

Danon disease is an underdiagnosed cause of advanced heart failure in young female patients: a LAMP2 flow cytometric study

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Abstract

Aims Danon disease (DD) is a rare X-linked disorder caused by mutations in the lysosomal-associated membrane protein type 2 gene (*LAMP2*). DD is difficult to distinguish from other causes of dilated or hypertrophic cardiomyopathy (HCM) in female patients. As DD female patients regularly progress into advanced heart failure (AHF) aged 20–40 years, their early identification is critical to improve patient survival and facilitate genetic counselling. In this study, we evaluated the prevalence of DD among female patients with non-ischemic cardiomyopathy, who reached AHF and were younger than 40 years.

Methods and results The study cohort comprised 60 female patients: 47 (78%) heart transplant recipients, 2 (3%) patients treated with ventricular assist device, and 11 (18%) patients undergoing pre-transplant assessment. Aetiology of the cardiomyopathy was known in 15 patients (including two DD patients). *LAMP2* expression in peripheral white blood cells (WBC) was tested by flow cytometry (FC) in the remaining 45 female patients. Whole exome sequencing was used as an alternative independent testing method to FC. Five additional female DD patients (two with different novel *LAMP2* mutations) were identified by FC. The total prevalence of DD in this cohort was 12%. HCM phenotype (57% vs. 9%, $*P = 0.022$) and delta waves identified by electrocardiography (43% vs. 0%, $**P = 0.002$) were significantly more frequent in DD female patients.

Conclusions Danon disease is an underdiagnosed cause of AHF in young female patients. *LAMP2* expression testing in peripheral WBCs by FC can be used as an effective screening/diagnostic tool to identify DD in this patient population.

Keywords Advanced heart failure; Danon disease; Lysosomal-associated membrane protein type 2; Screening; White blood cells

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Introduction

Danon disease (DD, OMIM 300257) is a rare X-linked disorder caused by mutations in the lysosomal-associated membrane protein type 2 gene (*LAMP2*, *Xq24*).¹ All three *LAMP2* isoforms (B, A, and C) contribute to lysosomal processing of autophagic substrates.² Almost all *LAMP2* mutations result in the absence of the protein [LAMP2 deficiency (LAMP2def)].

X-hemizygous male DD patients present with a complex phenotype that is dominated by cardiomyopathy with massive left ventricular hypertrophy, delta waves by electrocardiography, muscle weakness, and mild cognitive disability. Importantly, male DD patients have extremely poor prognosis due to end-stage congestive heart failure and malignant ventricular arrhythmias. Their mean age at heart transplantation or death is 18–19 years.^{3,4}

Unless modified by processes such as formation of syncytia,⁵ expression of the mutant *LAMP2* allele is mosaic (mosaic *LAMP2*def) because of X-chromosome inactivation (XCI) in the tissues of the X-heterozygous female DD patients. As a likely consequence, females present with a milder cardiac DD phenotype with an equal prevalence of (on many occasions isolated) dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM). DD female patients also become symptomatic approximately 15 years later than male patients, have an average survival ~35 years, and are less affected by skeletal myopathy and cognitive defects.³ However, as shown by the latest meta-analysis of published DD studies, similar proportions of DD females and males progress into end-stage heart failure.⁶ Some DD females are also at risk of sudden cardiac death.⁷ Timely identification of DD female patients is therefore important for their prognostic stratification and family counselling.

Peripheral white blood cells (WBCs) can be used to assess *LAMP2* expression/*LAMP2* deficiency in suspect male and female DD patients. *LAMP2* western blotting in WBCs homogenates was described by Fanin *et al.*⁸ who documented the protein deficiency in male DD patients. In their single female DD patient, however, the latter authors showed nearly normal *LAMP2* levels and highlighted the inefficiency of this approach due to problematic interpretation of the results in this particular patient group. Flow cytometric (FC) detection of *LAMP2* in WBCs was first reported by Regelsberger *et al.*⁹ Expanding these seminal studies, we optimized and presented a polychromatic FC protocol that allows quantitation of *LAMP2*def WBC populations not only in male DD patients but also in female DD patients and somatic mosaic carriers of *LAMP2* mutations.^{10–12}

Even though female patients are frequently the first affected individuals in DD families, many are identified retrospectively or even *post-mortem* after the diagnosis is established in their affected male relative(s) (brother or son). To the best of our knowledge, no large-scale study(ies) evaluating prevalence of DD among female patients with cardiologic pathologies has been presented. To fill this unfortunate information gap, we assessed the prevalence and evaluated the clinical characteristics of DD in female patients who reached advanced heart failure (AHF) due to non-ischemic cardiomyopathy and were younger than 40 years of age. *LAMP2* FC in WBCs was used as a screening diagnostic method. Whole-exome sequencing (WES) served as an alternative independent testing approach.

Methods

Study design and patient cohort

This was a two-centre cohort study. The study group was recruited from patients of the two Czech heart transplant

centres. From November 2016 to October 2018, 60 female patients with AHF due to non-ischemic cardiomyopathy were identified and agreed to inclusion in the study (Figure 1A). All patients were younger than 40 years at the time of heart transplantation or at pre-transplant assessment and were either living female heart transplant recipients, female patients on mechanical circulatory support or female patients referred to pre-transplant assessment. The selected cut-off age of 40 years was based on previously presented data of mean age of cardiac transplant (32.3 ± 14.5 years)³ and median age of end-stage cardiomyopathy [28 years (18.0–50.0)]⁶ in female DD patients.

The study was approved by the Ethics Committee of the authors' home institution and was conducted in accordance with the principles of the Declaration of Helsinki. All patients provided a written informed consent prior to participating in the study.

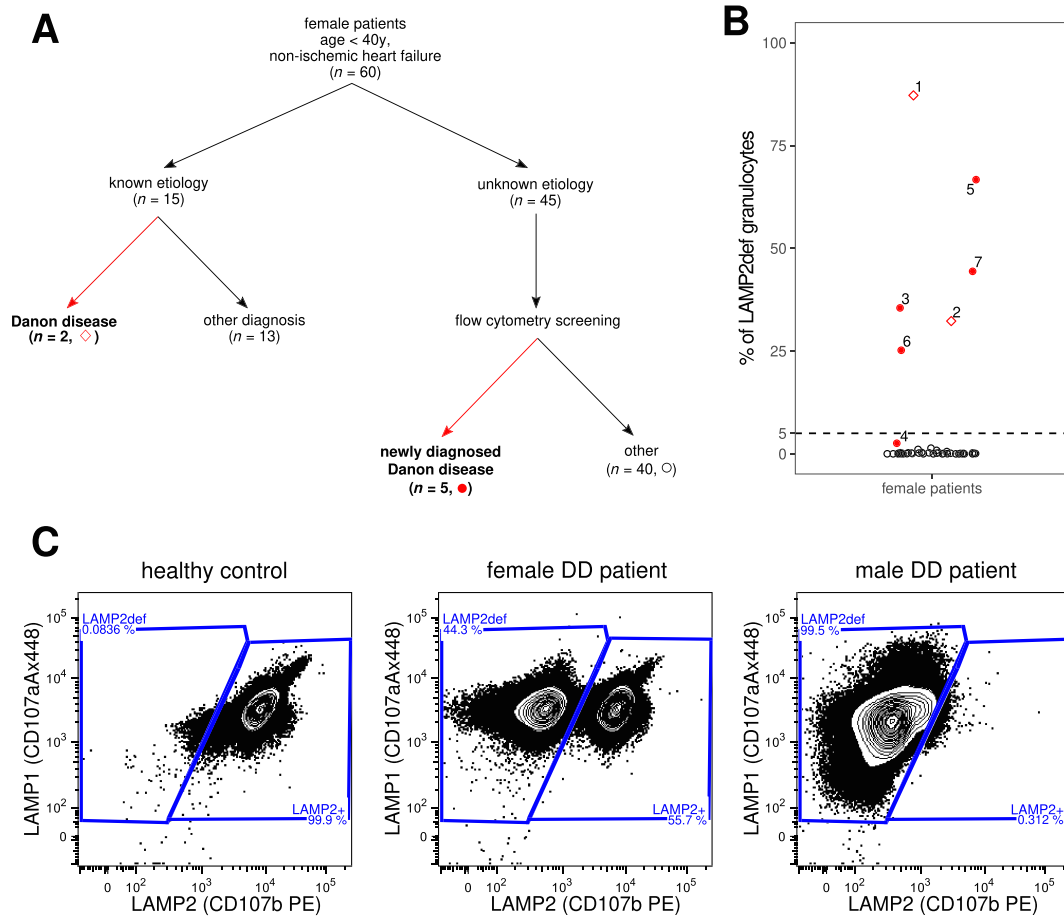
Study protocol

Medical records of the 60 female patients were reviewed, and their clinical data, electrocardiographic, and echocardiographic findings were collected. Abnormal electrocardiograms suggesting pre-excitation were reviewed by an electrophysiologist blinded to the aetiology of the cardiomyopathy, to confirm/exclude the presence of delta waves. Among the 60 patients (Figure 1A, Table 1), 15 female patients (25%) had a known aetiology of the cardiomyopathy (for details, see supporting information). Two patients (#1 and #2, Table 2) who were diagnosed with DD prior the start of this study were among these 15 patients. The remaining 45 patients (75%) underwent *LAMP2* FC in WBCs and WES analyses. For these analyses, 5 ml of peripheral venous blood were collected into an EDTA-containing tube(s). Samples for FC were maintained at room temperature and tested within 24 h of collection.

LAMP2 flow cytometry in white blood cells

Intracellular *LAMP1* and *LAMP2* protein content in peripheral WBCs was assessed by polychromatic FC based on previously reported protocols.¹¹ In short, leukocytes were isolated by sedimentation over 2% dextran, fixed and permeabilized (BD FACS Lyse and Perm solution (BD Bioscience, San Jose, CA, USA)), and then incubated with fluorochrome-conjugated antibodies anti-*LAMP2* (CD107b, clone H4B4) phycoerythrin, anti-*LAMP1* (lysosomal-associated membrane protein type 1, CD107a, clone H4A3) Alexa Fluor 488, anti-CD45 PerCP, anti-CD19 APC, anti-CD3 Alexa Fluor 700 and anti-CD14 Pacific Blue (all from Exbio Praha, Vestec, Czech Republic), anti-CD15 Brilliant Violet 510 (BioLegend, Inc, San Diego, CA, USA), for 30 min, washed twice, and measured using BD LSRII flow

Figure 1 LAMP2 flow cytometric screening in the patient cohort. (A) Schematic summary of the findings in the patient cohort. Symbols correspond to panel B. Summary of the whole-exome sequencing findings is provided in the supporting information. (B) % fractions of LAMP2 deficiency (LAMP2def) granulocytes identified by the LAMP2 flow cytometry screening in 45 female patients. The numbers correspond to individual female DD patients as listed in Table 2. Values shown for Patients #1¹⁰ and #2¹³ were measured prior the start of this screening study. The threshold of 5% of LAMP2def granulocytes is highlighted by the dashed line. The minute fraction of LAMP2def granulocytes (2.6%) in Patient #4 is a result of her unique Xq24 molecular pathology resulting in extremely skewed X-chromosome inactivation ratios in white blood cells (Table 2 and also Majer et al.¹⁴ for further details including the LAMP1/LAMP2 scatterplots in monocytes and granulocytes of patient #4). (C) LAMP1/LAMP2 flow cytometry scatterplots demonstrating the typical profiles seen healthy control, female DD patient (Patient #7 is shown), and male DD patient (patient III.3 from Majer et al.¹⁰ is shown). LAMP2def and LAMP2+ granulocytes are gated. The deficit is mosaic (LAMP2def and LAMP2+ cells are found) and corresponds to white blood cell X-chromosome inactivation ratios in the X-heterozygous female DD patient, whereas it is uniform (only LAMP2def cells are found) in X-hemizygous male DD patient. DD, Danon disease.



cytometer (BD Biosciences) equipped with 405, 488, and 633 nm lasers. Technical details (sample processing/staining protocol and gating strategy), interpretation, and representative examples of positive results in female and male DD patients are summarized in a Standard Operating Protocol, which is included in the supporting information (pages 7–9).

Populations of LAMP2def granulocytes and LAMP2def monocytes were analysed by FC specialists (T.K. and O.P.) blinded to the aetiology of cardiomyopathy and results of the WES analyses. Anti-LAMP1 staining served as a permeabilization control. A threshold of 5% of LAMP2def

granulocytes (or monocytes) was set to calculate the sensitivity and specificity of the FC assay. This value was selected because ~98.3% of healthy adult females have WBC XCI ratios within the >5:95/<95:5 range.¹⁵

Molecular genetic analyses

To detect causal genetic variants, WES was performed according to internationally accepted guidelines.¹⁶ Full technical details are provided in the supporting information. Analyses of

the *LAMP2* genomic DNA, full-length isoform *LAMP2* messenger RNAs/complementary DNAs and HUMARA XCI assays in DD patients #5, #6, and #7 were performed as reported previously.^{10,11}

Myocardial LAMP2 immunohistochemistry

In DD patients #5, #6 and #7, myocardial LAMP2 immunohistochemistry (IHC) was performed in endomyocardial biopsies, explanted hearts or excisions from the left ventricle obtained at implantation of ventricular assist devices. The staining protocol followed previous studies.^{10,14}

Statistical analyses of the clinical, electrocardiographic, and echocardiographic data

Categorical data were expressed as percentages and compared using a χ^2 -test or Fisher's exact test. Normally distributed continuous variables were expressed as a mean and

standard deviation. Abnormally distributed continuous variables were given as a median and interquartile range. Continuous variables were compared using the Student's *t*-tests or by the non-parametric Mann–Whitney *U* test, where appropriate. For all tests, a probability value of $P < 0.05$ was considered significant. All analyses were performed using the statistical software SPSS, version 17.0 (Chicago, Illinois, USA).

Results

Clinical findings in the patient cohort

The study group of 60 female patients included 47 (78%) heart transplant recipients, 2 individuals (3%) treated by a ventricular assist device, and 11 patients (18%) in the pre-transplant phase. *Table 1* summarizes the clinical findings. Median ages at disease onset, surgery or pre-transplant assessment, and the LAMP2 FC screening were

Table 1 Study group characteristics and comparison of clinical and instrumental findings between individuals with Danon disease and other aetiologies of non-ischemic heart failure

Characteristic	Danon disease (n = 7)	Non-Danon disease (n = 53)	Overall (n = 60)	P value
Age at first symptoms (year)	16 (15–24)	24 (12–32)	22 (12–31)	0.398
Age at progression (year)	25 (21–28)	30 (19–36)	28 (20–35)	0.228
Cardiomyopathy type				0.022 [*]
Hypertrophic	4 (57%)	5 (9%)	9 (15%)	
Dilated	3 (43%)	43 (81%)	46 (77%)	
Restrictive	0 (0%)	4 (8%)	4 (6%)	
Left-ventricular non-compaction	0 (0%)	1 (2%)	1 (2%)	
NYHA functional class	(n = 7)	(n = 45)	(n = 52)	0.754
I	1 (14%)	3 (7%)	4 (8%)	
II	1 (14%)	10 (22%)	11 (21%)	
III	4 (58%)	21 (47%)	25 (48%)	
IV	1 (14%)	11 (24%)	12 (23%)	
Arrhythmia				0.052
Atrial fibrillation	3 (43%)	4 (8%)	7 (12%)	
Ventricular tachyarrhythmia	0 (0%)	5 (10%)	5 (8%)	
Electrocardiogram	(n = 7)	(n = 42)	(n = 49)	
PR duration (ms)	154 (152–160)	166 (159–184)	162 (154–184)	0.268
QRS duration (ms)	144 (137–187)	96 (80–110)	102 (83–121)	0.001 ^{**}
Delta-waves	3 (43%)	0 (0%)	3 (6%)	0.002 ^{**}
LBBB	3 (43%)	5 (12%)	8 (16%)	0.068
Echocardiography	(n = 7)	(n = 46)	(n = 53)	
LVEDD (mm)	62 (49–67)	61 (56–69)	62 (56–68)	0.537
LVEF (mm)	20 (20–39)	25 (20–30)	24 (20–30)	0.749
Interventricular septum (mm)	14 (9–14)	8 (7–9)	8 (7–9)	0.001 ^{**}
Posterior wall (mm)	12 (10–14)	8 (7–9)	8 (7–9)	0.002 ^{**}
Mitral regurgitation	(n = 7)	(n = 50)	(n = 57)	0.899
None or trace	4 (58%)	20 (40%)	24 (42%)	
Mild	1 (14%)	10 (20%)	11 (19%)	
Moderate	1 (14%)	12 (24%)	13 (23%)	
Severe	1 (14%)	8 (16%)	9 (16%)	

LBBB, left bundle branch block; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.

^{*} $P < 0.05$.

^{**} $P < 0.01$.

22, 28, and 37 years, respectively. DCM was found in 46 patients (77%), HCM in nine patients (15%), restrictive cardiomyopathy in three patients (5%), and left ventricular non-compaction cardiomyopathy in one patient (2%).

LAMP2 flow cytometry in white blood cells

Patients #1¹⁰ and #2,¹³ who were diagnosed with DD prior the start date of this study, had fractions of LAMP2def granulocytes larger than 5% (Figure 1A,B, Table 2).

