

Role of natriuretic peptide to predict cardiac abnormalities in patients with hereditary transthyretin amyloidosis.

Running head: Cardiac biomarkers in FAP

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Abstract

Background: Familial amyloid polyneuropathy (FAP) mainly targets the peripheral nervous system and heart. Early noninvasive detection of cardiac impairment is critical for therapeutic management.

Aim: To assess if amino-terminal pro-brain natriuretic peptide (NT-proBNP) or troponin T (cTnT) can predict echocardiographic left-ventricle (LV) impairment in FAP.

Methods: 36 asymptomatic carriers and patients with FAP had echocardiographic measurement of left-ventricular (LV) systolic function, hypertrophy (LVH) and estimation of filling pressure (FP),

Results: Overall, median age, NT-proBNP, and LV ejection fraction were, respectively, 59 years (41–74), 323 pg/ml (58–1960), and 60% (51–66). 12 patients had increased in cTnT. Prevalence of ATTR gene mutations was 53% for *Val30Met*. Four individuals were asymptomatic, 6 patients had isolated neurological clinical signs, and 26 had echo-LV abnormalities. The ROC curve identified NT-proBNP patients with echo-LV abnormalities (area: 0.92; (0.83–0.99), $p=0.001$) at a threshold >82 pg/ml with a sensitivity of 92%, and a specificity of 90%. Increased in NT-proBNP occurred in patients with SD and/or LVH with or without increase in FP. Elevated cTnT (>0.01 ng/ml) was only observed in patients with LVH and systolic dysfunction, with or without FP.

Conclusion: In FAP, NT-proBNP was associated with cardiac impairment suggesting that NT-proBNP could be used in carriers or in FAP patients with only neurologic symptoms for identifying the appropriate time to start cardiac echocardiographic assessment and follow-up. cTnT identified patients with severe cardiac disease.

Keywords: cardiac amyloidosis, transthyretin, cardiac markers, natriuretic peptide, troponin, echocardiography, speckle tracking.

Abbreviations:

ATTR: transthyretin amyloidosis

E, A, EA: early, late, and mean transmitral diastolic peak flow velocities

FAP: familial amyloid polyneuropathy

FP: filling pressure

LR+ and LR–: positive and negative likelihood ratios

LV: left ventricle

LV-2D-strain: left ventricular peak systolic longitudinal strain

LVEF: left ventricular ejection fraction

LVEDD: left ventricular end-diastolic diameter

LVH: left ventricular hypertrophy

MRI: magnetic-resonance imaging

NT-proBNP: amino-terminal pro-brain natriuretic peptide

SD: systolic dysfunction

Introduction

Amyloidosis is characterized by the deposition of insoluble protein aggregates in the tissue interstitium[1]. Several causes of systemic amyloidosis have been described[2]. Primary amyloidosis is caused by a plasma-cell disorder, which produces abnormal amounts of light chains. Secondary amyloidosis is caused by chronic inflammatory disease and hereditary amyloidosis, of which familial amyloid polyneuropathy (FAP) is more frequent[3, 4]. FAP is an autosomal dominant disease characterized by the systemic deposition of amyloidogenic variants of transthyretin protein, especially in the peripheral nervous system and heart [5].

The natural history of interstitial deposition of cardiac transthyretin causes increased parietal stiffness and intra-ventricular pressure, followed by decreased systolic function. All of these alterations are measurable by echocardiography[6]. Cardiac involvement in FAP causes progressive heart failure and is associated with a poor prognosis[2, 4, 7].

Early detection of heart involvement is critical for the therapeutic management of FAP carriers and FAP patients with only neurological symptoms. However, expert echocardiography is not always accessible to neurologists who frequently first manage FAP patients. Furthermore, it is troublesome to repeat this procedure every year in asymptomatic carriers or in patients with isolated neurological symptoms because of costs and availability of echocardiography.

Two biomarkers, natriuretic peptide and troponin, are of interest in chronic heart failure and particularly in primary amyloidosis[8]. Natriuretic peptides are used in patients who have dyspnea to diagnose heart failure and for the prognosis of chronic heart failure[9, 10]. These biomarkers are secreted by overloaded ventricles. Cardiac troponins are also sensitive and specific markers for myocardial injury. These two biomarkers have demonstrated their potential benefits for the prognosis of primary amyloidosis[11, 12].

