into a non-compliant ventricle. For this reason, S_4 heart sound is more specific for diastolic heart failure.

Various valve related murmurs can be heard and can be either the cause or the consequence of heart failure. For instance a systolic murmur can signify aortic valve stenosis, in that case we can assume that the stenosis was the cause of heart failure; Similarly a systolic

murmur can also signify a mitral valve regurgitation and the assumption this time can be that the valve insufficiency was in fact secondary to heart failure and dilatation of the annulus fibrosus; this because dilation and remodeling of the heart may have led to valve insufficiency. Hepatomegaly and thus liver dysfunction come from congestion of blood in the liver. Borovac et.al found that RV FWS (right ventricle free wall longitudinal strain) is correlated to total bilirubin, INR and albumin (74). Splenomegaly and hypersplenism may follow hepatosplenomegaly. Symptoms related to hepatomegaly are nausea, vomiting and lack of appetite. All of that plus an increase in inflammatory mediators and metabolic rate will lead to cachexia, although this usually becomes an overt sign only in the end-stages of the disease. Peripheral edema, a pathognomonic sign of heart failure, is the result of an increase in hydrostatic pressure in the venous system and consequent extravasation of fluid into the interstitial spaces; it happens most commonly in the lower extremities in deambulatory patients, whereas it is found in the sacral region in bedridden patients.

1.6 Diagnosis

Diagnostic procedures include:

- Routine laboratory tests (complete blood count, electrolytes, BUN, creatinine, hepatic enzymes, urinalysis)
- Chest x-ray
- ECG
- Cardiac bio-markers
- Cardiac US
- Cardiac MRI
- CT coronary angiography
- Genetic testing

Routine laboratory tests give an idea of the general condition of the patient and may point to a possible etiology of the heart failure. They are also useful to monitor the response of the patient to therapy. Chest x-ray allows for detection of pulmonary edema and assessment the

dimension of heart chambers. Moreover, it may offer an alternative diagnosis for the patient's presenting symptoms. ECG is of outmost important for the detection of possible arrhythmias and heart chambers enlargement, most importantly LVH (left ventricle hypertrophy) (11).

NT-proBNP (N-terminal pro-B type natriuretic peptide) and BNP (B-type natriuretic peptide) are the two cardiac bio-markers that represent the pivot point in heart failure diagnosis (11). If the cardiac bio-markers are negative (NT-proBNP < 125 pg/ml, BNP < 35pg/ml) the diagnosis of heart failure is unlikely and prompts the clinician to look for another diagnosis. On the contrary, if bio-markers are positive, heart failure is likely and the next step is cardiac imaging namely Cardiac US and MRI. The most common, cheap and widely used examination is trans-thoracic 2-D ultrasound with color-doppler which can assess with great precision multiple parameters such as size of the left atrium and left ventricle, regional wall motion abnormalities etc. (12,14). In addition, it allows for detection of abnormalities in diastolic filling of the left ventricle. The most important indicator of heart function is EF (ejection fraction) which is calculated as stroke volume over end-diastolic volume. However, EF cannot detect sub-clinical LV dysfunction (14). In this regards it is useful to measure LV-GLS (left ventricle global longitudinal strain). In a study conducted by Park et.al it was shown that LV-GLS is effective in stratifying all-cause mortality rate from heart failure (7); the same result can't be achieved with LVEF determination (14). For instance, in patients taking chemotherapy it is advisable to use LV-GLS to monitor heart function given its capability of detection of subclinical LV dysfunction. Under scientific investigation there is Catestatin, a poorly understood peptide produced by autonomic neurons. Borovac et.al have found an association between Catestatin and cardiac disease (73). MRI is the gold standard for assessment of the heart anatomy but it is expensive and many times not necessary. The disease in which MRI has been shown to be very useful is infiltrative diseases such as cardiac amyloidosis (13).

CT coronary angiography is a non-invasive procedure that can be used to evaluate the coronary arteries in HF patients with low to intermediate pretest probability of CAD or equivocal noninvasive stress test results (14,16). According to the Heart Failure Society of America, genetic testing is recommended in patients with HCM (hypertrophic cardiomyopathy), NIDCM (non-ischemic dilated cardiomyopathy), ARVC (arrhythmogenic right ventricular cardiomyopathy), cardiomyopathy with other extracardiac manifestations and LVNC (left ventricular non-compaction cardiomyopathy) (17).

1.7 Treatment

Drugs for heart failure include:

- Diuretics
- ACE inhibitors
- ARBs (angiotensin receptor blockers)
- B-blockers
- MR antagonists (mineralocorticoid receptor antagonists)
- ARNI (angiotensin receptor-neprilysin inhibitor)
- Ivabradine
- Digoxin
- H-ISDN (hydralazine-isosorbide dinitrate)

Interventions for heart failure include:

- CRT (cardiac resynchronization therapy)
- LVAD (left ventricular assisting device)
- ICD (implantable cardioverter defibrillator)
- Heart transplantation

Therapy for heart failure is aimed at inhibiting cardiac remodeling and improving quality of life of the patient by decreasing symptomatology and workload of the heart. Initially diuretics are used to relieve signs and symptoms of congestion and reach euvolemia of the patient (18). Loop and thiazide diuretics are the drugs of choice and have been shown to improve exercise capacity, reduction in risk of disease progression and risk of death (19). Metra et al. have found that the degree of congestion in acute decompensation of heart failure drives renal function deterioration in these patients and that both these factors together have a significant prognostic value. Diuretics therefore are very useful in managing acute decompensations of heart failure and get rid of edema and improve prognosis (20).

