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The role of adiponectin in patients with non-ischemic dilated cardiomyopathy and chronic heart failure

DOCTORAL DISSERTATION

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CONTENT

ABBREVIATIONS

ACE	angiotensin converting enzyme
ADIPOQ	adiponectin gene
AdipoR1	adiponectin receptor 1
AdipoR2	adiponectin receptor 2
AHA	American Heart Association
AMPK	AMP- activated protein kinase
APN	adiponectin
APPL1	Adaptor protein containing a Pleckstrin homology domain, a Phosphotyrosine binding domain and a leucine zipper motif
BMI	body mass index
BNP	brain natriuretic peptide
CAD	coronary artery disease
CD3+	T cell receptor
CD4+	T helper cell receptor
CD45ro+	memory T cell receptor
CD68+	monocyte/macrophage receptor
CHF	chronic heart failure
CK2	protein kinase CK2
CO	cardiac output
Cox-2	cyclooxygenase 2
CRP	C reactive protein
CRT	Calreticulin
CVD	cardiovascular disease
DCM	dilated cardiomyopathy
EMB	endomyocardial biopsy
ERp46	Endoplasmic reticulum-resident protein 46
eNOS	endothelial nitric oxide synthase
FOXO1	Forkhead box O1
GFR	glomerular filtration rate
GLS	global longitudinal strain
GPI	glycosylphosphatidylinositol
HF	heart failure
HFpEF	heart failure with preserved ejection fraction
HFrEF	heart failure with reduced ejection fraction
HMW	high molecular weight
hsTnT	high sensitivity cardiac troponin T

HT	heart transplantation
ICAM-1	Intercellular adhesion molecule 1
ICD	implantable cardioverter defibrillator
iDCM	inflammatory dilated cardiomyopathy
IL-6	interleukin-6
IL-8	interleukin-8
iNOS	inducible nitric oxide synthase
I/R	ischemia/reperfusion
iPSCs	induced pluripotent stem cells
KO	Knockout
LMW	low molecular weight
ln	logarithm to the base of the constant e
LV	left ventricle
LVAD	left ventricular assist device
LVEDD	left ventricle end-diastolic diameter
LVEF	left ventricular ejection fraction
MACE	major adverse cardiac event
MEF-2	myocyte enhancer factor-2
MI	myocardial infarction
MMP	matrix metalloproteinase
MMW	medium molecular weight
MSC	mesenchymal stem cells
NF-κB	nuclear factor κB
NI-DCM	non- ischemic dilated cardiomyopathy
NO	nitric oxide
NT-pro-BNP	N-terminal pro b-type natriuretic peptide
NYHA	New York Heart Association functional class
PAP	pulmonary artery pressure
PAQR	progestin and adipo-Q receptor
PCR	polymerase chain reaction
PCWP	pulmonary capillary wedge pressure
PGE2	prostaglandin E2
PH	pulmonary hypertension
PPAR-α	Peroxisome proliferator-activated receptor α
PPAR-γ	Peroxisome proliferator-activated receptor γ
RACK1	Receptor for Activated C Kinase 1
RAP	right atrial pressure
SD	standard deviation
S1P	sphingosine 1 phosphate

ST2	soluble suppression of tumorogenicity-2
TAC	transverse aortic constriction
T-cad	T-cadherin
TLR	Toll-like receptor
TNF-α	tumor necrosis factor alfa
TNFR1	tumor necrosis factor receptor 1

1. INTRODUCTION

1.1 The research problem

Heart failure (HF) is one of the most common disorders, and contrary to other cardiac diseases, its prevalence is rising worldwide. Approximately 50% of all patients suffer from HF with reduced ejection fraction (HFrEF) typically considered as left ventricle ejection fraction (LVEF) <40%) (1). Despite the improvement in the yearly rate of major adverse cardiovascular events, non-ischemic DCM (NI-DCM) remains one of the substantial causes of chronic heart failure (CHF), eventually leading to heart transplantation (HT) (2). The course of the disease is not easily predictable: some of the patients remain stable while others deteriorate quickly (3). Moreover, cardiologists do not have reliable tools to differentiate early between these two disease courses. The accurate assessment of prognosis in patients with NI-DCM and advanced HF is critical, as patients with the most severe disease are facing the fact of the limited number of hearts available for transplantation. There is a pressing need for novel biomarkers or a combination of new biomarkers with the well-established ones, which would aid in patient counseling and transplantation list prioritization.

The understanding of CHF pathogenesis has changed fundamentally in recent years. It became clear that CHF is not merely a hemodynamic disorder and even not only a problem of impaired neuroendocrine activation. Nowadays, it is considered to be a complex systemic disorder, which involves the activation of the immune system, metabolic alterations, processes in skeletal muscles, and systemic as well as local inflammation (1, 4, 5). A better understanding of these processes might aid in the search for novel therapeutic targets as well as new diagnostic and prognostic tools in HF.

Adiponectin (APN) is an adipocyte-derived cytokine, which plays an important role not only in obesity-linked diseases, including insulin resistance/diabetes and atherosclerosis but also in advanced heart and renal failure (6). Experimental studies demonstrate insulin-sensitizing, anti-inflammatory, and cardioprotective properties of APN (7-10). This adipokine has a critical signaling function in the heart, which is particularly important in patients with CHF. APN was shown to exert antiapoptotic, antihypertrophic, antifibrotic, and antioxidative properties at the myocardial level (11-13). Reduced levels of APN compared to healthy controls is seen in metabolic syndrome and subclinical atherosclerosis (14, 15). Diminished serum APN concentration was linked to increased cardiovascular risk and inflammation in

hypertension, coronary artery disease, obesity, and insulin resistance (16-18), and correlated with left ventricular hypertrophy (19, 20). In light of these findings, APN was considered to be a purely salutary adipokine.

Data from clinical studies of patients with HFrEF have revealed that APN levels are elevated, correlate with disease severity, and even more, portend increased risk of recurrent cardiovascular events (21) and mortality (22-24). The underlying pathophysiologic mechanisms of increased APN concentrations in chronic HFrEF remain unclear. There are also conflicting data from studies concerning the role of APN in inflammation: with some studies demonstrating potent anti-inflammatory activity while others - the opposite effect of APN (25). It is still a question of debate whether APN loses its cardioprotective and anti-inflammatory activities, or maybe the increasing amount of APN fails to control for the progressing disease (1).

Although it seems that APN has an additive role in predicting the course of chronic HF (26-28), it has not been recognized as a biomarker in HFrEF (1). It has been demonstrated that APN can be an independent predictor of mortality in patients with ischemic CHF (28). However, the data regarding APN's role in NI-DCM was missing. To our knowledge, only a single study addressed the role of APN as an outcome predictor in a cohort of patients with NI-DCM (29). There are no clinical data about the long-term prognostic role of baseline APN in patients with NI-DCM and CHF.

In addition to the two classical APN receptors, adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2), T-cadherin (T-cad) is yet another APN receptor abundantly expressed in the heart and vascular tissues (30). Experimental studies suggest that T-cad sequesters APN to cardiovascular tissues and might act as a negative regulator of circulating APN levels (31). Accumulating data from animal models revealed that APN, through its association with T-cad, protects the heart from stress-induced pathological remodeling and is essential for mediating the vascular actions of APN (32, 33). Moreover, the cardiovascular-protective role of APN is abolished in T-cad-knockout (KO) mice despite the high concentration of plasma APN (32, 33). The recent study by Wang et al. has demonstrated the downregulation of cardiac APN receptors, including T-cad in the mice model of HF at a stage, corresponding to CHF (34). Downregulation of myocardial T-cad would explain the diminished cardio-protective activity of APN in patients with CHF. However, there are no corresponding data concerning the expression of cardiac T-cad and its changes depending on the severity of HF in humans.

In the present investigation, we evaluated the relation of circulating APN and cardiac T-cad with other biomarkers indicating the severity of HF and

assessed the impact of circulating APN and cardiac T-cad levels on a five-year outcome in patients with NI-DCM and CHF.

1.2 The aim of the study

Hypothesis: The levels of circulating APN and its cardiac receptor T-cad are biomarkers indicating HF severity and predicting the composite outcome (Left ventricular assist device (LVAD) implantation, heart transplantation (HT), or death from cardiovascular causes) in patients with NI-DCM and CHF.

The aim of the study: To evaluate the relationship of serum APN and cardiac T-cad levels with established biomarkers of HF severity and to assess the impact of their baseline levels on the five-year outcome of patients with NI-DCM and CHF.

1.3 The objectives of the study

1. To assess the connection of serum APN with serum B-type natriuretic peptide (BNP) levels and to evaluate its association with other established biomarkers of HF severity.
2. To investigate the role of baseline serum APN levels as a five-year outcome predictor in patients with NI-DCM and CHF.
3. To estimate the association of serum APN levels with the biomarkers of systemic and cardiac inflammation.
4. To assess the relationship between cardiac T-cad and myocardial inflammation, and evaluate its relevance as a biomarker and outcome predictor in patients with NI-DCM and CHF.

1.4 The scientific novelty of the study

The prognostic benefit of serum APN was evaluated in a cohort of patients with chronic NI-DCM followed-up for five years. To our knowledge, this is the first study evaluating the impact of serum APN on the 5-year outcome of patients with NI-DCM.

To the best of our knowledge, this is the first report about the association between APN and global longitudinal strain (GLS), suggesting that serum APN could be an additional marker of myocardial dysfunction.

Furthermore, the concentration of myocardial T-cad and its possible role in APN-mediated cardioprotection was evaluated for the first time in humans with CHF.

1.5 The practical value of the study

NI-DCM is a significant cause of HF, and HT and the progression of the disease defies easy prediction so, there is a substantial need for novel prognostic biomarkers aiding patient counseling and treatment decision making. Elevated serum APN levels and cardiac T-cad expression changes could serve as potential prognostic biomarkers, distinguishing patients with the worst prognosis matured for HT in end-stage HFrEF.

1.6 Defended statements of the dissertation

1. Circulating levels of APN are associated with HF severity and disease progression in patients with NI-DCM and CHF.
2. Elevated serum APN concentration is an independent risk factor of poor composite outcome in patients with NI-DCM and CHF.
3. Serum APN levels are related to systemic and cardiac markers of inflammation.
4. Cardiac T-cad levels are associated with myocardial inflammation, indicate the severity of HF, and portend outcome in patients with NI-DCM and CHF.

2. LITERATURE REVIEW

2.1 Dilated cardiomyopathy as a cause of heart failure

2.1.1 Epidemiology of dilated cardiomyopathy

DCM is a cardiac condition characterized by dilatation and systolic dysfunction of the left ventricle in the absence of coronary artery disease (CAD), hypertension, valvular heart disease, and congenital heart disease sufficient to cause the observed myocardial abnormality (32, 35). Regardless of the etiology, DCM manifests as progressive ventricular wall thinning and dilatation accompanied by gradual functional impairment (36). LV systolic dysfunction eventually results in the development of HF and frequently deteriorates towards heart transplant (HT) in relatively young patients. Amongst cardiomyopathies, DCM is the most prevalent cause of HF. HFrEF is the most frequent presenting manifestation, as well as the predominant cause of death in DCM. Also, advanced HF in DCM is one of the leading indications for heart transplantation. Randomized clinical trials report that patients with NI-DCM usually comprise about 30-40% of all DCM patients, compared to ischemic DCM.

In daily practice, all attempts should be made to identify a specific or combined etiology of DCM; therefore this syndrome should prompt further in-depth investigations. Although DCM is far less common than coronary artery disease (CAD) and arterial hypertension, it is the third cause of HF (37). DCM is not infrequently associated with an increased risk of severe arrhythmia, indicating the pathological involvement of the cardiac conduction system.

The data concerning the epidemiology of DCM comes from a study conducted between 1975 and 1984 in Olmsted County, Minnesota. In this study, the DCM cases were evaluated by echocardiography, angiography, or autopsy resulting in the prevalence of 36.5/100 000 inhabitants or 1 in 2700 with men to women ratio of 3:4 in a European-American population (38). The variance in the prevalence of DCM might be caused by geographic and ethnic differences, as well as the methodologies used (39). Studies from England (8.3/100000), Italy (7.0/100 000), and Japan (14/100 000) report similar DCM prevalence (39). Hershberger et al. specified a much higher prevalence of DCM (in the range of 1:250) using a different approach (40). Their methodology for the evaluation of DCM prevalence was based on the known ratio of idiopathic DCM to hypertrophic cardiomyopathy of ≈2:1 and the

estimates of the prevalence of HF and left ventricle dysfunction as a surrogate for DCM.

The annual incidence of DCM is about 5 to 8 cases per 100.000. DCM is a major cause of sudden death in young adults (41) and is the most frequent diagnosis in patients referred for heart transplantation. The course of the disease is unpredictable, from relatively mild to severe and rapid, progressing to death. Typically, the prognosis is poor, with a 1-year mortality of 25–30% and a 5-year mortality of 46% (41). The prognosis is the worst for patients with a left ventricular LVEF of less than 25%, a right ventricular involvement, poor New York Heart Association (NYHA) functional class, and poor hemodynamic status at cardiac catheterization. Worsened diastolic dysfunction based on echocardiography is linked to symptoms, exercise intolerance, the development of pulmonary hypertension, and a higher mortality rate. Up to 12% of patients with DCM experience sudden death typically caused by electromechanical dissociation or ventricular arrhythmias, and it accounts for 25-30% of all deaths.

2.1.2 Etiology and pathogenesis of dilated cardiomyopathy

The causes of DCM are heterogeneous. CAD and myocardial infarction (MI) are the causes of HF in approximately 50-75% of patients in developed countries. Idiopathic cardiomyopathy is a diagnosis of exclusion. Coronary angiography should be performed in all cases of LV ventricular wall thinning and chamber dilatation with decreased EF in order to rule out the presence of CAD. In up to 7% of patients with initially unexplained DCM, coronary angiography revealed CAD as the main cause of HF (42). Randomized clinical HF trials typically report 30% to 40% of subjects with non-ischemic DCM compared to ischemic DCM. The term “non-ischemic cardiomyopathy” has been used to describe dilated cardiomyopathy. In ischemic cardiomyopathy, ventricular dysfunction is a consequence of myocardial ischemia and infarction related to coronary arteriosclerosis. There are many potential causes of non-ischemic cardiomyopathy, including hemodynamic pathology, primary myocardial diseases, genetics, infection, immunologic abnormalities, toxic injury, or other unknown (idiopathic) etiology.

As the phenotype of DCM is common for many heart conditions, it is not easy to define the etiology of the disease. In most cases, DCM involves the inflammatory process and genetics. Toxic injury is also common. Still, the causes usually remain largely obscure. Generally, the inflammatory process, inherited forms, and idiopathic DCM cause more than 90% of cases. Less common forms of primary cardiomyopathies are peripartum, tachycardia-

induced, stress-provoked Takotsubo cardiomyopathy, and myocarditis, according to the definition and classification of the American Heart Association (AHA) (43). Differential diagnosis of DCM should always include connective tissue diseases, endocrinological disorders, infiltrative diseases, medications, toxins, and tachycardia-induced DCM. Most of these diseases can be diagnosed by a thorough medical history, laboratory parameters, and imaging studies. Some of them, however, require more sophisticated laboratory tests, cardiac magnetic resonance imaging, or histological studies (44). The further diagnostic approach includes endomyocardial biopsy (EMB) and genetic studies. The genetic nature of DCM is increasingly recognized. However, causative mutations are verified in only 35% of patients at this stage (37). Persistent myocardial inflammation post myocarditis is a frequent cause in non-genetic cases of DCM. In the light of recent basic and clinical studies, inflammation and autoimmunization are considered to be the primary cause of otherwise unexplained cardiomyopathy (45).

As reported by the study of Felker et al. and according to the Myocarditis Treatment Trial, myocarditis causes DCM in 9 and 10% of patients, respectively (37, 42). Inflammation and sub-sequent autoimmunization is probably a more frequent cause of DCM than previously thought since novel imaging modalities detect inflammatory processes in tissues that would have been previously described as normal (46). However, in 50% of patients with DCM, the etiology remained undefined (37).

Inflammatory dilated cardiomyopathy (iDCM) is a late sequela of the complex interplay of the infectious agent, usually a virus, and the (auto)-immune response, developing in susceptible individuals (47). The diagnosis of iDCM is confirmed by EMB, as there is no difference in the phenotype of DCM and iDCM. The evaluation of EMB should include not only histopathological examination but also immunohistochemical and molecular biology techniques to identify the DNA or RNA of infectious agents (48-50).

2.1.3 Pathophysiology of dilated cardiomyopathy

Various mechanisms are implicated in the pathophysiology of HF, including genetic causes, direct myocardial damage due to infectious agents, toxins, endocrine and metabolic abnormalities, immune-mediated processes, and vasculo-hormonal pathways such as in peripartum cardiomyopathy. Positive familial anamnesis is reported in 30-50% of cases, and a genetic determinant is confirmed in up to 40% of DCM patients. A vast array of genes are implicated in the pathogenesis of DCM. These genes code for sarcomere

proteins, cytoskeleton, nuclear envelope, sarcolemma, ion channels, intercellular junction molecules (51). The truncating titin mutation is one of the most prevalent mutations implicated in ~13% and 25% of non-familial and familial cases of DCM, respectively (37, 52). However, routine genetic testing has a low yield (30-50%) and does not significantly influence the patients' management.

Damage to the myocardium by a viral infection, followed by autoimmune activation in the myocardium, is probably one of the most important causes of DCM. The direct cytotoxic effect of the virus is implicated at the beginning of the disease, leading to cardiomyocyte necrosis. At the same time, the immune system is activated. In the subacute phase of the disease, the immune system may remain activated, targeting the viral as well as self-antigens (mitochondrial proteins, heat shock proteins, cardiac myosin) due to molecular mimicry (47). The inadequate (too weak or too strong) reaction of the host has an impact on the pathogenesis of DCM. Excessive immune reaction due to escape from control mechanisms might lead to chronic low-grade inflammation and increased cardiomyocyte apoptosis.

The imbalance of pro-inflammatory (tumor necrosis factor α) over anti-inflammatory (tumor growth factor β, interferon γ) cytokines is also typical (53) and has a detrimental effect on cardiomyocytes. In addition to the inflammatory myocardial damage, autoantibodies against ADP/ATP carrier might also contribute to LV dysfunction. Myocardial inflammation, as addressed by the presence of cytotoxic T-lymphocytes in the myocardium, is linked to the deterioration of LV function in the long-term (54).

On the other hand, persistent viral genomes have been found without DCM (55), demonstrating the relevance of host immune response in the disease pathogenesis. Activation of certain genes, including the ones for MMP-9 and type-1 procollagen in mast cells, have been related to more pronounced myocardial inflammation and necrosis (1, 56). In general genetic susceptibility might be the major factor influencing the progression of HF after autoimmune myocarditis (39, 40).

The development of HF following chemotherapy, alcohol abuse, or intoxication with certain drugs and toxins is mediated by direct cardiotoxicity as well as neurohormonal activation, changes in calcium homeostasis, and oxidative stress (2, 4). Depending on the toxic agent and patients' susceptibility, the myocardial injury may manifest soon after exposure, or it may become clinically evident years after treatment (late DCM), as a result of continuous cardiac injury (37).

LV failure is characterized by LV dilative remodeling, in which the LV acquires a spherical shape. Decreased stroke volume and cardiac output,

impaired ventricular filling, and increased LV end-diastolic pressure are typical pathophysiological changes. They are typically accompanied by compensatory changes in the vascular system, such as an increase in systemic vascular resistance, a decrease in arterial compliance, and an increase in venous pressure and circulating blood volume. Cardiac preload and afterload are concomitantly increased. The increased afterload results in elevated wall stress (57, 58). Diastole involves both active relaxation (early diastole) and passive compliance (mid-to-late diastole). Diastolic dysfunction affecting both components may appear with reduced systolic function in DCM. Reduced rapid ventricular filling is caused by impaired ventricular relaxation. Diminished ventricular compliance due to hypertrophy or fibrosis leads to restrictive pathophysiology, revealing itself in reduced ventricular filling and elevated end-diastolic pressures.

Neurohormonal changes include an increase in circulating catecholamines and vasopressin levels, as well as activation of the renin–angiotensin–aldosterone system. Reduced vagal activity in the heart is typically documented. The levels of natriuretic peptides are elevated. The combination of increased catecholamines, elevated cardiac afterload, fluid retention, and tachycardia further elevates wall stress and increases myocardial oxygen demand, as well as direct cardiotoxicity. These secondary adaptations promote the progressing myocyte damage and reduction in cardiac performance.

2.1.4 Diagnosis of dilated cardiomyopathy

Biomarkers. Advanced HF involves not only the adverse remodeling of cardiomyocytes and interstitial tissues but also changes in vasculature, kidney, and skeletal muscle. Biomarkers reflect different aspects of HF pathophysiology and provide direct clinical, diagnostic, and prognostic information, which is of great importance in clinical decision making. Moreover, they aid in search of novel therapeutic targets. Various mutually overlapping pathophysiological mechanisms play a role in HF. Braunwald proposed the following classification of biomarkers based on their pathogenetic role in HF (59): 1) neurohormonal activation (e.g., norepinephrine, renin, aldosterone), 2) cardiomyocyte injury (e.g., high sensitivity troponin [hs-TnT]), 3) cardiomyocyte stress (e.g., BNP/NT-proBNP), 4) inflammation (e.g., C-reactive protein (CRP), different cytokines), 5) oxidative stress (e.g., myeloperoxidase, uric acid); 6) fibrosis and extracellular matrix remodeling (e.g., matrix metalloproteinases (MMPs) and their inhibitors: MMP-2, tissue inhibitor of MMP-4, MMP-8 and collagen pro-peptides: procollagen type I amino-terminal pro-peptide; collagen III N-

terminal pro-peptide); 7) renal dysfunction (creatinine, blood urea nitrogen, Cystatin C) and 8) novel and/or multifactorial markers (e.g., soluble suppression of tumorigenicity-2 (ST2), galectin-3, adiponectin, tissue inhibitor of metalloproteinase-1, growth differentiation factor-15).

BNP and N-terminal pro–B-type natriuretic peptide (NT-pro-BNP) are the most studied biomarkers in HF, playing a significant role in diagnosis and outcome prediction. Evaluation of NT-proBNP or BNP levels to support the clinical diagnosis of HF and assess disease severity or predict prognosis is a Class I recommendation in current clinical practice guidelines (60, 61). Natriuretic peptides have been examined as biomarkers of clinical responsiveness to guideline-directed treatment, as their levels diminish with a wide range of medical therapies, indicating their therapeutic benefit. If NT-proBNP or BNP levels are not reduced with a medical therapy provided, this portends a worse prognosis and more adverse LV remodeling (62).

A combination of biomarkers derived from invasive and non-invasive imaging, serological parameters, and EMB evaluation is used in the Marburg Registry for the identification of patients with inflammatory cardiomyopathy and myocarditis (63). Such a multi-marker approach is useful for risk monitoring and clinical decision making regarding medical therapy as well as advanced HF treatment.

Echocardiography. The phenotype of DCM is established employing imaging studies. LV measurements can be determined by multiple imaging modalities (64). M-mode and 2-dimensional echocardiography are frequently applied for determining LV internal dimensions and volumes in systole and diastole, assessing LVEF, global and regional LV function, ventricular wall thickness, valvular abnormalities, and right ventricular involvement. The cardiomyopathy is considered dilated if it meets the following criteria: 1) left ventricular end-diastolic diameter (LVEDD) >117% of the predicted value (>2 standard deviations [SD] of 112% of the predicted value, corrected for age and body surface area) 2) left ventricular systolic dysfunction defined by LVEF <45%, and/or 3) fractional shortening < 25%. Familial DCM is defined by the presence of (1) ≥2 affected relatives with DCM meeting the above criteria, or (2) a relative of a DCM patient with sudden unexplained death before the age of 35 years (39).

Doppler evaluation aids in quantifying the severity of diastolic dysfunction, functional mitral regurgitation caused by annular dilatation as well as noninvasively measured hemodynamic parameters such as right ventricular systolic pressure, central venous pressure, and LV filling pressures. Tissue Doppler imaging can facilitate early detection of myocardial

dysfunction in NI-DCM (65). However, there is little data concerning the diagnostic usefulness of this mean in more advanced HF.

Serial echocardiographic investigations can help to make decisions regarding device therapy (implantable cardioverter-defibrillator, cardiac resynchronization therapy) or applying advanced therapies (ventricular assist device or HT). Strain and strain rate differences can be detected by echocardiography in newly diagnosed NI-DCM, indicating that LV dimensions are not the earliest detectable differences in DCM (37).

Cardiac magnetic resonance. Cardiac magnetic resonance imaging provides the means of accurately determining LV chamber dimensions and function, including strain measurements. In order to evaluate tissue fibrosis and myocardial tissue quality, contrast agents such as gadolinium can be utilized. Delayed gadolinium enhancement corresponds to the degree of myocardial fibrosis and predicts all-cause mortality and future hospitalizations (66). It is also related to increased risk of ventricular arrhythmias (67) and may reflect edema and inflammatory infiltrate (68).

Endomyocardial biopsy. Endomyocardial biopsy (EMB) has been used to confirm the diagnosis in some forms of DCM. Irregular myocyte hypertrophy, with or without areas of fibrosis and myocyte damage in EMB specimen, is a typical finding for NI-DCM. A lymphocytic infiltrate indicates the presence of inflammation that could be post-viral or immune-mediated.

Immunohistochemical diagnosis and quantification of the inflammatory process were endorsed by the European Society of Cardiology (ESC) Working Group on Myocardial and Pericardial Diseases and described by the World Heart Federation consensus definition of inflammatory cardiomyopathy (myocarditis) (45, 47, 55). According to these statements: 1) abnormal findings – more than 2 CD3+ T-lymphocytes per high-power field (400-fold magnification) or 7 per mm^2; 2) myocarditis – the presence of an inflammatory infiltrate of a minimum of 14 infiltrating leukocytes/mm^2; 3) active myocarditis – the presence of > 14 infiltrating leukocytes/mm^2 and/or the presence of more than 2 CD3+ T-lymphocytes per high-power field, which are adherent to the contour of cardiomyocytes and focally related to cell necrosis (55).

Myocarditis is an infectious/inflammatory disease mainly caused by a viral infection and consequent immune response. The course of the disease is determined by the individual genetic background, which might favor or inhibit the progression of the disease to chronic cardiomyopathy. This is largely dilated cardiomyopathy. The immune response is specific, and infiltrated immune cells produce ongoing myocardial damage despite the diminished viral titers. This finally ends-up in post-inflammatory cardiomyopathy.

Specific genotypes influence the LV remodeling in post-myocarditis dilated cardiomyopathy. It is questionable whether the viral infection is directly responsible for the development of dilated cardiomyopathy, or is an environmental trigger leading to the manifestation of the genetically determined disease (45).

EMB has been used to evaluate myocarditis and in the setting of unexplained HF. Viral genome in cardiomyocytes can be identified via PCR despite resolved inflammatory changes. The data concerning the impact of this finding on long-term prognosis is contradictory (69) (70-72)

However, it seems that not the persistence of the viral genome itself, but the stage of the viral infection and signs of active viral replication play a crucial role in long-term prognosis (70, 73).

Histological evaluation aids in the identification of specific disorders such as sarcoidosis and hemochromatosis. Abnormal mitochondria, lysosomes, or myocardial inclusions can reveal specific metabolic and storage disorders. EMB is the most beneficial in patients with a recent onset of HF, ventricular arrhythmia, or Mobitz type II second or third-degree atrioventricular block when a specific diagnosis, having an impact on diagnosis, is suspected (74).

