

Titin-Related Dilated Cardiomyopathy: The Sequence of Events and The Role of Circulating Biomarkers in The Clinical Assessment

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Abstract

Titin truncating variants (*TTMtv*) are known as the leading cause of inherited dilated cardiomyopathy (DCM). Nevertheless, the clinical course is not fully understood and it is unclear whether circulating cardiac biomarkers are helpful in the detection and risk assessment. We sought to assess: 1) early signs of cardiomyopathy including serum biomarkers: high-sensitivity cardiac troponin T (hs-cTnT) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) in clinically stable patients, and 2) indicators of outcome among *TTMtv* carriers. Our single-centre cohort included 108 carriers (including 70 DCM patients) from 43 families. Clinical, laboratory and follow-up data were analyzed. The earliest abnormality was left ventricular dysfunction, present in 8, 26 and 47% of patients in the 2nd, 3rd and 4th decade of life, respectively. It was followed by symptoms of heart failure and elevation of NT-proBNP, linked to severe (persistent or transient) left ventricular systolic dysfunction, and later by arrhythmias, preceding both malignant ventricular arrhythmia (MVA) and end-stage heart failure (esHF). Hs-cTnT serum levels were increased in the late stage of the disease only. During the median follow-up of 5.2 years both MVA and esHF occurred in 12% of *TTMtv* carriers. In multivariable analysis, NT-proBNP level ≥ 650 pg/ml was the best predictor of both composite endpoint (MVA and esHF), and of MVA alone. We conclude that assessment of circulating cardiac biomarkers is not useful in the detection of cardiomyopathies but may be helpful in the risk assessment.

1. Introduction

Dilated cardiomyopathy (DCM) is a major cause of heart failure (HF) and has a genetic basis in 40 to 50% of cases¹. Titin truncating variants (*TTMtv*) account for as many as 20–25% of the genetic background in DCM^{2–7} of European but not African ancestry⁸. *TTMtv* are also found in peripartum^{4,9}, alcoholic¹⁰ and chemotherapy-induced cardiomyopathy¹¹. Earlier studies showed good response to optimal medical therapy^{7,12,13}, impact of mutation location on the course of disease^{2,6} and similar prognosis as in other forms of DCM^{2,3}. Nevertheless, none of the studies involved circulating cardiac biomarkers in baseline characteristics. So, there are no data on their performance in the early stage of the disease or their role as markers of prognosis. The presence of arrhythmia in cardiomyopathies is not well characterized, either. Recently, the presence of *TTMtv* was shown to be an important risk factor for clinically significant arrhythmia in DCM patients (pts)^{14,15}.

In 2017, we published our data on cardiomyopathies including characteristics of 16 *TTMtv* identified in 17 probands and their 29 relatives³. Data on the probands were also included in the multicentre study by Akhtar et al¹⁶. Since then, we have identified next 29 DCM probands with *TTMtv* and their 39 relatives. In this study, we sought to assess the clinical characteristics including serum biomarkers, penetrance of abnormal clinical findings and prognostic risk factors in our cohort of *TTMtv* carriers.

2. Results

2.1. Molecular findings in the study cohort

We identified 41 different *TTM* variants in 46 unrelated probands: two variants were shared by two probands each and one was identified in four probands. As a result of analysis of other DCM-related genes, 3 additional variants classified as likely pathogenic according to the American College of Medical Genetics and Genomics (ACMG) criteria¹⁷ were identified in *MYH7*, *SCN5A*, and *TNNT2* in 3 probands. Of the 41 *TTM* variants, 30 were described before in ClinVar, Varsome or HGMD databases and 11 are novel. The identified variants are pathogenic (n = 40) or likely pathogenic (n = 1) according to ACMG criteria. Two of them are located in the Z-disc (both nonsense), 10 in the I-band (5 nonsense, 4 frameshift and 1 splice-site), 28 in the A-band (12 nonsense and 16 frameshift) and one in the M-band (nonsense). As a result of genetic screening in the families, 68 *TTM* carriers were identified among relatives. Three probands with likely pathogenic variants in other DCM-related genes and their 2 relatives as well as a patient after heart transplantation (HTx) performed at another centre were excluded from the study. The details of the identified variants in *TTN* and other DCM-related genes are shown in the supplementary Tables S1 and S2.

2.2. Clinical characteristics of the study population

The study cohort was composed of 108 subjects: 70 DCM patients (all 43 probands and 27 relatives), 13 subjects with other cardiac abnormalities, labelled as indeterminate cardiomyopathy (xCM) and 25 healthy relatives with no signs of cardiomyopathy. In two women DCM was diagnosed following symptoms of acute HF shortly after a delivery as peripartum cardiomyopathy. Four patients experienced fulminant HF at the onset of their disease; they all underwent left ventricular assist device (LVAD) implantation: as a bridge to urgent HTx in three cases and as a bridge to recovery in one case.

The baseline clinical characteristics of 108 *TTM* carriers is given in the Table 1 and in the supplementary Table S3. In case of three above mentioned patients who could be assessed only in the acute phase of the disease, the data on cardiac biomarker levels and medical therapy were not included in the analysis. The baseline data of the patient who had LVAD implanted as a bridge to recovery were collected during a consequent ambulatory visit.

Table 1
Baseline clinical characteristics of *TTN* truncating variant carriers.

	All N = 108	Probands N = 43 (39.8%)	Relatives N = 65 (60.2%)	p	Men N = 67 (62.0%)	Women N = 41 (38.0%)	p
Age, years	39.7 ± 15.5	40.1 ± 13.5	39.5 ± 16.9	0.832	37.4 ± 13.8	43.6 ± 17.6	0.044
Men	67 (62.0%)	33 (76.7%)	34 (52.3%)	0.010			
Probands					33 (49.2%)	10 (24.3%)	0.010
Symptoms							
Heart failure	54 (50.0%)	39 (90.7%)	15 (23.1%)	< 0.001	39 (58.2%)	15 (36.6%)	0.015
NYHA class ≥ 3	14 (13.0%)	9 (20.9%)	5 (7.7%)	0.045	11 (16.4%)	3 (7.3%)	0.172
Family history of SCD < 50 yrs	28 (25.7%)	7 (16.3%)	21 (31.8%)	0.063	16 (23.9%)	12 (29.3%)	0.535
Arrhythmias and CCD							
Atrial arrhythmias	25 (23.1%)	15 (34.9%)	10 (15.4%)	0.019	20 (29.8%)	5 (12.2%)	0.035
nsVT (n = 106)	42 (39.6%)	26 (60.5%)	16 (25.4%)	< 0.001	28 (42.4%)	14 (35.0%)	0.449
LBBB	11 (10.2%)	7 (16.3%)	4 (6.1%)	0.110	9 (13.4%)	2 (4.9%)	0.200
AV block (≥ 1st degree)	15 (13.9%)	8 (18.6%)	7 (10.8%)	0.249	11 (16.4%)	4 (9.8%)	0.331
Echocardiography							
LVEF < 50%	66 (61.1%)	40 (93.0%)	26 (40.0%)	< 0.001	52 (77.6%)	14 (34.1%)	< 0.001
LVEF, %	43.5 ± 13.8	34.7 ± 10.6	49.3 ± 12.6	< 0.001	39.8 ± 12.5	49.6 ± 13.9	< 0.001
LVEDD, mm	58.3 ± 9.6	64.2 ± 7.6	54.4 ± 8.9	< 0.001	61.2 ± 9.0	53.6 ± 9.0	< 0.001
LA _s , mm (n = 103)	40.6 ± 8.1	44.9 ± 7.2	38.1 ± 7.5	< 0.001	43.2 ± 7.9	36.2 ± 6.5	< 0.001
Diagnosis							