One of the defensive mechanisms of the body to maintain the blood pressure at physiological levels is the activation of the RAA system that on one side maintains the blood

pressure but on the other contributes to the heart failure locking the cardiovascular system in a vicious circle. Blockers of this neuro-hormonal pathway are ACE inhibitors and ARBs. These drugs have shown effectiveness in reduction of cardiovascular mortality and morbidity (21,22). Some studies have shown a beneficial additive effect of ACE inhibitors and ARBs (23) however it is a common consensus among clinicians that the risk of hyperkalemia most importantly and also hypotension and renal dysfunction is too high so their concomitant use is discouraged (23).

B-blockers acts on the Beta receptors of the heart and have a negative chronotropic and inotropic effect; they have been shown to reduce the mortality and morbidity in heart failure patients with reduced ejection fraction as shown in the COPERNICUS trial and others (25,26,27). Some of the B-blockers such as carvedilol have also an alpha blocking activity which aids in a decrease of TPR (total peripheral resistance) by peripheral vasodilation. Other B-blockers commonly used are metoprolol, bisoprolol and nebivolol. It was postulated that carvedilol due to its dual effect of directly decreasing the work of the heart and causing peripheral vasodilation, would improve mortality and that was tested and proven in the COMET trial (28). B-blockers are not to be used in acute heart decompensations due to their negative inotropic effect, therefore it is recommended to use them in stable and euvolemic patients. B-blockers are known to decrease the quality of life of the patient in the beginning of therapy but that soon resolves after initiation of therapy (29).

MR antagonists decrease the aldosterone effect by binding to the mineralocorticoid receptor. This leads to increased sodium excretion, resulting in decreased body fluid and lower blood pressure. The most common MR antagonists used are spironolactone and eplerenone and they are recommended in cases where diuretics, ACE inhibitors and B-blockers are not enough to manage the disease. MR antagonists also decreases mortality and hospitalizations. Common side effects of these drug are hyperkalemia and gynecomastia due to its affinity to progesterone and androgen receptor. Eplerenone have fewer side effects compared to spironolactone but it also requires higher dosages to obtain the same effect (30).

ARNI contains both an angiotensin receptor blocker and a neprilysin inhibitor. Inhibiting the neprilysin enzyme increases the levels of natriuretic peptides (NPs). The natriuretic peptide system counter regulates the detrimental effects of the up-regulation of RAA system that occurs in HF, inhibits secretion of arginine vasopressin and modulates the autonomic nervous system. Sodium and water retention and vasoconstriction caused by activation of RAAS and the sympathetic nervous system, and the action of vasopressin, lead to

increased ventricular preload and afterload and elevated wall stress which in turn lead to production of pre-pro B-type natriuretic peptide (BNP) which is cleaved to BNP and N-terminal proBNP (NT-proBNP). The peptide BNP acts to promote natriuresis and vasodilation whereas NT-proBNP is physiologically inactive. Atrial stretch leads to the production of ANP (atrial natriuretic peptide) which has similar biological properties to BNP. ARNI reduced mortality and hospitalizations more compared to ACE inhibitors in patients with NYHA II-IV with reduced ejection fraction (31). Ivabradine is a blocker of the "funny channels" (I_f) in the sinoatrial node. The I_f current is responsible for the spontaneous depolarization of the atrioventricular node cells. A mixed sodium/potassium inward current brings the AV node resting membrane potential to the threshold for action potential firing. Inhibition of this current has a negative chronotropic effect, it increases the time in which the ventricle is in diastole thus allowing more blood to flow inside the coronary arteries and decreases oxygen demand of the heart. Ivabradine is used in heart failure with reduced ejection fraction and resting heart rate ≥75 bpm after beta blocker optimisation (32). After having used Diuretics, Ace inhibitors, B-blockers and MR antagonist, Ivabradine is a good choice for patients that are in sinus rhythm and display a heart rate of at least 70 bpm. Another use of ivabradine is stable angina as a second line therapy to decrease chest pain (33,34). Unlike B-blockers, Ivabradine does not have a negative inotropic effect on the heart. Side effects are hypertension, Afib and bradycardia. Prognostic improvement has been shown with ivabradine (35).

Digoxin inhibits the Na/K+ exchanger in the heart muscle causing an increase in the intracellular calcium content of the cardiomyocytes thus increasing its contractility. Digoxin has been shown to decrease hospitalizations but not mortality (36). However, the DIG trial has shown a decrease in mortality in very high-risk patients with low dose digoxin. The current guidelines recommend the use of digoxin as a last line therapy, after all other medications combined have failed to control heart failure symptoms. Possible side effects of digoxin are arrhythmia, nausea, vomiting, diarrhea, visual and endocrine changes.

H-ISDN is a combination of hydralazine and isosorbide dinitrate. Hydralazine exerts its effects by dilating the arteriolar bed thereby decreasing the afterload and blood pressure. Isosorbide dinitrate (ISDN) is chemically related to glyceryl trinitrate and also has a similar mechanism of action; it directly produces dilation of the venous capacitance bed thus reducing the venous return and consequently the preload. ISDN also relaxes the arterioles thus reducing the afterload; the epicardial coronary vessels also get dilated. Reduction of preload and afterload

decreases the myocardial oxygen requirements. The effectiveness of H-ISND is limited; there is not benefit in terms of mortality or hospital readmissions (37). Possible benefits may come from higher dosages but that would increase its side effects so that only younger patients would be able to tolerate them (37).

