



Compensatory Upregulation of Anti-Beta-Adrenergic Receptor Antibody Levels Might Prevent Heart Failure Presentation in Pediatric Myocarditis

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Background: Myocarditis can be associated with severe heart failure and is caused by different inflammatory and autoimmune responses. The aim of this study was to describe the immunological response in children with myocarditis by analyzing anti-beta-adrenergic receptor antibodies (anti- β -AR Abs).

Methods: Sera of children who were hospitalized with biopsy-proven myocarditis were prospectively collected between April 2017 and March 2019. Anti- β 1-AR Ab, anti- β 2-AR Ab, and anti- β 3-AR Ab were quantified by a CE-certified ELISA kit. According to normal values for immunoglobulin G (IgG), three age groups, <1, 1–5, and >5–17 years, were defined. Children without inflammatory cardiac pathology and no heart failure signs were served as a control group.

Results: We compared 22 patients with biopsy-proven myocarditis and 28 controls. The median age (interquartile range) of the myocarditis group (MYC) was 12.1 (2.7–16.4) years, 13 men, left ventricular ejection fraction (LVEF) 51% and for control group, the median age was 5.0 (3.0–6.8) years, nine men, LVEF 64%. Myocarditis patients in the age group >5–17 years showed significantly higher anti- β 3-AR Ab levels as compared to controls ($p = 0.014$). Lower anti- β 2-AR Ab and anti- β 3-AR Ab levels were significantly correlated with higher left ventricular diameters in myocarditis patients. The event-free survival using a combined endpoint (mechanical circulatory support [MCS], transplantation, and/or death) was significantly lower in myocarditis patients with antibody levels below the median as compared to myocarditis patients with antibody levels \geq the median.

Conclusion: Anti- β -AR Ab levels are increased in children with myocarditis and >5 years of age. These antibodies might be upregulated compensatory to prevent further cardiac deterioration. A worse event-free survival in patients with lower anti- β -AR Ab levels might be a therapeutic target for immunoglobulin substitution.

Keywords: anti-beta-adrenergic receptor antibodies, myocarditis, autoimmune, pediatric, endomyocardial biopsy

INTRODUCTION

Myocarditis is an inflammatory disease of the myocardium that is caused by various different triggers (1). In children, it is one major cause of the development of acute or chronic heart failure (2). One of the most striking findings in pediatric patients with myocarditis is the high prevalence of heart failure with severely reduced systolic function in patients under 1 year of age (3, 4). This specific cohort often experiences adverse events, such as the need for mechanical circulatory support (MCS) or heart transplantation (HTx) and death, whereas adolescents mostly present with preserved or mild impaired left ventricular ejection fraction (LVEF) and angina pectoris (4, 5). At the same time, studies on the therapeutic use of immunoglobulins have failed to show any positive effects in adults and adolescents but showed encouraging effects in young children (6). Both findings might be related to the fact that the immunological activities, such as immunoglobulin production, only reach a mature state at the age of >5 years (7). Focusing on anti-beta-adrenergic receptor antibodies (anti- β -AR Ab), levels have been described as increased in patients with myocarditis and dilated cardiomyopathy (DCM) and are associated with heart failure and arrhythmic events (8–10). In children with DCM and poor clinical outcomes, high anti- β 1-AR Ab levels were described (11). However, a multicenter study in children with myocarditis could not verify this for anti- β 1- and anti- β 2-AR Ab (12). In adults with an ST-elevation myocardial infarct (STEMI), lowered anti- β 1-AR Ab levels led to higher rates of re-infarction and cardiovascular death pointing toward a possible protective effect of these antibodies (13). Referring to these divergent results, the impact of anti- β -AR Ab remains not fully understood, especially in children.

The aim of this study was to analyze anti- β -AR Ab levels in pediatric patients with biopsy-proven myocarditis and pediatric controls to gain knowledge on their distribution in the different age groups and their impact on the outcome.

Abbreviations: Anti- β -AR Ab, anti-beta-adrenergic receptor antibodies; ASD, atrial septal defect; CRP, C-reactive protein; CTRL, control group; DCM, dilated cardiomyopathy; DNA, Deoxyribonucleic acid; ELISA, enzyme-linked immunosorbent assay; EMB, endomyocardial biopsy; HTx, heart transplantation; IgG, immunoglobulin G; LVEF, left ventricular ejection fraction; LVIDd, left ventricular internal diastolic diameter; MCS, mechanical circulatory support; MYC, myocarditis group; NT-proBNP, N-terminal-pro brain natriuretic peptide; PDA, persistent ductus arteriosus; STEMI, ST-elevation myocardial infarct; RNA, Ribonucleic acid; (RT)-PCR, (real-time)-polymerase chain reaction.