We hypothesized that NT-proBNP and cardiac troponin T (cTnT) can detect and evaluate the involvement of cardiac amyloidosis in patients with FAP. Our objectives were, firstly, to

demonstrate that NT-proBNP and cTnT levels were correlated with LV echocardiographic abnormalities defined by the presence of LV hypertrophy and/or systolic dysfunction (SD) and/or increased filling pressure (FP); and, secondly, to define the best cut-off values for NT-proBNP and cTnT to predict cardiac involvement associated with FAP .

Materials and methods

Study population and procedures

Enrolled patients were referred for the diagnosis and management of FAP between August 2009 and September 2011 at the Amyloidosis Network in Henri Mondor Hospital, Créteil, France.

Patients were clinically evaluated and had an electrocardiogram, an echocardiogram, and blood tests for hematology, biochemistry profiles for NT-proBNP and cTnT. Both biomarkers were assessed on Roche Cobas 600E apparatus using commercially available kits as used in routine clinical procedures. NTproBNP and cTnT had a limit of detection respectively of 1pg/mL and 0.01 ng/ml. Values of cTnT >0.01 ng/ml were considered abnormal.

Patients were divided into three diagnostic groups based on clinical signs and echocardiographic findings. The first group had no neuropathy and no LV-echo abnormalities, and was called the asymptomatic group. The second group included those who had clinical signs of neuropathy without any LV echo-abnormalities, and were called the neurological phenotype. Those with LVH and/or FP and/or SD were considered to have LV echo-abnormalities whatever the clinical examination, and were called the cardiac with or without neurological phenotype.

Echocardiographic measurements

Echocardiograms were performed in accordance with the American Society of echocardiography (ASE) recommendations using a Vivid Five (GE Healthcare, UK) system operating at 3.4 MHz[13]. Echocardiograms were stored and reviewed by two operators (TD, SR), blinded to NT-

proBNP and troponin levels, using an EchoPAC station (GE Healthcare, UK). The average of three measurements was used for patients with a sinus rhythm and five for patients with atrial fibrillation. Systolic function was assessed by measuring the left ventricle ejection fraction (LVEF) using Simpson's biplane method and the mean LV peak systolic longitudinal strain (LV-2D-strain). LV-2D-strain was calculated from the three apical-chamber view (two, three, and four chambers) using speckle-tracking analysis with real-time tracking of frame-to-frame movements of naturally occurring echo-dense speckles (Echo-PAC software). The 2D strain values could be derived by comparing the displacement of the speckles relative to one another throughout the cardiac cycle.

For our study, the endocardial border was drawn manually and the region of interest was generated automatically to include the entire myocardium. The position and width of the region of interest were adjusted manually when necessary. Segments with poor-quality tracking were discarded. The software automatically tracked myocardial movements, dividing the myocardium into segments. QRS onset was detected from simultaneous ECG recordings to define the time point at which strain equaled zero. LV walls were divided into basal, middle, and apical segments to compute regional strain values; software obtained means per segment. Global longitudinal strain for the entire traced ventricular contour was computed and expressed as its median \pm interquartile (IQR). Higher values ($>-17\%$) of LV-2D-strain indicated decreased LV contractility. Our intra-observer and inter-observer reproducibility were respectively of 8% and 9% for left ventricle longitudinal global strain.

The LV mass indexed to body-surface area was calculated using a previously described formula for hypertensive patients [14]: $\text{LV mass} = (0.8 \times (1.04 \times (\text{LVEDD} + \text{IVST} + \text{LVPWT})^3 - \text{LVEDD}^3) + 0.6) / \text{body-surface area}$. Where LVEDD is left ventricular end-diastolic diameter, IVST is inter-ventricular septal thickness, and LVPWT is left ventricular posterior wall thickness. LV hypertrophy (LVH) was defined as a LV-mass indexed $>115 \text{ g.m}^{-2}$ in men and $>95 \text{ g.m}^{-2}$ in

women. It is important to note that, in cardiac amyloidosis, there is not a true LVH as the increase in LV wall thickness is caused by extracellular amyloid deposition rather than cardiomyocyte hypertrophy.

