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HIGHLIGHTS IN PEDIATRIC CARDIOLOGY: 2021

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MINI REVIEW
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Potential and Limitations of Atrial Natriuretic Peptide as Biomarker in Pediatric Heart Failure—A Comparative Review

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Although B-type Natriuretic Peptide (BNP), N-terminal-proBNP (NT-proBNP), and mid-regional-proANP (MR-proANP) are included in current guidelines on heart failure in adults, no guideline considering these biomarkers in pediatric heart failure is available. A new drug class of neprilysin inhibitors as fixed-dose combination (Sacubitril/valsartan) has been introduced and is currently being investigated in children suffering from heart failure. Atrial Natriuretic Peptide (ANP) is discussed as a more useful alternative to BNP because it may grants better insights into the effects of this treatment. Thus, this review aimed to provide an overview of the current knowledge concerning ANP in pediatric heart failure and compares its suitability regarding diagnosis and prognosis of heart failure. A literature search using PubMed resulted in 147 publications of which 22 studies were classified as relevant. The review presents available ANP, NT-proANP, and MR-proANP level data in children (0–18 years). Summarizing, ANP shows only minor differences as marker for diagnosing and monitoring pediatric heart failure if compared to BNP. Due to its fast release, ANP offers the advantage of displaying rapid changes during therapy or operation. ANP is -like the other natriuretic peptides- influenced by age, presenting with the highest levels in very young infants. ANP also correlates with atrial pressure and volume overload in children. In addition, ANP determination in saliva appears to be a promising alternative to blood sampling. Similarly to NT-proBNP, NT-proANP, and MR-proANP offer better stability but only few data has been published in children and thus their potential is only presumable so far.

Keywords: pediatric, natriuretic peptide, heart failure, ANP, BNP, sacubitril, preanalytical, saliva

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INTRODUCTION

Heart failure has been defined as a clinical and pathophysiologic syndrome that results from ventricular dysfunction, volume or pressure overload or a combination of these causes. The inability of the heart to supply the body sufficiently with oxygen results in an activation of the Renin-Angiotensin-Aldosterone-System and the sympathetic nervous system, leading to an upregulation of blood flow. Important counterregulatory hormones are the natriuretic peptides, among which Atrial Natriuretic Peptide (ANP) and B-type Natriuretic Peptide (BNP) play a major role. Both peptides mediate vasodilation, natriuresis, diuresis, and block renin, resulting in a down-regulation of the Renin-Angiotensin-Aldosterone-System (1, 2).

The guidelines published by the European Society of Cardiology (2016) recommend the clinical biomarkers BNP, N-terminal-proBNP (NT-proBNP), and mid-regional-proANP (MR-proANP) for diagnosis and prognosis of heart failure (3). Whereas, these natriuretic peptides are included in guidelines for adults, there is no guide considering BNP, NT-proBNP, and MR-proANP in pediatric heart failure. Different etiology in adults and children, the effect of ontogeny on clinical course and outcome are some of the reasons why recommendations given in the adult guidelines cannot be simply transferred to pediatrics (4).

The recently introduced drug class of neprilysin inhibitors to patients suffering from heart failure raised the question whether ANP is the better alternative compared to BNP to adequately reflect effects during therapy with sacubitril/valsartan (Entresto®) (5). ANP is more susceptible to degradation by neprilysin than BNP. Changes effected by this treatment may be more obvious than the observed variation of BNP and NT-proBNP, which was within the weekly fluctuation (6). Currently sacubitril/valsartan is being investigated in children suffering from heart failure within the PANORAMA-HF study (7).

This review provides an overview outlining the current knowledge on pediatric ANP levels focusing on its usefulness in diagnosis, monitoring and different etiology of heart failure. Moreover, ANP is compared to the standard clinical biomarker BNP/NT-proBNP. Since for the latter much more data is available and detailed reviews already exist, it will only be comparatively referred to. Finally, analytical issues concerning general measurement of ANP as well as difficulties occurring in the context of pediatric blood sampling will be discussed.

ANP IN PEDIATRIC HEART FAILURE

A literature search using PubMed resulted in 147 studies of which 22 were classified as relevant and included in this review (**Figure 1**). Publications had to meet the following inclusion criteria: original research papers regarding pediatric population with congenital heart defect and/or heart failure and measurement of ANP, NT-proANP, or MR-proANP in blood or urine. Those criteria were fulfilled by 19 publications which reported pediatric ANP levels and are presented in **Tables 1**, **2**. Another 3 studies determined MR-proANP or NT-proANP. All of them mainly evaluated the association between elevated ANP levels and diagnosis, severity, or etiology of heart failure as well as correlation with hemodynamic parameters, age or medical/surgical intervention.

Heart failure in children has a different etiology compared to adults. While in adulthood coronary artery diseases and hypertension are usually causative, it is cardiomyopathies (CMP) and congenital heart defects (CHD) in infants. While CMP leads to low output cardiac failure, CHD results in high output cardiac failure. Less common and more present in developing countries is heart failure originating from rheumatic heart disease (RHD) (4). It is possible that depending on the location of the cardiac malformation, differences in the diagnostic performance of natriuretic peptides arise (15).

Abbreviations: ANP, Atrial/A-type Natriuretic Peptide; AS, aortic stenosis; ASD, atrial septal defect; AVSD, atrioventricular septal defect; BNP, Brain/B-type Natriuretic Peptide; CHD, congenital heart defect; CMP, cardiomyopathy; CoA, Coarctation of the aorta; DCM, dilated cardiomyopathy; EDTA, ethylenediaminetetraacetic acid; MR-proANP, mid-region Atrial/A-type Natriuretic Peptide; MS, mitral stenosis; NT-proANP, N-terminal Atrial/A-Type Natriuretic Peptide; NT-proBNP, N-terminal Brain/B-type Natriuretic Peptide; NYHA, New York Heart Association; PDA, patent ductus arteriosus; PS, pulmonary stenosis; RHD, rheumatic heart disease; TA, tricuspid atresia; TOF, tetralogy of Fallot; VSD, ventricular septal defect.

ANP as a Diagnostic Marker in Pediatric Heart Failure

In heart failure, ANP secretion is extended from atria to ventricles. ANP can reflect changes faster since it is stored as a prohormone, whereas BNP has to be genetically translated before release (22, 27–29). ANP can indicate acute volume overload and hemodynamic changes, while BNP better reflects prolonged overload and shows increased stability (30). Nevertheless, ANP and BNP significantly correlate with each other in adults as well as in children, with ANP presenting higher levels than BNP (11, 15, 18).

Identifying Children With Heart Failure

Concerning the diagnosis of heart failure, ANP levels are significantly increased in diseased children compared to healthy controls (15, 26, 31). In children with dilated cardiomyopathy (DCM), CHD or RHD, ANP can differentiate symptomatic (heart failure) and asymptomatic children (e.g., mean 232.5 pg/mL vs. 48.4 pg/mL) (8–15).

In asymptomatic infants with CHD, levels of ANP compared to controls are elevated, but not necessarily significantly (e.g., 48.4 pg/mL vs. 24.1 pg/mL) (8, 13, 18). In children with ventricular septal defect (VSD), ANP, and BNP appeared to be comparable markers to identify children suffering from VSD. ANP displayed higher sensitivity, but lower specificity in identifying those with significant shunt or pulmonary hypertension (11). For further details, please see **Table 1.1**.

In the case of NT-proANP, Holmstrom et al. reported that CHD patients had significantly elevated serum levels (median 904 pmol/L vs. median 384 pmol/L) (32). In 2000, the same authors published higher level data, speaking of a 10-fold increase in heart failure patients (mean 4515 pmol/L) in comparison to controls or other diseases (urologic or malignant) (33). Samples were also measured with an in-house immunoassay but plasma instead of serum was used.

Similarly to ANP and NT-proANP, the marker MR-proANP can be utilized to diagnose heart failure in children with CHD and CMP (34). Median serum MR-proANP in healthy children was 40 pmol/L. In CHD patients levels increased up to 72.8 pmol/L and in CMP up to 76.2 pmol/L.

In comparison to NT-proBNP, and in contrast to adults, MR-proANP was slightly inferior regarding diagnostic accuracy (34, 35).

Altogether, ANP, NT-proANP, and MR-proANP can like BNP be used to separate children with heart failure from those without. Regarding asymptomatic pediatric CHD, ANP is equally to BNP not necessarily significantly elevated compared to healthy

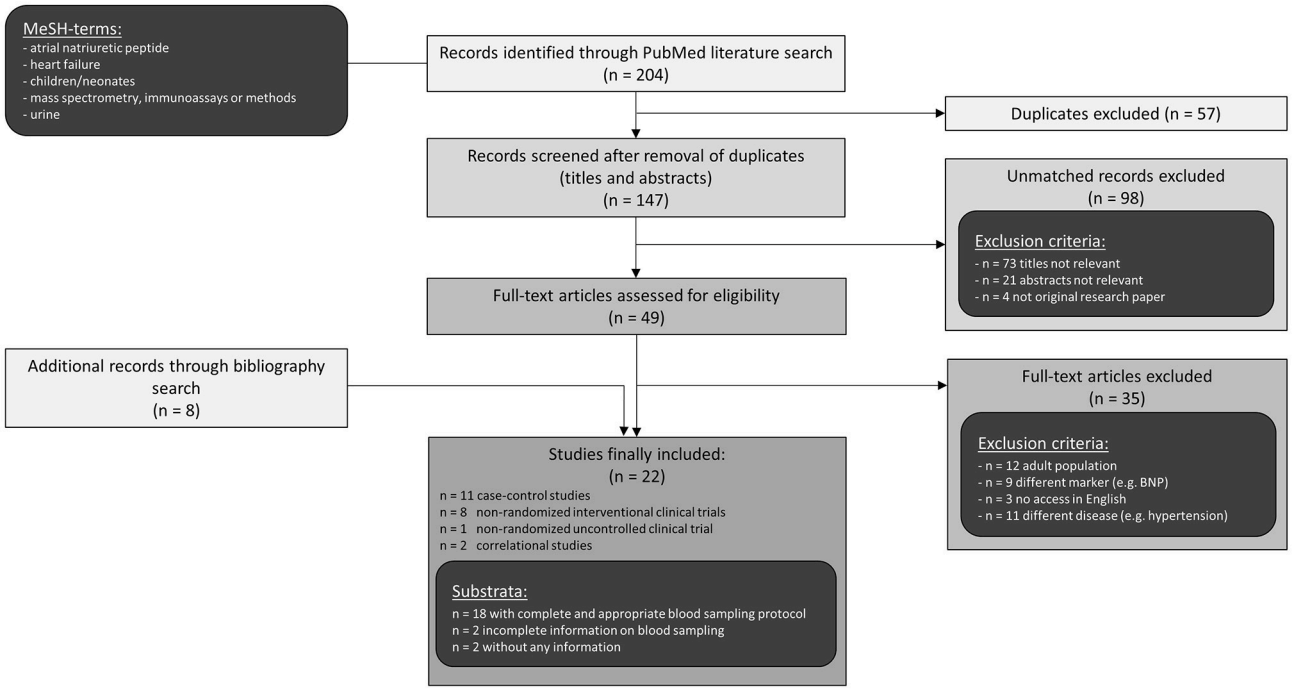

FIGURE 1 | Literature search strategy.

controls (36). Only 3 studies concerning NT-proANP/MR-proANP measurement in pediatric heart failure have been conducted, consequently further research needs to clarify their relevance and possible benefits in children.

Classification of Pediatric Heart Failure Severity

Levels of ANP cannot only serve for the diagnosis of heart failure but further correlate with the severity of heart failure. They increase stepwise with the New York Heart Association (NYHA) stage, irrespective of the underlying cause (**Table 1.2**, e.g., mean: NYHA I: 45 pg/mL, II: 77 pg/mL, III: 125 pg/mL) (17, 18). The same applies to NT-proANP which correlates positively with the severity of heart failure (32). There is no data for MR-proANP.

Thus, ANP and NT-proANP discriminate equally to BNP/NT-proBNP between different stages of heart failure severity (37–40).

Discrimination of Cardiac Load in Pediatrics

ANP is released in response to cardiac wall stretch from the atrial tissue. Consequently, in CHD, ANP correlates with right and left atrial pressure, mean pulmonary arterial pressure and pulmonary resistance (8, 22, 26, 41). Hayabuchi et al. observed a correlation between ANP and right ventricular pressure, but only BNP correlated significantly with right ventricular volume (16).

When comparing elevated ANP levels resulting from different etiologies of heart failure, the highest were observed in systolic ventricular dysfunction (median: 431 pg/mL) followed by volume overload with preserved systolic dysfunction (median: 93.0 pg/mL). Pressure overload showed the lowest increment (median: 51.9 pg/mL), whereas the group without overload (median: 28.6 pg/mL) did not differ from controls (median: 32.9 pg/mL) (9, 15). Also, significantly higher ANP and BNP levels could be observed in left ventricular volume overload (median: 164 pg/mL) compared to right ventricular volume overload (median: 57.2 pg/mL) or pressure overload (median left: 40.8 pg/mL, right: 69.3 pg/mL) (10). Kotby et al. confirmed that in left ventricular volume overload resulting from different etiologies, levels of ANP are significantly elevated compared to controls (17). Detailed levels are displayed in **Table 1.4**.

ANP can discriminate cardiac loading conditions equally to BNP showing the same tendencies in the different conditions (42). However, BNP might be superior to ANP in the right ventricular system since only BNP has been shown to significantly correlate with right ventricular volume so far.

Differentiation of Underlying Etiology

As previously mentioned, etiological factors attributable to pediatric heart failure have been identified as DCM, RHD and CHD.

Pediatric patients with DCM present with the highest levels of ANP and MR-proANP—fully in line with the BNP data (**Table 1.3d**) (9, 15, 17, 34, 43). The hypothesis of a resistance to natriuretic peptide effects in patients with DCM has been proposed, additionally to an increased ANP synthesis and secretion, which accounts for the highest observed levels.

In RHD, ANP levels are significantly higher than in controls. The values are in the range of other etiologies like DCM and CHD (**Table 1.3e**) (17).

Most studies have focused on CHDs. Those can be categorized into cyanotic [e.g., tetralogy of Fallot (TOF), tricuspid atresia

TABLE 1 | Overview of ANP levels in pediatric heart failure sorted in order of increasing age of diseased children in each section.

Children	Age	n	ANP in heart failure (pg/mL)	ANP in controls (pg/mL)	Statistical operator	Blood sampling procedure		References
1. DIFFERENTIATION OF SYMPTOMATIC (S) AND ASYMPTOMATIC (A) CONGENITAL HEART DISEASE								
Diseased	13.7 ± 14.1 mo	11	S: 232.5 ± 82.9*		mean ± SD	20 min supine Cubital or jugular vein Plasma	I	Agnoletti et al. (8)
	76.1 ± 49.4 mo	11	A: 48.4 ± 29.4$^{n/p}$					
Control	Age-matched	30		24.1 ± 19.2				
Diseased	2.9 (0.3–16.7) y	23	S: 285 (53.3–1990)*		median (range)	Awake Before procedures Peripheral veins Plasma	I	Westerlind et al. (9)
		114	A: 48.2 (8.6–449)$^{n/p}$					
Control	1.1 (0.1–8.3) y	23		32.9 (11.7–212.2)				
Diseased	3.1 (0.3–16.2) y	61	S: 259.1 (86.4–246)* A: 81.2 (31.8–164)$^{n/p}$		median (range)	Awake Before procedures Peripheral veins Plasma	I	Holmgren et al. (10)
Control	1.1 (0.1–8.3) y	23		32.9 (11.7–212.2)				
Diseased	3.1 (0.3–13) y	59	S: Hepatomegaly: 116 ± 51 ($n = 21$)* Respiratory retraction: 117 ± 37 ($n = 17$)* Growth failure: 118 ± 58 ($n = 17$)* A: Hepatomegaly: 72 ± 54 ($n = 38$)$^{n/p}$ Respiratory retraction: 45 ± 43 ($n = 28$)$^{n/p}$ Growth failure: 63 ± 47 ($n = 42$)$^{n/p}$	n/a	mean ± SD	During cardiac catheterization Femoral vein Plasma	I	Suda et al. (11)
Diseased	4.3 ± 3.9 y	17	S: 748 ± 439* A: 303 ± 71$^{n/p}$	n/a	mean ± SD	Plasma Supine, quite for 20 min or 20 min after last dose of sedative during catheterization Femoral vein and right ventricle	IIb	Ross et al. (12)
Diseased	5.0 ± 1.1 y	27	S: 35.5 ± 4.2*		mean ± SD	>20 min resting Peripheral venous blood Plasma	I	Yeh et al. (13)
	6.6 ± 1.1 y	41	A: 7.6 ± 0.6$^{n/p}$					
Control	3.5 ± 0.6 y	13		4.8 ± 1.1				
Diseased	5.2 (0.2–14.5) y	23	S: 284 (93–967)$^{n/p}$		mean (range)	Supine >15 min Peripheral vein, after eating Plasma	IIa	Weil et al. (14)
		17	A: 57 (15–118)$^{n/p}$					
Control	0.2–16 y	143		47 (2–109)				
Diseased	0.3–16 y	26	S: 303 (168–466)* A: 42.9 (13.7–189)$^{n/p}$	n/a	median (range)	Awake Before procedures Peripheral veins Plasma	I	Westerlind et al. (15)

(Continued)

TABLE 1 | Continued

Children	Age	n	ANP in heart failure (pg/mL)	ANP in controls (pg/mL)	Statistical operator	Blood sampling procedure		References
2. HEART FAILURE SEVERITY								
Diseased	4.2 ± 1.1 y	14	NYHA I/II: 114.2 ± 47.3 (79% NYHA I)*		mean ± SD	Plasma Venous blood	I	Hayabuchi et al. (16)
Control	4.8 ± 0.9 y	n/a		18.6 ± 4.7				
Diseased	7.4 ± 3.0 y	22 23 3	NYHA I: 20 (5)* NYHA II: 26 (6)* NYHA III: 30 (8)*		median (IQR) resp. mean ± SD	Serum	III	Kotby et al. (17)
Control	7.8 ± 5 y	12		5.54 ± 1.41				
Diseased	15.2 ± 5.5 y	221 60 16	NYHA I: 45 ± 31* NYHA II: 77 ± 49* NYHA III: 125 ± 111*		mean ± SD	Plasma	III	Ohuchi et al. (18)
Control	n/a	51		19.1 ± 10				
3. ETIOLOGIES								
(a) Acyanotic - left-to-right shunt								
Diseased	22 (20–23) wk, gestational	8 7	VSD: 48.1 (30–315)$^{n.s.}$ AVSD: 30 (12–76)$^{n.s.}$		mean (IQR)	Fetal umbilical cord blood Plasma	IIa	Bartha et al. (19)
Control	21 (20.75–22) wk, gestational	14		29 (8.75–37.75)				
Diseased	4.8 (2.5–9.9) mo	5	VSD ($n = 4$), AVSD ($n = 1$): 175.1 (115–437)$^{n/p}$	n/a	median (range)	Arterial catheter Plasma	I	Costello et al. (20)
Diseased	0.4 (9.3–4.5) y	11	VSD: 166 (31.8–346)$^{n/p}$	n/a	median (range)	Awake Before procedures Peripheral veins Plasma	I	Westerlind et al. (15)
Diseased	2 (1–3) y 2 (0.1–15) y 6 (1–16) y	1/4 14/20 13	PDA: S: 416.0/A: 116.3 ± 26.5* VSD: S: 350.6 ± 200.2*/A: 69.2 ± 36.5* ASD: 99.4 ± 40.7*		mean ± SD resp. median (range)	Supine position at rest Peripheral vein	I	Kikuchi et al. (21)
Control	4 (0.1–15) y	30		44.6 ± 22.3				
Diseased	2.7 ± 3.3 y	12	VSD ($n = 6$), PDA ($n = 4$), AVSD ($n = 2$): 28.5 ± 6.6*		mean ± SD	Serum	III	Kotby et al. (17)
Control	7.8 ± 5 y	12		5.54 ± 1.41				
Diseased	5.0 (0.6–13.1) y	24	LRS with pulmonary hypertension: IVC: 198 (10–1417)* PA: 456 (10–1697)* AO: 336 (10–1190)* RA: 360 (10–1655)*		mean (range)	Plasma	I	Oberhänsli et al. (22)
Control	6.2 (1.3–16.4) y	12		IVC: 56 (28–162) PA: 90 (46–147) AO: 78 (38–168) RA: 70 (35–122)				

(Continued)

TABLE 1 | Continued

Children	Age	n	ANP in heart failure (pg/mL)	ANP in controls (pg/mL)	Statistical operator	Blood sampling procedure		References
Diseased	6.8 ± 6.4 y	6	PDA: 102.3 ± 30.3*		mean ± SD	Inferior vena cava during catheterization Plasma	I	Zeevi et al. (23)
Control	6.8 ± 2.5 y	9		24.6 ± 4.6				
Diseased	6.9 ± 1.1 y 5.0 ± 1.1 y	26 27	A: VSD ($n = 15$), ASD ($n = 6$), PDA ($n = 4$), PDA+ASD ($n = 1$): 8.3 ± 1.2$^{n.s.}$ S: VSD ($n = 16$), ASD ($n = 7$), AVSD ($n = 4$): 35.5 ± 4.2*		mean ± SD	>20 min resting Peripheral venous blood Plasma	I	Yeh et al. (13)
Control	3.5 ± 0.6 y	13		4.8 ± 1.1				
Diseased	10.6 ± 3.6 y	14	ASD: 24 ± 9.8*		mean ± SD	Supine Peripheral vein Plasma	I	Muta et al. (24)
Control	6–18 y	10		17 ± 6.8				
Diseased	0.2–14 y	18 7 6	VSD: 221 ± 123* ASD: 65 ± 42$^{n/p}$ PDA: 124 ± 38*		mean ± SD	Peripheral vein Plasma	IIb	Matsuoka et al. (25)
Control	0.1–15 y	53		< 80				
(b) Acyanotic - obstructive defects								
Diseased	22 (20–23) wk, gestational	1	PS: 152$^{n.s.}$		mean (IQR)	Fetal umbilical cord blood Plasma	IIa	Bartha et al. (19)
Control	21 (20.75–22) wk, gestational	14		29 (8.75–37.75)				
Diseased	8.5 ± 5.5 d	3 1	PS: 193.0 ± 61.6$^{n.s.}$ AS: 192$^{n.s.}$		mean ± SD	Inferior vena cava during catheterization Plasma	I	Zeevi et al. (23)
Control	14 ± 11 d	7		220.8 ± 16.2				
Diseased	4.5 (0.3–16.2) y	9	CoA: 42.2 (13.7–63.2) $^{n/p}$	n/a	median (range)	Awake Before procedures Peripheral veins Plasma	I	Westerlind et al. (15)
Diseased	6.2 ± 1.2 y	15	PS ($n = 9$), AS ($n = 4$), CoA ($n = 2$): 5.2 ± 0.7$^{n.s.}$		mean ± SD	>20 min resting Peripheral venous blood Plasma	I	Yeh et al. (13)
Control	3.5 ± 0.6 y	13		4.8 ± 1.1				
Diseased	6.8 ± 6.4 y	16 8 4 3 1	125.2 ± 15.8* of which: PS: 126.4 ± 24.1$^{n/p}$ AS: 141.7 ± 46.2$^{n/p}$ CoA: 108.9 ± 42.5$^{n/p}$ MS: 241$^{n/p}$		mean ± SD	Inferior vena cava during catheterization Plasma	I	Zeevi et al. (23)
Control	6.8 ± 2.5 y	9		24.6 ± 4.6				

