Clinical features, etiology, and survival in patients with restrictive cardiomyopathy: A single-center experience

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ABSTRACT

Background: Numerous prognostic factors have been proposed for cardiac amyloidosis (CA). The knowledge about other subtypes of restrictive cardiomyopathy (RCM) is scant.

Aims: This study aimed to elucidate the etiology and prognostic factors of RCM as well as assess cardiac biomarkers: high-sensitive troponin T (hs-TnT), growth differentiation factor-15 (GDF-15), N-terminal pro-B-type natriuretic peptide (NT-proBNP), and soluble suppression of tumorigenicity 2, as mortality predictors in RCM.

Methods: We enrolled 36 RCM patients in our tertiary cardiac department. All patients were screened for CA. Genetic testing was performed in 17 patients without CA.

Results: Pathogenic or likely pathogenic gene variants were found in 86% of patients, including 5 novel variants. Twenty patients died, and 4 had a heart transplantation during the study. Median overall survival was 29 months (8–55). The univariate Cox models analysis indicated that systolic and diastolic blood pressure, GDF-15, hs-TnT, NT-proBNP, left ventricular stroke volume, the ratio of the transmitral early peak velocity (E) estimated by pulsed wave Doppler over the early mitral annulus velocity (e'), tricuspid annulus plane systolic excursion, early tricuspid valve annular systolic velocity, the presence of pulmonary hypertension, and pericardial effusion influenced survival (P <0.05). A worse prognosis was observed in patients with GDF-15 >1316 pg/ml, hs-TnT >42 ng/l, NT-proBNP >3383 pg/ml, and pericardial effusion >3.5 mm (Kaplan-Meier analysis, log-rank test, P <0.001).

Conclusions: Genetic testing should be considered in every RCM patient where light-chain amyloidosis has been excluded. Survival remains poor regardless of etiology. Increased concentrations of GDF-15, hs-TNT, NT-proBNP, and pericardial effusion are associated with worse prognosis. Further studies are warranted.

Key words: genetic testing, growth differentiation factor-15, light-chain amyloidosis, restrictive cardiomyopathy, soluble suppression of tumorigenicity 2

WHAT'S NEW?

This is the first report about clinical utility of new cardiac biomarkers, growth differentiation factor-15 (GDF-15) and soluble suppression of tumorigenicity 2, in the whole spectrum of restrictive cardiomyopathy (RCM). We have preliminarily identified GDF-15, high-sensitive troponin T, N-terminal pro B-type natriuretic peptide, and pericardial effusion as relevant predictors of death in RCM. We have described an easily applicable diagnostic workup for RCM, which may be useful in countries where there are no diagnostic centers for amyloidosis. We have presented an insightful analysis of RCM etiology including up-to-date genetic testing results with five novel genetic variants.

INTRODUCTION

Restrictive cardiomyopathy (RCM) occurs with an incidence of only 2% of all cardiomyopathies in adults, and although heterogeneous, is the rarest cardiomyopathy according to the European Registry [1]. Cardiac amyloidosis (CA) is a traditional paradigm of RCM [2]. Numerous prognostic factors in CA have been proposed. N-terminal pro-B-type natriuretic peptide (NT-proBNP) and troponin T have been included in the light-chain (AL) amyloidosis staging system since 2012 [3]. The role of the right ventricular (RV) dimension and function in CA is considered important [4, 5]. Growth differentiation factor-15 (GDF-15) and soluble suppression of tumorigenicity 2 (sST2) have proved to be encouraging biomarkers in AL amyloidosis [6, 7]. The following prognostic factors have been described in non-amyloid RCM (na-RCM): male sex, age >70 years, the New York Heart Association Functional Classification (NYHA class) ≥III, left atrial (LA) diameter >60 mm, and low cardiac output [8]. Recent studies underline the role of left ventricular end-diastolic diameter (LVEDD) and tricuspid regurgitation [9].

GDF-15 is considered a cardioprotective hormone due to its antioxidative, anti-inflammatory, and antiapoptotic properties [10]. In heart failure (HF) with preserved ejection fraction, a positive correlation of GDF-15 concentrations with echocardiographic parameters of left ventricular (LV) diastolic dysfunction was observed [11].

In patients with acute dyspnea assessed in the emergency unit, sST2 concentrations correlated with systolic pressure in the right ventricle and early diastolic myocardial velocities (e') [12].

Pathogenic mutations in nineteen different genes have been identified in patients with primary RCM [13]. On the other hand, Anderson-Fabry disease, transthyretin (ATTR) amyloidosis, or glycogenosis may also demonstrate RCM phenotype [2], so screening of genes associated with these diseases should be considered if clinically reasonable.

This study aimed to describe the etiology of RCM, including the genetic background of na-RCM, to identify prognostic factors and to assess whether new cardiac biomarkers, GDF-15 and sST2, may be useful in clinical evaluation of this group.

METHODS

The study enrolled 36 consecutive RCM patients diagnosed in a tertiary cardiac department from January 2015 to August 2016. Patients were followed up until April 2021. Features of RCM including biatrial enlargement, normal LV cavity size, and systolic function with severe diastolic dysfunction were assessed on echocardiography by an experienced cardiologist. Severe diastolic dysfunction was defined by criteria presented in Table 1 based on the current recommendations [14]. Patients with the following echocardiographic presentations of hypertrophic cardiomyopathy (HCM) were not included: profuse asymmetric LV hypertrophy without features of amyloidosis, systolic anterior motion of mitral valve leaflet, and LV outflow tract obstruction.