Early (E) and late (A) transmitral diastolic peak-flow velocities and filling deceleration time were measured using pulsed-wave Doppler imaging of the mitral inflow valve. Early lateral and septal peak diastolic acceleration of mitral annular velocity were also measured using tissue Doppler imaging (TDI). Their mean was calculated (Ea) as the E/Ea ratio. Maximal tricuspid regurgitation velocity was determined. Maximal peak velocity of tricuspid regurgitation (TR) was measured (Vmax TR). Right atrial pressure was estimated using the alterations of the inferior vena-cava diameter during respiration. Systolic pulmonary arterial pressure was calculated using the modified Bernoulli equation as follows: systolic pulmonary artery pressure = $4(V_{\max} \text{ TR})^2 + \text{right atrial pressure}$. Systolic right ventricle systolic function was evaluated as previously described with tricuspid plane systolic excursion measurement [15].

LV echo-abnormalities

Signs of increased LV FP was defined as E/A >2 and/or E/Ea ≥ 15 . SD was defined as a LV-2D-strain $> -17\%$ and/or a LVEF of $< 55\%$. LVH was defined as a LV-mass indexed of $> 115 \text{ g.m}^{-2}$ in men and $> 95 \text{ g.m}^{-2}$ in women. Prevalence of the different combinations of these three factors was also studied (for example: LVH-SD or LVH-SD-FP). The normal pattern was defined when none of these arrangements was observed.

Ethics

The investigation conformed to the principles outlined in the Declaration of Helsinki. It was approved by the Henri Mondor Research Ethics Committee. All subjects gave their written informed consent.

Statistical analyses

The population was divided into three groups depending on neurological signs and LV echo-abnormalities. Values are given as medians and interquartiles (IQR) for quantitative data, and as numbers and percentages for categorical data. NT-proBNP was logarithmically transformed prior to entry into the statistical models. Differences between continuous data were tested using the Kruskal–Wallis test for comparisons between the three groups. Proportions were compared using chi-square test or the Fisher’s exact test as the number of patients was less than five in one group.

NT-proBNP and cTnT values to predict LV-echo abnormalities were determined using a receiver-operating characteristic curve (ROC). The area under the ROC curve measured the accuracy of the diagnostic test: values near 0.5 indicated failure of the test and values near 1 indicated virtually perfect test accuracy. The cut-offs for NT-proBNP and cTnT were determined using Youden’s test[16]. Sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios (LR+ and LR–) were calculated using the thresholds previously defined. Data were considered significant if $p < 0.05$. Analyses were performed using SPSS 16.0 software (SPSS Inc., Chicago, IL).

Results

Baseline characteristics of the population

The median (IQR) age and LVEF of the whole cohort were, respectively, 59 (41–74) years and 60% (51–66%). A description of the cohort and pathogenic gene mutations is shown in Figure 1a. Fifty three percent had the ATTR *Val30Met* mutation. Baseline characteristics of the patients according to the clinical and echocardiographic classification are presented in Table 1. Briefly, four patients were asymptomatic, six had only isolated neurological clinical signs and 26 had LV echocardiographic abnormalities. The latter patients were older and, more frequently, had carpal

tunnel syndrome and a pacemaker implant. Patients with isolated neurological signs were all women and had normal but significant lower systolic blood pressure than the other two groups. In the asymptomatic group only one patient had a biopsy and it was normal. In the isolated neurological signs group, the six patients had a biopsy (4 salivary gland, 2 nerve) and they all showed amyloid deposits. In the last group with echocardiographic abnormalities 25 patients had a biopsy (17 salivary gland, 3 cardiac, 1 gut, 1 stomach) of whom, 20 showed amyloid deposits. The 5 patients without amyloid deposition had only salivary gland biopsy. No other biopsies were performed as liver transplant and/or heart transplant were not indicated (severe heart failure and/or patients over age 65 years).

Echocardiographic findings

Echocardiographic measurements are presented in Table 2 according to the clinical and echocardiographic classification. There was no difference in LVEDD, left ventricular end-systolic diameter, LVEF, E, A, E/A, and E/Ea between the three groups. Inter-ventricular septal diastolic thickness, left ventricle posterior wall diastolic thickness, LV-mass index, and aortic and left atrial diameters were greater in the group with cardiac involvement compared to the asymptomatic and neurologic phenotype groups. LV-2D strain was also higher in this last group showing impaired LV contractility. Tricuspid annular-plane systolic excursion was lower in the echo-LV abnormality group. Prevalence of LV-echo patterns defined as normal, SD, LVH, FP, LVH+SD, and LVH+SD+FP, are shown in Figure 1b. The LVH+SD+FP pattern was the most frequent. No patient had isolated FP or FP+SD or FP+LVH. The prevalence of SD, LVH and FP in the overall cohort were respectively of: 58%, 64% and 39%,. The prevalence of SD, LVH and FP in patients with an abnormal echocardiography were respectively 84%, 89%, and 56%.