In patients where the combination of diuretics, ACE inhibitors and MR antagonists hasn't worked CRT or cardiac resynchronization therapy is recommended if they are in sinus rhythm and present a QRS duration ≥ 130ms. In CRT two or three pacemaker leads are placed. One is placed in the endocardium at the distal tip of the right ventricle; the other is placed in the coronary sinus to pace the left ventricle. The placement of the third lead varies among patients and goes in the right atrial appendage to pace the right atrium. The use of CRT was associated with lower rates of mortality, all-cause readmission, and cardiovascular readmissions (38). The COMPANION trial compared optimal medical therapy alone or optimal medical therapy combined with CRT or CRT-D (CRT with a cardioverted defibrillator); They found that the implantation of a cardioverter defibrillator reduced mortality and cardiovascular readmissions although CRT-D shows a better survival advantage (39).

LVAD (left ventricular assisting device) is used in the end-stage heart failure when all other options haven't worked. It can be used as a bridging therapy or as destination therapy. Bridging therapy is temporary and is for those patients waiting for a heart transplant. Some patients are not eligible for heart transplantation such as patients with irreversible pulmonary hypertension/elevated pulmonary vascular resistance, active systemic infection or active malignancy (40); these patients will be implanted a LVAD as a destination therapy namely until the end of life. Ventricular assisting devices are usually subdivided into RVAD (right ventricular assisting device) , LVAD (left ventricular assisting device) and BIVAD (biventricular assisting device) which is RVAD and LVAD together. The RVAD helps the right ventricle to pump blood into the pulmonary artery and is not commonly in use (usually in cases of post-operative LVAD surgery for a limited amount of time). The LVAD is the most common type of ventricular assisting device. There are many different types of LVAD in the market but all of them follow the same principle namely help the left ventricle push blood into the aorta. One of the most common LVAD used is IMPELLA which can be inserted with a mini invasive procedure through the femoral artery; it is commonly used in the USA.

Cardiac transplantation is the treatment of choice for many patients with end-stage heart failure who remain symptomatic despite optimal medical therapy (11).

The American College of Cardiology/American Heart Association (ACC/AHA) include the following guidelines for heart transplantation (41):

- Refractory cardiogenic shock requiring intra-aortic balloon pump counterpulsation or left ventricular assist device
- Cardiogenic shock requiring continuous intravenous inotropic therapy (i.e., dobutamine, milrinone, etc.)
- Peak VO2 (VO2max) less than 10 mL/kg per min
- NYHA class of III or IV despite maximized medical and resynchronization therapy
- Recurrent life-threatening left ventricular arrhythmias despite an implantable cardiac defibrillator, antiarrhythmic therapy, or catheter-based ablation
- End-stage congenital HF with no evidence of pulmonary hypertension
- Refractory angina without potential medical or surgical therapeutic options

A meta-analysis by Theocari et.al compared the use of LVAD to heart transplant (42). More precisely the LVAD BTT (LVAD used as bridging therapy) and LVAD DT (LVAD used as destination therapy) were each compared to HT (heart transplant) and the 1-year mortality rates were calculated. No statistically significant different were found in 1-year mortality.

The use of LVAD vs HF highly depends on the patient condition so that, for instance, if pulmonary hypertension is present the patient is a candidate for LVAD but not for heart transplantation;

On the contrary if severe right ventricular failure is present a heart transplantation is more beneficial.

Therapeutic decision making may be facilitated also by the use of scores which may aid the physician to better understand the health risks inherent to heart failure. S2PLiT-UG score has shown potential in this regard (24). Another element to consider is weather the patient has preserved, mid-range or reduced ejection fraction; for instance, a mid-range EF prompts a more stringent therapy since it has been shown to have a significantly higher mortality rate compared to preserved EF (10).

1.8 Adropin and heart failure

Adropin is a 76 amino acids long peptide hormone which is encoded by the Enho gene and was discovered in 2008 by Kumar et al. during the microarray analysis of liver gene expression in mouse models of obesity (48). Two main tissues producing adropin are liver and brain. Adropin is found in blood and as a membrane protein in brain where it may function as a neuropeptide modulating cell to cell communication (60). Moreover, it has been seen to have an important role for normal motor activity and coordination, as well as for the proper development of the cerebellum (71).

Adropin was also found in cheese way and milk of dairy cows where its concentration was higher compared to the blood. This suggests that adropin may be produced by the mammary gland or that it is actively transported from the blood to the milk (64).

Adropin was found to improve the blood brain barrier (BBB) integrity after intracerebral haemorrhage (ICH); in short, ICH was induced in mice, subsequently adropin was administered and the brain water content, the BBB permeability and the neurological function were measured and found to be significantly improved (67).

An inverse correlation has been found between progression of knee osteoarthritis and levels or adropin in the blood; this leads us to hypothesize that adropin may be used in the future as a biomarker for early osteoarthritis (68).

Serum adropin levels and progression of Chronic kidney disease has shown a negative correlation but more data are needed on the matter (66).

Adropin was found to be linked to pre-term pregnancy compared to term pregnancy; indeed, pre-term babies had lower serum adropin levels compared to term babies (65).

Adropin has shown a significant correlation with many aspects of cardiovascular disease.