The complication rates of EMB procedure range from 1% to 3%, and serious complications such as perforation and tamponade occur at the rate of 0.5% (74).

Coronary angiography and right-sided heart catheterization. Perfusion deficits and segmental wall-motion abnormalities indicating CAD are commonly visualized with non-invasive imaging. However, only coronary angiography and coronary CT can reliably show the presence of coronary artery obstruction. According to some experts, CAD should be excluded whenever possible, as revascularization may improve LV function. It is especially important in patients with diabetes mellitus or other causes of silent myocardial ischemia as well as young patients who may possess congenital coronary anomalies.

Right heart catheterization provides much crucial hemodynamic information regarding filling pressures, vascular resistance, and cardiac output. It is of utmost importance in cases of HF refractory to initial treatment, considering the candidates for HT or assist device implantation, in questionable fluid status of the patient, in cases of significant hypotension (systolic blood pressure < 90 mm Hg or symptomatic low systolic blood pressure) and worsening of renal function during the initial treatment.

Pulmonary capillary wedge pressure (PCWP) helps to determine the severity of congestion and is essential for evaluating the origin of pulmonary artery hypertension (PAH). PCWP above 25 mmHg indicates impending

pulmonary edema. PAH is usually caused by increased pulmonary vascular resistance but may also result from increases in pulmonary venous pressure and pulmonary blood volume secondary to LV failure. Pulmonary blood flow (usually measured by the thermodilution technique), pulmonary artery pressure (PAP), and PCWP measurements are required for the calculation of pulmonary vascular resistance.

PCWP evaluation helps to differentiate cardiogenic causes of pulmonary edema from noncardiogenic ones, such as acute respiratory distress syndrome. A PCWP exceeding 18 mm Hg in a patient to have chronically elevated left atrial pressure indicates cardiogenic decompensated heart failure. In patients with chronic pulmonary capillary hypertension, PCWP exceeding 25 mm Hg is generally required to overcome the compensatory activity of the lymphatics and cause pulmonary edema.

Better awareness of filling pressures and hemodynamics might lead to cardinal changes in HF treatment.

2.1.5 Treatment of dilated cardiomyopathy

Medical treatment. Management of DCM is focused on (1) reversing LV remodeling, (2) arrhythmia surveillance and treatment, and (3) reducing congestive symptoms if present. The main goals of medical treatment are to prevent HF progression, to improve exercise capacity, to improve prognosis, and to prolong life-time. Symptomatic DCM and HFrEF are managed following current ESC and AHA/American College of Cardiology guidelines (60, 61). Guideline-directed medical treatment includes angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blocker, in association with β-blockers, aldosterone antagonists, and in individual cases, vasodilators. Medications are supposed to be titrated to the dose used in clinical trials unless limited by side effects. Patients having either high-risk features or a poor response despite optimal therapy should be considered for referral to an advanced HF specialist.

Guidelines recommend switching from ACE inhibitor/angiotensin receptor blocker to angiotensin receptor-neprilysin inhibitor in NYHA class II to III patients who are not responding to optimal medical therapy (61). AHA/American College of Cardiology/Heart Failure Society of America guidelines approve shifting to angiotensin receptor-neprilysin inhibitor even in those who respond to optimal therapy, because of the superior benefit of the latter over ACE inhibitor/angiotensin receptor blocker in terms of mortality and morbidity (60). Ivabradine can be added to optimal medical therapy to reduce morbidity in patients with sinus rhythm and a heart rate >70 bpm.

Mechanical circulatory support and surgery. In the case of refractory medical treatment, advanced therapies such as long-term mechanical circulatory support implantation or HT are considered (61, 75). Surgical correction of mitral regurgitation, LV remodeling, use of restraints to prevent progressive ventricular dilatation, or a combination of these approaches may also be applied. However, none of these methods are demonstrated to improve survival compared to appropriately matched controls.

Patients with NI-DCM comprise the biggest group of patients undergoing HT. Patients with DCM are referred to HT in case of persisting intractable advanced HF (NYHA class III-IV) despite the optimal medical and device treatment. These are usually younger people having fewer comorbidities compared to other patients with CHF. Cardiopulmonary stress testing is recommended as a part of the HT selection procedure to evaluate the functional limitation and risk stratification. Patients, referred to HT, have an expected survival of fewer than two years without transplantation or long-term mechanical support. After transplantation, the median survival reaches > 10 years (37).

In the case of intractable advanced HF, acute and long-term mechanical circulatory support devices are used. An extracorporeal membrane oxygenation device is typically used in case of acute circulatory failure. It can be used as a bridge therapy for critically ill patients. In these settings, HT or long-term mechanical circulatory support devices are considered only after improvement in multiorgan failure. Evolution to smaller continuous-flow left ventricle assist devices and associated improved outcomes have led to their more widespread use as well as earlier implantation. About 20% of patients require biventricular support. Mechanical circulatory support devices are implanted for bridging the DCM patient to HT. However, for patients with contraindications to HT, implantation of those devices could be considered as a destination treatment for advanced HF. With current devices, 6-month survival rates following implantation of LVAD is more than 80% in children and 90% in adults (60).

Implantable electrical devices. Prevention and treatment of ventricular tachyarrhythmias, treatment of symptomatic bradyarrhythmias, and cardiac resynchronization are the main indications for implantable electrical devices. Implantable cardioverter defibrillators (ICDs) are used for patients who have survived ventricular fibrillation or symptomatic ventricular tachycardia. Although it seems that there is no overall-survival benefit implanting ICD for primary prevention in NI-DCM, except for the diminished risk of sudden cardiac death, it might be useful for younger patients with hereditary DCM.

Modern imaging modalities such as MRI might help distinguish patients at the highest risk, which should be referred to an ICD implantation.

Patients with DCM might require pacing for symptomatic bradycardia. Biventricular pacing is the choice for patients with DCM and symptomatic bradycardia because traditional right ventricular pacing can induce LV dyssynchrony and precipitate HF (76).

Patients with DCM on optimal therapy with complete left bundle branch block may benefit from cardiac resynchronization therapy. The improvement of survival in patients with CAD with LVEF <35% is also well established. In well-selected patients, cardiac resynchronization therapy (with or without CAD) improves survival and quality of life, reduces hospital admissions, and induces reverse LV remodeling when added to optimal medical treatment (77).

Cardiac tissue regeneration for DCM. The human heart has a limited regenerative capacity, and DCM is associated with increased loss of cardiomyocytes. Various strategies for regeneration and repair of cardiac tissue have been developed, including the application of a stem cell suspension, growth factors, miRNAs, and engineered tissue implantation. Cardiac progenitor cells, bone marrow-derived mesenchymal stem cells (MSC) as well as induced pluripotent stem cell applications were tested in laboratory investigations and clinical trials. However, these interventions mainly failed to prove functional improvement (78). The potential effects of bone marrow MSC are likely to be caused by the release of paracrine factors and/or recruitment of cardiac progenitor cells because the injected cells do not remain in the cardiac tissue (79).

Stem cells have been used for cellular models of DCM. Induced pluripotent stem cells (iPSCs) can be more readily generated from patients with DCM and then differentiated into cardiomyocytes (79). Cardiomyocytes obtained from iPSC can be used to study cellular properties that reflect DCM features and aid in understanding the mechanisms of the disease and in testing treatment.

To sum up, the data mentioned above demonstrate the complicated nature of HF pathogenesis. It became evident that it is not only the sympathetic nervous system and renin-angiotensin-aldosterone system that are important for the progression of DCM to HF. Autoimmunity, genetic defects, MMPs, increased collagen deposition and degradation, beta 2-adrenoreceptors, and many other factors also seem to play an important role here. Some of them became the targets of novel treatment methods. One of the potential targets in the pathogenesis and progression of HF due to NI-DCM is serum APN and its cardiac receptors.

2.2 Structure of the adiponectin system

APN was, for the first time, described in 1995 (80). It is the most widely studied adipokine so far. APN drew much attention as a critical messenger for the crosstalk between adipose tissue and other metabolic-related organs, as well as a molecule granted with potential cardioprotective properties (81). In the last 25 years of research, scientists have put much effort into ascertaining the molecular mechanisms of action APN (Figure 1).

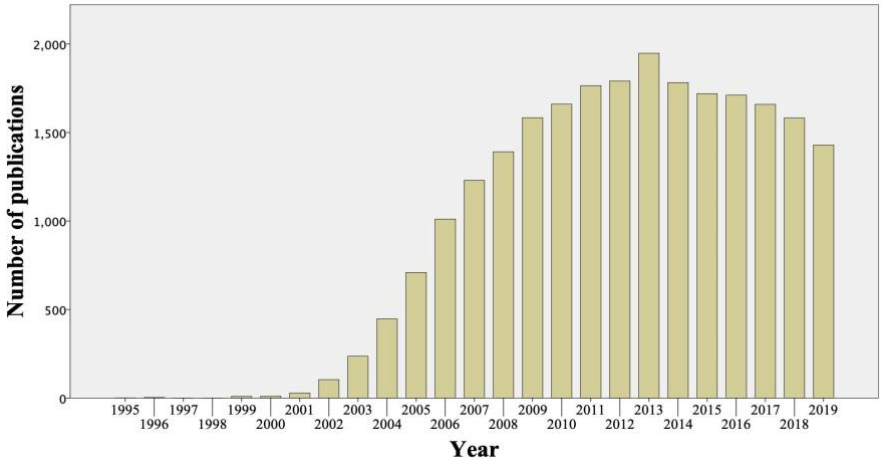

Figure 1. Yearly publications on adiponectin in the Pubmed database

Beneficial effects of APN on glucose homeostasis, chronic low-grade inflammation, apoptosis, oxidative stress, and atherosclerotic processes were demonstrated in basic studies. Despite that, APN remains a scientifically elusive molecule. Elevated APN levels are present in chronic inflammatory and autoimmune diseases as well as chronic systolic HF (24, 25, 82) and are linked to the worse outcome of the patients. Several recent genetic studies in patients with CAD and diabetes have even raised the hypothesis that higher APN levels might have a direct deleterious effect and add-up to the increased cardiovascular and all-cause mortality (83, 84). As concluded by Menzaghi and colleagues, "much has been done on adiponectin, but still more has to be done" (85).

2.2.1 Structure and synthesis of adiponectin

APN is the most abundant protein secreted from adipose tissue accounting for 0.01% of total plasma protein (86, 87). It circulates in plasma at mg/ml levels, compared to ng/ml of conventional hormones such as insulin and leptin (88). Circulating levels of APN range from 5 to 30 μg/ml in healthy individuals (1).

APN coding gene, ADIPOQ, is localized in chromosome 3q27 (89). The ADIPOQ promoter contains many transcription factor binding sites through which several factors modulate its activity, as reviewed by Caselli et al. (90). The biosynthesis of APN is regulated by various factors, including cytokines, transcription factors, and drugs (91). Activation of the transcription factors peroxisome proliferator-activated receptor-α and γ (PPARα and PPARγ) and Forkhead box O1 (FOXO1) plays a critical role in regulating the production of APN in adipocytes (25). Although many drugs have been implicated in modulating the production of APN, most of them act via PPARγ activation (91). Proinflammatory molecules such as TNF-α, interleukin 6 (IL-6), and CRP downregulate the synthesis of adiponectin (92, 93). Chronic low-grade inflammation has been shown to reduce the expression of APN gene (94). BNP activates ADIPOQ expression in cell cultures (95), and human adipose tissue explants (96). Kamigaki et al. demonstrated that reactive oxygen species, such as hydrogen peroxide, reduce ADIPOQ expression (97).

APN is mainly expressed as an adipocyte complement-related protein of 30 kDa (Acrp30) in adipose tissue and fully differentiated adipocytes (80). The monomer of APN has a collectin protein-like structure with a globular head and a collagenous tail and is called the full-length APN (12) (Figure 2). The monomeric subunits assemble into larger oligomeric structures. In peripheral circulation, APN is present as three distinct oligomeric complexes, including trimers, hexamers, and high molecular weight (HMW) isomers composed of 12-18 monomers (80, 98). The monomeric form of adiponectin is undetectable in natural conditions. The trimeric or low molecular weight (LMW) adiponectin represents the basic unit. HMW isoform is considered to be the most biologically active (99). Extensive post-translational modifications take place in adipocytes to assure the intracellular assembly and secretion of the HMW complex and maintain stability in the circulation (100, 101). The hexameric or middle molecular weight (MMW) isomer and HMW isoform are firstly reduced to the APN trimer, which then interacts with the receptors and exerts the downstream biological functions (81).

There exists a truncated form of APN, containing only the COOH-terminal portion, produced via proteolytic cleavage by leukocyte elastase. The so-called globular adiponectin is found in plasma and exerts biological activity (102). The isoforms of APN and molecular structure of APN are presented in Figure 2 (103).

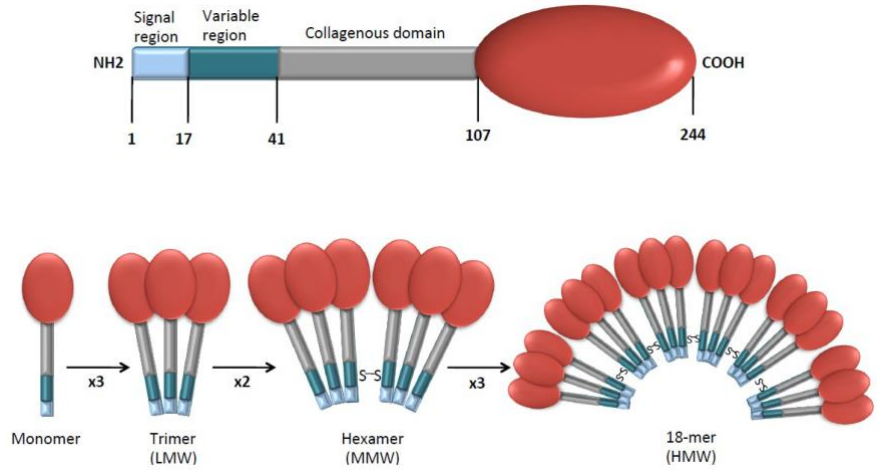

Figure 2. The structure of adiponectin monomers and isomers
From: "Adipose Tissue, Obesity and Adiponectin: Role in Endocrine Cancer Risk." Andrea Tumminia et al. Reproduction permitted given proper citing. Abbreviations: LMW; low molecular weight, MMW: middle molecular weight, HMW: high molecular weight.

There are similarities in the tertiary structure of the APN and the TNF family, indicating that they may have arisen from a common primordial molecule during evolution (104).

Although APN is produced in other cells including hepatocytes, osteoblasts, skeletal muscle cells, and cardiomyocytes (105-107), the primary source of this cytokine remains white adipose tissue (108). APN levels are the highest in lean subjects and decline as body mass increases (109). Its levels are reduced in obesity and insulin resistance (110). There is a negative correlation between circulating APN and body mass index (BMI) (111). APN is an exceptional adipokine as the majority of adipokines, including IL-6, interleukin-8 (IL-8), TNF-α, etc., are produced in direct proportion to fat mass, and mainly exhibit proinflammatory or otherwise adverse effects on the cardiovascular system (112). The levels of circulating APN are nearly twice as high in women compared to men. This sexual dimorphism is probably due to the effect of testosterone (113).

2.2.2 Adiponectin receptors and their signaling

APN acts via binding and activation of three main types of receptors: AdipoRs, T-cad, and calreticulin (CRT).

Adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2) have a seven-transmembrane topology, but they are not G protein-coupled receptors (81). They belong to the progestin and AdipoQ receptor (PAQR) family (114). AdipoR1 is abundantly expressed in skeletal muscle and endothelial cells, and AdipoR2 is mainly expressed in the liver (115). Both AdipoR1 and AdipoR2 are constitutively expressed in adult cardiomyocytes (115, 116). AdipoR1 can act as a high-affinity receptor for globular APN, but it can also bind full-length APN, whereas AdipoR2 shows an intermediate affinity for both globular and full-length adiponectin molecules (117). Finally, recent studies suggest that the actions of APN may also be mediated through additional receptors, including PAQR3 (renamed AdipoR3) (114) and AdipoRX (118).

AdipoR1- or AdipoR2-deficient mice have never been tested in *in vivo* cardiac or vascular models. To date, the majority of the AdipoR1 and AdipoR2 research has focused on their metabolic functions (116, 119). AdipoR1 gene ablation was associated with metabolic dysfunction and excessive adipose tissue accumulation in mice (117)**.** In murine genetic and diet-induced models of metabolic syndrome, an overexpression of AdipoR1 in macrophages improved glucose tolerance and insulin sensitivity (120, 121). Overexpression of the AdipoR1 and AdipoR2 receptors by adenovirus leads to the enhanced conversion of ceramide to sphingosine 1-phosphate (S1P), consequently protecting against insulin resistance (81). Reduced expression of either AdipoR1 or AdipoR2 individually did not inhibit the stimulatory actions of APN (122, 123).

T-cadherin as APN receptor. T-cad was shown to be an additional APN receptor binding the MMW and HMW isoforms of APN in smooth muscle cells, endothelial cells, and cardiomyocytes (32, 124). T-cad is a unique cadherin molecule that is anchored to the surface membrane via glycosylphosphatidylinositol (GPI) moiety (125). GPI anchor precludes direct T-cad interaction with cytoplasmic domains, and the signaling of APN via T-cad is still obscure. Another unusual property of T-cad is the simultaneous expression on the cell of its two forms (the mature protein and partially processed precursor containing an uncleaved pro-peptide), both binding APN (126). T-cad is highly expressed in the heart, smooth muscle, and endothelium, which are important targets of APN (30). T-cad expression was shown to be critical for APN sequestration to murine cardiovascular tissues and to human cell cultures (32, 33, 127, 128). T-cad ablation lead to markedly increased circulating levels of APN and abolished the cardioprotective role of APN in murine models of ischemia/reperfusion (I/R) injury, pressure overload, and ischemia of hindlimb (32, 33, 127). T-cad-KO mice

demonstrated AMP-activated protein kinase (AMPK) activation, the primary downstream signaling molecule of APN, suggesting that T-cad is critical for APN-mediated cardioprotection (32).

Calreticulin as APN receptor. Calreticulin (CRT), a multifunctional endoplasmic reticulum chaperone protein, was shown to be yet another APN receptor (129, 130). The interaction of APN with CRT/CD91 complex has been shown to exert anti-inflammatory and vasculo-protective effects (131). In the murine model of hind limb ischemia, adenovirus-mediated overexpression of APN enhanced Cox-2 expression and revascularization of ischemic limbs in wild type mice, but not in Cox-2-deficient mice (132). Ablation of CRT or its adaptor protein CD91 by siRNA resulted in reduced Cox-2 induction by APN, suggesting that vasculo-protective effects of APN are exerted via Cox-2-dependent signaling.

Furthermore, APN was shown to opsonize apoptotic cells and facilitate their uptake and removal by macrophages via binding to CRT/CD91 on the surface of macrophages in insect cells or E. coli. (129). It was assumed that APN protects from systemic inflammation by promoting the clearance of early apoptotic cells by macrophages.

Signaling by APN. Upon stimulation by APN, several intracellular molecules are activated, including Adaptor protein-containing pleckstrin homology domain, a phosphotyrosine binding domain and a leucine zipper motif (APPL1), Receptor for Activated C Kinase 1 (RACK1), protein kinase CK2β, and Endoplasmic reticulum-resident protein 46 (ERp46) connecting AdipoRs to their downstream molecules (133). APPL1 binds the cytoplasmic domain of AdipoRs (134). RACK1, protein kinase CK2β, and ERp46 may act as bridging factors in signal transduction interacting with AdipoR1 (133, 135, 136). The pleiotropic biological effects of APN are thought to be realized through the activation of two major downstream molecules such as AMPK (137, 138) or PPAR-α (116). The APN-induced activation of AMPK is blocked in mice with targeted deletion of AdipoR1, and disruption of AdipoR2, in turn, diminishes the APN-stimulated PPAR-α signaling (116). Activation of AMPK is a key step in mediating most of the effects of APN at the cellular level (139). AMPK is a ubiquitously expressed cellular energy sensor that is activated by an increase in the intracellular AMP/adenosine triphosphate (ATP) ratio (140). The metabolic, anti-inflammatory, and vasculo-protective effects of APN share the activation of AMPK signaling (131).

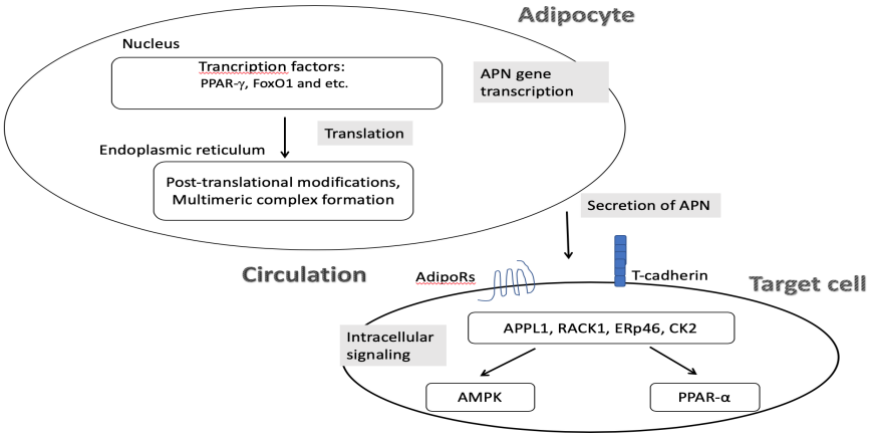

Figure 3. Gene regulation, synthesis, secretion, and signaling of adiponectin
Abbreviations: PPAR-γ: peroxisome proliferator activator receptor γ, FoxO1: Forkhead box O1, APN: adiponectin, AdipoRs: adiponectin receptors, APPL1: Adaptor protein-containing pleckstrin homology, a domain, a phosphotyrosine binding domain, and a leucine zipper, RACK1: Receptor for Activated C Kinase 1, ERp46: Endoplasmic reticulum-resident protein 46, CK2: protein kinase CK2, AMPK: Adenosine monophosphate-activated protein kinase, PPAR-α: Peroxisome proliferator-activated receptor α

However, some of the APN effects are mediated via AMPK-independent mechanisms (141). APN was also shown to diminish cardiac cell TNF-α production and reduce infarct size via activation of cyclooxygenase-2 (Cox-2)-dependent synthesis of prostaglandin E2 in the murine model of ischemia-reperfusion injury (8). The interaction of APN with calreticulin/CD91 complex has been shown to exert anti-inflammatory and vasculo-protective effects independently from AMPK: mainly via facilitating the removal of apoptotic bodies and Cox-2 mediated PGI2 synthesis (129, 132).

Holland and colleagues have demonstrated that activation of intracellular ceramidase activity might be critical to the actions of APN in various cell types, including the cardiomyocytes (142). Ceramidase is an enzyme converting harmful ceramides into a beneficial class of lipids, including the S1P (81), which is a potent inducer of proliferation and inhibitor of apoptosis (143). Ceramides are lipids that are involved in insulin resistance, cell death, inflammation, and atherosclerosis (144). APN catalyzes ceramide deacylation via activation of ceramidase and leads to the enhanced conversion of ceramide to S1P (Figure 4) (144).

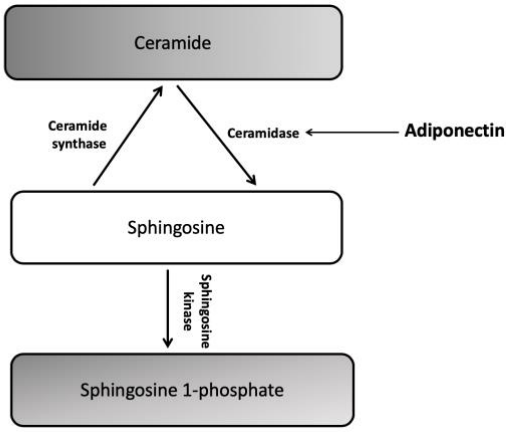

Figure 4. The intracellular ceramide decreasing activity of adiponectin

AdipoR1 and R2 possess inherent ceramidase activity, and APN/T-cad association was related to increased exosome biogenesis leading to more efficient elimination of intracellular ceramides (145). In the model of inducible apoptosis, APN overproduction decreased caspase-8-mediated death in cardiomyocytes, whereas genetic ablation of APN promoted apoptosis *in vivo* through a sphingolipid-mediated pathway (142). The antiapoptotic role of APN depended on its ability to activate ceramidase via activation of AdipoR1 and AdipoR2, leading to the formation of its antiapoptotic metabolite—S1P.

In conclusion, APN receptors are widely expressed, especially in the cardiovascular system, including endothelial cells, vascular smooth muscle cells, cardiomyocytes, and monocytes, leading to a host of effects on different target tissues (7).

2.2.3 The biological functions of adiponectin

APN is characterized by metabolic actions and signaling in different cells and tissues. APN targets the liver, heart, pancreatic beta cells, kidney, skeletal muscle, and many other cell types in various tissues (Figure 5).

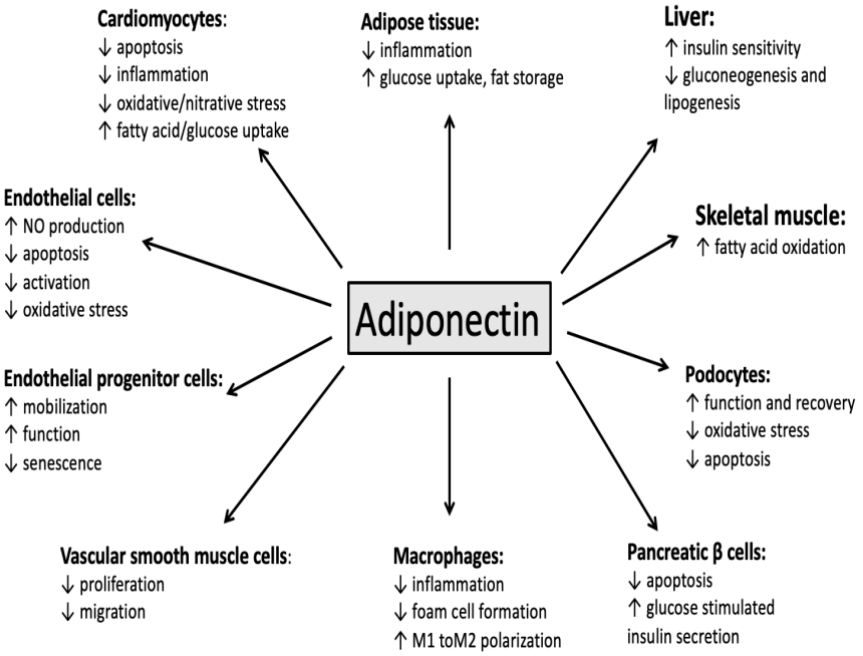

Figure 5. Summary of physiological and cellular changes in response to adiponectin

Four major biological functions of APN were identified by previous experimental and clinical studies as reviewed by Lau et al. They include metabolic regulatory function, vascular protective function, anti-inflammatory and cardioprotective/anti-ischemic function of APN (131).

