Supplementary material

Kwiatkowska J, Meyer-Szary J, Bazgier M, et al. Left ventricular volumes and function affected by myocardial fibrosis in patients with Duchenne and Becker muscular dystrophies: a preliminary magnetic resonance study. Kardiol Pol. 2020; 78: 331-334. doi:10.33963/KP.15223

Methods

Inclusion criteria:

a) genetically confirmed diagnosis of Duchenne and Becker muscular dystrophies with Multiplex Ligation-dependent Probe Amplification and Sanger sequencing techniques,

b) age 6–18 years,

c) patients legal guardians' consent for the study.

Exclusion criteria:

a) Not meeting inclusion criteria (n = 29),

b) Contraindications for cardiac magnetic resonance (CMR) according to current recommendations (n = 2),

c) autism (n = 3),

d) consent withdrawal (n = 4).

The patients were enrolled to the study during standard annual check-up. The protocol of the study did not require additional patient visits or diagnostic tests, and it did not influence the management of patients.

CMR imaging procedure:

CMR scans were performed on a 1.5T scanner (Aera, Siemens, Erlangen, Germany) according to a standard protocol (below). Left ventricular dimensions, volumes) and ejection fraction (LVEF) in a short axis cine stack were measured with Siemens proprietary analysis package (SyngoVia, Siemens Healthcare, Erlangen, Germany). Volumes were indexed to the body surface area (BSA) and normalized (z-score) according to the reference data published by Kawel-Boehm [7]. Left ventricular ejection fraction (LVEF) was calculated based on a short axis cine series. The presence of late gadolinium enhancement (LGE), (seen as areas of bright signal within the nulled signal of the preserved myocardium and reflecting myocardial fibrosis), was confirmed on a per segment basis in a visual assessment (Figure S1) performed independently by two experienced readers. Any discrepancy was reassessed by both readers to reach consensus. Normal, remote myocardium was defined as a region without wall motion abnormalities and/or LGE. The LGE-positive segment count was considered a proxy of fibrosis extensiveness. To analyze the prevalence of the findings the patients were divided into the following age groups: 6 - 10, 10 - 13, 13 - 16, 16 - 18.

Standard CMR protocol:

1. scout images
2. segmented steady-state free precession cines:
    a. standard long axis
    b. short axis cine stack covering the entire left ventricle.

3. breath-hold segmented phase-sensitive inversion recovery sequence acquired in the same orientation as the cine images performed 8 - 15 min after intravenous injection of gadolinium-based contrast agent (gadobutrol 0,1 mmol/kg).

The following CMR parameters were measured: left ventricular end-systolic and end-diastolic volumes (LVESV and LVEDV, respectively), their derivatives indexed to the body surface area (LVESVi and LVEDVi, respectively) and normalized (z-score) according to the reference data. Values of z-score less than -2 and more than +2 (equivalent of 5th and 95th percentile respectively) were considered abnormal. Decreased LVEF was defined as less than 55%. The presence of LGE, i.e. areas of bright signal within the nulled signal of preserved myocardium reflecting areas of fibrosis, was confirmed on a per segment basis in a visual assessment (Figure S2).