	All N = 108	Probands N = 43 (39.8%)	Relatives N = 65 (60.2%)	p	Men N = 67 (62.0%)	Women N = 41 (38.0%)	p
DCM	70 (64.8%)	43 (100%)	27 (41.5%)	< 0.001	55 (82.1%)	15 (36.6%)	< 0.001
indeterminate CM	13 (12.0%)	0 (0%)	13 (20.0%)		7 (10.4%)	6 (14.6%)	
no CM	25 (23.2%)	0 (0%)	25 (38.5%)		5 (7.5%)	20 (48.8%)	
Biomarkers in stable phase							
hs-cTnT, ng/l (n = 90)	4.4 [2.9; 8.6]	7.1 [4.1; 9.4]	3.6 [2.9; 7.1]	0.003	6.4 [3.1; 9.2]	3.6 [2.9; 7.0]	0.0498
hs-cTnT > 14 ng/l	9 (10.3%)	4 (11.8%)	5 (9.4%)	0.732	6 (11.3%)	3 (8.8%)	1.00
NT-proBNP, pg/ml (n = 69)	254 [80; 1276]	631 [186; 2034]	125 [40; 259]	< 0.001	244 [76; 1214]	292 [96; 2034]	0.500
NT-proBNP > 125 pg/ml	47 (69.1%)	32 (84.2%)	15 (50.0%)	0.002	34 (68.0%)	13 (72.2%)	0.739
NT-proBNP > 650 pg/ml	27 (39.7%)	20 (52.6%)	7 (23.3%)	0.014	19 (38.0%)	8 (44.4%)	0.632
Implantable devices							
PM for bradyarrhythmias	5 (4.6%)	3 (7.0%)	2 (3.1%)	0.385	2 (4.9%)	3 (4.5%)	1.000
CRT-D	2 (1.8%)	2 (4.6%)	0 (0%)	0.156	2 (3.0%)	0 (0%)	0.525
ICD in secondary prophylaxis	1 (0.9%)	0 (0%)	1 (0.9%)	1.000	0 (0%)	1 (2.4%)	0.380
ICD/CRT-D	14 (13.0%)	11 (25.6%)	3 (4.6%)	0.001	10 (14.9%)	4 (9.8%)	0.438

Legend: Number of subjects is expressed as n (%). Continuous variables are shown as mean ± standard deviation or median and quartiles [Q1:25th- Q2:75th percentiles]. AV block, atrioventricular block; CCD, cardiac conduction defect; CM, cardiomyopathy; CRT-D, cardiac resynchronization therapy defibrillator; DCM, dilated cardiomyopathy; HF, heart failure; hs-cTnT, high-sensitivity cardiac troponin T serum concentration; ICD, implantable cardioverter defibrillator; LAs, left atrial systolic dimension; LBBB, left bundle branch block; LVEDD, left ventricular end-diastolic dimension; LVEF, left ventricular ejection fraction; nsVT, non-sustained ventricular tachycardia; NT-proBNP, N-terminal pro-B-type natriuretic peptide

serum concentration; NYHA class, New York Heart Association functional class; PM, pacemaker; SCD, sudden cardiac death.

Compared between probands and relatives, we found no significant difference in age, suggesting incomplete penetrance and mild course of cardiomyopathy in some carriers. Indeed, among evaluated relatives there were not only 14 probands' offspring and 23 siblings, but also 16 parents and a grandmother. Understandably, HF in probands was more common (91% vs 23% in relatives, $p < 0.001$), and more advanced in terms of symptoms, as well as echocardiographic and biochemical exponents: New York Heart Association (NYHA) class ≥ 3 : 21 vs 8%, left ventricular ejection fraction (LVEF): 35 vs 49%, N-terminal pro-B-type natriuretic peptide (NT-proBNP) serum concentration: 631 vs 125 pg/ml, respectively). Probands had more arrhythmias, both atrial (AA) and ventricular (VA): 35 vs 14% and 61 vs 25%, respectively, but did not differ significantly with regard to cardiac conduction defects (CCD). Implantable cardioverter defibrillators (ICD) had been implanted more often in probands (30 vs 5%).

Median high-sensitivity cardiac troponin T (hs-cTnT) concentration measured in stable condition was higher in probands but at a low level (7.1 vs 3.6 ng/l, respectively).

There were more probands among male carriers (49 vs 24%). It may account partially for higher prevalence of DCM and HF in male vs female patients (82 vs 37% and 58 vs 37%, respectively) and for more demonstrable echocardiographic abnormalities (e.g. LVEF: 40 vs 50%, respectively). AA were more frequent and hs-cTnT concentration was higher in male patients (30 vs 12% and 6.4 vs 3.6 ng/l, respectively). No significant differences were found with regard to NT-proBNP concentration, VA, CCD or frequency of ICD implantation.

Of note, sudden cardiac death (SCD) under the age of 50 years in family history was found as often as in 26% of patients.

2.3. Penetrance of cardiomyopathy indicators

Penetrance of cardiac abnormalities in the course of cardiomyopathy was age-dependent (Fig. 1 and supplementary Table S4). The earliest abnormality was left ventricular dysfunction (LVD), defined as LVEF $< 50\%$ or left ventricular enlargement (LVE) $> 112\%$, and detected in the 2nd, 3rd and 4th decade of life in 8%, 26% and 47% of carriers, respectively. It anticipated by approx. 10 years the onset of symptoms of HF, accompanied by NT-proBNP elevation, transient or persistent severe left ventricular systolic dysfunction (LVSD), and VA. AA and atrioventricular block (AVB) appeared late, preceding occurrence of such adverse events as malignant ventricular arrhythmia (MVA) and end-stage heart failure (esHF). Elevated hs-cTnT concentration seems to be an indicator of end-stage phase of cardiomyopathy.

The rise in frequency of circulating cardiac biomarker concentrations, observed in the 6th and 7th decades of life may be attributable to co-existing conditions, such as atherosclerosis, rather than to cardiomyopathy itself.

2.4. Results of screening in carriers of cardiomyopathy-causing truncating variants

Observations on the sequence of occurrence of individual abnormalities may be biased due to the fact that the disease is often detected at an advanced stage with a number of anomalies present. Therefore, we analyzed the penetrance of cardiac abnormalities also in the group of 49 *TTM*tv carriers who came to our Unit for screening. The diagnosis of DCM was established in 11 of them, however only one patient developed HF during the follow-up. In this group, LVD was detected as the earliest indicator of *TTM*tv carriership in 21 (43%) pts (Fig. 2). Of note, in 16% of pts, LVE was accompanied by LVSD whereas LVE and LVSD were found as isolated deviations in 16% and 10% of pts, respectively. NT-proBNP serum concentration was elevated in only 27% of pts with LVD.