2.2.3.1 Regulation of metabolism by adiponectin

Considering the role of APN in metabolism, it was shown that APN promotes glucose uptake and suppresses hepatic glucose production (138, 146). Moreover, by increasing the expression and activity of PPAR-α, APN stimulates fatty acid oxidation and energy expenditure (117). Through its local action in key metabolic tissues, APN promotes insulin sensitization and therefore improves whole-body energy homeostasis. The level of circulating APN was shown to be inversely correlated with insulin resistance in humans (86). Prospective and longitudinal studies have shown that lower APN levels are associated with a higher incidence of diabetes in healthy individuals, patients with impaired glucose tolerance, and community-based patient cohorts (147-150) and are a marker for the risk of the onset of diabetes (149). Furthermore, considering the relationship between dyslipidemia and APN,

research showed a positive correlation between circulating APN and HDL-cholesterol levels, as well as an inverse association between APN and low-density lipoproteins as well as triglyceride levels in humans with CAD, metabolic syndrome, end-stage renal disease (151-153).

2.2.3.2 Vasculo-protective mechanisms by adiponectin

Most of the vasculo-protective benefits of APN are carried out by increasing the nitric oxide (NO) production through the activation of endothelial NO synthase (eNOS) in an AMPK-dependent manner (154, 155). APN was shown to prevent endothelial cell apoptosis in a dose-dependent manner in human umbilical vein endothelial cells (155). APN-KO mice present with endothelial dysfunction, defined by increased superoxide production, reduced eNOS phosphorylation, and aberrant vasodilatory response to acetylcholine (156). Supplementation with exogenous APN restores endothelial function in APN-KO in terms of the previously mentioned parameters.

2.2.3.3 The role of adiponectin in the regulation of inflammation

A series of previous experimental studies demonstrated the potent anti-inflammatory activity of APN. APN supplementation reduced TNF-α mediated vascular cell adhesion molecule-1 and interleukin-8 expression in endothelial cells (157, 158). APN was reported to promote apoptotic body clearance by CRT/CD91 receptors, thus inhibiting inflammation (129). The adipokine suppressed TNF-α production in cardiac cells (8). In macrophages, APN attenuated TNF-α and IL-6 production through suppression of nuclear factor κB (NF-κB) activation and enhanced IL-10 expression (159, 160).

However, other studies have demonstrated the opposite phenotype of APN. The proinflammatory activity of APN, especially in terms of activating NF-κB, was reported in endothelial cells (161), fibroblasts (162), as well as hepatocytes (163). Oher controversies concerning the dual APN role in inflammation are covered in the section "The dual nature of adiponectin in inflammation control."

2.2.3.4 The role of adiponectin in the protection of heart tissue

In the models of cardiac dysfunction, overexpression of APN mainly preserved function, whereas its deficiency exacerbated damage due to cardiac stress. The ability of APN to protect cardiac function may be explained by modulating inflammation, apoptosis, oxidative/nitrative stress, fibrosis, and hypertrophy as well as regulating cardiac metabolism (164). Experimental

models and clinical studies show that APN acts directly on cardiomyocytes to protect the heart from ischemic injury, hypertrophy, and adverse remodeling (90).

The role of APN in ischemic injury. APN inhibited cardiomyocyte apoptosis and diminished infarct size in the murine model of I/R injury (8). Besides, APN inhibited inducible NO synthase (iNOS) and NADPH-oxidase protein expression, thus resulting in decreased oxidative/nitrative stress during the I/R injury. Immediately after ischemic injury, serum APN was demonstrated to localize to injured cardiac tissue in wild type mice as well as APN-KO mice following systemic APN infusion (165). It was also noted that a decrease in serum levels of HMW APN was observed in parallel with the cardiac localization of APN, suggesting that this isoform predominantly accumulates in damaged cardiac tissue. Four weeks after left anterior descending artery ligation, APN-KO mice demonstrated increased LV dilation and diminished contractile dysfunction accompanied by myocyte hypertrophy, increased apoptosis, interstitial fibrosis, and reduced capillary density at the border zone of infarct compared to wild type mice (166). Similar data was corroborated in a preclinical porcine model of I/R injury. Intracoronary administration of APN resulted in reduced myocardial infarct size and improvement of left ventricular function associated with diminished inflammation, apoptosis, and oxidative stress (167).

In patients with DCM without overt HF, APN downregulation was related to severe endothelial/microvascular dysfunction (164). However, in the meta-analysis performed by Sook Lee and colleagues, no protective effect of serum APN regarding CAD was observed in clinical studies. It was concluded that elevated APN levels are related to increased risk of recurrent CAD as well as increased all-cause mortality in patients with previous CAD (168). It is possible that the relationship between APN levels and increased risk of recurrent cardiovascular events has been biased by the counter-regulatory increase of APN in patients with advanced renal dysfunction or HF, as concluded by the authors.

Nevertheless, the underlying mechanisms of this association are not clear and even raise questions about the direct deleterious impact of APN affecting the results. The association between serum APN levels and the new-onset and recurrent cardiovascular events in non-CAD disease subjects was less evident than that with pre-existent CAD. Several genetic studies have reported that genetically higher APN appears to be unrelated to CAD and even questioned the causative role of APN in CAD (169).

The role of APN in protection against hypertrophy and cardiac remodeling. APN exerted antihypertrophic effects and protected against

cardiac remodeling in the models of reactive oxygen species induced oxidative stress, or transverse aortic constriction (TAC) induced pressure overload. APN treatment attenuated fibrosis and significantly reduced MMP-2 and MMP-9 matrix remodeling in APN-KO mice in angiotensin II-induced oxidative stress (13). APN-KO mice subjected to TAC had attenuated AMPK levels and increased hypertrophy, systolic dysfunction, and reduced myocardial capillary formation compared to their wild type counterparts (170-172). The resulting adverse ventricular remodeling and reduced cardiac angiogenesis promoted the transition from hypertrophy to HF in the absence of APN (170, 172). APN supplementation would rescue both APN-KO and wild-type mice in models of I/R and pressure overload. Lower levels of APN were shown to correlate with LV hypertrophy in clinical studies (19, 20).

The anti-inflammatory, antihypertrophic, and anti-fibrotic activities of APN are considered to be important in cardio-protection. Unexpectedly elevated circulating levels of this apparently salutary adipokine have been documented in patients with chronic HF (173-176), correlating with disease severity and mortality (173, 174). As reviewed by Woodward et al., it is plausible that decreased plasma APN levels in a healthy population portend the onset of atherosclerosis. However, this association becomes less evident at later stages of the atheromatous disease, while HF is clearly associated with increased APN levels (177). All in all, further studies are warranted to elucidate the role of APN in maintaining cardiometabolic health under pathologic conditions.

2.3 The relevance of adiponectin as a biomarker of chronic heart failure severity

APN is a multifaceted biomarker. Low APN concentrations are associated with metabolic syndrome (14), subclinical atherosclerosis (15), and cardiovascular disease (14) and are implicated in the development of obesity-linked illness. However, when it comes to HFrEF, the higher the values of APN, the worse the outcome. Increased APN levels correlate with poor all-cause and cardiovascular mortality (82, 178, 179). APN values fluctuate during the course of the disease and may be used in the monitoring of the pathological process.

A series of previous studies have examined the role of serum APN as a biomarker of disease severity in patients with chronic systolic HF (24, 28, 174, 176, 180). Circulating APN was shown to be elevated in patients with chronic HFrEF compared to healthy controls (181-183). Circulating APN levels were correlated to other well-known markers of HF severity, such as NYHA

functional class, serum NT-proBNP and BNP levels, LVEF, and hemodynamic parameters. An increase in serum APN with each subsequent NYHA class, especially NYHA class IV, was well demonstrated by previous studies, proving that APN levels increase with clinical worsening of chronic HF (174, 176, 178-181, 184).

Huang et al. demonstrated that circulating levels of APN were significantly higher in particular in patients with NI-DCM and advanced HF compared to controls and that worsening of scores in the NYHA classification system were accompanied by an increase in serum APN concentration (181). BNP, NT-proBNP - the mostly established biomarkers of HF correlated positively with serum APN in series of studies of patients with CHF: APN levels accompanied the increase in serum NT-proBNP or BNP (28, 82, 176, 179, 185, 186). The correlation of APN with echocardiographic parameters was not unambiguous. Some studies demonstrated an inverse correlation between serum APN and LVEF in patients with CHF (179, 180, 187). However, other studies, analyzing the relevance of APN as outcome predictor, found no such correlation between APN and LVEF (28, 174, 186). The association between serum APN and hemodynamic parameters was covered in the study by Tsutsamoto et al.: APN did not correlate with PCWP but was positively associated with PAP, RAP (176). Those hemodynamic parameters were no longer significant predictors of plasma adiponectin levels in the models of multivariable analysis in the latter study.

In conclusion, APN was correlated positively with NYHA functional class, serum levels of BNP, NT-proBNP, and inversely correlated to LVEF, indicating that an increase in APN levels parallels the HF severity.

2.4 Adiponectin as an outcome predictor in patients with chronic heart failure

Serum APN levels were also tested as a predictor of worse outcome in patients with chronic HF. Increasing APN concentration was shown to be an independent predictor for major adverse cardiac events (death, HT, and hospitalization with deteriorating HF) (180, 187) and all-cause mortality (24, 28, 176, 184, 188-190), independent of other outcome affecting covariates, in patients with chronic HF. Most of those studies evaluated the impact of binary APN levels on the outcome and used different cutoff values. The optimal threshold of the APN level was not defined (191) (Table 1).

Table 1. APN impact on outcome in patients with chronic heart failure. The cutoff values.

Study	No. of patients	Outcome	APN cutoff value (μg/ml)	Adjusted for	Duration of follow-up
Kistorp C. (2005)	195	Overall mortality	11.6	Age, systolic blood pressure, LVEF<25%, duration of CHF, and creatinine clearance, NT-pro-BNP	A median of 2.6 years
George J. (2006)	175	Overall mortality, hospitalizations or a combination of both	14	Age, NYHA class, sex, LVEF, diabetes, serum TNF- α, IL-6, IL-10, IL-8, IL-12 CD-40 ligand, IL-1b, CRP and NT-pro-BNP	2 years
Tsutamoto T.	449	Overall mortality	8.8	In patient group with BMI 21-25 kg/m2	>5 years
Van Berendoncks AM. (2011)	73	Overall mortality and hospitalization due to heart failure deterioration combined	15.2	LVEF < 25%, impaired renal function, male gender, age and NYHA classes III-IV, NT-pro-BNP, peak VO_2 , BMI, total and HDL cholesterol, triglycerides, beta-blockers	A median of 7 years
Szabo T. (2014)	111	Overall mortality	13.5	Age, pro-ANP rezistin and for BMI, total fat, peak-VO_2, pro-ANP or rezistin in bivariate models.	2 years
Yu HP. (2017)	124	Death, Tx, hospitalization due to worsening HF (MACE) after free months of follow-up with optimal anti-HF treatment	8.138	Chronic kidney disease, stage 3, LVEF at 3-month, Log NT-pro-BNP at 3-month, use of diuretics.	3 months

Abbreviations: LVEF: left ventricle ejection fraction, CHF: chronic heart failure, NT-pro-BNP: N-terminal proB-type natriuretic peptide, TNF-α: tumor necrosis factor α, IL-6: interleukin 6, IL-10: interleukin 10, IL-8: interleukin 8: interleukin 8, IL-12: interleukin 12, IL-1b: interleukin 1b, CRP: C reactive protein, VO_2: oxygen consumption, BMI: body mass index, HDL: high-density lipoprotein

A recent meta-analysis by Menzhagi et al. concluded that the positive association between APN and all-cause mortality was consistent across various clinical settings, including CVD, peripheral artery disease, ischemic stroke, end-stage renal disease as well as chronic HF, with the strongest association in patients with type 2 diabetes (192). Considering the association of APN with cardiovascular mortality, there was a tendency that studies with longer follow-up showed a weaker association. It was suggested that part of the deleterious effect of APN on cardiovascular mortality might be lost over the years.

It seems that the correlation of circulating APN with clinical severity of HF and poor outcome is characteristic for patients with HF and reduced EF. This fact was illustrated by the study of Faxen et al., as no associations between serum APN and NT-pro-BNP, and study outcomes, were found in patients with HF with preserved ejection fraction (HFpEF) (193).

2.4.1 The impact of temporal changes in adiponectin levels on chronic heart failure outcome

A few studies evaluated the temporal changes of APN in the follow-up or after a particular intervention and the impact of those changes on patient outcomes (196-199). Khan et al. showed that mechanical unloading of the heart via LVAD implantation resulted in decreased circulating APN levels as well as an increase in cardiac AdipoR expression in patients with advanced HF (194). It was demonstrated that circulating APN levels were elevated with concomitant downregulation of cardiac AdipoR1 and AdipoR2 before the correction of hemodynamics, suggesting that hyperadiponectinemia might be a result of APN resistance in tissues. Serum APN levels were shown to decrease after treatment of acute decompensation in patients with prior HF in the study by Matsumoto et al. The study included 95 patients with acutely decompensated HF. APN levels were measured at the beginning of the study and at the discharge. The patients were followed up for an average of 14 months. The decrease in APN levels at the hospital discharge (after the mean of 3 weeks) was related to lower risk of hospitalization due to HF worsening as well as cardiac death (195). The patients were divided into tertiles according to the difference in serum APN values at baseline and discharge. The biggest treatment-induced changes in log APN concentration were associated with higher event-free survival, but this was not the case for the differences in BNP levels.

Yu and colleagues demonstrated that increased APN levels at a three-month follow-up of patients receiving guideline-recommended anti-HF

treatment were associated with a significant increase in the poor outcome (death, HT, or hospitalization due to worsened HF) (196). This study revealed that temporal changes in APN levels carry additional prognostic information on top of baseline APN concentration, and evaluation of both APN and BNP is superior to the use of APN or BNP levels alone. Moreover, serial measurements of APN levels might even guide the effectiveness of HF treatment.

2.4.2 The association between serum levels of adiponectin and natriuretic peptides

As demonstrated by previous studies, circulating APN levels increase with worsening of HF and are related to the rising of serum BNP concentration. Accumulating evidence suggests that natriuretic peptides increase the levels of circulating APN either directly or indirectly. Catabolic predominance, accompanied by enhanced lipolysis, is typical for CHF, and increased lipolysis is also linked to bodyweight loss and cardiac cachexia (197). APN is known to be inversely associated with body mass (198). Natriuretic peptides are one of the critical mediators of lipolysis (199), suggesting that systemic APN levels may be passively coupled to the degree of triglyceride hydrolysis and free fatty acid release from adipose tissue. However, data, provided by Tsukamoto and colleagues, substantiate the idea that natriuretic peptides directly promote APN expression/release from the adipocytes (95). They have demonstrated an enhanced production of APN in cultured adipocytes from patients with chronic HF treating them with natriuretic peptides. Also, the infusion of atrial natriuretic peptide increased the levels of serum APN in patients with decompensated heart failure (200, 201). Besides, natriuretic peptides are established biomarkers of increased cardiovascular and all-cause mortality (202, 203). However, most of the studies analyzing APN association with increased all-cause mortality, adjusted for BNP levels in CHF, showed that APN remains a significant predictor, even though the association is attenuated (174, 176, 184, 204).

In conclusion, it seems that elevated APN levels are at least partly driven by high BNP concentrations in advanced HF (96). So the stage of the cardiovascular disease, BNP levels as well as background inflammation should be taken into account evaluating the prognostic relevance of circulating APN.

2.4.3 Renal failure, body mass index, and medications as confounders in predicting chronic heart failure outcome

Progression of CHF towards the end-stage disease is also associated with weight loss as a part of wasting process and renal failure, which may add up to increased levels of circulating APN as it was shown in patients with ischemic heart disease (24, 205, 206). However, although reduced kidney function is an important cause of premature death (207), epidemiological studies failed to demonstrate it as a significant confounder in association between APN levels and mortality as reviewed by Menzaghi et al. (85). The decrease in body weight could also result in elevation of circulating APN levels, which might compensate for a state of impaired metabolic efficacy (a shift in substrate preferences from free fatty acid to glucose) and malnutrition (208). An inverse association between APN and BMI has been reported in CHF patients (24, 174). Besides, obesity portends better outcome in patients with established CHF, since wasting is strongly associated with the increased risk of death in the final stages of the disease (209). Elevated APN levels are predictors of mortality in patients with CHF, as discussed earlier. It seems that high body mass, hence low APN, favors survival in end-stage HF. However, there is also evidence that APN levels are increased regardless of the presence of cachexia, as demonstrated by Tengiz et al. in a cohort of patients with a newly diagnosed systolic HF and no cachexia (187). Various medications are also related to changes in APN levels. Beta-blockers are known to decrease APN concentration (188, 210), while renin-angiotensin blocking agents behave on the opposite, increasing the amount of circulating APN (211, 212). Thiazolidinediones are shown to upregulate the expression of APN via PPAR-γ (213).

To conclude, elevated levels of circulating APN in chronic HFrEF indicate a worsening prognosis. However, it is questionable if the prognostic value of circulating APN remains after adjustment for other covariates such as exercise capacity, type of HF, and medication. The etiology of hyper-adiponectinemia in progressing HF lacks a clear explanation, particularly considering the positive functions assigned to APN. Does APN lose its cardio-protective and anti-inflammatory activity or is unable to overcome the detrimental environmental changes in CHF.

2.5 The role of cardiac and systemic inflammation in the pathogenesis of chronic heart failure

Inflammation is usually a natural response to injury and an essential mechanism for healing and tissue repair. The inflammatory response initially facilitates tissue reparation, but when it becomes persistent, it promotes cardiac remodeling and dysfunction (214). Persistent inflammation underlies the pathogenesis and progression of many common cardiovascular diseases, including myocardial infarction (MI), atherosclerosis, hypertrophy, myocarditis, dilated cardiomyopathy, and HF as reviewed by Jenke et al. (215). During HF, the immune system is chronically and measurably activated (216). Mounting evidence supports the role of inflammation in the pathogenesis of any type of HF (54, 217, 218).

The reciprocal relation between inflammation and chronic heart failure. Long-lasting low-grade inflammation is supposed to be involved in the etiology of many chronic human diseases, including CHF (219-221). Low-grade inflammation or para-inflammation is an intermediate condition between basal and inflammatory states (222). It is based on tissue-resident macrophages and is an attempt to restore normal homeostasis when tissues undergo stress. However, the chronic inflammatory state in HF does not seem to be caused by the classic triggers of inflammation: infection and injury but instead is more related to the malfunction of tissue (222).

Chronic inflammation and HF seem to be mutually related and even augment each other (4). HF induces sterile inflammation in the myocardium triggered by wall stress and signals released by stressed, malfunctioning, or dead cells secondary to HF (4). On the other hand, mounting evidence suggests that inflammation-triggered myofibroblasts can induce the inflammatory response by expressing chemokines, which increase myocardial immune cell infiltration, via inducing endothelial adhesion molecules, stimulating monocytes, etc. (223, 224). Therefore a vicious circle maintaining chronic inflammation in the heart might be induced by persistent homeostatic disturbances underlying HF (224).

The role of systemic inflammation in chronic heart failure. Persistent systemic inflammation, as reflected by elevation of circulating inflammatory mediators: TNF-α, ST2, IL-6, galectin-3, and pentraxin-3, is a typical finding in chronic HF (225). The levels of proinflammatory cytokines in chronic HF are significantly lower than those reported in autoimmune diseases or acute infections, supporting the idea that it is the low-grade inflammation that plays an important role in the pathogenesis and progression of the disease in patients

with CHF (218). Various studies demonstrate the detrimental effects of proinflammatory cytokines on the heart. TNF-α and IL-1ß are known to exert a direct negative inotropic effect in myocardium caused by changes in intracellular Ca2+ homeostasis in the adult cardiac myocyte (226-228). TNF-α and IL-1ß promote hypertrophy (229), and IL-6 increases cardiomyocyte stiffness by reducing the phosphorylation of titin (230). TNF-α also triggers cardiomyocyte apoptosis (231). Systemic inflammation seems to play a role in the development and progression of HF not only by promoting myocardial dysfunction but also inducing pathogenic sequelae in other organs and tissues, leading to cachexia, endothelial dysfunction, and anemia as reviewed by Yndestad et al. (232).

Besides, increased levels of serum TNF-α, IL-6 are related to the severity of HF in clinical studies (225). The correlation between elevated serum proinflammatory cytokines and adverse clinical outcomes is also typical (233-236). Cytokines and their receptors were shown to be independent predictors of mortality in patients with advanced HF (237).

Thus, inflammatory biomarkers are supposed to be involved in the pathogenesis of worsening HF.

The role of cardiac inflammation in the pathogenesis of DCM. In patients with DCM, cardiac inflammation is linked to LV dysfunction that is mediated by cytotoxic T-lymphocytes, natural killer cells, and macrophages, as well as inflammatory mediators released by infiltrating lymphocytes and macrophages in patients with DCM (238). Baseline myocardial inflammation appeared to be an independent predictor for an adverse outcome in patients with DCM in the study by Kinderman et al. (70). Elevated baseline APN levels were associated with higher concentrations of circulating proinflammatory cytokines as well as increased cardiac infiltration of CD3+ and CD45ro cells in patients with iDCM (239).

In conclusion, the peculiar characterization of inflammation and the systemic and cardiac immune profile is meaningful for a better understanding of the process and search for new therapeutic targets.

2.6 The dual nature of adiponectin in inflammation control

The role of serum APN in inflammation is unambiguous. Although circulating APN seems to be granted with potent anti-inflammatory activity, there is also substantial data challenging the perspective of this adipokine as an exclusively anti-inflammatory one. The controversies underlying this dual nature of APN will be illustrated herein after considering the role of circulating APN in

inflammation at the cellular level, in animal models of heart disease, and finally in humans.

Inflammation regulation at the cellular level. At least several studies have demonstrated the anti-inflammatory role of APN in vitro. APN was associated with decreased proinflammatory cytokine synthesis in endothelial cells and cultured cardiac myocytes (8, 157). It inhibited TNF-α induced adhesion molecule expression and monocyte adhesion to endothelial cells in mouse aorta and human endothelial cell cultures (158, 240). APN diminished the TNF- α or lipopolysaccharide-induced activation of the prototypic inflammatory transcription factor-NF-κB in various cell types, including endothelial cells, macrophages, adipocytes (157, 158, 239, 241). This adipokine has been shown to modulate the inflammatory functions of macrophages via decreased phagocytic activity (242) and diminished proinflammatory cytokine synthesis (159, 242). Besides, APN enhanced the anti-inflammatory IL-10 production in human leukocytes and monocyte-derived macrophages (160, 243). Macrophages from APN-KO mice were shown to produce more TNF-α, MCP-1, and IL-6 demonstrating and activated phenotype, which can be reversed by delivering of recombinant APN (244). APN modulated the polarization of macrophages towards an alternatively-activated M2 (anti-inflammatory) phenotype (244). Besides, APN activates cellular ceramidase and, in that way, decreases the levels of proinflammatory ceramides (142).

At the same time, other studies have demonstrated the proinflammatory nature of APN. Globular APN was shown to induce the expression of proinflammatory cytokine and adhesion molecule genes in endothelial cells (161). A series of studies have shown the NF-κB activating effect of APN under basal (unstimulated) conditions demonstrating the controversial behavior of APN regarding inflammation (162, 245, 246). It is plausible that APN-induced suppression of NF-kB, activated by inflammatory stimuli, as well as APN-induced mild activation of NF-kB under a basal state coordinately maintain in vivo homeostasis.

Inflammation regulation in animal models. Studies investigating the role of APN in animal models of heart diseases were mostly focused on anti-inflammatory activity. Elevated TNF-alpha expression in cardiomyocytes was seen in APN-KO mice compared to wild-type mice in the model of ischemia-reperfusion injury, which was reduced by administration of APN (8).

APN seems to suppress Toll-like receptor (TLRs)-dependent innate immune response, modulate the polarization of macrophages towards the anti-inflammatory phenotype and reduce the number and activation of NK as

demonstrated in the murine model of coxsackievirus B3 myocarditis (247, 248). APN-KO mice demonstrated increased cardiac expression of TLR co-receptor and enhancer CD14+ as well as TNF-α, higher myocardial infiltration of proinflammatory M1 macrophages, and NK cells. Diminished expression of cardiac TLR 4 and its downstream targets (TNF-α, IL-6, Intercellular adhesion molecule 1 (ICAM-1)), as well as reduced myocardial immune infiltration, was documented in murine experimental myocarditis as a result of APN overexpression (215). Besides, TLR4 ligation prevented tissue injury by reducing the activation of immune cells and their interaction with cardiac myocytes.

The majority of the studies concerning the role of inflammation in HF have focused mainly on the outcomes in the acute setting of ischemic or non-ischemic tissue injury (8, 215, 248, 249), and only relatively few studies addressed the role of inflammation once HF is established (250, 251). There is a lack of mechanistic animal studies addressing the role of inflammation after chronic HF sets up (218).

Inflammation regulation in humans. The role of APN in inflammation in humans is also complicated. Systemic low-grade inflammation suppresses APN levels and might be a potential link between systemic inflammation and the early stages of CVD development (96). Inducible low-grade inflammation in healthy individuals caused by *S. typhi* vaccine, decreased circulating APN levels within 24 hours compared with placebo (252). Hypoadiponectinemia was related to a more signifant risk risk of acute MI in men without prior cardiovascular disease (253). Data from previous studies indicate that inflammation suppresses the production of APN in adipocytes and might lead to hypoadiponectinemia in metabolic diseases (7, 254, 255). Proinflammatory cytokines downregulate APN gene expression in cultured adipocytes (92, 93). So if inflammation is an essential negative regulator of APN synthesis, it would be reasonable to expect low APN levels in diseases accompanied by elevated inflammation such as HF (25). However, a positive association between inflammation and APN is usually reported in inflammatory/immune pathologies, as reviewed by Fantuzzi et al. (25). Immune-mediated diseases, including chronic HF, are associated with higher circulating APN, which are also related to poor outcomes (82, 178, 179, 256). Provided inflammation leads to diminished adipocyte-derived APN, which means that either other mechanisms must overcome the suppressive effect of inflammation on APN production or modulate the association in chronic HF (25).

It seems that after the development of advanced cardiovascular disease, the APN levels are no longer negatively controlled by low-grade inflammation, and it might lead to elevated circulating levels of APN (4). Instead, the

elevation of BNP levels might activate the release of APN from adipose tissue in advanced CVD and HF (96). Circulating APN levels might be regulated by the interplay between proinflammatory cytokines.

2.7 T-cadherin as a critical adiponectin receptor in cardio-protection

2.7.1 Signal transduction through T-cadherin

T-cad is one of APN receptors that is highly expressed in the human heart (30). T-cad is a glycosylphosphatidylinositol (GPI) anchored protein, lacking transmembrane and cytosolic domain (257). GPI anchor attaches T-cad to plasma membrane cholesterol and sphingolipid-rich microdomains known as lipid rafts, yet making the direct interactions with intracellular signaling adaptors impossible (32, 258). Therefore, it is not completely clear how T-cad initiates intracellular signaling. It is plausible that T-cad associates with transmembrane proteins such as integrins, which in turn join intracellular adaptor proteins activating APN signaling targets (259). The presence of a lipid GPI anchor suggests that T-cad may also employ some of the signaling mechanisms that depend on its localization within lipid rafts, which act as signal transduction platforms (260).

It was demonstrated that APN acting via T-cad enhances exosome biogenesis and secretion, leading to diminished levels of cellular ceramides (142, 145).

