

# Natriuretic Peptides Assessment in Dilated Cardiomyopathy in Patients with Emery-Dreifuss Muscular Dystrophy

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## Abstract

**Introduction:** Levels of natriuretic peptides in blood are often tested for as screening for heart disease and their progress assessed. Dilated cardiomyopathy (DCM) with conduction disturbances is one of the leading serious manifestations in genetically transmitted Emery-Dreifuss muscular dystrophy (EDMD). However, the potential significance of and variability to brain-natriuretic peptides (BNP) and atrial-natriuretic peptides (ANP) in this disease has not been tested hitherto. It seemed worth considering whether estimation of natriuretic peptides might help in defining cardiac dysfunction in the early stages of the disease, prior to the appearance of echocardiographic changes. This is perceived especially important in cardiologically asymptomatic patients, who are still at risk of cardiac sudden death.

**Patients and Methods:** Serum levels of BNP, NT-proBNP, ANP and NT-proANP were quantified by ELISA sandwich immunoassay in 25 EDMD patients (10 autosomal-dominant AD-EDMD, 15 X-linked EDMD), 8 X-EDMD carriers, 9 patients with dystrophinopathy as disease controls, and 20 age-matched healthy controls.

**Results:** Serum levels of BNP, NT-pro-BNP, ANP, and NT-proANP were elevated in the blood of about 50% of patients with both the AD-EDMD and the X-EDMD form. Values were distributed from normal through to highly elevated. In the X-EDMD group there was a marked increase in the ANP and NT-proANP values. The X-EDMD group also manifested a correlation between level of atrial natriuretic peptides, echocardiographic parameters and severity of cardiac symptoms.

**Conclusions:** The presented results indicate that assessment of circulating natriuretic peptides is of limited value in identifying cardiac involvement in EDMD. However, when included to the panel of other cardiologic biomarkers the estimation of natriuretic peptides may offer additional information in respect of proper diagnosis, prognosis, monitoring of appropriate treatment, prediction of outcome. and help to prevent cardiac decompensation and sudden death.

**Keywords:** Cardiac biomarkers; Detection of cardiac involvement; Natriuretic peptides in EDMD

**Abbreviations:** AF: Atrial Fibrillation; VT: Ventricular Tachycardia; AFL: Atrial Flutter; SVT: Supraventricular Tachycardia; AVB: Atrio-Ventricular Block; ICD: Implanted Cardioverter/Defibrillator

## Introduction

It is a deficit of lamins A/C or emerin in skeletal muscle and heart muscle that causes the rare, genetically transmitted disease known as Emery-Dreifuss muscular dystrophy (EDMD). The cell defect is generalized, but skeletal muscles, heart and joints are selectively affected. The clinical symptoms of EDMD are manifested as skeletal muscle atrophy and weakness, joint contractures and dilated cardiomyopathy (DCM) with conduction disturbances. DCM remains clinically silent even for prolonged periods, but unexpected death in still young patients is not a rare event. The pathogenesis of DCM in EDMD is not recognized, as yet.

Several biomarkers of cardiovascular risk are the subject of constant investigation. Cardiac specific troponins, CK-MB, brain natriuretic peptides (BNP) and N-terminal pro-hormone brain natriuretic peptide (NT-proBNP) are often tested. Less frequently atrial natriuretic peptide (ANP) and N-terminal pro-hormone atrial natriuretic peptide (NT-proANP) are examined. Generally there are greater or lesser reservations in regard to nearly all tested biomarkers, including natriuretic peptides. Assessment of the stage of the involvement and advancement of cardiac disease, control of its treatment and establishment of further prognosis

with the aid of cardiovascular biomarkers is difficult and still under debate.

The aim of our study was to compare the diagnostic value of circulating BNP, ANP, NT-proBNP and NT-proANP in order to decide which of the commonly tested natriuretic peptides would be of value in cardiological practice and specifically in detecting cardiomyopathy in EDMD in order to prevent unexpected cardiac decompensation and sudden death, in cardiac asymptomatic patients in particular.