Arrhythmias and CCD were detected less frequently than in the whole cohort, despite repeated ECG and Holter recordings. Interestingly, AA were detected in 4 (8%) pts, and they preceded LVD in all of them. Also VA in 2 cases and AVB in one case were detected in subjects without LVD.

2.5. Outcome and risk stratification in cardiomyopathy

The median follow-up in the group of 108 *TTM*tv carriers was 5.2 years [Q1: 2.1, - Q3: 7.9]. During the follow-up period, 13 (12%) patients developed esHF (Table 2): twelve men and only one woman ($p = 0.02$). Six (6%) of these pts died of HF (one of them shortly after HTx and another one after LVAD implantation) and seven (6.5%) were transplanted successfully (in 4 cases preceded by LVAD implantation). There was one SCD, two patients had sudden cardiac arrest (SCA) with successful resuscitation. Of the 27 (25%) patients with ICD implanted before the baseline visit or during follow-up, 13 (48%) experienced adequate ICD intervention.

Table 2
Clinical outcomes in the cohort of *TTN* truncating variant carriers.

Events during follow-up	Total N = 108	Men N = 67 (62.0%)	Women N = 41 (38.0%)	P
Implantable devices	(implanted before baseline visit or during follow-up)			
ICD in secondary prophylaxis	2 (1.9%)	1 (1.5%)	1 (2.4%)	1.00
CRT-D	7 (6.5%)	6 (9.0%)	1 (2.4%)	0.249
ICD/CRT-D implantation	27 (25.0%)	21 (31.3%)	6 (14.6%)	0.052
Malignant ventricular arrhythmia, n = 107	13 (12.1%)	9 (13.4%)	4 (10.0%)	0.763
Appropriate ICD intervention, n = 27	13 (48.1%)	9 (42.9%)	4 (66.7%)	0.384
Cardiopulmonary resuscitation, n = 106	2 (1.9%)	2 (3.0%)	0	0.530
Sudden cardiac death, n = 106	1 (0.9%)	0	1 (2.6%)	0.368
End-stage heart failure, n = 107	13 (12.1%)	12 (17.9%)	1 (2.5%)	0.024
LVAD	5 (4.6%)	5 (7.3%)	0	0.155
Heart transplantation	8 (7.4%)	8 (11.9%)	0	0.024
HF death, n = 106	6 (5.7%)	5 (7.5%)	1 (2.6%)	0.410
Death	10 (9.3%)	7 (10.3%)	3 (7.3%)	0.742

Legend: Number of subjects with events is expressed as n (%). ATP, antitachycardia pacing; CRT-D, cardiac resynchronization therapy defibrillator; HF, heart failure; ICD, implantable cardioverter defibrillator; LVAD, left ventricular assist device.

We examined the influence of pre-specified risk factors on the risk of occurrence of composite endpoint (end-stage HF or MVA) in the whole cohort of 108 *TTN* carriers (Table 3). The univariable analysis suggests possible influence of such factors, as severely reduced LVEF, dilated left atrium, elevated NT-proBNP and hs-cTnT, the presence of left bundle branch block (LBBB), non-sustained ventricular tachycardia (nsVT) or AA. In multivariable analysis, NT-proBNP level ≥ 650 pg/ml was the best predictor of the composite endpoint at 6 years of follow-up (Fig. 3). The model had good discrimination as evidenced by the C-index of 0.842 [95% CI: 0.776–0.908].

Table 3

Potential risk factors affecting the risk of occurrence of the composite endpoint of malignant ventricular arrhythmia and end-stage heart failure in cardiomyopathy.

	Cumulate incidence	P-value Log-rank	Univariable		Multivariable	
			HR [95% CI]	p-value Wald	HR [95% CI]	p-value Wald
MVA + esHF	at 6 years of follow-up					
Sex: male vs female	32 vs 14	0.033	3.08 [1.04–9.18]	0.043		
AA: yes vs no	50 vs 18	0.001	3.77 [1.56 ; 8.90]	0.002		
nsVT: yes vs no	46 vs 7	< 0.001	5.5 [1.9 ; 16.5]	0.002		
LAs: ≥ 45 vs < 45 mm	67 vs 5	< 0.001	28.9 [6.7 ; 124.8]	< 0.001		
LVEF: < 30 vs $\geq 30\%$	78 vs 9	< 0.001	14.0 [5.5 ; 35.7]	< 0.001		
LBBB: yes vs no	86 vs 16	< 0.001	8.5 [3.6 ; 20.4]	< 0.001		
NT-proBNP ≥ 650 vs < 650 pg/ml	68 vs 8	< 0.001	15.9 [3.6 ; 70.3]	< 0.001	29.4 [3.7 ; 231.6]	< 0.001
hs-cTnT : ≥ 18 vs < 18 ng/l	75 vs 14	< 0.001	7.6 [2.0 ; 28.0]	0.002		
Legend: AA, atrial arrhythmia; CI, confidence interval; esHF, end-stage heart failure; HF, heart failure; HR, hazard ratio; hs-cTnT, high-sensitivity cardiac troponin T serum concentration; LAs, left atrial systolic dimension; LBBB, left bundle branch block; LVEF, left ventricular ejection fraction; MVA, malignant ventricular arrhythmia; nsVT, non-sustained ventricular tachycardia; NT-proBNP, N-terminal pro-B-type natriuretic peptide serum concentration.						

Anticipating life-threatening arrhythmia episodes is even more important as they can be interrupted by ICD interventions. The univariable analysis of MVA events during follow-up in the subgroup of 107 patients with no history of SCA or sVT shows possible influence of such risk factors as severely reduced LVEF, dilated left atrium, elevated NT-proBNP, the presence of LBBB, AA or nsVT but no impact of sex, family history of SCD or elevated hs-cTnT (Table 4). In multivariable analysis, NT-proBNP level ≥ 650 pg/ml was again the best predictor of MVA at 6 years of follow-up (Fig. 4). The model had good discrimination as evidenced by the C-index of 0.787 [95% CI: 0.672–0.909].

Table 4
Potential risk factors affecting the risk of occurrence of malignant ventricular arrhythmia in cardiomyopathy.

	Cumulate incidence	P-value Log-rank	Univariable		Multivariable	
			HR [95% CI]	p-value Wald	HR [95% CI]	p-value Wald
MVA	at 6 years of follow-up					
Sex: Male vs Female	21 vs 14	0.362	1.72 [0.52; 5.59]	0.368		
SCD < 50 in family: yes vs no	13 vs 20	0.684	0.76 [0.21 ; 2.78]	0.685		
AA: yes vs no	35 vs 14	0.010	3.81 [1.28; 11.36]	0.016		
nsVT: yes vs no*	39 vs 2	< 0.001	12.5 [2.2 ; 72.7]	0.005		
LAs: ≥ 45 vs < 45 mm	52 vs 5	< 0.001	16.9 [3.7 ; 77.4]	< 0.001		
LVEF: <30% vs $\geq 30\%$	65 vs 8	< 0.001	9.9 [3.2–30.2]	< 0.001		
LBBB: yes vs no*	83 vs 10	< 0.001	14.6 [4.8 ; 44.3]	< 0.001		
NT-proBNP ≥ 650 vs < 650 pg/ml	59 vs 8	< 0.001	11.7 [2.6–53.6]	0.002	11.0 [2.3; 53.2]	0.003
hs-cTnT: ≥ 14 vs < 14 ng/l	25 vs 13	0.823	1.27 [0.16 ; 10.2]	0.823		

Legend: AA, atrial arrhythmia; CI, confidence interval; HF, heart failure; HR, hazard ratio; hs-cTnT, high-sensitivity cardiac troponin T serum concentration; LAs, left atrial systolic dimension; LBBB, left bundle branch block; LVEF, left ventricular ejection fraction; MVA, malignant ventricular arrhythmia; nsVT, non-sustained ventricular tachycardia; NT-proBNP, N-terminal pro-B-type natriuretic peptide serum concentration; SCD, sudden cardiac death; *, Firth's correction.