APN circulates at very high levels and has a complex structure, so a classical, high-affinity ligand/receptor interaction is barely possible because the receptor would be permanently saturated at physiological levels of this adipokine (33). Therefore, T-cad might be a low-affinity APN receptor or possibly function as a co-receptor acting in concert with AdipoR1 and AdipoR2 (33). In some cell types, a partially processed 130 kDa protein precursor is expressed on the cell surface along with 100-kDa T-cad, known as mature T-cad (125). The 130-kDa pro-domain-bearing T-cad is preferentially localized on the cell surface and bounds more APN than its 100-kDa form (261, 262).

2.7.2 The role of T-cadherin in the association of adiponectin with the tissues

Denzel and colleagues have demonstrated that T-cad is critically required for the association of APN with the myocardium in mice (32). An extensive colocalization of T-cad with APN on the sarcolemma of murine cardiomyocytes was shown by Denzel et al. (32). Fujishima and colleagues demonstrated that APN accumulates in healthy vasculature as well as in endothelial and smooth muscle cells of atherosclerotic plaques via T-cad in cellular and mice models (127). The finding that APN accumulates in tissues, such as the heart, muscle, and vascular endothelium, through binding with T-cad, was demonstrated in a series of murine studies and cell cultures (33, 128, 263). In T-cad-KO mice, APN failed to associate with cardiac and vascular tissue, and its levels dramatically increased in the circulation. Considering the human myocardium in DCM, Skurk and colleagues showed that the overall distribution pattern of AdipoR1 and AdipoR2 differed significantly from that of its ligand APN in cardiomyocytes, suggesting that an additional APN ligand exists (264).

In conclusion, it seems that T-cad is essential for APN binding in cardiovascular tissues.

2.7.3 The role of T-cadherin in mediating the cardio-protective effect of adiponectin

Denzel et al. demonstrated that TAC-induced pressure overload results in markedly exaggerated concentric hypertrophy in T-cad-KO mice, which was comparable to APN-KO hearts seven days after surgery. After extended exposure to pressure overload (4 weeks of TAC), myocardial hypertrophy was more expressed in APN-KO and T-cad-KO mice compared to their wild type counterparts. In the model of I/R injury significantly increased necrotic area in both T-cad-KO and APN- KO mice as well as in the double KO mutants was shown by Denzel et al. (32) In conclusion, murine models of pressure overload and ischemia-reperfusion injury revealed that T-cad-KO mice phenocopy the APN-KO mice. Moreover, T-cad ablation leads to diminished AMPK activation, despite the remaining expression of AdipoR1 and AdipoR2, suggesting that cardiovascular tissues become unresponsive to APN (265). Expression of T-cad was also critical for revascularization actions of APN in the murine model of chronic hind limb ischemia, which is an equivalent to peripheral artery disease. Mice deficient in both APN and T-cad failed to improve blood flow recovery after APN supplementation (33). APN–T-cad association played a protective role against the neointimal proliferation and atherosclerotic plaque formation in the mouse model of atherosclerosis (127). In conclusion, mice deficient for T-cad lacked tissue-associated APN,

accumulated APN in the circulation, and mimicked the APN-KO cardiovascular phenotype. Thus evidence from the above-mentioned animal and cell culture models suggest that APN binding to T-cad is critical for APN-mediated cardiovascular protection.

Provided T-cad is essential for tissue sequestration of circulating APN to the tissue, changes in T-cad expression might lead to APN resistance, diminished APN biological activity, and hyperadiponectinemia in CHF. There is a need to evaluate the changes in tissue T-cad expression in CHF. Future studies on T-cad would clarify the role of APN in HF and may guide the design of new anti-HF drugs.

To summarize the data from the literature review, several points that still lack explanation can be highlighted. The underlying pathophysiologic mechanisms of elevated serum APN levels in chronic HFrEF remain unclear. Several hypotheses explaining the elevation in serum APN concentration in advanced HF have been defined. Natriuretic peptides have been shown to be important drivers of APN concentration in previous studies. Furthermore, serum APN levels might be raised because of the hyper-catabolic state that marks the advanced HF. Finally, a possible APN resistance in tissues of patients with CHF has been demonstrated in skeletal muscles of patients with CHF as well as in the heart in the study by Khan et al. The reduced responsiveness of downstream signaling molecules to APN in the heart and vessels, would be yet another mechanism explaining the increase in APN levels in CHF.

The data concerning the role of serum APN in inflammation are also conflicting. Finally, although APN seems to have an additive role in predicting the course of CHF, it has not been recognized as a biomarker in HFrEF. Besides, there are no clinical data about the long-term prognostic role of baseline APN in patients with NI-DCM and CHF. There are no data concerning the levels of cardiac T-cad in human myocardium or the changes in T-cad concentration depending on the disease severity and its impact on long-term prognosis in patients with NI-DCM and CHF.

In the present study, we employed a prospectively enrolled, well-characterized, histology, and virology enriched NI-DCM cohort with long follow-up, which gives an advantage evaluating of the possible role of APN and T-cad as biomarkers.

3. MATERIALS AND METHODS

3.1 Definitions of dilated cardiomyopathy and heart failure used in the investigation

Advanced HF. As defined by the updated Heart Failure association of ECS criteria (266):

1. Severe and persistent symptoms of HF (NYHA class III or IV),
2. Severe cardiac dysfunction defined by a reduced LVEF ≤30%, isolated RV failure (e.g., arrhythmogenic right ventricular cardiomyopathy) or non-operable severe valve abnormalities or congenital abnormalities or persistently high (or increasing) BNP or NT-proBNP values and data of severe diastolic dysfunction or LV structural abnormalities according to the ESC definition of HFpEF and HFmrEF,
3. Episodes of pulmonary or systemic congestion requiring high-dose intravenous diuretics (or diuretic combinations) or episodes of low output requiring inotropes or vasoactive drugs or malignant arrhythmias causing >1 unplanned visit or hospitalization in the last 12 months,
4. Severe impairment of exercise capacity with an inability to exercise or low distance walked in 6 minutes (<300 m) or peak VO_2 (<12–14 mL/kg/min), estimated to be of cardiac origin.

NI-DCM: a cardiac condition characterized by dilatation and systolic dysfunction of the left ventricle in the absence of CAD, hypertension, valvular heart disease, and congenital heart disease sufficient to cause the observed myocardial abnormality (35, 45)

Myocarditis: Myocardial inflammation defined as immunohistochemical detection of significant focal or diffuse cellular infiltration in the endomyocardial biopsy (≥14 leucocytes/mm2, including up to 4 monocytes/mm2 with the presence of CD3 positive T-lymphocytes ≥7 cells/mm2) (47).

Acute myocarditis: a cardiac condition with symptoms of heart failure developing over three months or less (267), confirmed by myocardial inflammation in EMB.

iDCM: DCM (the presence of hemodynamic dysfunction, including LV dilation (LVEDD >55mm) and reduced LVEF (<50 %)) accompanying immunohistochemically-proved myocarditis (myocardial inflammation, confirmed by EMB, and fulfilling the ESC Working Group on Myocardial and Pericardial Diseases criteria (47)).

3.2 Patient selection and follow-up

The investigation was a prospective cohort study. All subsequent patients admitted to tertiary care Vilnius University Hospital Santaros Klinikos from 2010-2013 with a suspected NI-DCM were enrolled. The inclusion criteria were HF with unknown cause of LV dilation, evidenced by echocardiography and LV systolic dysfunction (LVEF <45%). The quantification of LV size was performed based on the reference ranges provided by the ESC (268). These were patients with indications for EMB. The patients were excluded in case of significant coronary artery disease (>50% stenosis on coronary angiography) or MI in the history and the presence of acute myocarditis or other heart diseases including primary valvular heart disease, toxic DCM, arterial hypertension as well as diabetes, advanced chronic kidney disease and abuse of alcohol or illicit drugs.

All patients underwent a careful history and physical examination. Routine laboratory studies, including common blood count, serum creatinine, glucose, high sensitivity CRP, BNP, and cardiac troponin T (hsTnT) were performed at baseline. Utilizing ELISA baseline levels of APN, tumor necrosis α (TNF-α), interleukin 6 (IL-6) were evaluated. Echocardiography was carried out upon admission for the evaluation of conventional echocardiographic parameters. NYHA class was assessed before echocardiographic investigation. Mandatory investigations included: coronary angiography to exclude significant coronary disease, right heart catheterization for hemodynamic evaluation, and EMB for evaluation of inflammatory infiltrates. If required, cardiac resynchronization therapy, radiofrequency ablation, or implantable cardioverter-defibrillator were planned after coronary angiography and right heart catheterization.

All in all, 59 patients were enrolled. Two of them were excluded from the subsequent analysis after coronary angiography because of CAD. The rest 57 patients had a long duration of HF symptoms before enrolment, significantly impaired systolic function (LVEF < 45%), and LV dilation (mean LV end-diastolic diameter 6.8±0.8 cm), consistent with chronic HFrEF due to NI-DCM. The median duration of observed symptoms before inclusion in the study was 12 months.

Patients were treated according to the HF guidelines of the European Society of Cardiology (36) receiving ACE inhibitors or angiotensin receptors blockers, β-blockers, mineralocorticoid receptors blockers, digoxin (in case of atrial fibrillation), diuretics, anticoagulant (in the case of atrial fibrillation) and antiarrhythmic (class III: amiodarone). Thiazolidinediones were not administered to any patients at the time of blood sample collection.

Patients were followed up for five years after inclusion in the study. The date of the EMB was considered to be the first day of the follow-up. The study outcome was composite involving three endpoints: death from cardiovascular causes, LVAD implantation, or HT. In the case of two or three endpoints in one patient, the time of the outcome was the time of the first event. Follow-up data were revised once a year (the exact date of the event was included in statistical analysis). The data concerning endpoint occurrence were retrieved from clinical records and the national death registry. Two patients were lost to follow-up after having participated in the study for three years.

In 2019 a sub-investigation was performed. 29 EMB biopsies that were gathered during the first part of the investigation and stored were subjected to the analysis of myocardial T-cad levels. All available EMB samples were involved. Myocardial T-cad concentration was evaluated by ELISA. Those 29 patients comprised the cohort of patients for the subsequent pilot investigation.

For comparative purposes, patients were subdivided into groups based on their outcomes: those who have reached the follow-up endpoint versus those who were event free. To compare cardiac T-cad expression levels in patients with different HF severity, patients were divided into two groups based on their mean PCWP value. Mean PCWP, a surrogate marker of LV end-diastolic pressure, was treated as a biomarker of HF severity for patients with chronic NI-DCM. The most appropriate mean PCWP cutoff point was found building a recursive partitioning survival tree using a party package in R (269). Survival tree analysis showed that patients with mean PCWP >19 mmHg had a significantly worse composite outcome than those with mean PCWP ≤19 mmHg ($p=0.003$). This cutoff value was used for further analysis. The impact of APN on long-term prognosis was also tested in the patients based on their myocardial inflammation: iDCM vs. non-inflammatory DCM.

3.3 Biochemical assays of adiponectin and other serological markers

2 blood samples data were obtained on the same day as cardiac catheterization and EMB.

Collected blood specimens for serum were placed in vacutainer tubes without anticoagulants and kept at room temperature for 30–45 min allowing to clot. Blood specimens for plasma were placed into EDTA-treated vacutainer tubes. Samples were centrifuged for 15 min at the manufacturer's recommended speed (1,000 - 2,000 RCF). The upper layer was carefully aspired, checked for turbidity, aliquoted into cryovials, labeled and stored at

−80°C. Before measurement, all serum samples were thawed on ice, centrifuged at 12,000 g for 5 min, and, if necessary, appropriately diluted.

The total APN was measured by human adiponectin ELISA assay according to manufacturers' recommendations (Millipore, USA). Absorbance was measured at 450 nm with a spectrophotometer (Spectramax®i3, Molecular Devises). The final concentration of the total APN was calculated from the calibration curve and expressed as μg/ml. The expected average values in healthy individuals, as provided by the manufacturer, were 10.2 μg/ml for women and 6.8 μg/ml for men.

The pro-inflammatory serum cytokines TNF-α and IL-6 were measured by solid-phase, chemiluminescent immunometric assays using IMMULITE/Immulite 1000 systems (Immulite, Siemens) according to manufactures instructions: TNF-α (Catalog No: LKNFZ (50 tests), LKNF1 (100 tests); IL-6 (Catalog No: LK6PZ (50 tests), LK6P1 (100 tests).
The level of high-sensitivity troponin T (hsTnT) was evaluated using an Elecsys 2010 analyzer (Roche Diagnostics, Indianapolis, Indiana) and expressed as pg/ml.

Brain natriuretic peptide (BNP) in plasma was measured with a two-step immunoassay using CMIA technology and protocols referred to as Chemiflex. Sample and anti-BNP coated paramagnetic particles were combined. After incubation, samples were washed and combined with secondary antigen and acridinium-labeled conjugate. Samples were incubated, washed again, and the chemoluminescence initiating mixture was added. The resulting chemiluminescent reaction was measured as relative light units by a chemo-luminometer.

3.4 Echocardiographic evaluation

Trans-thoracic echocardiography was accomplished upon admission by an investigator blinded for the study objectives. GE Vivid 7 and 9 ultrasound systems were used. The regular LV apical (apical 4, apical 2, and apical 3) views and parasternal short-axis views at the mid-papillary level were acquired at 70–90 frames/s. Routine echocardiographic parameters: LV EF, LV EDD, left ventricular end-systolic dimension (LV ESD) (268), velocities of E and A waves (E and A) and their ratio (E/A), and E deceleration time (DcT) were measured. All obtained images were stored digitally for offline analysis. 2D speckle tracking was performed for quantification of myocardial deformation parameters employing Echo-Pac PCBT08 (GE Healthcare) software. Following manual selection, speckles were assumed automatically and then confirmed by the investigator. The global strain was expressed as the

mean value of all valid segments. The intra-observer and inter-observer variability of global strain measurement was 1.1+0.9% and of 1.3+1.2%, respectively.

3.5 Cardiac catheterization and endomyocardial biopsies

Written informed consent for cardiac catheterization, including EMB and coronary angiography, was signed by all patients enrolled in the study. Coronary angiography was done for all participants to exclude significant coronary artery disease (stenosis >50%). Each patient underwent right heart catheterization in order to evaluate hemodynamic impairment and the level of pulmonary hypertension (PH). Intracardiac hemodynamic parameters: mean pulmonary artery pressure (PAP), right atrial pressure (RAP), PCWP, pulmonary vascular resistance, and cardiac output were assessed.

EMB's were collected using a flexible bioptome (West-med) via the right femoral vein. Biopsies were drawn from the inter-ventricular septum on the right ventricular side. At least 3 EMBs were sent for conventional histologic and immunohistochemical evaluation, and 2 EMBs were stored as retained bio-samples. They were promptly stored at - 80 C.

EMB samples directed to histological analysis were fixed in 10% buffered formalin and afterward paraffin-embedded. 3 μm-thick sections were produced for further analysis and stained with Hematoxylin and Eosin (H&E) according to the standard protocol for the routine histological evaluation. Endocardium (thickness, subendocardial fat, fibrosis and inflammation); myocardium (muscle fiber number, size and damage); interstitium (fibrosis, fat, edema, and inflammation) and intramural vessels (size, signs of inflammation, damage and luminal stenosis) were evaluated by an experienced pathologist.

For immuno-histological assessment of EMBs autoantibodies (Santa Cruz Biotechnology, Inc.) against CD3 (T lymphocytes; DAKO A0452 Rabbit 1, Hamburg, Germany), CD45Ro (active-memory T lymphocytes; DAKO Hamburg), CD68 (macrophages; DAKO M0876 Mouse 1, Hamburg), CD4 (T helper lymphocytes; DAKO Hamburg, Germany); CD54 (Intercellular adhesion molecule 1; NovocastraTM Lyophilized Mouse Monoclonal Antibody CD54 Clone 23G12) and HLA-DR (MHC class II cell surface receptor; DAKO Hamburg, Germany) were used. The positively stained cells in the EMB sample were scored by an experienced pathologist and expressed as a count of positive cells/mm^2.

Myocardial inflammation was confirmed if immunohistochemical staining revealed significant diffuse or focal inflammatory cellular infiltrate as outlined

by European Society of Cardiology Working Group on Myocardial and Pericardial Diseases an expert consensus group (≥14 leucocytes/mm2 including up to 4 monocytes/mm2 with the presence of CD 3 positive T-lymphocytes ≥7 cells/mm2) (47).

3.6 Estimation of T-cadherin in endomyocardial biopsies

Stored EMB's were kept at −80°C. Before the assay, tissue samples were weighed and finely minced thrice for 10 min on ice in appropriate proportions of cold PBS (according to ELISA kits manufacturer's recommendations) using FB15061 (Fisher brand) sonicator. They were centrifuged at 12,000 g for 15 min. The supernatant was discarded and utilized for the subsequent analysis. The amount of total protein in biopsy samples was estimated by the modified Lowry Protein Assay kit (Thermo Scientific Inc., USA). Human T-cad concentration in EMB was estimated by using commercially available ELISA assay kits (Abbexa, UK, Cat. No.: abx051689). Absorbance was measured at 450 nm with a spectrophotometer (Spectramax®i3, Molecular Devises). Total protein concentration was expressed as μg/ml. The final concentration of T-cad was expressed as ng/mg of protein. All procedures were performed according to the provided manufacturer's manuals.

3.7 Statistical analysis

Statistical analysis was carried out using the SPSS package (version 23.0 for Windows; IBM.SPSS statistics) and R studio package (version 1.1.463; 2018 R Studio, Inc.) at not higher than 5% significance level.

During the follow-up, two patients out of 57 were lost: they were right-censored in subsequent survival analysis. One patient with a T-cad value of 161.54 ng/mg was excluded from further analysis as an absolute outlier (z-score 3.45).

Shapiro-Wilk test was used for testing the normality of the data distribution. In the case of a normal distribution, continuous variables were expressed as means ± standard deviation. If variables did not follow a normal distribution, they were expressed as medians and interquartile ranges. Differences between continuous variables in the subgroups of patients stratified by the patient outcome, the severity of HF, mean T-cad were tested with the non-parametric Wilcoxon rank-sum test. Chi-squared test and Fisher exact test (in case of expected values less than 5 in the subgroups) were used for testing the differences between categorical variables. The association between continuous variables was tested, estimating the Spearman correlation

coefficient. Multivariate regression analysis was performed in order to test which variables influence the levels of circulating APN most of all. The best-fitting model was chosen.

Survival tree analysis was performed using the recursive partitioning method for building the decision tree. The root node of the survival tree included 57 in the case of serum APN and 28 patients considering the impact of T-cad on the outcome of patients. The log-rank test was used for node-splitting. The recursive procedure produces offspring nodes until no further statistically significant split is obtained. The decision tree received was depicted with each terminal node representing a group of patients with different survival outcomes.

Kaplan-Meier analysis was used to compare the cumulative survival rates between the subgroups of NI-DCM patients. Differences between survival times were tested using a log-rank analysis. Univariate Cox regression analysis was used to assess the impact of APN or T-cad level on the patient outcome. Multivariate Cox proportional hazards analysis was used to assess the significance of lnAPN as a predictor of the composite endpoint. The best-fitting model, decreasing the Akaike information criterion (AIC), was chosen. As several variables did not fulfill the proportional hazard assumption, the Cox model was extended to contain time-dependent variables. The stepwise selection procedure was used. The model was adjusted for serum ln BNP, ln IL-6 levels, and cardiac CD3+ count.

Factor analysis was used in order to find a set of variables which could be a reflection of some more global parameter and be a good combination for the multi-marker approach in predicting patient outcome. Factor analysis was performed using principal component analysis. The number of factors retained was determined using the Guttman-Kaiser criterion, which keeps only eigenvalues greater than 1, data from scree plot analysis, and the possibility to explain the factor by medical aspect. The variance maximizing rotation (varimax) was performed in order to make the interpretation of the results easier. Factor scores were evaluated using the weighted least squares method.

3.8 Ethical approval

The study was approved by the local Lithuanian Bioethics Committee (license No. 158200-09-382-l03; No. 158200- 382-PP1-23, No 158200-17-891-413). Informed consent approved by the Lithuanian Bioethics Committee was signed by all study patients. Our study was conducted in accordance with the Declaration of Helsinki.

4. RESULTS

4.1 Baseline patient characteristics

Patient baseline characteristics are depicted in Table 2. The average age of the patients was 47±11 years. Men comprised more than two-thirds of the cohort. 90% of patients were NYHA class III and IV. BNP values were elevated; the mean value was 727.7 (88.5-2074.6). The average LVEDD was 6.8 ± 0.9 cm, average LVEF - 26.08 ± 9.5 %, and average pulmonary artery wedge pressure - 21.8 ± 8.9 mm Hg. The median duration of HF symptoms before inclusion in the study was 12 months: all of the above consistent with chronic HFrEF due to NI-DCM (Table 2).

Table 2. Baseline characteristics and treatment of patients

Variable	**Value**	**Number of patients**
Age (years)	47±11	57
Male gender, n (%)	45 (79%)	57
BMI (kg/m2)	26.84 (23.05-31.66)	57
GFR (ml/min)	108.6±38.6	54
Duration of symptoms before enrolment (months)	12 (4.5-60)	57
NYHA class n, (%)		55
II	6 (11)	
III	38 (69)	
IV	11 (20)	
iDCM n, (%)	30 (55)	55
Advanced HF n, (%)	19 (33.3%)	57
Serum biomarkers of HF		
APN (μg/ml)	14.2 (7.9-29)	55
BNP (pg/ml)	727.7 (88.5-2074.6)	56
Serum biomarkers of inflammation		
Il-6 (pg/ml)	2.4 (2.0-6.9)	55
TNF α (pg/ml)	8.6 (6.6-8.6)	55
CRP (μg/ml)	4.6 (1.4-16.4)	52
Biomarkers of myocardial necrosis		
hsTnT (pg/ml)	29.9 (18.4-50.8)	55
Echocardiographic parameters		
LV EF (%)	26.08±9.5	57
LV EDD (cm)	6.8±0.8	57
Global longitudinal strain	-8.07±3.5	41
Hemodynamic parameters		
Mean RAP (mmHg)	11 (6.25-13.75)	51
Mean PAP (mmHg)	29 (21-40)	53
Mean PCWP (mmHg)	21.8±8.9	54
CO (l/min)	4.00±1.88	51
CI (l/min/m2)	2.16±1.14	51
Markers of immune infiltration in the myocardium (cells/mm2)		
CD3+	10 (7-16)	55
CD4+	4 (2-6)	55

CD45ro+	7 (5-10)	55
CD68+	4 (3-5)	55
CD54+	0 (0-1)	55
HLA-DR+	5 (4-6)	55
Cardiac adiponectin receptors (ng/mg)		
T-cadherin	41.16 (21.37-55.33)	29
Medications used		
ACEI/ARB n, (%)	36 (63)	57
Beta-blockers n, (%)	54 (95)	57
Diuretics n, (%)	55(96)	57
Mineralocorticoid receptor blockers n, (%)	51(90)	57

Data presented as mean±SD, median (interquartile range)* or n (%). Abbreviations: BMI: body mass index, GFR: glomerular filtration rate, APN: adiponectin, iDCM: inflammatory dilated cardiomyopathy, BNP: Brain natriuretic peptide, Il-6: interleukin 6, TNF-α: Tumor necrosis factor α, CRP: C reactive protein, hsTnT: high sensitivity troponin T, LVEF: left ventricular ejection fraction, LVEDD: left ventricle end-diastolic diameter, RAP: right atrial pressure, PAP: pulmonary artery pressure, PCWP: pulmonary capillary wedge pressure, CO: cardiac output, CI: cardiac index, CD3+: T cell receptor, CD4+: T helper cell receptor, CD45ro+: memory T cell receptor, CD68+: monocyte/macrophage receptor, CD54+: Intercellular Adhesion Molecule 1, HLA-DR+: Human Leucocyte Antigen isotype DR, ACEI: angiotensin-converting enzyme inhibitors, ARB: angiotensin receptor blockers.

4.2 The association of serum adiponectin levels with biomarkers indicating heart failure severity

One of the hypotheses that have been tested in the present study was that higher circulating APN levels are associated with the worse outcome in patients with NI-DCM. The assumption that higher serum APN levels correlate with other well-established biomarkers of HF, indicating a more advanced disease was tested, and the results are provided in Table 3.

Table 3. Spearman correlation between serum APN and biomarkers of HF severity

Variable	**rho**	**p value**	**No of cases**
Serum biomarkers			
BNP (pg/ml)	0.651	**<0.001**	54
Echocardiographic parameters			
LVEF (%)	-0,206	0,139	53
GLS	0.472	**0.002**	40
Hemodynamic parameters (mmHg)			
Mean RAP	0.31	**0.03**	49
Mean PAP	0.434	**0.001**	51
Mean PCWP	0.388	**0.005**	51
CI (l/min/m2)	-0.336	**0.018**	49

Statistically significant values marked in bold. Abbreviations: BNP: B-type natriuretic protein, LVEF: left ventricular ejection fraction, GLS: global

longitudinal strain, RAP: right atrial pressure, PAP: pulmonary artery pressure, PCWP: pulmonary capillary wedge pressure, CI: cardiac index.

There was a strong direct correlation between serum APN and the serum "gold standard" biomarker of HF - BNP (rho=0.651, p≤0.001) (Figure 6).

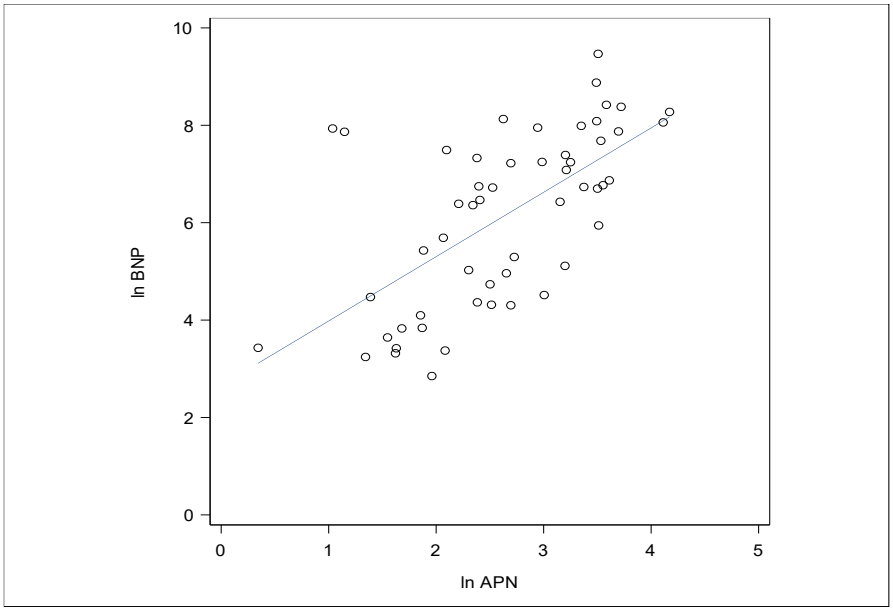

Figure 6. Correlation between serum log-transformed APN and BNP values

APN was also significantly correlated with mean PCWP and other hemodynamic parameters indicating HF severity and pulmonary hypertension. The more impaired the function of myocardium, the higher the concentration of serum APN (Table 3).

There was no significant correlation with LVEF. However, a significant association between serum APN and another echocardiographic parameter global longitudinal strain was seen (rho=0.472, p=0.002). Worsening of the global myocardial deformation seems to be accompanied by elevated serum levels of APN (Figure 7).