MATERIALS AND METHODS

Patient Data and Follow-Up

Sera from patients under 18 years of age with suspected myocarditis and enrollment within the MYKKE registry between April 2017 and March 2019 were collected at the Pediatric Cardiology Departments of the German Heart Center Berlin and the Charité - Universitätsmedizin Berlin, Berlin, Germany. The study was approved by the institutional ethics committee (Charité - Universitätsmedizin Berlin, EA2/131/10, EA2/074/13). All parents or guardians of patients <18 years gave written informed consent.

Serum was collected at the time of admission, centrifuged at 20°C and 3,800 g for 10 min, and frozen at -80°C. Clinical parameters, diagnostic cardiac imaging, and endomyocardial biopsy (EMB) were assessed routinely. Initial clinical and follow-up data were entered into the online MYKKE study database (4). Patients without EMB or not proven myocarditis in EMB were excluded from further analyses.

Patients with proof of myocarditis in EMB were called the myocarditis group (MYC). Patients under 18 years of age, administered for elective cardiac catheterization, and without inflammatory cardiac pathology served as a control group (CTRL). See the study flow chart for further details (**Figure 1**).

Regarding outcomes, the occurrence of adverse events, such as MCS, HTx, and/or all-cause death, was defined as a combined endpoint.

Detection of Anti- β -Adrenergic Antibodies

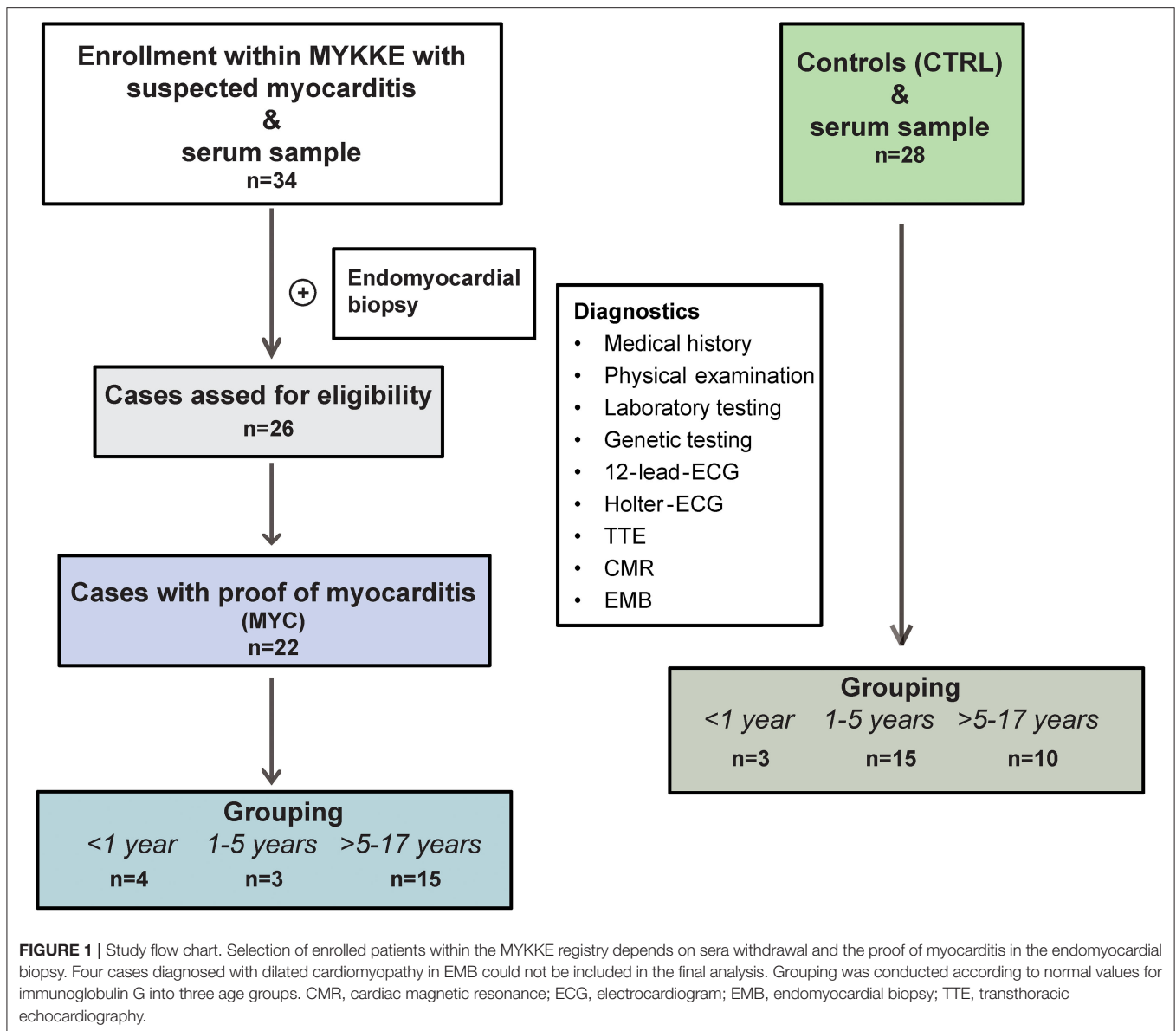
Anti- β 1-AR Ab, anti- β 2-AR Ab, and anti- β 3-AR Ab were measured with commercially available enzyme-linked immunosorbent assays (ELISA; CellTrend GmbH, Luckenwalde, Germany) according to the instructions of the manufacturer (14). All these assays provided native receptors presented in their physiological membrane environment as immunogenic targets of immunoglobulin G (IgG) binding.