Of the 45 female patients tested by FC in this study, populations of LAMP2def granulocytes larger than 5% were identified in four female patients (Patients #3, #5, #6, and #7) (Figure 1A,B, Table 2). Additional five female patients had distinct populations of LAMP2def granulocytes that were smaller than 5% (0.17–2.6%). The most suspect of DD was the values of 2.6% LAMP2def granulocytes and 3.9% LAMP2def monocytes in a single patient. This result was later explained by identifying a very complex *LAMP2* molecular pathology¹⁴ that skewed WBC XCI ratios in DD patient #4 (Figure 1B and Table 2). No *LAMP2* mutation was detected in the remaining four patients harbouring the very small LAMP2def cellular subsets. The remaining 36 samples did not contain any distinct populations of LAMP2def granulocytes.

Including patients #1 and #2, the total prevalence of DD in this cohort of female patients with non-ischemic heart failure was 12% (7/60). At a threshold of 5% LAMP2def granulocytes or monocytes, DD female patients were identified with a sensitivity of 86% and specificity of 100%. Positive predictive value (PPV) and negative predictive value (NPV) of the test were 100% and 98%, respectively. The presence of any distinct subpopulation of LAMP2def granulocytes identified DD female patients with a sensitivity of 100%, specificity of 92%, PPV 63%, and NPV 100%.

Whole-exome sequencing analyses, *LAMP2* mutations, HUMARA X-chromosome inactivation ratios, and immunohistochemistry assessment of the myocardial samples

Whole-exome sequencing results are summarized in the supporting information. ACMG class 3–5 variants in disease-related genes were found in 24 patients (53%) including the five newly identified DD patients (Figure 1A,B). Importantly, characterization of the *LAMP2* mutations in DD patients #3 and #4 mandated an individualized methodological approach that was far beyond the standard WES analytics in both patients.^{13,14} WES testing was negative or inconclusive in 21 patients (47%).

Table 2 summarizes the molecular genetic analyses, WBC XCI, and myocardial LAMP2 IHC findings in the female DD

Table 2 LAMP2 flow cytometry, *LAMP2* molecular genetic analyses, and LAMP2 myocardial expression in Danon disease female patients

Patient	LAMP2def granulocytes (%)	LAMP2def monocytes (%)	XCI in WBCs (HUMARA)	LAMP2 mutation/ LAMP2 cardiac IHC	Note
#1 ^b	87.2	84.9	Not informative	p.[Ala314Glnfs*32];[=] / mosaic expression	III.2 in Majer et al. ¹⁰
#2 ^b	32.3	38.0	30:70	g.19925_45401[del25477];[=], deletion of <i>LAMP2</i> exons 4–9C/no tissue available	II.1 (family 2) in reference Majer et al. ¹³
#3 ^c	35.5	49.1	40:60	g.17916_29069[del11154];[=], deletion of <i>LAMP2</i> exons 4–8/no tissue available	II.1 (family 1) in Majer et al. ¹³
#4 ^c	2.6	3.9	96:4 ^a	Heterozygous deletion of <i>CUL4B</i> , <i>LAMP2</i> , <i>ATP1B4</i> , <i>TMEM255A</i> , and <i>ZBTB33</i> genes/mosaic expression	II.1 in Majer et al. ¹⁴
#5 ^c	66.7	70.8	57:44	p.[Asp149Phefs*2];[=] / mosaic expression	Novel <i>LAMP2</i> mutation (Supporting Information, Figure S1A)
#6 ^c	25.2	28.9	20:80	p.[Gln240*];[=] / mosaic expression	Known <i>LAMP2</i> mutation (Figure S1B) ¹⁷
#7 ^c	44.3	52.7	46:54	p.[Leu139Phefs*8];[=] / mosaic expression	Novel <i>LAMP2</i> mutation (Figure S1C)

DD, Danon disease; HUMARA, human androgen receptor assay; IHC, immunohistochemistry

^aSkewed WBC XCI ratios are an effect of the parallel mutation in the *CUL4B* gene.

^bDD diagnosis established prior the LAMP2 FC screening study.

^cDD diagnosis established by the LAMP2 FC screening study.

Table 3 Clinical findings in Danon disease female patients

Patient	Age at disease onset (years)	Age at HTx (years)	Age at DD diagnosis (years)	Cardiac phenotype	QRS width (ms)	Delta waves	LVEDD (mm)	LVEF (%)	IVS (mm)	PW (mm)
#1	15	29	33	DCM	140	I, aVL, V4-6	67	15	10	10
#2	11	—	17	HCM	134	I, II, III, aVF, V5-6	33	60	22	21
#3	12	21	42	HCM	208	Absent	68	20	15	15
#4	25	26	36	DCM	112	Absent	62	20	8	7
#5	25	28	48	HCM	200	Absent	68	20	14	14
#6	16	27	25	HCM	174	I, II, III, aVF, V4-6	40	55	14	12
#7	23	24	23	DCM	144	Absent	59	23	9	10

DCM, dilated cardiomyopathy; DD, Danon disease; HCM, hypertrophic cardiomyopathy; HTx, heart transplantation; IVS, interventricular septum end-diastolic thickness; LVEDD, left-ventricular end-diastolic diameter; LVEF, left-ventricular ejection fraction; PW, left-ventricular posterior wall end-diastolic thickness.

patients. Patient #1 was heterozygous for a frame-shift c. del940G *LAMP2* mutation.¹⁰ Patients #2 and #3 carried heterozygous deletion exon copy number variants encompassing *LAMP2* exons 4-9C and 4-8, respectively.¹³ Patient #4 had an *Xq24* re-arrangement that caused a heterozygous deletion of several C-terminal exons of *CUL4B* and the complete deletion of *LAMP2*, *ATP1B4*, *TMEM255A*, and *ZBTB33* genes.¹⁴ Patient #5 was heterozygous for a novel frameshift c.445_449delGACCT mutation in the *LAMP2* exon 4 (Supporting Information, *Figure S1A*). A previously reported¹⁷ heterozygous c.718C>T non-sense mutation (*Figure S1B*) in the *LAMP2* exon 5 was found in patient #6. Patient #7 was heterozygous for a novel frameshift c.418delC mutation in the *LAMP2* exon 4 (*Figure S1C*).

White blood cell XCI ratios were within the >5:95/<95:5 range and corresponded to percentages of *LAMP2*def granulocytes and monocytes in patients #2, #3, #5, #6, and #7. The extremely skewed WBC XCI ratios in patient #4 (96:4) were an effect of the parallel mutation in the *CUL4B* gene.¹⁴ *LAMP2* expression in cardiomyocytes was mosaic in all DD patients from whom tissue samples were available (*Table 2*).

Family screening in newly identified Danon disease patients

Patients #3,¹³ #4,¹⁴ and #7 (*Figure S1C*) were the only affected individuals in their families. Patient #5 transmitted the *LAMP2* mutation to her daughter (III.3, *Figure S1A*). At 30 years of age, the daughter had a sinus rhythm with a short PQ interval (98 ms) and discrete delta waves in leads I, II, aVF, and V4-V6. Her echocardiography showed a borderline systolic function of the non-dilated left ventricle (end-diastolic diameter 49 mm, ejection fraction 50-55%, and interventricular septum thickness 10 mm). The populations of her *LAMP2*def granulocytes and monocytes were 22.1% and 27.2%, respectively. XCI ratios by HUMARA were 33:67. Patient #6 had the most complex family history (*Figure S1B*). Her mother (an obligatory heterozygote for the *LAMP2* mutation) had a DCM and died at 27 years of age. Hypertrophic cardiomyopathy was diagnosed in the patient's brother who carried the same *LAMP2* mutation as Patient #6 and died aged 18 years. For additional clinical details see supporting information.

Clinical characteristics of Danon disease female patients

Hypertrophic cardiomyopathy phenotype (57% vs. 9%, **P* = 0.022) and delta waves by electrocardiograms (43% vs. 0%, ***P* = 0.002) were significantly more frequent in DD female patients in comparison with the rest of the patients in

the cohort. The presence of HCM identified DD female patients with a sensitivity of 57%, specificity of 91%, PPV of 44%, and NPV of 94%. Delta waves identified DD female patients with a sensitivity of 43%, a specificity of 100%, PPV of 100%, and NPV of 93%. The sensitivity of these clinical variables was thus much lower than sensitivity of the LAMP2 FC test. The increased thickness of the left ventricular walls and broader QRS complexes in DD female patients corresponded to higher prevalence of HCM and delta waves in these individuals (Table 1). Electrocardiograms of DD female patients are shown in Supporting Information, Figure S2.

Discussion

To the best of our knowledge, we present the first study specifically assessing the prevalence of DD among female patients with AHF. This is also the first large-scale study that uses LAMP2 flow cytometry as a primary screening tool in this particular patient group.

Our key findings are (i) the prevalence of DD among young female patients (≤ 40 years of age) with AHF due to non-ischemic cardiomyopathy is relatively high (12%), (ii) LAMP2 FC in WBCs is an effective screening/diagnostic tool to detect female (and also male) DD patients, and (iii) despite more frequent than in non-DD patients, DD female patients cannot be reliably identified based on the presence of hypertrophic cardiomyopathy and/or delta waves in their electrocardiograms.

Danon disease screening studies

The overall population frequency as well as population ratio of male and female DD patients is, to the best of our knowledge, not known. The recent extensive review of the available DD literature documented 90 male and only 56 female patients.⁶ It is very likely that female patients heterozygous for pathogenic LAMP2 variants remain an underdiagnosed DD patient group despite they are often the first affected family members. Furthermore, DD affects female patients in their reproductive age. Timely and correct diagnosis is therefore also critical for genetic counselling in their families.

There are several studies that used molecular genetic testing and evaluated the frequency of DD in specifically selected patient cohorts. Again, male DD patients predominated among probands identified by most of these studies.

Prevalence of DD was suggested to be ~ 1 –3% among patients with HCM.^{18,19} The values are higher among similarly affected paediatric patients (4–6%)²⁰ or patients with end-stage HCM (7.7%).²¹ DD is, nonetheless, most frequent ($\sim 33\%$) among patients presenting with both HCM and preexcitation.²² When tested among patients, who underwent endomyocardial biopsy because of suspected

cardiac storage disease, the prevalence of DD reached 8% after exclusion of patients with amyloidosis.²³ Interestingly, a large scale study that combined oligonucleotide hybridization-based and dideoxy-based DNA sequencing techniques of selected genes including LAMP2 did not identify any DD individual among 558 HCM patients.²⁴

Almost all reported LAMP2 mutations (106–108 in December 2019 by the authors' review of literature) putatively result in the absence of the protein. LAMP2 missense mutations are very rare in DD patients.⁶ Residual LAMP2 was unambiguously documented for very few variants (e.g. a leaky splice-site mutation²⁵ or a mutation in the isoform specific exon 9B²⁶). Given this mutation spectrum, LAMP2 protein testing identifies uniform LAMP2 deficiency in cells and tissues of male DD patients (Figure 1C).

XCI triggers mosaic expression of the wild-type and mutant LAMP2 alleles in tissues of female DD patients (Figure 1B, C).^{10,12–14} Setting thresholds for skewing or extreme skewing of XCI is arbitrary. Importantly, $\sim 98\%$ of females have WBC XCI ratios within the $>5:95/<95:5$ range.¹⁵ A large-scale survey evaluating XCI ratios in DD female patients has never been reported; however, a systematic extreme skewing beyond the aforementioned range is unlikely in these patients based on the dataset presented in this manuscript. The only patient in our cohort (#4), who had extremely skewed WBC XCI ratios resulting in fractions of LAMP2def granulocytes (and monocytes) $<5\%$, had a complex Xq24 re-arrangement that deleted not only the entire LAMP2 but also several C-terminal exons of the neighbouring CUL4B gene.¹⁴ Although clinically asymptomatic, female patients heterozygous for mutations in the latter gene have extremely skewed WBC XCI ratios as a result of selective pressure, most likely tissue-specific, against the CUL4B deficient cellular clones.¹⁴

Critical for interpretation of the sensitivity and specificity values of the LAMP2 FC assay in WBCs, all six female DD patients (#1–3 and #5–7) with mutations impacting solely the LAMP2 gene had unambiguously detectable LAMP2def populations larger than 5%. Identifying patient #4 among the individuals with LAMP2def fractions $<5\%$ supports the robustness of the method. Rather than expression-related, we attribute the LAMP2def populations $<5\%$ in the other four patients with no LAMP2 molecular abnormality to sample collection/processing-induced errors.

Clinical implications

Prognostic stratification of patients with non-ischemic heart failure is difficult. It is particularly problematic in patients with recent-onset DCM, because clinical outcomes in these patients may range from full recovery to sudden death or progressive heart failure.²⁷ Clarification of a monogenic aetiology (DD in this case) may considerably improve the risk assessment and facilitate family counselling. Although no

specific therapy is currently available, further development of efficient diagnostic algorithms for DD is substantiated by the currently ongoing *LAMP2B* gene-therapy trial in male patients.²⁸

It is not easy to establish the diagnosis of DD in male patients. However, identification of female patients is even more complicated.⁶ As we show in this study, hypertrophic cardiomyopathy and/or the presence of delta waves may suggest DD. Documenting these clinical clues, however, does not have the sufficient sensitivity and specificity to effectively support the diagnosis in female patients. A study by Boucek *et al.*³ evaluated 18 DD female patients and reported pre-excitation in 27% of them. Brambatti *et al.*⁶ identified pre-excitation in 32% of 56 DD female patients included into their review of previously published DD reports. The slightly higher fraction of patients with delta waves (43%), that we observed in our study, potentially reflects a combined effect of the relatively limited number of evaluated DD female patients and stringent electrocardiogram assessment criteria.

Laboratory diagnostic implications

Massively parallel sequencing technologies increased the efficiency of molecular genetic DD diagnostics.¹⁷ However, pathogenicity validation/prediction of many of the variants identified by these methods is often inconclusive and certain *LAMP2* mutation types (e.g. copy number variants) can be easily missed.¹³ Contrary to these high-throughput genomic analytical techniques, the outlined FC method is a selective protein-level expression test. Unlike to analyses of WBCs homogenates by western blotting,⁸ the fraction of LAMP2def cells is directly quantified by the FC method. The FC test identifies uniform LAMP2 deficiency in X-hemizygous male patients as well as mosaic LAMP2 deficiency in X-heterozygous female DD patients (*Figure 1C*). In specific pedigree situations, the test may also be modified to allow identification of individuals with extremely small LAMP2def populations (<1%) resulting from somatic mosaicism.^{11,12}

Sample collection is minimally invasive, the FC test uses commercially available reagents and the turnover time is short (<24 h). Flow cytometers are a standard laboratory equipment and the overall cost of the test is low. Most importantly, the test is easy to set-up—Standard Operating Protocol is part of the supporting information (pages 7–9) for those interested in using the methodology in their local diagnostic practice.

Given the high prevalence of DD among young female patients with non-ischemic cardiomyopathy, that we identified in this study, we suggest screening of similarly affected patients by flow cytometry since this technique allows direct assessment of the (XCI-driven) mosaic expression of the mutant *LAMP2* allele. Additionally, the information about the

presence or absence of LAMP2def cells may facilitate molecular genetic testing and expedite the correct diagnostic classification.

Study limitations

Patients with AHF constitute ~5% of the patients with heart failure.²⁹ AHF is also rare among female patients aged 20–40 years (e.g. 6–23 cases per one million people in a study by authors from Northeast France³⁰). Genetic cardiomyopathies, myocarditis, and peripartum cardiomyopathy are the most common causes of heart failure in young female patients. Contrary to infectious and pregnancy-related insults, genetic defects almost exclusively result in irreversible cardiac damage. The high prevalence (12%) of DD seen in our cohort likely reflects a combination of the listed reasons—that is, small number of young female AHF patients in the general population and possible proportional increase of patients with genetic causes of cardiomyopathy in the tested population.

Patients included in our study were gathered from two centres that participate in the nation-wide heart transplantation program and jointly serve the entire population of the Czech Republic (~10 million). The studied population of female patients with non-ischemic heart failure younger than 40 years is not composed of patients who were followed long-term to reach the inclusion criteria but rather is a snapshot group of young female patients recruited within 24 months, who lived with a heart transplant, ventricular assist device or were undergoing a pre-transplant assessment.

Lastly, almost all of currently known *LAMP2* pathogenic variants result in the absence of the protein. The set-up of the LAMP2 FC assay allows identification of LAMP2 deficient cells. Given the spectrum of *LAMP2* variants identified in the Czech DD patient cohort, samples of patients with residual LAMP2 were not tested.

Conclusions

Danon disease is an underdiagnosed cause of AHF in young female patients. LAMP2 flow cytometry in peripheral WBCs can be used as an effective screening method to facilitate the timely diagnosis, treatment, and family counselling in these patients.

Acknowledgements

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Conflict of interest

None declared.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Family pedigrees and *LAMP2* mutations.

Figure S2. ECG curves in female DD patients.