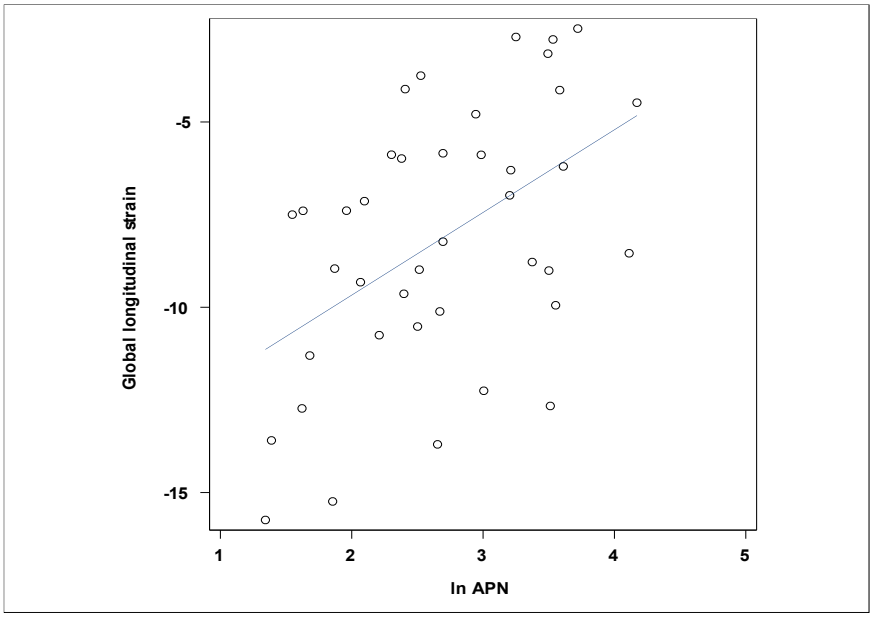

Figure 7. Spearman correlation between serum log-transformed APN and global longitudinal strain

An increase in APN levels in each subsequent NYHA functional class, especially NYHA class IV, was demonstrated. Circulating APN levels were higher in more advanced HF, as indicated by higher NYHA functional class (Table 4).

Table 4. Serum APN levels based on NYHA class

	Serum APN level (μg/ml)			
NYHA class	Mean	SD	Median (interquartile range)	No of patients
II	11.125	7.716	9.43 (5.65-13.47)	6
III	16.486	11.692	12.35 (7.3-24.75)	38
IV	31.413	18.447	32.00 (16.9-33.86)	11

As serum APN levels were not normally distributed, the concentration of APN was log-transformed (ln APN), and ANOVA analysis run. A significant difference between serum ln APN in NYHA functional classes II, III, and IV was seen (p=0.0114). Pairwise comparisons showed that APN concentration was significantly higher in NYHA class IV compared to NYHA class III (p=0.0186) and NYHA class IV compared to NYHA class II, p=0.0114. No difference in APN levels was demonstrated for NYHA class III vs. NYHA class II (p=0.650) (Figure 8).

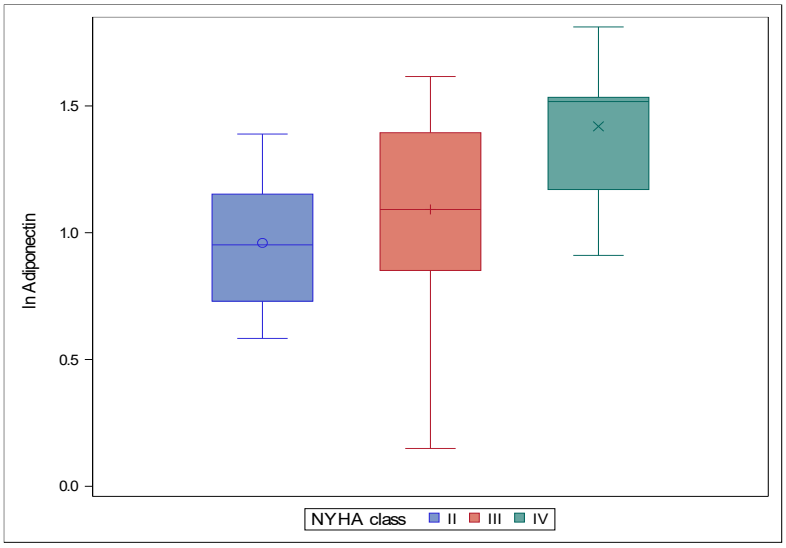

Figure 8. Levels of serum ln APN based on different NYHA classes
Abbreviations: ln APN: logarithm of serum adiponectin concentration to the base of the constant e.

Circulating APN levels directly correlate with plasma BNP concentration, average global longitudinal strain, and mean PCWP values and are related to NYHA functional class. Therefore, serum APN might be considered as an additional biomarker of disease severity in patients with NI-DCM and CHF.

4.3 The predictive potential of baseline serum adiponectin levels

4.3.1 Serum levels of adiponectin are higher in poor outcome patient group

In order to test the role of serum APN levels in predicting outcome, patients were divided into two groups according to their outcome: those who experienced composite outcome (LVAD implantation, HT or death from cardiovascular causes) versus those who were event-free after 5 years of follow-up.

28 (49.12%) patients out of 57 patients experienced a composite outcome. 18 patients died during the five years of follow-up. 8 patients experienced LVAD implantation, and 11 were heart transplanted. The date of the outcome was the date of the first event (HM implantation, heart transplantation, or cardiovascular death). Some patients experienced several events (Figure 9).

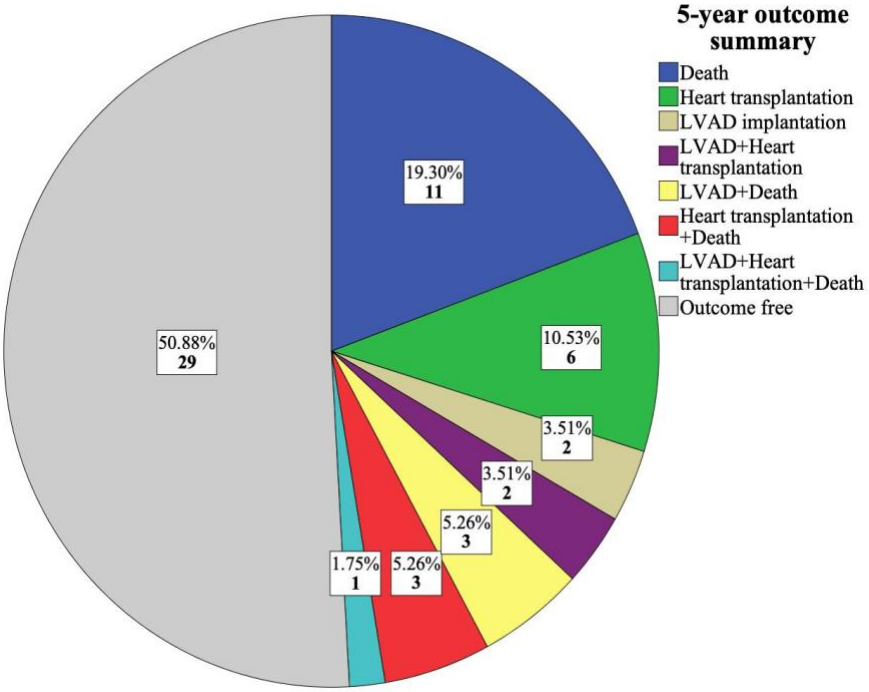

Figure 9. Summary of 5-year outcome. LVAD: left ventricular assist device.

The differences in patient baseline characteristics are shown in Table 4.

Table 4. Differences in patient groups based on their composite outcome

	Good outcome		**Bad outcome**		
Variables	Median (IQR)	No. of pts.	Median (IQR)	No. of pts.	p value
Age (years)	48 (46-53)	29	46 (37.8-54.8)	28	0.192
BMI (kg/m2)	27.8 (22.6-32.7)	29	26.6 23.5-31.2	28	0.811
GFR (ml/min)	104.3(91.1-122.8)	26	111.2 (98.1-138.5)	27	0.292
Echocardiographic parameters					
LVEF(%)	30 (20-36)	29	23 (19-30)	28	**0.029**
LVEDD (cm)	6.5 (6.2-7.2)	29	6.9 (6.5-7.5)	28	0.177
GLS	-9.64 (-12.70- (-7.87)	21	-5.86 (-7.33- (-3.84)	20	**<0.001**
Hemodynamic parameters (mmHg)					
Mean AoP	100 (90-104.8)	24	93 (83.3-99.3)	24	**0.028**
Mean RAP	9 (5.3-12)	28	13.0 (7-19)	24	0.070
Mean PAP	25 (21-37.8)	28	34.0 (26-43)	25	0.064
Mean PCWP	18 (14.3-26.3)	28	23 (15.5-34.0)	25	0.117
Serum biomarkers of heart failure					
BNP (pg/ml)	247.7 (46.1-939.0)	28	1280.0 (266.3-2786.8)	28	**0.007**
APN (µg/mL)	10.80 (5.24-19.94)	29	21.80 (11.04-33.23)	26	**0.01**
Serum markers of systemic inflammation					
CRP (µg/mL)	2.4 (1.3-17.8)	25	6.6 (1.9-16.8)	27	0.272

IL-6 (pg/ml)	2.01 (2.00-4.11)	29	5.24 (2.01-14.30)	26	**0.002**
TNF-α (pg/ml)	7.54 (6.01-9.41)	29	9.02 (7.91-13.01)	26	**0.034**
Markers of cardiac inflammation (cells/mm2)					
CD3+	10 (7-14)	27	10 (5-11)	28	0.394
CD4+	4 (2-6)	27	3 (1-8)	28	0.570
CD45Ro+	7 (5-10)	27	7 (5-11)	28	0.715
CD68+	4 (3-5)	27	4 (3-5)	28	0.966
Cardiac adiponectin receptors (ng/mg)					
T-cad	38.01 (22.06-54.83)	18	41.42 (18.72-75.37)	11	0.707
Cardiac adiponectin receptors (ng/mg)					
ACEI/ARB n, (%)	19 (65.5)	29	17 (60.7)	28	0.707
Beta-blockers n, (%)	28 (96.6)	29	26 (92.9)	28	0.611
Mineralcorticoid receptor blockers n, (%)	24 (82.8)	29	27 (96.4)	28	0.08
Diuretics n, (%)	26 (89.7)	29	27 (96.4)	28	0.306

Significant differences are bolded (Wilcoxon rank-sum test, χ^2 test for equality of proportions, Fisher exact test when expected frequencies <5). Significant at the p level 0.05 (2-tailed).

Abbreviations: SD: standard deviation, IQR: interquartile range, BMI: Body mass index, GFR: glomerular filtration rate, LVEF: left ventricular ejection fraction; LVEDD: Left ventricle end-diastolic diameter, GLS: global longitudinal strain, mean AoP: mean aortic pressure, mean RAP: mean right atrial pressure, mean PAP: mean pulmonary artery pressure, mean PCWP: mean pulmonary capillary wedge pressure, BNP: B-type natriuretic protein, APN: Adiponectin, CRP: C-reactive protein, IL-6: interleukin-6, TNF-α: tumor necrosis factor α, CD3+: T cell receptor, CD4+: T helper cell receptor, CD45Ro+: memory T cell receptor, CD68+: monocyte/macrophage receptor, T-cad: T-cadherin, ACEI: angiotensin receptor inhibitors, ARB: angiotensin receptor II receptor blockers.

Patients in poor composite outcome group had a significantly lower LVEF, worse average global longitudinal strain, as well as higher levels of serum BNP, APN, and systemic inflammatory markers, such as IL-6, TNF-α, compared to event-free patients (Table 4). There were no significant differences in the medications used in patients with either good or poor outcome (Table 4). Serum APN levels at baseline were significantly higher in the poor outcome group: 21.8± 21.498 vs. 10.8± 9.910, p=0.01 (Figure 10).

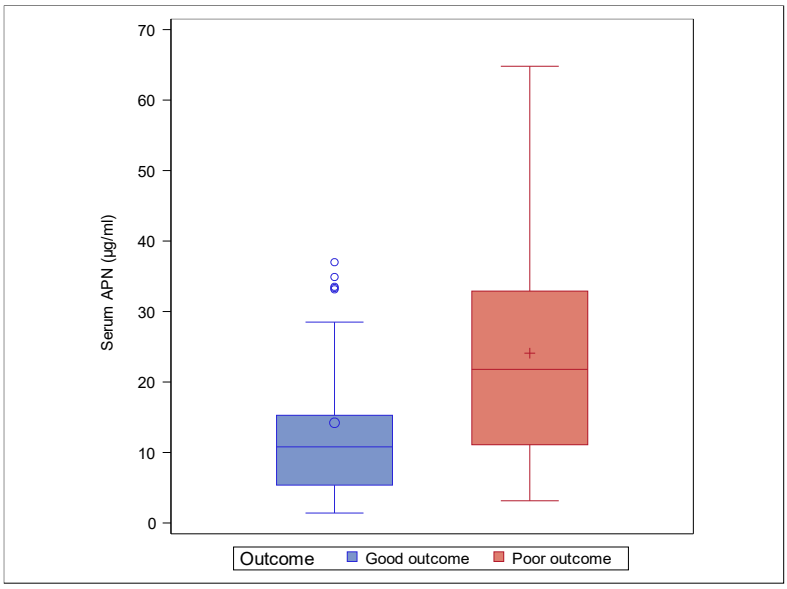

Figure 10. The difference in baseline serum APN levels based on patient outcome

4.3.2 Serum levels of adiponectin predict the poor 5-year composite outcome

The Kaplan-Meier survival curve for the composite 5-year outcome is depicted in Figure 11.

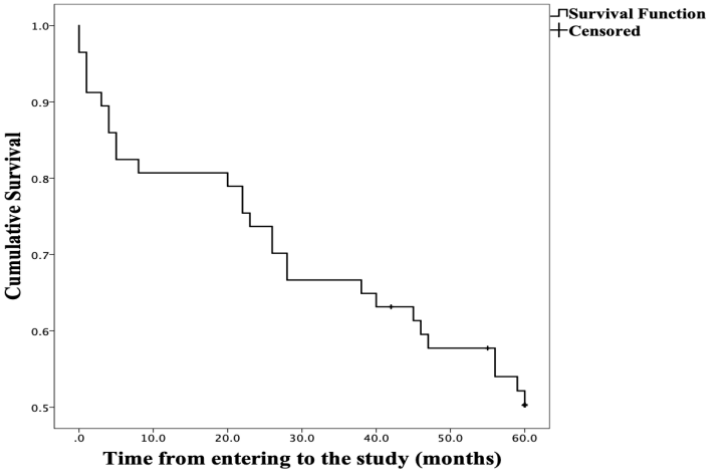

Figure 11. Kaplan-Meir survival curve for five years of follow-up

Univariate Cox-regression analysis revealed that higher serum log-transformed APN levels are associated with a significantly higher 5-year relative risk of the composite outcome (HR 5.991, p=0.006, 95% CI 1.671-

21.482). The effects of other variables on the composite outcome were also evaluated (Table 5). As some of them did not fulfill the proportional hazard assumption, the Cox model was extended to contain time-dependent variables. Serum ln BNP value, and the amount of cardiac infiltration of ln CD3+ significantly increased the relative risk of poor outcome. However, their impact on relative risk was decreasing with time. Therefore, parameters serve as predictors of poor outcome for a limited time after inclusion in the study.

Table 5. The association of unadjusted variables with the composite outcome, five years after inclusion to the study. Cox univariate analysis.

Variable	HR	p value	95% confidence limits for HR
ln APN	5.991	**0.006**	1.671-21.482
ln BNP	7.98	**<0.001**	2.581-24.708
ln BNPt	0.956	**0.007**	0.926-0.988
ln IL-6	3.627	**0.001**	1.655-7.945
ln LVEF	0.259	**0.007**	0.096-0.697
ln CD3+	41.567	**0.004**	3.276-527.369
ln CD3+t	0.905	**0.020**	0.832-0.984

Abbreviations: logarithm to the base of the constant e, HR: Hazard ratio, BNP: Brain natriuretic peptide, IL-6: interleukin 6, LVEF Left ventricle ejection fraction, CD3+: T cell receptor. Significant values marked in bold.

To test whether the association between APN and poor outcome remains after adjusting for other relevant covariates, multivariate Cox regression analysis was performed (Table 6).

Table 6. The association of serum ADP levels with the composite outcome, adjusted for other covariates.

Model: input variables	Hazard ratio	p value	95% confidence limits for Hazard Ratio
Model 1:			
ln APN	5.089	**0.026**	1.215-21.317
ln BNP	4.434	**0.035**	1.114-17.649
ln BNP_t	0.954	**0.009**	0.920-0.988
ln IL-6	4.197	**0.007**	1.469-11.992
Model 2:	**Hazard ratio**	**p value**	**Hazard Ratio 95% Confidence Limits**
ln APN	5.528	**0.031**	1.169-26.153
ln BNP	3.814	0.069	0.903-16.106
ln BNP_t	0.959	**0.024**	0.924-0.995
ln IL-6	3.849	**0.012**	1.344-11.021
CD3+	1.072	**0.028**	1.008-1.140
CD3+_t	0.997	0.131	0.994-1.001

Multivariate Cox regression analysis. Abbreviations: ln: logarithm to the base of the constant e, APN: adiponectin, BNP: Brain natriuretic peptide, BNP_t: BNP time-dependent variable, IL-6: interleukin 6, CD3+: T cell receptor, CD3+_t: CD3+ time-dependent variable

Multivariate Cox regression analysis revealed that ln APN levels remain a significant prognostic marker after adjusting for serum ln BNP, ln IL-6 levels, and cardiac CD3+ counts (CD3+_t). Ln IL-6 remained an independent prognostic marker too. Moreover, higher levels of baseline lnAPN and ln IL-6 significantly increase the relative risk of the poor composite outcome.

The role of APN as an outcome predictor was also tested in the subgroup analysis of patients with inflammatory DCM (iDCM) vs. non-inflammatory DCM (DCM). Their baseline characteristics are depicted in Table 7.

Table 7. Differences in patient groups based on myocardial inflammation.

	iDCM		**DCM**		
Variable	**Median (IQR)**	No of pts.	**Median (IQR)**	No of pts.	p value
Age (years)	48 (43-53)	31	47 (42-55)	24	0.64
Male gender, n (%)	26 (83.49%)	31	17 (70.8)	24	0.328
BMI (kg/m2)	25.7(22.7-31.6)	31	27.3 (23.1-34.0)	24	0.54
GFR (ml/min)	112.4(91.9-138.2)	28	104.9 (91.4-120.9)	23	0.256
NYHA class, n (%)		31		24	0.455
II	2 (6.5)		4 (16.7)		
III	23 (74.2)		15 (62.5)		
IV	6 (19.4)		5 (20.8)		
Echocardiographic parameters					
LVEF (%)	25(21-30)	31	23 (16-34)	24	0.428
LVEDD, cm	6.9 (6.4-7.3)	31	7.0 (6.2-7.5)	24	0.966
GLS	-6.2 (-8.99-(-4.48)	23	-9.63 (-2.70-(-7.27)	17	**0.012**
Hemodynamic parameters (mmHg)					
Mean AoP	90 (81-100)	23	98 (90-103)	23	0.051
Mean RAP	11 (5-16)	27	10 (7-13)	23	
Mean PAP	29 (23-38)	28	20 (20-43)	23	0.538
Mean PCWP	21 (16-31)	28	19 (13-32)	23	0.489
Serum biomarkers					
BNP (pg/mL)	1017.1(260.0-2802.5)	30	213.7 (49.9-1696.2)	24	0.051
CRP (μg/mL)	4.5 (1.4-17.3)	29	5.2 (1.4-11.5)	21	0.738
APN (μg/ml)	14.8 (10.6-32.85)	29	13.2 (6.5-27.5)	24	0.372
IL-6 (pg/mL)	2.85 (2.01-11.83)	29	2.23 (2.00-5.35)	24	0.215
TNF-α (pg/mL)	8.87 (6.76-10.85)	29	7.95 (6.62-9.74)	24	0.372
hsTnT (pg/mL)	35.4 (21.4-52)	29	21.5 (17.8-40.9)	24	0.108
Markers of immune infiltration (cells/mm2)					
CD3+	15 (12-20)	31	7 (5-8)	24	**<0.001**
CD4+	4 (2-8)	31	4 (2-6)	24	0.521
CD45Ro+	10 (6-12)	31	5 (3-6)	24	**<0.001**
CD68+	5 (3-7)	31	3 (2-4)	24	**0.001**
Myocardial adiponectin receptors (ng/mg)					
T-cad	26.81 (16.80-44.1)	13	50.36 (34.87-56.33)	15	0.058
Medications used n (%)					
ACEI/ARB n, (%)	18 (58.1)	31	17 (70.8)	24	0.329

Beta-blockers n, (%)	28 (90.3)	31	24 (100)	24	0.117
Mineralocorticoid receptor blockers n, (%)	30 (96.8)	31	20 (83.3)	24	0.081
Diuretics n, (%)	30 (96.8)	31	21 (87.5)	24	0.189

Significant differences are bolded (Wilcoxon rank-sum test). Significant at the p level 0.05 (2-tailed). Abbreviations: IQR: interquartile range, BMI: Body mass index, GFR: glomerular filtration rate, LVEF: left ventricular ejection fraction; LVEDD: Left ventricle end-diastolic diameter, GLS: global longitudinal strain, mean AoP: mean aortic pressure, mean RAP: mean right atrial pressure, mean PAP: mean pulmonary artery pressure, mean PCWP: mean pulmonary capillary wedge pressure, BNP: B-type natriuretic protein, CRP: C-reactive protein, APN: Adiponectin, IL-6: interleukin-6, TNF-α: tumor necrosis factor α, hsTnT: high sensitivity troponin T, CD3+: T cell receptor, CD4+: T helper cell receptor, CD45Ro+: memory T cell receptor, CD68+: monocyte/macrophage receptor, CD54+: Intercellular Adhesion Molecule 1, HLA-DR+: Human Leucocyte Antigen isotype DR, T-cad: T-cadherin, ACEI: angiotensin converting enzyme inhibitors, ARB: angiotensin receptor blockers.

Most of the baseline characteristics, except for GLS, did not differ between the groups. Baseline APN levels were not significantly different either (p=0.372). The number of immune cells (CD3+, CD45Ro+, CD68+) was significantly higher in the iDCM group as expected.

Cumulative 5-year survival curves did not differ significantly either (Figure 12).

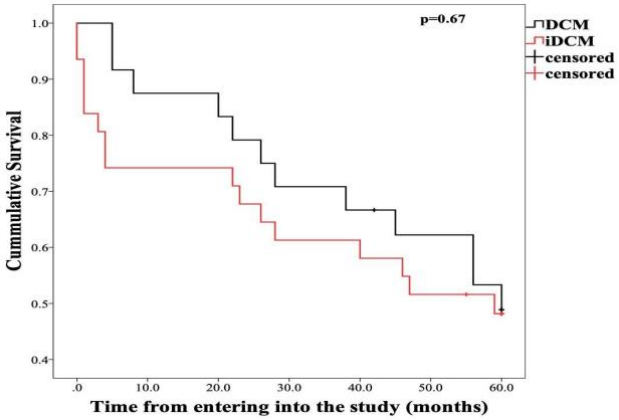

Figure 12. Caplan-Meier survival curves for patients with inflammatory DCM vs. non-inflammatory DCM. Survival function compared using the Log-rank test.

Univariate Cox regression analysis revealed that higher lnAPN levels predict worse 5-year composite outcome in patients with iDCM (HR=2.708,

p=0.017, 95% CI 1.197-6.125). Serum lnAPN levels were not shown to increase the relative risk of the composite outcome in patients with non-inflammatory DCM (HR=1.762, p=0.169, 95% CI 0.787-3.947).

To sum up, the results mentioned above suggest that baseline levels of serum APN portend poor 5-year outcome. This was valid even after adjusting for serum BNP and IL-6 levels and the extent of cardiac CD3+ infiltration.

4.3.3 Survival tree analysis and the cutoff value of serum adiponectin

Survival tree analysis reveals which variables divide the cohort into groups with a maximally different outcome. The method was applied to find the cutoff value of the APN and demonstrate the possible non-linear associations between variables. An increasing number of variables were included as an input. Three different models with increasing input were constructed:

1. Single APN.
2. APN plus covariates, known to impact survival in CHF.
3. APN, covariates known to impact survival plus global longitudinal strain (GLS)

Survival tree analysis involving single serum APN showed that patients with APN values above 15.28 μg/ml had a significantly worse outcome (p=0.003) than those with their APN values ≤15.28 μg.ml (Figure 13). This value was used for the subsequent Kaplan-Meier analysis illustrating the use of this cutoff value at different periods of follow-up.

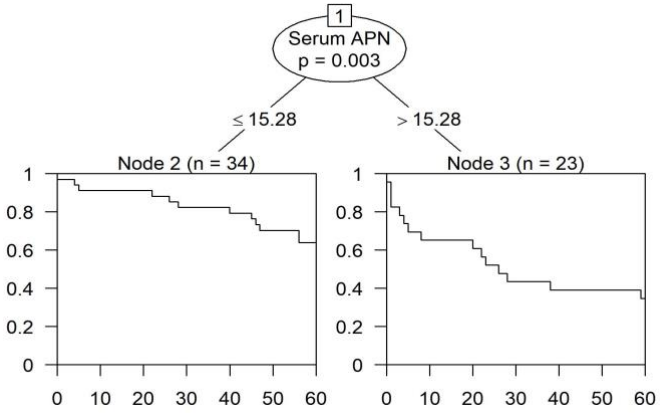

Figure 13. Survival tree analysis for the composite outcome, 5-year follow-up. Input: serum APN.

Entering into the model other variables that are known to be associated with patient outcome and those that were significant in Cox univariate model

revealed that the worst outcome remained in patients with serum APN >15.28μg/ml. In APN ≤15.28μg/ml group, patients experienced a worse outcome in the case of lower LVEF <=30% (Figure 14). APN level was still the most informative predictor after including other relevant variables such as age, gender, NYHA class, GFR, BMI, serum BNP, serum IL-6, TNF-α, LVEF, mean PCWP, cardiac CD3+ count and medications used.

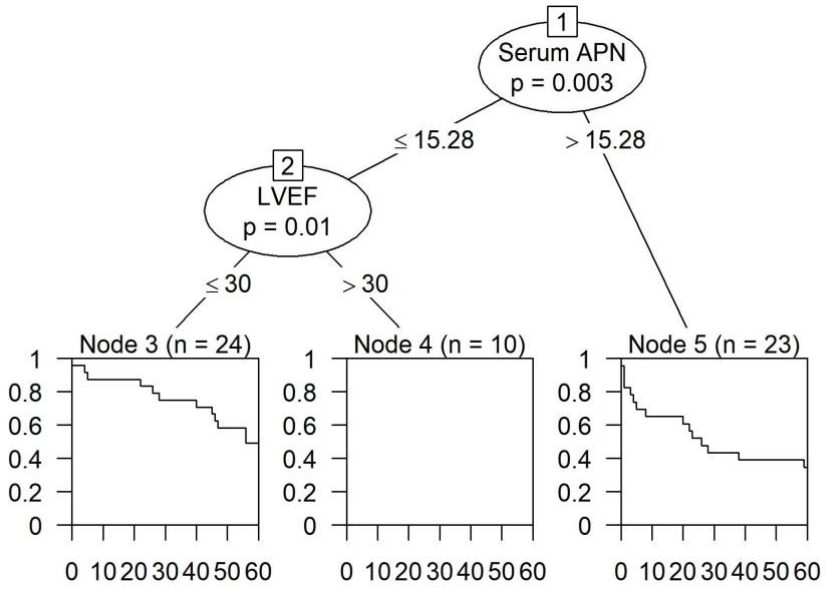

Figure 14. Survival tree analysis for the composite outcome, 5 years of follow-up. Input: age, gender, NYHA class, GFR, BMI, serum BNP, serum IL-6, serum TNF-α levels, LVEF, mean PCWP, and cardiac CD3+ count, medications (angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, beta-blockers, mineralocorticoid receptor blockers, diuretics).