## Materials and Methods

### Patients

A total of 25 patients (19 males and 6 females) with EDMD were included in this study. Ten patients had autosomal-dominant EDMD associated with laminopathy (AD-EDMD), with one patient in this

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Received June 04, 2012; Accepted July 04, 2012; Published July 15, 2012

**Citation:** Niebroj-Dobosz IM, Sokołowska B, Madej-Pilarczyk A, Marchel M, Hausmanowa-Petrusewicz I (2012) Natriuretic Peptides Assessment in Dilated Cardiomyopathy in Patients with Emery-Dreifuss Muscular Dystrophy. J Clin Exp Cardiol 3:206. doi:10.4172/2155-9880.1000206

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group being after heart transplantation (age  $31 \pm 4$  years), 15 had X-linked EDMD (X-EDMD) associated with emerinopathy (age  $28 \pm 3$  years), and 8 were X-EDMD carriers (age  $37 \pm 5$  years). The diagnosis of AD-EDMD and X-EDMD was established clinically and confirmed genetically by reference to the genetic defect in the *LMNA* and *EMD* gene, respectively. The group of disease controls included 9 patients with Duchenne/Becker progressive muscular dystrophy (DMD/BMD). The diagnosis in these cases was based on clinical status, creatine kinase activity in blood and a lack/decrease of dystrophin expression noted on immunohistochemical/immunochemical examination of skeletal muscles biopsy.

Cardiomyopathy was diagnosed in all the EDMD patients (Table 1). In the AD-EDMD group the progression of cardiac symptoms was either mild/moderate in 5 cases, severe/very severe in the other 5. Two patients died, and one patient who was not taken into account in the statistical calculations of natriuretic peptides was after heart transplantation. The cardiac parameters on echocardiography were out of the normal range in only two AD-EDMD patients. In 5 cases a pacemaker, while in a further 2 a cardiac cardioverter-defibrillator had been implanted. In the X-EDMD group the cardiac involvement was

either moderate (in 12 cases), or mild (in 3 cases). The echocardiographic parameters had been changed in 3 cases. A pacemaker was implanted in 12 of the 15 patients. Mild/moderate cardiac symptomatology was also found in the X-EDMD carriers. In 2 of the 8 carriers pacemaker had been implanted. In the DMD/BMD group dilated cardiomyopathy was manifested as echocardiographic abnormalities, while in some patients severe heart failure symptoms were present. In neither of the EDMD groups was it a straightforward matter to track the development of cardiomyopathy. Even patients with evident bradycardia usually had their cardiac symptoms detected once the first neurological diagnosis of EDMD had been established. The control group consisted of 20 age-matched normal subjects with no history of cardiac disease.

Skeletal muscle atrophy in AD-EDMD was mild in 2 cases, moderate in 4 and severe/very severe in further 4. In X-EDMD skeletal muscle atrophy was present in 12 cases, being either mild/moderate, or severe (3 cases).

### Analytical measurements

Blood was collected for routine biochemical analyses, and centrifuged at 3000 rpm for 10 minutes. Serum was separated and

Description	X-EDMD (n=15)	AD-EDMD (n=10)	X-EDMD carriers (n=8)
Family history	familial (14) sporadic (1)	familial (7) sporadic (3)	familial (8)
Age (yrs)	25 (18-36)	29 (22-31)	33 (28-51)
Skeletal muscle involvement	elbow, ankle contractures and spine rigidity (15) generalized muscle atrophy (3) arms, calf atrophy (5) arm atrophy (2)	elbow, ankle contractures and spine rigidity (9) generalized atrophy (6) arms, tights (2) arms, calf atrophy (1) wheelchair-bound (2)	no skeletal muscle involvement
Cardiac symptoms	AVB 2/3 (7) AF/AFL (5) tachy-brady (3)	heart failure (3) (including NYHA III (1), NYHA II/III (2)) AVB 3 (3) AF (4) SVT (3) VT (1)	AVB 2/3 (2)
Cardiac device/age of implantation	pacemaker/14-33 yrs (11)	Pacemaker/20-41 yrs (5), then ICD/28-29yrs (2 of these 5)	Pacemaker/42-44 yrs (2)
Mutation	1A>G (2) 3G>A (1) 153delC (6) 192G>T, 194insC (1) 256C>T (1) 266-27del18 (1) 397C>T (1) 399+1G>C (1) 450insG (1)	334_336del (1) 788T>C (1) 743T>C (1) 1072G>A (1) 1337A>T (1) 1357C>T (4)	153delC (4) 399+1G>C (2) 450insG (2)
CK-MB (%) A	3.2 (2.6-6.4) (n=11)	3.4 (2.5-7.7) (n=7)	9.9 (2.0-10.5) (n=5)