Lack of informed consent (n = 1), dialysis treatment (n = 1), and neoplastic disease other than AL amyloidosis (n = 0) were the exclusion criteria (see Figure 1).

On presentation, levels of serum NT-proBNP, high-sensitive troponin T (hs-TnT), GDF-15, sST2, and creatinine were measured. Concentrations of GDF-15 and sST2 were measured using R&S Quantikine ELISA Kits (Minneapolis, MN, US).

Echocardiography

In order to obtain a reliable assessment of LV diastolic dysfunction, echocardiography with tissue Doppler imaging (TDI) was performed. The dimensions and functions of both ventricles and the LA volume index (LAVI) were assessed according to current guidelines [15, 16]. Atrial dimensions were also presented as their areas.

Table 1. Criteria for severe diastolic dysfunction

Sinus rhythm + LVEF ≥50%	Sinus rhythm + LVEF <50%	Atrial fibrillation
E/e' ratio >14	E/e' ratio >14	E/e' septal ≥11
e' septal <7 cm/s and e' lateral <10 cm/s	TRPV >2.8 m/s	IVRT ≤65 ms
TRPV >2.8 m/s	LAVI >34 ml/m^2	DT <160 ms
LAVI >34 ml/m^2		

Abbreviations: DT, deceleration time; e' lateral, mitral annular early diastolic lateral velocity; e' septal, mitral annular early diastolic septal velocity; E/e' ratio, the ratio of the transmitral early peak velocity (E) estimated by pulsed wave Doppler over the early mitral annulus velocity (e'); IVRT, isovolumic relaxation time; LAVI, left atrial volume index; LVEF, left ventricular ejection fraction; TRPV, tricuspid regurgitation peak velocity

Left ventricular function was assessed by modified Simpson's method as LV ejection fraction (LVEF). Maximal systolic velocities of LV longitudinal fibers (LV S') were assessed in apical views by TDI [17]. RV function was described by tricuspid annular plane systolic excursion (TAPSE) measured in M-mode presentation in a four-chamber view and by the longitudinal myocardial velocity of the right ventricle (RV S') measured on TDI. The ratio of the transmitral early peak velocity (E) estimated by pulsed wave Doppler over average e' velocity estimated by TDI (E/e' ratio) was used to assess LV diastolic dysfunction.

Pericardial effusion was defined as an echo-free space between the two layers of the pericardium. The amount of pericardial effusion was assessed as small (<10 mm) or moderate (10–20 mm).

Pulmonary hypertension (PH) was assessed using tricuspid regurgitation peak velocity (TRPV) [18]. Values of TRPV >3.4 m/s indicated a high PH possibility. Additional criteria were used for values of TRPV between 2.8 and 3.4 m/s: inferior vena cava (IVC) diameter >21 mm with decreased inspiratory collapse; RV/LV basal diameter or area ratio >1.0; interventricular septum flattering; pulmonary artery (PA) diameter >25 mm or PA diameter >aortic root; RV outflow tract acceleration time <105 ms and/or mid-systolic notching.

Cardiac amyloidosis screening

Every patient enrolled in the study was screened for CA. The full list of analyzed amyloidosis features is presented in the Supplementary material, *Table S1*. The main clinical data taken into consideration were as follows: age >30 years, no family history of cardiomyopathy, a short history of HF symptoms (≤12 months), rapidly progressing HF, early satiety, loss of appetite (particularly aversion to meat dishes), weight loss (at least 10 kg), persistent diarrhea or constipation, hoarseness, and macroglossia. Medical history and typical features of amyloidosis, such as a low amplitude and pseudo-infarct pattern on 12-lead electrocardiogram, hyperechogenicity of the myocardium on echocardiography, right atrial enlargement, thickening of the interatrial septum and valve leaflets, or characteristic late gadolinium enhancement and abnormal gadolinium kinetics on cardiovascular magnetic resonance (CMR) resulted in further investigations for amyloidosis, including biopsy.

Amyloid typing

Cardiac AL amyloidosis was diagnosed using free light chain (sFLC) concentrations in the serum (Binding Site test, Birmingham, United Kingdom), the serum and urine immunofixation, and at least two biopsies: endomyocardial biopsy, labial salivary gland biopsy, gastric biopsy, surgical fat tissue biopsy, and hematologic consultation including bone marrow biopsy.

Immunohistochemistry (IHC) was performed for amyloid typing. Four monoclonal antibodies were used: against serum amyloid A, transthyretin, kappa, and lambda light chains (DAKO, Glostrup, Denmark).

Cardiac ATTR amyloidosis was confirmed with tissue biopsy and Technetium-based diphosphono-1,2-propanodicarboxylic acid (Tc-99m-DPD) scintigraphy. In every patient with ATTR amyloidosis, genetic analysis was performed and coding regions of the transthyretin (*TTR)* gene were screened by Sanger sequencing (SGS).

Genetic testing

Commercial testing of the galactosidase alpha (*GLA*) gene was performed in two patients with clinical features of Anderson-Fabry disease (SGS, CENTOGENE, Rostock, Germany). DNA was extracted from the peripheral blood by phenol extraction or salting-out method in 15 patients. In 12 patients, Next Generation Sequencing (NGS) was performed using the TruSight One (TSO) sequencing panel consisting of >4800 disease-associated genes (Illumina, San Diego, California, CA, US) on Illumina HiSeq 1500. Whole exome sequencing (WES) was performed in 2 patients. WES libraries were prepared using the TruSeq Exome Enrichment Kit (Illumina San Diego, CA, US) and sequenced on Illumina HiSeq1500. Library preparation, sequencing, and data analysis were performed as described previously [19]. SGS was performed in one patient and the presence of a gene variant detected previously in a relative was confirmed (Patient 18 in Table 2).