Analysis of Endomyocardial Biopsies

All EMB specimens were analyzed histopathologically and immunohistologically and by polymerase chain reaction [(RT)-PCR] for myocardial detection of viral RNA/DNA by one specialized center for Cardiopathology (Institute for Pathology and Neuropathology, University Hospital Tübingen, Tübingen, Germany) as previously described (15).

The diagnosis of myocarditis was confirmed according to the established criteria and grouped in accordance with the WHO definition in (16, 17):

(a) Acute myocarditis: Infiltrate of ≥ 14 leucocytes/mm² and presence of myocyte damage.



(b) Healing/chronic myocarditis: Infiltrate of ≥ 14 leucocytes/mm² and absence of myocyte damage but the presence of fibrosis.

(c) Healed myocarditis: Multifocal fibrosis or scarring without inflammation (0–3 leucocytes/mm²).

Statistical Analysis

Categorical variables were summarized by frequencies and percentages. For continuous measures, data were presented as median values with interquartile range (IQR). Pearson's chi-square test and Fisher's exact test were used to compare dichotomous variables. For comparison of independent groups, the Mann-Whitney U and Kruskal-Wallis test were applied. For correlation of antibody levels and laboratory and clinical parameters, Spearman's rho test was used. Kaplan-Meier curves and log-rank tests were employed for survival analysis. Therefore,

the groups $<$ median and \geq median were built according to the median anti- β -AR Ab levels of the MYC. A probability value of <0.05 was considered statistically significant. Data were analyzed using IBM Corp. SPSS Version 24.0 (Armonk, NY, USA).

RESULTS

Basic Characteristics

We enrolled 22 patients with biopsy-proven myocarditis and median age (IQR) of 12.1 (2.7–16.4) years, 13 were men (MYC). Twenty-eight patients served as controls with a median age of 5.0 (3.0–6.8) years, 9 were men (CTRL).

The control group was administered due to the following diagnoses: atrial septal defect (ASD; $n = 19$), persistent ductus arteriosus (PDA; $n = 6$), a combination of ASD and PDA ($n = 1$), aortic isthmus stenosis ($n = 1$), and mild pulmonary

TABLE 1 | Basic characteristics of the myocarditis (MYC) and control group (CTRL).

	MYC group <i>n</i> = 22	CTRL group <i>n</i> = 28	<i>p</i> -Value
Gender, male	13 (59)	9 (32)	0.086
Age (years)	12.1 (2.7–16.4)	5.0 (3.0–6.8)	0.042
BSA (kg/m ²)	1.6 (0.6–1.8)	0.8 (0.6–1.2)	0.063
Echocardiography			
Z-score LVIDd (mm)	1.9 (0.1–5.3)	−0.8 (−1.6–0.4)	<0.001
LVEF (%)	51.0 (28.0–60.0)	63.5 (57.8–72.3)	<0.001
Antibody levels			
Anti-β1-AR Ab (U/ml)	7.7 (3.8–24.3)	5.0 (3.7–8.2)	0.125
Anti-β2-AR Ab (U/ml)	6.0 (2.4–19.9)	3.6 (2.6–4.7)	0.077
Anti-β3-AR Ab (U/ml)	6.4 (2.9–18.8)	3.5 (3.0–6.8)	0.035

Values are given as *n* (%) or median (interquartile range).