Values are presented as medians and interquartile ranges

Number of patients is shown in parentheses

A Values in normals: 2.2 (0.0-2.8) (n=20)

Abbreviations: AF - atrial fibrillation; VT - ventricular tachycardia; AFL - atrial flutter; SVT - supraventricular tachycardia; AVB atrio-ventricular block; ICD - implanted cardioverter/defibrillator

**Table 1:** Summary of clinical and laboratory data in patients with Emery-Dreifuss muscular dystrophy

frozen at -30°C until used. The level of the biomarkers was determined using an ELISA sandwich enzyme immunoassay for BNP, ANP and NT-proBNP using the USCN Life Science INC kit, to test for NT-proANP a Biomedica Gruppe kit was used. Absorbance at 450 nm was assessed using a Sigma Diagnostics EIA Microwell Reader II.

### Statistical analyses

The analysis was performed using a commercial statistical program (Statistica 5, StatSoft, Poland). The medians and interquartile ranges are presented, because data distributions were non-normal. Use was made of analysis of variance on ranks with the Kruskal-Wallis test. Differences in variable values were assessed using the Mann-Whitney U test, while the relationships between variables were analyzed using Spearman's correlation coefficient ( $\rho$ ). The ROC analysis was also included. The  $p < 0.05$  value was considered to be statistically significant.

### Results

Serum levels of different natriuretic peptides were significantly elevated in the EDMD patients, through values were distributed across a wide range-from normal to highly elevated in both EDMD forms (Table 2). An elevated level of ANP and NT-proANP appeared more often in X-EDMD, as compared with the AD-EDMD group (Figure 1). To assess the usefulness of natriuretic peptides levels estimation in diagnostics the areas under the ROC curves and (AUC) were calculated (Figure 2 and 3). It is indicated that both the sensitivity and specificity of natriuretic peptides, although limited, allows for the detection of the disease status in some of the EDMD patients. In the X-linked EDMD patients there was a significant correlation between BNP and ANP ( $\rho = 0.77$ ,  $p = 0.003$ ), ANP and LVDD ( $\rho = 0.73$ ,  $p = 0.016$ ), NT-proANP and LVDD ( $\rho = -0.69$ ,  $p = 0.014$ ) and NT-proANP and LAD ( $\rho = -0.71$ ,  $p = 0.006$ ). In 8 X-EDMD carriers certain values were raised (in 4 for BNP, in 5 for NT-pro-BNP, in 1 for ANP, and in 2 for NT-proANP). Very much raised values for all natriuretic peptides were present in all cases with dystrophinopathies, also the asymptomatic ones (in five of the nine examined) (Table 2). Raised natriuretic peptides were detected also in carriers of X-EDMD (in two of the eight tested). Values for natriuretic peptides did not correlate with age. A correlation with the severity of cardiac symptoms was observed when atrial natriuretic peptides were assessed. The results were at the threshold of significance in AD-EDMD ( $\rho = 0.55$ ,  $p = 0.050$ ), while in X-EDMD the correlation achieved significance ( $\rho = 0.65$ ,  $p = 0.023$ ).

### Discussion

Several biomarkers of heart diseases of various etiologies are currently introduced in the cardiological practice. Detection of heart diseases in an early stage may arrest their progress, delay the development of serious heart failure symptoms, allow for the introduction of appropriate cardiological treatment and provide

for predictions of cardiovascular mortality Among biomarkers brain natriuretic peptide (BNP) and N-terminal prohormone brain natriuretic peptide (NT-proBNP) are already accepted in respect of the early diagnosis and prognosis of left ventricular dysfunction [1]. It is also proposed that B-type natriuretic peptide is a strong prognostic indicator for both asymptomatic patients and those with heart failure at all stages of disease [2]. However, controversies about usefulness of natriuretic peptides in clinical practice exist, as their diagnostic credibility is still not settled. NT-proBNP is thought to be even more useful in screening for latent heart disease [3] and the earlier diagnosis of cardiac dysfunction as compared with BNP [4]. Although promising, atrial natriuretic peptide (ANP) and N-terminal prohormone atrial natriuretic peptide (NT-proANP) are so far seen as of more limited value, and still under debate.