- of Danon disease among patients with left ventricular hypertrophy and concomitant electrocardiographic preexcitation. *Mol Genet Genomic Med* 2019; **7**: e638.
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Standard operating protocol

LAMP-2 protein deficiency test in peripheral blood

1. add 100µl whole blood to each FACS tube
2. add 3ml BD Lyze (10x diluted in dH₂O) → incubate 10 min/RT
3. spin 2000 rpm/5 min
4. add 3ml BD Perm (10x diluted in dH₂O) → incubate 10 min/RT
5. spin 2000 rpm/5 min
6. wash with 3ml PBS/BSA
7. spin 2000 rpm/5 min
8. add antibodies: **Tube 1** and **Tube 2**
9. incubate 30 min in dark at room temperature
10. wash with 3ml PBS/BSA
11. spin 2000 rpm/5 min
12. analyze cells using cytometer with Violet 405nm and Blue 488nm laser

Compensation (instrument specific): use single stained UltraComp beads with each reagent below

Tube 1

	Exbio	Exbio	Exbio	Exbio	BD
cat. no	A4-671-T100	1P-672-T100	PC-222-T100	PB-293-T100	561585
fluorochrome	A488	PE	PerCP	PB	H-V500
mAb	LAMP1 (CD107a)	LAMP2 (CD107b)	CD45	CD14	CD15
ul/Tube	1	0.5	5	2	2

Tube 2

	Exbio	Exbio	Exbio	Exbio	BD
	A4-671-T100	1P-632-C100	PC-222-T100	PB-293-T100	561585
fluorochrome	A488	PE	PerCP	PB	H-V500
mAb	LAMP1 (CD107a)	IgG1	CD45	CD14	CD15
ul/Tube	1	1	5	2	2

Reagents BD Lyze, cat no. 349202 (BD Biosciences, San Jose, CA, USA)
BD Perm, cat no. 340973 (BD Biosciences, San Jose, CA, USA)
UltraComp eBeads™ Compensation Beads, cat no. 01-2222-41 (Thermofisher, Waltham, MA, USA)

Suppliers Exbio Praha, Vestec, Czech Republic, www.exbio.cz
BD Biosciences, San Jose, CA, USA, www.bdbiosciences.com
Thermofisher, Waltham, MA, USA www.thermofisher.com

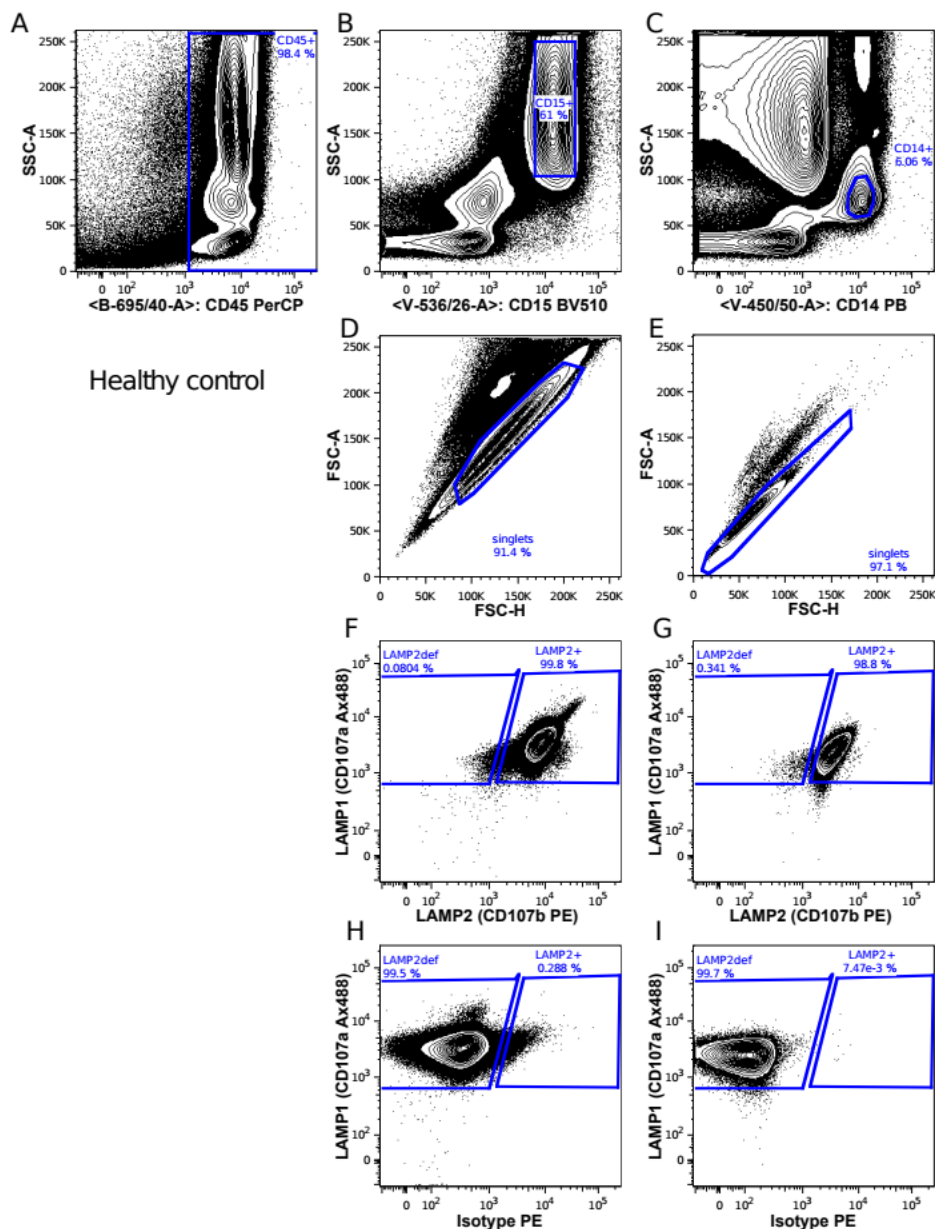
Further reading: Cossarizza A et al. Guidelines for the use of flow cytometry and cell sorting in immunological studies (second edition). Eur J Immunol. 2019 Oct;49(10):1457-1973

Analysis and gating:

Leukocytes are selected as CD45 positive (**panel A**), further split to CD15 granulocytes (**panel B**) and CD14+ monocytes (**panel C**) in tube 1. Cell doublets are removed by singlets gate (**panels D and E**). Healthy donor's granulocytes are LAMP-2 positive (**panel F**), as well as healthy donor's monocytes (**panel G**).

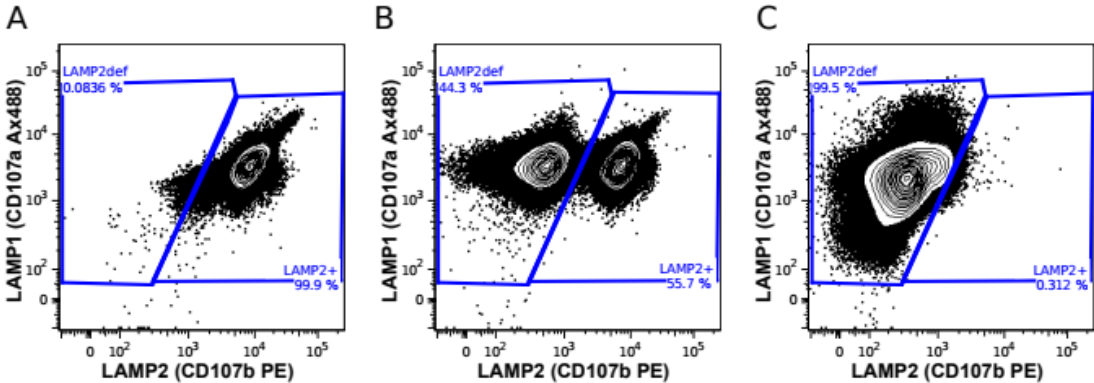
Tube 2 contains isotype control instead of LAMP2 (CD107b PE) and thus provides a control for nonspecific interaction of target cells with mouse antibodies (**panels H and I**).

Tube 1 and Tube 2 serve as a gating control for LAMP2 (CD107b) positive (LAMP2+) and LAMP2 (CD107b) deficient (LAMP2def) cells respectively, in each subset analyzed. LAMP1 (CD107a) detection provides a positive control for proper permeabilisation. LAMP2 protein deficiency should be concluded only in the presence of the LAMP1 protein detection (absence of both LAMP proteins suggests technical artifact=inappropriate permeabilisation of the target cells).




Interpretation - expected findings (granulocytes are shown):

(A) uniform LAMP2 positivity - healthy control, **(B) mosaic LAMP2 deficiency (44.3 %)** – female (XX) DD patient, **(C) uniform LAMP2 deficiency** – male (XY) DD patient



CLINICAL REPORT

***Alu*-mediated *Xq24* deletion encompassing *CUL4B*, *LAMP2*, *ATP1B4*, *TMEM255A*, and *ZBTB33* genes causes Danon disease in a female patient**

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Abstract

Cullin 4B (*CUL4B*), lysosomal-associated membrane protein Type 2 (*LAMP2*), *ATP1B4*, *TMEM255A*, and *ZBTB33* are neighboring genes on *Xq24*. Mutations in *CUL4B* result in Cabezas syndrome (CS). Male CS patients present with dysmorphic, neuropsychiatric, genitourinary, and endocrine abnormalities. Heterozygous CS females are clinically asymptomatic. *LAMP2* mutations cause Danon disease (DD). Cardiomyopathy is a dominant feature of DD present in both males and heterozygous females. No monogenic phenotypes have been associated with mutations in *ATP1B4*, *TMEM255A*, and *ZBTB33* genes. To facilitate diagnostics and counseling in CS and DD families, we present a female DD patient with a *de novo* *Alu*-mediated *Xq24* rearrangement causing a deletion encompassing *CUL4B*, *LAMP2*, and also the other three neighboring genes. Typical to females heterozygous for *CUL4B* mutations, the patient was CS asymptomatic, however, presented with extremely skewed X-chromosome inactivation (XCI) ratios in peripheral white blood cells. As a result of the likely selection against *CUL4B* deficient clones, only minimal populations (~3%) of *LAMP2* deficient leukocytes were identified by flow cytometry. On the contrary, myocardial *LAMP2* protein expression suggested random XCI. We demonstrate that contiguous *CUL4B* and *LAMP2* loss-of-function

copy number variations occur and speculate that male patients carrying similar defects could present with features of both CS and DD.

KEYWORDS

Cabezas syndrome, cullin 4B, Danon disease, female heterozygotes, lysosomal-associated membrane protein 2

1 | INTRODUCTION

Cullin 4B (*CUL4B*), lysosomal-associated membrane protein type 2 (*LAMP2*), ATPase Na⁺/K⁺ transporting family member beta 4 (*ATP1B4*), transmembrane protein 255A (*TMEM255A*), and KAISO (*ZBTB33*) are neighboring genes that occupy ~300 kb of Xq24.

Cabezas syndrome (CS, MIM #300354) is caused by mutations in *CUL4B* (Tarpey et al., 2007; Zou et al., 2007). Male CS patients express developmental, neuropsychiatric, genitourinary, endocrine, and dysmorphic symptoms. Females heterozygous for *CUL4B* mutations are clinically asymptomatic but skewed ratios of X-chromosome inactivation (XCI) in their peripheral blood suggest a selection against *CUL4B* deficient leukocyte clones (Ravn, Lindquist, Nielsen, Dahm, & Tumer, 2012; Zou et al., 2007).

Danon disease (DD, MIM #300257) results from mutations in *LAMP2*. DD is characterized by cognitive deficit, cardiomyopathy, and myopathy in male patients while delayed progression and cardiomyopathy dominate in heterozygous females (Brambatti et al., 2019).

No monogenic clinical phenotypes have been linked to variants in *ATP1B4*, *TMEM255A*, and *ZBTB33* genes.

Loss-of-function copy number variations (CNVs) in either *CUL4B* or *LAMP2* were reported in CS and DD families, respectively (Brambatti et al., 2019; Isidor, Pichon, Baron, David, & Le Caignec, 2010; Ravn et al., 2012). Despite their proximity, a simultaneous deficiency of the two genes has not yet been documented (Isidor et al., 2010).

To demonstrate the pitfalls of diagnostic assessment and facilitate counseling in CS and DD families, we present clinical, tissue and molecular findings in a female DD patient who carried a unique de novo *Alu*-mediated Xq24 rearrangement causing a deletion encompassing *CUL4B*, *LAMP2*, *ATP1B4*, *TMEM255A*, and *ZBTB33* genes.

2 | CLINICAL REPORT

Aged 25 years, the proband (II.1) was diagnosed with dilated cardiomyopathy and severe bilateral heart failure. The left ventricle (LV) had normal wall thickness by echocardiography. LV ejection fraction was 20%. Moderate mitral and tricuspid valve regurgitation were also detected. A pulsatile biventricular assist device was implanted after 10 months of pharmacological treatment due to refractory cardiogenic shock with liver and kidney failure. Heart transplantation was performed 3 months later. Reevaluation (at the age of 36 years) of the

proband's pretransplantation electrocardiograph identified discrete delta waves in lateral leads (Figure S1a).

The Wechsler Adult Intelligence Scale-III test in the proband (performed at the age of 37 years) suggested mild mental retardation (verbal IQ—66, performance IQ—68, full-scale IQ—64), with verbal scores worse than performance subtest scores (see Supporting Materials and Methods for index and subtest scores). No additional psychiatric abnormalities were identified by the evaluation of anamnestic data.

No abnormalities were found by neurological examination and brain MRI (Figure S1b-e).

The patient has no subjective visual complaints. Best-corrected visual acuity is 1.0 in both eyes. Fundus examination in dilatation revealed salt and pepper retinopathy (Figure S2a-d), altered autofluorescence distribution that spared the foveal and parafoveal zones (Figure S2e,f) and mild visual field defects (Figure S2g,h). High-resolution spectral-domain optical coherence tomography detected deposits in the retinal pigment epithelium/Bruch's membrane layer and hyper-reflective foci in the outer nuclear layer. OCT angiography revealed normal retinal vasculature (Figure S2k,l). The hyperreflective signal was noted in the avascular central zone (Figure S2m,n) by automated segmentation. Retinal nerve fiber layer thickness of the right and left eye was normal (Figure S2o,p). The patient has a normal perception of colors, but her contrast sensitivity is bilaterally decreased to 1.20.

The cardiologic examination was normal in the proband's mother and brother. The father has mild hypertrophy (13 mm) of the interventricular septum as a likely consequence of arterial hypertension.

3 | MATERIALS AND METHODS

3.1 | Editorial policies and ethical considerations

The study was approved by the Ethics Committee of the authors' home institution. Informed consent (also approved by the Ethics Committee of the authors' home institution) for presentation of the results was obtained from all participants.

Standard laboratory protocol based on *LAMP2* protein assessment and molecular genetic studies (Majer et al., 2014; Majer et al., 2018; Sikora, Majer, & Kalina, 2015) confirmed DD in the clinically highly suspect proband (II.1, Figure 1a). Multicolor flow cytometry (FC) of the lysosomal-associated membrane protein Type 1 (*LAMP1*) and *LAMP2*, PCR and sequence analyses of the coding gDNA and full-length *LAMP2* mRNA/cDNAs, quantitative PCR (qPCR) *LAMP2* exon-copy number testing, and XCI HUMARA assay were performed in