Lian et.al reported elevated levels of serum adropin in patients with heart failure. Moreover, elevated levels of serum adropin in heart failure patients was correlated with the severity of heart failure according to NYHA class and BNP levels (56).

As mentioned above, the gene encoding for adropin is the Enho gene which is expressed in many tissues of the body including liver, brain, pancreas and kidney. Up-regulation of Enho gene was observed in mice put under high fat diet wherein adropin caused a decreased in insulin resistance and hepatosteatosis (48). This suggests a possible role of adropin in the therapy of diabetes type 2. However chronic intake of high fat diet decreased the levels of adropin in the blood suggesting that the liver loses its regulatory function when damaged. Moreover, adropin

stimulates carbohydrate oxidation over fat oxidation and increase insulin sensitivity in mice with diet induced obesity (63).

Patients with diseases connected with a higher insulin resistance such as gestational diabetes insipidus and PCOS were found to have lower amounts of serum adropin (61,62).

NAFLD (non-alcoholic fatty liver disease) patients have lower levels of adropin in blood, thus adropin may be a predictor of NAFLD (63).

Adropin also protects from endothelial dysfunction via the upregulation of NO synthase through the VEGFR2-phosphatidylinositol 3-kinase-akt and VEGFR2-extracellular signal regulated kinase pathways (49,51). Other than causing vasodilation NO inhibits monocytes, leukocyte adhesion to endothelium, aggregation of platelets, oxidation of LDL and smooth muscle proliferation; people with cardiac syndrome X with endothelial dysfunction have significantly lower levels of serum adropin (52).An inverse correlation has been found between serum adropin levels and children with obstructive sleep apnea (58).

Adropin has also been shown to be an independent predictor of coronary atherosclerosis so that low serum adropin corresponds to higher atherosclerosis (50). Hou-you-Yu et al. Found that adropin can be an independent predictor of acute myocardial infarction (AMI) and that there is an inverse proportionality between the levels of adropin in the blood and BMI and TG. This suggests that serum adropin might be a biomarker to predict AMI in CAD patients (59).

As mentioned earlier adropin is expressed also in the brain tissue and is thought to have regulatory properties of the central nervous system (46). This notion plus its vasodilatory effects suggest that adropin might also have a role in blood pressure regulation. Patients with hypertension have significantly lower levels of serum adropin compared to normotensive patients (61).

2. OBJECTIVES

The aim of this study is to determine serum adropin levels in patients with heart failure in comparison with healthy controls.

Hypothesis:

1. Serum adropin levels will be higher in patients with heart failure in comparison with control group.
2. There will be positive correlation between serum adropin levels and severity of heart failure.

3. MATERIALS AND METHODS

3.1 Subjects

In this cross-sectional study, a total of 30 HF patients that were hospitalized at the Department of Cardiology, University Hospital of Split were consecutively enrolled. This study was approved by Ethical Committee of University Hospital of Split and University of Split School of Medicine, while all included participants signed an informed consent form.
Framingham criteria for HF were used for clinical examination of patients, while final diagnosis was made by ESC-certified specialist for HF that was involved in the study.
Exclusion criteria were age<35 and >90 years, severe valvular disease, cardiomyopathy, chronic *cor pulmonale*, pulmonary disease, Diabetes Mellitus type 1, severe renal disease, severe hepatic disease, acute infective disease, autoimmune disease, coagulopathy, chemotherapy, recent acute coronary syndrome, drug or alcohol use.

All involved patients were evaluated in the first 24 h of hospital admission. Evaluation included detailed physical examination, medical history, venous blood sampling, blood pressure measurement and transthoracic echocardiography (TTE).

3.2 Anthropometric characteristics of study participants

Calibrated scale (Seca, Birmingham, UK) was used for body weight and height measurements, while measurements of neck, waist and hip circumference was measured with tape measure.

3.3 Collection of data

American Society of Echocardiography (ASE) guidelines were used for TTE examination, that was made on the same day as blood samples were obtained. Patients were positioned at left lateral decubitus position, and examination was performed by expert cardiologist in the field of ultrasonography. Vivid 9 ultrasound system (GE Medical Systems, Milwaukee, WI, USA) was used for all examinations.

Laboratory analyses of collected venous blood samples was performed at Department of Medical Laboratory Diagnostics by an experienced biochemist that was blinded to the group

assignment of the subject. High-sensitivity cardiac troponin I (hs-cTnI) was determined with Abbot Diagnostics hs-cTnI assay (Abbott ARCHITECT ci16200, Abbott, Chicago, USA), while N-terminal pro brain natriuretic peptide (NT-proBNP) levels were determined with Eclesys®Cobas e601 NT-proBNP assay. Furthermore, electrochemiluminescence (ECLIA) method was used for this measurement.

Serum adropin levels were determined with dual enzyme-linked immunosorbent assay (ELISA) (Phoenix Pharmaceuticals, Burlingame, USA). Coefficient of variation (CV) within the probe was stated <10%, while between probes was <15%. All other included biochemical parameters were analysed according to standard laboratory procedures.

3.4. Statistical analysis

Statistical software MedCalc for Windows (MedCalc Software, Ostend, Belgium, version 19.1.2) was used for statistical analysis. Mean ± standard deviation or median and interquartile range were used for describing quantitative data, according to normality of data distribution (tested with Kolmogorov-Smirnov test). Whole numbers and percentages were used for qualitative data description, with chi-square test used for testing differences between variables. T-test for independent samples was used for testing differences between anthropometric and biochemical parameters between patient and control group, with exception of NT-proBNP and CRP levels, in which cases Mann-Whitney test was conducted. Estimation of correlation between serum adropin levels and other parameters was done with Pearson's or Spearman's correlation. The level of significance was set at value of $P<0.05$.