(Continued)

TABLE 1 | Continued

Children	Age	n	ANP in heart failure (pg/mL)	ANP in controls (pg/mL)	Statistical operator	Blood sampling procedure		References
Diseased	0.2–14 y	7	PS: 36 ± 21$^{n/p}$		mean ± SD	Peripheral vein Plasma	IIb	Matsuoka et al. (25)
Control	0.1–15 y	53		< 80				
(c) Cyanotic defects								
Diseased	22 (20–23) wk, gestational	5 3 2 1	Hypoplastic left heart: 15 (7.5–46)$^{n.s.}$ Hypoplastic right heart: 44 (19–44)$^{n.s.}$ TOF: 44 (19–44)$^{n.s.}$ Univentricular heart: 53$^{n.s.}$		mean (IQR)	Fetal umbilical cord blood Plasma	IIa	Bartha et al. (19)
Control	21 (20.75–22) wk, gestational	14		29 (8.75–37.75)				
Diseased	8.5 ± 5.5 d	2	ToGA: 344 ± 6.3$^{n.s.}$		mean ± SD	Inferior vena cava during catheterization plasma	I	Zeevi et al. (23)
Control	14 ± 11 d	7		220.8 ± 16.2				
Diseased	4 (0.1–15) y	10	TOF: 69.4 ± 38.8$^{n.s.}$		mean ± SD resp. median (range)	Supine position at rest Peripheral vein	I	Kikuchi et al. (21)
Control	4 (0.1–15) y	30		44.6 ± 22.3				
Diseased	6.8 (1.8–14.7) y	21	TOF: IVC: 68 (10–169)$^{n.s.}$ PA: 139 (10–262)$^{n.s.}$ AO: 133 (10–365)$^{n.s.}$ LA: 128 (24–210)$^{n.s.}$ RA: 114 (10–308)$^{n.s.}$		mean (range)	Plasma	I	Oberhänsli et al. (22)
Control	6.2 (1.3–16.4) y	12		IVC: 56 (28–162) PA: 90 (46–147) AO: 78 (38–168) RA: 70 (35–122)				
Diseased	0.2–14 y	7 5	TOF: 25 ± 11$^{n/p}$ TA: 221 ± 90$^{n/p}$		mean ± SD	Peripheral vein Plasma	IIb	Matsuoka et al. (25)
Control	0.1–15 y	53		< 80				
(d) Dilated cardiomyopathy								
Diseased	1.44 ± 1.4 y	12	29.25 ± 4.52*		mean ± SD	Serum	III	Kotby et al. (17)
Control	7.8 ± 5 y	12		5.54 ± 1.41				
Diseased	3.4 (0.3–14.8) y	6	412 (148–553)$^{n/p}$	n/a	median (range)	Awake Before procedures Peripheral veins Plasma	I	Westerlind et al. (15)
(e) Rheumatic heart disease								
Diseased	13.2 ± 1.6 y 12.4 ± 2.9 y	12 12	S: 28.33 ± 5.78* A: 26.5 ± 4.91*		mean ± SD	Serum	III	Kotby et al. (17)
Control	7.8 ± 5 y	12		5.54 ± 1.41				

(Continued)

TABLE 1 | Continued

Children	Age	n	ANP in heart failure (pg/mL)	ANP in controls (pg/mL)	Statistical operator	Blood sampling procedure		References
4. CARDIAC LOADING CONDITION								
Diseased	4.5 (0.3–16.2) y 0.4 (9.3–4.5) y 3.4 (0.3–14.8) y	9 11 6	Pressure overload: 42.2 (13.7–63.2)$^{n/p}$ Volume overload: 166 (31.8–346)$^{n/p}$ LV dysfunction: 412 (148–553)*	n/a	median (range)	Awake Before procedures Peripheral veins Plasma	I	Westerlind et al. (15)
Diseased	3.7 (1.6–16.7) y 4.3 (0.3–16.2) y 1.0 (0.3–16.4) y 3.3 (0.3–15.0) y	35 29 60 13	No overload: 28.6 (8.6–105)$^{n.s.}$ Pressure overload: 51.9 (8.7–210)* Volume overload: 93.0 (15.9–346)* Systolic ventricular dysfunction: 431 (43.8–1990)*		median (range)	Awake Before procedures Peripheral veins Plasma	I	Westerlind et al. (9)
Control	1.1 (0.1–8.3) y	23		32.9 (11.7–212.2)				
Diseased	6.8 (0.3–16.2) y 1.9 (0.3–14.7) y 0.5 (0.3–4.9) y 4.0 (0.5–13.3) y	15 11 16 18	LV pressure overload: 40.8 (12.6–219)$^{n.s.}$ RV pressure overload: 69.3 (8.7–182)* LV volume overload: 164 (31.8–346)* RV volume overload: 57.2 (11.3–234.1)*		median (range)	Awake Before procedures Peripheral veins Plasma	I	Holmgren et al. (10)
Control	1.1 (0.1–8.3) y	23		32.9 (11.7–212.2)				

A, asymptomatic; ANP, Atrial/A-type Natriuretic Peptide; AO, aorta; AS, aortic stenosis; ASD, atrial septal defect; AVSD, atrioventricular septal defect; CoA, Coarctation of the aorta; d, days; IQR, interquartile range; IVC, inferior vena cava; LA, left atrium; LRS, left-to-right shunt; LV, left ventricle; min, minutes; mo, months; MS, mitral stenosis; n, number; n/a, not available; NYHA, New York Heart Association; PA, pulmonary artery; PDA, patent ductus arteriosus; PS, pulmonary stenosis; RA, right atrium; RV, right ventricle; S, symptomatic; SD, standard deviation; TA, Tricuspid atresia; TOF, tetralogy of Fallot; ToGA, transposition of great arteries; VSD, ventricular septal defect; wk, weeks; y, years.

** Statistically significant to reference group/controls, $^{n/p}$ significance not provided, $^{n.s.}$ non-significant.*

Sample collection protocol:

I: detailed information available regarding: blood sampling procedure, used inhibitors, storage conditions.

IIa: detailed information available regarding: blood sampling procedure, storage conditions; incomplete information regarding used inhibitors.

IIb: detailed information available regarding: blood sampling procedure, used inhibitors; incomplete information regarding storage conditions.

III: without any information regarding: blood sampling procedure, used inhibitors, storage conditions.

TABLE 2 | ANP levels influenced by medical or surgical intervention sorted in order of increasing age.

Intervention	Age	n	ANP before intervention (pg/mL)	ANP after intervention (pg/mL)	ANP in controls (pg/mL)	Statistical operator	Blood sampling procedure		References
Catheterization	8.5 ± 5.5 d	6	243.0 ± 42.1	30 min: 243.6 ± 48.4* FU: 62.1 ± 12.7*		mean ± SD	IVC during catheterization Plasma	I	Zeevi et al. (23)
None	14 ± 11 d	7			220.8 ± 16.2				
Catheterization	6.8 ± 6.4 y	22	125.2 ± 15.8	30 min: 75.6 ± 11.4* FU: 42.9 ± 5.0*					
None	6.8 ± 2.5 y	9			24.6 ± 4.6				
Cardiopulmonary bypass	4.8 (2.5–9.9) mo	5	175.1 (115–437)	1 day: 44 (28–81)*	n/a	median (range)	Arterial catheter Plasma	I	Costello et al. (20)
Cardiac surgery	41.11 ± 25.39 mo	27	Normal PBF: 175.9 ± 253.2	24 h: 149.3$^{n.s.}$ 48 h: 155.8$^{n.s.}$		mean ± SD	15 min supine 9:00 am Before surgery: peripheral vein After surgery: central venous catheter Plasma	I	Alvarez Kindelan et al. (26)
	30.61 ± 40.74 mo	38	High PBF: 229.9 ± 311.2	24 h: 183.8$^{n.s.}$ 48 h: 187.2$^{n.s.}$					
None	0.2–14 y	48			46.5 ± 25.5				
Catheterization	3.1 (0.3–13) y	59	99.8 ± 60.2	8 mo: 23.8 ± 10.1*	n/a	mean ± SD	During cardiac catheterization Femoral vein Plasma	I	Suda et al. (11)
Right venricular angiography	6.8 (1.8–14.7) y	21	TOF before: IVC: 68 (10–169) PA: 139 (10–262) AO: 133 (10–365) LA: 128 (24–210) RA: 114 (10–308)	After: IVC: 217 (42–612)* PA: 33 (49–805)* AO: 360 (95–910)* LA: 228 (77–525)* RA: 298 (21–875)*		mean (range)	Plasma	I	Oberhänsli et al. (22)
	5.0 (0.6–13.1) y	24	LRS with pulmonary hypertension before: IVC: 198 (10–1417) PA: 456 (10–1697) AO: 336 (10 −1190) RA: 360 (10–1655)	After: IVC: 412 (63–1568)* PA: 883 (74–3220)* AO: 760 (116–1960)* RA: 594 (45–2275)*					
Right venricular angiography	6.2 (1.3–16.4) y	12		Controls after: IVC: 181 (70–346)* PA: 207 (105–266)* AO: 221 (66–567)* RA: 260 (122–378)*	Before: IVC: 56 (28–162) PA: 90 (46–147) AO: 78 (38–168) RA: 70 (35–122)				
Transcatheter closure of ASD	10.6 ± 3.6 y	14	24 ± 9.8	5 min: 34 ± 18* 24 h: 19 ± 11$^{n.s.}$		mean ± SD	Supine Peripheral vein Plasma	I	Muta et al. (24)
None	6–18 y	10			17 ± 6.8				

(Continued)

TABLE 2 | Continued

Intervention	Age	n	ANP before intervention (pg/mL)	ANP after intervention (pg/mL)	ANP in controls (pg/mL)	Statistical operator	Blood sampling procedure		References
Captopril 0.5 mg/kg	13.2 ± 1.6 y	12	RHDF: 28.33 ± 5.78	RHDF: 15.3 ± 5.3*		mean ± SD	Serum	III	Kotby et al. (17)
	12.4 ± 2.9 y	12	RHD: 26.5 ± 4.91	RHD: 10.7 ± 2.5*					
	2.7 ± 3.3 y	12	CHD: 28.5 ± 6.6	CHD: 11.5 ± 3.8*					
	1.44 ± 1.4 y	12	DCM: 29.25 ± 4.52	DCM: 15.7 ± 10.7*					
None	7.8 ± 5	12			5.54 ± 1.41				

ANP, Atrial/A-type Natriuretic Peptide; AO, aorta; ASD, atrial septal defect; CHD, congenital heart disease; d, days; DCM, dilated cardiomyopathy; FU, follow-up after 7-30 days; h, hours; IVC, inferior vena cava; LA, left atrium; LRS, left-to-right shunt; min, minutes; mo, months; n, number; n/a, not available; PA, pulmonary artery; PBF, pulmonary blood flow; RA, right atrium; RHD, rheumatic heart disease; RHDF, rheumatic heart disease in failure; SD, standard deviation; TOF, tetralogy of Fallot; y, years.

**statistically significant decrease through intervention, n.s., non-significant.*

Sample collection protocol:

I = detailed information available regarding: blood sampling procedure, used inhibitors, storage conditions.

III = without any information regarding: blood sampling procedure, used inhibitors, storage conditions.

(TA)] and acyanotic defects. In the latter, obstructive defects [e.g., pulmonary stenosis (PS), aortic stenosis (AS), coarctation of the aorta (CoA), mitral stenosis (MS)] and left-to-right shunts [e.g., VSD, atrioventricular septal defect (AVSD), atrial septal defect (ASD), patent ductus arteriosus (PDA)] are further differentiated. ANP levels in each group can be seen in **Tables 1.3a,b,c**.

Levels in VSD, PDA, or TA are significantly elevated compared to other malformations like CoA, ASD or TOF and also to controls (15, 21, 23, 25). It further has been shown that in VSD, ANP as well as BNP correlate with the shunt size of the defect [ANP: small: 59.1 ± 28.9 pg/mL ($n = 17$), moderate: 130.6 ± 12.2 pg/mL ($n = 5$), large: 386 ± 194.1 pg/mL ($n = 12$)] (11, 21).

Significantly elevated levels of ANP compared to controls in ASD were measured by Muta et al. whereas Yeh et al. only reported increased levels in symptomatic children but pooled children with VSD, ASD, AVSD, and PDA (13, 24). In TOF patients, Oberhänsli et al. and Kikuchi et al. observed insignificantly elevated levels of ANP in comparison to controls, whereas Matsuoka et al. measured levels in the range of controls (21, 22). While Zeevi et al. reported a significant increase of ANP in PS, AS, CoA and MS compared to controls, Matsuoka et al. and Yeh et al. could not confirm this (13, 23, 25). By examining whether fetuses with cardiac malformations already show increased ANP levels, different types of CHD did not significantly differ (19). Furthermore, no significant difference to fetal controls could be detected. NT-proBNP can identify fetal cardiac defects but the results may be influenced by a higher mean age of 6 weeks (44).

Only one study assessed pediatric NT-proANP levels regarding the etiology and stated highest levels in AVSD followed by PDA > AS > ASD > VSD (32). These results contrast to measurements of NT-proBNP or ANP, where higher concentrations are reported in VSD and PDA than in ASD (32, 45).

In conclusion, DCM, RHD and CHD show elevated levels of ANP as it is seen for BNP. Nevertheless, ANP levels are inhomogeneous if different types of CHDs are compared. This might be attributed to the small and heterogeneous pediatric study populations. Generally, more complex CHDs present higher levels of ANP, in line with measurements of BNP (42).

Monitoring the Cardiac Status With ANP in Pediatric Heart Failure

ANP levels decreased after treatment with the Angiotensin-Converting-Enzyme inhibitor captopril in patients with left volume overload, reflecting the improvement of NYHA stage and left ventricular remodeling (**Table 2**) (17). Infusion of the short-acting sympathomimetic dobutamine given to children with surgically repaired TOF decreased ANP and BNP significantly from elevated levels. Larger changes in ANP reflect atrial and ventricular pressure changes and the larger decline of ANP might be attributed to its faster release and clearance compared to BNP. After stopping the infusion, levels rose back to the baseline (16).

Regarding surgical interventions in children with CHD, interventional catheterization significantly lowered preoperative

values already 30 min subsequent to the operation and further after 7–30 days (**Table 2**). The ANP levels stayed elevated compared to age-matched controls (23). Setting the follow-up after a longer period, in infants with VSD, preoperative ANP and BNP levels also decreased significantly compared to measurement at 8 months postoperative (11). Cardiopulmonary bypass in children with a left-to-right shunt in failure resulted in a decrease of baseline ANP levels one day after. Surprisingly, in the same population BNP levels were normal at baseline and rose during operation (20). In 14 children undergoing transcatheter closure of ASD, significantly elevated levels of ANP compared to controls were reported before closure (24). Those increased significantly 5 min after closure, but declined within 24 h to levels insignificantly different from controls, maintaining these levels during follow-up at 1 and 3 months. Contrastingly, BNP showed a prolonged elevation. At the time-point of 24 h ANP levels were higher than preoperative or 5 min after operation. The authors suggested that this might be reflective of the mechanistic differences of the atria and the right ventricle as compared to the left ventricle during the remodeling process after correction.

Cardiac surgery of various CHDs in 65 children with significantly increased baseline levels of ANP compared to controls, resulted in a non-significant decrease of ANP at 24 and 48 h post-operative (26). Details of each intervention are presented in **Table 2**.

In conclusion, anatomical correction of malformations, as well as medical treatment of heart failure, reduce ANP levels in children. Differences between ANP and BNP can be seen due to their diverging secretion and clearance reflecting the faster acting of ANP.

Effect of Age and Body Weight on ANP Levels in Healthy Infants

There is conflicting data on the correlation of ANP and age. Klar et al. obtained significantly different levels between the age groups of 3 months to 3 years on the one hand and 3 to 14 years on the other hand. Ohuchi et al. and Matsuoka et al. also observed an inverse correlation in control subjects from 1 to 40 years, respectively 1 month to 15 years. (18, 25, 46).

Few studies included neonates, in which levels of ANP were significantly elevated. But beyond neonatal period up to adulthood, these levels did not vary significantly (14, 23). Hemodynamic changes after birth are being held responsible for increased natriuretic peptide levels (14, 23, 47).

By contrast, there are also some studies that measured ANP in healthy children older than 1 month without observing any correlation (8, 9, 41, 48). In comparison, BNP is high during the first days of life, decreases rapidly soon afterward and slowly during the remaining period of childhood (49).

In case of NT-proANP and MR-proANP, significantly negative connections to age were seen (32–34). NT-proANP levels are significantly elevated during the first days of life with a peak on the first day reaching a more pronounced increase than NT-proBNP but displaying constant levels within few days (47). Other studies observed the highest levels of NT-proANP in newborns until 1 month of age, passing into slightly elevated levels during the first year of life and subsequently resulting in consisting ones (32, 33). In case of pro-ANPs, not only adapting mechanisms of the heart are causative, but also—due to their renal clearance mechanism—the changing of the glomerular filtration rate with age (33). However, only in children with severe renal dysfunction (glomerular filtration rate <30–40 mL/min/1.73 m^2) did increased levels of NT-proANP occur and thus the efficiency in terms of diagnosing heart failure is usually not influenced (32, 33).

In conclusion, levels of ANPs in children are relatively constant after passing the peak caused by adaptional processes after birth, equivalently to BNPs. Following from these peak levels, ANP concentrations in newborns with pulmonary stenosis and aortic stenosis did not significantly differ from levels in controls as already physiologically high levels of ANP can mask the elevation induced by heart failure (**Table 1.3b**) (23). In neonates with transposition of great arteries, ANP levels were elevated (**Table 1.3c**). Therefore, the use of ANP and generally natriuretic peptides in neonates in a diagnostic manner has to be carefully applied.

Furthermore, ANP and BNP significantly negatively correlated with bodyweight in connection with symptoms of heart failure (11). Therefore, infants with growth failure, a clinical sign of heart failure, present also higher levels of ANP (11, 50).

Meaningful Alternatives to Blood Sampling

The European Medicines Agency demands considering non-invasive alternatives to blood sampling whenever possible for pediatric studies (51).

Urine

ANP is found in urine as well and was determined in children to assess the possibility of circumventing stressful blood sampling in children (8). But ANP was not detectable in healthy controls ($n = 30$). Urine levels did not correlate with plasma levels in CHD patients ($n = 22$). Further, it was not possible to distinguish patients with ($n = 11$, mean 13.7 months) and without clinical signs ($n = 11$, mean 76.1 months) by the means of urinary ANP. As the main metabolism of ANP is not organ-specific (e.g., kidney), measurement of ANP in urine cannot make up as an alternative to plasma measurement. Hence, NT-pro-peptides which are renally excreted could rather be an alternative, but currently there is no data for NT-proANP, NT-proBNP, or also BNP in urine in pediatric heart failure.

Saliva

In healthy men, ANP can be measured in saliva which correlates with plasma levels (52). So far, no studies evaluated the significance of salivary ANP in heart failure, but for NT-proBNP and BNP first studies were conducted. Those indicate that adult heart failure patients present higher levels than controls (53, 54). Saliva offers the advantages of being non-invasive, less demanding and easier to handle than blood. Thus, further research should be conducted since it seems to offer a promising alternative approach to the determination of biomarkers in heart

failure, an approach which would be especially advantageous in pediatrics.

PREANALYTICAL AND ANALYTICAL ISSUES DURING MEASUREMENT OF ANP

Collection and Processing of Blood Samples

ANP has a short half-life (2–5 min) and has to be treated carefully after blood sampling (55). Despite its short half-life, it is proven that ANP with EDTA and aprotinin is stable for 2 months at −20°C and for 2 h at room temperature which allows for analysis (56, 57). Plasma should be obtained as fast as possible as ANP clearance receptors are located on platelets and therefore concentrations in whole-blood diminish (58). Hemolyzed samples should be excluded as falsely low concentrations of ANP will be measured (59). However, hemolytic samples can be used for the determination of NT-proANP (60, 61).

Difficulties Concerning Pediatric Sampling

The position of the patient during blood sampling affects ANP levels substantially. Moving into supine position causes levels of ANP to rise, probably as a consequence of venous return and the following increment in atrial pressure (62–64). In severe heart failure, a change from supine to upright position is associated with further increase of supine ANP levels, likely caused by tachycardia or increased sympathetic nervous activity (62). Therefore also crying, which is common in infants during venipuncture, is most likely influencing levels of ANP and also excitement may play a role. Further the site of blood collection matters because central and peripheral plasma concentrations differ (65). Thus, a well-standardized protocol is needed and deviations have to be recorded in order to be able to interpret findings adequately.

CONCLUSION

ANP serves as a helpful marker for the diagnosis of pediatric heart failure and follow-up of treatment and after operation in children. Due to its fast release, ANP offers the advantage of displaying rapid changes during therapy or operation. Nevertheless, it altogether offers no major advantage over BNP and NT-proBNP. The perspective of establishing ANP as a standard clinical biomarker for diagnosis and prognosis seems to be low, but it has high potential in research for a better understanding of the natriuretic peptide system and the impact of drugs (e.g., sacubitril/valsartan). So far, no measurement of ANP/NT-proANP has been reported in humans taking sacubitril/valsartan. Thus, it remains to be seen whether it turns out to be an alternative. ANP is susceptible to a lot of preanalytical and analytical issues, especially in pediatric sampling. Finally, saliva is suggested to be a promising alternative to blood sampling enabling non-invasive measurement of ANP.

AUTHOR CONTRIBUTIONS

Conception and design of the work was developed in close collaboration by TG and BB. Data collection and analysis was performed by TG. The interpretation and drafting of the article was done by TG and BB. Critical revision and final approval of the version to be published was given by BB.

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Thirty Years of Kawasaki Disease: A Single-Center Study at the University Hospital of Lausanne

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Kawasaki disease is an acute vasculitis with a particular involvement of the coronary arteries. Coronary artery aneurysms develop in 20% of untreated children. It has been shown that early treatment with intravenous immunoglobulins and aspirin decreases this risk to 5%, but the medium to long term prognosis of children with Kawasaki disease is still unclear. To determine the outcome of the disease and risk factors for poor evolution, we reviewed retrospectively the medical records of all patients with a diagnosis of Kawasaki disease at our Institution between 1981 and 2014. Among the 207 patients included in the study, 96 patients had coronary diameter anomalies (46.4%) at diagnosis and children with atypical ages for Kawasaki disease (<1 year or >10 year of age) were more often affected with aneurysms or dilatations. Eighty-four of them had complete regression of coronary aneurysms during the follow-up (87.5%) Absence of immunoglobulins in the acute phase was associated with less regression rate (57.1 vs. 92.2%), and boys had greater *z*-scores at last echocardiography, statistically significant for the left anterior descending artery. We found rare complications after the acute phase documented in our patient charts (only 3.8%). Recurrence of the disease occurred in 5 children (2.4%) and myocardial ischemia in 3 patients (1.4%), all with initial coronary aneurysm.

Conclusion: Medium to long term prognosis after Kawasaki disease is excellent. Boys, patients not treated with immunoglobulins or outside the usual age range are more at risk for an unfavorable outcome.