Results were inspected for rare (minor allele frequency <0.001 for dominant and <0.05 for recessive disorders) protein-coding or splicing variants in HCM- and RCM-associated genes including genes causative for genetic amyloidosis and storage diseases (Supplementary material, *Table S2*). The identified variants were classified according to the American College of Medical Genetics and Genomics guidelines [20]. Pathogenic and likely pathogenic variants identified with NGS were followed up in probands and their relatives with SGS using BigDye Terminator v3.1 or v1.1 Cycle Sequencing Kit (Life Technologies, Carlsbad, CA, US) according to the manufacturer's instructions and the 3500xL or 3130xl Genetic Analyzer (Life Technologies, Carlsbad, CA, US). The results were analyzed with Variant Reporter 1.1 Software (Life Technologies, Carlsbad, CA, US).

Statistical analysis

The Shapiro-Wilk test was used for data distribution assessment. Data were presented as mean with SD or as medians with interquartile ranges (IQR) depending on data distribution. The quantitative variables of two groups (the AL amyloidosis group and the na-RCM group) were compared with the independent samples t-test or the Mann-Whitney test. Fisher's exact test was used for the comparison of categorical variables. The Pearson or the Spearman correlation analysis was used depending on data distribution. All-cause mortality was the only one analyzed endpoint. Four na-RCM patients who underwent heart transplantation were excluded from the survival

Table 2. Diagnostics of 18 patients with non-amyloid restrictive cardiomyopathy

No.	Age (yrs) and sex	Amyloidosis diagnostics			Genetic testing		Survival (mos.)
		sFLC	CMR	biopsy	Gene and ACMG classification	Variant position (hg38), nucleotide, and amino acid change	
1.	45, F	N/A	(–)	N/A	*GLA*[c] Pathogenic	chrX-101407766-G-T, NM_000169.3:c.138C>A (p.His46Gln)	1, OHT
					GLA Pathogenic	chrX-101407751-C-G, NM_000169.3:c.153G>C (p.Met51Ile)	
					GLA Pathogenic	chrX-101407737-C-A, NM_000169.3:c.167G>T (p.Cys56Phe)	
2.	27[a]	N/A	(–)	(–) EMB	*TTN*[c] Likely pathogenic	chr2-178534401-A-G, NM_001267550.2:c.102214T>C (p.Trp34072Arg), rs375159973	11, OHT
3.	35[a], F	(–)	(–)	(–) EMB	*MYH7* Pathogenic	chr14-23429005-G-A, NM_000257.4:c.1357C>T (p.Arg453Cys), rs121913625	10, OHT
4.	65	N/A	(–)	N/A	N/A (systemic sclerosis – genetic testing not performed)		28[d]
5.	57[a], F	N/A	(–)	(–)	*GLA* Pathogenic	chrX-101403846-G-A, NM_000169.3:c.334C>T (p.Arg112Cys)	70
6.	20[a], F	N/A	(–)	N/A	*MYH7* Likely pathogenic	14:23418243-G-T, NM_000257.4:c.4136C>A (p.Ala1379Asp)	69
7.	33, F	(–)	(?)	(–) EMB	*TNNI3*[c] Likely pathogenic	19:55151904-A-C, NM_000363.5:c.563T>G (p.Val188Gly)	66
8.	42, F	N/A	(–)	N/A	*FLNC*[c] Likely pathogenic	7:128851562-T-G, NM_001458.5:c.5776T>G p.Tyr1926Asp	10[d]
					TTN[c] VUS	2:178776534-C>T, NM_001267550.2:c.5330G>A (p.Cys1777Tyr)	
9.	55[a]	N/A	(–)	N/A	*FLNC*[c] VUS	7:128849405-G>A, NM_001458.5:c.5026G>A (p.Gly1676Arg)	36[d]
10.	49, F	N/A	(–)	(–) EMB	*PRKAG2* Likely pathogenic	7:151576440-A>G, NM_016203.4:c.877T>C (p.Phe293Leu)	58
					BAG3 VUS	10:119676965-G>A, NM_004281.4:c.1411G>A (p.Glu471Lys), rs778496291	
11.	63	(?)	(–)	(–)	MYBPC3 Pathogenic	11:047332813-C>A, NM_000256.3:c.3490+1G>T, rs397516020	5[d]
12.	44[a]	(–)	(–)	N/A	*MYH7*[c] Likely pathogenic	14:023425363-A>T, NM_000257.4:c.2342T>A (p.Leu781Gln)	58
13.	63	N/A	(–)	(–) EMB	*MYBPC3* Pathogenic	chr11-47341990 C-G, NM_000256.3:c.1790+1G>C	18[d]
					ACTN2[c] VUS	chr1-236762528 G-C, NM_001103.4:c.2594G>C	
14.	40, F	(–)	(–)	(–)		Nothing to report	57
15.	50, F	N/A	(–)	(–) EMB	*TNNI3*[c] Likely pathogenic	19:055151859-C-T, NM_000363.4:c.608G>A (p.Gly203Asp)	7, OHT[d]
16.	52	N/A	(–)	(–) EMB	*TNNI3*[c] Likely pathogenic	19:055154073-A-G, NM_000363.5:c.506T>C (p.Leu169Pro)	55[d]
17.	18, F	N/A	(–)	N/A	*BAG3* Pathogenic	chr10-119672373 C-T, NM_004281.4:c.626C>T (p.Pro209Leu) rs121918312	50[d]
18.	37[a,b]	N/A	(–)	N/A	*MYBPC3* Pathogenic	11:047332813-C>A, NM_000256.3:c.3490+1G>T, rs397516020	61