Anti-β-AR Ab, anti-beta-adrenergic antibodies; BSA, body surface area; LVEF, left ventricular ejection fraction; LVIDd, left ventricular internal diastolic diameter. Bold values represents significant values.

valve stenosis (*n* = 1). The MYC had significantly lower LVEF in echocardiography and higher Z-scores of the left ventricular internal diastolic diameter (LVIDd; *p* < 0.001, respectively). Detailed basic characteristics are given **Table 1**.

Basic Characteristics of MYC Patients and Controls Within Age Groups

According to known values for overall IgG and its age-depending distribution in childhood, three age subgroups (<1 year, 1–5 years, and >5–17 years) were defined for both groups (MYC and CTRL, see **Table 2**) (7).

Four patients with myocarditis and three in the control group belonged to the age group < 1 year. The MYC group was presented with a severely reduced LVEF and dilated left ventricles. They were presented with signs of heart failure and a median (IQR) value of N-terminal-pro brain natriuretic peptide (NT-proBNP) of 133,389 (103,375–177,557) ng/l. Troponin T_{hs} was increased with 1,425 ng/l. No pathologies in the blood count or C-reactive protein (CRP) increment were detected. Two had the diagnosis of healing/chronic myocarditis and one of acute myocarditis in EMB. The control group (*n* = 3) was presented with normal LVEF and had no dilated ventricles.

The age group of 1–5 years consisted of 3 MYC patients and 15 controls. Again, patients with MYC showed signs of heart failure with severely reduced LVEF and left ventricular dilatation. The median NT-proBNP was 35,000 ng/l, Troponin T_{hs} was 85 ng/l. Leucocytes, thrombocytes, and hemoglobin were in a normal range. The CRP was slightly increased at 17.0 mg/dl. Two patients with MYC were diagnosed with acute myocarditis in EMB, one with a chronic/healing myocarditis. The controls in the age groups 1–5 years had a normal LVIDd and LVEF.

Fifteen patients with myocarditis and a median age of 15.6 (11.9–16.9) years belonged to the age group >5–17 years, 12 were men. All had biopsy-proven lymphocytic myocarditis: 12 reported chronic/healing myocarditis, 2 with acute, and 1 with healed myocarditis (see **Table 2**). They presented with a median

Z-score of the LVIDd of 0.5 (−0.4 to 2.4) and an LVEF of 57.0 (50.0–61.0)% (18). NT-proBNP was increased at 577.0 (175.8–1885.8) ng/l and Troponin T_{hs} with 672.0 (523.5–1427.0) ng/l. Leucocytes, thrombocytes, and hemoglobin were in a normal range, whereas CRP was increased with 37.6 (1.4–137.0) mg/l. Ten patients served as a control group with a median age of 10.5 (6.0–17.0) years, 5 were men.

The myocarditis patients in the age group >5–7 years did not differ significantly in LVEF and Z-scores of the LVIDd as compared to controls (see **Table 2**; *p* = 0.071, respectively).

Further, MYC patients with Anti-β3-AR Ab level ≥10 U/ml had a significantly lower Z-Score of LVIDd than MYC patients with Anti-β3-AR Ab <10 U/ml [−0.1 (−1.4 to 0.5) vs. 2.3 (0.2–3.5); *p* = 0.036]. There was no significant difference in age, body surface area, sex, LVEF, NT-proBNP, the combined endpoint, or the diagnosis in the EMB.

Antibody Levels Within Age Groups

The comparison of anti-β-AR Ab levels between myocarditis patients and controls within the first two age groups (<1 year and 1–5 years) did not differ significantly. Patients >5 years of age showed significantly higher anti-β3-AR Ab levels as compared to controls (*p* = 0.014; see **Table 2**). The distribution of the different anti-β-AR Ab levels over age are shown in **Figure 2**.

Correlations of Antibody Levels and Clinical Parameters

Lower anti-β2-AR Ab and anti-β3-AR Ab levels were significantly correlated with increased Z-score of the LVIDd in patients with MYC across all ages (β2: *p* = 0.029; β3: *p* = 0.045, see **Figure 3**). An inverse correlation was detected between anti-β2-AR Ab levels and NT-proBNP (*p* = 0.034, see **Figure 3**). No significant correlations between anti-β-AR Ab levels and other inflammatory laboratory parameters were found in the myocarditis patients.

Impact of Antibody Levels on the Combined Endpoint

Seven out of 22 myocarditis patients reached the combined endpoint of MCS, HTx, or death. All seven patients needed MCS, three were transplanted. No patient had died. Four patients were belonged to the age group <1 year, two to the age group 1–5 years, and one was in the age group >5–17 years.