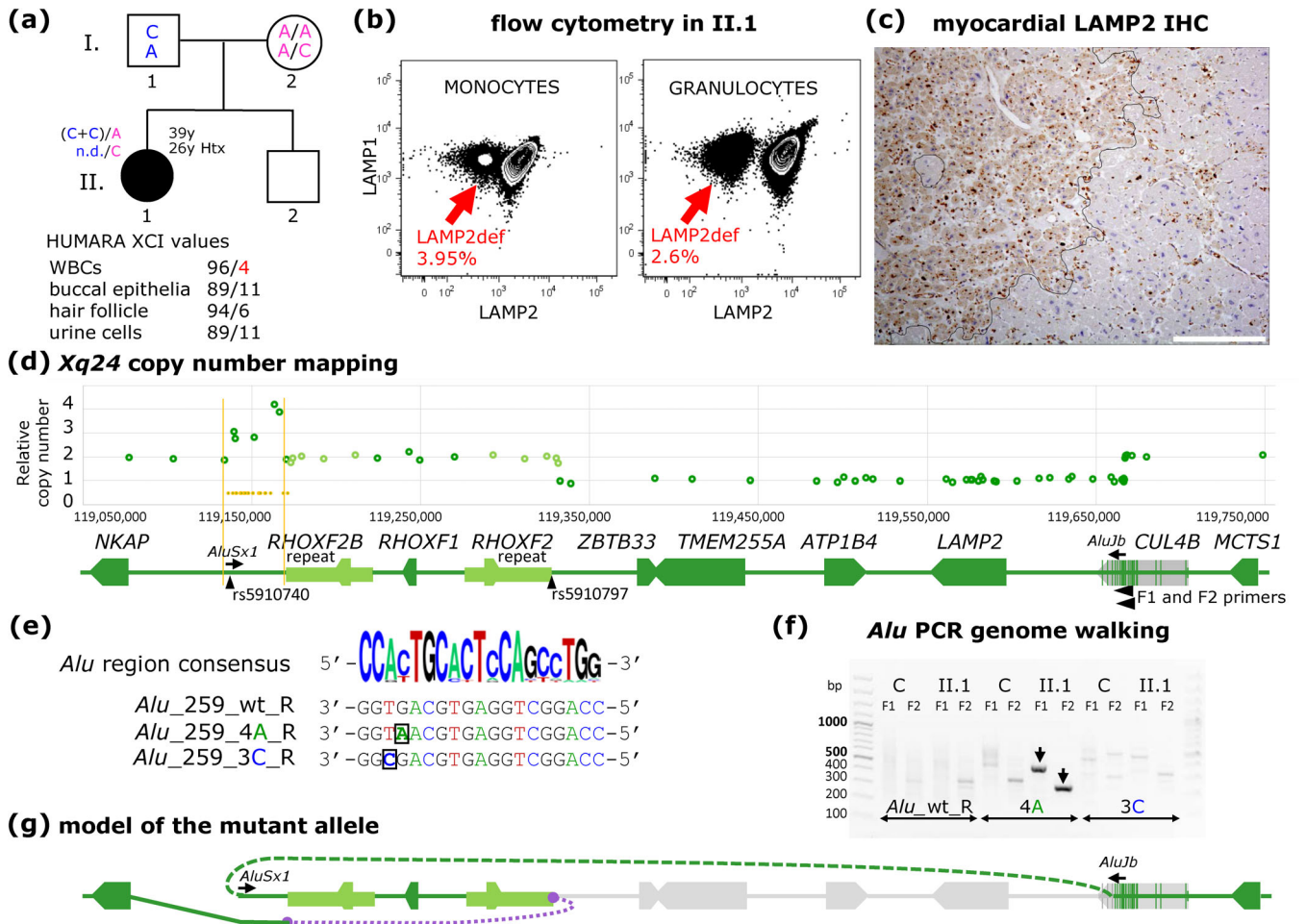


FIGURE 1 (a) Family pedigree. Current age and age at heart transplantation (Htx) are shown for II.1. Two SNPs (rs5910740 and rs5910797—positions are highlighted in Figure 1d and Figure S7) helped to identify, which of the parental alleles was affected by the rearrangement in the proband. The genetic setup of rs5910740 (upper) and rs5910797 (lower) is color-coded in both parents and the proband. rs5910740 localizes to a region with relative copy number 3 (Figure 1d). Paternal variant of rs5910797 was not detected (n.d.) in the proband (see also Figure S7). XCI ratios were assessed by the HUMARA assay. (b) FC dot plots show LAMP2^{def} monocytes and granulocytes (red arrows) with normal presence of LAMP1 in II.1. (c) representative image of the mosaic LAMP2 expression (brown signal) in the patient's myocardium of the interventricular septum (borderline of LAMP2^{def} and LAMP⁺ patches is highlighted, for further details see Figure S3), scale bar = 200 μ m. (d) qPCR relative copy number values (green circles) in the critical Xq24 region. *Alu* elements in the region with abnormally increased copy number values 3 and 4 are highlighted in orange. Black arrowheads indicate the position and orientation of the forward (F1 and F2) *Alu* PCR genome walking primers used to map the *CUL4B* breakpoint/junction site. Positions and orientation of *AluJb* and *AluSx1* elements are shown by arrows. (e) Sequences of *Alu* elements in the Xq24 region with the copy number 3 or 4 were aligned (Figure S5) and three reverse primers for *Alu* PCR genome walking were designed. *Alu_259_wt_R* aligned to the most abundant and conserved part of the *Alu* sequence. *Alu_259_4A_R* and *Alu_259_3C_R* aligned to the most frequent 3' *Alu* variants. (f) Agarose gel of *Alu* PCR genome walking reactions. Specific products (black arrows) were gained only with the *Alu_259_4A_R* primer (4A). The size difference (~177 bp), corresponds to the difference in position of primers F1 and F2 (Figure S6 shows the breakpoint junction sequence in detail). C—control sample. (g) A model of the complex rearrangement at the mutant allele. The orientation of the *AluSx1* element that participates in the *CUL4B* breakpoint junction highlights the presumed inversion of the *RHOXF* gene cluster. Violet dotted line marks the breakpoint junction that was not characterized [Color figure can be viewed at wileyonlinelibrary.com]

peripheral white blood cells (WBCs) as previously described (Majer et al., 2012, 2014). Primers used for all the PCR-based analyses are listed in Table S1. Cytogenetic analyses of peripheral blood samples followed standard protocols (Verma & Babu, 1995).

Quantification of the ratio of LAMP2⁺ and LAMP2 deficient (LAMP2^{def}) cardiomyocytes was performed on immunohistochemically (IHC) stained sections from the formalin-fixed and paraffin-embedded explanted heart. For additional details see Supporting Materials and Methods.

4 | RESULTS

4.1 | FC, XCI analyses, and myocardial (immuno) histopathology

FC detected small but distinct populations of LAMP2^{def} monocytes (~4%) and granulocytes (~3%) in the proband (Figure 1b). This corresponded with XCI ratios assessed by HUMARA (Figure 1a). HUMARA also showed that the inactive androgen receptor allele was of paternal origin.

Myocardial histopathology showed patchy hypertrophy and vacuolization of cardiomyocytes and fibrosis (Figure S3a,b). LAMP2 expression in cardiomyocytes was mosaic (Figure 1c) by IHC. The cell counts of the LAMP2^{def} and LAMP2⁺ cardiomyocytes were comparable (Figure 1c, Supplementary Materials and Methods and Figure S3c–f).

4.2 | LAMP2 molecular studies

Profile and sequence of the three full-length *LAMP2* isoform mRNAs/cDNAs were normal in the proband (Figure S4). gDNA qPCR analyses identified ~300 kb long *Xq24* region with abnormally reduced relative copy number (1 instead of 2). The deletion started in *CUL4B* intron 19 (see Figure S6 for details) and encompassed the distal part of *CUL4B* (including exons 20–22 that code the last 140 amino acids of the protein and also the 3' untranslated sequences with poly(A) sites), and the entire *LAMP2*, *ATP1B4*, *TMEM255A*, and *ZBTB33* genes. Five qPCR probes downstream of the *RHOXF2/RHOXF1/RHOXF2* homeobox gene cluster showed abnormally increased copy number values of 3 or 4 (Figure 1d). *Alu* PCR genome walking method was used to characterize the *CUL4B* breakpoint/junction site (Figure 1e,f). We identified residual sequences of *AluJb* (strand-) from intron 19 of *CUL4B* and *AluSx1* (strand+) (Figure S6) from the *Xq24* region with relative copy numbers of 3 and 4 (Figure 1d). G-banded chromosome analysis identified a normal female (46, XX) karyotype. Genotyping of two *Xq24* single nucleotide polymorphisms (SNPs; rs5910740 and rs5910797) in the proband and her parents (Figure 1a, Figure S7, and Table S1) suggested that the *Xq24* rearrangement affected the proband's paternal allele. The mutation-specific sequences were not identified in WBCs of either parent or the father's sperm (Figure S8).

5 | DISCUSSION

We present clinical findings and results of laboratory analyses in a female proband with a unique complex de novo *Xq24* rearrangement (Figure 1g) that caused a deletion impacting the distal part of *CUL4B* and the complete sequences of *LAMP2*, *ATP1B4*, *TMEM255A*, and *ZBTB33* genes.

The ophthalmic symptoms, myocardial histopathology, and mosaic LAMP2 expression corresponded to findings in DD female patients (Brambatti et al., 2019). In contrast to the latter study, however, the patient reached the end-stage heart failure more than 10 years earlier than DD females presenting with dilated cardiomyopathy.

Instability (and most probably also decay) of the putative *CUL4B* transcript is expected in the proband. Any truncated *CUL4B* protein (773 instead of 914 amino acids in length) would also likely not be stable, not complex with its partners, and fail the ubiquitination function (see Figure S9). Similar to other *CUL4B* female heterozygotes, our patient was asymptomatic in nonpsychiatric clinical domains that are usually impacted in male CS patients (Tarpey et al., 2007; Zou et al., 2007). Her mild mental retardation could be caused by the pathogenic effects of both *LAMP2* and *CUL4B* mutations. However, the

contribution of the pathogenic variants in either of the two genes to this particular phenotype is impossible to establish.

An additional finding typical for female CS heterozygotes, XCI ratios were extremely skewed (presumably due to selection against *CUL4B* deficient cellular clones) in several of the proband's tissues including peripheral blood (Zou et al., 2007). This phenomenon was reflected by minimal but distinct LAMP2^{def} populations of peripheral WBCs. On the contrary, the fraction of LAMP2^{def} cardiomyocytes was comparable to the number of cardiomyocytes that expressed LAMP2 normally. This tissue-specific discrepancy suggests that the selection may not be universal to all cell types/tissues. Interestingly, a similar phenomenon was suggested in heterozygous female *Cul4b* knock-out mice (Jiang et al., 2012).

Little is known about the function of the three other deleted genes (*ATP1B4*, *TMEM255A*, and *ZBTB33*). *ATP1B4* is a muscle-specific protein of the inner nuclear envelope (Zhao, Pestov, Korneenko, Shakhparonov, & Modyanov, 2004), *TMEM255A* is a predicted transmembrane protein (UniProtKB), and *ZBTB33* (KAISO) is a zinc-finger containing transcriptional factor involved in cell cycle regulation and cancer progression (Schackmann, Tenhagen, van de Ven, & Derksen, 2013). Variants in these three genes have not been associated with any defined human genetic phenotype. Contribution of their reduced copy number to the proband's phenotype is thus speculative.

As repetitive elements often contribute to *LAMP2* CNVs (Majer et al., 2018), we used the *Alu* PCR genome walking technique and identified residual *AluJb* and *AluSx1* sequences in the breakpoint junction (Figure 1e and Figures S5 and S6). Detailed analysis of this junctional sequence suggested an inversion of the *RHOXF2/RHOXF1/RHOXF2* homeobox cluster as part of the complex *Xq24* rearrangement. The full characterization of the other breakpoint(s) (violet dots in Figure 1g) was not completed because of the high content of repetitions in the region.

Overall, our data demonstrate that deletion CNVs contiguously affecting *CUL4B* and *LAMP2* occur. In reference to findings in the presented proband, the clinical phenotype in heterozygous females with similar genetic setup is expected to be dominated by cardiac DD symptoms. The values of XCI ratios in WBCs and quantitation of the LAMP2^{def} leukocyte populations in these patients should, however, be interpreted cautiously, because the results of these tests may be impacted by the (likely tissue-specific) effects of the *CUL4B* mutation. In male patients, on the contrary, a contiguous *CUL4B* and *LAMP2* loss-of-function CNV could result in a phenotype combining symptoms of CS and DD.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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Pigmentary retinopathy can indicate the presence of pathogenic *LAMP2* variants even in somatic mosaic carriers with no additional signs of Danon disease

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ABSTRACT.

Purpose: Danon disease (DD) is a rare X-linked disorder caused by pathogenic variants in *LAMP2*. DD primarily manifests as a severe cardiomyopathy. An early diagnosis is crucial for patient survival. The aim of the study was to determine the usefulness of ocular examination for identification of DD.

Methods: Detailed ocular examination in 10 patients with DD (3 males, 7 females) and a 45-year-old asymptomatic female somatic mosaic carrier of a *LAMP2* disease-causing variant.

Results: All patients with manifest cardiomyopathy had pigmentary retinopathy with altered autofluorescence and diffuse visual field loss. Best corrected visual acuity (BCVA) was decreased (<0.63) in 8 (40%) out of 20 eyes. The severity of retinal pathology increased with age, resulting in marked cone-rod involvement overtime. Spectral-domain optical coherence tomography in younger patients revealed focal loss of photoreceptors, disruption and deposition at the retinal pigment epithelium/Bruch's membrane layer (corresponding to areas of marked increased autofluorescence), and hyperreflective foci in the outer nuclear layer. Cystoid macular oedema was seen in one eye. In the asymptomatic female with somatic mosaicism, the BCVA was 1.0 bilaterally. An abnormal autofluorescence pattern in the left eye was present; while full-field electroretinography was normal.

Conclusions: Detailed ocular examination may represent a sensitive and quick screening tool for the identification of carriers of *LAMP2* pathogenic variants, even in somatic mosaicism. Hence, further investigation should be undertaken in all patients with pigmentary retinal dystrophy as it may be a sign of a life-threatening disease.

Key words: autofluorescence – Danon disease – *LAMP2* – pigmentary retinopathy – somatic mosaicism – spectral-domain optical coherence tomography

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Introduction

Danon disease (DD; OMIM #300257) is a rare X-linked disorder caused by mutations in the lysosomal-associated membrane protein type 2 gene (*LAMP2*). DD is characterized by cardiomyopathy, skeletal muscle myopathy and mild cognitive impairment; with male patients having earlier onset and more severe disease. Most patients require heart transplantation, which in males is usually before the age of 25 years (Boucek et al. 2011; Brambatti et al. 2019).

The majority of *LAMP2* causal variants result in the absence of the protein. *LAMP2* deficiency is uniform in tissues of male individuals. In heterozygous female patients, cellular expression of the alternative *LAMP2* alleles is mosaic (unless influenced by events like formation of syncytia; Wu et al. 2014) as a result of X-chromosome inactivation (XCI). Although not fully mechanistically understood at the molecular level, *LAMP2* deficiency is presumed to compromise lysosomal processing of autophagic substrates (Rowland et al. 2016; Chi et al. 2019).

A growing number of reports suggest that females, who are somatic (and possibly also germinal) mosaics for *LAMP2* variants represent a specific and under-detected patient/carrier group. Some of these carrier females have been reported to express the phenotype (Meinert et al. 2019) but others remain free of clinical DD symptoms (Chen et al. 2012; Majer et al. 2014).

Although a number of individual case reports demonstrate impaired vision in patients with DD, detailed ocular findings have been, to the best of our knowledge, reported in less than 20 patients, and of these only 10 underwent imaging with spectral-domain optical coherence tomography (SD-OCT) and 7 had fundus autofluorescence imaging (Prall et al. 2006; Schorderet et al. 2007; Thiadens et al. 2012; Mack 2014; Thompson et al. 2016; Fukushima et al. 2020; Majer et al. 2019; Meinert et al. 2019).

Based on functional and clinical imaging testing, DD has a variable ocular phenotype, but a pigmentary retinopathy is commonly present, associated with generalized loss of cone and rod function (Schorderet et al. 2007).

Occasionally fine lens opacities can be observed (Prall et al. 2006).

In this study, we report detailed ophthalmic examination including SD-OCT imaging in a cohort of 10 Czech patients with DD and one asymptomatic female with somatic mosaicism for a *LAMP2* disease-causing variant. Documented ocular findings highlight the fact that the pigmentary retinopathy may be the only sign of this potentially life-threatening cardiac condition, warranting further clinical and laboratory investigation in all cases with a phenotype suggestive of an inherited retinal disease (IRD).

Methods

The study was approved by the Institutional Ethical Committee and adhered to the tenets of the Declaration of Helsinki. All patients or their legal guardians provided signed, informed consent prior to inclusion in the study.

Ten Czech patients with DD (three males and seven females) and one asymptomatic female with somatic mosaicism were included (Table 1). In all affected individuals, the diagnosis of DD was based on the following criteria:

clinical (primarily cardiological) symptoms, cardiac histopathology if endomyocardial biopsy or explanted heart were available, absence of the *LAMP2* protein in peripheral blood granulocytes and monocytes and/or cardiac tissue, and presence of a pathogenic variant in the *LAMP2* gene. The detailed description of clinical and molecular findings in individuals 1, 5, 6, 8, 9, 10 and 11, as well as protocols of cardiac tissue histopathological and *LAMP2* immunohistochemical (IHC) analyses, HUMARA XCI assay in peripheral white blood cells (WBC), flow cytometric (FC) *LAMP2* expression testing in WBCs, and *LAMP2* mRNA/cDNA and gDNA analyses are provided in previous reports (Majer et al. 2012, 2014, 2018).

Briefly, *LAMP2* deficit in granulocytes and monocytes was uniform in all three male patients (8–10) and mosaic in females (1–7). WBC XCI ratios were in all females within or close to the prevalent 20:80–80:20 range (Amos-Landgraf et al. 2006). Only one of the females (5) was not informative for the HUMARA assay (Majer et al. 2012). Myocardial

histopathological abnormalities and IHC *LAMP2* expression patterns in four females (2, 3, 5 and 7), and one male patient (10) were compatible with changes observed in male and female DD patients (Majer et al. 2012). Detailed information about *LAMP2* expression profiles in WBCs and *LAMP2* pathogenic variants in patients 2, 3, 4 and 7 have not yet been reported but are available upon request from the authors.

Female 11 was previously reported to be a clinically asymptomatic mother of two sons affected by DD (Majer et al. 2014). Her genotype was characterized as somatic (and presumably also germinal) mosaicism for a tandem duplication of *LAMP2* exons 4 and 5. Her WBC HUMARA XCI ratio value was 30:70. The population of her *LAMP2*def leukocytes was, however, extremely small (0.06% of granulocytes and 0.13% monocytes) (Majer et al. 2014). She underwent ocular examination at the age of 45 years. She is currently 48 years old and remains asymptomatic with normal heart function.

All eleven individuals underwent comprehensive ophthalmic examination comprising best corrected visual acuity (BCVA) tested using ETDRS charts (extrapolated to decimal values), standard slit-lamp biomicroscopy including dilated retinal assessment. Colour, red-free and autofluorescence photographs of the fundus were taken using FF 450 plus IR and Visucam 200 (Carl Zeiss Meditec AG, Jena, Germany), and automated perimetry was performed with the M700 (Medmont International, Nunawading, Australia). Macular architecture and BluePeak blue laser autofluorescence imaging were studied using SD-OCT (Spectralis, Heidelberg Engineering GmbH, Heidelberg, Germany). Colour vision was assessed with Lanthony desaturated D-15 test, more than one diametrical crossing was considered as abnormal (Shoji et al. 2009).