[a]Family history of cardiomyopathy; [b]Patient 18 is a relative of Patient 11; [c]Novel variant; [d]Patient died during the study

Abbreviations: ACMG, American College of Medical Genetics and Genomics; *ACTN2*, actinin alpha 2; *BAG3*, BAG cochaperone 3; F, female; *FLNC*, filamin C; *GLA*, galactosidase alpha; hg38, Genome Reference Consortium Human Build 38; *MYBPC3*, myosin binding protein C3; *MYH7*, myosin heavy chain 7; N/A, not applicable — the test was not performed; OHT, orthotopic heart transplantation; *PRKAG2*, protein kinase AMP-activated non-catalytic subunit gamma 2; *TNNI3*, cardiac troponin I; *TTN*, titin; (–), the test excluded amyloidosis; (?), the test did not exclude amyloidosis; other — see Figure 1

analysis. Survival was defined as the time between entry to the study and death for deceased patients (medical documentation or relatives' reports) and time to last follow-up in April 2021 for patients who stayed alive (personal contact or phone call). The univariate Cox proportional models were prepared. Kaplan-Meier curves analysis with log-rank tests were performed for overall survival predictors provided that the sample size was large enough. Patients were assigned into two subgroups using the median values of predictors. Statistical analysis was performed with MedCalc v. 22.009 and PQstat v.1.8.2.

The study conformed to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Cardinal Wyszynski National Institute of Cardiology in December 2014. All patients provided written informed consent to participate in the study.

RESULTS

Thirty-six patients (median age 52 years, 18 females) were enrolled (Figure 1). The clinical details of the 8 patients with negative CA screening results are presented in the Supplementary material, *Table S3*. Fourteen patients were