Adding to the model GLS, which is not a routinely used outcome measure, changed the situation. The main parameter dividing patients into two groups with a maximally different outcome appeared to be GLS (-5.98). Mean PCWP was important for further subdivision of patients in the GLS≤-5.98 group. Serum APN, however, lost its significance (Figure 15).

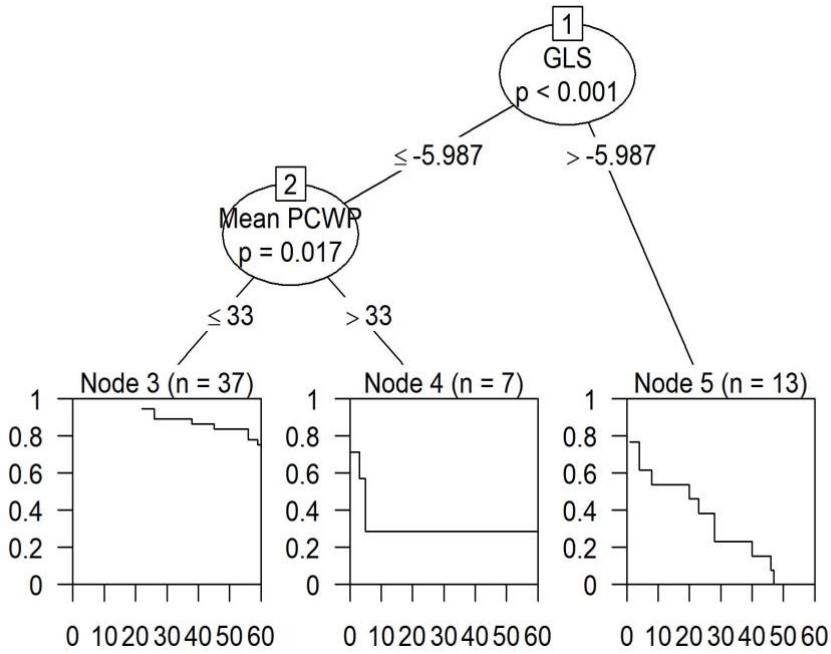

Figure 15. Survival tree analysis. Input: age, gender, NYHA class, GFR, BMI, serum APN, serum BNP, serum IL-6, serum TNF-α level, LVEF, mean PCWP, and cardiac CD3+ count, medications (angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, beta-blockers, mineralocorticoid receptor blockers, diuretics) plus GLS.

All in all, APN remained valid even after taking into account a vast array of parameters, that in turn have an impact on survival, such as age, sex, NYHA class, glomerular filtration rate, body mass index, serum BNP, IL-6, TNF-α, LVEF, mean PCWP and cardiac CD3+ count and main medications used in HF. GLS seems to be the most potent poor outcome predictor.

4.3.4 The differences in patient outcome based on serum adiponectin cutoff value

Further, patients were subdivided into two groups based on the APN cutoff value (15.28 μg/ml), determined from the survival tree analysis (Figure 13). The differences in their baseline characteristics are revealed in Table 8.

Table 8. Differences in baseline characteristics of patients stratified by APN cutoff (15.28μg/ml).

	APN ≤15.28 μg/ml		**APN>15.28 μg/ml**		
	Median (IQR)	No. of pts.	Median (IQR)	No. of pts.	p value
Age (years)	48 (45-52.8)	32	47 (40-57)	23	0.918
BMI (kg/m2)	28.23 (25.18-32.23)	32	24.41 (21.06-29.96)	23	0.058
GFR (ml/min)	110.4 (94.3-130.9)	30	107.97 (80.1-124.4)	21	0.605
Echocardiographic parameters					
LVEF (%)	28 (21-35)	32	24 (20-30)	23	0.208
LVEDD (cm)	6.7 (6.2-7.3)	32	6.9 (6.5-7.4)	23	0.321
GLS	-8.99 (-11.3-(-7.1))	23	-6.20 (-8.89-(-3.65))	17	**0.016**
Hemodynamic parameters (mmHg)					
Mean AoP	100 (89.5-104.3)	26	93 (84.5-98.0)	21	**0.03**
Mean RAP	11 (5.3-13)	28	10.5 (7-20.5)	22	0.332
Mean PAP	26 (21-34)	29	38 (23.2-43.7)	22	**0.038**
Mean PCWP	19 (14.5-23.5)	29	25.5 (14.7-34.0)	22	0.135
Serum biomarkers of heart failure					
BNP (pg/ml)	142.8 (46.0-829.8)	31	1619.2 (839.9-3245.7)	23	**<0.001**
Markers of systemic inflammation					
CRP (μg/mL)	2.4 (0.95-7.90)	29	6.5 (2.7-16.5)	21	0.099
IL-6 (pg/ml)	2.01 (2.00-6.42)	32	4.41 (2.0-8.6)	23	0.069
TNF-α (pg/ml)	7.65 (5.70-9.42)	32	9.25 (7.9-10.9)	23	**0.039**
Markers of cardiac immune infiltration (cells/mm2)					
CD3+	10 (7-15)	30	10 (7-16)	23	0.746
CD4+	4 (2-6)	30	4 (1-8)	23	0.850
CD45Ro+	7 (5-8)	30	6 (4-12)	23	0.691
CD68+	4 (3-5)	30	4 (3-6)	23	0.797
Markers of myocardial necrosis (pg/ml)					
TnT	22.88 (14.93-44.38)	32	32.98 (24.2-62.0)	23	0.180
Cardiac adiponectin receptors (ng/mg)					
T-cad	32.13 (19-52.4)	18	49.39 (36.48-78.38)	10	0.119
Medications used					
AKFI/ARB, n (%)	22 (66.8)	32	12 (52.5)	23	0.212
Beta-blockers, n (%)	31 (96.9)	32	21(91.3)	23	0.565
Mineralocorticoid receptor blockers n, (%)	27 (84.4%)	32	22 (95.7)	23	0.163
Diuretics, n (%)	30 (93.8%)	32	23 (100%)	23	0.136

Significant differences are bolded (Wilcoxon rank-sum test, χ^2 test for equality of proportions, Fisher exact test when expected frequencies <5). Significant at the p level 0.05 (2-tailed)

Abbreviations: IQR: interquartile range, LV EF: left ventricular ejection fraction; LV EDD: Left ventricle end-diastolic diameter, GLS: global longitudinal strain, mean AoP: mean aortic pressure, mean RAP: mean right atrial pressure, mean PAP: mean pulmonary artery pressure, mean PCWP: mean pulmonary capillary wedge pressure, BNP: B-type natriuretic protein, CRP: C-reactive protein, IL-6: interleukin-6, TNF-α: tumor necrosis factor α, CD3+: T cell receptor, CD4+: T helper cell receptor, CD45Ro+: memory T cell receptor, CD68+: monocyte/macrophage receptor, hsTnT:

high sensitivity troponin T, T-cadherin: T-cad, ACEI: angiotensin-converting enzyme inhibitors, ARB: angiotensin receptor blockers.

Patients with serum APN levels above the cutoff have also had a significantly worse myocardial function a more positive GLS, higher intracardial pressures as well as increased levels of serum BNP. Patients with higher circulating APN levels had elevated markers of systemic inflammation (TNF-α and IL-6).

Those with baseline serum APN levels >15.28 μg/ml had a significantly worse outcome for various periods of follow-up. Worse survival in patients with APN> 15.28 μg/ml was seen as early as 6 months of follow-up and persisted until 5 years of follow-up (Figure 16).

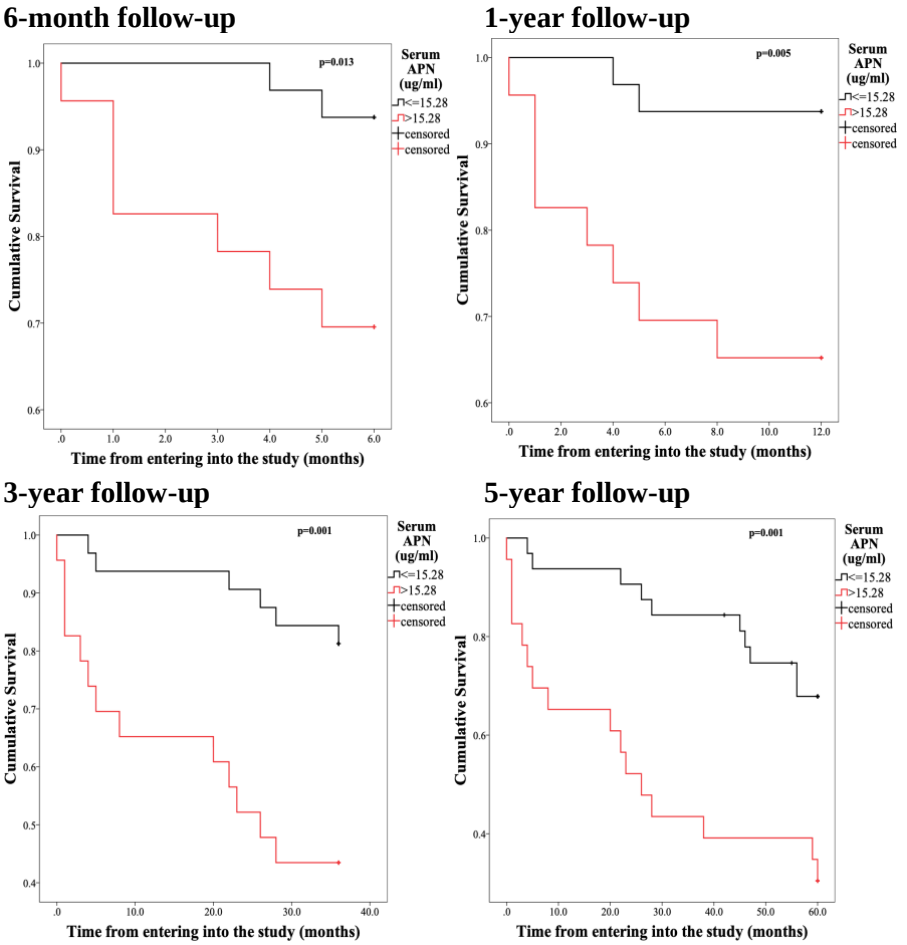

Figure 16. Kaplan-Meier survival curves for binary APN value for various periods of follow-up. Survival functions compared by the Log-rank test.

Univariate Cox regression analysis for binary APN value revealed that the relative risk of composite outcome for patients above the APN cutoff (>15.28μg/ml) is more than three folds higher compared to those below the cutoff value (Table 9).

Table 9. Univariate Cox regression analysis for binary serum APN. The relative risk for patients above the cutoff value (>15.28μg/ml).

Duration of follow-up	Hazard ratio	p value	CI 95%
6 months	5.680	0.030	1.178-27.382
1 year	6.591	0.017	1.397-31.083
3 years	4.239	0.004	1.604-11.200
5 years	3.364	0.003	1.520-7.444

Abbreviations: CI 95%: 95% confidence interval for hazard ratio.

4.3.5 Serum adiponectin as an additive prognostic factor. Factor analysis

Factor analysis revealed three factors with eigenvalues above 1. Altogether they explain 69.34 % of the overall variance in the observed variables (Figure 17). Each factor captures a certain amount of the overall variation. Factors are listed in order of how much variance they explain.

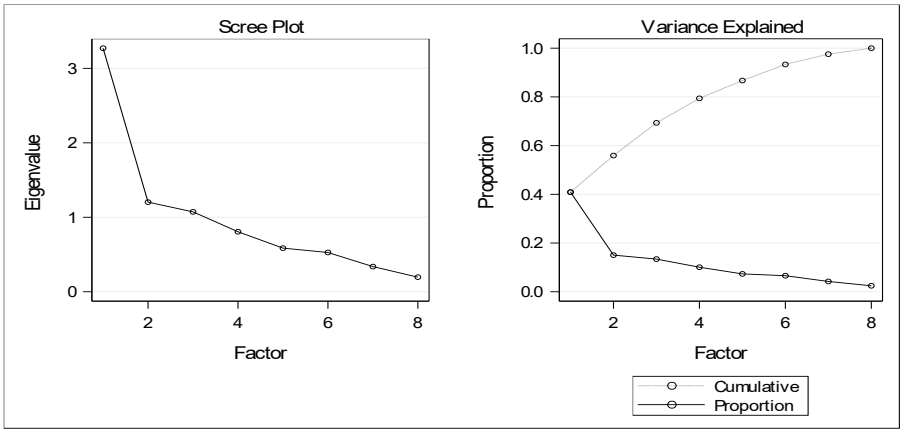

Figure 17. Factor analysis: a line plot of eigenvalues of factors (on the left) and the plot depicting the proportion of variance explained by the factors (on the right)

All three factors marked in different colors are depicted in Figure 18.

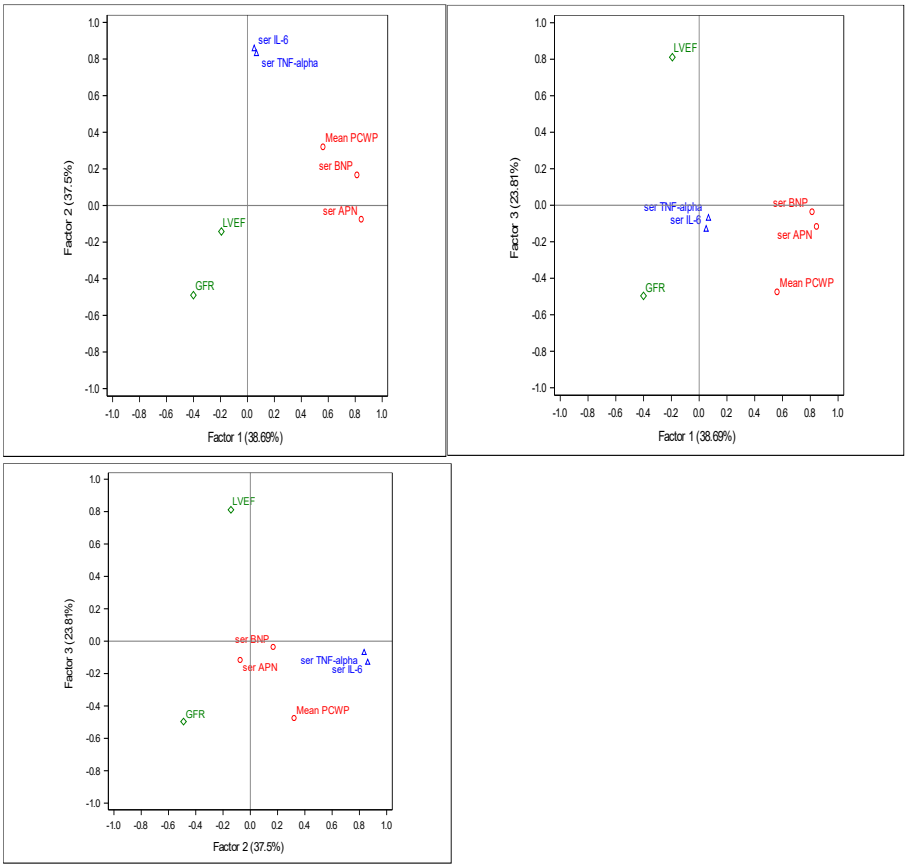

Figure 18. Factor analysis: variables defining each Factor are depicted. Factor 1: colored in red, Factor 2: colored in blue, Factor 3: colored in green.

Factor 1 was best described by serum BNP, APN, and mean PCWP values, as they had the maximal loadings. Factor 1 represents the markers of HF failure severity in patients with NI-DCM and advanced HF. It was involved in the subsequent survival analysis. Factor 1 cutoff value was determined, and the patients were divided into two groups based on their factor 1 cutoff value. Kaplan-Meier analysis revealed that patients with their factor 1 above the cutoff value had a significantly worse outcome (Figure 19). Median BNP, APN, mean PCWP values were significantly higher in Factor 1 above the cutoff group (Table 10). Higher BNP, APN, mean PCWP values were associated with a significantly higher risk of poor outcome, HR= 8.14, CI 95% 1.48-44.68, p=0.0041.

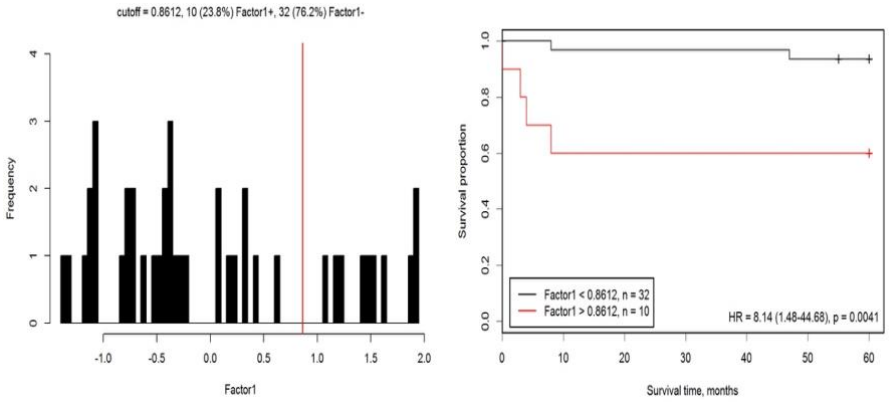

Figure 19. Factor 1 cutoff value and survival curve comparison based on this cutoff

Table 10. Differences in median APN, BNP, and PCWP values based on Factor 1 cutoff.

Variables defining Factor 1	**Factor 1< cutoff**	**Factor 1 > cutoff**	**p value**
APN (µg/mL)	19.92 (6.53-16.21)	35.45 (33.56-41.05)	0.0000003
BNP (pg/ml)	338.6 (74.6-1244.3)	3541.7 (2707.7-4491.2)	0.000007
Mean PCWP (mmHg)	18 (15-23)	34 (32-35)	0.002

Data presented as median (interquartile range). Significant differences bolded (Wilcoxon rank-sum test). Significant at the p level 0.05 (2-tailed). Abbreviations: APN: adiponectin, BNP: brain natriuretic protein, PCWP: pulmonary capillary wedge pressure.

A combination of serum BNP, APN, and mean PCWP values, representing HF severity in patients with NI-DCM and advanced HF, might be a better prognostic marker compared to any of those parameters in single.

4.4 The relation between the serum levels of adiponectin and the markers of systemic and cardiac inflammation

Table 11. The correlations between serum APN and systemic and cardiac inflammatory markers

Variable	Rho	p value	No. of patients
Serum markers of inflammation			
CRP (µg/mL)	0.1706	0.2361	50
IL-6 (pg/ml)	0.2575	0.058	55
TNF-α (pg/ml)	0.311	**0.021**	55
Galectin-3 (ng/ml)	0.303	0.092	32
Markers of cardiac inflammatory infiltration (cells/mm2)			
CD3+	0.144	0.305	53
CD4+	-0.023	0.71	53
CD68+	0.051	0.715	53
CD45ro+	0.113	0.422	53
Markers of immune infiltration (cells/mm2)			
HLA-DR+	0.127	0.366	53
CD54+	0.016	0.910	53

Abbreviations: CRP: C reactive protein, IL-6: interleukin 6, TNF-α: Tumor necrosis factor α, CD3+: T cell receptor, CD45ro+: memory T cell receptor, CD68+: monocyte/macrophage receptor, HLA-DR+: Human Leucocyte Antigen isotype DR, CD54+: Intercellular Adhesion Molecule 1

A positive correlation between serum APN levels and proinflammatory cytokine TNF-α was shown (Table 11). There was a statistically significant increase in serum ln TNF-α as well as serum ln IL-6 concentrations in each subsequent NYHA functional class ($p \leq 0.001$ for each variable) in Figure 9.

Pairwise comparisons demonstrated a significant difference in the levels of both ln TNF-α and ln IL-6 between NYHA functional classes II and IV as well as III and IV (Table 12).

Table 12. Pairwise comparisons of circulating proinflammatory markers in different NYHA classes

NYHA class	**p values**	
	ln TNF-α	ln IL-6
II vs III	0.149	0.95
II vs IV	**0.000**	**0.000**
III vs IV	**0.005**	**0.001**

Abbreviations: ln: logarithm to the base of the constant e, TNF-α: Tumor necrosis factor α, IL-6: interleukin 6

Progressing HF was associated with higher serum proinflammatory cytokine levels, indicating increasing systemic inflammation (Figure 20). Rising serum APN levels paralleled the increase of proinflammatory cytokines and the clinical worsening of HF.

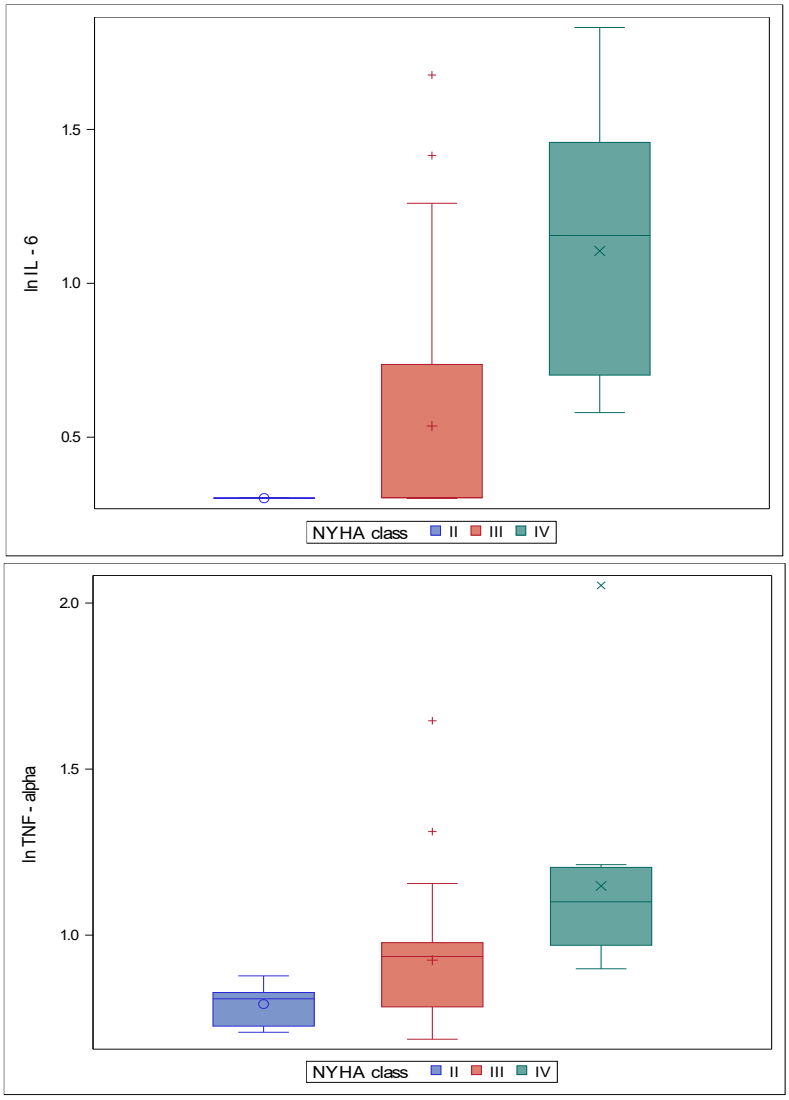

Figure 20. Increase in serum ln IL-6 and ln TNF-α in each subsequent NYHA functional class
Abbreviations: ln: logarithm to the base of the constant e, Il-6: interleukin 6, TNF-α: tumor necrosis factor α.

There was a positive correlation between serum IL-6 and serum TNF-α levels, as well as serum BNP values (Table 13).

Table 13. Correlation matrix between serum APN and biomarkers of inflammation in serum and myocardium.

	APN	BNP	CRP	IL-6	TNF-α	Gal-3	CD3+	CD4+	CD45Ro+	CD68+	HLA-DR+	CD54+
APN	1.0000 55											
BNP	**0.651** **<.0001** **54**	1.000 56										
CRP	0.171 0.236 50	**0.328** **0.018** **52**	1.000 52									
IL-6	0.258 0.058 55	**0.660** **<.001** **54**	**0.431** **0.002** **50**	1.000 55								
TNF-α	**0.311** **0.021** **55**	**0.505** **<0.001** **54**	0.212 0.139 50	**0.488** **<0.001** **55**	1.000 55							
Gal-3	0.303 0.092 32	**0.421** **0.015** **33**	**0.533** **0.003** **30**	**0.699** **<.001** **32**	**0.433** **0.013** **32**	1.000 33						
CD3+	0.144 0.305 53	**0.310** **0.023** **54**	0.194 0.177 50	0.215 0.122 53	0.207 0.137 53	0.185 0.309 32	1.000 55					
CD4+	-0.023 0.871 53	-0.004 0.978 54	0.003 0.987 50	-0.222 0.110 53	-0.066 0.640 53	-0.005 0.469 32	0.226 0.0973 55	1.000 55				
CD45Ro+	0.113 0.4220 53	**0.2759** **0.0434** **54**	0.225 0.116 50	0.093 0.509 53	0.012 0.936 53	0.003 0.988 32	**0.759** **<.001** **55**	**0.306** **0.023** **55**	1.000 55			
CD68+	0.051 0.715 53	0.219 0.112 54	0.258 0.070 50	**0.355** **0.009** **53**	0.202 0.148 53	0.280 0.120 32	**0.402** **0.002** **55**	-0.014 0.919 55	**0.433** **0.001** **55**	1.000 55		
HLA- DR+	0.127 0.366 53	0.121 0.385 54	0.124 0.393 50	0.088 0.534 53	0.097 0.490 53	-0.021 0.909 32	**0.288** **0.033** **55**	0.100 0.46 55	0.158 0.249 55	**0.348** **0.009** **55**	1.000 55	
CD54+	0.016 0.910 53	0.151 0.277 54	-0.005 0.972 50	-0.029 0.890 53	-0.051 0.717 53	**-0.093** 0.612 32	0.007 0.960 55	**0.307** **0.023** **55**	0.152 0.268 55	0.228 0.094 55	0.059 0.671 55	1.000 55

Abbreviations: APN: Adiponectin, BNP: B-type natriuretic protein, CRP: C-reactive protein, IL-6: interleukin-6, TNF-α: tumor necrosis factor α, Gal-3: galectin 3, CD3+: T cell receptor, CD4+: T helper cell receptor, CD45Ro+: memory T cell receptor, CD68+: monocyte/macrophage receptor, HLA-DR+: Human Leucocyte Antigen isotype DR, CD54+: Intercellular Adhesion Molecule 1

Plasma BNP levels positively correlated with markers of cardiac inflammatory infiltration - CD3+, CD45Ro+. Progressing HF seems to be associated with both increasing systemic as well as cardiac inflammation. No association between serum APN and cardiac markers of immune infiltration was found (Table 13). We were interested in finding out whether markers of systemic inflammation are associated with LVEF and hemodynamic parameters. LVEF correlated inversely with CRP and IL-6. Higher mean PCWP was associated with increased levels of IL-6 (Table 14).