3. Discussion

A major finding of the study is that the earliest marker of the carrier status in *TTMv*-related DCM is left ventricular dysfunction defined as LVEF < 50% or LVE > 112% which preceded the development of overt HF and transient or persistent severe LVSD by approximately 10 years which, in turn, was followed by a variety of arrhythmia, both ventricular and atrial, as well as AVB. Of note, among screened relatives (n = 49) isolated LVE with normal LVEF was the first sign of cardiomyopathy in 16% of subjects, reduced

LVEF without LVE in 10%, and both abnormalities were detected simultaneously in 16% of subjects. LVE is known as the first sign of early DCM¹⁸⁻²⁰. *TTMtv* were also associated with eccentric cardiac remodeling in analysis of cardiac magnetic resonance in healthy humans²¹. This is in agreement with a proposal of a new definition of DCM which recognizes 3 forms of preclinical phase of DCM, including isolated left ventricular dilation²². However, in a zebrafish model, serial echocardiography showed significant LVEF reduction in *ttnatv/+* vs *ttna+/+* zebrafish preceding LVE by 3–6 months²³. Furthermore, comprehensive genomics-first studies on the impact of *TTMtv* on cardiac phenotype by Haggerty et al.⁸ and Pirruccello et al.²⁴ showed that *TTMtv* carriers are characterized by lower LVEF but not larger left ventricular (LV) diastolic dimensions or volumes. Our DCM-dedicated study shows that both LVE and LVSD may be the first detectable abnormality.

To the best of our knowledge, circulating cardiac biomarkers in relation to either the disease penetrance or prognosis have not been reported in cardiomyopathies. The role of circulating cardiac biomarkers in the detection of HF is widely recognized²⁵. In community based studies, multiple cardiac biomarkers are detectable in ambulatory individuals and add prognostic value to standard risk factors for predicting mortality, overall cardiovascular events, and HF^{26,27}. However, little is known about the significance of circulating biomarkers in early stage of DCM in humans^{26,28}. We showed recently that elevated hs-cTnT is the earliest marker of the carrier status in cardiomyopathies²⁹ and might be a “red flag” in asymptomatic or mildly symptomatic carriers.

Our data show that determination of widely available serum biomarkers cannot replace echocardiography in detection of affected *TTMtv* carriers. NT-proBNP serum level is rarely elevated in subjects with mildly reduced LVEF or isolated LVE and it usually exceeds the normal range when HF symptoms and advanced LVSD are present. In contrast to cardiomyopathies, elevated hs-cTnT concentration seems to be an indicator of end-stage phase of cardiomyopathies.

In our cohort, AA was found during baseline evaluation in 23% of *TTMtv* carriers and VA in 40%. The frequencies are lower than in the largest to date study by Akhtar et al. who found AA in one third and VA in one-half of *TTMtv* carriers¹⁶. The difference can be explained by younger population and less advanced LVSD in our study. The timing of the appearance of VA seems similar in both cohorts, in conjunction with LVSD progression.

Authors of several recent papers report significant arrhythmic burden characteristic of *TTMtv*-associated DCM, most often found in a relatively advanced disease stage. In the study by Corden et al., having a *TTMtv* was associated with a higher risk of receiving appropriate ICD therapy in the group of 148 DCM patients with implanted ICDs (hazard ratio (HR) 4.9; $p < 0.001$)¹⁵. In addition, *TTMtv* was a risk factor for developing new persistent atrial fibrillation (AF)¹⁵. Tayal et al. found that patients with *TTMtv* are more likely to have a history of AA or VA at the time of DCM diagnosis¹⁴. In a Danish study on 115 *TTMtv*-related DCM patients with mean LVEF 28%, AF and MVA occurred in 43% and 23% of patients,

respectively³⁰. Of note, AF preceded the DCM diagnosis in 16% of pts, and in 11% the presenting symptom of DCM was MVA³⁰.

In cardiomyopathies, conduction disease and arrhythmias precede the onset of HF by seven years³¹⁻³³. However, *LMNA* mutations are not a common cause of lone AF³⁴. Arrhythmias are also reported in *TTMv* carriers with normal cardiac function. In a case control study that included 2,781 participants with early-onset AF and normal LVEF, and 4,959 controls, there was a statistically significant association between *TTMv* and AF (odds ratio, 1.76)³⁵. Associations with arrhythmias, including AF, were also observed in the genomics-first study by Haggerty et al., even when conditioning on DCM diagnosis⁸. Among screened relatives in our study, AA or VA was found in 8/50 (16%) subjects and it was the earliest detected abnormality in 6 (12%) of them. This shows that in the course of cardiomyopathies various clinical scenarios are possible: arrhythmias appear typically in the late disease stage but they can also precede the DCM diagnosis.

As shown previously by others^{7,15} and us³, LBBB is relatively uncommon among cardiomyopathy patients and therefore CRT-D requirement is less pronounced.

Another major finding of our study was strong, independent association between NT-proBNP level ≥ 650 pg/ml and the occurrence of the composite endpoint of MVA and esHF among *TTMv* carriers. Recently several studies defining prognostic factors in cardiomyopathy have been published, however none of them included impact of circulating biomarkers^{7,14-16}.

NT-proBNP serum concentration ≥ 650 pg/ml was also the best predictor of MVA in our study. Excellent prognostic role of NT-proBNP in patients with HF is widely recognized³⁶. The association of raised levels of NT-proBNP and MVA in HF patients was shown previously in general HF cohorts^{37,38}. NT-proBNP provides also information regarding the risk of SCD in a community-based population beyond other traditional risk factors³⁹.

There is great need for identification of prognostic factors that may help in decision making with regard to ICD therapy. Our study suggests that NT-proBNP, a commonly available circulating biomarker, may be useful in the setting of clinically stable *TTMv* carriers.

The hearts of patients with *TTMv* and DCM have thinner LV walls and lower indexed LV mass compared to *TTMv*-negative controls⁶. It results in higher LV wall stress and release of NT-proBNP, and is associated with increased risk of VA^{6,15,40}. It explains why NT-proBNP may be a good predictor of both MVA and esHF in *TTMv*-related DCM.

Unlike in cardiomyopathies⁴¹, *TTMv*-positive DCM patients have midwall replacement fibrosis detected in CMR with similar frequency as *TTMv*-negative DCM controls^{15,40} but interstitial fibrosis is found at endomyocardial biopsy significantly more often⁴⁰. We hypothesize that hs-cTnT leakage, detectable from early stages of cardiomyopathy²⁹, may reflect cardiomyocyte death and replacement fibrosis, prevalent

in *LMNA*-related cardiac disease, but it may be undetectable in interstitial fibrosis, characteristic of cardiomyopathy. This might explain why hs-cTnT level raises significantly only in the end-stage of *TTMtv*-related DCM whilst in earlier stages, when interstitial fibrosis and increased risk of life-threatening arrhythmias are already present, it remains within normal range.