4. RESULTS

The heart failure group and the control group did not differ in age (70.7 ± 9.9 vs 71.8 ± 11.5 years, P=0.693), BMI (28.9 ± 3.8 vs 29.8 ± 4.1 kg/m^2, P=0.381), systolic blood pressure (135.1 ± 29.8 vs 138.2 ± 24.4 mmHg, P=0.661) and diastolic blood pressure (82.6 ± 14.8 vs 84.8 ± 12.1 mmHg, P=0.531). In the heart failure group, the NYHA functional classification was 3.1 ± 0.41 and LVEF 48.8 ± 14.2% (Table 1).

Table 1. Baseline characteristics of study population

Parameter	**Control group (N=30)**	**HF group (N=30)**	***P****
Age (years)	70.7 ± 9.9	71.8 ± 11.5	0.693
Males (N, %)	17 (56.6)	18 (60.0)	0.793
BMI (kg/m^2)	28.9 ± 3.8	29.8 ± 4.1	0.381
Neck circumference (cm)	39.2 ± 3.7	39.9 ± 4.2	0.496
Waist circumference (cm)	103.2 ± 10.2	104.8 ± 11.4	0.569
Hip circumference (cm)	107.1 ± 10.8	108.2 ± 10.4	0.689
Systolic arterial pressure (mmHg)	135.1 ± 29.8	138.2 ± 24.4	0.661
Diastolic arterial pressure (mmHg)	82.6 ± 14.8	84.8 ± 12.1	0.531
NYHA Functional Classification	-	3.1 ± 0.41	-
LVEF (%)	-	48.8 ± 14.2	-
Therapy			-
ACEI or ARB	-	20 (66.6)	-
Sacubitril-valsartan	-	8 (26.6)	-
Beta blocker	-	28 (93.3)	-
Calcium blocker	-	5 (16.6)	-
MRA	-	12 (40.0)	-
Diuretics	-	26 (86.6)	-
Warfarin	-	9 (30.0)	-
NOAC	-	10 (33.3)	-

Data are presented as mean ± standard deviation or as stated otherwise. **HF-**heart failure; **BMI**-body mass index; **NYHA-** New York Heart Association; **LVEF-** Left ventricular ejection fraction; **ACEI-** Angiotensin-converting-enzyme inhibitors; **ARB-** Angiotensin II receptor blockers; **MRA-** Aldosterone receptor antagonists; **NOAC-**New oral anticoagulants

***** t-test for independent samples or Chi-square test

Creatinine was significantly higher in the heart failure group compared to the control group (95.1 ± 31.4 vs 147.9 ± 76.4 μmol/L, P<0.001), and total cholesterol was significantly higher in the heart failure group in comparison with the control group (5.1 ± 1.02 vs 5.8 ± 1.4 mmol/L, P<0.031). Additionally, the heart failure group was found to have a significantly higher NT-proBNP compared to the control group. There were no other significant differences between groups in laboratory parameters (Table 2).

Table 2. Laboratory parameters of study population

Parameter	**Control group (N=30)**	**HF group (N=30)**	***P****
Creatinine (μmol/L)	95.1 ± 31.4	147.9 ± 76.4	<0.001
Urea (mmol/L)	3.9 ± 2.1	4.2 ± 1.9	0.564
Uric acid (μmol/L)	519.1 ± 158.2	528.1 ± 187.4	0.841
Total proteins (g/L)	68.4 ± 10.7	71.2 ± 8.4	0.264
Fasting glucose (mmol/L)	6.8 ± 2.9	7.5 ± 3.1	0.371
Triglycerides (mmol/L)	1.56 ± 0.55	1.68 ± 0.48	0.342
Total cholesterol (mmol/L)	5.1 ± 1.02	5.8 ± 1.4	0.031
HDL cholesterol (mmol/L)	1.28 ± 0.3	1.31 ± 0.5	0.779
LDL cholesterol (mmol/L)	3.33 ± 1.5	3.97 ± 1.8	0.141
NT-proBNP (pg/mL)	23 (15-48)	4182 (2735-7812)	<0.001
hs-cTnI (ng/L)	-	29.2 (15.5-65.0)	0.661
CRP (mg/L)	6.6 (2.0-9.5)	10.8 (5.2-21.4)	0.187

Data are presented as mean ± standard deviation or median (IQR).
HF-heart failure; HDL-High-density lipoprotein; LDL- Low-density lipoprotein; NT-proBNP-N-terminal proBrain Natriuretic Peptide; hs-cTnI-High sensitivity Troponin I; CRP-C-reactive protein
* t-test for independent samples or Mann-Whitney test

Measurements of serum adropin in the control and heart failure group showed significantly higher levels of serum adropin in the heart failure group compared to the control group (7.1 ± 0.56 vs 4.23 ± 0.31 ng/mL, P<0.001) (Figure 1).