Relationship between NT-proBNP and echo-LV abnormalities

NT-proBNP was significantly different between the three groups. The LV-echo-abnormality subgroup had the highest level of NT-proBNP compared to the other two groups (Table 1). NT-proBNP was significantly correlated to severity of amyloid deposition estimated by LV mass index (Figure 2a), LV-2D strain (Figure 2b) and E/Ea (Figure 2c). Patients with a ATTR Val30Met mutation had less frequent LVH and signs of elevated LV FP compared to those with other mutations (Figure 2a–c).

The ROC curve (Figure 3a) and the Youden test identified a threshold of 82 pg/ml for NT-proBNP (AUC: 0.92; 95% CI (0.82–0.99); $p=0.0001$) as the best threshold for predicting LV-echo-abnormalities when all patients were considered (Figure 3a).

Sensitivity and specificity of the 82-pg/ml NT-proBNP threshold to predict LV abnormalities were, respectively, 92% and 90%, with positive and negative predictive values of, respectively, 96% and 82% and LR+ and LR– of, respectively, 9.23 and 0.08. This means that NT-proBNP had a high accuracy to predict cardiac involvement. The prevalence of patients with NT-proBNP > 82 pg/ml, depending on the LV echo-patterns, is shown in Figure 3b. Briefly, prevalences of NT-proBNP > 82 pg/ml in Normal, SD, LVH, LVH+SD, LVH+SD+FP were respectively of: 10%, 100%, 80%, 75% and 100% (Figure 3b). These indicate that increase in NTproBNP can occur independently of increase in estimated LV filling pressure in FAP (ie in SD or LVH or LVH+SD patterns).

Relationship between cTnT, NT-proBNP, and LV echo-abnormalities

cTnT values are shown in Table 1 for the three groups. cTnT was abnormal in 12 patients from the overall cohort, all of whom were in the LV-echo-abnormality subgroup. Elevation of cTnT was associated with increased NT-proBNP for all patients but one (Figure 4). Accordingly, patients with elevated cTnT levels had a higher NT-proBNP than those without elevated cTnT (2859 (770–

4899) 82 (35–266); $p=0.0001$). Increased cTnT was observed in all patients with ATTR mutations. The three patients with ATTR–*val30met* and increased in cTnT had late onset disease (aged 77, 80, and 74 years).

The ROC analysis and Youden test identified a threshold of 0.01 $\mu\text{g/ml}$ for cTnT (figure not shown; AUC: 0.77; 95%CI (0.61–0.93); $p=0.015$) as the best threshold for predicting LV-echo-abnormalities when all patients were considered (Figure 2a). The cTnT threshold (0.01 ng/ml) had a sensitivity, specificity, and positive and negative predictive values to predict LV abnormalities of, respectively, 54%, 100%, 100% and 58%. The LR+ of the cTnT cut-off could not be calculated (infinite as the specificity was equal to 100%) and the LR– was 0.45. cTnT was elevated in all the patients with LVH+SD+FP. cTnT was normal in Normal LV, SD and LVH (Figure 5).

Discussion

Our study demonstrated: 1-that LV echo-abnormalities were frequent in hereditary ATTR, 2-that NT-proBNP was correlated with the severity of cardiac involvement and predicted echocardiographic LV abnormalities when $>82\text{pg/ml}$ and 3-that elevated cTnT indicated severe amyloid cardiac involvement

Prevalence of LV-echo-abnormalities

We have shown that ~80% of patients in our study had LV-echo-abnormalities (SD, LVH and FP); of these, 50% had two or three pathologic echo-patterns. Such high prevalences of LV involvement have been already reported in cardiac amyloidosis Italian cohort known to have a high prevalence of pathogenic mutations inducing cardiac involvement[7]. In contrast, the most frequent ATTR mutation in our cohort was the ATTR *Val30Met*(known as the Portuguese mutation) [17]. The high rates of LV echo-abnormalities reported here are mainly due to the use of new sensitive echocardiographic parameters, such as the E/Ea ratio for estimating LV FP [18] and

the 2D-strain for LV contractility [19]. We have shown that LV contractility was impaired despite normal or subnormal LVEF suggesting that global LV-2D strain was a useful tool for detecting early LV systolic dysfunction in FAP. This suggests that LV-2D strain is more sensitive than LVEF to detect early alteration of LV contractility and could be useful in clinical practice in FAP[20].