Male patients were younger (37 vs 44 years, $p = 0.04$) and had more advanced HF, similarly as in earlier studies^{3,16}. We found more AA among male patients (30 vs 12%, $p = 0.04$) but no significant differences with regard to nsVT. In largest to date study on phenotypic characterization of *TTMtv* carriers male probands had a higher prevalence of AF at baseline (20 vs 5%, $p = 0.01$), the difference with regard to nsVT was less pronounced (58 vs 42%, $p = 0.046$)¹⁶.

As Giudicessi et al stated⁴², the prevalence of *TTMtv* in the Genome Aggregation Database (1.8%) is more than 4 times higher than the estimated prevalence of DCM in general population (0.4%). This underlines the role of *TTMtv* as susceptibility variants and suggests that strong environmental effects or additional genetic factors contribute to the development of a cardiac phenotype⁴³.

In this study we showed that pathogenic or likely pathogenic *TTMtv* in DCM patients were located in all domains of the gene and had high proportion spliced-in index, as in the study by Akhtar et al.⁴⁴. Early studies², including ours³, showed A-band location of *TTMtv* mutations as more specific for DCM patients. With many more *TTMtv* identified and more accumulated data, no statistically significant differences in baseline clinical phenotypes or LVSD penetrance associated with *TTMtv* location across different TTN bands were identified¹⁶.

4. Materials And Methods

4.1. Study design

On a prospective basis, genetic testing was offered to all DCM probands in the care of our Unit and cascade screening was offered to all probands' families. All *TTMtv*, considered to be pathogenic or likely pathogenic according to the American College of Medical Genetics and Genomics (ACMG) criteria^{16,17}, were identified between 2012 and 2021.

Data from all persons (probands and relatives) with *TTMtv* in the care of the National Institute of Cardiology, Warsaw, were retrospectively collected, including baseline clinical information from the first documented visit to the Institute and follow-up data, in particular data for all major cardiovascular events. In case of patients whose first contact to the Institute took place during HF exacerbations the baseline evaluation was moved to latter ambulatory visits whenever possible. We analyzed the baseline data, comprising medical history, clinical examination, 12-lead electrocardiography, two-dimensional Doppler echocardiography, 24-hour Holter ECG monitoring, as well as the serum biomarkers - high-sensitivity cardiac troponin T (hs-cTnT) and N-terminal pro-B-type natriuretic peptide (NT-proBNP), measured during ambulatory visits in patients in stable condition (data on serum biomarkers measured in unstable

patients were not included). In all probands, coronary angiography or coronary computed tomography angiography was performed.

We sought to examine the order of appearance of abnormal signs in cardiomyopathy, including elevated circulating biomarkers. We also wanted to evaluate the prognostic value of circulating cardiac biomarker concentrations with regard to occurrence of MVA and esHF during the follow-up period.

The study was funded by the statutory grant 2.56/II/14 from the National Institute of Cardiology, Warsaw, Poland, the grant 2011/01/B/NZ4/03455 from the National Science Centre, Poland, and the recent grant ERA-CVD DETECTIN-HF/2/2017 from the National Centre for Research and Development, Poland. All methods were carried out in accordance with relevant guidelines and regulations. All patients and relatives signed written informed consent in accordance with the Declaration of Helsinki. The study received the approval of the Bioethics Committee of the National Institute of Cardiology.

4.2. Definitions

Left ventricular enlargement (LVE) was ascertained when the left ventricular end diastolic diameter (LVEDD) exceeded 112% of the predicted value, corrected for age and body surface area according to Henry's formula, while left ventricular systolic dysfunction (LVSD) was ascertained when left ventricular ejection fraction (LVEF) was $< 50\%$. We used the term left ventricular dysfunction (LVD) when one of the above mentioned criteria was met and the diagnosis of DCM was made when both criteria were met. When no LVE but more distinct LVSD was present (LVEF $< 45\%$), hypokinetic non-dilated cardiomyopathy (HNDC) was diagnosed; for the purpose of this study, patients with HNDC were included in DCM cohort. When LVEF fell below 35%, we used the term severe LVSD. In the presence of relevant abnormalities, not sufficient for the diagnosis of DCM/HNDC, such as LVE $> 117\%$, LVEF 45–49%, cardiac conduction defect (CCD), atrial or ventricular arrhythmias unexplained by other conditions, we used the term indeterminate cardiomyopathy (xCM).

CCD included atrioventricular block (AVB) and left bundle branch block (LBBB). First degree AVB was defined by a PR interval > 200 ms on standard 12-lead ECG. High degree AVB included type II second degree or third degree AVB. Atrial arrhythmias (AA) included atrial fibrillation (AF), flutter, and paroxysmal atrial tachycardia lasting ≥ 30 s. Ventricular arrhythmias (VA) included ventricular ectopy burden of $> 500/24$ hours on Holter monitoring or non-sustained ventricular tachycardia (nsVT), defined as ≥ 3 consecutive ventricular beats at > 120 bpm and a duration for < 30 seconds on Holter monitoring. If the VT lasted over 30 sec., it was considered sustained (sVT).

HF was recognized in the presence of typical symptoms (e.g. breathlessness or fatigue), accompanied by structural and/or functional cardiac abnormalities, resulting in a reduced cardiac output and/or elevated intracardiac pressures. The symptoms of HF were assessed using New York Heart Association classification (NYHA classes 1–4). The HF condition was considered stable when there had been no worsening of HF for ≥ 3 months. Peripartum cardiomyopathy was defined by the development of maternal systolic HF late in the pregnancy or early in the postpartum period⁴. When the symptoms of HF

had acute onset, were refractory to medical therapy and led directly to heart transplantation (HTx) or implantation of left ventricular assist device (LVAD) we used the term fulminant HF. End-stage HF was defined as HTx, LVAD implantation or death caused by HF.

MVA was defined as sudden cardiac death (SCD), cardiopulmonary resuscitation (CPR), or appropriate implantable cardioverter defibrillator (ICD) intervention, i.e. an ICD discharge or anti-tachycardia pacing (ATP) for termination of ventricular fibrillation/VT. Death was classified as sudden if it occurred within 1 hour of the onset of cardiac manifestations, or during sleep (in the absence of previous hemodynamic deterioration), or within 24 hours after the patient was last seen apparently stable clinically. Sudden cardiac arrest (SCA) was defined as occurring within 1 hour of the onset of acute symptoms and reversed by CPR. Relatives included all probands' family members with *TTMtv* identified as a result of cascade screening, irrespective of the degree of kinship.

A family history of SCD was considered positive if ≥ 1 first degree relative had died suddenly before the age of 50 years.

4.3. Biomarkers' measurements

The plasma levels of NT-proBNP were measured by the electrochemiluminescent immunoassays Elecsys 2010 (Roche, Germany) with the upper limit of normal values defined by the manufacturer at 125 pg/ml. The plasma levels of cardiac troponin T were measured by the troponin T hs-STAT (Roche, Germany) with the upper limit of normal values defined by the manufacturer at 14 ng/l. All measurements were performed in the National Institute of Cardiology laboratory.