Full-field electroretinography (ERG), multifocal ERG (mfERG) and pattern ERG were performed in the somatic mosaic female (11) according to the standards recommended by International Society for Clinical Electrophysiology of Vision (Hood et al. 2012; Bach et al. 2013; McCulloch et al. 2015).

Table 1. Demographic data and ocular findings in 10 individuals with Danon disease.

ID/Gender/ Age when examined (yrs)	BCVA		Refraction (DS/DC)		Colour vision		Lens		Localization of pigmentary retinopathy/Other retinal findings		Comment
	RE	LE	RE	LE	RE	LE	RE	LE	RE	LE	
1/F/19	1.0	1.0	0/−0.75	+0.75/−1.25	N	N	Clear	Clear	Peripheral	Peripheral	II.1 (family 2) in Majer et al. 2018; Htx at 23 yrs
2/F/23	0.58	0.60	+0.5/−1.0	−0.25/−0.5	N	N	Clear	Clear	Panretinal/ peripheral band of chorioretinal atrophy	Panretinal/ peripheral band of chorioretinal atrophy	Htx at 24 yrs
3/F/27	0.63	1.0	+1.5/+2.5	+0.25/+2.5	N	N	Nuclear white dots	Nuclear white dots	Peripheral	Peripheral	Htx at 27 yrs
4/F/28	1.0	0.95	−0.25/−1.0	−0.25/−1.25	N	N	Clear	Clear	Peripheral	Peripheral	Daughter of 7 III.2 in Majer et al. 2012; mother of 8; sister of 10; Htx at 29 and 41 yrs; death at 41 yrs
5/F/39	0.63	0.48	−3.75/−2.0	−5.0/−1.0	N	N	Mild nuclear opacification	Mild nuclear opacification	Panretinal	Panretinal/ peripheral larger area of chorioretinal atrophy	
6/F/41	0.35	0.83	−3.25/−1.0	−1.75/−1.0	N	N	Nuclear white dots	Nuclear white dots	Panretinal	Panretinal	II.1 (family 1) in Majer et al. 2018; Htx at 21 yrs
7/F/47	0.55	0.2	Nil	−2.0/−2.25	NP	NP	Mild cortical opacification	Mild cortical opacification	Panretinal	Panretinal	Mother of 4; Htx at 28 yrs
8/M/17	0.87	0.69	−11.0/−0.5	−10.5/−0.5	N	N	Clear	Clear	Peripheral/ RPE clumping in the fovea	Peripheral/ RPE clumping in the fovea	IV.2 in Majer et al. 2012, son of 5; Htx at 20 yrs; death at 21 yrs
9/M/18	0.76	0.8	−0.5/−2.0	0/−1.75	N	N	Clear	Clear	Peripheral/ panretinal yellow dots, RPE clumping in the fovea	Peripheral/ panretinal yellow dots, RPE clumping in the fovea	III.2 in Majer et al. 2014, son of 11; Htx at 20 yrs
10/M/35	0.25	0.1	−5.75/−0.5	−6.5/−0.5	N	I	Clear	Clear	Panretinal/ RPE clumping in the fovea	Panretinal/ RPE clumping in the fovea	III.3 in Majer et al. 2012, brother of 5; Htx at 28 yrs

BCVA = best corrected visual acuity, DC = dioptre cylinder, DS = dioptre sphere, F = female, HTx = heart transplantation, I = impaired, LE = left eye, M = male, N = normal, NP = not performed, RE = right eye, RPE = retinal pigment epithelium, yrs = years.

Results

Summary of demographic and ophthalmic findings in the 10 patients with DD is shown in Table 1. The detailed ocular imaging and static perimetry findings are provided in Supporting Information.

The mean age of female subjects at ocular examination was 32.0 ± 9.6 years, and the mean age of male subjects was 23.3 ± 8.3 years.

None of the patients reported nyctalopia and only one male (10) admitted subjective symptoms; reduced visual acuity and impaired perception of colours. Visual acuity was normal, that is ≥0.63 in 12 (60%) eyes of 7

individuals. The most severe BCVA decrease down to 0.1 was observed in the left eye of male 10, aged 35 years, this eye also had impaired colour vision (Table 1).

Dilated fundus examination revealed mainly symmetrical pathological findings. Typically, pigmentary retinopathy (punctate areas of hypopigmentation and hyperpigmentation) was observed (Table 1, Fig. 1). Additional findings included a bilateral peripheral band of chorioretinal atrophy in female 2, a large area of peripheral chorioretinal atrophy in the left eye of female 5, cystoid macular oedema (confirmed by SD-OCT) in the right eye of male 6 (Fig. 2D), and bilateral foveal retinal

pigment epithelial (RPE) clumping in all three male individuals – the severity of which seemed to increase with age (Fig. 1, Supporting Information).

Fundus autofluorescence imaging demonstrated an abnormal pattern with hypo- and hyperautofluorescent mottling in all eyes of patients with DD, except for male 8 (aged 17 years) who had normal autofluorescence bilaterally. The foveal region was relatively spared, except for the right eye of female 6 (Fig. 1C), left eye of female 7 and both eyes of male 10 (Fig. 1F, Supporting Information). Individuals 2, 3, 5, 6, 7, 9 and 10 had markedly hyperautofluorescent dots located predominantly in the macular region and

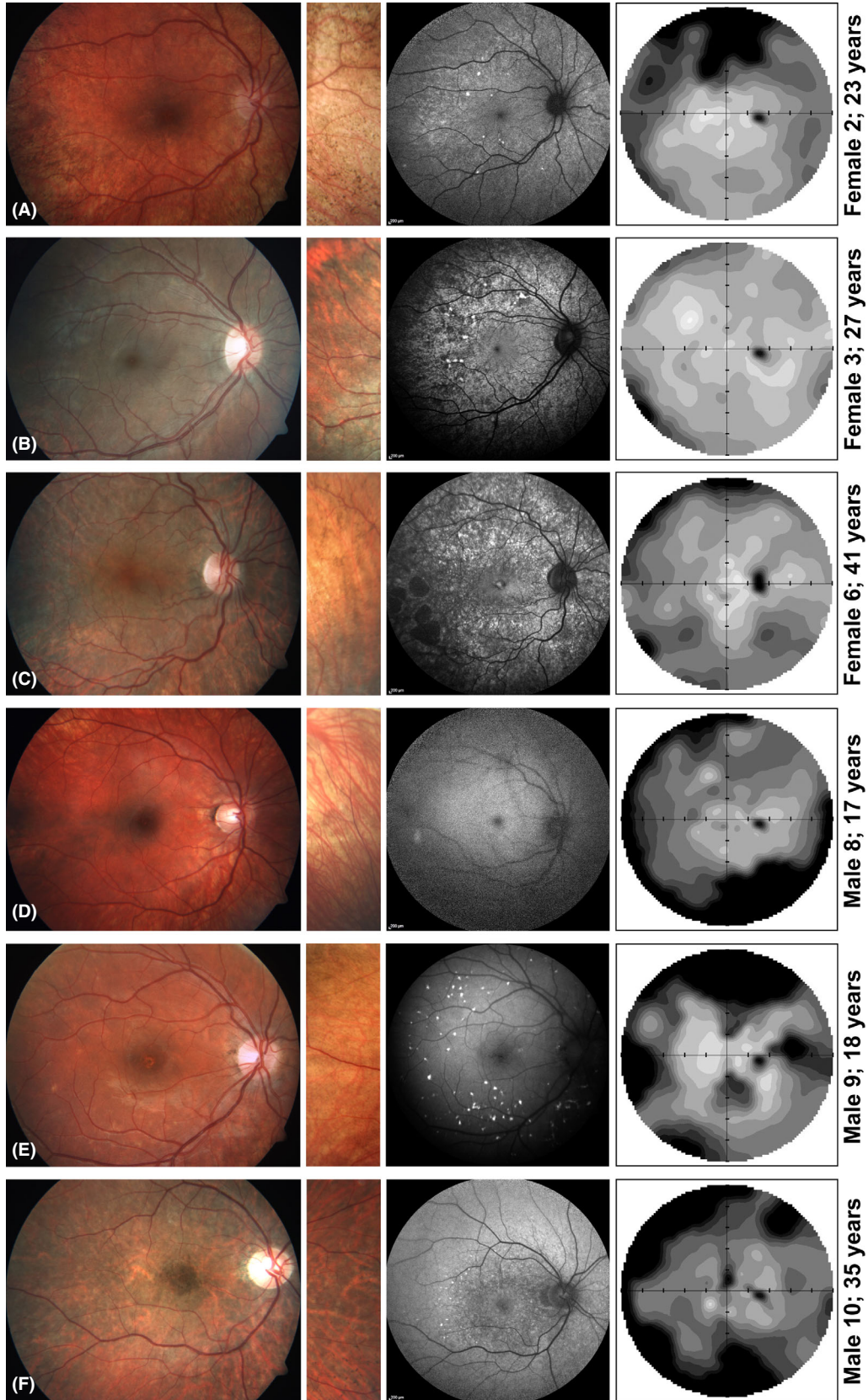


Fig. 1. Ocular imaging and static perimetry in selected patients with Danon disease. For each subject fundus photographs (left column), magnified areas of the retinal periphery (left middle column), fundus autofluorescence (right middle column) and 50° visual field sensitivity map (right column), of the right eye is shown. Images are ordered by increasing age and sex. Female 2 (A), female 3 (B), female 6 (C), male 8 (D), male 9 (E), and male 10 (F). Note the pigmentary retinopathy, predominantly in the peripheral retina. Fundus autofluorescence imaging demonstrates hypo- and hyperautofluorescent changes corresponding to the mottled areas of retinal pigment epithelium. Diffuse loss of sensitivity, more profound in males, can be observed on static perimetry. The severity of findings increases with age.

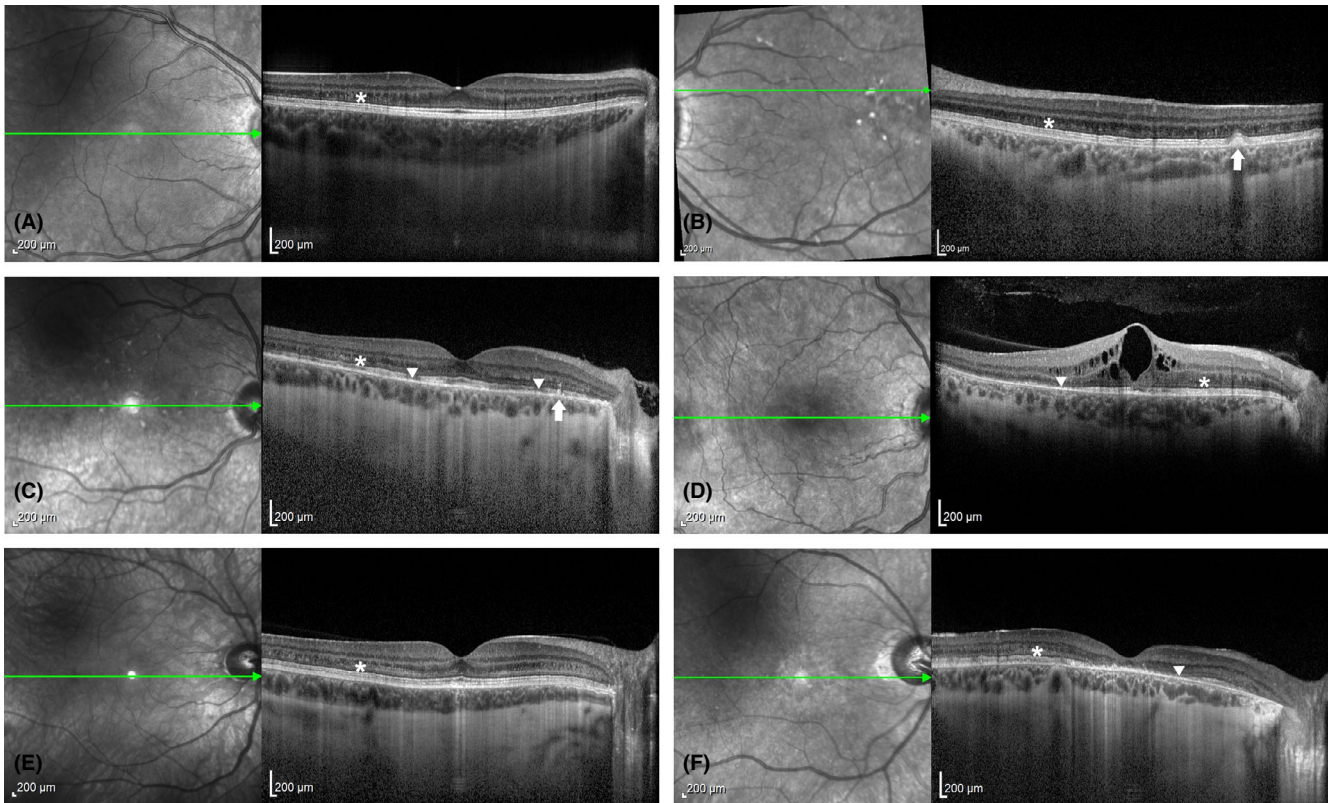


Fig. 2. Variability of spectral-domain optical coherence tomography (SD-OCT) findings in Danon disease. Female 1 (19 years) (A), female 3 (27 years) (B), female 5 (39 years) (C), female 6 (41 years) (D), male 8 (17 years) (E) and male 10 (35 years) (F). Note the hyperreflective areas in the outer nuclear layer (asterisks), focal disruptions of the ellipsoid zone (EZ) (triangles), and hyperreflective deposits (arrows) at the interface of the retinal pigment epithelium and EZ layers.

in the mid-peripheral retina (Fig. 1A–C,E,F, Supporting Information). On SD-OCT, these dots corresponded to round and oval deposits at the interface of the RPE and ellipsoid zone (EZ) layers, measuring from 60 to 294 µm in diameter at their base, and from 43 to 87 µm in height (Fig. 2C,F, Table 2).

In addition to deposits in the RPE/EZ layers, SD-OCT documented a range of findings from variation of signal intensity of the interdigitation and EZ in mildly affected individuals with normal or nearly normal BCVA; to focal disruptions of these two bands in more advanced disease. Atrophy of the neuroretina, with total loss of the photoreceptor layers and RPE, was observed in the most severely affected individuals; female 7 aged 47 years and male 10 aged 35 years. Correspondingly, the total central subfield retinal thickness was below normal values in these two patients (Table 2). An interesting finding was foci of increased signal in the outer nuclear layer in all examined patients. A summary of SD-OCT findings and representative

images is provided in Table 2 and Fig. 2.

Static automated perimetry was abnormal in all 10 patients with DD. Visual field changes were characterized by patchy areas of decreased retinal sensitivity, more pronounced in the periphery. In males, the loss of sensitivity was more profound and extensive compared with similarly aged females (Fig. 1, Supporting Information).

Except for tiny and visually insignificant lens opacities found in 8 eyes of 4 females, the anterior segment appeared normal. Intraocular pressure was also within the normal range (under 21 mmHg) in all eyes.

The BCVA of individual 11 was 1.0 in both eyes. She denied any subjective eyesight-related problems including nyctalopia. The right fundus appeared normal (Fig. 3A), in the left eye there were few scattered yellow deposits (Fig. 3C). Static perimetry was performed but was unreliable due to poor fixation and her underlying anxiety disorder. Autofluorescence imaging identified one hyperautofluorescent

spot in the right eye (Fig. 3B), whereas in the left eye, there were a number of hypoautofluorescent and a few hyperautofluorescent spots (Fig. 3D). SD-OCT imaging (Fig. 3E,F) revealed only discrete irregularities of the RPE band in areas corresponding to these hyperautofluorescent foci (Fig. 3F). The central retinal thickness was 277 and 275 µm in the right and left eye, respectively (normal values 280.1 ± 35 µm) (Invernizzi et al. 2018). Light and dark-adapted full-field ERGs were bilaterally normal (Fig. 3G–J). No abnormalities were detected on pattern ERG (Fig. 3K, M). Multifocal ERG showed mild bilateral decreased foveal responses (75% of normal) which potentially could be attributed to poor fixation (Fig. 3L,N).

Discussion

Herein, we describe ocular findings of 10 patients with DD and one asymptomatic female with an extremely low-level somatic mosaicism for a pathogenic *LAMP2* variant (Majer et al. 2014). We show that pigmentary retinopathy associated with DD is

Table 2. Spectral-domain optical coherence tomography and autofluorescence imaging in 10 individuals with Danon disease.