Keywords: Kawasaki disease, coronary aneurysm, complications, cardiac sequelae, follow-up, prognosis

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INTRODUCTION

Kawasaki disease (KD) is an acute systemic vasculitis of early childhood considered the leading cause of acquired heart disease in children in developed countries. Cardiac lesions are a hallmark of KD and coronary artery aneurysms (CAA) develop in 20% of untreated children and can lead to coronary stenosis, myocardial infarction (MI), or sudden death. Pericarditis complicated by cardiac tamponade or myocarditis associated with myocardial dysfunction can also occur during the acute phase (1, 2).

Despite almost 50 years of research, the etiology of KD remains unknown. An infectious trigger which causes an excessive inflammatory response in genetically predisposed children is a widespread hypothesis, but no specific pathogen has been identified yet (3).

KD has been reported in almost every country, with variable incidence rates (4). Over the past decades, the worldwide incidence has been increasing in most countries. KD occurs predominantly in children <5 years of age, with a peak incidence between 12 and 24 months (5–10).

Without a specific diagnostic test, the diagnosis of KD is based on clinical criteria, established by the Japanese Ministry of Health Research Committee and adopted by the American Heart Association, which include fever >39°C for at least 5 days (or less if shortened by treatment for KD) plus four of the five main clinical features: rash, conjunctivitis, oral mucosal changes, changes in the extremities, lymphadenopathy. In some cases, KD presentation is incomplete and echocardiographic and laboratory findings have an important role in the diagnosis (11–14).

Conventional therapy associates intravenous immunoglobulin (IVIG) and high-dose aspirin (ASA). Early administration of IVIG reduces the risk of CAA to 5% (15–18). About 15–20% of patients are unresponsive to this initial treatment and will need repeat or additional treatment such as corticosteroids or infliximab (19, 20).

Mortality rates are low and the majority of patients are considered to have an excellent prognosis but long-term cardiovascular outcome is not totally defined (21–25). Only males with cardiac sequelae are known to have a higher mortality rate than the general population (26). However, even for patients with regressed CAA, there may be a possible cardiovascular dysfunction years after the acute phase, secondary to increased arterial stiffness, abnormal endothelial function, intimal thickening or calcification of coronary arteries (1, 11, 27–31). The clinical relevance of those changes is unclear and long-term follow up of patients depends on the degree of coronary involvement.

Because the prognosis of patients with a diagnosis of KD is not well defined, we decided to conduct this study to determine the characteristics and outcome of all patients at our Institution between 1981 and 2014 and to highlight risk factors for unfavorable evolution.

MATERIALS AND METHODS

Patients and Study Design

This is a retrospective study including all patients with a diagnosis of pediatric KD, followed in the Pediatric Cardiology Unit at the University Hospital of Lausanne.

We analyzed retrospectively the medical records of all patients up to 18 years of age at diagnosis, with complete or incomplete KD and followed in the Pediatric and Adult Cardiology Unit of the University Hospital of Lausanne from January 1981 to March 2014. The following variables were documented: epidemiologic and clinical features, laboratory and echocardiographic data, treatment regimen, complications during the acute phase (shock, heart failure, cerebral injury, aseptic meningitis, uveitis, renal dysfunction, haemolysis, and pancreatitis) and during the follow-up (death, recurrence, ischemia, surgical or catheter coronary interventions).

Patients were excluded from the study if the diagnosis of KD was rejected or if there were missing information in their medical records.

The study protocol was approved by the Cantonal Ethics Committee Vaud.

Definitions

A KD case was defined as: fever for 5 days or more (unless IVIG treatment was begun before the 5th day of fever), exclusion of any other diagnosis, and any four of the five principal clinical features. Incomplete KD was defined according to the guidelines of the American Heart Association as fever for 5 days or more, <4 diagnosis criteria and echocardiographic abnormalities or a set of suspect laboratory criteria (1).

First day of fever was defined as day 1 of illness.

Cardiovascular Assessment

Cardiovascular complications, including coronary artery lesions, pericarditis, myocarditis, valvular regurgitations and heart failure, were assessed during the acute phase and during the follow-up at 3 different periods of time (6 month to 18 months, 3 to 6 years, 8 years and more). The coronary involvement (dilatation, small, medium, giant CAA) was determined by the z-scores adjusted to patients' body surface area (BSA) (Boston formula), if available. We used the z-score stratification proposed by AHA guidelines (2017) to determine the degree of coronary involvement (32):

1. No involvement: Z score always <2
2. Dilation only: Z score 2 to <2.5; or if initially <2, a decrease in Z score during follow-up ≥1
3. Small aneurysm: Z score ≥2.5 to <5
4. Medium aneurysm: Z score ≥5 to <10, and absolute dimension <8 mm
5. Large or giant aneurysm: Z score≥10, or absolute dimension ≥8 m

If z-scores could not be calculated because of missing details about coronary diameters, the degree of involvement was based on cardiologist report.

Statistical Analysis

Statistical analysis was performed using the statistical software STATA 14. Continuous variables are reported as median and range (minimum, maximum). Categorical data are reported by percentage. Pearson's chi-squared test was used to compare distribution between groups, separated by sex, age, treatment regimen, KD presentation (complete vs. incomplete). A $p < 0.05$ was considered statistically significant.

Abbreviations: ASA, Aspirin; BSA, Body Surface Area; CAA, Coronary Artery Aneurysm; IVIG, Intravenous Immunoglobulin; KD, Kawasaki Disease; LAD, Left Anterior Descending Coronary Artery; LMCA, Left Main Coronary Artery; MI, Myocardial Infarction.

RESULTS

Patients' Characteristics

During the study period, 229 patients with KD were diagnosed at the University Hospital of Lausanne. Twenty-two were excluded because of missing information in their medical file.

Of the 207 children included, 58.9% were male ($n = 122$), with a sex ratio of 1.43.

The median age at onset of KD was 32 months (range 1 month to 15 years). The majority of patients were less than 5 years of age (76.8%, $n = 159$). Age distribution is shown in **Figure 1**.

KD was complete in 146 cases (70.5%) and incomplete in 61 cases (29.5%). The annual number of incomplete KD increased after 1996: only 7 (17%) children were diagnosed with incomplete KD from 1981 to 1996 compared to 54 (32.5%) cases after 1996. Patients with incomplete KD were younger at diagnosis than patients with complete KD, but with no statistically significant difference (26 vs. 35 months, $p = 0.08$). The diagnosis, and therefore treatment, of patient with incomplete KD was delayed compared to complete KD (median duration before diagnosis 6.5 vs. 8 days, $p = 0.007$).

The diagnostic criteria and other clinical features are summarized in **Table 1**. There was no statistically significant difference in disease presentation according to sex or age.

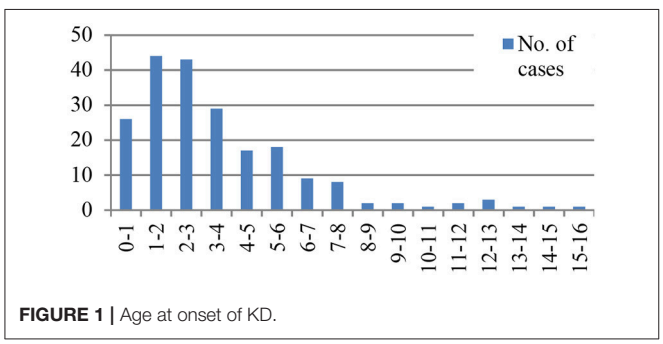

FIGURE 1 | Age at onset of KD.

TABLE 1 | Clinical features at presentation.

Diagnostic criteria		Other clinical features	
Polymorphus rash	$n = 176$ (85%)	GI tract (diarrhea, vomiting, abdominal pain, hematemesis)	$n = 121$ (58.4%)
Changes in lips and oral cavity	$n = 161$ (77.8%)	Respiratory/ORL (cough, dyspnea, pneumonia, sinusitis, epistaxis, pharyngitis)	$n = 60$ (30.4%)
Conjonctival injections	$n = 159$ (76.8%)	Musculoskeletal (arthralgia, myalgia, joint effusion)	$n = 30$ (14.6%)
Changes of the extremities	$n = 144$ (69.9%)	Neurological (seizure, meningism, headache, dizziness, lethargy, photophobia)	$n = 24$ (11.6%)
Cervical adenopathy	$n = 136$ (65.7%)	Genito-urinary (hematuria, proteinuria, oliguria, urinary infection)	$n = 18$ (8.7%)
		Cardiac (murmur)	$n = 2$ (1%)

KD Occurrence

The University Hospital of Lausanne is a leading tertiary center that covers an overall population of around 1,400,000 person. Every child within this area with a suspected diagnosis of KD is referred for echocardiographic evaluation to its pediatric cardiology unit.

As shown in **Figure 2**, KD was diagnosed at least in one child every year except in 1984, and there is an increase in the number of patients identified over the years with a peak of 22 patients in 2012.

A non-significant seasonal variability was noted, with a highest incidence in winter (29.5% of KD occurred in winter, which represents 61 patients).

Treatment

Treatment during the acute phase of KD is summarized in **Figure 3**. All patients received a first infusion of IVIG, except 16 children (7.7%). Half of them were diagnosed before IVIG was available at our Institution. The other half was diagnosed after more than 7 days of evolution.

One hundred and Sixty Five patients (86.4%) received a single IVIG infusion of 2 g/kg, the other patients received 0.4 g/kg for 4–5 days (13.1%, $n = 25$) or 1 g/kg for 2 days (0.5%, $n = 1$). IVIG therapy was well tolerated and only 8 patients (3.9%) developed side effects (allergic reaction, hypotension).

A majority of children also received ASA (97.1%, $n = 201$). High dose of ASA (80–100 mg/kg/day) was given in the majority of cases (86.1%, $n = 173$). This dose was usually reduced to 5 mg/kg/day after the acute phase.

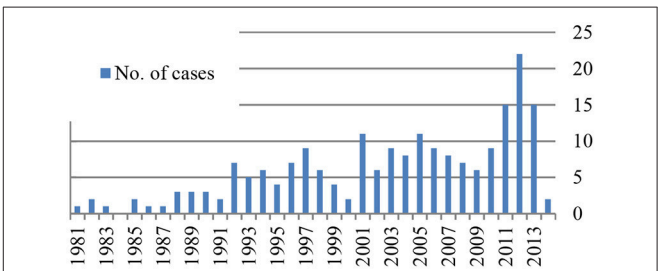

FIGURE 2 | Occurrence rates.

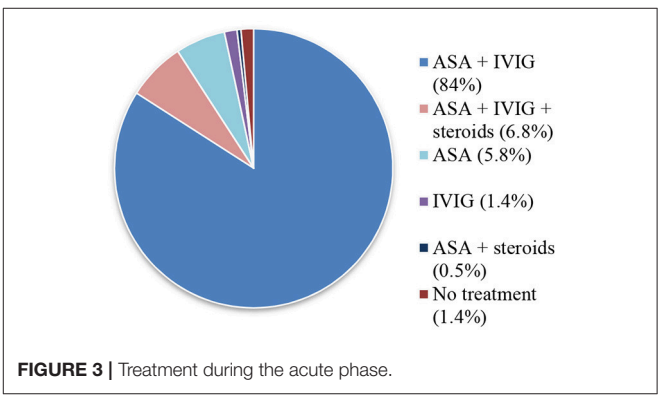

FIGURE 3 | Treatment during the acute phase.

Fifteen treated patients (7.2%) also received steroids during their hospitalization. Reasons for using steroids were either severe coronary anomalies or associated severe complications such as shock, respiratory distress, pancreatitis, renal insufficiency, ascites, pleural effusion, and pancytopenia.

Antibiotics were given in 31 cases (15% of treated patients), concomitantly or before diagnosis.

Three patients (1.4%) were diagnosed with KD after more than 2 weeks of evolution and were not treated either with IVIG or ASA since they had no residual inflammatory changes.

The majority of patients (58.5%, $n = 121$) were treated before day 7 of illness (median: day 7; range: day 1–39). However, 22.7% of patients ($n = 47$) did not received a treatment before the 10th day of illness.

Thirty-four children (16.4%) needed a 2nd therapy because of persistent or recrudescent fever after the initial treatment: 24 patient a 2nd IVIG infusion, 8 patients received steroids associated to IVIG. Infliximab was added to this latter treatment once. Steroids alone were used in 1 case (3%).

Three children needed a 3rd treatment for persistent fever (1.4%): a 3rd IVIG infusion ($n = 1$), corticosteroid associated to Anakinra ($n = 1$) and Infliximab ($n = 1$).

There was no difference in gender, age distribution or KD presentation between IVIG responders and non-responders.

Information about treatment after the acute phase was available for 200 children (96.6%). Patients were treated with ASA more than 1 year after the onset of KD in 29 cases (14%). The median duration of treatment after the acute phase was 2 months (range 0 month to 34 years). At the end of the study period, 11 patients (5.3%) were still under treatment. Every patient received low-dose (5–10 mg/kg/day) ASA and additional acenocoumarol was given in 1 child with persistent giant CAA.

Echocardiography

Results of echocardiography during the acute phase of KD were available for all patients except for 3 Swiss children who were diagnosed with KD and treated abroad during their holidays. The echocardiography findings at diagnosis are summarized in **Table 2**.

Z-scores could be calculated in 171 echocardiograms (82.6%), the other 36 did not report the exact diameters of the coronary arteries. After the z-score calculation, the degree of coronary involvement was modified from the echocardiographic report in 90 cases (52.6% of the patients with z-score). Cardiologists underestimated the coronary artery lesions in 70 patients (40.9%) and overestimated them in 20 patients (11.7%).

The initial echocardiography showed anomalies in 136 cases (65.7%). Perivascular brightness of coronary arteries was the most common finding (52.6%, $n = 109$), followed by changes in coronary arteries diameter (46.4%, $n = 96$).

Isolated left coronary artery lesions (25.6%, $n = 53$) were more frequent than isolated right coronary artery lesions (4.8%, $n = 10$). Bilateral abnormalities occurred in 33 patients (15.9%).

In order of decreasing frequency, the sites of left coronary involvement were the left anterior descending coronary artery (LAD) (51.1% of left coronary artery lesions, $n = 44$), both LAD and left main coronary artery (LMCA) (25.6%, $n = 22$), isolated LMCA (23.2%, $n = 20$). LMCA did not present any giant CAA. Multiple CAA on the same coronary artery occurred in only one patient (0.5%).

No patient suffered from MI during the acute period.

Patients with atypical ages for KD (<1 or >10 year of age) had a higher incidence of changes in coronary arteries diameter (atypical age: 62.8% of coronary artery lesion vs. age 1–10 year: 43%, $p = 0.01$, see **Table 3**). Giant CAA occurred in 11.4% of patients with an atypical age and 1.7% in the 1–10 year old group.

Valvular involvement occurred in 19.8% of patients ($n = 41$) and most often affected the mitral valve (87.8% of valvular dysfunction, $n = 36$). Fifty patients (27%) suffered from pericarditis.

Death

Of the 207 patients, 1 died during the acute phase. This corresponds to a mortality rate of 0.5%. The deceased patient was a 33 months old boy. He developed cardiac tamponade, pulmonary hypertension and vena cava thrombus treated with thrombolysis and heparin. He died of subsequent cerebral hemorrhage 13 days after onset of KD.

Follow-Up

Echocardiography report after the acute phase of KD was available in the majority of cases (97.1%, $n = 201$). The patients were followed up for a median duration of 21 months (range 0 month to 30 years). The patients without echocardiography follow-up were 6 children diagnosed between 1982 and 2010 who did not have any CAA and no subsequent follow-up appointment was planned for them.

Three different time frames from the time of diagnosis were selected for the echocardiographic follow-up: 6 to 18 months, 3

TABLE 2 | Echocardiography results at diagnosis.

Perivascular brightness of coronary arteries	$n = 109$ (52.6%)
Coronary dilatation	$n = 16$ (7.7%)
Coronary aneurysm:	
- Small	$n = 41$ (19.8%)
- Medium	$n = 32$ (15.4%)
- Giant	$n = 7$ (3.4%)
Pericarditis:	
- Pericardial effusion	$n = 54$ (26.1%)
- Cardiac tamponade	$n = 2$ (1%)
Valvular disease	$n = 41$ (19.8%)
Myocarditis:	
- Decreased ejection fraction of the left ventricle	$n = 12$ (5.8%)
- Congestive heart failure	$n = 3$ (1.4%)
Arrhythmia	$n = 4$ (1.9%)

TABLE 3 | Coronary involvement according to age, sex and IVIG treatment.

		Study population ($n = 207$)	Age 1-10 year ($n = 172$)	Atypical age ($n = 35$)	*P*-value	Males ($n = 122$)	Females ($n = 85$)	*P*-value	IVIG ($n = 191$)	No IVIG ($n = 16$)	
CAA at diagnosis	Normal	111 (53.6%)	98 (56.9%)	13 (37.1%)	**0.01**	61 (50%)	50 (58.8%)	NS	100 (52.3%)	11 (68.7%)	NS
	Dilatation	16 (7.7%)	14 (8.1%)	2 (5.7%)	**0.01**	8 (6.5%)	8 (9.4%)	NS	15 (7.8%)	1 (6.2%)	NS
	Small CAA	41 (19.8%)	34 (19.7%)	7 (20%)	**0.01**	26 (21.3%)	15 (17.6%)	NS	39 (20.4%)	2 (12.5%)	NS
	Medium CAA	32 (15.4%)	23 (13.4%)	9 (25,7%)	**0.01**	22 (18%)	10 (11.7%)	NS	32 (16.7%)	0 (0%)	NS
	Giant CAA	7 (3.4%)	3 (1.7%)	4 (11.4%)	**0.01**	5 (4.1%)	2 (2.3%)	NS	5 (2.6%)	2 (12.5%)	NS
CAA at last follow-up	Normal	194 (93.7%)	162 (94.2%)	32 (91%)	NS	111 (90.9%)	83 (97.6%)	NS	181 (94.7%)	13 (81.2%)	NS
	Dilatation	1 (0.5%)	1 (0.58%)	0 (0%)	NS	0 (0%)	1 (1.2%)	NS	1 (0.5%)	0 (0%)	**<0.001**
	Small CAA	5 (2.4%)	4 (2.3%)	1 (2.9%)	NS	5 (4.1%)	0 (0%)	NS	5 (2.6%)	0 (0%)	**<0.001**
	Medium CAA	3 (1.4%)	3 (1.74%)	0 (0%)	NS	3 (2.4%)	0 (0%)	NS	3 (1.6%)	0 (0%)	**<0.001**
	Giant CAA	3 (1.4%)	2 (1.16%)	1 (2.9%)	NS	2 (1.6%)	1 (1.2%)	NS	1 (0.5%)	2 (12.5%)	**<0.001**
	Tortuous	1 (0.5%)	0 (0%)	1 (2.9%)	NS	1 (0.8%)	0 (0%)	NS	0 (0%)	1 (6.2%)	**<0.001**

Bold values are values that are statistically significant.

to 6 years, 8 years and more. A follow-up echocardiogram at 6 to 18 months was performed in 137 patients (66.2%, median time 12 months). Forty-five patients had an echocardiography follow-up at the second time frame (21.7%, median time 4 years) and 34 patients after 8 years or more (16.4%, median time 10 years).

Comparison between initial coronary artery diameter and at different time frames is shown in **Figure 4**. 89.8% of patients ($n = 123$) had normal coronary artery at first follow-up (6–18 months) echocardiography, compared to 82.3% ($n = 28$) at the last one. This is not related to recurrence of cardiac abnormalities but to a longer duration of the follow-up of patients with cardiac sequelae.

The first follow-up echocardiography (6–18 months) showed normal coronary artery in almost 90% of patients, compared to 34.3% in the initial one. This large difference is related to CAA and coronary dilatation or perivascular brightness regression: of the 96 patients with initial changes in coronary arteries diameter, 84 (87.5%) had only a transient abnormality and CAA or dilatation regressed over time. Regression occurred mostly within 3 months (range 2 weeks to 19 years) and only 7 of the 84 patients (8.3%) with transient abnormality had regression 2 years or more after the acute phase. Dilatations were transient in 93.7% of cases (15 patients of the 16 with initial dilatation), small CAA in 92.6% (38 of the initial 41), medium CAA in 84.3% (27 of the initial 32). Of the seven patients with giant CAA, two showed complete resolution, three partial regression, and the last two stayed giant.

Absence of IVIG in the acute phase was associated with less regression rate (57.1 vs. 92.2%, $p = 0.07$), and this association was especially important for giant CAA persisting at last echocardiography (0.5% of giant CAA in IVIG group vs. 12.5% if no IVIG was given during the acute phase, see **Table 3**). In addition, boys had greater z-score at last follow-up, statistically significant for the LAD (boys: median z-score 1.28, range 0.06–18.4/girls: median z-score 0.9, range 0.01–2/$p = 0.03$).

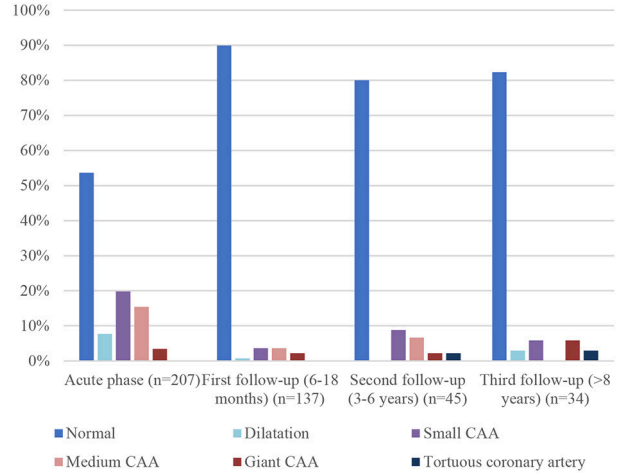

FIGURE 4 | Comparison between initial coronary artery diameter and at different time.

Complications of KD during follow-up occurred in 8 patients (3.8%). The most frequent complication was recurrence of KD ($n = 5$, 2.4%). Of those with recurrence, the 2nd onset of KD happened within 1 year of the acute phase in 3 cases (60%) and within 1 to 5 years for the 2 (40%). MI occurred in 3 patients (1.4%): one patient had antero-septal MI within 1 year of the onset of KD, the other two had signs of ischemia on stress-MRI or myocardial perfusion scintigraphy 5–10 years after KD. Surgery was performed in one of them (coronary artery reimplantation). MI affected only children with initial CAA. Twenty-nine patients were treated with ASA for 1 year or more (14%) and 7 for more than 10 years (3.4%). We found no statistically significant predictive factor for the occurrence of long term complication. However, the absence of IVIG treatment during the acute phase was associated with more complications during the follow-up, but with no statistically significant

difference (No IVIG: 12.5% of complications vs. IVIG: 3.6%, $p = 0.09$).

DISCUSSION

This is the 1st study of medium to long-term follow-up of children diagnosed with KD at our Institution. The epidemiological characteristics, with a majority of patients <5 years of age with predominance of male gender and the seasonal trends, were similar to those reported in other studies from other countries (9, 14, 33–35). Incomplete KD rates were higher than expected, with 29.5% of incomplete cases compared to 20% in other studies. This difference may be associated with a high index of suspicion of this presentation of the disease at our Institution. However, patients with incomplete KD were identified and treated later than the other, highlighting the difficulty in identifying these patients. In any case, it is crucial for physicians to maintain a high index of suspicion for every child presenting with symptoms suggestive of KD. Increase in incomplete KD over time was also reported in other articles (5, 10).