4.4. DNA sequencing and TTN mutation analysis

DNA was extracted from the peripheral blood by phenol extraction, salting out method or using Genomic Maxi AX kit (A&A Biotechnology, Gdynia, Poland). Next generation sequencing (NGS) was performed in 46 probands using: whole exome sequencing (WES) in 27 probands, TruSight One Sequencing Panel Kit (TSO) in 4 probands or TruSight Cardio Sequencing Kit (TSC) in 15 probands (Illumina, San Diego, CA, USA). WES libraries were prepared using TruSeq Exome Enrichment Kit (Illumina), Nextera DNA Sample Preparation Kits (Illumina), or Twist Human Core Exome Kit (Twist Bioscience, South San Francisco, CA, USA). TSO sequencing was performed similarly to WES using Nextera DNA Sample Preparation Kit (Illumina) with only difference in the enrichment probes used. WES and TSO libraries were paired-end sequenced (2x100 bp) on Illumina HiSeq1500 or NovaSeq 6000 and TSC libraries on Illumina MiSeqDx. Library preparation, sequencing, and data analysis were performed as described previously⁴⁵. *TTMtv* identified with NGS were followed-up in probands and relatives with Sanger sequencing using BigDye Terminator v3.1 or v1.1 Cycle Sequencing Kit (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions and the 3500xL or 3130xl Genetic Analyzer (Life Technologies). The results were analyzed with Variant Reporter 1.1 Software (Life Technologies). Other genes with strong or moderate evidence of causative relationship with DCM (*ACTC1, ACTN2, BAG3, DES, DSP, FLNC, JPH2, LMNA, MYH7, NEXN, PLN, RBM20, SCN5A, TNNC1, TNNI3, TNNT2,, TPM1, VCL*)⁴⁶ were also inspected for

presence of rare variants. Identified variants were classified according to ACMG criteria^{17,47}. Carriers of pathogenic or likely pathogenic variants in other DCM-related genes were excluded from the study.

4.5. Statistical analysis

All results for categorical variables were presented as counts and percentages and for continuous variables as mean and standard deviation (SD) or median and quartiles (Q1:25th, Q2:75th percentiles). The chi-square independence or Fisher exact test was used for comparison of categorical variables. The differences between continuous variables were tested by Student's t-test (for two independent samples, normally distributed data), or in the case of skewed distribution, non-parametric Mann-Whitney tests. A receiver-operating characteristic curve (ROC) analysis was used to assess the cut-off point of the markers for the prediction of events. The optimal cut off was defined as the value with the maximal sum of sensitivity and specificity. Event analysis over time was made by using the univariable and multivariable Cox proportional-hazards regression model. In order to indicate independent predictors of events the stepwise (backward) variable selection procedure was used. All variables with significant prognostic impact in univariate analysis ($p \leq 0.10$) were included in the multivariable model. Then a backward selection was used to creating the final model. The proportionality of hazards was verified using the weighted Schoenfeld residuals. Model discrimination was assessed using Harrell's Concordance Statistics (C-index). Risk was quantified as a hazard ratio (HR) with 95% confidence interval (CI). In case of a zero number of events in one of subgroups, HRs were calculated using the Firth's penalized likelihood approach. Survival curves were constructed by the Kaplan-Meier method and compared by the log-rank test. In subjects without an event, the follow-up period extended to the most recent evaluation or the date of May 31st, 2021. All hypotheses were two-tailed with a 0.05 type I error. All statistical analyses were performed using SAS statistical software, version 9.4 (SAS Institute, Cary, NC, USA).

4.6. Study limitations

The major limitation of the study is small sample size due to its single-centre character and hence, small number of major cardiovascular events, precluding the use of multivariate analysis models. The retrospective observational design of the study may include confounders. This study comes from a tertiary referral centre, one of two leading cardiology centers in Poland performing HTx, therefore patients may present with more severe disease than patients usually admitted in other centers.

Declarations

Author contributions:

Conceptualization, P.C. and Z.T.B.; data curation, P.C., I.K., G.T.; formal analysis, P.C., and I.K.; investigation, P.C., G.T., E.M., M.S.-M., M.F., P.S., M.S.-W., A.O., M.L., P.L., M.B., T.Z., and Z.T.B.; methodology, P.C., M.R., I.K., R.P., and Z.T.B.; supervision, R.P. and Z.T.B.; writing—original draft, P.C., G.T., I.K., and Z.T.B.; writing—review and editing, P.C., M.R., E.M., M.S.-M., M.F., P.S., M.S.-W., A.O., M.L., P.L., M.B., T.Z., and R.P. All authors have read and agreed to the published version of the manuscript.

Competing interests.

The authors declare no competing interests.