ID	Outer retinal deposits (base/height in μm)		Layer integrity at the macula		Focal signal(s) of increased reflectivity in ONL		Central subfield thickness (μm)		Autofluorescence pattern	
	RE	LE	RE	LE	RE	LE	RE	LE	RE	LE
1	N	N	Disturbance of RPE/EZ intensity	Disturbance of RPE/EZ intensity	Y	Y	268	263	Mottled	Mottled
2	Y (258/87)	Y (186/74)	Disturbance of RPE/EZ intensity	Disturbance of RPE/EZ intensity	Y	Y	302	299	Mottled, $\uparrow\text{F}$ foci	Mottled, $\uparrow\text{F}$ foci
3	Y (184/62)	Y (294/73)	Disturbance of RPE/EZ intensity	Disturbance of RPE/EZ intensity	Y	Y	305	303	Mottled, $\uparrow\text{F}$ foci	Mottled, $\uparrow\text{F}$ foci
4	N	N	Disturbance of RPE/EZ intensity	Disturbance of RPE/EZ intensity	Y	Y	303	299	Mottled	Mottled
5	Y, (134/60)	Y (150/64)	Partial disruption of RPE/EZ, including the fovea	Partial disruption of RPE/EZ sparing the fovea	Y	Y	266	269	Mottled, $\uparrow\text{F}$ foci	Mottled, $\uparrow\text{F}$ foci
6	Y (100/45)	Y (135/45)	CME, loss of photoreceptors and RPE temporally, partial disruption of RPE/EZ nasally	Disturbance of RPE/EZ intensity	Y	Y	478	299	Mottled, $\uparrow\text{F}$ foci, marked $\downarrow\text{F}$ areas in the periphery	Mottled, $\uparrow\text{F}$ foci
7	Y (61/43)	Y (60/28)	Loss of photoreceptors and RPE temporally, partial disruption of RPE/EZ in the fovea and nasally	Loss of photoreceptors and RPE	Y	Y	248	175	Mottled, $\uparrow\text{F}$ foci	Mottled, $\uparrow\text{F}$ foci, marked $\downarrow\text{F}$ areas in the periphery
8	N	N	Disturbance of RPE/EZ intensity	Disturbance of RPE/EZ intensity	Y	Y	284	278	Normal	Normal
9	Y (140/56)	Y (135/70)	Intact	Intact	Y	Y	283	284	$\uparrow\text{F}$ foci, $\downarrow\text{F}$ fovea	$\uparrow\text{F}$ foci, $\downarrow\text{F}$ fovea
10	Y (131/51)	Y (150/64)	Loss of photoreceptors and RPE in the fovea, disruption of RPE/EZ nasally and temporally	Loss of photoreceptors and RPE in the fovea, disruption of RPE/EZ nasally and temporally	Y	Y	132	105	Mottled, $\uparrow\text{F}$ foci, $\downarrow\text{F}$ ring in the fovea	Mottled, $\uparrow\text{F}$ foci, $\downarrow\text{F}$ ring in the fovea and parafoveal region

CME = cystoid macular oedema, EZ = ellipsoid zone, LE = left eye, N = no, ONL = outer nuclear layer, RE = right eye, RPE = retinal pigment epithelium, Y = yes. $\downarrow\text{F}$ hypoautofluorescent, $\uparrow\text{F}$ = hyperautofluorescent, normal range of central subfield thickness $280.1 \pm 35 \mu\text{m}$ (Invernizzi et al. 2018).

readily visible on autofluorescence imaging in all individuals and that there are detectable changes on SD-OCT even when the fundus appears normal on clinical examination.

At the cellular level, pigmentary retinopathy in DD has been suggested to be a sequela of the accumulation of outer segment remnants, ineffective mutant mitochondria and undegradable lipofuscin in lysosomes of the RPE cells (Thompson et al. 2016). Furthermore, a recent experimental study in *Lamp2-KO* mice demonstrated altered autophagy and phagocytosis in RPE cells, age-dependent accumulation of lipids, basal laminar deposits and thickening of Bruch’s membrane, which resulted in RPE and photoreceptor cell loss (Notomi et al. 2019). Thus, available evidence supports that outer retinal deposits and both rod and

cone degeneration are typical clinical findings in DD patients.

The severity of retinal pathology in our cohort generally increased with age, although females, when compared to similarly aged male patients, seemed to be less severely affected. The most advanced findings were present in the oldest male (10), who had the lowest BCVA, marked cone-rod involvement, in addition to pigmentary retinopathy. Bearing in mind the limits of XCI testing in WBCs for predicting pathology in non-haematologic tissues, we presume that the ocular/retinal abnormalities in female patients with DD reflect the XCI-induced mosaic expression of the mutant *LAMP2* allele.

Females with non-homogenous distribution of heterozygous *de novo LAMP2* mutations in their tissues (somatic mosaicism) represent a specific

and diagnostically neglected patient/cARRIER group (Majer et al. 2014; Sikora et al. 2015). Clinical presentation in these individuals is likely very variable. A *de novo* somatic mosaic for a *LAMP2* variant in a symptomatic female with DD presenting with cardiomyopathy and also exhibiting peripheral pigmentary retinopathy has been recently reported by Meinert et al. (2019). In contrast, in female 11 from our cohort, retinal pathology was the only detectable DD-associated abnormality.

High-resolution SD-OCT allowed for more detailed visualization of retinal pathology. We demonstrate for the first time, that DD manifests, apart from previously reported abnormalities of the RPE and photoreceptor layers, also with focally increased signals in the outer nuclear layer corresponding

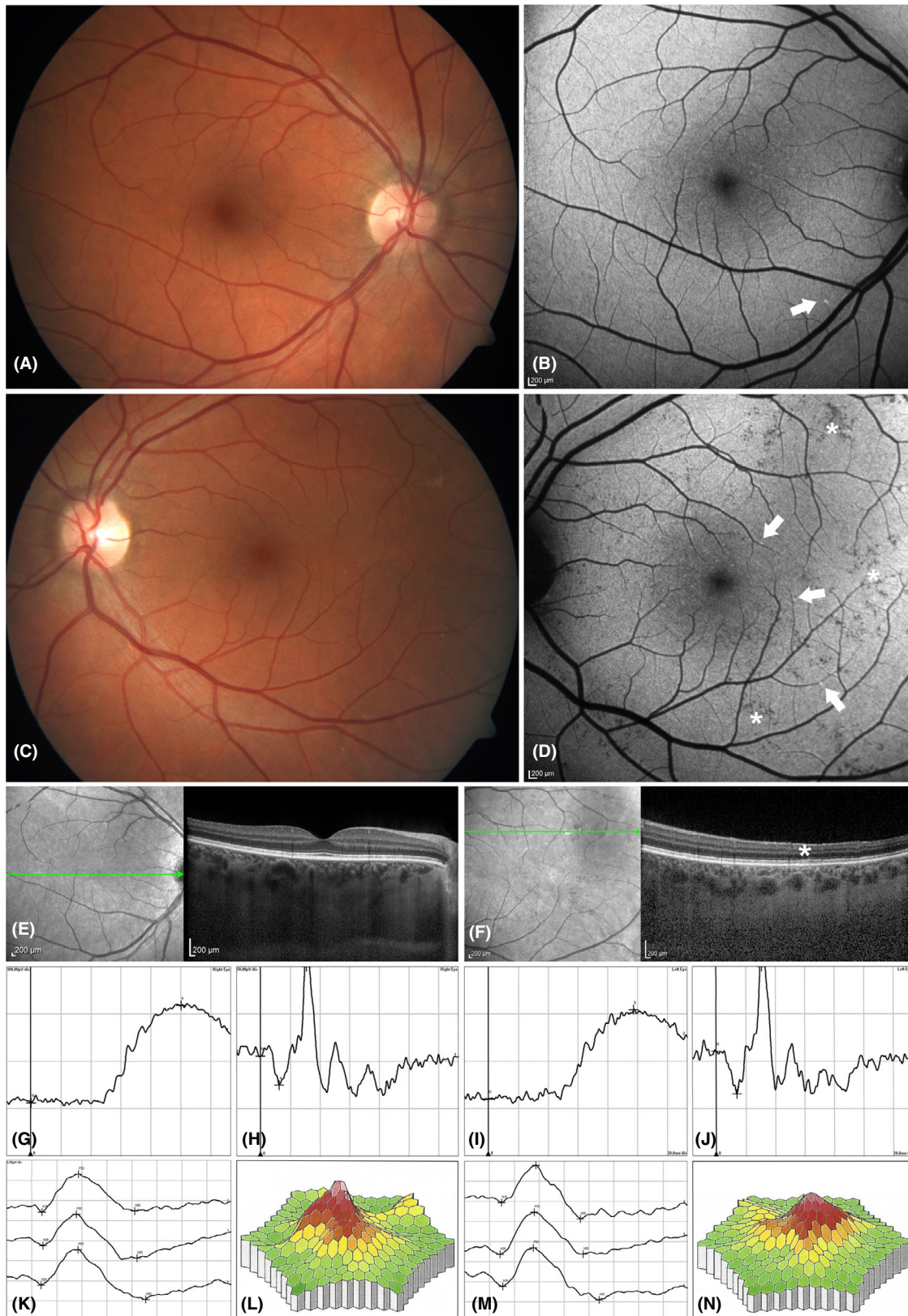


Fig. 3. Ocular phenotyping in an asymptomatic female (11) – a somatic mosaic for a *LAMP2* pathogenic variant. Normal appearance on fundus photography of the right (A) and left (C) eye. Fundus autofluorescence of the right (B) and left (D) eye, note only one hyperautofluorescent spot in the right eye (arrow), abnormal fundus autofluorescence pattern in the left eye with a number of hypoautofluorescent (asterisks) and a few hyperautofluorescent (arrows) spots. Spectral-domain optical coherence tomography images of the right (E) and left (F) eye, with the area corresponding to the hyperautofluorescent spot denoted by an asterisk. Normal full-field ERG responses in the right (G – scotopic), (H – photopic), (K – pattern) and left (I – scotopic), (J – photopic), (M – pattern) eye, and decreased foveal responses, to a lesser extent in the right (L) than left (N) eye on multifocal ERG.

histologically to photoreceptor nuclei. The biological explanation of these observations is currently uncertain. We also extend the spectrum of autofluorescence imaging abnormalities associated with DD.

Unlike non-syndromic retinitis pigmentosa (Strong et al. 2017), the finding of cystoid macular oedema in pigmentary retinopathy associated with DD is uncommon. In this study, it was present in only one eye and previously has been observed bilaterally in one other individual with DD (Mack 2014).

In summary, this study provides deep ocular phenotyping in the largest cohort of patients with DD reported to date, highlighting the utility of detailed ophthalmic examination and imaging in the identification of *LAMP2* variant carriers, including individuals with very low somatic mosaicism. It also provides a rationale for molecular genetic and/or further functional investigation in individuals with pigmentary retinopathy since this may be the only sign of a life-threatening condition such as DD. High-resolution imaging with SD-OCT and autofluorescence imaging should become an integral part in the overall diagnostic multidisciplinary approach in families with DD.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Těhotenství u pacientek se srdečním selháním na podkladě preexistující kardiomyopatie nebo po transplantaci srdce

(Outcomes of pregnancy in pre-existing cardiomyopathy and after heart transplantation)

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INFORMACE O ČLÁNKU

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Dilatační kardiomyopatie

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Transplantace srdce

SOUHRN

Úvod: Těhotenství u pacientek s preexistující kardiomyopatií se systolickou dysfunkcí levé komory srdeční nebo po ortotopické transplantaci srdce (OTS) je rizikové pro matku i dítě. Proto je ve většině případů nedoporučujeme. Avšak ve vybraných případech lze těhotenství nechat proběhnout s vědomím zvýšeného rizika. Cílem této práce je analyzovat soubor pacientek se srdečním selháním nebo po transplantaci srdce, které úspěšně absolvovaly těhotenství a porod živého plodu během sledování na našem pracovišti.

Metody: Pacientky jsme retrospektivně identifikovali v klinickém informačním systému IKEM a zpracovali jsme jejich klinická data a výsledky pomocných vyšetření.

Výsledky: Celkem jsme zaznamenali devět těhotenství u osmi žen s preexistující kardiomyopatií. Ve většině případů šlo o dilatační kardiomyopatii (šest pacientek, 75 %). Ejekční frakce levé komory (EF LKS) byla v mezích normy nebo lehce snižená v 56 % případů (pět těhotenství), středně snižená ve dvou případech (22 %) a v pásmu těžké dysfunkce ve dvou dalších případech (22 %), kde jsme těhotenství nedoporučovali. Nebylo doporučeno ani těhotenství u ženy s hypertrofickou kardiomyopatií a silně pozitivní rodinnou anamnézou zahrnující úmrtí bratra na srdeční selhání a OTS u matky. Později byla v rodině zjištěna Danonova nemoc. Během těhotenství s preexistující kardiomyopatií a v následujícím roce po porodu jsme zaznamenali dvě dekompenzace srdečního selhání, jednu dekompenzaci arteriální hypertenze a dvě tranzitorní ischemické ataky. Kardiiovaskulární příhoda komplikovala pět těhotenství (55 %). Ve čtyřech případech (44 %) byl pozorován pokles EF LKS ≥ 10 % po roce sledování. V pozdějším období byla u ženy s Danonovou nemocí nutná srdeční transplantace, nedošlo k žádnému úmrtí. Dále jsme zaznamenali čtyři porody po OTS, které měly kromě jedné epizody preeklampsie nekomplikovaný průběh s narozením čtyř hypotrofičkových, ale jinak zdravých dětí. Medián od OTS do porodu byl 68 měsíců. Další sledování bylo bez významnějších komplikací, ženy jsou naživu se sledováním 49–118 měsíců po porodu.

Závěry: Díky moderní léčbě srdečního selhání je možné nechat proběhnout těhotenství i u žen s neischemickými kardiomyopatiemi a dysfunkcí levé komory srdeční. Těhotenství je možné také u vybraných žen po OTS, nicméně vzhledem k složitě biologické situaci a prognostickým aspektům jej u těchto pacientek nedoporučujeme.

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ABSTRACT

Introduction: Pregnancy in females with pre-existing cardiomyopathy with left-ventricular systolic dysfunction and/or after heart transplantation is associated with risks for both the mother and the child. Thus, it is not recommended in the majority of patients. However, in selected cases, with awareness of the risks, pregnancy may be considered. We aimed to analyse a group of patients with heart failure and/or after heart transplantation (HTx) who were pregnant and gave birth to a living new-born during follow-up in our institution.

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Methods: Patients were found in the clinical database of IKEM retrospectively. Clinical and para-clinical data were analysed.

Results: Nine pregnancies in eight females with pre-existing cardiomyopathy were identified. Most patients had dilated cardiomyopathy (75%), their left-ventricular ejection fraction (LVEF) was normal or mildly reduced in 56%, moderately reduced in 22%, and severely reduced in 22%. Pregnancy was not recommended in females with severe left-ventricular systolic dysfunction and also in a female with hypertrophic cardiomyopathy and a strong family history with death due to heart failure in her brother and necessity of HTx in her mother, which was subsequently diagnosed with Danon disease. During pregnancies with pre-existing cardiomyopathy and twelve months postpartum we recorded two episodes of decompensated heart failure, one episode of decompensated arterial hypertension and two transient ischemic attacks. Taken together, cardiovascular events complicated 55% of these pregnancies. We observed a decrease in LVEF $\geq 10\%$ in 44% of pregnancies after one year. Subsequently, the female with Danon disease required HTx. There was no maternal death. In addition, we recorded four deliveries after HTx, which were except of one episode of preeclampsia uncomplicated and gave birth to four hypotrophic, but healthy babies. Median time from HTx to delivery was 68 months, subsequent period was uneventful, all four females are alive with a follow-up of 49–118 months after delivery.

Conclusions: Contemporary therapeutic modalities of heart failure lead in most patients with dilated cardiomyopathy to at least temporary improvement of left-ventricular systolic function outside the range of severe systolic dysfunction, which enables in selected cases birth of living fetus. Pregnancy is possible also in highly selected females after HTx. However, due to their complicated biological situation and prognostic aspects, we do not recommend pregnancy in this setting.

Keywords:

Dilated cardiomyopathy
Heart transplantation
Pregnancy

Úvod

První úspěšné těhotenství dva roky po ortotopické transplantaci srdce (OTS) pro dilatační kardiomyopatii (DKMP) bylo popsáno v roce 1988 u dvacetileté ženy.¹ Zlepšení prognózy mladších nemocných s kardiomyopatiemi, po korekci vrozených srdečních vad nebo po OTS vedlo v posledních letech k nárůstu populace žen v reprodukčním věku, které uvažují o těhotenství i v přítomnosti srdečního onemocnění.² Některé typy kardiomyopatií jako například neobstrukční formy hypertrofické kardiomyopatie nebo kompletně korigované vrozené srdeční vady nepředstavují významnější překážku k úspěšnému dokončení těhotenství. Výrazná rizika však přetrvávají u pacientek s preexistující systolickou dysfunkcí levé komory srdeční (LKS), nejčastěji při DKMP nebo u pacientek po OTS.^{3,4} Problematické gravidity u těchto nemocných je věnována tato práce.