Table 14. Correlation between the markers of systemic inflammation and LVEF and hemodynamic parameters.

	CRB	IL-6	TNF-α	LVEF	Mean PAP	Mean PCWP
CRP	1.0000 52					
IL-6	**0.431** **0.002** **50**	1.000 55				
TNF-α	0.212 0.139 50	**0.48762** **0.0002** **55**	1.0000 55			
LVEF	**-0.311** **0.025** **52**	**-0.439** **0.001** **55**	-0.235 0.084 55	1.000 57		
Mean PAP	0.189 0.192 49	**0.483** **0.000** **51**	**0.391** **0.005** **51**	**-0.344** **0.012** **53**	1.000 53	
Mean PCWP	0.172 0.237 49	**0.416** **0.002** **51**	0.247 0.081 51	**-0.330** **0.016** **53**	0.902 0.000 53	1.000 53

Abbreviations: CRP: C reactive protein, IL-6: interleukin 6,TNF-α: Tumor necrosis factor α, LVEF: left ventricle ejection fraction, PAP: pulmonary artery pressure, PCWP: pulmonary capillary wedge pressure.

Multivariate regression analysis was performed to test which variables impact the variance of serum APN levels. As the number of cases was small, only a limited amount of independent variables could be included in the models. Various models, combining different input variables, such as age, NYHA class (NYHA II-III vs. NYHA IV), lnLVEF, IL-6, TNF-alpha, CRB, GLS, mean PCWP were tested. The best-fitting model (R-square of 0.55) was chosen (Table 15). NYHA class, logarithmically transformed BNP (ln BNP), and IL-6 (ln IL-6) were the parameters that were significantly associated with the changes of serum APN (Table 15).

Table 15. Multivariate regression analysis. APN as a dependent variable.

	Estimate	Standard error	t value	p value
Intercept	0.206	0.452	0.46	0.65
NYHA class II-III	-0.278	0.11	-2.51	**0.015**
ln BNP	0.374	0.059	6.31	**<0.0001**
ln IL-6	-0.397	0.111	-3.56	**0.001**
ln LVEF	0.293	0.246	1.19	0.241

Abbreviations: ln: logarithm to the base of the constant e, BNP: Brain natriuretic peptide, IL-6: Interleukin-6, LVEF: Left ventricle ejection fraction

In conclusion, higher levels of circulating APN were significantly associated with NYHA functional class IV, higher circulating BNP, and IL-6 levels. Serum APN levels correlated directly with the markers of systemic inflammation and were elevated in parallel with worsening HF, as indicated by the NYHA class. Worsening of HF, as reflected by higher NYHA class,

BNP concentration, and elevated mean PAP and PCWP, is, in turn, related to increasing proinflammatory milieu. No correlation between plasma APN and cardiac inflammatory infiltrates was found.

4.5 Myocardial T-cadherin as a biomarker and outcome predictor in patients with NI-DCM and chronic heart failure

T-cad concentration was evaluated from the stored EMB samples that were gathered between the years 2010-2013. 29 EMB samples that were still available were used for further analysis. Those patients comprised the cohort of the subsequent retrospective analysis. Their baseline data are depicted in Table 16.

Table 16. Baseline patient characteristics.

Variable	Value	No. of patients
Age (years)	53 ± 8	29
Male/gender, n (%)	22 (75.9)	29
BMI (kg/m2)	28.8 ± 5.6	29
GFR (ml/min)	104.2 ± 32	25
Duration of symptoms before enrollment (months)	12 (6-60)*	29
NYHA class, n (%)		29
II	4 (13.79)	
III	19 (65.52)	
IV	6 (20.69)	
iDCM, n (%)	13 (46.4)	28
Permanent atrial fibrillation, n (%)	8 (27.59%)	29
Echocardiographic parameters		
LVEF (%)	28.28 ± 11.44	29
LVEDD (cm)	6.5 (6.2-7.3)*	29
Average global strain	-9.97 ± 3.67	17
Hemodynamic parameters		
Mean AoP (mmHg)	100 ± 13	27
Mean RAP (mmHg)	11 (7-14)*	29
Mean PAP (mmHg)	28 (21-38)*	28
Mean PCWP (mmHg)	19 (15-30)*	29
Serum biomarkers		
BNP (pg/mL)	305 (56.8-1496.2)*	28
APN (μg/ml)	10.6 (5.30-27.54)*	28
Markers of inflammation in serum		
CRP (μg/mL)	2.4 (1.3-11.5)*	27
TNF-α (pg/mL)	8.73 (6.62-9.82)*	28
Il-6 (pg/mL)	2.38 (2-5.14)*	28
Markers of myocardial immune infiltration (cells/mm2)		
CD3+	9 (7-14.5)*	28
CD4+	4 (3.75-6)*	28
CD45Ro+	6 (4.75-8)*	28
CD68+	4 (2.75-5)*	28
Myocardial adiponectin receptors (ng/mg)		
T-cad	41.16 (22.75-54.34)*	29
Medications used		
ACEI/ARB blockers n, (%)	24(82.8)	29
Beta blockers n, (%)	28 (96.5)	29
Mineralocorticoid receptor blockers n, (%)	26 (89.7)	29
Diuretics n, (%)	27 (93.1)	29

Data presented as mean±SD, median (interquartile range)* or n (%). Abbreviations: BMI: Body mass index, GFR: glomerular filtration rate, iDCM: inflammatory dilated cardiomyopathy, NYHA: New York Heart Association functional class LVEF: left ventricular ejection fraction, LVEDD: left ventricular end-diastolic diameter, AoP: aortic pressure, RAP: right atrial pressure, PAP: pulmonary artery pressure, PCWP: pulmonary capillary wedge pressure, BNP: B-type natriuretic protein, APN: adiponectin, CRP: C-reactive protein, TNF-α: tumor necrosis factor α, IL-6: interleukin-6, CD3+: T cell receptor, CD4+: T helper cell receptor, CD45Ro+, memory T cell receptor, CD68+: monocyte/macrophage receptor, T-cad: T-cadherin, ACEI: angiotensin-converting enzyme inhibitors; ARB: angiotensin receptor blockers.

4.5.1 The association of T-cadherin with other biomarkers of heart failure

To investigate the relevance of T-cad levels in cardiac tissue as a biomarker in patients with NI-DCM and advanced HF, the association between cardiac T-cad and other biomarkers of chronic HF was assessed (Table 17).

Table 17. Spearman correlations between T-cadherin and other biomarkers

Variables	**rho**	**p value**	**No. of patients**
Age (years)	0.015	0.940	28
Echocardiographic parameters			
LVEF (%)	-0.098	0.621	28
LVEDD(cm)	-0.079	0.689	28
Average global strain	-0.297	0.248	17
Hemodynamic parameters (mmHg)			
Mean AoP	0.015	0.943	26
Mean RAP	-0.047	0.814	28
Mean PAP	-0.221	0.257	28
Mean PCWP	-0.397	**0.036**	28
Serum biomarkers of HF			
BNP (pg/ml)	-0.013	0.947	27
APN (µg/mL)	-0.027	0.897	27
Biomarkers of myocardial necrosis			
hsTnT (pg/ml)	0.016	0.937	27
Markers of systemic inflammation			
CRP(µg/mL)	0.354	0.076	26
IL-6 (pg/ml)	0.185	0.356	27
TNF-α (pg/ml)	0.124	0.537	27
Markers of cardiac inflammation (cells/mm$_2$)			
CD3+	-0.423	**0.028**	27
CD4+	0.032	0.874	27
CD45Ro+	-0.220	0.279	26
CD68+	-0.074	0.719	26

Abbreviations: LVEF: left ventricular ejection fraction; LVEDD: Left ventricle end-diastolic diameter, Average global strain: left ventricular Average global strain, mean AoP: mean aortic pressure, mean RAP: mean right atrial pressure, mean PAP: mean pulmonary artery pressure, mean PCWP: mean pulmonary capillary wedge pressure,

BNP: B-type natriuretic protein, APN: adiponectin, hsTnT: high sensitivity troponin T, CRP: C-reactive protein, IL-6: interleukin-6, TNF-α: tumor necrosis factor α, CD3+: T cell receptor, CD4+: T helper cell receptor, CD45Ro+: memory T cell receptor, CD68+: monocyte/macrophage receptor.

There was an inverse correlation between T-cad levels and mean PCWP (rho=-0.397, p=0.037): more advanced HF, as defined by higher mean PCWP, was associated with lower levels of cardiac T-cad (Figure 21).

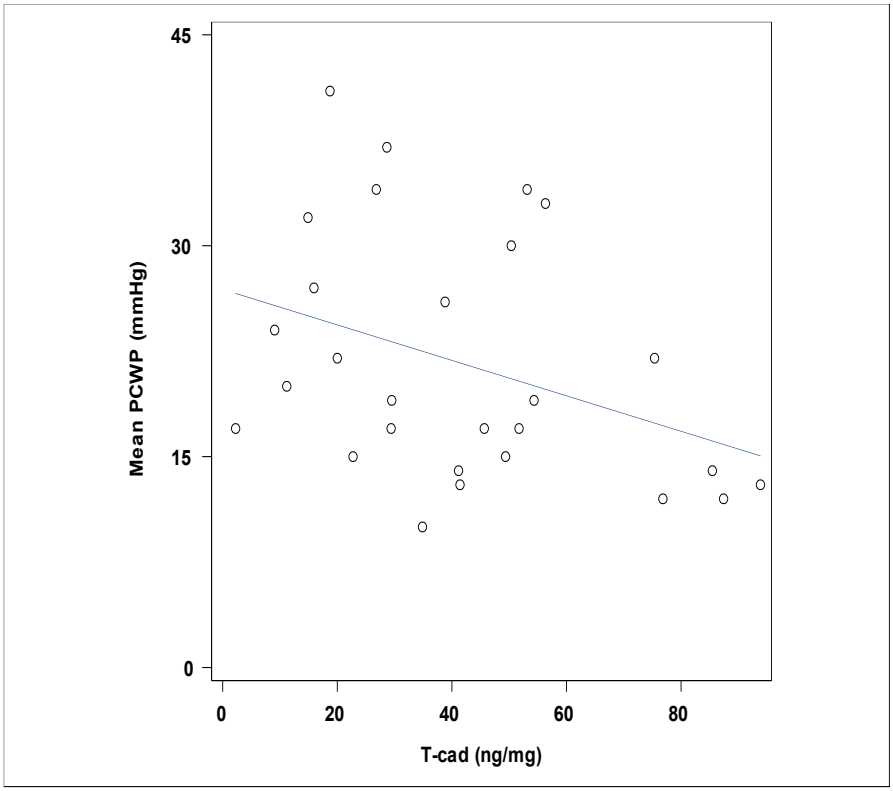

Figure 21. Correlation between cardiac T-cad and mean PCWP

Knowing the well-described anti-inflammatory activity of APN, it was assumed that T-cad expression might have an impact on cardiac inflammation. Correlation analysis demonstrated that T-cad expression is inversely associated with myocardial infiltration of T cells (CD3+) (Figure 22).

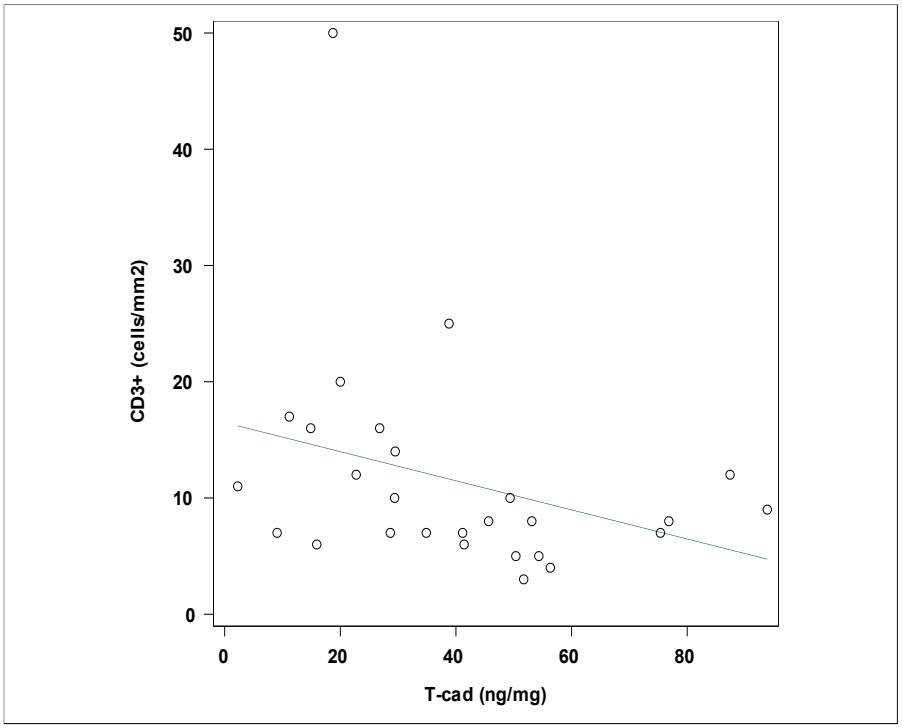

Figure 22. Correlation between cardiac T-cad and amount of CD3+ cells in the myocardium

Correlation analysis revealed no significant correlation between the levels of serum APN and myocardial T-cad. The correlation coefficient was low and insignificant (rho -0.027, p=0.892) (Table 17).

In conclusion, elevated cardiac T-cad levels are associated with lower cardiac CD3+ infiltration indicating its role in local inflammation control. An inverse correlation between cardiac T-cad and mean PCWP suggests that T-cad levels might be lower in more severe HF in patients with NI-DCM and advanced HF.

4.5.2 T-cadherin as a biomarker indicating heart failure severity in patients with NI-DCM and chronic heart failure

In order to test if myocardial T-cad concentration differs in patients stratified by HF severity, patients were divided into two groups based on their mean PCWP cutoff value (19 mmHg). The cutoff value was received from the survival tree analysis (Figure 23). Mean PCWP, a surrogate marker of LV end-diastolic pressure, was treated as a biomarker of HF severity for patients with chronic NI-DCM.

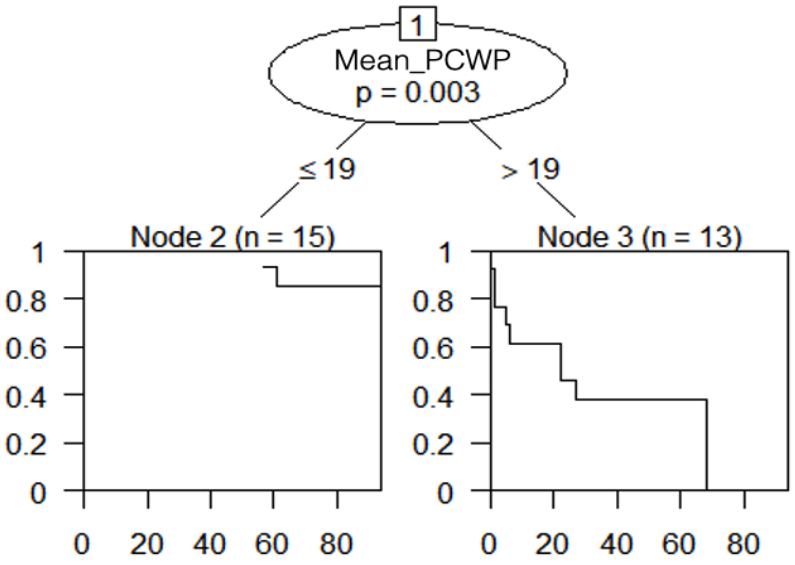

Figure 23. Survival tree analysis. Input: mean PCWP.

The baseline patient characteristics based on mean PCWP value are depicted in Table 18.

Table 18. Differences in patients groups divided by mean PCWP.

	Mean PCWP ≤19 mmHg		**Mean PCWP >19 mmHg**		p value
Variable	Value	No. of patients	Value	No. of patients	
Age (years)	52 (48-54)	15	53 (48-58)	13	0.474
Male gender, n (%)	11 (73.3)	15	10 (76.9)	13	1
BMI (kg/m2)	28 (25.3- 34.9)	15	28 (25.2- 31.8)	13	0.872
GFR (ml/min)	103.7 (91.5- 119.7)	14	104 (74.8- 132.9)	10	0.931
NYHA class, n (%)		15		13	0.731
II	3 (20.0)		1 (7.7)		
III	10 (66.7)		9 (69.2)		
IV	2 (13.3)		3 (23.1)		
iDCM, n (%)	6 (46.2)	14	6 (42.9)	13	1
Echocardiographic parameters					
LVEF (%)	35 (30-42)	15	20 (17-30)	13	**0.003**
LVEDD, cm	6.3 (6.2-6.9)	15	6.9 (6.5-7.5)	13	**0.047**
GLS	-12.46 (-13.38-9.58)	10	-8.54 (-9.45 -5.15)	7	**0.007**
Serum biomarkers					
BNP (pg/mL)	75 (30.75-304.7)	15	1134 (335.8-2653.4)	12	**0.005**
CRP (µg/mL)	3.0 (1.2-13.3)	14	3.3 (1.4-9.45)	12	0.959
APN (µg/ml)	6.6 (5.1-16.3)	15	14.9 (7.73-27.57)	12	0.213
IL-6 (pg/mL)	2.00 (2-3.3)	15	4.83 (2-14.44)	12	0.056

TNF- α (pg/mL)	6.72 (6.01-9.6)	15	9.21 (8.65-10.61)	12	0.075
hsTnT (pg/mL)	25.8 (13.5-45.7)	15	23.4 (13.5-63.6)	12	0.867
Markers of immune infiltration (cells/mm2)					
CD3+	9 (7-11)	14	8 (7-17)	13	0.450
CD4+	5 (4-6)	14	4 (3-6)	13	0.472
CD45Ro+	6 (4-8)	14	6 (5-7)	13	0.961
CD68+	5 (2-5)	14	3 (3-5)	13	0.920
CD54+	0 (0-1)	14	0 (0-2)	13	0.324
HLA-DR+	5 (4-5)	14	5 (4-6)	12	0.921
Myocardial adiponectin receptors (ng/mg)					
T-cad	45.654 (32.184-65.583)	13	26.805 (15.926-50.362)	15	**0.058**
Medications used n, (%)					
ACEI/ARB n, (%)	13 (86.7)	15	10 (76.9)	13	0.512
Beta-blockers n, (%)	15 (100)	15	13 (100)	13	
Mineralocorticoid receptor blockers	13 (86.7)	15	12 (92.3)	13	1
Diuretics n, (%)	13 (86.7)	15	12 (92.3)	13	0.627

Data are presented as median (interquartile range) or n (%). Significant differences are bolded (Wilcoxon rank-sum test, χ^2 test for equality of proportions).

Abbreviations: LVEF: left ventricular ejection fraction; GLS: global longitudinal strain, BNP: B-type natriuretic protein, CRP: C-reactive protein, APN: adiponectin, IL-6: interleukin-6, TNF-α: tumor necrosis factor α, hsTnT: high sensitivity troponin T, CD3+: T cell receptor, CD4+: T helper cell receptor, CD45Ro+: memory T cell receptor, CD68+: monocyte/macrophage receptor, CD54+: Intercellular Adhesion Molecule 1, HLA-DR+: Human Leucocyte Antigen isotype DR, T-cad: T-cadherin, ACEI: angiotensin-converting enzyme inhibitors, ARB: angiotensin receptor blocker.

Patients with mean PCWP values above 19 mmHg had a tendency towards lower cardiac T-cad (26.805±34.436 vs. 45.654±33.398), p=0.058 (Figure 24).

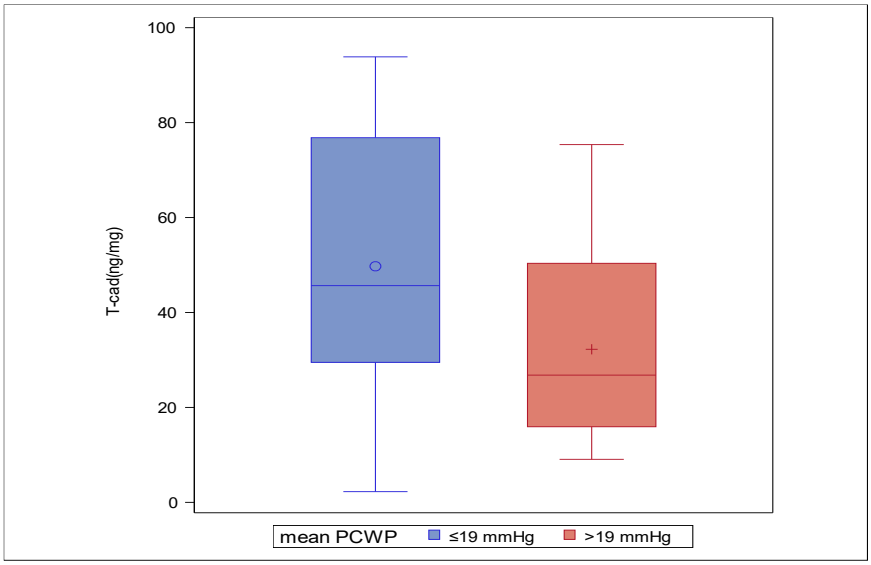

Figure 24. Cardiac T-cad expression in patients based on their mean PCWP cutoff value

Cardiac T-cad levels are lower in more advanced HF concomitantly with decreased LVEF, LV average global strain, and higher BNP. However, the association between mean PCWP and other already established biomarkers of chronic HF (LVEF, BNP, GLS) seems to be stronger, as shown in Table 18.

Patients with mean PCWP above 19 mmHg, and herewith lower myocardial T-cad level, had a higher circulating IL-6 and circulating TNF-α concentration **(**4.83 vs. 2.00 pg/mL, p=0.056 and 9.21vs 6.72 pg/mL, p=0.075 respectively) compared to patients with mean PCWP below 19 mmHg, although the differences did not reach statistical significance (Table 18).

4.5.3 The role of myocardial T-cadherin levels in outcome prediction

Univariate Cox regression analysis did not prove T-cad to be an outcome predictor: HR for T-cad was 1.00. p=0.944, CI 95% (0.973-1.026). The impact of bivariate T-cad on the patient outcome was also tested. Patients were divided into two groups, based on the median T-cad value (39.984 ng/mg). Differences in baseline characteristics of patient groups stratified by median T-cad value are depicted in Table 19.

Table 19. Differences in patient groups divided by T-cad median (39.984 ng/mg)

	T-cad≤39.984 ng/mg		**T-cad >39.984 ng/mg**		**p value**
Variable	**Median (interquartile range)**	**No. of pts.**	**Median (interquartile range)**	**No. of pts.**	
Age (years)	53 (50-58)	14	50.5 (46-55)	14	0.322
Male gender, n (%)	11 (78.6)	14	10 (71.4)	14	1
BMI (kg/m2)	27.8 (25.2-31.7)	14	28.6 (25.6-33.9)	14	0.696
GFR (ml/min)	105.8 (83.7-122.2)	11	103.1 (91.4-120.6)	13	0.733
NYHA class, n (%)		14		14	0.540
II	3 (21.4)		1 (7.1)		
III	8 (57.1)		11 (78.6)		
IV	3 (21.4)		2 (14.3)		
iDCM, n (%)	10 (71.4%)	14	2 (15.4%)	13	**0.003**
Serum biomarkers					
BNP (pg/mL)	381 (74.8-1793.6)	13	160 (38.2-764.1)	14	0.43
CRP (µg/mL)	1.8 (1.1-4.2)	13	6.7 (1.5-20.6)	13	0.106
Adiponectin (µg/ml)	10.8 (6.5-15.3)	13	7.3 (4.3-30.71)	14	0.867
IL-6 (pg/mL)	2.00 (2-4.62)	13	2.38 (2-4.86)	14	0.8
TNF α (pg/mL)	8.47 (6.72-9.80)	13	8.94 (6.62-9.74)	14	0.981
Echocardiographic parameters					
LVEF (%)	30 (21-34)	14	30 (20-39)	14	0.889
LVEDD (cm)	6.7 (6.3-7.2)	14	6.5 (6.2-6.9)	14	0.503
GLS	-8.97 (-10.41-6.14)	8	-12.25 (-13.59-9.01)	9	0.093
Hemodynamic parameters					
Mean AoP (mmHg)	97 (90-107)	12	101 (100-104)	14	0.587
Mean PCWP (mmHg)	23 (18-31)	14	16 (13-21)	14	0.062
Mean PAP (mmHg)	34 (28-38)	14	24 (21-34)	14	0.112
Mean RAP (mmHg)	10 (7-16)	14	10 (6-13)	14	0.612
Markers of immune infiltration (cells/mm2)					
CD3+	13 (8-17)	14	7 (5-8)	13	**0.005**
CD4+	5 (4-6)	14	4 (3-6)	13	0.503
CD45Ro+	7 (5-8)	14	5 (3-8)	13	0.13
CD68+	4 (2-5)	14	4 (3-5)	13	0.67
Medications used					
ACEI/ARB n, (%)	11 (78.6)	14	12 (85.7)	14	1
Beta-blockers n, (%)	14 (100)	14	14 (100)	14	
Mineralocorticoid receptor blockers n (%)	13 (92.9)	14	12 (85.7)	14	1
Diuretics n, (%)	13 (92.9)	14	13 (92.9)	14	1

Data are presented as median (interquartile range) or n (%). Significant differences are bolded (Wilcoxon rank-sum test or χ^2 test for equality of proportions). Significant at the p level 0.05 (2-tailed).

Abbreviations: BMI: body mass index, GFR: glomerular filtration rate, iDCM: inflammatory dilated cardiomyopathy, BNP: B-type natriuretic protein, CRP: C-reactive protein, IL-6: interleukin-6, TNF-α: tumor necrosis factor α, hs TnT: high sensitivity troponin T, LVEF: left ventricular ejection fraction, LVEDD: left ventricle end-diastolic diameter, AoP: aortic pressure; PCWP: pulmonary capillary wedge

pressure; PAP: pulmonary artery pressure; RAP: right atrial pressure, CD3+: T cell receptor, CD4+: T helper cell receptor, CD45Ro+: memory T cell receptor, CD68+: monocyte/macrophage receptor, CD54+: Intercellular Adhesion Molecule 1, HLA-DR+: Human Leucocyte Antigen isotype DR, Adipo R1: adiponectin receptor 1, ACEI: angiotensin-converting enzyme inhibitors, ARB: angiotensin receptor blocker.

Patients with the T-cad value above the median had a lower myocardial CD3+ infiltration (Table 19). Patients with lower cardiac T-cad levels (≤39.984 ng/mg) experienced a worse outcome during five years of follow-up in the cohort studied (Figure 25).