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Figures

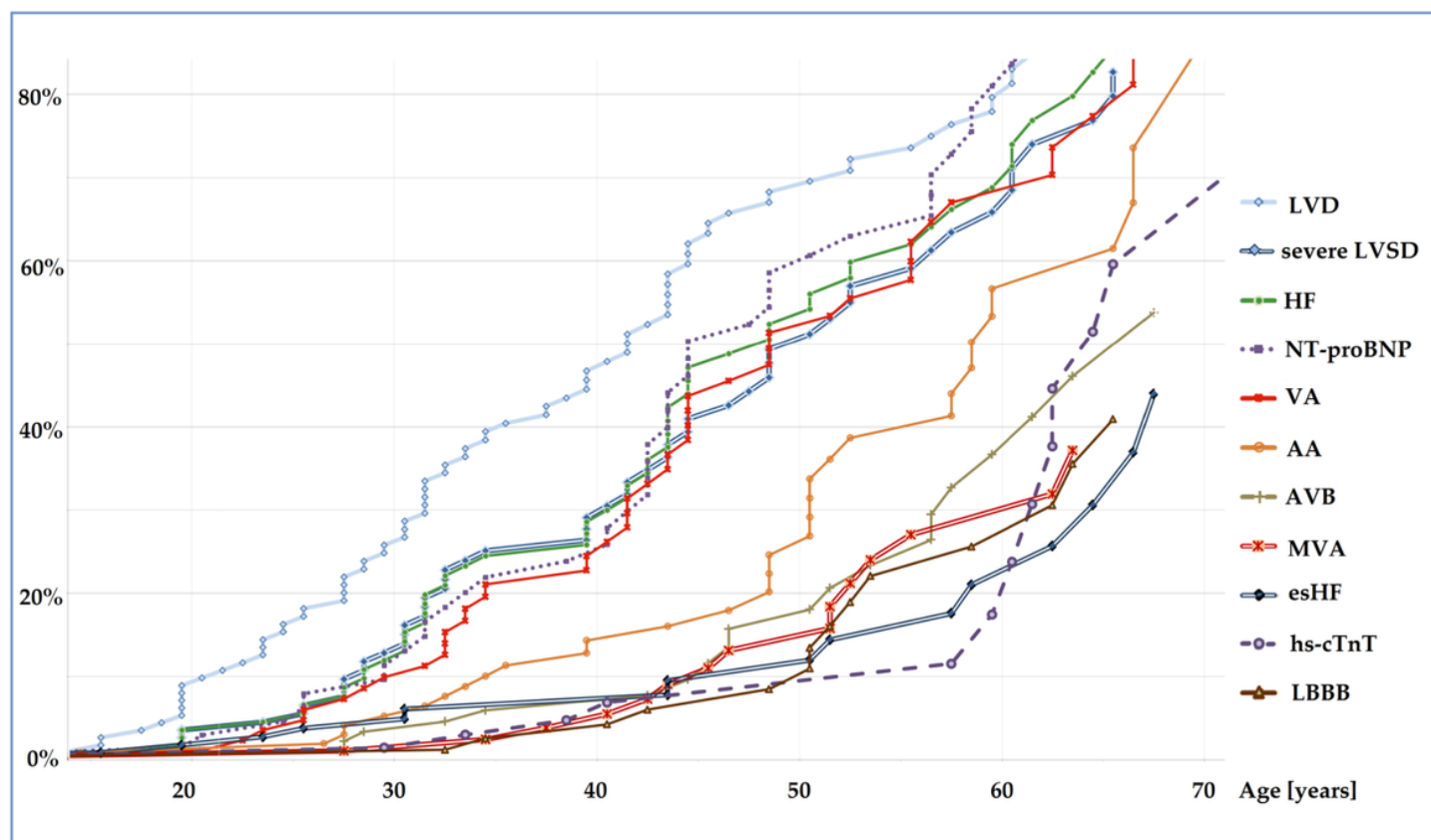


Figure 1

Penetrance of disease symptoms in the whole study cohort of TTNtv carriers. Legend: AA, atrial arrhythmia; AVB, atrioventricular block; esHF, end-stage heart failure; HF, heart failure; hs-cTnT, high-sensitivity cardiac troponin T concentration >14 ng/l; LBBB, left bundle branch block; LVD, left ventricular dysfunction; severe LVSD, severe left ventricular systolic dysfunction; MVA, malignant ventricular arrhythmia; NT-proBNP, N-terminal pro-B-type natriuretic peptide serum concentration >125 pg/ml; TTNtv, titin truncating variants; VA, ventricular arrhythmia.

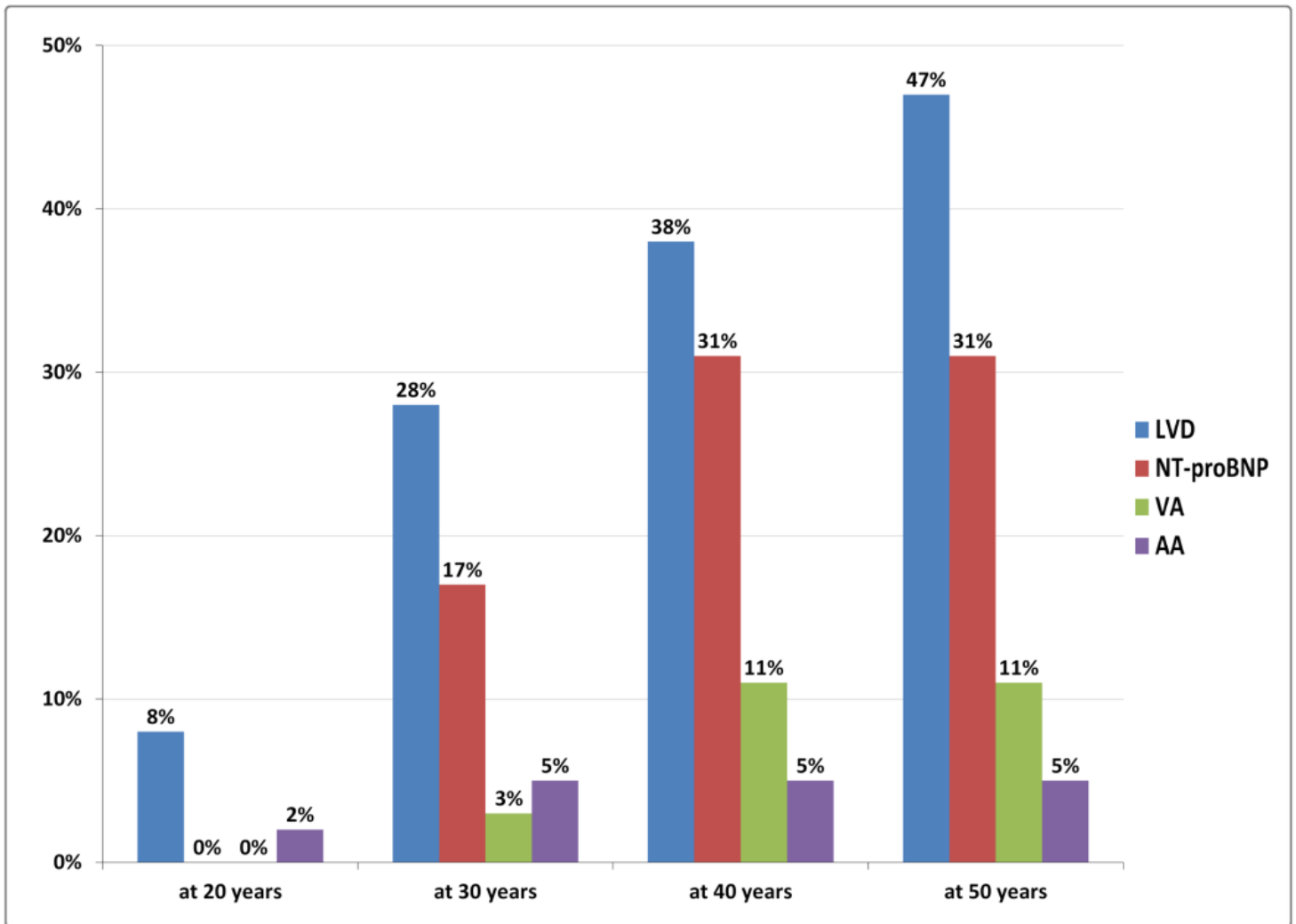


Figure 2

Penetrance of cardiomyopathy indicators in asymptomatic TTNtv carriers identified through familial screening. Legend: AA, atrial arrhythmia; LVD, left ventricular dysfunction, NT-proBNP, N-terminal pro-B-type natriuretic peptide serum concentration >125 pg/ml; VA, ventricular arrhythmia.