Pacientky s DKMP ohrožuje v těhotenství především zvýšená hemodynamická zátěž a retence tekutin. Dalším problémem je také nutnost vysazení teratogenních léků nezbytných pro léčbu srdečního selhání, především inhibitorů systému renin-angiotenzin. Z těchto důvodů mají pacientky v těhotenství zvýšené riziko srdečního selhání, závažných arytmií, tromboembolických nebo krvácivých komplikací.^{5–8} U žen po OTS může navíc dojít k imunizaci ženy nebo k poklesu biologické dostupnosti imunosupresiv s rozvojem akutní, případně chronické rejekce transplantátu, k vzniku preeklampsie a renálního selhání.^{9–13} Rizika pro plod zahrnují zejména možnost spontánního potratu (15–30 % těhotenství po OTS), hypotrofii plodu, předčasný porod a teratogenní působení léků jak během těhotenství, tak laktace.¹³ Pokud je matka nositelkou geneticky podmíněné kardiomyopatie, onemocnění může zdědit také plod a některé typy kardiomyopatie se mohou časně manifestovat.¹⁴ Při plánování těhotenství je třeba zvážit kromě klinických a laboratorních nálezů také odhadovanou životní prognózu matky a její sociální zázemí.

Těhotenství je proto jednoznačně kontraindikováno u žen s DKMP v přítomnosti těžké systolické dysfunkce LKS (s ejekční frakcí $< 30\%$), výraznější symptomatologie (NYHA III a IV) nebo mechanické srdeční podpory.^{2,5} U žen po OTS těhotenství ve většině případů také nedoporučujeme. Těhotenství lze po OTS zvažovat jen u vybraných žen, které mají několik let po operaci normální funkci štěpu, nemají evidenci o proběhlé nebo přítomné akutní nebo chronické rejekci transplantátu, jsou normotenzní, mají normální renální funkci a funkční rodinné zázemí. U obou skupin pacientek je třeba vysadit teratogenní léky. Po OTS je imunosuprese udržována kombinací tacrolimu a prednisonu s vysazením mykofenolát mofetilu.^{9,13}

Cílem této práce je analyzovat soubor pacientek se srdečním selháním nebo po OTS, které úspěšně absolvovaly těhotenství a porod živého plodu během sledování na našem pracovišti.

Metodika a soubor pacientek

V klinické databázi pacientů IKEM jsme identifikovali 12 pacientek, které porodily životaschopný plod se základní diagnózou kardiomyopatie nebo po OTS. Jednalo se o osm pacientek se známou diagnózou kardiomyopatie, které jsme sledovali během devíti těhotenství. Pokud bylo těhotenství plánované, redukovali jsme u nich medikaci srdečního selhání pouze na beta-blokátor a v případě potřeby furosemid již při plánování těhotenství. V ostatních případech byla medikace upravena okamžitě při zjištění těhotenství. Během těhotenství absolvovaly pacientky minimálně tři kontroly na našem pracovišti, a to na začátku těhotenství, v posledním trimestru a v prvním měsíci po porodu s provedením echokardiografie a většinou i se stanovením natriuretického peptidu typu B. Dále byly sledovány v minimálně měsíčních intervalech v poradně pro riziková těhotenství – ve většině případů na Gynekologicko-porodnické klinice 1. LF UK a VFN v Praze. Porod byl u nich zaznamenán v letech 2014 až 2020, průměrná

doba sledování po porodu byla 54 měsíců. Těhotenství úspěšně donosily také 4 pacientky po OTS. Ve třech případech proběhla OTS v roce 2008, poslední v roce 2013. Porod jsme u nich zaznamenali v letech 2011 až 2016, průměrná doba sledování od porodu byla 70 měsíců. Pokud byla těhotenství plánována, byla u pacientek redukována imunosuprese na kombinaci tacrolimus a prednison s vysazením mykofenolát mofetilu. Následně v odstupu alespoň jednoho měsíce byla provedena kontrolní endomyokardiální biopsie k vyloučení rejekce srdečního štěpu a dále byla provedena selektivní koronarografie k vyloučení koronární nemoci štěpu. Pacientky na našem pracovišti absolvovaly minimálně čtyři kontroly během těhotenství a hodnota tacrolimu u nich byla kontrolována minimálně jednou měsíčně. Pacientky byly kontrolovány také v poradně pro riziková těhotenství gynekologického pracoviště (ve většině případů na Gynekologicko-porodnické klinice 1. LF UK a VFN v Praze).

U pacientek jsme retrospektivně vyhledali základní klinická, laboratorní a echokardiografická data. Zhodnotili jsme riziko těhotenství podle modifikované klasifikace Světové zdravotnické organizace (WHO), která je založena na konsenzu expertů^{4,15} a je vhodnější pro pacientky s vrozenými vadami srdce. Pacientkám s nejvyšším stupněm WHO IV je pro extrémně vysoké riziko doporučeno přerušení těhotenství. Dále jsme u pacientek spočítali skóre CARPREG II, které vychází z kohortové studie a lépe reflektuje rizika těhotenství u žen se srdečním selháním a kardiomyopatiemi. Skóre koreluje lineárně s incidencí kardiovaskulárních příhod u těhotných s kardiomyopatií. Při skóre nad 4 dosahuje riziko kardiovaskulární příhody až 41 %.^{16,17} Pacientky souhlasily s anonymním zpracováním dat o zdravotním stavu. Výzkum proběhl se schválením etické komise. Data jsme zpracovali pomocí statistického programu Excel a SPSS (Chicago, Illinois, USA) pro Windows, verze 17.0.

Výsledky

Pacientky s preexistující kardiomyopatií

Celkem jsme zaznamenali devět porodů deseti zdravých dětí (v jednom případě dvojčata) u osmi žen s preexistující kardiomyopatií. Základní charakteristiky souboru ukazuje tabulka 1. Většina pacientek měla diagnózu dilatační kardiomyopatie (75 %) a byly ve funkční třídě NYHA I (78 %). Po předchozí farmakoterapii srdečního selhání byla před porodem ejekční frakce LKS normální nebo lehce snížená v 56 %, středně snížená ve 22 % a v pásmu těžké dysfunkce ve 22 % (dva případy). Těhotenství jsme jednoznačně nedoporučovali u žen s těžkou dysfunkcí LKS (případ 2 a 5, tabulka 2). Dále jsme nedoporučovali třetí těhotenství u ženy s familiární dilatační kardiomyopatií a anamnézou abúzu pervitinu, která měla sice lehkou systolickou dysfunkci LKS, ale závažné komplikace během předchozích těhotenství (případ 4, tabulka 2). Neúměrně vysoké riziko jsme konstatovali také u pacientky s hypertrofickou kardiomyopatií a normální ejekční frakcí LKS, která měla silně pozitivní rodinnou anamnézu s údajem o OTS u matky a úmrtí bratra na srdeční selhání v 18 letech (případ 1, tabulka 2). V této rodině byla později diagnostikována

Danonova nemoc, pro kterou je typická progresse do terminálního srdečního selhání v mladém věku.

Celkem 89 % porodů bylo vedeno císařským řezem. Tabulky 1 a 2 ukazují výskyt komplikací – dekompenzace srdečního selhání komplikovala těhotenství u dvou matek a u jedné matky došlo k zhoršení arteriální hypertenze. U dvou matek jsme zaznamenali v prvním roce po porodu tranzitorní ischemickou ataku. Ve sledovaném období se vyskytla kardiovaskulární komplikace u 55 % těhotenství žen s preexistující kardiomyopatií. Ve 44 % případech jsme zaznamenali snížení ejekční frakce LKS ≥ 10 %. Vývoj end-diastolického rozměru a ejekční frakce LKS a dále natriuretického peptidu typu B v graviditě a následujícím období ukazuje obrázek 1.

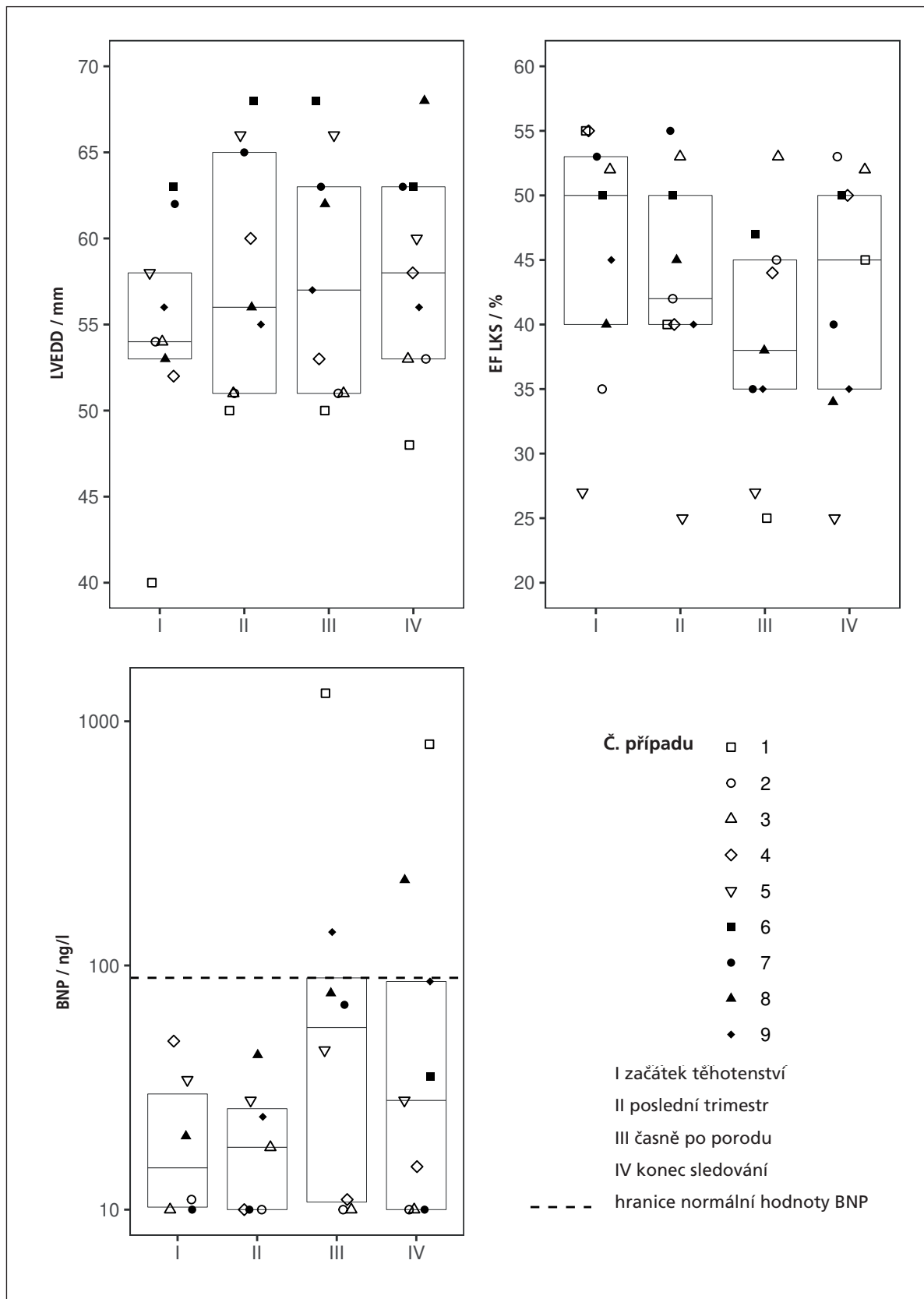
Průměrná porodní hmotnost novorozenců dosáhla $2\,505 \pm 511$ gramů, byla tedy na hranici hypotrofie plodu. U dětí však nedošlo v časném období ani během dalšího sledování k významnějším komplikacím.

Pacientky po transplantaci srdce

Těhotenství donosily čtyři ženy po OTS, a to průměrně 67 ± 33 měsíců po operaci. Podrobnější informaci podávají tabulky 1 a 2. Dvě těhotenství byla plánována (50 %). U pacientky s familiární dilatační kardiomyopatií a mutací *RBM20* (případ 11) bylo těhotenství plánováno s použitím preimplantační diagnostiky, po redukcii imunosupresivní léčby (vysazení mykofenolátu a další teratogenní medicace) byla předem provedena endomyokardiální biopsie k vyloučení rejekce a byla vyloučena koronární nemoc štěpu. Podobně bylo postupováno i u druhé ženy po OTS (případ 12). Ve zbývajících dvou případech (případ 10 a 13) byla teratogenní medicace vysazena až po zjištění těhotenství. Kromě epizody preeklampsie (případ 11) byla těhotenství těchto čtyř žen po OTS nekomplikovaná. Ženy jsou naživu se sledováním 49–118 měsíců po porodu. U pacientky s Friedreichovou ataxií dochází neurologicky k progresi stavu při základním onemocnění. V žádném z případů nedošlo během sledování k rejekci srdečního štěpu vyššího stupně, ani zhoršení funkce štěpu po porodu, ve všech případech byla na začátku těhotenství normální ejekční frakce LKS. Všechny čtyři děti byly bezprostředně po porodu zdravé, ale hypotrofné. Průměrná porodní hmotnost dosáhla pouze $1\,883 \pm 494$ gramů. Dcera jedné pacientky s nonkompaktní kardiomyopatií zdědila patogenní variantu genu *MYH7*, později u ní byla diagnostikována počínající kardiomyopatie (případ 10). U dalšího dítěte byla pozorována několik měsíců po narození porucha sluchu (případ 12).

Diskuse

V této práci prezentujeme poprvé v českém písemnictví unikátní soubor osmi pacientek s preexistující kardiomyopatií a čtyř pacientek po OTS, které úspěšně absolvovaly těhotenství a porod živého plodu během sledování na našem pracovišti. Naše pozorování lze shrnout následovně: 1) Většina žen s preexistující kardiomyopatií měla dilatační kardiomyopatii (75 %). Předchozí léčba srdečního selhání přispěla k tomu, že ejekční frakce LKS byla v době těhotenství v mezích normy nebo lehce snížená v 56 % a středně snížená ve 22 % případů. 2) Otěhotněly i ženy,



Obr. 1 – Vývoj individuálních hodnot end-diastolického rozměru levé komory (LVEDD), ejekční frakce levé komory srdeční (EF LKS) a natriuretického peptidu typu B (BNP) v průběhu těhotenství pacientek s preexistující kardiomyopatií. Zachyceny jsou hodnoty na počátku těhotenství, v posledním trimestru, při první kontrole po porodu a na konci sledování. Čísla případů korespondují s tabulkou 2. Graficky jsou znázorněny hodnoty mediánu a interkvartilové rozmezí. Při porovnání párovými parametrickými a neparametrickými testy jsme nezjistili statisticky významné rozdíly.

Tabulka 1 – Charakteristika pacientek a průběh těhotenství u skupiny pacientek s preexistující kardiomyopatií nebo po transplantaci srdce

Charakteristika pacientek (n = 12)	Preexistující kardiomyopatie (8 pacientek, 9 porodů)	Transplantované srdce (4 pacientky, 4 porody)
Průměrný věk v době porodu (roky)	30 ± 6 (medián 28)	31 ± 2 (medián 31)
Hypertenze	1 (12,5 %)	75 % (n = 3)
Diabetes mellitus	1 (12,5 %)	25 % (n = 1)
BMI > 30	3 (37,5 %)	0 % (0)
Základní diagnóza	n = 8	n = 4
Dilatační kardiomyopatie	6 (75 %)	3 (75 %)
Hypertrofická kardiomyopatie	1 (12,5 %)	0
Arytmogenní kardiomyopatie LKS	1 (12,5 %)	0
Nonkompaktní kardiomyopatie	0	1 (25 %)
Klasifikace NYHA před porodem	n = 9	n = 4
NYHA I	6 (77 %)	4 (100 %)
NYHA II	2 (22 %)	0
NYHA III–IV	0	0
Dysfunkce LKS/EF LKS před porodem	n = 9	n = 4
Těžká (EF LKS ≤ 35 %)	2 (22 %)	0
Střední (EF LKS 36–45 %)	2 (22 %)	0
Lehká (EF LKS 46–54%)	3 (34 %)	0
Normální (EF LKS ≥ 55 %)	2 (22 %)	4 (100 %)
LVEDD na začátku gravidity (mm)	55 ± 6	46 ± 5
LVEDD rok po porodu (mm)	58 ± 6	46 ± 4
Mitrální regurgitace střední/významná	0	0
Laboratorní diagnostika		
eGFR na začátku gravidity (ml/s/1,73 m ²)	1,97 ± 0,18	1,66 ± 0,21
eGFR při poslední kontrole (ml/s/1,73 m ²)	1,88 ± 0,16	1,28 ± 0,4
BNP na začátku těhotenství (ng/l)	16 (10–31)	–
BNP za rok po porodu (ng/l)	28 (10–86)	–
Riziková klasifikace	n = 9	n = 4
mWHO I	0	–
mWHO II	0	–
mWHO II–III	3 (33 %)	–
mWHO III	4 (45 %)	–
mWHO IV	2 (22 %)	–
CARPREG II 1-2 (5–10% riziko)	3 (33 %)	–
CARPREG II 3 (15% riziko)	3 (33 %)	–
CARPREG II 4 (22% riziko)	0	–
CARPREG II > 4 (41% riziko)	3 (33 %)	–
Charakteristika porodu	n = 9	n = 4
Doba od transplantace do porodu	–	67 ± 33 měsíců (medián 68)
Porodní hmotnost dítěte (g)	2 505 ± 511	1 883 ± 494
Porod v týdnu těhotenství	36 ± 4 (medián 37)	31 ± 3 (medián 30)
Porod císařským řezem	8 (89 %)	4 (100 %)
Peripartální komplikace	n = 9	n = 4
Preeklampsie	1 (11 %)	1 (25 %)
Akutní dekompenzace srdečního selhání	2 (22 %)	0
Časné tromboembolické komplikace	2× TIA (22 %)	0
Zhoršení EF LKS ≥ 10 procentních bodů jeden rok po porodu	4 (44 %)	0
Závažný další průběh	4 (44 %)	0

BMI – body mass index; BNP – natriuretický peptid typu B, CARPREG II – rizikové skóre „the CARDiac disease in PREGnancy“; EF LKS – ejekční frakce levé komory srdeční; eGFR (dle CKD-EPI) – odhadovaná glomerulární filtrace; LVEDD – end-diastolický rozměr levé komory; mWHO – modifikovaná klasifikace WHO rizikivosti gravidity; NYHA – klasifikace dušnosti New York Heart Association; TIA – tranzitorní ischemická ataka.