The occurrence of KD has increased over time, as found in other studies (4, 5). Part of this increase may be related to a better diagnosis of KD associated with a highest awareness of the disease and an improvement in the imaging modalities.

Many non-specific clinical features were present in addition to the diagnostic criteria. This polymorphous presentation of KD makes the diagnosis even more challenging, with a large differential diagnosis.

The majority of children received treatment before the 7th day of illness, but there were still 22.7% of patients with a late therapy due to late diagnosis. This rate in delayed treatment is similar to other studies, but it is crucial to improve diagnosis and early therapy as we found that IVIG treatment during the acute phase is associated with greater regression rate and, thus, with less cardiac sequelae.

Other therapies were used in the acute phase, such as corticosteroids, IL-1 receptor antagonist or TNF inhibitor. The role of those treatments is not adequately assessed yet and it would be useful to clarify their indications.

The proportion of IVIG non-responders was comparable to other studies (32). We found no differences in terms of gender or age between IVIG responders and non-responders, as seen in other studies (29, 36).

Treatment of patients who fail to respond to initial therapy is not fully standardized but American Heart Association recommend retreatment with à 2nd infusion of IVIG (evidence level B). In our study, the majority of patients received a 2nd dose of IVIG but further studies would be needed to define clearly the best therapy for the non-responders.

The proportion of patients with abnormal echocardiography at diagnosis was high (65.7%). This number is high because it includes perivascular brightness. If we look only at coronary artery diameters, 46.4% patients developed abnormalities, with 7.7% of dilatation and 38.6% of CAA. This number of dilatation and CAA is higher than expected (8, 18, 33) and is a consequence of an increased recognition of these lesions, as we used z-score in this study. Indeed, it has been established that z-score allow for better evaluation of the severity of coronary artery changes by correcting for BSA, unlike the Japanese criteria that underestimate coronary involvement (1, 32, 37, 38). Z-score calculations changed the degree of coronary artery involvement from the initial echocardiographic report in more than 50% of patients, which had underestimated the coronary artery lesion in the majority of cases. This fact shows how essential the z-score calculations is in KD. However, z-score use has to be done with caution, as it is proved that small error in measurement of the coronary artery diameter can produce large difference in z-score calculation (32). Furthermore, accurate measurement of BSA can be difficult in irritable young children. As seen in other studies, patients with atypical ages for KD (<1 or >10 year of age) had a higher incidence of coronary artery dilatations and aneurysms (10, 34). Sites of coronary artery lesions were comparable to other study: the left coronary artery (LAD > LMCA) was more affected than the RAD (39).

Pericarditis rate was in agreement with other studies (18). As expected, pericardial involvement was transient and no chronic pericarditis occurred.

Valvular involvement was found in almost 20% of patients in this study. Valvular disease rate varies widely in literature (from 1 to 25%) (2, 32). This large difference is probably related to variable definitions of valvular involvement. In our study, we considered even slight abnormalities without clinical consequences as valvular disease. As expected, mitral valve was affected in the vast majority of cases (32).

The majority of our patients with coronary diameter changes had good outcome and more than 85% of CAA and dilatations regressed after the acute phase. This regression rate was higher than expected, with 50–67% reported in literature (1). This difference is probably due to an increased recognition of coronary artery changes, associated with the use of z-scores in spite of diameters with no correction for BSA. As seen in other studies, CAA resolution occurred mostly within 2 years after onset of KD.

Absence of IVIG in the acute phase was associated with less regression rate, which highlight IVIG importance in KD treatment. Additionally, male gender was associated with greater z-score on LAD. Thus, close follow-up of male and patients who did not received IVIG is crucial.

Complications after the acute phase documented in our patient charts were rare (3.8%) and only 3 patients suffered from MI during the study. Recurrence of KD was also rare, as seen in other countries (21). Furthermore, mortality after the acute-phase of KD was not higher than in the general population, and the increase in mortality rate among males with cardiac sequelae reported in a study was not confirmed here (26). However, it would be useful to continue follow-up of patients with a history of KD to confirm that their long-term prognosis remains good. Indeed, at the end of the study period, the oldest patient was only 36 years of age. It is therefore difficult to define exactly the cardiovascular prognosis of patients with a history of KD when they reach middle age. Further trials would be crucial to determine whether patients with prior KD have higher cardiovascular risk and could benefit from aggressive prevention

of known and modifiable risk factor for MI (diabetes mellitus, hypertension, dyslipidemia, smoking, obesity).

The present study must be viewed in the light of some limitations. First, it has the limitations of a retrospective study, with lacking or incomplete information in the patient's medical record. Second, the guidelines for KD, and therefore the management of patients at our Institution, changed throughout the study period, which makes the follow-up of the patients included in this article not totally standardized. And as the study time frame is long, the follow-up of some children is incomplete, prematurely interrupted due to patients moving or transferred to adult cardiologist at a different center as the patients grow-up. Third, there is a bias of selection during the follow-up at the different times frames, as the follow-up of patients with transient CAA was shorter than the one with cardiac sequelae.

Finally, it is a single-center study and extrapolation of our results needs cautious interpretation. However, as it describes all patients at a single institution during a long-time frame, it offers a global picture of KD and its evolution in our region.

CONCLUSION

In conclusion, this study shows that medium to long-term prognosis after usual treatment of KD is excellent and the majority of children do not have any cardiac sequelae or suffer from complication during the follow-up. Risk factors for poorer outcome are male gender, atypical age and absence of IVIG infusion during the acute phase. It is therefore crucial for physicians to have a high index of suspicion among children having those characteristics and offer them a strict and standardized follow-up.

Despite numerous published studies, there remains a lot unknown about the clinical features and medium to long terms outcomes in children with KD. Thus, caution is necessary during follow-up and minimization of known cardiovascular risk factor is recommended for every patient with a history of KD. Further prospective multicenter studies are warranted in order to elucidate all residual interrogations about this disease and to clarify long-term outcome of KD survivors.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

This study was approved by the institutional ethics committee (Commission cantonale d'éthique de la recherche sur l'être humain). Informed consent was waived by ethics committee as this is a retrospective study based only on medical files, that covers a period longer than 30 years.

AUTHOR CONTRIBUTIONS

MdL performed the study and wrote the paper. SdB did statistical analysis and reviewed the paper. MH contributed to the design of study and reviewed the paper. NS designed the study with MdL supervised the study and reviewed the paper.

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Heart Rate Response During Treadmill Exercise Test in Children and Adolescents With Congenital Heart Disease

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Background: Impaired exercise capacity is a common feature of congenital heart disease (CHD). In adults with CHD, it has been shown that impaired heart rate response during exercise may contribute to exercise limitation. Systematic data in children and adolescents on this topic is limited. We therefore purposed to assess heart rate response during treadmill exercise testing in children and adolescents with CHD compared to healthy controls.

Methods: One hundred and sixty three children and adolescents (103 with CHD, median age 15 years and 60 age-matched controls) performed cardiopulmonary exercise testing and were included in this study. Beyond peak oxygen consumption, increase in heart rate from resting level to peak exercise (heart rate reserve) and decrease of heart rate after peak exercise (heart rate recovery) were measured. Chronotropic index was defined as percentage of age predicted maximal heart rate reserve. According to data from adults on bicycle exercise, chronotropic incompetence was assumed for chronotropic index below 0.8.

Results: While resting heart rate was similar between both groups, peak heart rate, heart rate reserve as well as chronotropic index were lower in the CHD group than in controls. Chronotropic index was lowest in patients with single ventricle hemodynamics and correlated with peak oxygen consumption. Heart rate recovery was impaired in the CHD group 1 and 2 min after peak exercise compared to controls and correlated with peak oxygen consumption. Chronotropic index below 0.8 was a relatively frequent finding even in the control group suggesting that the threshold of 0.8 appears inadequate for the identification of chronotropic incompetence using treadmill exercise testing in children. After normalizing to the 2.5th chronotropic index percentile of the control group we obtained a chronotropic incompetence threshold of 0.69.

Conclusion: As an adjunct to measurement of peak oxygen consumption, heart rate response to exercise appears to be a physiologically important diagnostic parameter in

children and adolescents with CHD. However, interpretation of heart rate response needs to consider specific age characteristics and the mode of exercise test. Our data may help future studies on chronotropic incompetence using treadmill ergometer protocols in children and adolescents.

Keywords: heart rate response, chronotropic incompetence, heart rate recovery, cardiopulmonary exercise testing, congenital heart disease

INTRODUCTION

Congenital heart disease (CHD) are among the most common congenital defects. Every year ∼1.5 million children worldwide are born with CHD (1). Progress in medical care has led to an improved survival of patients after surgical repair of CHD. Nowadays ∼90 percent of CHD patients have the prospect to reach adulthood (2, 3).

However, many CHD patients suffer from impaired exercise capacity. The assessment of peak oxygen consumption is the gold standard for the assessment of exercise tolerance (4) and has been widely used for evaluation of exercise capacity in individuals with CHD (5). In adults with CHD, it has been shown that impaired heart rate response during bicycle exercise may contribute to exercise limitation (6). Heart rate response during exercise is a complex composition of chronotropic reserve during exercise and heart rate recovery after cessation of exercise. Interaction of the sympathetic and parasympathetic nervous system plays an important role in the modulation of heart rate during exercise (7). In CHD patients this may be affected for different reasons including ischemia and/or denervation resulting from surgical procedure or in case of cyanotic CHD from chronic hypoxemia (8).

Systematic data in children and adolescents on this topic are limited and might differ from adult data due to different reasons: Firstly, differences in cardiorespiratory response to exercise between adults and children exist. In children a higher total peripheral resistance is seen. Smaller muscle mass and smaller heart size in children result in lower venous return and contribute to a lower stroke volume making the increase in heart rate the most important compensatory mechanism to increase cardiac output (9, 10). Therefore, heart rate response during exercise may play a more crucial role in the assessment of exercise limitation in children and adolescents than in adults.

Secondly, the mode of exercise testing may play a relevant role, as most frequently exercise testing in children is performed using a treadmill rather than a bicycle as in adults (11). Hereby upright body posture could result in higher resting heart rates, a phenomenon known from tilt table test, which may have impact on the interpretation of chronotropic response (12). We therefore pursued to assess heart rate response during treadmill exercise test and its impact on exercise performance in children and adolescents with CHD compared to healthy controls.

METHODS

Study Population

We retrospectively analyzed all evaluable cardiopulmonary exercise tests (CPET) performed in an outpatient clinic setting at our referral center between January 2015 and December 2016. Patients were referred for exercise testing as part of routine clinical follow-up protocols used for patients with CHD at our institution. Informed consent was obtained from all patients undergoing exercise testing. Almost all patients underwent only one test during the period; for those who underwent two tests we addressed only the first test. Patients treated with betablockers or pacemakers were excluded. A main diagnosis was determined for every patient from hospital records.

One hundred sixty-three examinations of children and young adults were eligible for analysis, including 103 examinations in CHD patients with varying underlying heart disease.

Sixty age-matched normal pediatric patients served as controls who had no cardiac defects or family history of cardiac disease or they were patients who were evaluated in the cardiology clinic for heart murmurs, chest pain, or syncope with normal structure hearts.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the local ethics committee.

Cardiopulmonary Exercise Testing

CPET was performed on an h/p/cosmos mercury med (Nussdorf-Traunstein, Germany) treadmill according to a modified Bruce protocol as described previously by Dubowy et al. (11). All subjects were encouraged to exercise until exhaustion regardless of the maximal heart rate achieved. Ventilation, oxygen uptake, and carbon dioxide production were measured continuously. Heart rate was assessed with 12-lead electrocardiography. Resting heart rate was measured after at least 2 min in an upright standing position and peak heart rate was defined as the maximal heart rate achieved during exercise. All patients with a respiratory exchange ratio (RER) at peak exercise $\geq$1.01 while reaching the second ventilatory threshold or a RER $\geq$1.04 as previously described by other study groups (13, 14) were considered to have performed a maximal CPET.

Heart Rate Reserve, Chronotropic Index, and Chronotropic Incompetence

Heart rate reserve was calculated as the difference between peak and resting heart rate. Chronotropic index was calculated by a

TABLE 1 | Study group characteristics.

Controls $n = 60$ (47% female)	Congenital heart disease (CHD) $n = 103$ (46% female)	
	Fontan circulation	$n = 12$ (42% Female)
	Pulmonary arterial hypertension (PAH)	$n = 12$ (58% Female)
	Complex anatomy, biventricular corrected	$n = 18$ (33% Female)
	Tetralogy of Fallot	$n = 21$ (67% Female)
	D-Transposition of the great arteries (TGA)	$n = 12$ (50% Female)
	Septal defects	$n = 13$ (46% Female)
	Valvular defects	$n = 15$ (20% Female)

formula [(peak heart rate–resting heart rate)/(220–age–resting heart rate)] derived from the chronotropic metabolic relationship concept introduced by Wilkoff and Miller (15). Thus, facilitating comparability of a normal chronotropic response irrespectively of age, resting heart rate, and functional state. In a group of 410 healthy adults Wilkoff and Miller reported 95% limits of normality for chronotropic index to be 0.8–1.3. Based on this finding, chronotropic incompetence is usually defined as failure to achieve a chronotropic index of 0.8 or higher (i.e., falling below 97.5 percent of healthy adults).

Heart Rate Recovery

Heart rate recovery (HRR) was recorded as decrease of heart rate 1, 2, and 3 min after cessation of peak exercise. Relative HRR was calculated dividing the heart rate after 1, 2, and 3 min by the heart rate at peak exercise.

Statistical Analysis

All values are given as mean ± standard deviation unless otherwise stated. Comparison between CHD- and non-CHD (sub)-groups was made using Mann-Whitney-*U*-test. For correlation analysis Spearman-Rho test was applied. Statistical analysis was performed using SPSS Statistics for Windows, version 24.0 (IBM Corp. Released 2016, Armonk, NY). *P*-values ≤ 0.05 were considered statistically significant.

RESULTS

Study Group Characteristics

Subgroup apportionment for the CHD cohort is shown in **Table 1**. Control and CHD group showed similar gender distribution. Age, weight, height, and body mass index did not differ substantially. Main demographic characteristics and CPET findings are listed in **Tables 2A,B**.

Chronotropic Index and Chronotropic Incompetence

Resting heart rate was similar between study groups. Peak heart rate, heart rate reserve as well as chronotropic index were lower in the CHD group than in controls, details shown in **Table 3**. Chronotropic index was lowest in patients with

TABLE 2 | Demographic and CPET data.

	Controls	CHD	
A DEMOGRAPHIC DATA			
Age [years]	13.0 ± 3.8	14.8 ± 4.8	
Weight [kg]	50.1 ± 15.7	54.4 ± 20.6	
Height [cm]	157.2 ± 16.3	159.6 ± 17.9	
BMI [kg/m²]	19.8 ± 3.4	20.5 ± 4.5	
	Controls	**CHD**	***p***
B CPET DATA			
Loading time [min]	11 : 53 ± 1 : 35	10 : 39 ± 2 : 02	<0.001
Distance [m]	905.9 ± 217.6	803.5 ± 214.0	<0.01
Speed [km/h]	6.2 ± 0.5	5.7 ± 0.7	<0.001
METS	14.8 ± 1.8	13.1 ± 2.7	<0.001
Peak VO_2 [l/min]	2.1 ± 0.73	1.8 ± 0.75	<0.05
Indexed peak VO_2 [ml/min/kg]	41.7 ± 7.0	34.3 ± 7.7	<0.001
V'/E [l/min]	63.5 ± 21.7	62.0 ± 22.9	n.s.
$V'E/V'O_2$	28.8 ± 3.6	32.3 ± 6.0	<0.001
$V'E/V'CO_2$	27.6 ± 2.8	30.6 ± 5.4	<0.01
RER	1.04 ± 0.08	1.06 ± 0.08	n.s.
VT	1.49 ± 0.50	1.50 ± 0.67	n.s.
RR [min^{-1}]	43.2 ± 7.3	43.6 ± 10.1	n.s.

CPET, Cardiopulmonary exercise testing; METS, Metabolic equivalent; Peak VO_2, Peak oxygen consumption; V'/E, minute ventilation; $V'E/V'O_2$, Ventilatory equivalent of oxygen; $V'E/V'CO_2$, Ventilatory equivalent of carbon dioxide; RER, Respiratory exchange ratio; VT, Ventilatory threshold; RR, Maximum respiratory rate. All parameters given at peak VO_2.

TABLE 3 | Heart rate characteristics during CPET.

	Control	CHD	*p*
Resting HR [bpm]	85.0 ± 14.6	83.3 ± 13.3	0.81
Peak exercise HR [bpm]	189.0 ± 8.3	180.0 ± 13.3	<0.001
HR reserve [bpm]	104.0 ± 13.6	96.2 ± 18.0	0.004
Chronotropic index	0.85 ± 0.07	0.79 ± 0.11	0.001
1 min HRR			
Absolute [bpm]	−37.8 ± 15.0	−30.2 ± 12.4	0.002
Relative	0.80 ± 0.08	0.83 ± 0.07	0.01
2 min HRR			
Absolute [bpm]	−57.1 ± 12.8	−50.3 ± 14.5	0.004
Relative	0.70 ± 0.07	0.72 ± 0.08	0.05
3 min HRR			
Absolute [bpm]	−64.0 ± 13.2	−59.1 ± 13.7	0.10
Relative	0.66 ± 0.07	0.67 ± 0.07	0.58

Heart rate characteristics during cardiopulmonary exercise testing. Values as mean ± standard deviation. CHD, Congenital heart disease; HR, heart rate; HRR, heart rate recovery (HR decrease from peak exercise); bpm, beats per minute.

Fontan circulation as shown in **Figure 1**. Chronotropic index was related to peak oxygen consumption (Spearman-rho correlation coefficient 0.25). Frequency of chronotropic incompetence in the study subgroups is displayed in **Figure 2**. Defining a chronotropic index of 0.8 as chronotropic incompetence threshold revealed a relatively high rate of chronotropic incompetence even in the control group (**Figure 2**), most likely due to an overestimation of the maximal heart rate using the (220-age) formula. Therefore, **Figure 3** is normalized to a chronotropic incompetence threshold defined as the 2.5th percentile of chronotropic index in the control group.

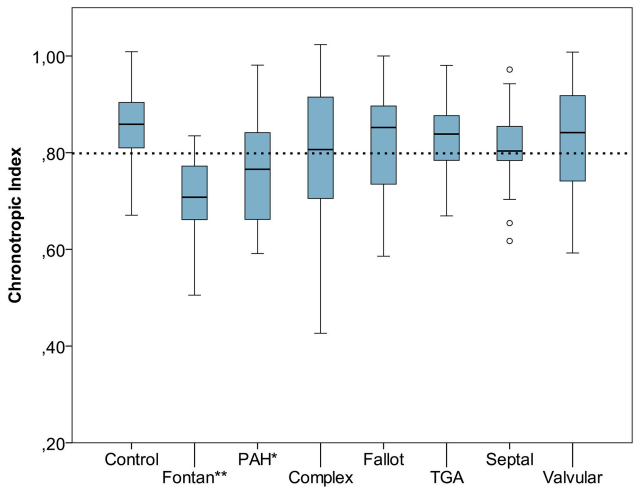

FIGURE 1 | Chronotropic index [Chronotropic index = (peak heart rate–resting heart rate)/(220–age–resting heart rate)] of control group vs. congenital heart disease (CHD) subgroups. Dashed line marks chronotropic incompetence threshold of 0.8. $^{*}p<0.05$, $^{**}p<0.001$. Boxplot bottom indicates 25th, top of the box 75th percentile. Whiskers extend to 1.5 times of box height or to minimum/maximum value. Outliers are shown as points.

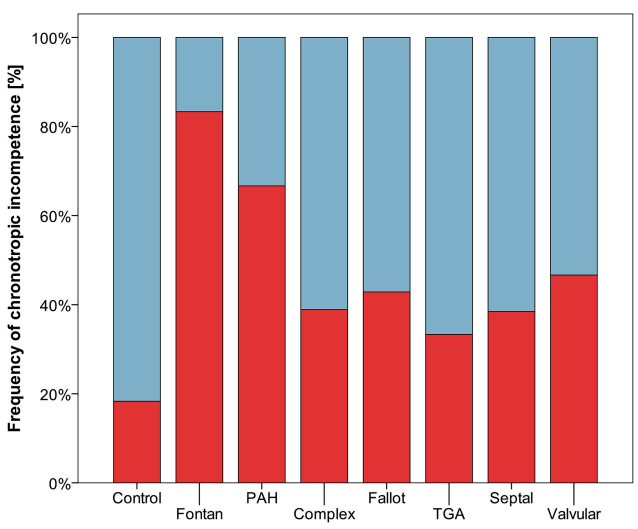

FIGURE 2 | Frequency of chronotropic incompetence (threshold defined as chronotropic index < 0.8) marked red.

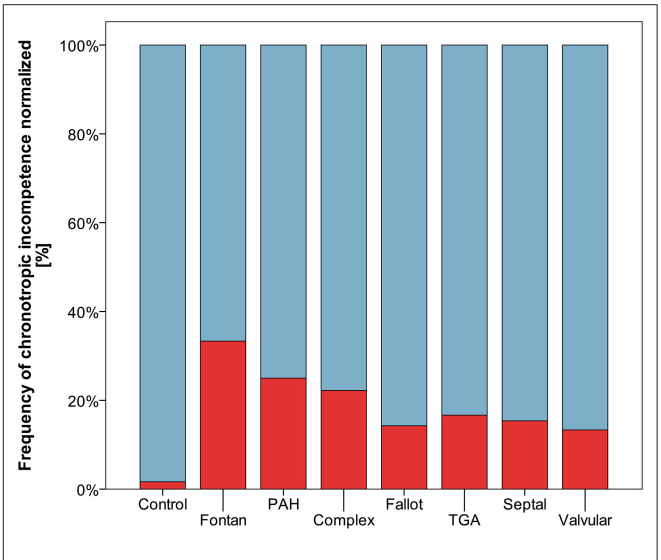

FIGURE 3 | Frequency of chronotropic incompetence normalized to healthy controls (threshold defined as chronotropic index < 0.69 corresponding to the 2.5th percentile of chronotropic index in controls) marked red.

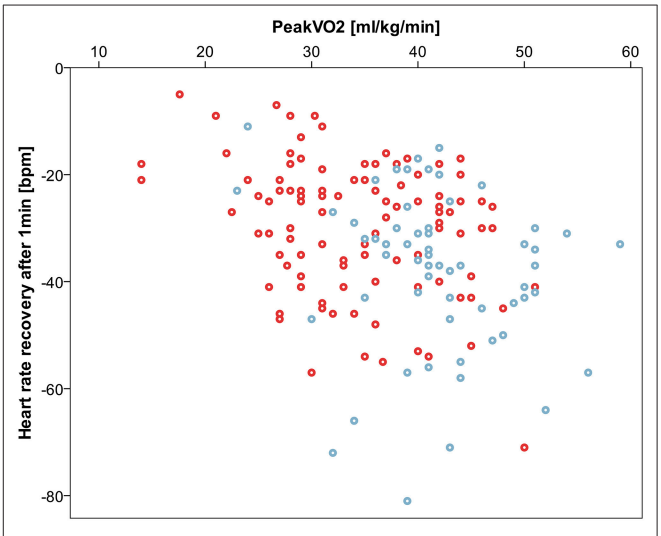

FIGURE 4 | Correlation between 1 min heart rate recovery (HRR) and peak oxygen consumption (peak VO_2). Spearmans-rho correlation coefficient −0.35. Controls displayed blue, congenital heart disease red.