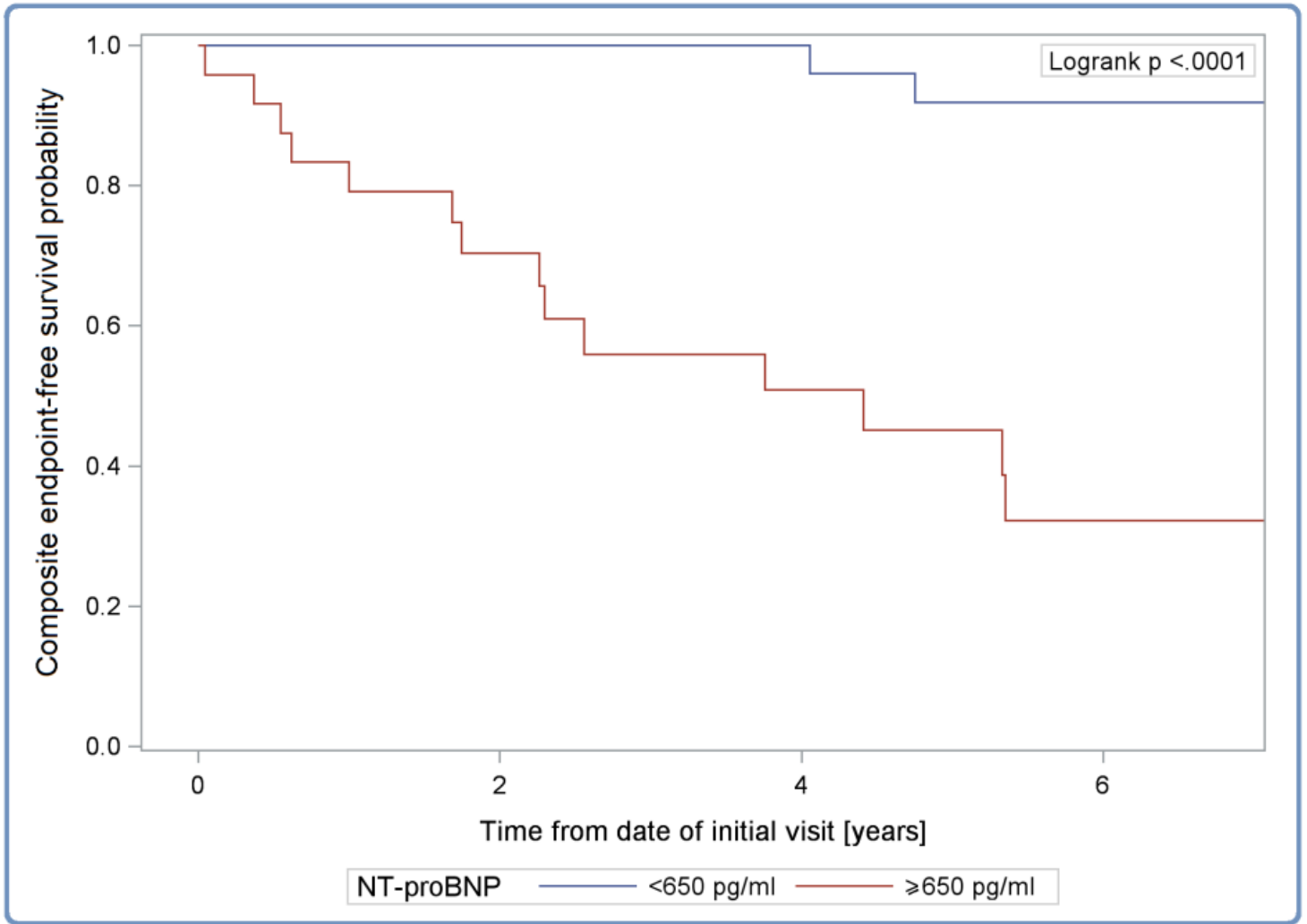


Figure 3

Kaplan-Meier composite endpoint-free survival curves in cardiomyopathy according to NT-proBNP serum concentration. Legend: NT-proBNP, N-terminal pro-B-type natriuretic peptide serum concentration.

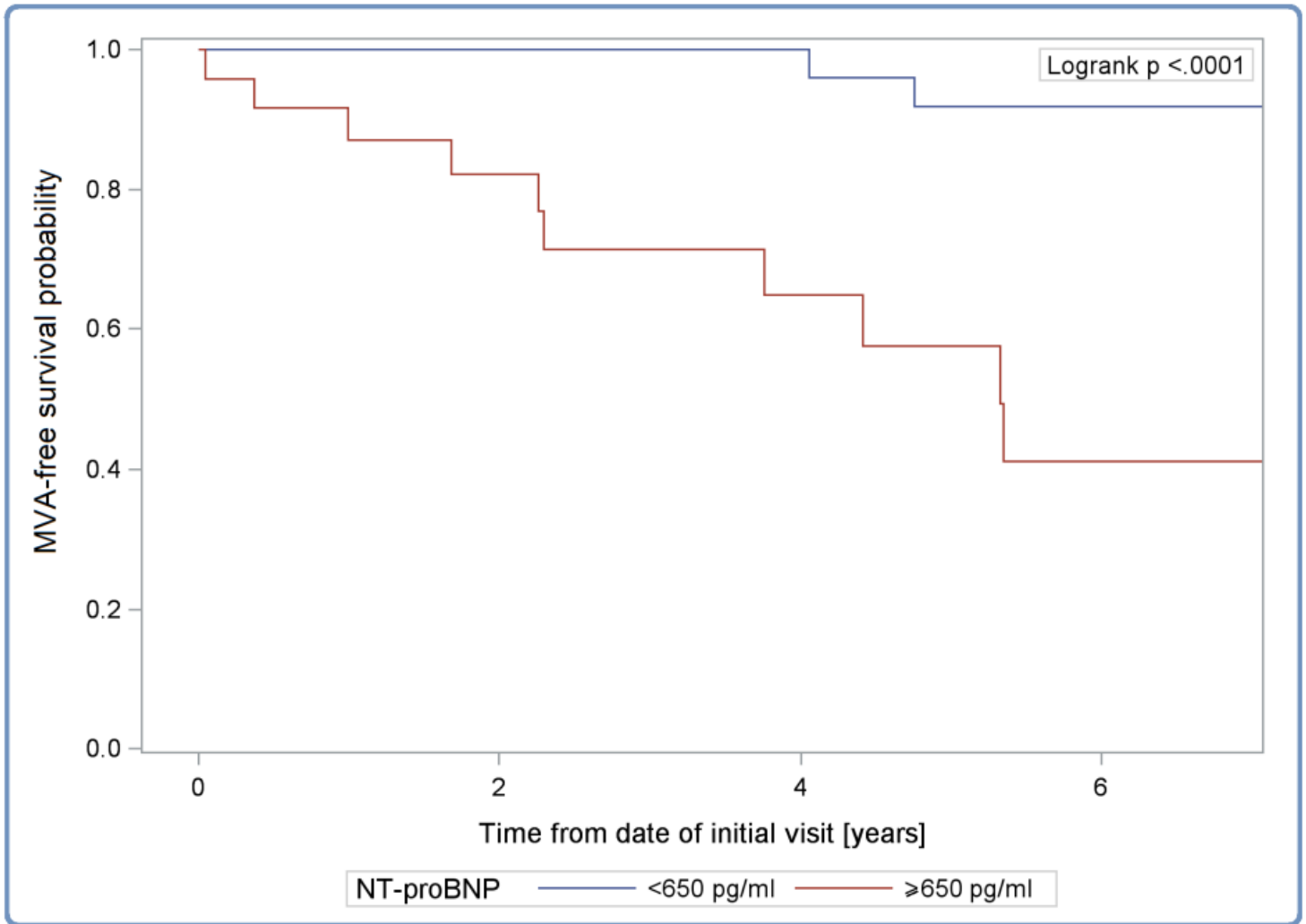


Figure 4

Kaplan-Meier malignant ventricular arrhythmia-free survival curves in cardiomyopathy according to NT-proBNP serum concentration. Legend: MVA, malignant ventricular arrhythmia; NT-proBNP, N-terminal pro-B-type natriuretic peptide serum concentration.

Supplementary Files

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