Five out of 11 patients with anti-β-AR Ab levels under the median reached the combined endpoint. Patients with antibody levels ≥ median experienced less adverse events, only 2 out of 11 patients (*p* = 0.361). The event-free survival using the combined endpoint of MCS, HTx, or death was significantly lower in MYC patients with anti-β-AR Ab levels below the median as compared to myocarditis patients with anti-β-AR Ab levels ≥ median (anti-β1/2/3-AR Ab: *p* = 0.049; **Figure 4**).

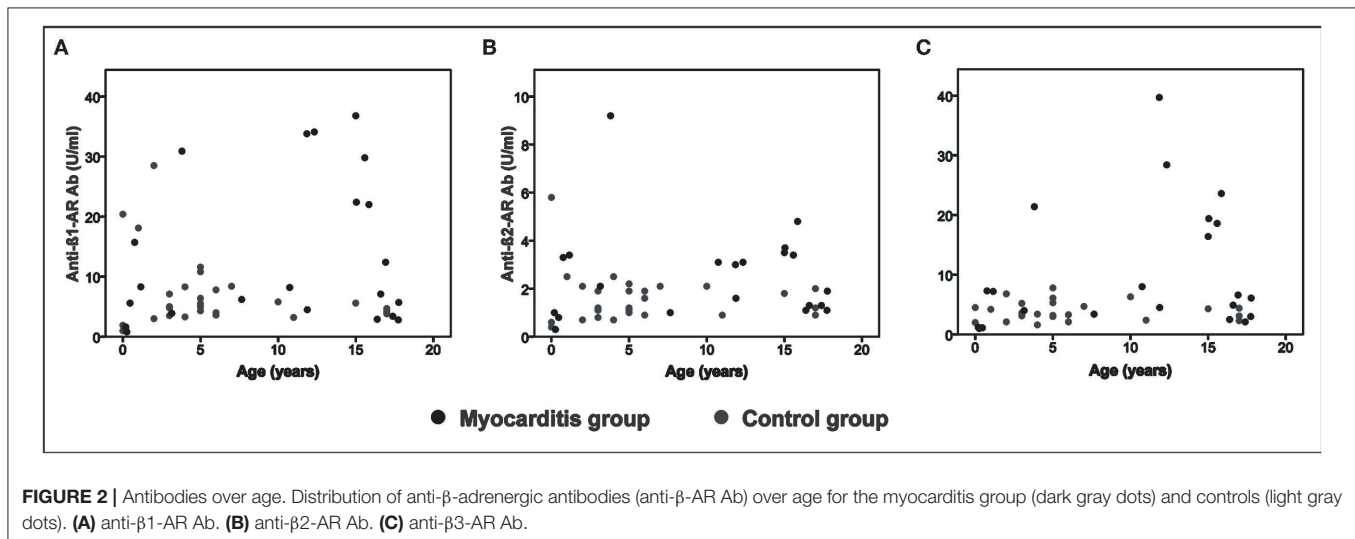
DISCUSSION

In this study, we investigated the anti-β-AR Ab levels in pediatric patients with biopsy-proven myocarditis according to different age groups and related outcomes.

TABLE 2 | Anti-beta-adrenergic antibody levels and echocardiographic parameters of controls (CTRL) and myocarditis (MYC) patients in different age groups.

	Age group <1 year			Age group 1–5 years			Age group >5–17 years		
	MYC group n = 4	CTRL group n = 3	p-value	MYC group n = 3	CTRL group n = 15	p-value	MYC group n = 15	CTRL group n = 10	p-value
Gender, male	0 (0)	1 (33)	0.429	3 (100)	5 (33)	0.069	12 (71)	5 (28)	0.111
Age (years)	0.4 (0.2–0.7)	0.0*	0.057	3.2*	4.0 (3.0–5.0)	0.426	15.6 (11.9–16.9)	10.5 (6.0–17.0)	0.015
Echocardiography									
Z-score LVIDD (mm)	6.0 (1.5–7.9)	−1.0*	0.057	6.2*	−0.7 (−1.6–0.3)	0.005	0.5 (−0.4–2.4)	−1.1 (−3.1–0.9)	0.071
LVEF (%)	25.5 (14.3–36.8)	72.0*	0.057	22.0*	65.0 (57.5–73.0)	0.004	57.0 (50.0–61.0)	59.5 (57.0–68.5)	0.071
Antibody levels									
Anti-β1-AR Ab (U/ml)	3.6 (1.0–13.2)	1.9*	0.857	8.3*	5.5 (4.3–10.8)	0.498	8.2 (4.5–29.8)	4.5 (3.8–6.3)	0.071
Anti-β2-AR Ab (U/ml)	1.7 (0.7–6.1)	2.7*	0.629	6.6*	3.6 (2.6–5.5)	0.130	6.2 (2.9–22.7)	3.8 (2.6–4.5)	0.080
Anti-β3-AR Ab (U/ml)	1.2 (1.0–5.8)	4.5*	0.400	7.2*	3.5 (3.0–5.3)	0.076	6.6 (3.4–19.4)	3.3 (2.4–4.5)	0.014
EMB									
Acute myocarditis	1 (25)	n.a.		2 (67)	n.a.		2 (13)	n.a.	
Chronic/healing myocarditis	3 (75)	n.a.		1 (33)	n.a.		12 (80)	n.a.	
Healed myocarditis	0 (0)	n.a.		0 (0)	n.a.		1 (7)	n.a.	