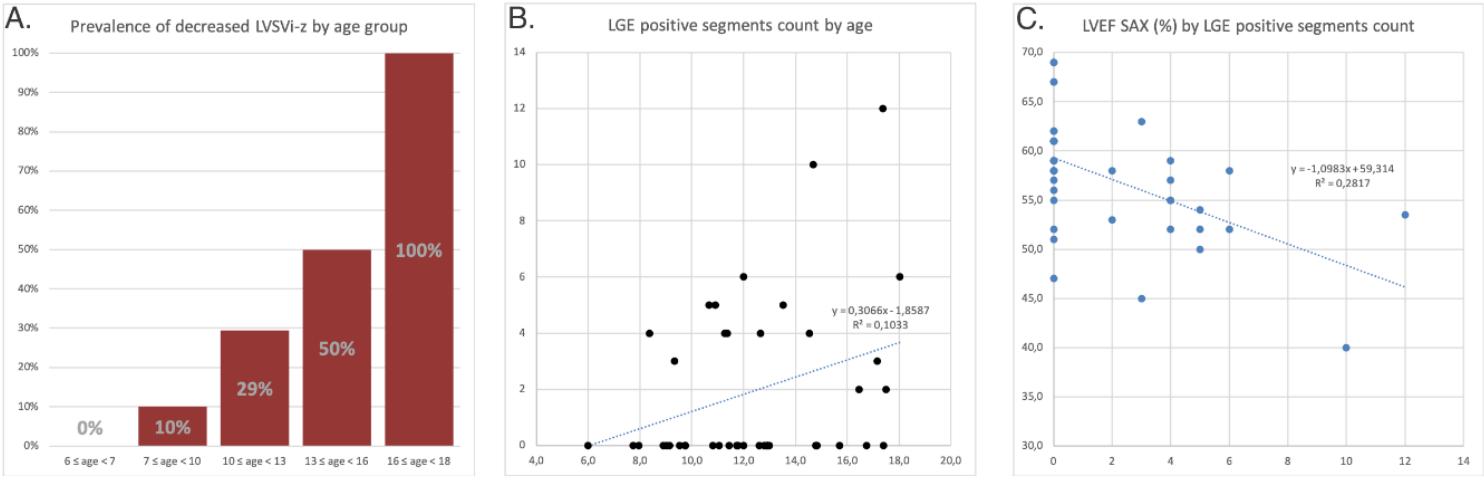

Figure S1. Chart A. represents the prevalence of decreased (z-score < -2.0) left ventricle stroke volume in different age groups. Plot B. depicts dependence of the number of affected segments with patient age and plot C. shows how the number of affected segments correlates with decrease of left ventricular ejection fraction.

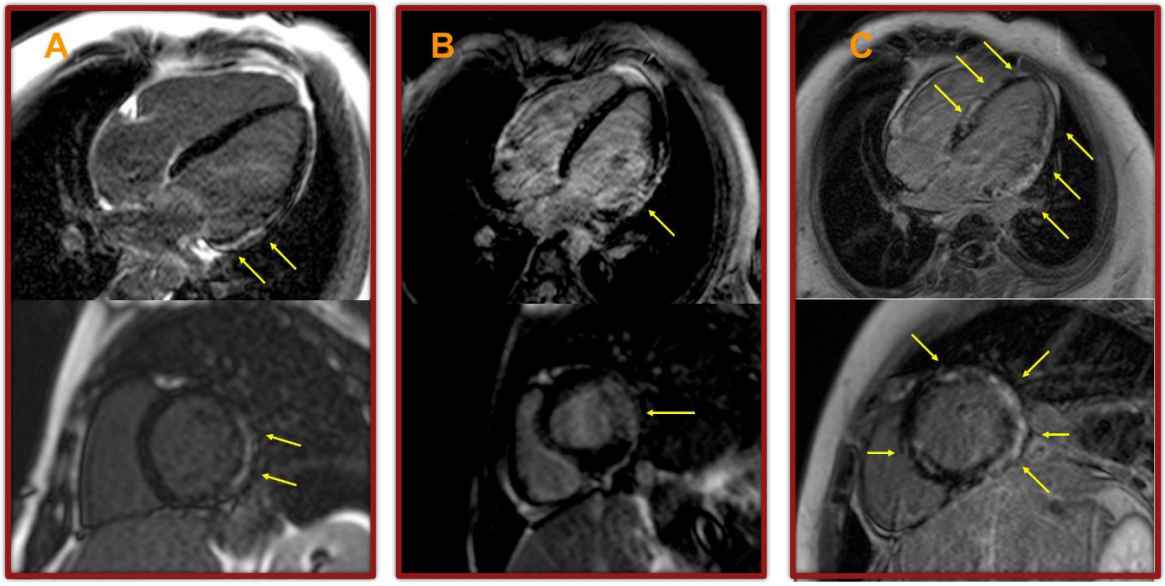

Figure S2. Examples of late gadolinium enhancement (LGE) extent and distribution in DMD/BMD patients. A. Minor, predominantly subepicardial LGE extending in infero-lateral LV segments, 10 yo DMD patient. B. Locally transmural LGE in mid-anterolateral and mid-inferolateral LV segments, 11 yo DMD patient. C. Extensive generalized subepicardial/transmural LGE in a 15 yo DMD patient. Magnitude images acquired with a phase-sensitive inversion recovery sequence on a 1,5T scanner (Siemens Aera, Erlangen, Germany). *LV - left ventricular.*