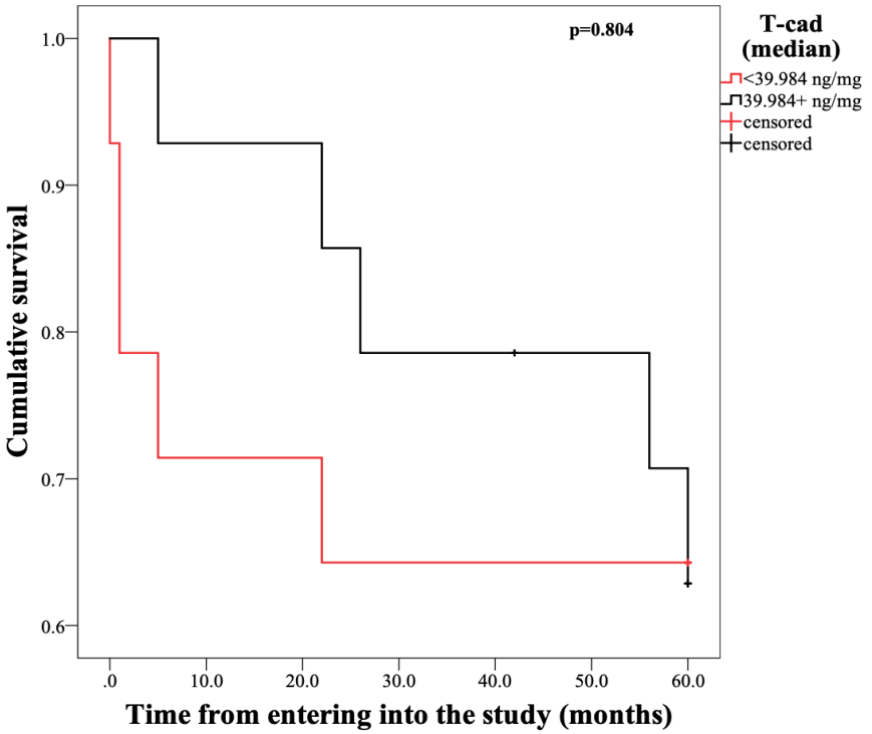

Figure 25. Kaplan-Meier curves categorized by the T-cadherin median. 5-year follow-up.

However, as the difference between the curves did not reach statistical significance (p=0.804), the data cannot be applied to all patients with NI-DCM and chronic HF.

5. DISCUSSION

In the present investigation, it was shown that baseline APN levels are positively associated with already established parameters of chronic HF, such as BNP, IL-6, hemodynamic parameters and parallel the clinical worsening of HF. Baseline APN levels above 15.28 ug/ml were linked to a three-fold increased relative risk of poor composite 5-year outcome in patients with chronic NI-DCM. Serum APN remained the main outcome predictor after having adjusted for other relevant covariates such as age, gender, NYHA class, GFR, BMI, serum BNP, serum IL-6, serum TNF-α levels, LVEF, mean PCWP, and cardiac CD3+ count. GLS appeared to be the most powerful outcome predictor.

5.1 Adiponectin as a biomarker of heart failure severity in patients with NI-DCM and chronic heart failure

In the present investigation, the relevance of APN as a biomarker indicating HF severity was tested in the first place. We were interested if elevated circulating APN levels may provide similar information about worsening HF compared to other well-established serum, echocardiographic, and hemodynamic parameters. There was a strong positive association between circulating APN and BNP levels in the present study comprised of patients with chronic NI-DCM that fully corresponds to the findings of other studies concerning chronic HF (28, 176, 270). As suggested by previous studies, elevated levels of serum BNP may at least partly drive the levels of circulating APN upwards (95, 96). The increase in serum levels of APN might be caused either by an indirect lipolytic effect of natriuretic peptides or directly through stimulating APN secretion in adipose tissue.

No association between circulating APN and LVEF was found, corresponding to the findings of several previous studies (28, 174, 186). However, there was a strong correlation between circulating APN and another echocardiographic parameter-GLS. LVEF represents the percentage change of LV chamber volume, but not the myocardial contractility. The myocardial strain is based on the speckle-tracking method and can be used for the objective and reliable assessment of systolic function independently of LVEF (271). Moreover, Park JJ. et al. have shown that GLS has a higher prognostic value than LVEF in patients with acute HF, but it was unclear whether the current study findings can be applied to patients with NI-DCM and advanced HF (272). The present study revealed that patients with elevated APN levels

have a more positive GLS estimate. As far as we know, this association is demonstrated for the first time. The strong positive correlation lets us assume that APN might be a surrogate marker of LV dysfunction.

APN correlated positively with intracardiac pressures, such as mean RAP, PAP, and PCWP in the present study. Worsening of HF, as indicated by higher mean PCWP, which is an indirect marker of LV end-diastolic pressure, was associated with higher serum APN concentration. The positive correlation between APN and mean RAP and PAP was also demonstrated in patients with chronic HFrEF by Tsutamoto et al. (176). However, no correlation between mean PCWP and circulating APN was found in the latter study. APN levels are also related to the clinical severity of HF: APN levels were elevated in more pronounced HF as defined by NYHA functional class in the present study. This is in agreement with other studies demonstrating elevated APN levels in patients with worsening chronic HF (174, 178, 180, 181, 184) and is fully concordant to the data provided by Huang et al. in the cohort of patients with NI-DCM and advanced HF (181).

In conclusion, the present investigation reveals that circulating APN levels are elevated in patients with more severe HF as defined by higher NYHA functional class, higher serum BNP levels, and increased mean PCWP values. In patients with chronic NI-DCM, higher serum APN levels are related to the worsening of myocardial function, as indicated by more positive GLS.

5.2 Adiponectin levels predict poor 5-year outcome in patients with NI-DCM and chronic heart failure

To our knowledge, none of the clinical studies has demonstrated the association between baseline serum APN levels and 5-year outcome in the cohort of patients with NI-DCM and chronic HF. Tamura et al. showed that the increase in the serum APN level was associated with higher mortality exclusively in the ischemic CHF patients, but not in the sub-group of NI-DCM patients after a median of 1.8 years of follow-up (28). However, the subgroups in this study were small, limiting generalization of conclusions regarding patients with NI-DCM. Wojciechowska and colleagues analyzed the cohort of 127 NI-DCM patients and concluded that elevated baseline serum APN levels were associated with a higher 3-year relative risk of urgent transplantation and composite outcome (urgent transplantation and death) (29).

The present investigation examined the role of circulating APN as a long-term outcome predictor in a well-described cohort of patients with chronic NI-DCM. Baseline APN levels were significantly higher in patients who experienced composite outcome in the present study. Unadjusted APN

increased the risk of composite outcome (cardiovascular death, HT, and LVAD implantation) in patients with NI-DCM and CHF. This finding is in agreement with studies demonstrating the association between poor outcome and elevated APN levels in CHF patients unstratified with respect to disease etiology (24, 174, 180, 184, 188). Multivariate Cox regression analysis demonstrated that APN is an independent outcome predictor in patients with NI-DCM and CHF, irrespective of other outcome affecting covariates, such as serum IL-6, BNP levels, and cardiac CD3+ count. IL-6 levels have also remained a significant outcome predictor in the model mentioned above. These findings are consistent with the previous studies where BNP and IL-6 have been shown to be independently related to cardiovascular events and death in CHF (202, 273-275).

The association between APN and the composite outcome was adjusted for main HF medications consumed by the patients, including ACEI/ARB, beta-blockers, mineralocorticoid receptor blockers, and diuretics in the regression tree analysis. APN remained valid as a prognostic marker despite the inclusion of the medications into the model.

The findings of the present study show that APN can be used as a long-term outcome predictor in patients with chronic NI-DCM alongside with the well-established serum biomarker BNP.

The role of serum adiponectin in predicting the outcome of patient groups based on cardiac immune infiltration

In the subgroup analysis, we tested the impact of baseline APN on the outcome of the patients with iDCM and non-inflammatory DCM separately. Elevated serum APN levels increased the relative risk of the composite 5-year outcome in patients with iDCM. This finding is contradictory to the one revealed by Bobbert and colleagues, who demonstrated the positive impact of higher baseline APN levels on myocardial inflammation and cardiac performance parameters, indicating a better outcome in patients with iDCM (239). These were the patients with a short duration of the symptoms (34.5±5.65 days) prior to enrolment to the study, classified as NYHA class II-III. iDCM patients with higher baseline APN levels had a lower cardiac immune infiltration (CD3+, CD45Ro+), smaller heart chambers, and higher LVEF after 6 months of follow-up. No association between circulating APN levels and outcome was found in patients with non-inflammatory DCM in this study.

In search of adiponectin cutoff value.

The cutoff point of serum APN varied in earlier studies of patients with chronic HF with no optimal threshold, portending poor outcome, being

determined. The cutoff point revealed in the present study (15.28 μg/ml) corresponds to the one determined by the study of Van Berendoncks et al. in patients with CHF (188). The threshold value of serum APN determined by Van Berendoncks and colleagues was 15.2 μg/ml. The relative risk experiencing composite outcome was more than three folds higher in those with their APN value above the current investigation's cutoff. The difference in survival function between the two groups was demonstrated, starting from 6 months of follow-up and was present after one, three, and five years of follow-up. Survival tree analysis including covariates linked to the outcome (age, sex, NYHA class, glomerular filtration rate, BMI, serum BNP, IL-6, TNF-α levels, LVEF, mean PCWP, cardiac CD3+ cell count) revealed that APN levels above 15.28 μg/ml remain the most significant marker indicating poor outcome.

Adiponectin as a candidate for a multi-marker approach in outcome prediction.

Having in mind that multi-marker approaches containing different combinations of established and novel biomarkers might improve HF risk prediction, factor analysis was performed. It revealed that higher serum APN, BNP levels and mean PCWP count combined indicate a bigger relative risk for a poor outcome. The combination of those parameters at baseline might be a more precise outcome predictor compared to single parameters.

Altogether, our findings suggest that circulating APN levels at baseline can serve as a biomarker of HF severity and predict long-term composite outcome in patients with NI-DCM and chronic HF. The adipokine might also serve as one of the candidates in a multi-marker approach predicting outcome in these patients. However, the particular mechanisms relating APN to the progression of HF remain elusive. There are at least three possible explanations of the association between increased serum APN and a poor outcome. First of all, it is possible that elevated APN levels merely reflect the increase in natriuretic peptides and is simply a marker of disease severity. Secondly, APN levels might increase as a consequence of tissue APN resistance, and in this case, APN is unable to realize its full cardioprotective effect, which might add up to a poor outcome. Finally, elevated APN levels may directly add to the worsening of HF, for example, by the possible proinflammatory behavior in chronic and advanced HFrEF. Those theories should be ruled out by extensive prospective clinical studies, including genetic data, as well as coordinated basic science and physiological studies in humans, as it was proposed by Menzaghi and colleagues (85).

5.3 The role of serum adiponectin and its myocardial receptor T-cadherin in inflammation control of patients with NI-DCM and chronic heart failure

In the present investigation serum APN positively correlated with TNF-α. Serum levels of APN paralleled the increase of other systemic inflammatory markers, such as IL-6, TNF-α with increasing NYHA functional class, indicating an increased proinflammatory milieu in patients with more advanced HF. No correlation between serum APN and cardiac inflammatory infiltrate was found. Myocardial T-cad levels correlated negatively with cardiac CD3+, indicating their role in local inflammation control mediated through APN.

The connection between systemic inflammation and heart failure severity.

A series of previous studies have demonstrated the positive correlation between increased serum levels of proinflammatory cytokines and the worsening of functional (NYHA class) and cardiac performance (LVEF) in CHF (276-278). In addition to being involved in the pathogenesis of CHF, inflammatory cytokines and related mediators are also used for risk stratification and outcome prediction in patients with HF. Raised proinflammatory cytokine and their receptor levels (i.e., TNF-α, TNF, Il-6, galectin-3) were related to poor outcome in patients with CHF in a series of studies (234, 235, 237, 274, 275). IL-6 was shown to be an independent outcome predictor in particular in patients with chronic NI-DCM (270). In the latter study, patients with NI-DCM mainly in NYHA class II-III and were followed for a mean of 42 months. Increased IL-6 levels were associated with a higher relative risk of cardiac events.

The findings of the present study fully correspond to the findings mentioned above as TNF-α, and IL-6 levels were higher in more advanced HF and increased in parallel with NYHA functional class. Besides, TNF-α and IL-6 levels correlated positively to each other as well as serum BNP substantiating the idea of increasing systemic inflammation with worsening of CHF due to NI-DCM. Serum levels of proinflammatory mediators IL-6, CRP correlated inversely with the cardiac performance parameter LVEF, and the amount of circulating IL-6 was positively associated with mean PCWP values. Baseline IL-6 levels were a significant 5-year composite outcome predictor in the present study, even after adjusting for other possible confounders (i.e., APN, BNP, NYHA, cardiac CD3+ count).

To sum up, the worsening of HF, together with increasing neurohormonal activation, is accompanied by higher levels of proinflammatory markers in patients with NI-DCM and CHF. It is plausible that homeostatic disturbances in tissues due to the worsening of CHF add to the growing proinflammatory milieu and eventually lead to a vicious circle.

The relationship between circulating levels of adiponectin and serum inflammatory mediators

Circulating APN levels are positively associated with serum TNF-α in the present study. The correlation between serum APN and IL-6 did not reach statistical significance. The findings are in line with the data of previous studies in patients with chronic HF, which have demonstrated a positive association between serum APN and TNF-α levels (178, 185). Bobbert et al. have shown a positive correlation between serum APN and TNF-α receptor 1(TNFR1), which is a marker for activated TNF-α signaling, in patients with iDCM. (239).

Considering the relationship between serum inflammatory mediators and APN, no correlation between serum APN and hsCRB was found in the present study. Nevertheless, CRB was positively correlated to serum BNP, IL-6 levels, and galectin-3 levels. The positive correlation between baseline serum APN and CRB levels was shown in iDCM patients by Bobbert et al. (239). Ishikawa et al. demonstrated that serum hsCRP was correlated to IL-6, but not BNP levels and was an independent predictor of cardiac events (death from cardiovascular causes and hospitalization) as well as mortality in patients with NI-DCM (270). Galectin-3 did not correlate to serum APN in the present study but was strongly associated with other circulating markers of inflammation (CRP, IL-6, and TNF-α).

Having in mind the data of the current study, the production of APN by adipocytes seems to parallel the activation of neurohormonal and inflammatory axes in chronic NI-DCM and CHF, simultaneously indicating poor outcome.

The relationship between circulating adiponectin levels and cardiac inflammation

No correlation between circulating APN levels and cardiac immune infiltration was found in the present study. This is contrary to the findings of Bobbert et al., who demonstrated a positive correlation between baseline APN and myocardial CD3+, CD45 count in patients with iDCM (239). A worse outcome was indicated in patients with DCM and higher cardiac inflammatory infiltrates in the study of Kinderman et al. (70). In agreement with this study, unadjusted CD3+ was also shown to be associated with poor composite

outcome in our study. However, myocardial CD3+ count lost its significance after adjusting with APN, BNP, and IL-6 values in the multivariable model provided in the present investigation.

In conclusion, no association between serum APN and markers of cardiac immune infiltration was demonstrated in the current cohort of patients with NI-DCM. Unadjusted CD3+ count was linked to the poor outcome in these patients.

The role of myocardial T-cadherin levels in cardiac inflammation

T-cad has a significant role in the expression of the APN cardioprotective effect (32, 33, 127), so changes in T-cad expression or functionality might also lead to changes in APN activity. A negative correlation between myocardial T-cad level and CD3+ count was demonstrated in the present investigation. Lower levels of T-cad seem to be associated with more abundant cardiac T lymphocyte infiltration, cardiac inflammation. We hypothesize that APN, through its receptor T-cad, might execute the local anti-inflammatory activity in patients with NI-DCM and CHF. Having in mind that cardiac inflammation in DCM might lead to LV dysfunction (47), higher cardiac T-cad expression might also be associated with local inflammation control and a better preserved cardiac function. Larger studies are needed to confirm the hypothesis. Moreover, it was shown that TNF-α antagonizes APN action by downregulating AdipoR1 expression in endothelial cells (240). Having in mind the increased systemic inflammation in progressing HF it would be reasonable to test if TNF-α could have the same effect on cardiovascular T-cad expression.

In conclusion, APN levels increase simultaneously with the levels of proinflammatory cytokines (Il-6, TNF-α) and parallel the clinical worsening of HF in patients with NI-DCM and CHF. APN and IL-6 appear to be independent poor long-term outcome predictors. Furthermore, a negative correlation between cardiac T-cad count and myocardial CD3+ infiltration suggests that diminished anti-inflammatory (cardio-protective) APN activity might be related to reduced T-cad levels in the myocardium.

5.5 The relation between T-cadherin levels and heart failure severity and outcome in patients with NI-DCM and chronic heart failure

In the current investigation, there was a negative correlation of T-cad with mean PCWP value, suggesting a possible decrease in T-cad levels with worsening of HF, increasing congestion. However, the differences between T-cad levels were not significantly different in the patient groups stratified by a mean PCWP cutoff (19 mmHg). Based on the data of the present study, the hypothesis that T-cad levels are diminished in patients with more advanced HF, could not be proved. T-cad was not related to significant changes in the relative risk of poor outcome in the current investigation. No correlation between serum APN levels and cardiac T-cad concentration was found.

The differences in myocardial T-cadherin levels based on HF severity

Growing APN levels have been repeatedly documented in patients with progressing CHF and are linked to the poor outcome (24, 28, 174, 188). Data suggest that increased APN levels in CHF patients might be compensatory due to diminished biological activity of APN in tissues, "APN resistance" (186, 194, 279). One of the possible mechanisms explaining APN resistance is the diminished expression or functionality of APN receptors. Wang and colleagues have demonstrated that different molecular mechanisms might be involved in APN resistance (280). Phosphorylation and desensitization of AdipoR1, blocking the anti-oxidative, anti-nitrative, anti-inflammatory, and cardio-protective function of APN, was documented in the early period (peaking at day 7) after MI in the murine model of ischemic HF. A significant decrease in the APN receptor (AdipoR1, AdipoR2, and T-cad) expression was documented 28 days post-MI, which would correspond to CHF. Therefore, it seems that APN receptors, including cardiac T-cad, are downregulated in CHF, leading to the diminished cardio-protective activity of APN (34).

Data concerning the changes in myocardial APN receptors in clinical studies of HF are quite contradictory. Khan et al. demonstrated decreased APN receptor expression in the failing myocardium, which was reversed after mechanical unloading (194). On the opposite, the increase in AdipoR1 and AdipoR2 expression was documented in patients with end-stage HF by Kreth and colleagues compared to healthy controls (281). Nevertheless, none of the studies mentioned above evaluated the changes in myocardial T-cad expression. To our knowledge, the present study is the first clinical study evaluating the levels of T-cad in human myocardium in patients with NI-DCM and chronic HF as well as presenting the results of 5 years of follow-up.

In the present study, a significant negative correlation between the levels of cardiac T-cad and mean PCWP values was demonstrated. In the subgroup analysis, patients with more advanced HF (mean PCWP >19 mmHg) had a tendency towards lower levels of myocardial T-cad. Cardiac T-cad levels were reduced in more advanced HF group concomitantly with lower LVEF, worse GLS higher BNP values, indicating that lower T-cad levels might be related to disease severity. However, the association between mean PCWP and other biomarkers of CHF (LVEF, BNP, GLS) seemed to be stronger. The present study showed that APN levels are elevated in patients who experienced the study outcome (LVAD implantation, HT, or death from cardiovascular causes) and increased with clinical worsening of HF, indicating a worse 5-year outcome. These findings are in line with the concept of the "adiponectin paradox", described in patients with CHF. Cox regression analysis did not prove T-cad levels to be a significant prognostic factor in patients with NI-DCM and CHF.

In the subgroup analysis, patients with T-cad concentration below the median had a worse outcome. However, the difference between the curves was not statistically significant.

In light of the findings mentioned above, we could hypothesize that more advanced HF, accompanied by higher serum APN levels, might be associated with lower cardiac T-cad levels. Considering the critical role of T-cad for the sequestration of APN to the tissues, it is plausible that diminished myocardial T-cad levels lead to reduced APN signaling in the heart, diminishing its cardio-protective effect.

The association between the levels of circulating APN and myocardial T-cad concentration

Accumulating data suggests that serum APN and tissue T-cad reciprocally influence their expression levels. In the setting of APN deficiency, tissue expression of T-cad was also reduced, suggesting a regulatory axis between the two proteins (32, 33, 128). APN positively regulated the levels of its own receptor/binding protein, T-cad in animal models, and endothelial cell cultures: (32, 128, 261). This feed-forward mechanism was demonstrated by administrating recombinant adenoviral APN to APN-KO mice, which lead to the accumulation of APN in aorta, heart, skeletal muscle concomitantly upraising the T-cad protein levels. Matsuda et al. demonstrated that circulating APN enhances the stability of T-cad in tissues by protecting it from the enzymatic cleavage with phospholipases (128). In turn, T-cad seems to be a critical modulator of APN levels. It acts as a negative regulator of circulating APN inducing APN clearance from the blood and sequestering it to the tissues (31). A significant elevation in plasma APN levels has been demonstrated in

T-cad-KO mice (32, 128). Genome-wide association studies in humans have linked variants in the T-cad gene to elevated or decreased circulating APN levels, supporting the idea that circulating APN is influenced by the presence and functionality of T-cad (282, 283).

Despite our expectations, no positive correlation between circulating APN and cardiac T-cad concentration was detected in our patient cohort. We suggest that it might be caused by the impairment of the feed-forward regulation mechanism, as suggested by Sternberg et al. (265). Dysregulation of the positive feedback mechanism could lead to lower myocardial levels of T cad, diminished tissue sequestration of APN, and increased levels of circulating APN. On the other hand, the positive relationship between circulating APN and cardiac T-cad, which is well documented in experimental studies, might not apply to humans with NI-DCM and advanced HF.

In the present study, we retrospectively assessed the myocardial T-cad concentration of patients with NI-DCM and looked for its possible role in APN mediated cardioprotection as well as NI-DCM progression. The findings suggest that T-cad levels are lower in patients with increasing mean PCWP values and might be related to HF severity. However, because of the small cohort of patients, the hypothesis warrants further testing in a more numerous study or even multicenter study. Post-hoc power analysis revealed that at least 30 patients in each subsequent group are required in order to confirm/reject the assumption that T-cad is lower in patients with more severe HF.

6. CONCLUSIONS

1. APN might be considered as an additional biomarker of HF severity in patients with NI-DCM and CHF. Circulating levels of APN positively correlated with plasma BNP, GLS, mean PCWP count, and were related to HF severity.
2. The increased baseline levels of serum APN portend poor 5-year outcome in patients with NI-DCM and CHF. The obtained threshold value of APN was associated with increased (by three folds) relative risk of the composite outcome (LVAD, HT, and death from cardiovascular causes).
3. The increased serum levels of APN positively correlated with a marker of systemic inflammation TNF-α in patients with NI-DCM and CHF. The concentration of APN paralleled the increase of circulating proinflammatory cytokines (IL-6, TNF-α) with clinical worsening of HF. No correlation between plasma APN and myocardial inflammation was confirmed.
4. Elevated levels of myocardial T-cad were associated with lower cardiac CD3+ infiltration indicating its role in local inflammation control. Myocardial T-cad was not proved to be a biomarker or outcome predictor in patients with NI-DCM and CHF.

7. LIMITATIONS

The study design might limit the strength of our conclusion. The present study was a single-center study analyzing a small number of subjects. Considering the prevalence of NI-DCM and the invasiveness of the investigation, it is complicated to accumulate enough participants in one center. Therefore, a multicenter study is warranted to confirm the relationship between worse prognosis and increased serum APN levels in the NI-DCM and chronic HF patients. Furthermore, there was no control group in the current investigation. The retrospective T-cad sub-investigation contained even a smaller number of patients and lacked a control group. It was a pilot investigation, aimed at preliminary hypothesis testing. A posthoc power analysis revealed that at least 30 patients should be involved in each respective group for testing the hypothesis that T-cad concentration is lower in patients with more severe HF (considering that the study power is 80% and α level - 0.05, with the mean value of T-cad is 30.00 ng/mg, SD 20.00 ng/mg and the significant change in T-cad expression 15.00 ng/mg).

8. PRACTICAL RECOMMENDATIONS

1. Elevated baseline levels of serum APN (>15.28mg/ml) can serve as an additional prognostic marker for selecting patients with the worst prognosis and the need for HT in patients with chronic NI-DCM.
2. A further step would be a trial comparing NI-DCM patients with mild vs. advanced HF. Such trial would test the hypothesis that myocardial T-cad levels are reduced in advanced HF. The data received could provide insights into the pathogenesis of diminished APN activity in advanced HF, and T-cad might even become a potential therapeutic target.

9. LIST OF PUBLICATIONS

List of original papers:

1. **Baltruniene V**, Bironaite D, Kazukauskiene I, Bogomolovas J, Vitkus D, Rucinskas K, et al. **The Role of Serum Adiponectin for Outcome Prediction in Patients with Dilated Cardiomyopathy and Advanced Heart Failure**. BioMed research international. Hindawi. 2017, Vol. 2017, Article ID 3818292, [p. 1-13]. **ISSN:** 2314-6133 ; **eISSN:** 2314-6141 ; **doi:** 10.1155/2017/3818292.
2. **Baltruniene V**, Rinkunaite I, Bogomolovas J, Bironaite D, Kazukauskiene I, Simoliunas E, et al. **The Role of Cardiac T-Cadherin in the Indicating Heart Failure Severity of Patients with Non-Ischemic Dilated Cardiomyopathy**. Medicina (Kaunas). 2020 Jan 9; 56(1):27. DOI: 10.3390/medicina56010027 PMCID: PMC7023024; PMID: 31936691
3. **Baltruniene V**, Vitkus D, Jablonskiene V, Grabauskiene V. **Serumo adiponektinas - galimas lėtinio sistolinio širdies nepakankamumo prognozės žymuo.** Laboratorinė medicina. Vilnius : Lietuvos laboratorinės medicinos draugija. ISSN 1392-6470. 2019, t. 21, Nr. 4, p. 187-193. [DB: Index Copernicus (IC)]

Presentations:

1. **Baltrūnienė V**, Žąsytytė I, Bironaitė D, Grabauskienė V. Adiponectin can predict mortality and readmission for heart failure. In: The 8th Baltic Morphology Conference: Interdisciplinary Nature of Contemporary Morphology.- P.76; 2015, Vilnius
2. Bironaitė D, **Baltrūnienė V**, Žąsytytė I, Grabauskienė V. The role of collagen and adiponectin in virus induced dilated cardiomyopathy. In: The 8th Baltic Morphology Conference: Interdisciplinary Nature of Contemporary Morphology.- P.79; 2015, Vilnius.
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4. **Baltruniene V,** Bironaite D, Kažukauskiene I, Augulis R, Grabauskiene V. Serum adiponectin level as an independent predictor of mortality in patients with inflammatory cardiomyopathy and advanced heart failure. In: Baltic Conference Series 2017 (online). Published online by the VBRI Press in 2017, page 7. http://www.vbripress.com/bcs17winter/abstractsbook/ch7tissueengineeringandemhealthcaretechnology.pdf
5. Kažukauskienė I, Balčiūnaitė G, **Baltrūnienė V**, Ručinskas K, Maneikienė V, Grabauskienė V. Left ventricle global longitudinal strain is an independent predictor of poor outcome in patients with nonischemic dilated cardiomyopathy. In: EuroEcho Imaging, 2018, Milan, Italy. European heart journal cardiovascular imaging. Oxford : Oxford University Press. **ISSN** 2047-2404. **eISSN** 2047-2412. 2019, vol. 20, suppl. 1, i627. **DOI:** 10.1093/ehjci/jey267.
6. Kažukauskienė I, **Baltrūnienė V**, Bironaitė D, Čibiras S, Ručinskas K, Grabauskienė V. Prognostic significance of carboxy-terminal telopeptide (ICTP) and Caspase-3 in patients with non-ischemic dilated cardiomyopathy. In: ESC Congress 2019 365 : Paris, France, 31 August-4 September 2019 / European Society of Cardiology. Paris. 2019, abstract no. P5447, p. [1-2].

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