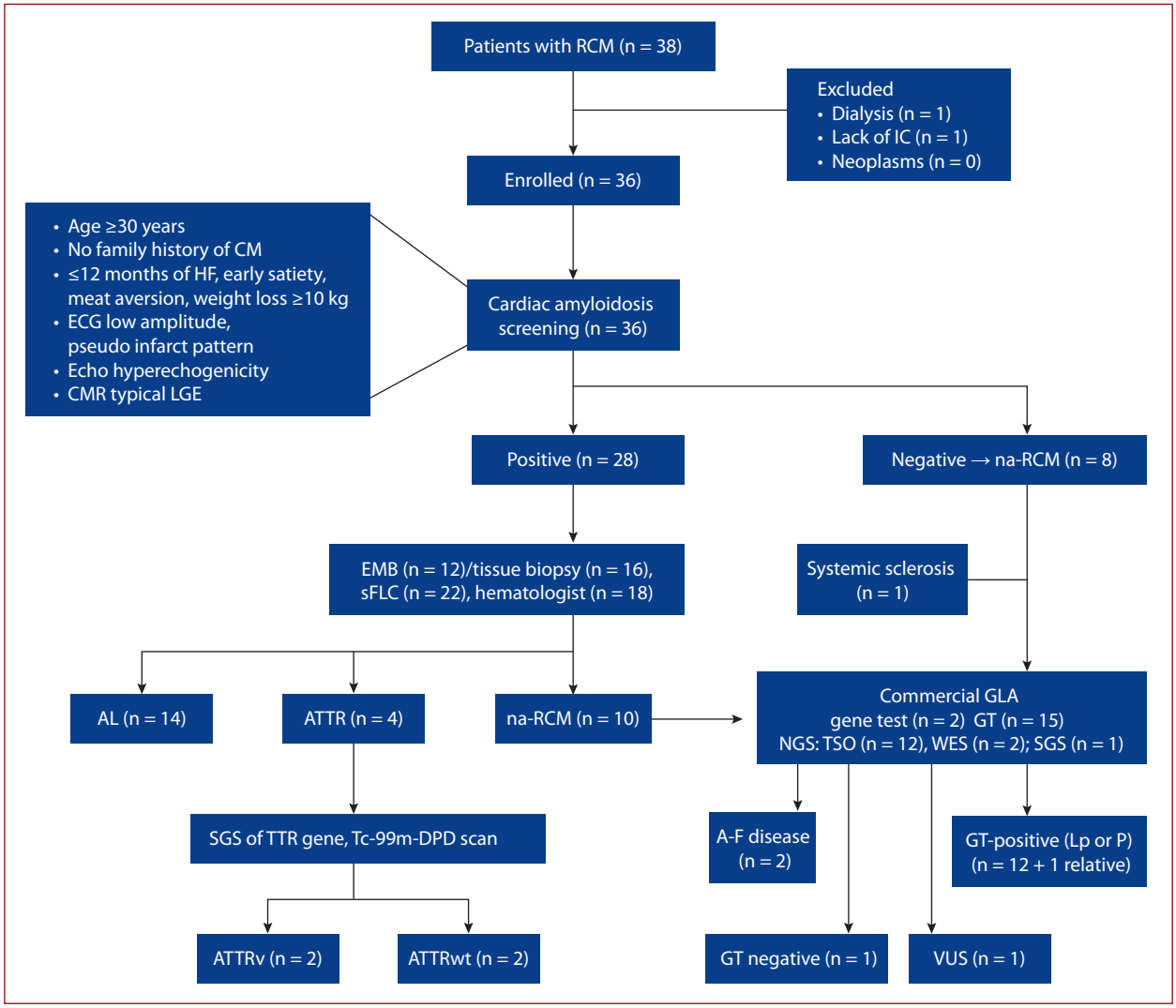

Figure 1. Patient flowchart

Abbreviations: A-F, Anderson-Fabry; AL, light-chain amyloidosis; ATTR, transthyretin amyloidosis; CM, cardiomyopathy; CMR, cardiovascular magnetic resonance; EMB, endomyocardial biopsy; sFLC, serum free light chains; *GLA*, alpha-galactosidase A; GT, genetic testing; HF, heart failure; IC, informed consent; LGE, late gadolinium enhancement; Lp, likely pathogenic gene variant; n, number of patients; na-RCM, non-amyloid RCM; NGS, next generationsequencing; P, pathogenic gene variant; RCM, restrictive cardiomyopathy; SGS, Sanger sequencing; Tc-99m-DPD scan, Technetium-based diphosphono-1,2-propanodicarboxylic acid scintigraphy; TSO, TruSight One; *TTR*, transthyretin; VUS, gene variant of uncertain significance; WES, whole exome sequencing

diagnosed with AL amyloidosis, 4 patients had ATTR amyloidosis, and 18 patients had na-RCM.

Two patients were diagnosed with variant ATTR amyloidosis, and the following gene variants were identified: NM_000371.4:c.157T>C(p.Phe53Leu) known as Phe33Leu, and NM_000371.4:c.302C>T(p.Ala101Val) known as Ala81Val, rs1555631417. Two patients were diagnosed with wild-type ATTR amyloidosis.

Genetic testing (GT) was performed in 15 patients with na-RCM, excluding two commercial tests of the *GLA* gene used for confirmation of Anderson-Fabry disease. Systemic sclerosis was the cause of na-RCM in one patient, so no GT was indicated (Figure 1). Detailed diagnostics of the na-RCM group with GT results are depicted in Table 2 (additional information — see the Supplementary material, *Table S4*). Positive GT results were observed in 12 of 14 patients (86%), who underwent NGS, and in one relative (Patient 18 see Table 2). Five novel pathogenic or likely pathogenic gene variants were detected in 5 patients.

The median follow-up time was 31.5 months (interquartile range [IQR]: 6.7–58.5) for all patients included in the survival analysis (n = 32) and 63.5 months (IQR: 58–67.5) for surviving patients (n = 12). At data cutoff, 20 patients (56%) had died with a median survival time of 9 months (IQR: 3.5–28.5). The median overall survival for all 32 patients included in the survival analysis was 29 months (IQR: 8–55). The main cause of death was advanced HF, and the main cause of death in AL amyloidosis was pulseless electrical activity.

General characteristics of the total cohort and comparison of the AL and na-RCM groups are presented in Table 3.

Table 3. General characteristics of the total cohort and comparison of the non-amyloid restrictive cardiomyopathy (na-RCM) group and the light-chain (AL) amyloidosis group