EMB, endomyocardial biopsy; MYC, patients with biopsy proven inflammatory myocardial disease; CTRL, patients without inflammatory myocardial disease. Values are given as n (%) or median (interquartile range). *Only median. Anti-β-AR Ab, anti-beta-adrenergic antibodies; BSA, body surface area; LVEF, left ventricular ejection fraction; LVIDD, left ventricular internal diastolic diameter. Bold values represents significant values. n.a.; not applicable.

**FIGURE 2** | Antibodies over age. Distribution of anti-β-adrenergic antibodies (anti-β-AR Ab) over age for the myocarditis group (dark gray dots) and controls (light gray dots). (A) anti-β1-AR Ab. (B) anti-β2-AR Ab. (C) anti-β3-AR Ab.

We could detect anti-β-AR Ab in all pediatric myocarditis patients and controls, which underlines their pre-existence also in healthy or in patients without heart failure (19, 20). In the age group >5 years, anti-β3-AR Ab levels were significantly increased in patients with MYC as compared to CTRL. Throughout all age groups, anti-β-AR Ab levels were consistently higher in patients with MYC as compared to CTRL, supporting the thesis of antibody increment in heart failure (21). However, the comparisons did not reach statistical significance in most age groups, this may be due to the small sample sizes. These results are in conflict with the ones of Simpson et al., where they did not find increased anti-β-AR Ab levels in children with myocarditis. An explanation could be that they did not group their cohort according to age, which might have resulted in a non-significant difference in the whole cohort as compared to controls (12).

A possible mechanism in older myocarditis patients with higher anti-β-AR Ab levels might be an innate Ab upregulation with an agonistic effect on the beta-adrenergic receptors in order to overcome cardiac dysfunction triggered by the myocardial inflammation. The initial upregulation might represent a normal immunological response rather than an autoimmunity process resulting in lower receptor expression and receptor desensitization and finally chronic heart failure (22–24). This could be supported by the fact that after ventricular assist implantation, anti-β1-AR Abs were undetectable in patients with DCM and increased anti-β1-AR Ab before implantation (25).

Our data cannot absolutely support the thesis of a protective effect of these antibodies described in other studies, but we found higher levels in the myocarditis age group of >5–17 years, which presented with less severe adverse events and better LVEF as compared to the other MYC age groups (19, 26). On the other