Tabulka 2 – Informace k základnímu onemocnění, průběhu těhotenství a dalšímu vývoji onemocnění u jednotlivých pacientek					
Číslo případu	Pacientky s kardiomyopatií	Průběh těhotenství	Medikace v těhotenství	Porod a vývoj dítěte	Dlouhodobý vývoj onemocnění
1	Hypertrofičká neobstrukční kardiomyopatie při Danonově nemoci	Neplánované, nedoporučeno vzhledem k rodinné zátěži. EF LKS 55 %. Ukončeno v 36. týdnu pro dušnost, pokles EF LKS.	Pouze selektivní beta-blokátor	SC, 36. týden, hmotnost 1 940 g.	Významná rodinná zátěž, matka vedena s dg. těhotenské KMP, podstoupila OTS, bratr zemřel na srdeční selhání, později v rodině dg. Danonova nemoc. Po porodu další progresse srdečního selhání, trombus v LKS, v r. 2015 OTS. Sledování 84 měsíců od porodu
2, 3	DKMP s reverzní remodelací LKS (susp. toxonutritivní podíl)	Dvě neplánovaná těhotenství bez komplikací, první nedoporučeno pro vstupní EF LKS 35 %.	Pouze beta-blokátory, při druhé graviditě bez léků (sama vysadila beta-blokátor)	SC, 39. týden, 3 250g SC, 36. týden, dvojčata, hmotnost 2 260 + 2 700 g, děti zdravé.	Paradoxně během prvního těhotenství a po porodu normalizace EF LKS, nekomplikovaný průběh, trvá normální EF LKS. Sledování 80 měsíců od prvního porodu
4	Familiární DKMP (patogenní varianta titinu), abúzus pervitinu v anamnéze	Třetí těhotenství Doporučeno přerušeni těhotenství. Pokles EF LKS během těhotenství na 40 %.	Beta-blokátor, LMWH, redukováná dávka furosemidu	SC, 36. týden, větší krevní ztráta, podvaz vejcovodů. Dcera, 2 200 g, zdědila vlohu v genu pro TTN, zdráva.	Manifestace srdečního selhání dva týdny po 2. těhotenství, kdy zároveň embolizační CMP s nutností katetrizační trombektomie. Tehdy i abúzus pervitinu. Při 3. těhotenství přechodný pokles EF LKS, následně opět normalizace. Sledování 52 měsíců
5	DKMP diagnostikována od dětství, implantován ICD	Dvě těhotenství při těžké dysfunkci LK, zde popsáno 2. těhotenství. Nedoporučeno, komplikováno oboustrannou kardiální dekompenzací.	Vysazeny potenciálně teratogenní léky (sartan, spironolacton)	SC, 27. týden, 1 520 g, zdravé dítě	Obě těhotenství komplikovány kardiální dekompenzací. Tromboembolická příhoda (st.p. TIA), v dalším sledování onkogynekologický zákrok (kónizace čípku, CIN 3). Sledování 47 měsíců
6	DKMP (mutace v genu <i>RBM20</i>)	Plánované těhotenství s nekomplikovaným průběhem, vstupní EF LKS 50 %	Pouze beta-blokátor	SC, 37. týden, 2 700 g, porod bez komplikací, zdravé dítě	Recidivující kardioembolizační CMP na antikoagulaci, bidirekční PFO, trvá lehký neurologický deficit. Sledování 80 měsíců
7	DKMP s podílem hypertenzní kardiomyopatie	Plánované, vstupní EF LKS 50–55 %, na konci těhotenství a po porodu dekompenzace arteriální hypertenze	Pouze beta-blokátor	SC, 37. týden, 2 660 g, zdravá dívka, u matky porucha hojení rány po SC	Po porodu pokles EF LKS, střední dysfunkce, dlouhodobě stabilní. Sledování 25 měsíců
8	DKMP, nezjištěna příčinná genetická varianta	Neplánované těhotenství, nedoporučeno, průběh bez komplikací, vstupně EF LKS 40 %	Monoterapie beta-blokátorem i 6 měsíců po porodu (trvala na kojení, laktace nepozastavena)	SC, 38. týden, hmotnost 2 900 g, zdravé dítě	Pro další pokles funkce levé komory nutnost implantace BiV ICD. Sledování 13 měsíců
9	Arytmogenní KMP s postižením levé komory (prokázaná patogenní mutace <i>PKP2</i>), přítomnost ICD	Plánované těhotenství, vstupně EF LKS 45 %, preindukce porodu z kardiologické indikace	Beta-blokátor, LMWH	Vaginální porod, 40. týden, 3 230 g, zdravé dítě	Po porodu další pokles EF LK, úprava medikace, postupně stabilizace až k NYHA I. Sledování 17 měsíců

Tabulka 2 – Informace k základnímu onemocnění, průběhu těhotenství a dalšímu vývoji onemocnění u jednotlivých pacientek (Dokončení)

	Stav po OTS, základní diagnóza	Průběh těhotenství	Medikace v těhotenství	Porod a vývoj dítěte	Dlouhodobé sledování
10	Nonkompaktní kardiomyopatie (patogenní varianta MYH7)	Neplánované těhotenství. Nekomplikovaný porod 31 měsíců po OTS	Imunosupresivní terapie kombinací tacrolimu a kortikosteroidu, MMF dlouhodobě vysazen pro dřeňový útlum	SC, 36. týden, hmotnost 2 480 g, dcera nese stejnou variantu MYH7, incipientní KMP.	Četné onkogynekologické zákroky pro neoplazii krčku dělohy, radioterapie, následně ovariectomie I.dx. Sledování 118 měsíců.
11	Familiární DKMP (patogenní varianta v genu RBM20)	Plánované těhotenství, vzhledem k rodinné zátěži IVF s preimplantační diagnostikou. Porod 101 měsíců po OTS	Při plánování těhotenství předem vyloučena rejekce a koronární nemoc, po předchozím vysazení MMF a kortikoidů, ponechán tacrolimus a LMWH	SC, 29. týden, ukončeno předčasně pro riziko eklampsie, syn 1 500 g, zdravý.	Gestační diabetes, dále DM na inzulinu, opakované pankreatitis, žije. Sledování 54 měsíců
12	DKMP (po proběhlé myokarditidě)	Plánované těhotenství, s nekomplikovaným průběhem. Porod 38 měsíců po OTS	MMF vysazen plánovaně 90 dní předem, dále podáván tacrolimus a kortikoidy	SC, 30. týden, 1 300 g, syn, několik měsíců po porodu porucha sluchu	Opakované onkogynekologické intervence (kónizace čípku, odstranění kondylomat – z histologie <i>ca in situ</i>), žije. Sledování 49 měsíců
13	DKMP při Friedreichově ataxii	Neplánované těhotenství. Nekomplikovaný průběh. Porod 98 měsíců po OTS	MMF vysazen dlouhodobě, během těhotenství tacrolimus a kortikoidy	SC, 28. týden, 2 250g, zdravý syn, nezdědil vlohu.	Recidivující infekce, neurologicky progresivní stavu matky, žije. Sledování 59 měsíců

DKMP – dilatační kardiomyopatie; EF LKS – ejekční frakce levé komory srdeční; ICD – implantabilní kardioverter-defibrilátor; IVF – *in vitro* fertilizace; KMP – kardiomyopatie, LMWH – nízkomolekulární heparin; MMF – mykofenolát mofetil; OTS – ortotopická transplantace srdce; SC – císařský řez (sectio caesarea); TIA – tranzitorní ischemická ataka.

kde jsme graviditu nedoporučovali, a to pro těžkou systolickou dysfunkcí LKS (22 %) a pro vysoké riziko dané rodinnou anamnézou nebo průběhem předchozích těhotenství. 3) Během těhotenství s preexistující kardiomyopatií a v následujícím roce po porodu jsme zaznamenali dvě dekompenzace srdečního selhání, jedno zhoršení arteriální hypertenze a dvě tranzitorní ischemické ataky. Jakákoliv kardiovaskulární příhoda komplikovala 55 % těhotenství těchto žen. U 44 % žen jsme pozorovali pokles ejekční frakce o deset a více procentních bodů po roce sledování. U ženy s prokázanou Danonovou nemocí byla později nutná OTS. 4) Těhotenství překonaly bez větších problémů čtyři ženy po OTS. Kromě jedné epizody pre eklampsie byly všechny gravidity nekomplikované a narozené děti byly zdravé s nižší porodní hmotností. Jejich matky jsou naživu se sledováním v rozmezí 49–118 měsíců po porodu.

Zlepšení prognózy pacientek s dilatační kardiomyopatií nebo po transplantaci srdce

Významné změny v léčbě srdečního selhání se sníženou systolickou funkcí vedly v posledních dekádách k zlepšení prognózy nemocných. V 80. letech minulého století, tedy v době před zavedením moderní farmakoterapie srdečního selhání, bylo roční a pětileté přežívání nemocných se symptomatickou dilatační kardiomyopatií 25 %, respektive 50 %.^{18,19} Řada prací dokumentovala postupné snižová-

ní mortality. Například Merlo a spol. ukázali v kohortové studii zlepšení osmiletého přežívání pacientů s dilatační kardiomyopatií diagnostikovanou v dekádách 1979–1987, 1988–1997 a 1998–2007 z 55 % na 71 % a 87 %.²⁰ Současná terapie dokáže onemocnění nejen stabilizovat, ale často navodit alespoň částečnou remisi onemocnění v podobě reverzní remodelace LKS až u poloviny nemocných s recentním záchtem srdečního selhání.^{21,22}

V posledních dvou dekádách došlo také k zlepšení přežívání u pacientů po OTS. Je to důsledek lepší imunosupresivní léčby a zlepšené péče zaměřené na prevenci infekčních komplikací a nádorů. Statistiky z posledních let referují roční a pětileté přežívání kolem 90 %, respektive 70 %, s mediánem přežívání kolem 12 let.^{9,23}

Výše uvedené zlepšení prognózy žen v obou uvedených skupinách umožňuje ve vybraných případech těhotenství.

Komplikace u žen s preexistující kardiomyopatií, porovnání s předchozími studii

První větší skupinu žen s těhotenstvím při preexistující kardiomyopatií analyzovala v roce 2010 práce kanadské skupiny.⁷ Referováno bylo 36 těhotenství u 32 žen s dilatační kardiomyopatií, z nichž 72 % mělo lehkou nebo středně těžkou dysfunkci LKS. Srdeční komplikace se objevila v těhotenství a následujících šesti měsících po porodu celkem v 39 % případů. Nejčastěji šlo o dekompenzaci srdečního

selhání (celkem devětkrát), arytmie (celkem sedmkrát) a jednu mozkovou příhodu. Nebylo zaznamenáno žádné úmrtí. Tyto komplikace nastaly u žen s pokročilejším selháváním (se vstupní klasifikací NYHA III/IV a s těžkou systolickou dysfunkcí LKS) nebo s anamnézou kardiálních příhod v předcházejících těhotenstvích. Francouzská skupina popsala v roce 2017⁸ celkem 43 těhotenství u 36 žen s preexistující kardiomyopatií. Pouze deset z nich (28 %) mělo dilatační kardiomyopatii s průměrnou ejekční frakcí LKS kolem 36 %. Výskyt kardiovaskulárních komplikací dosáhl 35 %, mateřská mortalita činila 7 %. Náš soubor se větším zastoupením pacientek s dilatační kardiomyopatií více podobal kanadské práci. Výskyt kardiovaskulárních komplikací byl sice numericky vyšší (55 %), ale ve většině případů se jednalo o reverzibilní příhody s nulovou mateřskou mortalitou. Tato příznivá data odrážejí skutečnost, že většína pacientek (56 %) měla normální nebo lehce sníženou ejekční frakci LKS jako důsledek předchozí optimalizované léčby srdečního selhání.

Komplikace těhotenství u žen po transplantaci srdce

Od roku 1988 byly celosvětově referovány desítky případů těhotenství u žen po OTS.^{1,10-13} Recentní pohled přináší práce z registru těhotenství u pacientek po OTS.¹² Analyzovala 157 těhotenství u 91 žen, průměrně $7 \pm 6,1$ roku po OTS. Těhotenství bylo úspěšně donošeno v 69 %. Nejčastější komplikací byla preeklampsie (23 %), dále infekce (14 %) nebo rejekce štěpu (9 %). Důležité je zjištění asociace mezi podáváním mykofenolátu v těhotenství a výskytem potratů. Průměrná doba sledování po porodu dosahovala $8,9 \pm 6,5$ roku. Z uvedeného souboru zemřelo 30 žen, a to průměrně $9,4 \pm 6,2$ roku po porodu. Nejčastější příčinou úmrtí byla koronární nemoc štěpu nebo rejekce. Naše výsledky zapadají i do tohoto kontextu. Složitý management pacientek v těhotenství, významná imunologická i neimunologická rizika pro matku a plod, zohlednění dlouhodobé prognózy žen po OTS a jejich sociálního zájmu představují významné výzvy při případném plánování těhotenství v této populaci žen. Ve většině případů těhotenství po OTS nedoporučujeme. U žen ve fertlím věku po transplantaci srdce tedy doporučujeme prevenci otěhotnění. U pacientek s nekomplikovaným potransplantačním průběhem, bez evidence o akutní nebo chronické rejekci štěpu nebo koronární nemoci štěpu můžeme při respektování kontraindikací použít většinu účinných antikoncepčních metod, kromě nitroděložních tělísek. U žen s komplikovaným průběhem nebo přítomností závažných komorbidit jsou naše možnosti většinou omezeny na monoterapii progestiny ve formě tablet, depotních injekcí nebo podkožních implantátů.²⁴ Recentně bylo popsáno úspěšné použití nitroděložních tělísek s levonorgestrellem, které bylo u malých kohort pacientek po orgánových transplantacích účinné a bezpečné, bez výskytu infekcí malé pánve. Tyto výsledky odpovídají ztlustění endometria a sníženému výskytu infekcí gynekologické oblasti při použití této antikoncepce u pacientek bez imunosuprese.^{25,26} Po orgánových transplantacích však zatím nejsou doporučovány pro rutinní užití.²⁴

Genetické poradenství v graviditě

Pokroky v molekulárně-genetické diagnostice příčin kardiomyopatií umožňují v řadě případů identifikaci patogenních mutací způsobující dané onemocnění. U devastujících onemocnění, která vedou v časně morbiditě nebo mortalitě, je možné po konzultaci s genetikem uvažovat o tzv. preimplantační diagnostice. S použitím fertilizace *in vitro* je ve vybraných případech možné identifikovat embrya, která nenesou danou patogenní mutaci a mají výrazně redukované riziko vzniku příslušné choroby.²⁷ V ostatních případech se může uplatnit stanovení genotypu u dítěte s pečlivým klinickým sledováním nosičů patogenních variant po narození. V rodinách s familiárním výskytem kardiomyopatií a neúspěšnou genotypizací je doporučeno klinické a echokardiografické sledování dítěte.²⁷ Před plánovanou graviditou je třeba zvažovat nejen možná prenatální a postnatální rizika pro matku a dítě, ale také upozornit, obzvláště u transplantovaných žen, na sníženou dobu přežívání oproti zdravým vrstevnicím, a tedy nutnost sociální stability rodiny.⁴

Limitace

Výpovědní hodnota naší práce je limitována malým souborem pacientek, který ale odpovídá nízké frekvenci těhotenství ve sledovaných skupinách nemocných. Dalším omezením je, že jsme se soustředili na pacientky s úspěšným průběhem těhotenství a donošením zdravého plodu. V klinické databázi se nám nepodařilo zachytit pacientky, které překonaly potrat nebo porodily mrtvý plod. Při retrospektivním zpracování dat nemůžeme vyloučit, že některé z těchto případů nebyly zaznamenány. Dále jsme si vědomi limitace chybějícího dlouhodobého sledování po těhotenství u žen s preexistující kardiomyopatií. K dispozici máme pouze střednědobá data. Po desetiletích sledování by se tyto ženy mohly významně lišit od netěhotných vrstevnic se stejnou diagnózou.

Závěr

Moderní postupy léčby srdečního selhání vedou u řady pacientek s dilatační kardiomyopatií k stabilizaci stavu a často k zlepšení systolické funkce LKS. To dovoluje za určitých podmínek úspěšný průběh těhotenství. Naopak těžká dysfunkce LKS a přítomnost mechanické srdeční podpory by měly být jasnou kontraindikací gravidity. Těhotenství je možné také u vybraných žen po OTS. Nicméně vzhledem k složité biologické situaci a prognostickým aspektům je však u těchto pacientek nedoporučujeme.

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