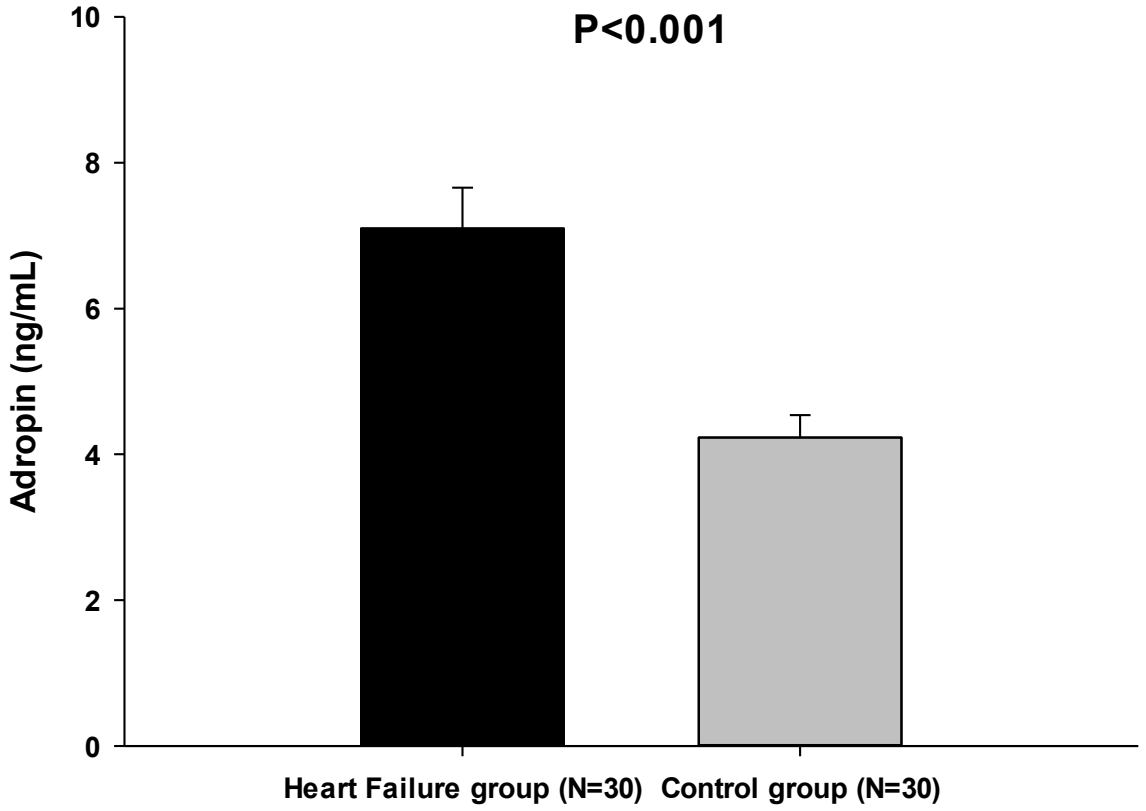

Figure 1. Adropin levels in heart failure group and control group

A negative correlation was found between CRP and serum adropin (r = -0.259, P=0.045) and a positive correlation was found between BMI and serum adropin (r = 0.256, P=0.035) (Table 3).

Additionally, a significant positive correlation was found between serum adropin and NYHA score (r = 0.389, P=0.002) and serum adropin and NT-proBNP (r = 0.393, P<0.001). Moreover, a significant negative correlation was noticed between serum adropin and LVEF (r = -0.325, P=0.011) (Table 4).

Table 3. Correlation analysis between serum adropin levels and different biochemical and anthropometric parameters in total study population

Parameter	Adropin (ng/mL) Total population (N=60) r*(*P*)
Age (years)	-0.089 (0.498)
BMI (kg/m^2)	0.256 (0.035)
Creatinine (µmol/L)	0.236 (0.069)
Urea (mmol/L)	0.089 (0.498)
CRP (mg/L)	-0.259 (0.045)

BMI- Body Mass Index**; CRP**- C-reactive protein
* Pearson's or Spearman's correlation coefficient

Table 4. Correlation analysis between serum adropin levels and different biochemical parameters in Heart failure patient group (N=30)

Parameter	Adropin (ng/mL) Heart failure group (N=30) r*(*P*)
NYHA Functional Classification	0.389 (0.002)
LVEF (%)	-0.325 (0.011)
NT-proBNP (pg/mL)	0.393 (0.001)
hs-cTnI (ng/L)	0.124 (0.345)

NYHA- New York Heart Association; **LVEF-** Left ventricular ejection fraction; **NT-proBNP-** N-terminal proBrain Natriuretic Peptide; **hs-cTnI**-High sensitivity Troponin I
* Pearson's or Spearman's correlation coefficient

5. DISCUSSION

The results of our study showed that patients with heart failure have a higher concentration of serum adropin compared to the control group. Furthermore, a highly significant positive correlation was found between serum adropin and NYHA score and between serum adropin and NT-proBNP. We also registered a negative correlation between serum adropin and LVEF.

Analogously Lian et.al reported elevated levels of serum adropin in patients with heart failure. They compared 56 patients with HF and 20 control subjects who were divided into 4 subgroups according to New York Heart Association (NYHA) functional classification. Their results showed that serum levels of adropin increases with higher NYHA classifications. Furthermore, they found a positive correlation between BNP, serum adropin and IL-6 whereas there was a negative correlation between serum adropin and LVEF (56). The author concluded that the augmented release of adropin may play a role in the pathogenesis of heart failure but couldn't establish any causality. Our study shows similar results with the only difference that we measured NT-proBNP rather than BNP. However, our results can be considered in full accordance with the aforementioned study since BNP and pro-BNP are both gold standards in the definition and diagnosis of heart failure (11).