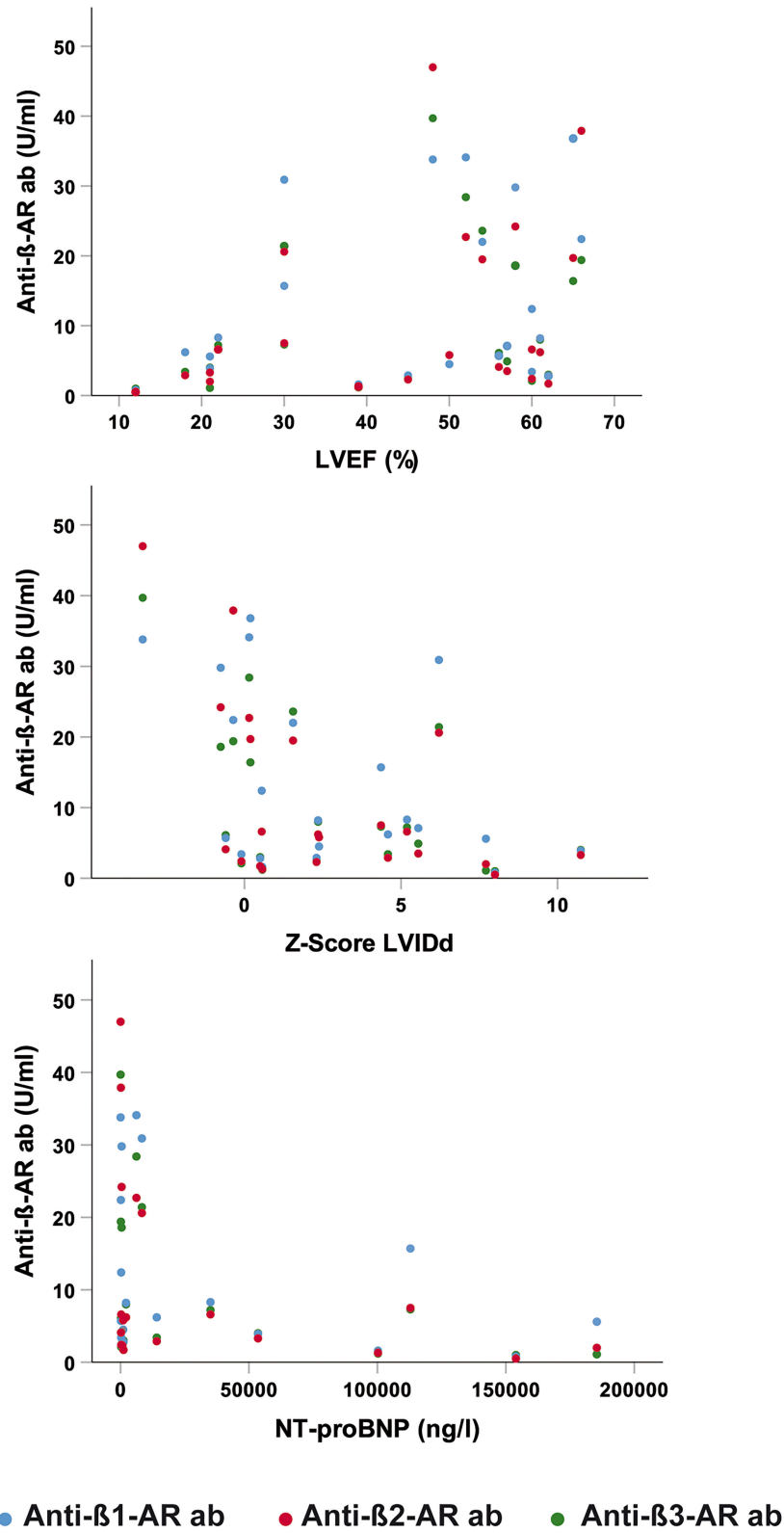
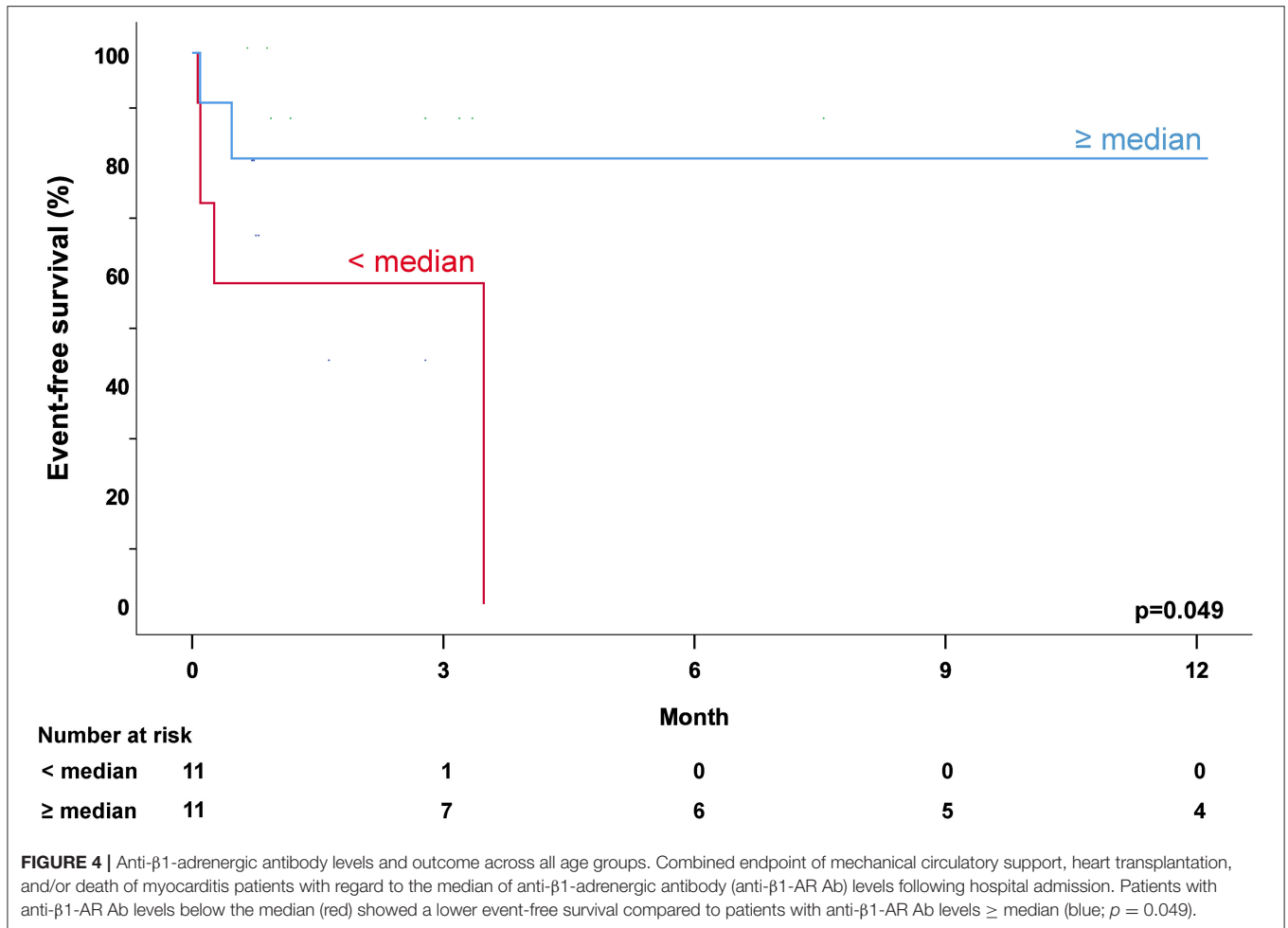