The pathophysiological effects of natriuretic peptides are vasodilatation, natriuresis, inhibition of the rennin-angiotensin-aldosterone system, a shift of fluids from intravascular into extravascular space, diuresis, inhibition of adrenergic stimulation, increase of intraglomerular pressure and glomerular filtration, inhibition of sodium and water transport in proximal tubules and the blocking of sodium reabsorption. BNP exerts its action through high affinity receptors on the target cells and is stored mainly in the ventricular myocardium. In both healthy subjects, and patients with left ventricle dysfunction, BNP and NT-proBNP are secreted mainly from the left ventricle, but in small amounts also from the atria [5].

BNP and NT-proBNP are used currently by cardiologists as diagnostic biomarkers of heart failure [6], LV dysfunction [7], severity of heart failure, and as indicators of regional conditions and structural change in myocytes [8], the risk of cardiovascular events and death [9-12]. BNP assessment is also useful in predicting the long-term risk of redecompensation in non-ischemic DCM, even in low-risk outpatients [13]. Its secretion is regulated by wall tension of the left ventricle and severity of heart failure [3,14], left ventricular wall thickness, left ventricular mass [15], ischemia caused by local hypoxia [16], local pH changes, the presence of arrhythmia, thrombosis of the pulmonary artery, and other factors of right ventricle dysfunction [16,17]. It improves diagnosis in acute dyspnea [9,18], gives prognostic information [2,13,19-23] and offers an evaluation of left-ventricular function and effectiveness of therapy in congestive heart failure [24,25]. In chronic heart failure there is a correlation between BNP and NT-proBNP with disease severity and prognosis [26]. In hypertrophic cardiomyopathy NT-proBNP and ANP are associated with severity of LV diastolic dysfunction and hypertrophy of LV, as well as of hemodynamic and functional disturbances [27]. BNP is suggested to be important in predicting sudden death in chronic heart failure and is an independent risk factor for heart failure events [28]. BNP is also indicated as a simple and useful marker in predicting of progressive ventricular remodeling within the first 30 days of acute myocardial infarction [29].

Groups	BNP [pg/ml]	NT-proBNP [pg/ml]	ANP [pg/ml]	NT-proANP [nmol/l]
AD-EDMD	42 (18-54) *,#	85 (17-195) *	273 (1.3-366)	0.50 (0.28-0.58) ##
X-EDMD	24 (6-105) #	53 (14-192)	366 (0.9-391)	0.54 (0.37-0.60) ##
X-EDMD carriers	22 (9-62)	87 (33-400)	8.1 (0.9-8.1)	0.34 (0.29-0.65)
Dystrophinopathies	163 (50-337) ***	103 (9-457) *	344 (286-362) ***	2.5 (1.6-2.6) ***
Healthy controls	15 (9-20)	29 (15-49)	4.9 (2.3-5.6)	0.38 (0.32-0.42)

Medians and interquartile ranges are presented

\*  $p < 0.05$ , \*\*  $p < 0.005$ , \*\*\*  $p < 0.0005$  for the patient groups vs. healthy controls

#  $p < 0.05$ , ##  $p < 0.005$ ; AD-EDMD or X-EDMD patients vs. disease controls

Table 2: Natriuretic peptide concentrations in EDMD patients and controls

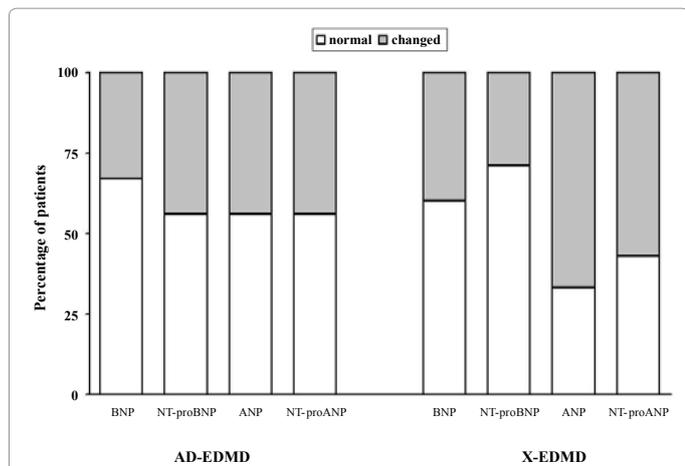


Figure 1: Percentage of changed values of serum natriuretic peptides in EDMD.