Heart Rate Recovery

HRR measured as heart rate decrease from peak exercise heart rate within a defined interval was impaired in CHD patients compared to controls after 1 and 2 min and converged 3 min after peak exercise level. Concordantly, relative HRR was higher in the CHD group after 1 and 2 min confirming a deferred heart rate decrease. Details are listed in **Table 3**. Correlation of HRR and peak oxygen consumption was noteworthy with Spearmans-rho correlation coefficients of −0.35 after 1 min, −0.43 after 2 min, −0.37 after 3 min. **Figure 4** shows a scatterplot of 1 min HRR and peak oxygen consumption. HRR was most impaired in the PAH subgroup compared to healthy controls with a HRR of −22.2 ± 13.7 bpm (relative HRR 0.87) after 1 min, −36.7 ± 15.2 bpm (relative HRR 0.79) after 2 min and −44.1 ± 15.5 bpm (relative HRR 0.75) after 3 min, all p-values < 0.01 compared to healthy controls.

DISCUSSION

To the best of our knowledge, this study is the first to assess heart rate response during treadmill exercise in children and

adolescents with CHD. Our results show that as an adjunct to the measurement of peak oxygen consumption, heart rate response during exercise appears to be a physiologically important diagnostic parameter in children and adolescents with CHD.

Our data may help to interpret future studies on chronotropic incompetence using treadmill ergometer protocols in children and adolescents.

Chronotropic Incompetence

The limited increase in heart rate during exercise (chronotropic incompetence) is known as a predictor of mortality in adult patients with coronary artery disease and in healthy adult population (16–18). Diller and colleagues detected impaired chronotropic response during exercise also in an adult CHD cohort, even with a prevalence of up to 62% (6).

Data regarding chronotropic response during exercise in children and adolescents is scarce. Our results highlight the importance to consider age specific characteristics and the mode of exercise test when interpreting heart rate response in children and adolescents. This is particularly apparent for the calculation of chronotropic index. The used formula to calculate chronotropic index (CI) is intended to normalize chronotropic response independently of age but it was developed for an adult cohort. Threshold definition for CI is variable throughout literature (19). In the original publication Wilkoff and Miller reported 95% limits of normality for chronotropic index to be 0.8–1.3 in a group of 410 healthy adults (15). Hereby CI had been defined as failure to achieve a chronotropic index of 0.8, i.e., falling below the 2.5th percentile of healthy adults, while 2.28th percentile represents −2 standard deviations, however rounding up to 2.5th percentile has been done before when reporting age related normal ranges (13). Whether the CI threshold of 0.8 is applicable in children and young adults has not been demonstrated so far and raises doubt. Using the (220-age) formula it is likely to overestimate the maximum heart rate of young subjects (20, 21). In addition, the above named reference dataset was assessed using a bicycle ergometer. The use of a treadmill ergometer as in our study might theoretically result in a higher resting heart rate level by recording the heart rate in an upright standing position and not in a sitting position. The resting heart rate is included in the calculation of the chronotropic index and thus a higher resting heart rate results in lower values for chronotropic index. This might explain the relatively high rate of CI in our control group applying a CI threshold of 0.8.

To cope with this bias, we adopted the above mentioned concept of Wilkoff and Miller, and normalized our study cohort to the 2.5th chronotropic index percentile of the control group, thus obtaining a CI threshold of 0.69. Still markedly higher frequencies of CI persist throughout the CHD spectrum. However, our data may help to interpret future studies on chronotropic index and CI using treadmill ergometer protocols in children and young adults.

As expected, in our study prevalence of CI was lowest in patients with minor lesions such as small shunt defects, or valvar defects, and was most present in patients with single ventricle circulation. Remarkably, the increase in prevalence of CI parallels the decline in peak oxygen consumption across the spectrum of CHD. Impaired heart rate response to exercise may therefore in part account for the diminished exercise capacity seen in these patients (22).

Heart Rate Recovery

A deferred decrease of heart rate after cessation of exercise (heart rate recovery, HRR) is associated with increased mortality in adult patients with coronary artery disease and is a marker for poor outcome after pediatric heart transplantation (23). An increased risk of death with impaired HRR was even found in a subgroup of patients without heart failure or myocardial perfusion defects (24). Diller and colleagues revealed heart rate recovery as predictor of mortality also in an adult CHD cohort (6).

In this study we also detected lower HRR in children and adolescents with CHD and revealed an inverse correlation to peak oxygen consumption. Underlying mechanisms responsible for impaired HRR are not fully understood but may at least partly be attributed to an impaired autonomic function. Especially the immediate phase of HRR after cessation of exercise is thought to be promoted by vagal reactivation, which is followed by a sympathetic withdrawal during subsequent minutes (25).

This study highlights the relevance of heart rate response during exercise as an important piece of physiological response to extract from CPET alongside with conventional parameters. Whether specifically targeting abnormal heart rate reserve could improve exercise performance and affect prognosis remains unknown. Cardiac rehabilitation with structured training has been described to improve peak exercise capacity as well as early HRR in patients with repaired complex CHD (26). Preliminary data in pediatric pulmonary arterial hypertension patients suggest that exercise training may improve exercise performance at least in part by improved chronotropic competence (27). The impact of heart rate response targeting rehabilitation programs on longterm outcome needs to be evaluated in future studies.

Study Limitations

The results of this study are limited by the lack of randomization and the relatively small number of patients. A referral bias where patients doing well have been preferentially selected cannot be ruled out with certainty, especially as we excluded patients on betablocker therapy and with pacemakers. However, in this study CPET was performed as part of routine evaluation in our pediatric cardiology outpatient clinic. Patients therefore were not restricted to any particular, narrow diagnostic group but rather the whole spectrum of CHD diagnoses was covered (including patients with mild, modest and complex lesions, as well as single ventricle and biventricular hemodynamics) and patients regardless of age, gender, history, and nature of surgery were included.

The formula for the chronotropic index by Wilkoff and colleagues might not be ideal for children and adolescents as it leads to an overestimation of chronotropic incompetence in young participants by using [220 − age] as formula to determine predicted maximum heart rate. Nevertheless, the formula by

Wilkoff and Miller is to our knowledge the best concept for the assessment of chronotropic incompetence. To obtain a realistic cut-off for detection of chronotropic incompetence in childhood we adapted the threshold to the 2.5th percentile of our healthy population (in accordance with the methods used by Wilkoff and colleagues in their original publication). Clearly, the presented study is not adequately powered to obtain a new formula for the use in children and adolescents but our findings highlight the need for future prospective studies investigating heart rate response and chronotropic incompetence in this age group.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the supplementary files.

ETHICS STATEMENT

The study protocol was approved by the ethics committee of the University of Ulm, Helmholtzstr. 20, 89081 Ulm (reference number 77/18).

AUTHOR CONTRIBUTIONS

FvS: concept, data interpretation, drafting article, statistics, approval of the final version of this manuscript. SM: concept, data acquisition, statistics, critical revision, approval of the final version of this manuscript. JK: concept, data acquisition, critical revision, approval of the final version of this manuscript. AA, JS, PB, and MK: data acquisition, critical revision, approval of the final version of this manuscript. CA: concept, data interpretation, drafting article, approval of the final version of this manuscript.

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Neutrophil-to-Lymphocyte Ratio Predicts Intravenous Immunoglobulin-Resistance in Infants Under 12-Months Old With Kawasaki Disease

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Objective: We evaluated the ability of peripheral blood neutrophil-to-lymphocyte ratio (NLR) to predict the intravenous immunoglobulin (IVIG) resistance in Kawasaki disease (KD) patients under 1-year of age.

Methods: A total of 92 KD patients under the age of 1-year and who were hospitalized in Peking University First Hospital from June 2007 to August 2016 were recruited in this study. The clinical and laboratory data were analyzed to see if peripheral blood NLR was useful for predicting the IVIG-resistance in KD.

Results: Totally 81 out of 92 patients were IVIG responders while 11 resistant to IVIG, with no significant difference in age, gender, ratio of the number of the incomplete to the number of complete KD, and the number of patients with coronary artery lesion between two groups ($p > 0.05$). Peripheral blood NLR was increased significantly in IVIG-resistant children compared to the IVIG responders [2.6 (interquartile range: 1.4, 3.8) vs. 1.7 (interquartile range: 0.9, 2.3), $p = 0.039$]. A cut-off value of NLR of 2.51 in KD patients younger than 1-year old yielded a sensitivity of 0.545 and specificity of 0.840, respectively, in the prediction of IVIG resistance. An area under the curve of 0.692 (95% confidence interval 0.526–0.859, $p = 0.039$) was determined.

Conclusions: The peripheral blood NLR $\geq$ 2.51 is useful to predict the IVIG resistance in KD patients younger than 1-year old.

Keywords: Kawasaki disease, immunoglobulin, resistance, neutrophil-to-lymphocyte ratio, prediction

INTRODUCTION

Kawasaki disease (KD) is an acute systemic vasculitis first reported in 1967 with unknown etiology affecting small and medium-size arteries (1). 10–15% patients have persistence or reoccurrence of fever 36–48 h after the first dose of intravenous immunoglobulin (IVIG), which is defined as intravenous immunoglobulin resistance (2–7). In recent years, studies showed that 5–38.3% of KD

patients were resistant to IVIG (3, 8–12). The prevalence of IVIG resistance was increased with greater incidence of KD (5, 9, 13, 14). Patients unresponsive to the IVIG therapy experienced a longer duration of fever or other systemic inflammatory symptoms (15). Importantly, the prevalence of coronary artery abnormalities is higher in the IVIG resistance group than in the IVIG responding group (2, 5, 8, 12, 16–21). Egami et al. and Kobayashi et al. reported a 32% incidence of coronary artery lesion (CAL) in KD cases with IVIG non-responders and only 0.8–2% with IVIG responders (9, 10). The remaining reported prevalence of CAA ranged from 17 to 43.3% for KD patients who were resistant to IVIG, while IVIG responders experienced a significantly lower incidence of CAA (2, 6, 9, 10, 13, 22, 23). Although no evidence was found to support a linear relationship between IVIG resistance and CAL, Burns et al. found an 8-fold increase in CAL prevalence in the IVIG resistant group compared to the IVIG responding group (8).

Since IVIG resistance is associated with poor outcomes in KD, especially in infants, it is important to predict IVIG resistance as early as possible to guarantee initial or rescue treatment in time. In previous studies, risk factors for IVIG non-response included male sex, age <12 months, IVIG after day 10 of illness, low hemoglobulin, thrombocytopenia, high neutrophil count, low albumin, low sodium, high C-reactive protein, impaired liver function, and high N-terminal-pro-b-type natriuretic peptide (NT-pro-BNP) (6, 9, 10, 13, 19, 20, 23–31). However, it remains necessary to search for inexpensive, easy-to-perform and useful predictors with high sensitivity and specificity for KD infants <1-year old to predict the IVIG resistance and help the selection of suitable treatment modalities for IVIG resistant cases in clinics.

Peripheral blood neutrophil-to-lymphocyte ratio (NLR) is an indicator combining two independent inflammation markers. It serves as a useful predictor of clinical outcomes of some diseases such as surgery stress and cardiovascular diseases in recent studies (32–34). Ha et al. first reported the correlation between peripheral blood NLR and Kawasaki disease outcomes. When NLR > 5.49, the sensitivity of predicting IVIG non-response was 39% and specificity 86% (35). However, the sensitivity of prediction in Ha's paper was not very high. Cho et al. also confirmed that high NLR was associated with IVIG resistance in Kawasaki disease (36). Takeshita et al. used a combination of NLR and platelet-to-lymphocyte ratio to predict resistance to IVIG (37). In Hua's article, a new scoring system was developed to predict IVIG resistance, which included NLR as a scoring variable (38). Since there is crossover of neutrophil and lymphocyte percentage at 4–6 days and 5-years old, the range of NLR varies significantly with age, which would evidently affect its ability of prediction (39). Therefore, we attempted to determine if NLR could be used as a predictor of IVIG resistance in infants <12 months old with Kawasaki disease.

SUBJECTS AND METHODS

Subjects and Grouping

We retrospectively analyzed Kawasaki disease patients within 1-year old admitted to Peking University First Hospital from June 2007 to August 2016. All of the subjects fulfilled the diagnostic criteria of *Diagnosis, Treatment, and Long-Term Management of Kawasaki Disease* by AHA Scientific Statement (3), and were initially treated with IVIG (2 g/kg) plus oral aspirin. Categorization of patients into IVIG responders and IVIG resistance cases was dependent upon their persistence or reoccurrence of fever 48 h after the first dose of IVIG (5, 6).

Methods

Basic demographic information including gender, age, and the clinical presentations were recorded. Total blood routine analysis and serum biochemistry were evaluated at a median of 5 and 6th day of fever in all patients prior to IVIG treatment, including white blood cell count, neutrophil and lymphocyte counts, platelet count, and levels of hemoglobin, C-reactive protein, liver function, serum albumin, pre-albumin, albumin-to-globulin ratio, and electrolytes. Echocardiography was performed before or after IVIG treatment, and the follow-up assessment of echocardiography was scheduled along with the course of disease. Patients experiencing persistence or reoccurrence of fever ($\geq$38°C) 48 h after the first dose of IVIG were categorized into the IVIG resistance group after excluding other possible reasons. Otherwise, patients were grouped into the IVIG responder group (5–8). This study was approved by the Regional Ethics Committee of our hospital.

Patient and Public Involvement

No patients were included in the design or implementation of the study. Neither were them involved in the interpretation of study results or draft of the manuscript. There are no plans to involve patients in the dissemination of results.

Statistics

Categorical variables such as gender were analyzed by a two-sided χ^2 test and continuous variables such as age and NLR were compared by the Student's *t*-test or Mann-Whitney *U*-test depending on whether a normal distribution was followed. For variables showing a significant difference between IVIG resistance and responder groups, cutoff values were determined by receiver operating characteristics (ROC) curves. All analyses were performed by the SPSS statistical software package ver. 18.0.

RESULTS

Demographic Characteristics and Clinical Outcomes

A total of 92 patients (male 64, female 28), with 81 responding to IVIG and 11 resistant to IVIG, were enrolled in this study (**Table 1**). Twenty-three out of 81 IVIG responders were diagnosed as incomplete KD, while one was found in the resistant group. The proportion of incomplete KD did not differ between IVIG resistant and responder groups ($p = 0.316$). The age of patients in the IVIG resistance group was 8.2 ± 3.2 months old, while the age of patients in the IVIG responder group was 7.6 ± 3.1 months old. The main clinical manifestations of these patients consisted of fever $\geq$5 days, bilateral bulbar conjunctival injection, erythema, and cracking of lips and strawberry tongue. Other symptoms and signs included rash, erythema and edema of hands

TABLE 1 | Demographic and laboratory characteristics of patients.

Groups	No. M/F	Days of fever (day)	Days of CBC (day)	Age (month)	WBC ($\times 10^9$)	Neutrophil Count ($\times 10^9$)	Lymphocyte Count ($\times 10^9$)	NLR	CRP (mg/L)	RDW (%)	PLT count ($\times 10^9$)	iKD/cKD	No. CAL
IVIG resistance	10/1	11 (9, 16)	5 (4, 15)	8.2 ± 3.2	17.6 ± 7.6	10.8 ± 4.1	5.1 ± 3.4	2.6 (1.4, 3.8)	89 ± 39	13.3 (12.6, 14.7)	304 (208, 585)	1/10	6
IVIG responders	54/27	7 (6, 8)	6 (5, 7)	7.6 ± 3.1	15.3 ± 6.6	8.4 ± 5.0	5.5 ± 2.5	1.7 (0.9, 2.3)	68 ± 43	13.2 (12.6, 13.9)	412 (348, 540)	23/58	26
p-value	0.163	0.000	0.756	0.501	0.281	0.130	0.691	0.039	0.139	0.499	0.258	0.316	0.142

CAL, coronary artery lesion; CBC, complete blood count; CRP, C-reactive protein; F, female; iKD/cKD, ratio of incomplete Kawasaki disease and complete Kawasaki disease, IVIG: intravenous immunoglobulin; M, male; NLR, neutrophil-to-lymphocyte ratio; No., number of patients; PLT count, platelet count; RDW, Red cell distribution width; WBC, peripheral white blood cell count.

and feet, periungual desquamation, cervical lymphadenopathy, etc. The incidence of CAL did not differ significantly between IVIG responder and resistant groups ($p = 0.142$).

There was no significant difference in age and gender between the two groups ($p = 0.501$ and $p = 0.163$).

The Changes of Peripheral Blood NLR Between the 2 Groups of KD Infants

Peripheral NLR was significantly greater in the IVIG resistance group than in the IVIG responder group [2.6 (interquartile range: 1.4, 3.8) vs. 1.7 (interquartile range: 0.9, 2.3), $p = 0.039$]. However, no statistical difference in peripheral blood white blood cell count (WBC), neutrophil count and lymphocyte count was observed between the IVIG responder and resistance group ($p = 0.281$, $p = 0.130$, and $p = 0.691$).

Cut-Off Value of NLR Predicting IVIG Resistance

The area under the curve (AUC) for predicting IVIG resistance with peripheral blood NLR was 0.692 (95% confidence interval 0.526–0.859, $p = 0.039$) (**Figure 1**). An NLR cut-off value of 2.51 yielded a sensitivity of 0.545 and specificity of 0.840 for predicting IVIG resistance in infants with KD.

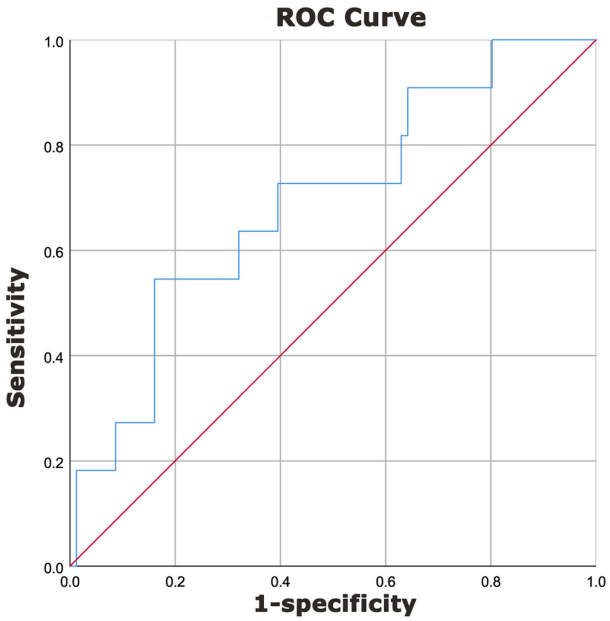

FIGURE 1 | Diagnostic characteristics of NLR for prediction of IVIG resistance in KD patients <1-year of age. A receiver operating characteristics curve was performed to determine the cut-off value of NLR to predict IVIG resistance. A cut-off value of 2.51 yielded a sensitivity of 0.545 and a specificity of 0.840 for predicting IVIG resistance in patients with KD. The area under the curve (AUC) was 0.692 (95% confidence interval 0.526–0.859, $p = 0.039$).

DISCUSSIONS

CAL is a major complication of Kawasaki disease, which was found to be correlated with IVIG resistance. According to Miura et al. IVIG resistance was found to be significantly associated with both coronary events and major adverse cardiac events, with a hazard ratio of 2.2 (95% CI 1.4–3.6) and 3.1 (95% CI 1.5–6.3), respectively (40). This might be due to more severe inflammation-induced damage to the vascular wall in the IVIG-resistant group than in the IVIG responder group.

Around 5–38.3% of KD patients were reported resistant to IVIG in recent studies conducted (2, 5, 8, 12, 16–21). Predictors for IVIG resistance included variables in blood routine, liver function, serum electrolytes, cardiac enzymes, as well as age, gender and illness days when the initial treatment was applied (2, 5, 6, 19, 20, 23, 27, 29, 30, 35, 36, 41). There were several scoring models to predict IVIG resistance in different countries (5, 9, 10, 31). Egami scoring involved age, illness days, platelet count, ALT and CRP, yielding a sensitivity, and specificity of 78 and 75% in predicting IVIG resistance. As for Kobayashi scoring, which consisted of seven variables, the sensitivity and specificity in the prediction of resistance to IVIG were 86 and 68%, respectively. When applying Sano criteria, a sensitivity of 77% and specificity of 86% were expected in predicting the resistance to IVIG in KD (5, 9, 10, 31).

In younger age groups, the prognosis and outcome of KD have attracted great attention. As reported by Uehara and Fu et al. 19.8–32% younger patients were not responsive to IVIG (10, 12, 19). In the study by Kobayashi et al. ages <1-year old were regarded as a risk factor for IVIG resistance (9). Meanwhile, the study by Egami et al. showed that the age range within 6 months old was considered a risk factor for IVIG resistance (10). Although the clinical symptoms and signs are less typical for young patients with Kawasaki disease, reports by several groups including McCrindle et al. showed that the CAL incidence was common compared to

overall data in younger-aged patients, with fewer symptoms than listed in diagnosis criteria presented by the patients when diagnosed (4, 42–48). Therefore, the early prediction of IVIG resistance is necessary in young infants with Kawasaki disease to prevent further CAL during the course. However, for younger patients, especially that of infant patients with KD who would have poorer prognosis if resistant to IVIG, no validated, and easy-to-perform biomarkers were found for IVIG resistance prediction in clinical practices (38). Therefore, it is urgent that predictive biomarkers of IVIG resistance, especially for patients <1-year old, be identified to determine a proper treatment modality for infant KD cases and therefore improve their prognosis.

The peripheral white blood cell count and its subpopulations are classic markers of inflammation. Recently however, NLR was reported to be a powerful indicator of systemic inflammation, sepsis, surgical stress and cardiovascular diseases, and cancers (32–34). Neutrophil counts reflect ongoing inflammation, while lymphocyte counts are considered a marker of immune regulatory response. NLR, a combination of neutrophils and lymphocytes, serves as a marker of balance between inflammation and immune regulation (32, 49). At the pathological level, infiltration of neutrophils in the coronary arteries is observed at the early phase, while lymphocytes dominate in the late phase. Therefore, NLR increases at the acute stage and decreases gradually during later stages of Kawasaki disease. This pattern indicates an accelerated inflammatory response in which patients resistant to IVIG might have a more severe inflammatory course.

In our study, we found that patients resistant to IVIG had a significantly higher NLR than patients <1-year of age responding to IVIG ($p = 0.039$). If NLR is no <2.51, patients with KD younger than 1-year can be predicted to be resistant to IVIG, with the sensitivity and specificity of 0.545 and 0.840, respectively. Compared to a previous study, where a predictive model involving four variables was used (38), we showed a relatively favorable specificity in the prediction of NLR which is easy-to-use and inexpensive in clinical practice.

Collectively, peripheral blood NLR $\geq$ 2.51 can be used as a marker to predict IVIG resistance in Kawasaki disease in patients within 1-year old. This indicator can help in predicting IVIG resistance in KD in an economical way, and it is convenient to use since blood cell count is a routine laboratory examination for both outpatients and inpatients.