Parameter	Value (SD or range)
Age	12.0 (3.1)
BSA	1.3 (0.3)
LVds [cm]	2.7 (0.4)
LVdd [cm]	4.1 (2.7 – 5.4)
IVS [mm]	6.7 (5.0 – 10.0)
LVw [mm]	6.3 (5.0 – 10.0)
LVEF SAX [%]	58.0 (6.4)
LVEDV [ml]	81.9 (21.6)
LVESV [ml]	39.2 (18.0 – 87.7)
LVSV [ml]	48.0 (12.1)
LVEDVi [ml/m2]	63.6 (17.4)
LVESVi [ml/m2]	30.0 (9.0)
LVSVi [ml/m2]	37.0 (10.8)
LVEDVi [z]	-1.4 (1.5)
LVESVi [z]	0.3 (1.5)
LVSVi [z]	-1.9 (1.2)
LA [cm2]	13.0 (2.7)

Table S1. Sample characteristics and CMR results (LVds - Left Ventricle dimension in systole, LVdd - Left Ventricle dimension in diastole, IVS - Intraventricular Septum, LVw - Left Ventricle wall, LVEF SAX - Left Ventricle Ejection Fraction in Short Axis, LVEDV(i) - Left Ventricle End Diastolic Volume (indexed), LVESV(i) - Left Ventricle End Systolic Volume (indexed), LVSV(i) - Left Ventricle Stroke Volume (indexed), LA - Left Atrium

Parameter	**vs Age**	**vs Fibrosis**
LVEDVi[z]	r = 0.41 (p = 0.008)	no correlation (p = 0.305)
LVESVi[z]	no correlation (p = 0.162)	no correlation (p = 0.896)
LVSVi[z]	r = 0.50 (p < 0.001)	negative (p = 0.022)
LVEF SAX[%]	r = -0.36 (p = 0.363)	negative (p < 0.001)

Table S2. Correlations of CMR variables vs Age and presence of fibrosis (LGE)

Variable	**n (%)**
N	41 (100%)
Age group	
Age 6–10	11 (27%)
Age 10–13	17 (41%)
Age 13–16	6 (15%)
Age 16–18	6 (15%)
Diagnosis	
DMD	37 (90.2%)
BMD	4 (9.8%)
LVEDVi	
Low	10 (24%)
Normal	31 (76%)
LVESVi	
High	5 (12%)
Low	1 (2%)
Normal	35 (85%)
LVSVi	
Low	16 (39%)
Normal	25 (61%)
LGE presence	
Negative	24 (62%)
Positive	15 (38%)

Table S3. Study results by categories. Values of z-score less than -2 and more than +2 (equivalent of 5th and 95th percentile respectively) were considered abnormal.

Rank	n	%	Segment affected
1	15	38%	mid anterolateral
2	14	36%	basal anterolatera
3	12	31%	basal inferolateral
4	10	26%	mid inferolateral
5	7	18%	apical lateral
6	4	10%	mid inferoseptal
7	3	8%	apical septal
8	2	5%	basal inferoseptal
9	2	5%	basal inferior
10	2	5%	mid anteroseptal
11	2	5%	mid inferior
12	1	3%	basal anterior
13	1	3%	basal anteroseptal
14	1	3%	mid anterior
15	1	3%	apical anterior
16	1	3%	apical inferior
17	1	3%	apex

Table S4. Prevalence and rank by prevalence of segments affected by fibrosis based on positive LGE