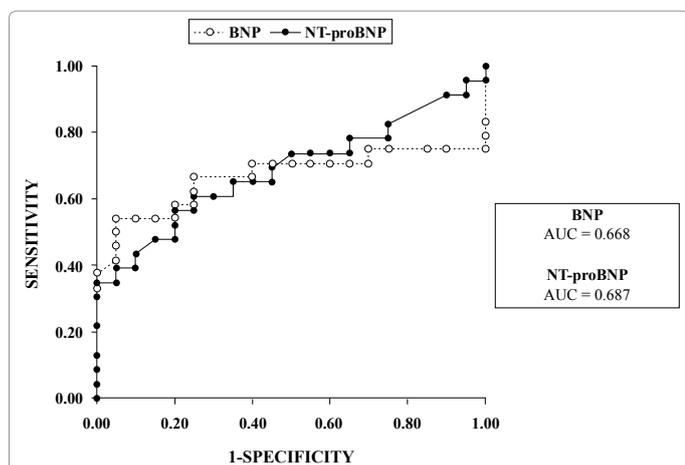


Figure 2: Receiver operating characteristics (ROC) analysis of serum concentrations of BNP and NT-proBNP in EDMD patients.

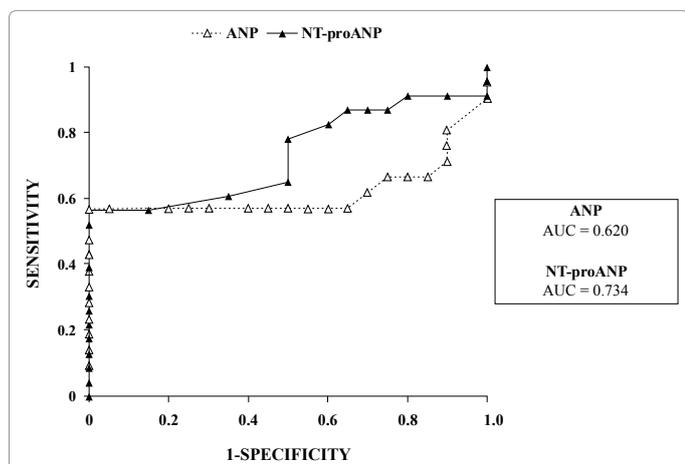


Figure 3: Receiver operating characteristics (ROC) analysis of serum concentrations of ANP and NT-proANP in EDMD patients.

Serial determinations of BNP and ANP may provide for the recognition of hemodynamic deterioration [30]. Both can be used as simple clinical markers for risk stratification in congenital heart disease in adults [31] and chronic heart failure [32-34].

BNP in blood is also advocated in the study of Duchenne dystrophy with a view cardiac dysfunction either manifest or latent [35]. Data on BNP, NT-proBNP, ANP and NT-proANP in Emery-Dreifuss muscular were not published, yet.

NT-proBNP may be an alternative marker for the detection of left ventricle dysfunction [36] and it is even preferable to BNP for the detection of heart failure, when it comes to the detection of heart failure, especially in asymptomatic patients [4]. Its concentrations are more elevated in acute left ventricular dysfunction as compared with stable chronic DCM [37]. It is a good marker by which to discriminate patients for transplantation and prognosis and is a predictive marker for the consequences of treatment in chronic heart failure. Very high levels are found in patients who went on to die. A strong correlation has been noted between NT-proBNP and NYHA class and HFSS [38]. Cardiac fibrosis correlates with elevated NT-proBNP and left ventricular remodeling and diastolic function in patients with non-ischemic DCM [39]. Marked reductions in the level of NT-proBNP and also BNP are indicative of an improved outcome in chronic heart diseases [40].

From the set of BNP, NT-proBNP and NT-proANP used as screening tests for heart failure, BNP is indicated as the best marker to detect patients with impaired LVEF, and NT-proBNP might be an alternative marker of deteriorated cardiac function [36,41]. However, it needs to be kept in mind that increased BNP/NT-proBNP levels are not strictly specific for heart diseases [42] and that BNP and NT-proBNP are influenced by such extracardial factors as gender, age, glomerular filtration [43] and obesity [44]. Elevated NT-pro-BNP better predicts the risk of adverse events in long-term systolic dysfunction [45]. A combination of left atrial enlargement and high NT-proBNP increase the risk of sudden death [46]. BNP and NT-proBNP are indicated as superior to ANP and NT-proANP as diagnostic and prognostic markers in heart insufficiency [1].