However, our study had several limitations. It was of a retrospective study in nature and the sample size was small. In the future, we still need further multi-center analysis to optimize the predictor and better fit the clinical situation.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

YbC, YH, CT, and JD conception and design of the study. YbC, PL, YW, YH, CZ, QZ, YL, HY, JQ, XL, HJ, and JD acquisition of the data. YbC, YH, CZ, SC, QZ, YL, HY, JQ, CT, HJ, and JD analysis and interpretation of the data. YbC, YH, XL, YhC, SC, HJ, and JD drafting the manuscript. CT, HJ, JD, YbC, XL, SC, and YhC accountable for all aspects of the work, critically revising the manuscript and approval of the final manuscript. All authors had full access to all study data, read and approved the final version of the manuscript.

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Accuracy of Micro-Computed Tomography in Post-mortem Evaluation of Fetal Congenital Heart Disease. Comparison Between Post-mortem Micro-CT and Conventional Autopsy.

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Aims: Early prenatal diagnosis of congenital heart disease is feasible. Conventional autopsy is the current gold standard method for post-mortem confirmation. Radiologic techniques alternative to conventional autopsy, such as post-mortem micro-computed tomography, have been proposed in case of limited diagnostic accuracy (i.e., early termination of pregnancy, samples of small dimension or of low weight). The aim of the present study was to define accuracy of micro-computed tomography for post-mortem diagnosis of congenital heart disease in gross anatomy samples.

Methods and Results: Fetal heart underwent *in-utero* prenatal echocardiography and *ex-vivo* post-mortem evaluation by 9 μm resolution micro-computed tomography and conventional autopsy. For each case, 25 indices of cardiac anatomy were studied by post-mortem micro-computed tomography and conventional autopsy; these were used to compare the two post mortem techniques. Ten samples were examined (gestational age between 12 + 4 and 21 + 6 weeks of gestation). Considering comparable indices, agreement between post-mortem micro-computed tomography and conventional autopsy was of 100% and sensitivity and specificity were of 100%. In "challenging specimens," post-mortem micro-computed tomography diagnoses more indices as compared to conventional autopsy and 84% of "not-diagnostic" indices at conventional autopsy would be diagnostic at post-mortem micro-computed tomography.

Conclusion: Micro-computed tomography can be a valid diagnostic alternative to conventional autopsy for post-mortem evaluation of human fetal heart. In addition, it may prove superior to conventional autopsy particularly in cases coming from early termination of pregnancy or in samples of small dimension or of low weight.

Keywords: congenital heart disease—cardiac, post mortem micro-computed tomography, prenatal diagnosis, fetal echocardiography, early prenatal diagnosis

INTRODUCTION

Congenital heart disease is the most frequent congenital malformations. Early prenatal diagnosis of congenital heart disease is feasible, thanks to progress in technology, and to better understanding of risk factors for heart malformations (1–6). By combining 2D analysis and color Doppler techniques, the four chambers, the outflow tracts and the aortic and ductal arches can be seen in a percentage of cases as early as 8–9th week while a complete, or near complete, fetal cardiac assessment can be reached at 11th week (1). Early diagnosis of congenital heart disease is important because it promotes the research of extra-cardiac pathology, it allows physicians to study the possible progression of the disease in order to potentially offer earlier intervention and it enables families to have multidisciplinary counseling. In case of fetal demise or parental decision for termination of pregnancy, post-mortem examination of the fetal heart is important for familial counseling, educational and research purposes. Conventional autopsy, the current gold standard technique for *ex vivo* confirmation of congenital heart disease, is an invasive and destructive tool and it can be challenging, up to impracticable, even for expert pathologists, especially in case of specimens low for gestational age, dimension, or weight (7–9). To overcome possible gaps in post-mortem evaluation of fetal heart due to limitations of conventional autopsy, different radiologic techniques have been proposed [post-mortem contrast-enhanced micro computed tomography (micro-CT) (10–13), post-mortem cardiac magnetic resonance imaging (10, 14–16), post-mortem CT-angiography (10), post-mortem X-ray phase-contrast tomography (10)]. Post-mortem micro-CT of the fetal heart has been tested in *ex vivo* murine and human cardiac samples, showing feasibility and high concordance rate with conventional autopsy. Given its high resolution power (up to 9 μm), it is feasible even at very early gestational age (7th week) and in samples of small dimension or of low weight, where autopsy can miss diagnosis and cardiac details (11–13). Though currently not validated, they may become valid alternative to autopsy for *ex vivo* cardiac examination, even in case where autopsy lacks of diagnostic power.

This study compares post-mortem micro-CT and conventional autopsy of the human fetal heart in case of prenatal suspected congenital heart disease. The primary aim of the study is to define whether micro-CT is at least equal to conventional autopsy in post-mortem diagnosis of congenital heart disease. The secondary aim of the study is to define if micro-CT is more accurate as compared to conventional autopsy in analyzing samples coming from early termination of pregnancy (<16 weeks of gestation) or in samples of small dimension (1 cm or less) or low weight (1 g or less). In this subgroup of patients, we hypothesize that micro-CT has greater diagnostic power when compared to conventional autopsy.

METHODS

Selection and Preparation of Specimens

Cases were selected prospectively. Inclusion criteria for study were fetal echocardiography performed at our Center, prenatal diagnosis of congenital heart disease, decision for termination of pregnancy, agreement to participate to study. For each case, there was fully informed parental consent for use of samples for research. For each case, anamnestic data on pregnancy, fetal extra-cardiac malformations and genetics were collected and anonymized. Especially in case of suction aspiration (termination of pregnancy before the 14th week of gestation), fetal body could be too damaged to permit whole body fetal micro-CT and autopsy. Therefore, in order to be able to compare specimens coming from all gestational ages, samples consisted only in isolated fetal hearts (or heart-lungs in case of prenatal suspicion of heterotaxy syndrome). A single pathologist with expertise in the field of fetal-placental disease isolated the samples. Post-mortem evaluation consisted in micro-CT followed by conventional autopsy. Between termination of pregnancy and micro-CT and between micro-CT and conventional autopsy, all specimens should be left in solution with formalin 10% to preserve anatomic characteristics and tissue properties. Post-mortem micro-CT can easily image calcified tissue but cannot differentiate between soft tissues because of their similarity in x-ray attenuation. As soft tissues adsorb different concentrations of iodine solution, contrast agent enables micro-CT to differentiate between soft structures. Therefore, in order to prepare samples for post-mortem micro-CT, they were removed from formalin 10%, weighted, immersed in Lugol solution at 20, 25, or 50% for 72 h and then washed to remove excess surface liquid just before micro-CT scan, according to previously described protocols (12). After micro-CT scan, they were re-weighted to estimate shrinking effect on tissue and then re-immersed in formalin 10%.

Techniques

Prenatal *in vivo* fetal echocardiographies were performed by a single pediatric cardiologist with expertise in the field of fetal cardiology using a Voluson E8 with a 2–7 MHz convex probe (General Elettrics Healthcare). Each exam consisted in all requested scans according to Italian guideline (2). Segmental approach (17) was used to study the heart.

Post-mortem micro-CTs were performed using a micro-CT scanner SkyScan 1176 (Bruker, Kontich, Belgium) by a single radiologist with expertise in the field of human heart analysis. Smaller voxel reached resolution of 9 μm. Acquisition dataset changed for each specimen, according to sample properties. Post-processing analysis was performed using CTVox volume rendering 64 bit version, DATAVIEWER 64 bit version (Bruker, Kontich, Belgium) and Horos v2.4 software (free and open source code software at Horosproject.org). Segmental approach (17) was used to study the heart.

Conventional autopsies were performed by a single senior pediatric cardiac surgeon using 3.5 magnification loops and neonatal surgical instruments. A standard technique to dissect the heart was adopted (inflows, atrioventricular valves, ventricles, semilunar valves, great arteries, aortic arch, and branches). Segmental approach (17) was used to study the heart.

Data Analysis

For each case, twenty-five indices of cardiac anatomy derived from conventional segmental analysis of the heart (17) were studied and were used to compare post mortem micro-CT and

conventional autopsy (atrial situs, ventricular loop, relationship of the great arteries, atrio-ventricular connection, ventricular-arterial connection, systemic venous returns, pulmonary venous returns, atrial septum, right atrium, left atrium, tricuspid valve, mitral valve, ventricular septum, right ventricle, left ventricle, right ventricular outflow tract, left ventricular outflow tract, pulmonary valve, main pulmonary artery, right pulmonary artery, left pulmonary artery, aortic valve, aortic root, aortic arch, arterial duct). For each index, a technique is defined "diagnostic" when it is able to define that index as normal or abnormal and "not-diagnostic" when it is inconclusive and it can neither confirm nor deny the presence of that index. Pulmonary venous returns was defined normal if at least 2 pulmonary veins were seen terminating in left atrium. Conventional autopsy was used as gold standard technique. For indices that are evaluable by both techniques, we defined "concordance" when the two compared techniques gave the same result for the studied index. Therefore, we included in "concordance" the true positive and the true negative indices. On the other hand, we defined "discordance" when the two compared techniques gave different result for the studied index. Therefore, we included in "discordance" the false positive and the false negative indices. For "not-diagnostic" indices, we define "missense of micro-CT" if that index was deemed "not-diagnostic" with micro-CT and "diagnostic" with conventional autopsy and "apparent advantage of micro-CT" if that index was deemed "diagnostic" with micro-CT but "not-diagnostic" with conventional autopsy.

We arbitrarily defined "challenging specimens" samples coming from early termination of pregnancy (gestational age $\leq$16th week), samples of small dimension (1 cm or less) or low weight (1 g or less).

As little is known about comparison between post-mortem micro-CT and conventional autopsy in *ex vivo* human fetal heart affected by congenital heart disease coming from early and late termination of pregnancy, we decided not to blind prenatal data to radiologist and pathologist and results of micro-CT to pathologist.

Statistical analysis relates only to concordant and discordant indices. Data are given as agreement, sensitivity and specificity. "Not-diagnostic" indices are only described in the text. "Diagnostic power" refers to the ability of a technique in defining indices. We define "greater diagnostic power" of micro-CT when it is able to define more indices as compared to conventional autopsy.

RESULTS

Ten cases met the eligibility criteria. Technical data on post-mortem micro-CT are summarized in **Table 1**. Data on prenatal information and macroscopic dissection are summarized in

TABLE 1 | Post-mortem micro-CT properties for images acquisition.

Case	% Lugol	Hours in lugol	Filter	Resolution (μm)	Exposure time (msec)	Energy range (V)	Current range (μA)	Rotational step (°)
1	25	72	Cu+Al	18	500	89	264	0.50
2	25	72	Cu+Al	18	500	89	264	0.50
3	25	72	Cu+Al	18	500	89	264	0.50
4	20	72	Cu+Al	18	500	89	264	0.50
5	25	72	Cu+Al	18	500	89	264	0.50
6	20	72	Al 0.5 mm	18	210	50	500	0.50
7	20	72	Al 0.5 mm	9	900	50	500	0.30
8	20	72	Al 0.5 mm	18	210	50	500	0.50
9	25	72	Cu+Al	18	500	89	264	0.50
10	20	72	Al 0.5 mm	9	900	50	500	0.30

TABLE 2 | Data on macroscopic dissection.

Case	GA diagnosis (w+d)	GA TOP (w+d)	Longitudinal diameter (cm)	Transverse diameter (cm)	Weight pre micro-CT (g)	Weight post micro-CT (g)
1	20	20+4	1.50	1.30	3.37	2.42
2	16+2	18+6	1.50	1.30	3.07	2.21
3	20+2	20+4	1.50	1.20	3.33	2.18
4	16+1	16+5	1.00	0.80	1.32	0.83
5	21+2	21+6	1.70	1.80	4.00	3.21
6	16+2	16+3	1.10	0.80	0.65	0.45
7	12+4	13+6	0.60	0.50	0.39	0.18
8	14+3	14+3	1.00	0.70	0.49	0.37
9	17+2	18+2	1.40	1.30	1.80	1.55
10	13+2	13+4	0.50	0.50	0.40	0.12

GA, gestational age; w, weeks; d, days; TOP, termination of pregnancy; cm, centimeter; g, gram.

Table 2. Median gestational age at prenatal diagnosis was 16 weeks (17 ± 3 weeks, range 13–21 weeks). Median gestational age at termination of pregnancy was 18 weeks (17 ± 3 weeks, range 14–22 weeks). Mean transverse and longitudinal diameters were respectively 1.02 ± 0.42 cm (range 0.5–1.8 cm) and 1.18 ± 0.41 cm (range 0.5–1.7 cm). Mean weight before and after micro-CT were respectively 1.88 ± 1.43 g (range 0.39–4 g) and 1.35 ± 1.11 g (range 0.12–3.21 g). Mean time interval between termination of pregnancy and post-mortem micro-CT was 80 ± 94 days (range 16–260 days). Mean time interval between termination of pregnancy and conventional autopsy was 127 ± 99 days (range 51–313 days). Mean time interval between post-mortem micro-CT and conventional autopsy was 47 ± 7 days (range 35–53 days).

Table 3 summarizes distribution of indices and statistical data.

Overall, 174/250 (69.6%) indices were evaluable by both techniques. They comprise concordant (174/174, 100%) and discordant (0/174, 0%) indices. The agreement between micro-CT and conventional autopsy was 100%. There were no false positive and false negative indices in micro-CT as compared to conventional autopsy (sensitivity and specificity of post-mortem micro-CT 100%).

76/250 (30.4%) indices were deemed "not-diagnostic" at micro-CT, conventional autopsy or both techniques. They comprise 5 "missense of micro-CT" indices (4 indices "not-diagnostic" at micro-CT and abnormal at conventional autopsy and 1 index "not-diagnostic" at micro-CT and normal at conventional autopsy), 58 "apparent advantage of micro-CT" indices (3 indices abnormal at micro-CT and "not-diagnostic" at conventional autopsy and 55 indices normal at micro-CT and "not-diagnostic" at conventional autopsy) and 13 indices "not-diagnostic" at both micro-CT and conventional autopsy (**Table 4**).

Post-mortem micro-CT could not distinguish the integrity of ventricular septum from the presence of small or medium ventricular septal defect (case 1, 4, 6, 8, 9). On the other hand, it was able to define large ventricular septal defect (case 2, 3, 7, 10) and integrity of the ventricular septum (case 5). Post-mortem micro-CT could not define the ductus arteriosus in all except from 2 cases (case 5, 9).

Conventional autopsy could not define systemic and pulmonary venous returns (possibly due to specimen harvest), atrio-ventricular connection (case 7, 10), atrial septum (case 3, 4, 8, 9), ventricular septal defect (case 1, 4, 8, 9), right ventricular outflow tract and left ventricular outflow tract (case 7,10), pulmonary valve (case 7, 8, 9, 10), main pulmonary artery (case 3, 7, 10), left and right pulmonary arteries (case 3, 7, 10), aortic valve (case 7, 10), aortic root, and aortic arch (case 7, 10).

5 cases met the criteria for "challenging specimens" (case 4, 6, 7, 8, 10). In case 4, post-mortem micro-CT defined 23 out of 25 indices while conventional autopsy defined 20 out of 25 indices. In case 6, post-mortem micro-CT defined 23 out of 25 indices while conventional autopsy defined 22 out of 25 indices. In case 7, post-mortem micro-CT defined 23 out of 25

TABLE 3 | Statistical analysis.

Micro-CT	Conventional autopsy		
	Positive (abnormal)	**Negative (normal)**	**"Not-diagnostic"**
Positive (abnormal)	24	0	3
Negative (normal)	0	150	55
"Not-diagnostic"	4	1	13
	Sensitivity	Specificity	Agreement
	100%	100%	100%

TABLE 4 | Diagnoses and missing data.

Case	ND micro-CT /abnormal autopsy	ND micro-CT /normal autopsy	Abnormal micro-CT /ND autopsy	Normal micro-CT /ND autopsy	ND micro-CT /ND autopsy
1				SVR, PVR	AD, VS
2				SVR, PVR	AD
3	RVOT, LVOT, PV		AS, RPA, LPA	SVR, PVR	AD
4				SVR, PVR, AS	AD, VS
5				SVR, PVR, AD	
6	VS			SVR, PVR	AD
7		relationship of the great arteries		SVR, PVR, ventricular loop, AV and VA connection, RV, LV, RVOT, LVOT, PV, MPA, RPA, LPA, aortic valve, aortic root, aortic arch	AD
8				SVR, PVR, AS, PV	AD, VS
9				SVR, PVR, AS, PV	AD, VS
10				SVR, PVR, AD, ventricular loop, AV and VA connection, RV, LV, RVOT, LVOT, PV, MPA, RPA, LPA, aortic valve, aortic root, aortic arch	

ND, "not-diagnostic"; SVR, systemic venous returns; PVR, pulmonary venous returns; AD, arterial duct; VS, ventricular septum; RVOT, right ventricular outflow tract; LVOT, left ventricular outflow tract; PV, pulmonary valve; AS, atrial septum; RPA, right pulmonary artery; LPA, left pulmonary artery; AV, atrio-ventricular; VA, ventricular-arterial; RV, right ventricle; LV, left ventricle; MPA, main pulmonary artery.

indices while conventional autopsy defined 8 out of 25 indices. In case 8, post-mortem micro-CT defined 23 out of 25 indices while conventional autopsy defined 19 out of 25 indices. In case 10, post-mortem micro-CT defined 24 out of 25 indices while conventional autopsy defined 8 out of 25 indices. 50/76 (65.7%) "not-diagnostic" indices belong to the group of the "challenging specimens." For 42/50 (84%) indices, conventional autopsy was "not-diagnostic" whether micro-CT was diagnostic ("apparent

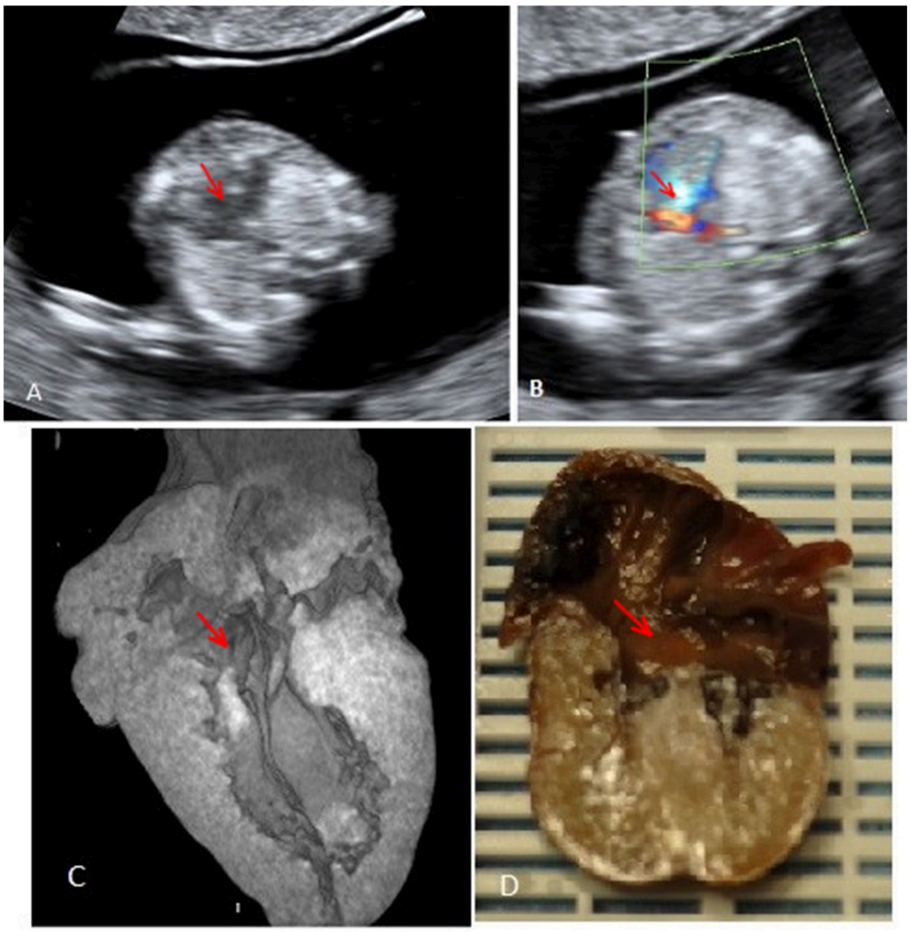

FIGURE 1 | Case 1 (atrioventricular septal defect): **(A)** Prenatal fetal echocardiography: four chamber view showing the atrioventricular septal defect. **(B)** Prenatal fetal echocardiography: four chamber view with color Doppler confirmation of the defect. **(C)** Post-mortem micro-CT: four chamber view showing the atrioventricular septal defect. **(D)** Conventional autopsy: coronal section showing the four chamber view and the atrioventricular septal defect. Red arrows mark the atrioventricular septal defect.

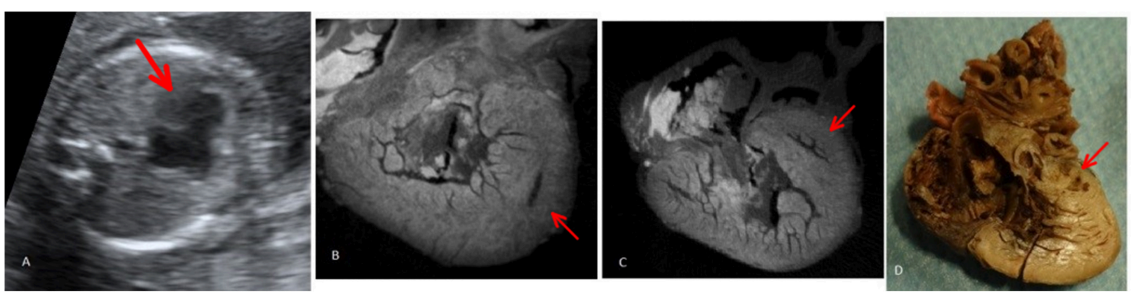

FIGURE 2 | Case 2 (hypoplastic left heart syndrome): **(A)** Prenatal fetal echocardiography: four chamber view showing hypoplastic/atretic mitral valve and hypoplastic left ventricle. **(B)** Post-mortem micro-CT: short axis view at the level of the ventricle comparing right ventricle with hypoplastic left ventricle. **(C)** Post-mortem micro-CT: four chamber view showing the hypoplastic left ventricle. **(D)** Conventional autopsy: four chamber view showing hypoplastic left ventricle. Red arrows mark the hypoplastic left ventricle.