NT-proBNP as marker of cardiac involvement.

In chronic heart failure, natriuretic peptides are secreted by heart ventricles in response to myocardial stretching and have been shown to be correlated with LV filling pressure. Natriuretic peptides are now routinely used for the bedside diagnosis of heart failure [9, 10].

In our study on FAP patients, NT-proBNP above 82 pg/ml was able to accurately identify patients with left ventricle echo-abnormalities with a high sensitivity of 92%, and a high specificity of 90%. NT-proBNP is easily measured and accessible almost in every care facility. In contrast, access to echocardiography is limited, time consuming and more expensive than biomarkers measurement. For these reasons, echocardiography is difficult to repeat in asymptomatic FAP carriers or in FAP patients with only neurological clinical signs usually followed by neurologist. Choosing the good time for echocardiographic referral is challenging. That is why NT-proBNP may be useful to raise physician attention about the need to refer patient for echocardiography.

Therefore, our NT-proBNP threshold was under normal values distribution that has been characterized in the community. Defining abnormal values is a critical step in the clinical use of a biomarker [21]. But values that lie within statistically defined reference limits may not indicate health in a given individual, especially when the person comes from a group, such as TTR-FAP, inherently different from the one used to derive the reference limits (the community). In another way, a change in values within the reference range defined in the community may indicate

pathology in a specific disease. This study suggested that the reference interval should be moved down in TTR-FAP to identify patients who need echocardiographic assessment.

NT-proBNP correlation with LV echo-cardiographic abnormalities

In our study, including FAP patients, NT-proBNP was highly correlated to echocardiographic signs of elevated filling pressure, systolic dysfunction and severity of amyloid deposition. Correlation of NT-proBNP was strongly correlated to E/Ea which has been reported as an acceptable estimate of LV filling pressure[22-24]. We observed elevated NT-proBNP in patients presenting only SD, LVH or LVH+SD i.e. independently of signs of elevated filling pressure. This suggests that transthyretin amyloid deposits have a direct effect on cardiomyocyte secretion of BNP independently of mechanical stress induced by overload pressure. Interestingly, Takemura *et al* in a histological study including patients with AL amyloidosis showed that myocytes neighboring amyloid deposits tended to show more intense peptide natriuretic (BNP and ANP) staining[25]. This suggests that AL amyloid deposits may produce regional mechanical stress by restricting myocyte motion and interfering with their cooperative movement [25]. Correlation between severity of cardiac AL amyloid deposition and natriuretic peptides was also observed in an echocardiographic where BNP levels did not differ between patients without or with clinical signs of heart failure (suggesting elevated LV filling pressure) [26]. Similarly, Lerke S *et al.* showed a correlation between BNP and amyloid deposition estimated by cardiac MRI measurement of LV mass including different types of cardiac amyloidosis (AL, ATTR...)[27]. To date and to our knowledge, only one study has compared BNP and echo-LV abnormalities in FAP patients[28]. Including, 28 Swedish patients with ATTR *Val30Met* mutations Suhr *et al.* found a correlation between brain natriuretic peptide and inter-ventricular septal thickness [28]. Our study further extends this finding by correlating global cardiac amyloid deposition (LV mass) to NT-proBNP and including other ATTR mutations. This last study and our together suggest that transthyretin amyloid deposits have a direct cardiac

cytotoxicity which might be also responsible of alteration of LV contractility as demonstrated by high prevalence (58%) of LV-2D strain alteration in our entire study cohort. Nevertheless this cellular toxic effect might be less important in FAP than in AL patients as FAP patients with cardiac amyloidosis have usually better survival outcome than AL patients[7].

cTnT was associated with severe cardiomyopathy

In our study, only patients with severe cardiomyopathy had increased serum cTnT. This may be because cardiac troponin T is a highly specific and sensitive marker of myocardial injury[29-32]. Increased troponin level in cardiac amyloidosis is caused by myonecrosis and small-vessel ischemia induced by amyloid deposits[33]. Suhr et al., in their Swedish population, found similar rates of increase of troponin[28]. However serum troponin is not sensitive enough to identify patients with LV echo-abnormalities. The prognostic value of troponin for FAP patients, as has been demonstrated in primary amyloidosis[11], remains to be elucidated.