ANP plays an important role in homeostasis of body fluids and blood pressure [47-49]. ANP is secreted from atria in normal humans and from the left ventricle in patients with left ventricle dysfunction, and correlates with the severity of left ventricle dysfunction [14] ANP is stored in atrial myocytes, its secretion being stimulated by atrial dilatation. In DCM ANP is also secreted from ventricles [50]. Blood ANP level is elevated in atrial fibrillation [51].

Blood levels of ANP like BNP correlate with hemodynamic parameters of patients with chronic heart failure. Relations between ventricular structure and secretion pattern of BNP and ANP point to differences in patients with idiopathic dilated cardiomyopathy and hypertrophic cardiomyopathy (HP). The levels of ANP and BNP are higher in DCM as compared with HP. The main factor determining the secretion of ANP as BNP is the intraventricular cavity size [52].

ANP is related positively to the clinical status and to the left ventricle dysfunction and dilatation [53], correlates with left ventricular dimensions [14], and reflects the severity of heart failure [54]. Successful cardioversion in persistent atrial fibrillation leads to ANP reduction [55]. Increase in plasma ANP concomitantly with BNP are sensitive markers confirming atrial standstill in the setting of congestive heart failure [56]. ANP is elevated in blood in various cardiac diseases, including valve diseases, coronary heart diseases and cardiomyopathy, where a linear relationship is found between mean pulmonary artery pressure and blood ANP concentration [28].

NT-proANP blood levels may be a more sensitive marker of left ventricular dysfunction than ANP [57]. It correlates with left atrial diameter, left ventricular ejection fraction and Doppler derived E/A

ratio on transmitral inflow. In DCM there is a relation between NT-proANP and systolic and diastolic parameters [58]. Both NT-proANP and ANP are elevated in patients with chronic heart failure and reflects the severity of heart failure [56]. NT-proANP is less variable than ANP and hence it is more suited for diagnostic and prognostic purposes [59]. It is elevated in cases with persistent atrial fibrillation [60] and is associated with increased cardiovascular risk [61].

Notwithstanding numerous reports and even introduction into clinical practice the diagnostic credibility of natriuretic peptides as biomarkers of heart diseases, despite numerous reports and their introduction in clinical practice, is limited. It should be kept in mind that there are several factors as several medicines should come [62], which could influence their level in the blood stream. Their concentration increases with of ages of patients and gender [62,63].

Biomarkers might be helpful in establishing of an early pharmacological treatment, in decisions regarding cardiac device implantation and in consideration of heart transplantation. As there are limitations caused by the influence of extracardial factors, a single biochemical marker accompanied by cardiological parameter assessments, cannot be treated as a definitive marker as regards the recognition of cardiomyopathy at an early stage, a decision relating to severity of the disease, the monitoring of treatment efficiency or the predicting of sudden death in these patients. By combining data from natriuretic peptides and other biomarkers testing as osteopontin [64], tenascin-C [65] and MMP-2 [66] it might be possible to develop an assay system with optimal diagnostic and prognostic significance, indicate potential therapeutic intervention and allow to start an early pharmacological resynchronizing therapy and/or implantation to start.

## Conclusions

- 1) The value BNP and NT-pro-BNP estimation in regard to the detection rate for dilated cardiomyopathy in patients with AD-EDMD and X-EDMD is limited, as their concentration in serum in about half of all patients is normal.
- 2) A high level of ANP and NT-proANP and a correlation with echocardiographic parameters in the majority of X-EDMD cases might indicate atrial involvement as a primary cardiac defect in this form of EDMD.
- 3) The correlation between the atrial natriuretic peptides and the severity of the disease in cases of X-EDMD corresponds with the deteriorating function of atria over time.
- 4) Elevated level of natriuretic peptides is present also in dystrophinopathies, exceeding even the values present in EDMD, also in cardiologically asymptomatic patients and in the majority of the X-EDMD carriers.
- 5) Although the credibility of natriuretic peptides as cardiologic biomarkers in EDMD is limited, it is, however, worth to include them in the panel of cardiological tests. They may offer additional information in respect of proper diagnosis, prognosis, the commencement and monitoring of appropriate treatment, prediction of the outcome and help to prevent cardiac decompensation and sudden death.

## Acknowledgement

The study protocol was approved by the Bioethics Committee of the Medical University of Warsaw.

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