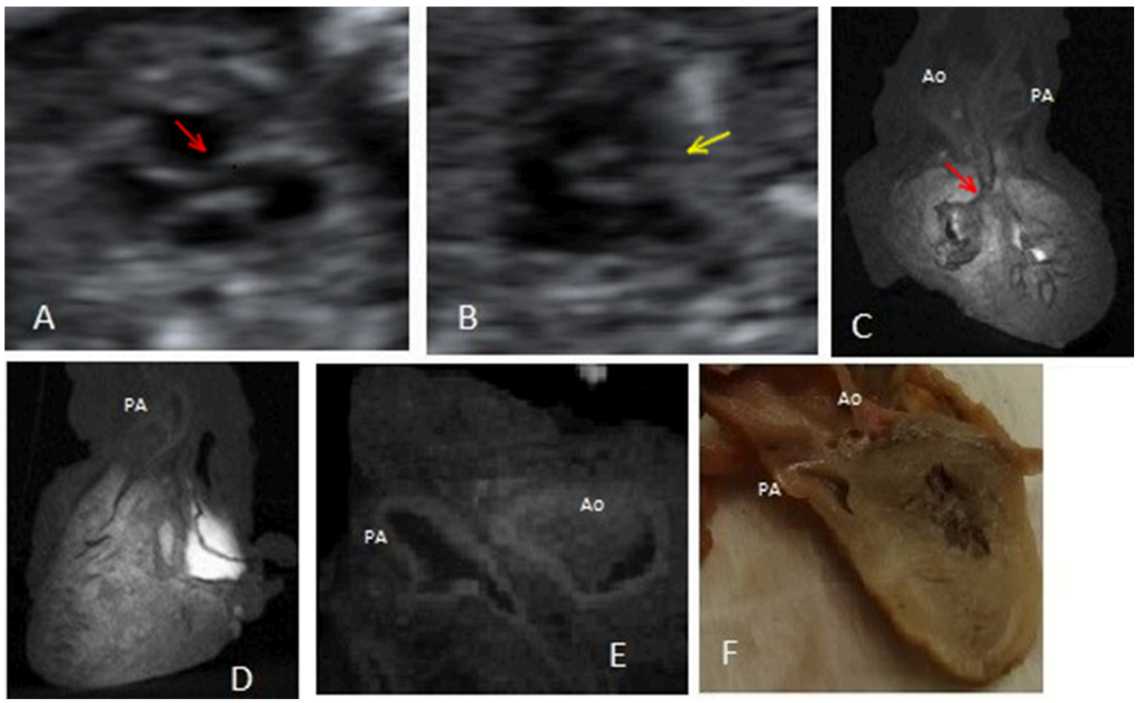

FIGURE 3 | Case 3 (pulmonary atresia and ventricular septal defect): **(A)** Prenatal fetal echocardiography: conoventricular septal defect and an overriding vessel of unclear morphology. **(B)** Prenatal fetal echocardiography: short axis view at the level of the semilunar valve showing the absence of the pulmonary artery structure. **(C)** Post-mortem micro-CT: presence of a ventricular septal defect, a posterior overriding vessel and another anterior vessel. **(D)** Post-mortem micro-CT: the anterior vessel exits from the anterior, morphological right ventricle. **(E)** Post-mortem micro-CT: bifurcation of the anterior vessel. **(F)** Conventional autopsy: presence of two different outflow tracts. Red arrows mark the ventricular septal defect. Yellow arrow marks the absent pulmonary artery. Ao, aorta; PA, pulmonary artery.

advantage of micro-CT"). For 6/50 (12%) indices, nor micro-CT nor conventional autopsy were diagnostic (cases 4, 6, 7, 8: ventricular septum, arterial duct). For 2/50 (4%) indices, micro-CT was "not-diagnostic" whether conventional autopsy was diagnostic ("missense of micro-CT"; case 3, ventricular septum, relationship of the great arteries).

Figures 1–3 give some examples of prenatal suspected congenital heart disease and post-mortem evaluation of samples.

DISCUSSION

To the best of our knowledge, this is the first study where human fetal hearts, with early and late prenatal diagnosis of congenital heart disease, undergo post-mortem evaluation by micro-CT and conventional autopsy by using segmental approach (10 cases, gestational age >13 weeks of gestation, prenatal diagnosis available for each case). Previous studies defined the feasibility of post-mortem micro-CT in evaluating murine (18) and human *ex vivo* pathological fetal heart (12, 13). Lombardi and colleagues demonstrated that post-mortem micro-CT provides similar information to conventional autopsy in case of normal heart and of prenatal suspected congenital heart disease by analyzing the four chamber view, the crux cordis, the atrioventricular valves and the three vessel cross sectional view of pulmonary artery, aorta, and systemic venous returns (6 cases, gestational age >15 weeks of gestation, prenatal diagnosis) (12). Hutchinson and colleagues showed that post-mortem micro-CT provide accurate definition of congenital heart disease by studying 21 indices of cardiac anatomy (5 cases, gestational age >17 weeks of gestation, no prenatal diagnosis) (13). Segmental approach is worldwide used to study the heart and to diagnose congenital heart disease. Therefore, we applied it to both post-mortem micro-CT and conventional autopsy and we compare the two techniques on its base.

The primary finding of this study is that post-mortem micro-CT, when compared to conventional autopsy, affords good agreement, sensitivity, and specificity in post-mortem evaluation of human fetal heart affected by congenital heart disease. Therefore, we can conclude that it has equal diagnostic power as compared to gold standard technique.

The secondary finding of this study is that post-mortem micro-CT defines morphological data even in challenging specimens, where autopsy lacks to. Indeed, for each case belonging to this subgroup of samples, post-mortem micro-CT was invariably able to define more indices as compared to conventional autopsy. Moreover, this subgroup of samples includes the majority (65.7%) of "not-diagnostic" indices, of which 84% are judged not evaluable by conventional autopsy but would be evaluable by micro-CT ("apparent advantage of micro-CT").

Furthermore, post-mortem micro-CT is able to identify potentially prognostic important anatomic features (i.e., origin

and position of the coronary arteries, anatomy of atrioventricular and semilunar valves) which are not dependably identified by conventional autopsy.

Technically speaking, post-mortem micro-CT does not jeopardize the diagnostic power of a subsequent conventional autopsy. After iodine preparation for micro-CT, we observed a slight reduction in weight of samples without modifications of tissue architecture and anatomic relation of intra-cardiac and extra-cardiac structure, as previously reported (12, 13). In addition, post-mortem micro-CT may guide pathologist in performing conventional autopsy, especially in small samples. In fact, images of post-mortem micro-CT allow the pathologist to decide where to cut and what to search for during the execution of challenging conventional autopsies. All this notwithstanding, conventional autopsy may not reach sufficient diagnostic power in all specimens.

Another possible advantage of micro-CT is represented by its non-destructive nature. It acquires a data set of images that can be reviewed and reconstructed many times and by different specialists, facilitating multidisciplinary discussion and comparison.

There are factors which lower diagnostic power of post-mortem micro-CT, as previously reported (18), including: poor quality of the specimens; post-mortem native tissue modification; and suboptimal preparation of samples. Poor tissue quality reduces the diagnostic accuracy of post-mortem micro-CT for small extra-cardiac structure (i.e., systemic and pulmonary venous returns). Post-mortem modifications of the muscular structure reduce the diagnostic accuracy of post-mortem micro-CT for small and medium ventricular septal defect. The definition of systemic and pulmonary venous returns and ventricular septal defect remains challenging also for conventional autopsy. Some of these indices (i.e., small ventricular septal defect) have no prognostic impact on postnatal care.

Limitations to the present study exist, including: the small population; the heterogeneity of samples, the absence of a control group (normal human fetal heart coming from early and late termination of pregnancy); the non-blinded comparison between techniques.

Nonetheless, the present pilot experience is the largest to date employing this innovative technique for post-mortem diagnosis of human congenital heart disease detected early in pregnancy.

In conclusion, we have preliminary evidence that post-mortem micro-CT gives at least equivalent information on structural heart defects as compared to conventional autopsy in *ex vivo* isolated human fetal hearts. In small specimens, where conventional autopsy has limited accuracy, post-mortem micro-CT provides more information on cardiac anatomy.

We conclude that micro-CT is at least equal to conventional autopsy for post-mortem confirmation of congenital heart disease and that it is superior to conventional autopsy in small specimens. More cases are needed to reach statistical power to confirm this finding and to define the threshold of weight and/or dimension under which conventional autopsy will be no more indicated. For further project, study population should consist in normal and pathological specimens and it should comprise enough samples from different gestational ages and enough samples of the same heart malformations. Double blinded study would be desirable for future investigations.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of local ethic committee (Comitato etico per la sperimentazione clinica delle province di Verona e Rovigo) with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the local ethic committee (Comitato etico per la sperimentazione clinica delle province di Verona e Rovigo).

AUTHOR CONTRIBUTIONS

CS contributed to conception and design of study, analysis and interpretation of data, drafting of manuscript. LR contributed to conception and design of study, drafting of manuscript, and final approval. VZ, RZ, FB, RS, CDP, and SH contributed to analysis and interpretation of data. FLR and GF contributed to final approval. CL and GBL contributed to conception and design of study, drafting of manuscript, funding, and gave final approval.

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ORIGINAL RESEARCH
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Oxygen Availability in Respiratory Muscles During Exercise in Children Following Fontan Operation

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Introduction: As survival of previously considered as lethal congenital heart disease forms is the case in our days, issues regarding quality of life including sport and daily activities emerge. In patients with Fontan circulation, there is no pump to propel blood into the pulmonary arteries since the systemic veins are directly connected to the pulmonary arteries. The complex hemodynamics of Fontan circulation include atrial function, peripheral muscle pump, integrity of the atrioventricular valve, absence of restrictive, or obstructive pulmonary lung function. Therefore, thoracic mechanics are of particular importance within the complex hemodynamics of Fontan circulation.

Methods: To understand the physiology of respiratory muscles, the aim of this study was to examine the matching of auxiliary respiratory muscle oxygen delivery and utilization during incremental exercise in young male Fontan patients ($n = 22$, age $= 12.04 \pm 2.51$) and healthy Controls ($n = 10$, age $= 14.90 \pm 2.23$). All subjects underwent a cardiopulmonary exercise test (CPET) to exhaustion whereas respiratory muscle oxygenation was measured non-invasively using a near-infrared spectrometer (NIRS).

Results: CPET revealed significantly lower peak power output, oxygen uptake and breath activity in Fontan patients. The onset of respiratory muscle deoxygenation was significantly earlier. The matching of local muscle perfusion to oxygen demand was significantly worse in Fontans between 50 and 90% $\dot{V}O_{2peak}$.

Findings: The results indicate that (a) there is high strain on respiratory muscles during incremental cycling exercise and (b) auxiliary respiratory muscles are worse perfused in patients who underwent a Fontan procedure compared to healthy Controls. This might be indicative of a more general skeletal muscle strain and worse perfusion in Fontan patients rather than a localized-limited to thoracic muscles phenomenon.

Keywords: muscle oxygenation, Fontan, CHD, pediatrics, NIRS, respiratory muscles

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INTRODUCTION

Functional univentricular congenital heart defects (CHD) entails different morphological diagnoses, the most common are hypoplastic left heart syndrome, tricuspid atresia, and double inlet left ventricle (1). In unpalliated univentricular CHD, cyanosis occurs because of mixing of unsaturated and saturated blood in the heart. The univentricular heart is exposed to volume overload as it drains both systemic and pulmonary venous return at the same time (2).

Palliation is achieved with the Fontan circulation (2). General principle is that the systemic venous return bypasses the subpulmonary ventricle with a separation of the systemic and pulmonary circulation and reduction of ventricular volume overload. Contemporary modifications of surgical techniques have significantly improved survival (2). A growing number of children and adults with congenital heart defects are living with a Fontan circulation with their limitations of chronic elevation of central venous pressure and restricted ventricular preload (3).

Patients with Fontan circulation are limited during exercise with a decreased peak VO_2 compared to healthy controls (4). Healthy individuals increase their pulmonary blood flow during exercise by a reduction in pulmonary vascular resistance due to vasodilation and recruitment of segments and increased right ventricular work consisting of flow acceleration coupled with increased systolic pressures (5). In the Fontan patients, no pump ventricle exists to increase and accelerate pulmonary blood flow. Beyond this, pulmonary vascular reactivity and recruitment of vessels are limited or even absent (6). That leads to a restricted ability to boost cardiac output during exercise (6). The leading force of pulmonary blood flow in the absence of a pump ventricle is namely the negative pressure of the systemic atrium during ventricular systole and atrial volume increase due to the apical descent of a non-insufficient atrioventricular valve. Therefore, this physiology is even more dependent on the work of breathing to generate cardiac output (7). Active muscle contraction including the diaphragm increases the thoracic dimensions during inspiration generating a negative pressure and thus systemic blood flow into the thorax and of a passive decrease in thoracic dimensions based on the elastic recoil of lung itself. MRI studies estimated that ∼30% of resting cardiac output is "respiratory dependent" while this dependency relies on the "thoracic pump" to increase cardiac out during exercise (8).

While understanding the hemodynamic differences between healthy individuals and Fontan patients, we know little about the peripheral muscle oxygenation in patients with univentricular circulation and the possible impact of an impaired ability to increase the oxygen supply adequately to the exercising muscles.

Few studies assessed the exercise capacity and respiratory muscle oxygenation in children with congenital heart diseases or studied the training effects on peripheral skeletal muscle oxygenation in children with congenital heart disease (9, 10). Till now, no study exists, which examined the respiratory muscle deoxygenation exclusively in children with Fontan circulation.

The aim of the study was to spot on the potential differences of respiratory muscle deoxygenation in children with Fontan circulation compared to healthy children during cardiopulmonary exercise testing (CPET) with near-infrared spectroscopy (NIRS).

TABLE 1 | Characteristics of fontans and controls.

	Fontans ($n = 22$)	Controls ($n = 10$)	*p*-value
Age (y)	12.04 ± 2.51	14.90 ± 2.23	0.004
Body mass (kg)	39.68 ± 13.38	52.80 ± 11.08	0.011
Height (cm)	149.77 ± 14.83	167.00 ± 12.78	0.003
BMI (kg^*m^{-2})	17.18 ± 2.42	18.68 ± 1.59	0.087
Skinfold thickness (cm)	6.50 ± 1.99	5.46 ± 1.80	0.173

BMI, Body Mass Index; data in mean ± standard deviation.

MATERIALS AND METHODS

Patients

From April 2014 to December 2016, 22 male patients (12.04 ± 2.51 year, range 12–18 year) with Fontan circulation (see **Table 1**) participated this study. They underwent a preliminary medical screening. From those 30 patients, 8 patients had to be excluded after the preliminary screening.

Underlying heart defects were hypoplastic left heart syndrome ($n = 9$), double outlet right ventricle (DORV) with transposition of the great arteries (TGA) and left ventricle outflow obstruction ($n = 1$), DORV with atrioventricular discordance and pulmonary stenosis ($n = 1$), DORV with hypoplastic left ventricle ($n = 1$), TGA with hypoplastic right heart and pulmonary stenosis ($n = 1$), double inlet left ventricle ($n = 4$), tricuspid atresia ($n = 2$), mitral atresia ($n = 2$), and pulmonary atresia with intact ventricular septum and coronary fistula ($n = 1$).

Fontan patients had at least two surgical procedures (mean 3.36, range 2–5). Median age at the Fontan procedure was 21.5 months (range 17–54 months). Median time interval from the Fontan procedure to the study was 9.12 years (range 6.62–14.51 years).

Two Fontan patients had a pacemaker due to bradycardia caused by sinus node dysfunction. Hemoglobin values were in a normal range in all Fontan patients (15.32 ± 1.25 gdl).There were no skeletal abnormalities as scoliosis in the Fontan patients.

All Fontan patients were in NYHA class II ($n = 22$). All Fontans participated to regular school sport activity, no one was excluded.

Transthoracic echocardiography revealed normal single ventricular function in 19 Fontan patients, while it was reduced in three patients. Four patients demonstrated moderate atrioventricular valve regurgitation, while the rest was mild or less. All patients were under oral anticoagulation and no thrombus could be detected by echocardiogram. ACE inhibitors were given in four, diuretics in three, digoxin and beta blockers in two patients each. None had pulmonary vasoactive medication.

Patients were in stable condition allowing exhaustive exercise.

The Controls consisted of 10 male healthy children (14.90 ± 2.23 year, range 10–17 year, **Table 1**) who underwent a routine check-up for participating in a sports club programme

(**Table 1**). Each healthy subject did at least 2 h of physical exercise per week at school. Fontan patients and Controls did not suffer from other relevant diseases that might lead to an impaired muscle performance. None of the subjects were in any family relationship. Anthropometric data of Fontan patients and Controls is listed in **Table 1**.

The study conforms to the principles outlined in the Declaration of Helsinki 1975 and was approved by the Institutional Review Board of the Technical University of Munich (project number 52/14). Written informed consent was obtained from all study participants and parents.

Cardiopulmonary Exercise Test (CPET)

All subjects underwent a CPET with a cycle ergometer (Corival, Lode, Groningen, The Netherlands) in upright position or in patients with a rate responsive pacer with a treadmill (CareFusion, LE 200 CE) according to international guidelines (11), with one of the authors present at all times.

After a 3 min baseline measurement at rest and further 3 min of unloaded cycling as warm-up, load was increased ramp-wise with 5, 10, 15, 20, or 30 W/min depending on the expected individual physical capacity estimated by the investigator. The aim was to reach cycle duration of 8–12 min after warm up until exhaustion was reached. The end of the CPET was marked by symptom limitation and was followed by a 5 min recovery period with the first 3 min cycling with minimal load and another 2 min at rest.

The following criteria considered the tests to be of maximal effort: a respiratory exchange ratio >1.0; exhaustion of the patient with an inability to maintain a cycle pedaling rate of $60 \cdot \text{min}^{-1}$.

Respiratory gas-exchanges were measured by a breath-by-breath analysis using a metabolic chart (Vyasis Healthcare Vmax Encore 29, Hochberg, Germany). The device was calibrated prior to each test according to manufacturer's recommendations.

Blood pressure was measured by an automated, ECG-triggered acoustic device (SunTech Medical Inc.: Tango M2, Morrisville, NC, USA) every 2 min during examination time. Heart rate and rhythm was monitored with a continuous ECG during the entire examination. Oxygen saturation (SpO_2) was monitored in the Fontans continuously using a forehead sensor.

The ventilatory threshold (VT) was determined from the gas exchange data using the V-slope method validated by Beaver and colleagues (12). VT is defined as the breakpoint of the linearity of the curve carbondioxide elimination ($\dot{V}CO_2$) vs. oxygen uptake ($\dot{V}O_2$). This point was identified by two independent experienced observers.

Peak oxygen uptake ($\dot{V}O_{2peak}$) was defined as the highest moving average 30 s interval during the exercise period. Reference values ($mL^*kg^{-1*}min^{-1}$) were calculated according to Cooper and Weiler-Ravell (13).

For adolescents 12 years and older, sex-specific reference values (mL/kg/min) were calculated:

$$\text{Female: } \dot{V}O_{2peak} = (22.5 \bullet \text{height (cm)} - 1837.8)/\text{weight (kg)}$$

$$\text{Male: } \dot{V}O_{2peak} = (43.6 \bullet \text{height (cm)} - 4547.1)/\text{weight (kg)}$$

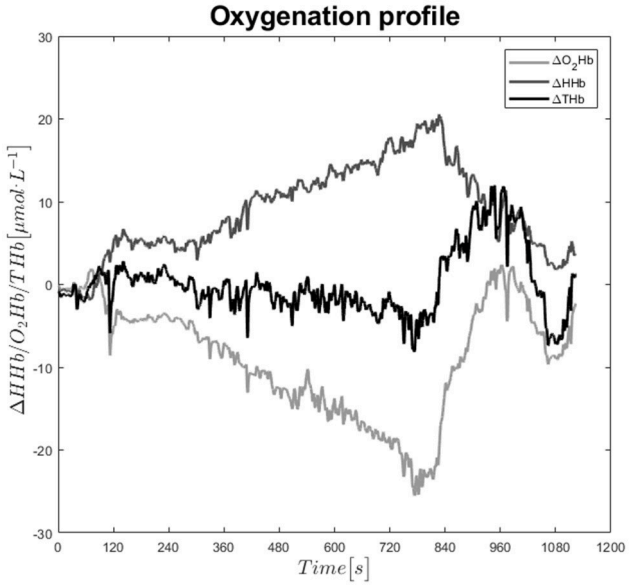

FIGURE 1 | Exemplary time-course of oxygenated, deoxygenated, and total hemoglobin during the CPET.

For patients younger than 12 years of age, reference values ($mL^*kg^{-1*}min^{-1}$) were calculated from pooled data from both sexes:

$$\dot{V}O_{2peak} = (37.1 \bullet \text{height (cm)} - 3770.6)/\text{weight (kg)}$$

Near-Infrared Spectroscopy (NIRS) Measurement

The principles behind the NIRS technique is described in detail elsewhere (14, 15). In brief, NIRS measurements are based on the relative tissue transparency for light in the near-infrared spectrum and on the O_2-dependent absorption changes of oxygenated and deoxygenated hemoglobin and myoglobin (ΔO_2Hb+Mb, ΔHHb+Mb) based on the Lambert-Beer-law. Data was measured continuously using a wireless, continuous-wave near-infrared spectrometer (PortaLite, Artinis B.V., Zetten, NL) with a sample rate of 10 Hz. A differential pathlength-factor of 4 was used, as this has been reported as appropriate factor for muscle measurements previously (15). Accordingly, all NIRS data are expressed as $\Delta\mu\text{mol}\cdot\text{L}^{-1}$ and adjusted to pre-exercise values that were obtained during the 3 min baseline measurement prior to the cardiopulmonary exercise test.

The NIRS device operates with NIR wavelengths of 760 and 850 nm, while the analyzed emitter-detector distance was 3.5 cm. This results in a penetration depth of ∼1.75 cm (15).

The NIRS probe was placed on the right side of the upper body above the sixth intercostal space at the anterior axillary line over the serratus anterior muscle. This position has been used previously to monitor oxygenation of this muscle (8). This muscle was chosen because it serves as an auxiliary inspiratory muscle during the ventilation as a "rib elevator".

We measured the skinfold thickness by a fat caliper (GPM, DKSH Inc., Zürich, CH) over the serratus anterior muscle for interpretation the quality of the gained NIRS data.

Data Processing

First, NIRS and CPET data were time-aligned. Therefore, the data were converted to 10 s bins and processed with a 30 s moving average. As mentioned previously NIRS data was calculated by subtracting-individual baseline values to be expressed for example as ΔHHb. Furthermore, the NIRS data was normalized to the individual peak values, which had been reached in the trial, to be expressed as %HHbmax. This means that in all subjects %HHbmax started at 0% at baseline and reaches 100% at the point of maximal respiratory muscle desoxygenation. As an estimate for the adjustment of microvascular perfusion, $\Delta HHb/\Delta \dot{V}O_2$ ratio was calculated according to $\%HHbmax/\%\dot{V}O_{2peak}$ for each time point during the test. This parameter has been used in several previous studies (16–19).

Statistical Analysis

A repeated-measures-design ANOVA with one group-factor *Fontans* vs. *Controls* was used to evaluate the effect of *intensity* on NIRS data and CPET data. Data was analyzed at 20, 30, 40, 50, 60, 70, 80, 90, and 100% $\dot{V}O_{2peak}$. Repeated contrasts were used to analyze possible interaction-effects of *intensity x Fontans* vs. *Controls*. All *post-hoc* tests were Bonferroni-Holm corrected. Power output at VT, $\dot{V}O_{2peak}$, peak power output, skinfold thickness were compared among the two groups using independent *t*-tests. Additionally, we divided the Fontans into two subgroups of left and right single ventricular morphologies and compared VO_{2peak}, peak power output and muscle deoxygenation among both morphologies. The level of statistical significance was set to $p < 0.05$.

RESULTS

Fontans and Controls

The detailed characteristics of the patients and control group are given in **Table 1**.

CPET

We obtained resting, submaximal, and maximal cardiopulmonary variables in all Fontans and Controls while submaximal variables were taken at the similar exercise intensity.

At rest, Fontans had similar heart rate, $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$, and blood pressure compared to healthy Controls.

$\dot{V}O_{2peak}$, peak power output, $\dot{V}CO_{2peak}$, $\dot{V}E_{peak}$, and HR_{peak} were significantly reduced in the Fontans compared to healthy Controls ($p < 0.01$).

At VT, all parameters showed significant lower values in Fontans compared to the Controls. The healthy Controls had larger absolute values and reached VT later than the Fontans.