Variable	Total cohort n = 36	na-RCM n = 18	AL amyloidosis n = 14	*P*-value
Demographical and clinical data				
Age, years, median (IQR)	52 (43–63)	45 (35–55)	58 (50–65)	0.008
HF symptoms, months, median (IQR)	16 (12–37)	57 (34–84)	12 (6–14)	<0.001
Heart rate, n/min, median (IQR)	77 (67–90)	69 (60–80)	80 (75–96)	0.1
Atrial fibrillation, n (%)	15 (42)	10 (56)	3 (21%)	0.08
Systolic blood pressure, mm Hg, median (IQR)	113 (99–125)	112 (100–123)	106 (90–125)	0.3
Diastolic blood pressure, mm Hg, median (IQR)	74 (61–80)	71 (60–80)	68 (60–80)	0.5
Laboratory investigations				
Creatinine, µmol/l, median (IQR)	91 (80–110)	81 (78-107)	93 (81–117)	0.5
eGFR, ml/min/1.73 m^2, median (IQR)	66 (51–77)	69 (55–83)	64 (49–70)	0.3
GDF-15, pg/ml, median (IQR)	1316 (654–2204)	972 (420–2188)	1656 (1344–2472)	0.04
hs-TnT, ng/l, median (IQR)	39 (24–90)	25 (18–42)	112 (36–145)	<0.001
NT-proBNP, pg/ml, median (IQR)	3384 (1998–6578)	2074 (1064–3288)	7091 (4048–10028)	<0.001
sST2, ng/ml, median (IQR)	23 (17–39)	19 (12–27)	37 (23–62)	0.01
Echocardiography				
e' lateral, cm/s, median (IQR)	7 (5–9)	9 (6–11)	5 (3–7)	0.02
e' septal, cm/s, median (IQR)	5 (3–6)	5 (3–6)	4 (4–6)	0.4
E/e' ratio, median (IQR)	15 (12–21)	14 (11–18)	20 (12–25)	0.1
Left atrial area, cm^2, median (IQR)	29 (25–35)	35 (32–41)	27 (23–28)	<0.001
LAVI, ml/m^2, median (IQR)	67 (46–76)	86 (61–108)	48 (42–57)	0.02
LV IVS, mm, median (IQR)	16 (14–19)	14 (12–16)	18 (15–19)	0.02
LV posterior wall, mm, median (IQR)	14 (13–16)	13 (10–14)	16 (15–16)	<0.001
LV s', cm/s, median (IQR)	6 (5–7)	6 (5–7)	5 (4–8)	0.2
LVEF, %, median (IQR)	55 (45–65)	59 (45–65)	55 (50–70)	0.5
LV stroke volume, ml, median (IQR)	35 (31–52)	48 (33–62)	34 (22–40)	0.03
LV end-diastolic diameter, mm, median (IQR)	45 (41–48)	48 (43–49)	42 (39–45)	0.02
PASP, mm Hg, median (IQR)	45 (40–55)	55 (43–73)	44 (40–48)	0.07
Pulmonary hypertension, n (%)	25 (69)	11 (61)	12 (86)	0.2
Right atrial area, cm^2, median (IQR)	24 (20–30)	28 (24–39)	20 (18–21)	0.002
RV s', cm/s, median (IQR)	9 (8–11)	10 (8–11)	9 (7–11)	0.6
RV end-diastolic diameter, mm, mean (SD)	38 (6)	39 (8)	37 (4)	0.2
RV wall, mm, median (IQR)	8 (5–9)	5 (5–8)	8 (6–9)	0.08
TAPSE, mm, mean (SD)	16 (5)	18 (5)	14 (5)	0.03
TRPV, m/s, mean (SD)	3.1 (0.6)	3.3 (0.7)	2.8 (0.4)	0.04
Pericardial effusion, n (%)	19 (53)	6 (33%)	13 (93%)	<0.001
Pericardial effusion, mm, median (IQR)	3.5 (0.0–8.0)	0.0 (0.0–6.4)	8.0 (5.4–11.0)	0.009

Abbreviations: eGFR, estimated glomerular filtration rate; GDF-15, growth differentiation factor-15; hs-TnT, high-sensitive troponin T; IQR, interquartile range; LV, left ventricle; LV s', early mitral valve annular systolic velocity; NT-proBNP, N-terminal-proB-type natriuretic peptide; PASP, pulmonary artery systolic pressure; RV, right ventricle; RV s', early tricuspid valve annular systolic velocity; sST2, soluble suppression of tumorigenicity 2; TAPSE, tricuspid annulus plane systolic excursion; others — see Table 1

The prognostic factors for overall survival identified by univariate Cox models are presented in Table 4.

The sample size was big enough to perform Kaplan-Meier analysis with the log-rank test for four predictors of overall survival: GDF-15, hs-TnT, NT-proBNP, and pericardial effusion, considered as continuous variables (Figure 2).

The Kaplan-Meier analysis of the total cohort, excluding the recipients of heart transplants (n = 32), is presented in Figure 3.

Significant correlations of GDF-15 and sST2 with other variables observed in the entire group of 36 patients are presented in the Supplementary material, *Table S5*.

DISCUSSION

We describe a thorough analysis of the etiology, clinical characteristics, and prognostic factors of the rarest cardiomyopathy subtype. Cardiac amyloidosis screening should start diagnostic workup for every RCM patient [21]. We preferred an early invasive strategy and performed a biopsy in 77.8% of patients. Today, CA screening should include sFLC assessment with serum and urine immunofixation and DPD scan in the event of negative laboratory results. However, positive hematological tests should result in a prompt biopsy.

It is worth emphasizing that a high percentage of positive GT results was observed in the na-RCM group (86%). This justifies the inclusion of GT in diagnostic management of na-RCM. Such a high proportion of positive GT results, namely the identification of pathogenic or likely pathogenic gene variants, may have resulted from careful group selection and identification of specific heart muscle diseases. Notably, in that group, there were two patients

Table 4. Univariate Cox models analysis in 32 patients with restrictive cardiomyopathy

Univariate Cox model	Hazard ratio	95% confidence interval of hazard ratio	*P*-value
Systolic blood pressure	0.97	0.95–0.99	0.03
Diastolic blood pressure	0.95	0.91–0.99	0.01
GDF-15 (per 1000 pg/ml increase)	1.45	1.12–1.88	0.004
hs-TnT (per 10 ng/l increase)	1.10	1.04–1.16	<0.001
NT-proBNP (per 1000 pg/ml increase)	1.17	1.08–1.28	<0.001
LV stroke volume	0.95	0.92–0.99	0.007
E/e' ratio	1.06	1.01–1.11	0.01
TAPSE	0.84	0.75–0.94	0.002
RV s'	0.74	0.59–0.92	0.006
Pulmonary hypertension (yes vs. no)	4.33	1.26–14.90	0.02
Pericardial effusion (yes vs. no)	5.49	1.94–15.51	0.001
Pericardial effusion (per 1 mm increase)	1.13	1.04–1.22	0.003