Moreover, we speculate that adropin may have a role in inflammation given the significant correlation between serum adropin and IL-6 found by Lian et.al and the pivotal role of IL-6 in the pathophysiology of inflammation studied by Tanaka et.al (56,78). Supporting this claim is the work of Akcilar et.al which reported that adropin possibly inhibits the expression of TNF-α and interleukin-6 (IL-6) of the pancreas (79). Thus, optimization of serum adropin levels may improve inflammation elucidating a possible role of adropin in the therapy of inflammatory diseases.

Accordingly, in our study we found a negative correlation between CRP and serum adropin.

More evidence between the inverse correlation of adropin with inflammatory parameters can be found in a study conducted by Ha-Neul Choi et.al, where hs-CRP (high sensitivity CRP) was measured in 36 patients with diabetes mellitus type 2, and a highly significant negative correlation was found between hs-CRP and serum adropin (70). Furthermore, a study by Bozic et al. where adropin and inflammatory biomarkers levels in people with obstructive sleep apnea where studied, showed an inverse relationship between serum adropin and C-reactive protein although this data was not statistically significant (r =

−0.182, P = 0.119)(72).

Moreover, Shuyu Zhang et al., in a recent review article on adropin and its effect on inflammation, found that adropin may have immunomodulatory effects (77). More precisely they reviewed that adropin upregulates the expression of eNOS (endothelial nitric oxide synthase) by upregulating PI3K/Akt and extracellular signal-regulated kinase (ERK) signal transduction pathways. The increase of NO (nitric oxide) has multiple benefits on the endothelium such as the preservation of its integrity through vasodilation and prevention of atherosclerotic plaque formation through the decrease of adhesion of monocytes to the endothelium. Moreover, they found a negative correlation of serum adropin with homocysteine, high sensitivity C-reactive protein (hs-CRP), and levels of inflammatory cytokines. The mechanism behind the decrease of the latter is thought to be connected to the upregulation of eNOS. Additionally, mice with adropin gene knockout were noticed to exhibit decreased amounts of Treg cells, highlighting a possible connection between adropin and autoimmune diseases (77).

BMI and serum adropin were found to be positively correlated in our study. The link between BMI and serum adropin was elucidated in a study by Kumar et al. where lipid metabolism and energy homeostasis in relation to adropin were thoroughly investigated (48). More precisely, a group of mice were fed a high fat diet for a short period of two days and another group were fed a high fat diet for a longer period of 3 months. It was noticed that the former group of mice had an increase in expression of the adropin gene in the liver (Enho gene) whereas, in comparison, the latter group showed a decreased amount of adropin production. Additionally, the mice were put in a fasting state and it was noticed that adropin production would decrease compared to chow-fed animals. Thus, they found that adropin gene is up-regulated if a high fat diet is administered for a short period of time but it is down-regulated in case of a chronic high fat containing diet intake. Kumar et al. also elucidated the role of adropin in lipogenesis and a possible therapeutic role of adropin in obese patients (48). In essence, mice were put on high fat diet for a period of three months thus creating a state of insulin resistance and hepatosteatosis; afterwards the mice were treated with adropin and it was shown that adropin would improve insulin resistance and decrease hepatosteatosis. Indeed, adropin was found to decrease lipogenesis (48). In a study conducted by Oya Sayın et.al serum adropin levels were significantly lower in obese children than healthy controls. In addition, serum adropin levels of patients with NAFLD were found to be significantly lower than in patients without

NAFLD and healthy controls. In essence, it was shown that serum adropin levels may be an independent factor for fatty liver disease in obese children (63).

Yu et al. found that serum adropin levels are negatively correlated with BMI and TG levels in AMI patients (73). However, BMI and serum adropin may also be positively correlated as shown in the present study and in the study mentioned above by Kumar et al. (48).

We speculate that the reason behind this apparently contradicting relationship between BMI and adropin resides in the gravity of obesity-related liver disease. Indeed, it seems that an obese state can cause an increase in serum adropin levels reflecting the physiologic response to the body in activating a defence mechanism against endothelial dysfunction and atherosclerosis that are two of the main mechanisms behind obesity-related mortality. Supporting our claim are two studies by Lovren et.al and Kuloglu et.al that show the importance of adropin in maintenance of endothelial homeostasis (49,51).

On the other hand, if obesity has caused a significant dysfunction of the liver (e.g. clinically significant NAFLD), this may be reflected in a decreased production of adropin by the liver.

Adropin shows potential as an independent predicting factor of myocardial infarction. Indeed, Yu et al. have found that serum adropin levels are decreased in patients with myocardial infarction. In addition, patients with acute myocardial infarction had lower serum adropin compared to stable angina pectoris patients (59).

Moreover, in our study we have registered a positive correlation between hs-cTnI (high sensitivity Troponin I) and serum adropin levels in heart failure patients, yet this correlation was statistically insignificant. However, we speculate that serum adropin levels may help in the diagnosis of myocardial infarction. In a study conducted by Aydin et al. the relationship between adropin expression and isoproterenol-induced myocardial infarction was examined. Changes in adropin synthesis in rat heart, kidney and liver tissues in isoproterenol (ISO)-induced MI were demonstrated immunohistochemically and levels of adropin in the blood were calculated. The results showed a significant increase in serum adropin after myocardial infarction and a positive correlation between serum adropin and troponin-I concentration (75).