Limitations of the study

The number of patients included in our study was small, although FAP is a rare disease. Five patients in the group with LV echocardiographic abnormalities did not have the histologic proof of amyloid deposition (negative salivary gland biopsy). These patients had predominant cardiac amyloidosis form with strong late gadolinium enhancement at the cardiac magnetic resonance imaging and/or strong cardiac tracer fixation at 99Tc DPD scintigraphy and genetic pathogenic ATTR mutation. All these patients were contraindicated for liver or combined heart-liver transplant. So, we considered other biopsies than salivary gland biopsy (such as cardiac) too invasive and not requested as it would not have affected the therapeutic strategy. Furthermore, we did not have systematic cardiac histopathology analysis for the patients with cardiac involvement. It would have been useful to correlate with the echocardiographic findings. Because

endomyocardial biopsies are invasive, it was not ethical to perform this procedure in patients known to have a genetic ATTR mutation or with already less invasive positive biopsy such as salivary gland. Invasive haemodynamic cardiac catheterization data were not available in our study because catheterization was not clinically indicated and has side effects.

Conclusion

In cardiology setting, NT-proBNP and troponin are used to diagnose respectively heart failure and cardiac ischemia. In this study, NT-proBNP was associated with cardiac echocardiographic involvement in FAP patients (LV hypertrophy and/or systolic dysfunction and/or signs of elevated filling pressure). This suggests that asymptomatic carriers or FAP patients with NT-proBNP value above 82pg/ml should be referred for an echocardiography to assess cardiac function and detect eventual cardiac involvement as early diagnosis of ATTR-cardiomyopathy in FAP is important for adapting treatments and monitoring. In our study, troponin was an indicator of the severity of cardiac involvement and was not helpful to identify early cardiac involvement.

The prognostic role of these cardiac biomarkers in FAP patients and their value in monitoring the response to newly developed ATTR treatments need to be further defined.

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Declaration of Interest statement

Pr. Thibaud Damy, Pr. Violaine Planté-Bordeneuve, and Pr. Jean-Pascal Lefaucheur have received speakers' honoraria from Pfizer-Synergy.

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Figures legends

Figure 1: a) Study plan; b) Prevalence of LV echo-patterns.

FP: isolated increase in filling pressure; SD: isolated LV systolic dysfunction; LVH: isolated LV hypertrophy as marker of amyloid deposition; LVH–SD: combination of LVH and SD; LVH–SD–FP: combination of the three previously described patterns.

Figure 2: Correlation of NT-proBNP with : a) LV mass index (LVMI) as marker of amyloid deposition, b) LV 2D-strain as an indicator of LV contractility, and c) E/Ea as a marker for LV filling pressure.

Specific mutations are presented as follows :ATTR*Ser77Tyr* as a black filled circle; ATTR*Val122Ile* as a black filled square; ATTR*Val30Met* as an open square; other mutations as black filled triangles. ATTR*Val30Met* presented less LV hypertrophy and increased filling pressure; however, it had similar LV contractility impairment as other mutations.

Figure 3: a) Receiver-operator curves for NT-proBNP (AUC: 0.92; 95% CI (0.82–0.99); $p=0.0001$) and b) prevalence of LV echo-patterns in the subgroup with NT-proBNP >82 pg/ml.

Figure 4: Correlation of cTnT and log NT-proBNP.

Specific mutations are presented as followed :ATTR*Ser77Tyr* as a black filled circle; ATTR*Val122Ile* as a black filled square; ATTR*Val30Met* as an open square; other mutations as black filled triangles. ATTR*Val30Met* presented less LV hypertrophy and

increased filling pressure; however, it has similar LV contractility impairment as other mutations.

Figure 5: Prevalence of LV echo-abnormalities in the subgroup with cTnT >0.01 ng/ml.