Abbreviations: see Tables 1 and 3

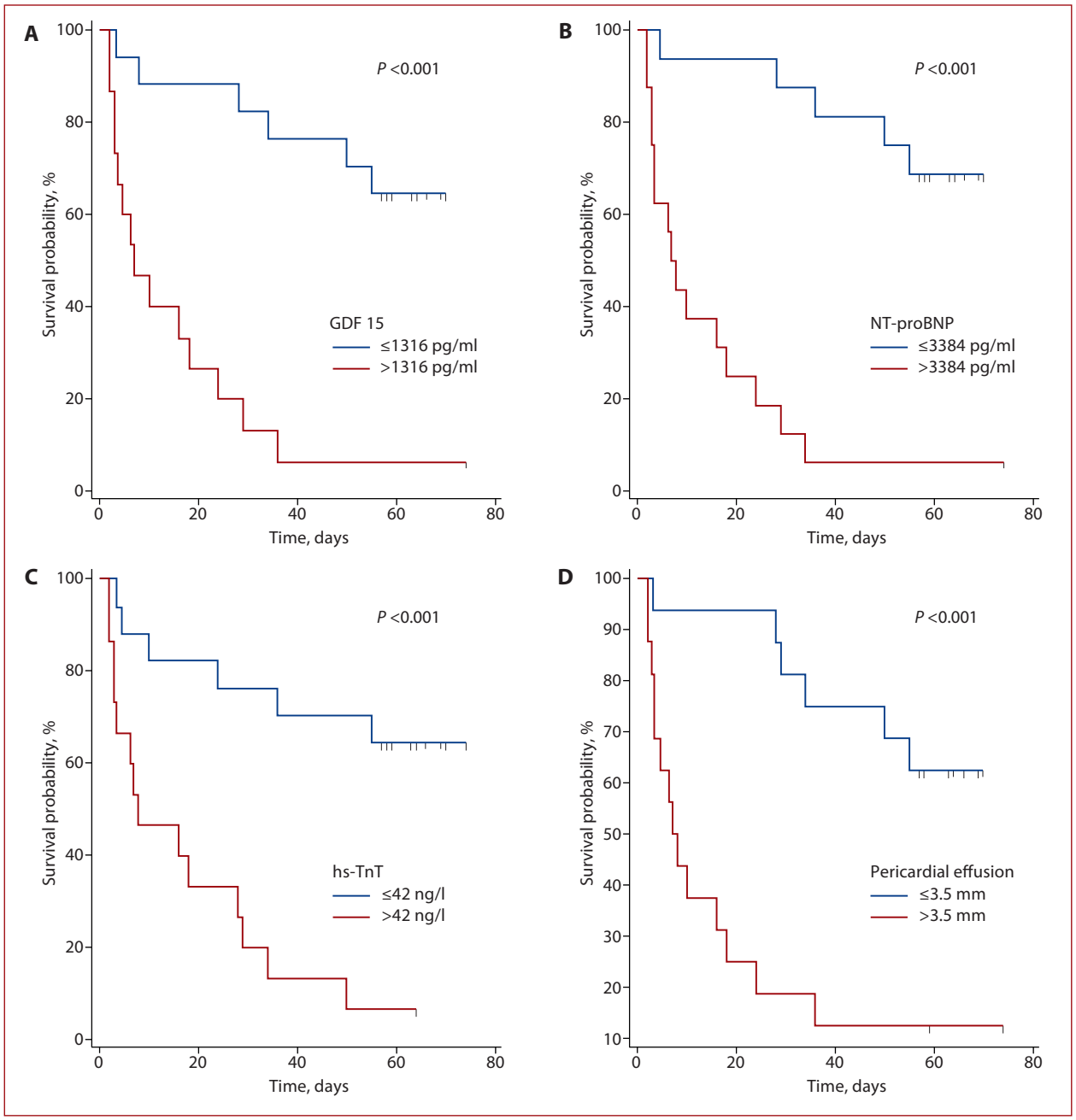

Figure 2. Prognostic factors for survival in restrictive cardiomyopathy. Kaplan-Meier survival curves stratified by the median of GDF-15 (**A**), NT-proBNP (**B**), high-sensitive troponin T (**C**), and pericardial effusion (**D**)

Abbreviations: see Table 2

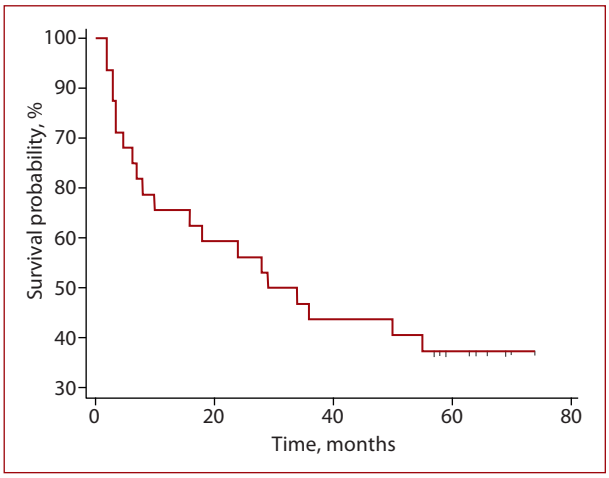

Figure 3. Overall survival in restrictive cardiomyopathy. Kaplan-Meier survival curve for 32 patients

with Anderson-Fabry disease – one with protein kinase AMP-activated non-catalytic subunit gamma 2 (*PRKAG2*) cardiomyopathy and one with myofibrillar myopathy related to the Bcl2-associated athanogene 3 (*BAG3*) Pro209Leu variant. Similarly, as in the literature, pathogenic sarcomeric variants were the most common. The study was conducted in a tertiary cardiac department, so patient selection bias should also be considered.