Additionally, in an observational study conducted by Aydin et al. the levels of saliva adropin were assessed in patients with Enzyme-positive acute coronary syndrome. At a cut off of 4.12 ng/ml the saliva adropin concentration indicated EPACS with 91.7% sensitivity and 57% specificity. Thus, adropin shows potential in diagnosis of myocardial infarction (76).

The limitation to our study is that the patients were from one Medical Centre only, University Hospital of Split. In addition, this is a cross-sectional study therefore we cannot conclude any causality between adropin and heart failure. Another limitation is the small sample size. Besides, given the positive correlation between serum adropin and BMI and the current scientific evidence supporting it, we consider adropin an important factor against obesity-related endothelial dysfunction and atherosclerosis. Moreover, adropin may have potential therapeutic benefits in inflammatory diseases. Lastly, adropin may be an independent risk factor for myocardial infarction and aid in its diagnosis. We feel that more studies are needed on adropin given its multiple roles in physiology and pathophysiology of multiple diseases.

6. CONCLUSIONS

1. Patients with heart failure have significantly higher levels of serum adropin compared to control group
2. A positive correlation was observed between serum adropin levels and NYHA class and serum
3. A positive correlation was observed between serum adropin and serum NT-ProBNP levels
4. A positive correlation was observed between serum adropin and BMI
5. A negative correlation was observed between LVEF and serum adropin
6. A negative correlation was observed between serum adropin and CRP levels

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8. SUMMARY

Objectives: The aim of this study is to determine serum adropin levels in patients with heart failure in comparison with healthy controls.

Patients and methods: This study enrolled a total of 30 heart failure patients and 30 healthy control subjects. The heart failure patients were evaluated in the first 24 hours of hospital admission. Evaluation included physical examination, medical history, venous blood sampling, blood pressure measurement, and transthoracic echocardiography (TTE). American Society of Echocardiography (ASE) guidelines were used for TTE examination. Laboratory analyses of collected venous blood samples was performed at Department of Medical Laboratory Diagnostics by experienced biochemist that was blinded to subject group assignment. Serum adropin levels were determined with dual enzyme-linked immunosorbent assay (ELISA) (Phoenix Pharmaceuticals, Burlingame, USA).

Results: Patients with heart failure had significantly higher serum levels of adropin compared to the control group (7.1 ± 0.56 vs 4.23 ± 0.31 ng/mL, $P<0.001$). We found a positive correlation between serum adropin and NYHA score ($r = 0.389$, $P=0.002$), NT-proBNP ($r = 0.393$, $P=0.001$) and BMI ($r = 0.256$, $P=0.035$). A negative correlation was found between serum adropin and LVEF ($r = -0.325$, $P=0.011$) and CRP ($r = -0.259$, $P=0.045$).

Conclusions: Serum adropin is significantly increased in people with heart failure compared to the control group which implies possible involvement of adropin in heart failure pathophysiology. However, future larger multicentric studies are needed to address these findings.

9. CROATIAN SUMMARY

Naslov: Adropin u bolesnika sa zatajenjem srca

Ciljevi: Cilj ovog istraživanja je utvrditi razinu adropina u serumu u bolesnika sa zatajenjem srca i usporediti je s razinom adropina u serumu zdravih kontrolnih ispitanika.

Metode: Ovo je istraživanje obuhvatilo ukupno 30 bolesnika sa zatajenjem srca i 30 zdravih ispitanika. Bolesnici sa zatajivanjem srca procjenjivani su u prva 24 sata po prijemu u bolnicu. Procjena je uključivala fizikalni pregled, povijest bolesti, uzimanje uzorka venske krvi, mjerenje krvnog tlaka i transtorakalnu ekokatiografiju (TTE). Za ispitivanje TTE korištene su smjernice Američkog društva za ehokardiografiju (ASE). Laboratorijske analize uzetih uzoraka venske krvi napravljene su na Odjelu za medicinsku laboratorijsku dijagnostiku od strane iskusnog biokemičara, koji je bio zaslijepljen za skupine ispitanika. Razine adropina u serumu određene su ELISA metodom (Phoenix Pharmaceuticals, Burlingame, SAD).

Rezultati: Pacijenti sa zatajivanjem srca imali su značajno više razine adropina u serumu u usporedbi s kontrolnom skupinom (7,1 ± 0,56 naprema 4,23 ± 0,31 ng/mL, P <0,001). Analizom je utvrđena pozitivna povezanost između serumskog adropina i NYHA zbira (r = 0,389, P = 0,002), NT-proBNP-a (r = 0,393, P = 0,001) i BMI-a (r = 0,256, P = 0,035). Pronađena je negativna povezanost između serumskog adropina i LVEF-a (r = -0,325, P = 0,011) i CRP (r = -0,259, P = 0,045).

Zaključak: Serumski adropin je značajno povišen u bolesnika sa zatajenjem srca u odnosu na kontrolnu skupinu, što implicira moguću uključenost adropina u patofiziologiju zatajenja srca. Međutim, potrebne su buduća veća multicentrična ispitivanja kako bi se razjasnili ovi nalazi.

10. CURRICULUM VITAE

Personal Data

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Internships

- Physician Shadowing – Dr. David C. Liu, Cardiothoracic Surgery, Beth Israel Deaconess Medical Center, Boston (United States)
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Awards

Winner of the Dean's Award as best student of the Year 2017/2018 - School of Medicine, University of Split

Language Skills

Italian (mother tongue)

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