FIGURE 3 | Correlation of anti-β-AR ab levels and clinical parameters. (Upper) Correlation of anti-β-AR ab levels and left ventricular ejection fraction (LVEF). (Middle) Correlation of anti-β-AR ab levels and Z-score of the left ventricular internal diastolic diameter (LVIDd). (Lower) Correlation of anti-β-AR ab levels and N-terminal-pro brain natriuretic peptide (NT-proBNP). Blue dots: anti-β1-AR ab. Red dots: anti-β2-AR ab. Green dots: anti-β3-AR ab.



end, the lowest antibody levels and worst disease courses with severely reduced left ventricular function were seen in patients <1 year of age. In this age group, the absence of a specific anti-β-AR Ab upregulation might be explained by inherently low overall IgG levels at this age. Therapeutic substitution of immunoglobulins might help to promote an immune response that resembles that of subjects with mature immune systems when applied early in this age group (2).

CONCLUSION

There are age-dependent different anti-β-AR Ab levels in children with biopsy-proven myocarditis and controls. In myocarditis patients >5 years of age, anti-β3-AR Ab levels are significantly increased as compared to controls which might rather be compensatory to trigger a receptor agonistic effect than primarily upregulated as they do not present with severe heart failure. Especially children <5 years with lower anti-β-AR Ab levels experience more often adverse events, which might be a missing compensatory effect due to lower innate IgG levels and a potential therapeutic target for immunoglobulin substitution.

Limitations

We analyzed, especially in the young age groups, a small number of patients and controls group. The size of these groups does not allow for drawing conclusions on the effects of anti-β-AR Ab on their outcome. Moreover, the children with myocarditis in the age group >5–17 years were older than the controls in this group. Additionally, even there was no myocardial inflammation or sign of heart failure, all controls had a simple isolated congenital heart defect, which could also have an influence on anti-β-AR Ab levels.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Ethics Committee Charité - Universitätsmedizin Berlin (EA2/131/10, EA2/074/13). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

FS, DM, and SS put down the concept and designed the study. FS analyzed the datasets, did the statistical analysis, wrote the initial draft, and finalized the article. CS helped design the study, analyze the datasets, and reviewed the manuscript draft. HH helped design the study, performed the measurements, analyze the dataset, and reviewed the manuscript draft. BO-R enrolled patients and reviewed the manuscript draft. TP was responsible for the ethics approval and biobanking. KK analyzed the biopsies. KK, FB, DM, and SS have reviewed, critically revised the initial, and final drafts of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: HH is CEO and employed by CellTrend GmbH.

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