Importantly, we identified five novel gene variants, with further genetic research on RCM. Restrictive cardiomyopathy is still a lesser-known cardiomyopathy subtype. Data about the genetic background of RCM in Polish patients are scant.

As LVEF remains normal or slightly decreased in RCM patients for a long time [2], it is important to include amyloidosis [22] and na-RCM in differential diagnosis of HF with preserved ejection fraction. Although LVEF is commonly used in clinical practice, it is less suitable for assessing prognosis in RCM than RV function. Only ten patients (28%) in the study had decreased LVEF <50%. The lowest LVEF value was 40%, and this was observed in four patients. Whereas decreased RV function defined as TAPSE <17 mm [16] was present in 19 patients (53%).

Results concerning atrial areas were serendipitous. Atrial dilation is considered a reliable marker of increased pressure in the left ventricle and diastolic dysfunction [14]. Meanwhile, in our study, patients with AL amyloidosis had smaller LA dimensions than in the na-RCM group; they had also lower lateral mitral annular e' velocities. The latter variable suggested more advanced diastolic dysfunction. Similarly, the higher values of the E/e' ratio in the AL group could indicate a more restrictive filling pattern although the latter difference was not significant. We suggest that amyloid deposits gathering in the atrial wall prevent further atrial dilation. Thus, LA dimension in AL amyloidosis may not actually reflect diastolic dysfunction.

Troponin T, NT-proBNP [23], GDF-15 [6], and pericardial effusion [24] were proposed as prognostic factors in AL amyloidosis. We observed a relevant prognostic value of these parameters in RCM regardless of disease etiology. Small (<10 mm; n = 12) to moderate (10–20 mm; n = 7) amounts of pericardial effusion were observed, with no cases of cardiac tamponade. Interestingly, just 3.5 mm of pericardial effusion indicated worse survival.

Recent studies showed that GDF-15 levels may be used in early determination of anthracycline-induced cardiomyopathy during treatment of childhood cancers [25] and are positively correlated with myocardial fibrosis parameters in systemic sclerosis [26]. Soluble ST2 predicts negative outcomes for patients with Chagas disease [27].

However, according to our knowledge, this is the first analysis of GDF-15 and sST2 concentrations in the whole spectrum of RCM. The advantage of GDF-15 above sST2 in RCM prognosis needs to be confirmed in further studies. Correlations observed between GDF-15 and RV function demonstrate the utility of this biomarker in right ventricular assessment in RCM (Supplementary material, *Table S5*).

Limitations

Patients were diagnosed from January 2015 to August 2016. Current guidelines concerning amyloidosis [28, 29] were not available at that time, so we needed to refine our own diagnostic algorithm to achieve an indisputable diagnosis. The early invasive strategy with endomyocardial or surrogate tissue biopsy, as presented in the study, is gaining ground today, especially in the case of high AL amyloidosis suspicion [29, 30].

Amyloid typing was performed by immunohistochemistry instead of mass-spectrometry, which is considered the gold standard. Still, it is accepted that referral centers use a method with which they are familiar [28, 29]. Each amyloidosis diagnosis was confirmed by two tissue/organ biopsies and further testing: DPD scan and genetic evaluation of the *TTR* gene, or hematological assessment for AL amyloidosis.

The limited size of the study group is a serious drawback. Prognostic factors for overall survival identified by the univariate Cox model may be thrown into question. The study group was not large enough to perform multivariate Cox model analysis (n <33), which reduced the value of survival analysis. However, given limited data about RCM, which is an extremely rare disease, we decided to report the results of our study. For comparison purposes, the Cardiomyopathy Registry of the EURObservational Research Programme included 66 RCM patients [1].

Nevertheless, it should be emphasized that all patients met the recent RCM criteria proposed by the European Society of Cardiology, which require several months of persistence of a restrictive filling pattern to confirm RCM diagnosis [2]. However, we suggest being wary of postponing RCM diagnosis because any delay in confirming AL amyloidosis may be fateful.

It would be very interesting to include CMR parameters in survival analysis. Unfortunately, performing CMR in five

patients with AL amyloidosis was unfeasible due to clinical and technical difficulties.

Treatment analysis was not performed because of the limited size of the study group and the different schemes of chemotherapy for AL amyloidosis.

Since 2015, when the study started, new variants in RCM-associated genes have been identified [13]. We re-analyzed our data in 2023 in light of these new findings. However, only two of our patients had WES performed (Patients 3 and 9 in Table 2) and the discoidin CUB and LCCL domain-containing protein 2 (*DCBLD2)* gene variants assessment since the TSO panel does not allow this analysis. Variants of unknown significance of the filamin C (*FLNC)* gene were detected in two patients, and further studies would be warranted.

CONCLUSIONS

Light-chain amyloidosis is the most common cause of RCM. Primary RCM with genetic background is the second most frequent cause of RCM, what justifies including genetic testing in the diagnostic workup of RCM patients after exclusion of AL amyloidosis. Although we have found relevant differences in clinical pictures of AL amyloidosis and non-amyloid restrictive cardiomyopathy, the prognosis of both RCM subtypes remains poor. GDF-15 concentration upon hospital admission, but not sST2 concentration, should be considered as prognostic factor in RCM patients. Remaining predictors of death that are worth further studies include NT-proBNP and hs-TnT concentrations and pericardial effusion.

Supplementary material

Supplementary material is available at https://journals.viamedica.pl/kardiologia_polska.

Article information

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