All data for CPET in comparison are given in **Table 2**.

Respiratory Muscle Oxygenation by NIRS

ΔHHb was significantly influenced by exercise intensity in both groups [$F_{(3.8,\ 120.3)} = 91.96$, $p \leq 0.001$], whereas the onset of muscle deoxygenation occurred differently: in the Controls, ΔHHb remained stable up to 50% $\dot{V}O_{2peak}$. Above

TABLE 2 | Parameters of CPET in fontan patients and healthy controls measured at the ventilatory threshold and at peak level.

	Fontan patients ($n = 22$)	Healthy controls ($n = 10$)	*p*-value
Workload ($W \cdot kg^{-1}$) max	3.18 ± 1.13	4.35 ± 0.24	0.003*
Workload ($W \cdot kg^{-1}$) at VT	1.50 ± 0.34	1.53 ± 0.63	0.875
$\dot{V}O_2$ ($ml \cdot min^{-1} \cdot kg\text{-}^{1}$) peak	35.97± 6.84	44.76 ± 5.09	0.001*
% VO_{2peak} at VT	36.14 ± 16.71	62.94 ± 9.61	<0.001*
$\dot{V}O_{2peak}$ % predicted	73.61 ± 17.69	86.87 ± 12.61	0.044*
$\dot{V}E_{peak}$ ($L \cdot min^{-1}$)	60.29 ± 25.19	74.70 ± 18.12	0.010*
$\dot{V}E_{peak}$ ($L \cdot min^{-1}\ m^{-2}$)	38.99 ± 9.97	47.12 ± 8.58	0.024*
$\dot{V}E$ ($L \cdot min^{-1}$) at VT	29.29 ± 10.86	26.05 ± 7.80	0.404
$\dot{V}E/\dot{V}CO_2$ Slope	32.04 ± 3.31	27.25 ± 5.41	0.004*
HR (beats·min) peak	171.20 ± 21.20	187.66 ± 5.16	0.002*
HR (beats·min) at VT	120.10 ± 16.17	118.50 ± 20.95	0.816
S_PO_2 (%) peak	87.59 ± 5.19	n.d.	
S_PO_2 (%) at VT	88.55 ± 7.37	n.d.	
Respiratory rate peak	51.68 ± 8.31	48.95 ± 11.13	0.444
Respiratory rate at VT	34.86 ± 5.99	26.25 ± 9.64	0.004

Data in mean ± standard deviation.
Asterisks indicate significant differences between Fontans and Healthy Controls.

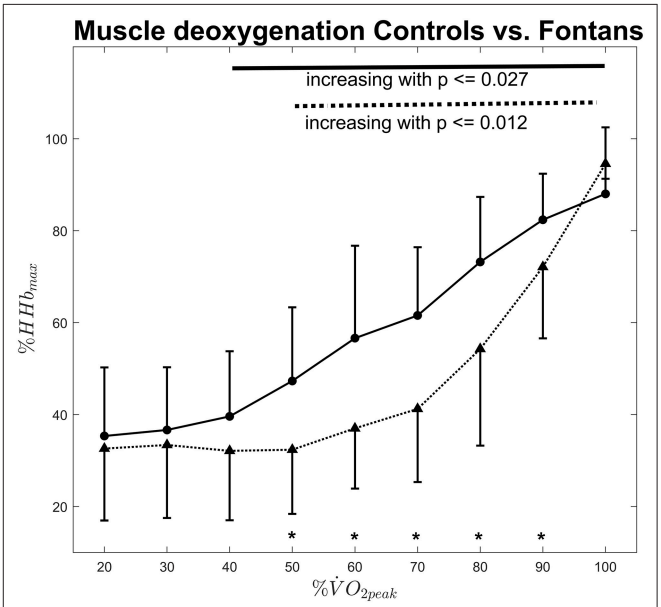

FIGURE 2 | Normalized muscle deoxygenation vs. exercise intensity for Fontans (solid line) and Controls (dashed line). The solid and dashed lines in the upper section indicate significant differences between intensities among Fontans and Controls, respectively. Significant differences ($p < 0.05$) between Fontans and Controls within one exercise intensity are marked with asterisks.

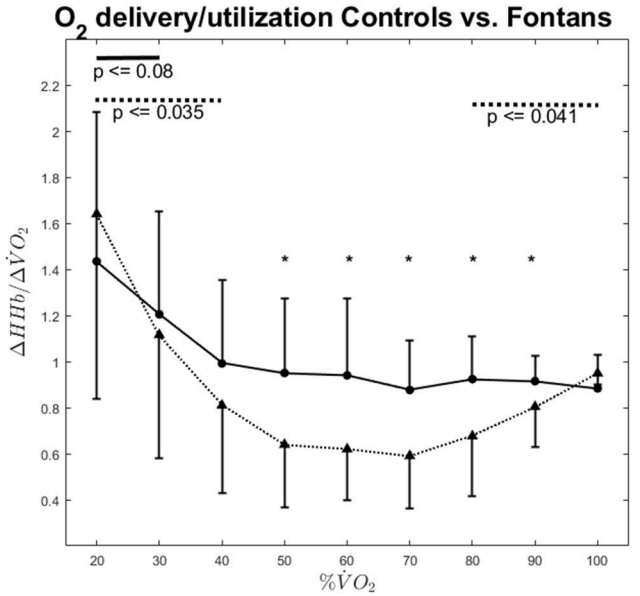

FIGURE 3 | ΔHHb/Δ VO_2 ratio vs. exercise intensity for Fontans (solid line) and Controls (dashed line). The solid and dashed lines in the upper section indicate significant differences between intensities among Fontans and Controls, respectively. Significant differences ($p < 0.05$) between Fontans and Controls within one exercise intensity are marked with asterisks.

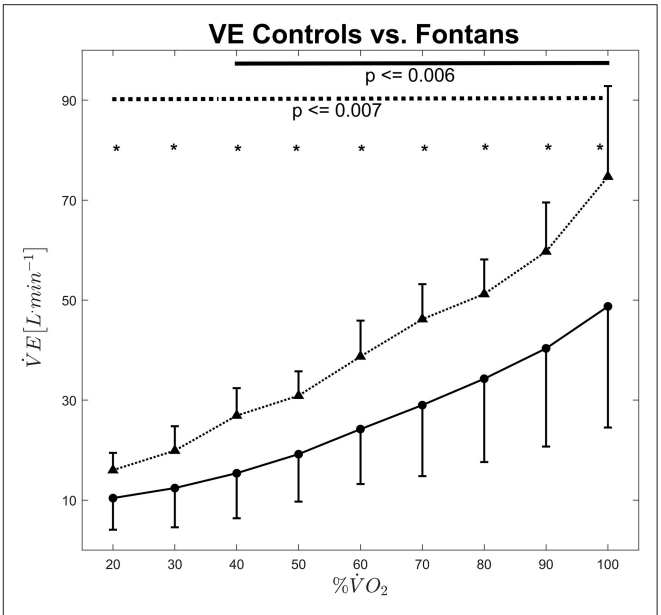

FIGURE 4 | Ventilation vs. exercise intensity for Fontans (solid line) and Controls (dashed line). The solid and dashed lines in the upper section indicate significant differences between intensities among Fontans and Controls, respectively. Significant differences ($p < 0.05$) between Fontans and Controls within one exercise intensity are marked with asterisks.

this intensity, ΔHHb increased significantly ($p \leq 0.012$) until exhaustion (see **Figure 2**). In the Fontans, ΔHHb evolved similar, but ΔHHb increased significantly already above 40% $\dot{V}O_{2peak}$ ($p \leq 0.027$). The temporal dissociation between the increase of ΔHHb in the Fontan and control group was supported by a significant interaction effect of *intensity x Fontans* vs. *Controls* [$F_{(3.8,\ 120.3)} = 4.7$, $p = 0.002$]. Those results were confirmed by *post-hoc* tests. Between 50 and 90% $\dot{V}O_{2peak}$, ΔHHb values were significantly higher for the Fontans compared to the Controls ($p \leq 0.028$).

Exercise intensity also had a significant influence on $\Delta HHb/\Delta\dot{V}O_2$ ratio [$F_{(2.3,\ 75.0)} = 91.96$, $p \leq 0.001$]. In Fontans, $\Delta HHb/\Delta\dot{V}O_2$ ratio decreased significantly up to an exercise intensity of 40% $\dot{V}O_{2peak}$ whereas no significant changes occurred at higher exercise intensities (see **Figure 3**). Contrary, $\Delta HHb/\Delta\dot{V}O_2$ ratio evolved "u-shaped" for the control group: After a significant decrease with increasing exercise intensity up to 50% $\dot{V}O_{2peak}$ ($p \leq 0.035$), $\Delta HHb/\Delta\dot{V}O_2$ ratio remained stable up to 70% $\dot{V}O_{2peak}$ but increased thereafter ($p \leq 0.041$). $\Delta HHb/\Delta\dot{V}O_2$ ratio did not differ among both groups at low (20–40% $\dot{V}O_{2peak}$) and maximum exercise intensity. In between, the Fontan group showed significantly higher values compared to those of the control group ($p \leq 0.031$).

Ventilation increased significantly with exercise intensity [$F_{(1.6,\ 52.5)} = 127.1$, $p \leq 0.001$] whereas the stepwise increases were significant above 40% $\dot{V}O_{2peak}$ ($p \leq 0.006$) for the Fontans and right from the beginning ($p \leq 0.007$) for the Controls (see **Figure 4**). While no interaction could be observed for *intensity x Fontans* vs. *Controls*, ventilation was greater in the control group at all tested intensities [ANOVA: $F_{(1.6,\ 52.5)} = 4.7$, $p = 0.018$; *post-hoc* tests: $p \leq 0.013$]. We analyzed ventilation also relatively to body surface area (L^*m^{-2}). Body surface area was calculated according to Dubois (20). Relative ventilation also increased significantly above 40% $\dot{V}O_{2peak}$ in Fontans ($p \leq 0.006$) and right from the beginning in the control group ($p \leq 0.007$). Again, no interaction could be shown but relative ventilation was significantly higher in seven out of the nine examined intensities (40–100% $\dot{V}O_{2peak}$; $p \leq 0.031$).

Peak power was significantly lower in Fontans compared to healthy Controls (3.18 ± 1.13 vs. 4.35 ± 0.24 $W{\cdot}kg^{-1}$, $p = 0.003$), just as $\dot{V}O_{2max}$ (35.97 ± 6.84 vs. 44.76 ± 5.09 $ml{\cdot}min^{-1}{\cdot}kg^{-1}$, $p = 0.001$).

After dividing the Fontans into two subgroups of right and left single ventricular morphologies (RVs: 8 subjects, LVs 14 subjects), VO_{2peak} (34.67 ± 5.11 $ml{\cdot}min^{-1}{\cdot}kg^{-1}$ vs. 38.15 ± 9.04 $ml{\cdot}min^{-1}{\cdot}kg^{-1}$) and Peak Power were higher (98 ± 25.80 W vs. 141.88 ± 56.25 W) in patients with a LV morphology, while this difference was only significant for Peak Power ($p = 0.029$). No significant effect of the heart morphology could be found on muscle oxygenation during exercise.

Skinfold thickness at the NIRS probe position was 3.49 ± 1.36 mm. The interoptode distance that we used in the study was 3.5 cm which corresponds to an approximate penetration depth of 1.75 cm. Hence, the NIRS signal can be referred to muscle tissue in the intercostal space.

DISCUSSION

The results of our study clearly show that there is high aerobic demand during cycling exercise, not only on working muscles but

also on respiratory muscles. This was indicated by an inflection point in the ΔHHb signal, representing the onset of muscle deoxygenation (**Figure 1**). This inflection point was significantly different in children with Fontan palliation compared to healthy controls (40 $\dot{V}O_{2peak}$ vs. 50% $\dot{V}O_{2peak}$). NIRS signal represents the dynamic balance of local oxygen extraction and blood flow (i.e., oxygen supply). Therefore, an increase of ΔHHb indicates a higher local oxygen extraction in relation to oxygen supply. Our data hence reveal that this dynamic balance alternates earlier in Fontans compared to Controls.

These results are similar compared to those of Moalla et al. (9), who investigated 12 patients with various congenital heart defects. By normalizing data to the maximum deoxygenation that was reached in the graded exercise test, we focused more on the relationship of muscle deoxygenation kinetics rather than oxygen saturation as an absolute value. Therefore, we could identify the different onsets of muscle deoxygenation in Fontans vs. healthy Controls. Moalla et al. found the onset of pronounced muscle deoxygenation to be concomitant to VT (21). The onset on muscle deoxygenation that was measured in this study therefore could indicate somehow the "local" VT for the serratus anterior muscle.

When deoxygenation is expressed relatively to oxygen uptake ($\Delta HHb/\Delta\dot{V}O_2$-ratio), the matching of oxygen delivery to utilization can be estimated (16, 17, 22–24). $\Delta HHb/\Delta\dot{V}O_2$-ratio was significantly lower in Controls between 50 and 90% $\dot{V}O_{2peak}$, suggesting a significantly higher local oxygen provision. In brief, the idea behind this parameter is that ΔHHb, which represents the dynamic balance between local oxygen extraction and desoxy-Hb removal (i.e., blood flow) is assumed to increase when $\dot{V}O_2$ rises if this can be (partially) referred to the muscle of interest. If local blood flow would rise correspondingly, no increase in ΔHHb would be visible. However, local oxygen extraction usually exceeds the increase in local blood flow. Consequently, the smaller ΔHHb at a given workload/$\dot{V}O_2$ is, the higher should be the local muscle perfusion and, hence, oxygen provision. However, the concept of $\Delta HHb/\Delta\dot{V}O_2$ originally was used for the estimation of microvascular oxygen provision in working muscles (24). We are aware that the serratus anterior muscle does not account for the major amount of consumed oxygen during cycling exercise. Hence, $\Delta HHb/\Delta\dot{V}O_2$ as an estimate for microvascular perfusion in the serratus anterior muscle has to interpreted with caution. However, data show clearly that ventilation increases consistently with exercise intensity. Concomitantly to exercise intensity, thigh muscle work increases. Consequently, a drop in $\Delta HHb/\Delta\dot{V}O_2$ it is very likely due to an enhanced local muscle perfusion. The m. serratus anterior is an auxiliary respiratory muscle that is supposed to support respiration especially under increased respiratory activity (25). Therefore, we think that $\Delta HHb/\Delta\dot{V}O_2$-ratio can be applied as a measure for respiratory muscle oxygen provision in the m. serratus anterior.

The reasons for this earlier onset of muscle deoxygenation and impaired matching of oxygen delivery and utilization might be explained by the response of the heart rate during exercise, as chronotropic incompetence could potentially explain part of this findings with the lack of increasing the cardiac output and thereby increased ΔHHb and decreased $\Delta HHb/\Delta\dot{V}O_2$-ratio earlier on exercise compared to healthy controls. Beside this, pulmonary vascular reactivity might be impaired, pulmonary vessel recruitment limited or even absent compared to healthy Controls (3).

In both groups, the matching of oxygen delivery to utilization improved during the initial phase of the incremental exercise test. However, this improvement stopped above 30% $\dot{V}O_{2peak}$ in Fontans and above 40% $\dot{V}O_{2peak}$ in Controls, respectively. This was close to VT (Fontans: 36.14 ± 16.71% $\dot{V}O_{2peak}$; Controls: 62.94 ± 9.61% $\dot{V}O_{2peak}$). Previous research showed that local oxygen availability in working muscles is improved following exercise bouts above VT (18), presumably due to greater local vasodilation. In the current study, $\Delta HHb/\Delta\dot{V}O_2$-ratio does improve further at intensities above VT.

The reasons for the improved $\Delta HHb/\Delta\dot{V}O_2$-ratio at low exercise intensities are likely due to the presence of more vasoactive substances, causing a greater local vasodilation (17). Various endothelial mediated pathways contribute to local vasodilation (26–28). Especially shear stress is to mention as important trigger for vasoactive substance release at higher workloads (29). Endothelial shear stress occurs when perfusion increases. In respiratory muscles, this can happen when breathing activity increases in response to the onset of exercise and therefore could explain why the matching of oxygen delivery and utilization improves across the lower exercise intensities. However, the $\Delta HHb/\Delta\dot{V}O_2$-ratio of the serratus anterior muscle stopped improving at exercise intensities close to VT. It therefore shows an inverted shape compared to the $\Delta HHb/\Delta\dot{V}O_2$-ratio that was measured previously in in working muscles during cycling exercise (18). One could speculate that this is due to the increasing exercise induced vasodilation in the working muscles above VT. It therefore would represent the blood flow redistributions to body regions with higher metabolic demands. Above VT, lactate is considered to support local vasodilation (30, 31) which could be an explanation for the promotion of local blood supply above VT e.g., to thigh muscles compared to respiratory muscles.

Besides the differences in microvascular hemodynamics, Fontan patients showed lower exercise tolerance and $\dot{V}O_{2peak}$, which is in accordance to previous literature (32). Ventilation was also lower in Fontan patients, even when it was expressed relatively to body surface. Former studies showed significantly reduced lung volume and weaker respiratory muscles (33). Beside a lack of exercise compared to healthy Controls, the number of thoracotomies has been suggested to account for this restricted lung patterns (34). The higher respiratory muscle activity in healthy subjects could have contributed to the better estimated microvascular perfusion by greater endothelial mediated vasodilation caused by higher shear stress on the one hand and by a greater muscle-pump-effect (28) on the other hand.

Of course, the muscular under-performance might be due to suboptimal hemodynamics like failure to increase cardiac output, reduced increase in heart rate and indicative of a general muscular under-performance. If we would postulate

this, the clinical implication of respiratory muscle oxygenation study would be of less clinical significance. Clinically, all our Fontans were in a good condition classified as NYHA II. However, training of respiratory muscles in the Fontan patients might improve the thoracic cavity mechanics under suboptimal hemodynamics, their pulmonary capacity and potentially also their microvascular perfusion. This could have a substantial effect in the management of patients with Fontan circulation. The next step would be to study the training effects on respiratory muscles in Fontans to figure out if there might be an improvement in microvascular perfusion.

Following a Fontan procedure, the heart's morphology results in an univentricular heart, either with the original left ventricle or the original right ventricle as the systemic ventricle. Because the left ventricle appears to be more powerful, the resulting heart morphology is of great importance. However, although VO_{2peak} and peak power output was significantly greater in Fontans with a left ventricle as the systemic ventricle, no differences could be observed regarding the muscle deoxygenation.

Study Limitations

The control group was significantly older than the Fontans which also indicates an unbalanced distribution of sexual maturity in both groups. This was due to dropouts, which substantially distorted the original distribution. We do not think that this bias affects the outcome too much for two reasons. First, the subjects age range of both groups overlaps significantly, indicating that both groups are recruited out of the same age-group. Of course, this does not solve the issue that the age distribution is unbalanced. Second, we addressed this issue by normalizing all relevant measures. Furthermore, we correlated relative respiratory muscle deoxygenation ($\%HHb_{max}$) with age at 50% $\dot{V}O_{2peak}$. This intensity evolved as the crucial intensity, where the onset of muscle deoxygenation has already happened in the Fontan group, but not in the control group. However, age was not significantly correlated with relative muscle deoxygenation ($r = -0.14$ for Fontans, $r = -0.37$ for Controls). Therefore, we assume that results were not influenced by the slightly different age distribution in both groups. In general, NIRS-derived signals are influenced by subcutaneous adipose tissue thickness (ATT) (35). To measure muscle oxygenation, the penetration depth, which is roughly half of the optode distance (15, 35), has to substantially exceed ATT. We used an inter-optode distance of 3.5 cm in this study, while ATT was 6.50 mm in Fontans and 5.46 mm in healthy Controls, respectively. Therefore, ATT should not impair NIRS-derived measures in this study.

Compared to adults, young children are mainly diaphragmatic breathers, meaning that when they face increased breathing work, they depend also to diaphragmatic contraction. The diaphragmatic function as major contributor to ventilation mechanics was not studied in our study. With the serratus anterior muscle, we analyzed an accessory muscle, probably not so much utilized in Fontan patients but comes to the fore when patients follow a respiratory muscle training.

A main issue is whether the oxygen saturation changes in the auxiliary respiratory muscles is indicative for a local response or it is indicative of a very similar or identical response of other muscles involved during the treadmill test. We did not compare the performance of respiratory muscles and skeletal muscles by using the NIRS technique on the same subjects in both cases and controls.

CONCLUSION

This study shows that that Fontan patients limitation in exercise capacity is not only due to limited oxygen provision in primary working muscles e.g., in arms and legs but also to auxiliary muscles like respiratory muscles. Therefore, both peripheral working muscles and respiratory muscles should be trained in order to improve local vascular function. Furthermore, respiratory muscle training could be beneficial especially for Fontan patients because in could support the passive blood flow for the pulmonary circulation.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

FS supervised the NIRS measurements, did the data processing and was mainly responsible for drafting the manuscript. RN and CF conducted the CPETs. RO and PE provided resources for research. AH was responsible for the general experimental procedures and was revised the drafting process. NN was the project leader and coordinated the study and co-wrote the manuscript. All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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Efficacy and Safety of Tranexamic Acid in Pediatric Patients Undergoing Cardiac Surgery: A Single-Center Experience

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Aims: This study evaluated the efficacy and safety of tranexamic acid (TXA) undergoing cardiac surgery.

Methods: Using a retrospective cohort study design, 2,026 consecutive pediatric patients who underwent surgical repair of atrial or ventricular septal defect or complete repair of Tetralogy of Fallot were included, and divided into a control group and a TXA group.

Results: Compared with that in the control group, there were statistically significant reduction of both the 12-h and total postoperative blood loss in the TXA group [6.573 ± 0.144 vs. 5.499 ± 0.133 ml kg^{-1}, mean difference (MD) 1.074 ml kg^{-1}, $p < 0.001$; 12.183 ± 0.298 vs. 9.973 ± 0.276 ml kg^{-1}, MD, 2.210 ml kg^{-1}, $p < 0.001$]. There was a statistically significant reduction of the MD of 12-h postoperative blood loss due to TXA in patients aged <1 year compared with that in patients aged ≥1 year (MD, 1.544 vs. 0.681 ml kg-1, $P = 0.007$). There were statistically significant reduction of the MD of both the 12-h and total postoperative blood loss due to TXA in patients weighing <10 kg compared with that in patients weighing ≥10 kg (MD, 1.542 vs. 0.456 ml kg-1, $P < 0.001$, and MD, 2.195 vs. 0.929 ml kg-1, $P = 0.036$, respectively). There was a statistically significant reduction of the MD of total postoperative blood loss due to TXA in cyanotic patients compared with that in acyanotic patients (MD, 3.381 vs. 1.038 ml kg^{-1}, $P = 0.002$). There was no significant difference in the postoperative volume or exposure of allogeneic transfusion, in-hospital morbidity or mortality between the groups.

Conclusions: TXA took effects in reduction of postoperative blood loss but not the allogeneic transfusion requirement in pediatric patients undergoing cardiac surgery, particularly in infants weighing <10 kg and cyanotic children. Moreover, the study suggested the use of TXA was safe in pediatric cardiac surgery.

Keywords: congenital heart disease, tranexamic acid, infant, cyanosis, safety, blood loss

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INTRODUCTION

Congenital heart disease (CHD) has been associated with abnormal coagulation, including low levels of fibrinogen and platelet dysfunction. Moreover, during the process of cardiopulmonary bypass (CPB), exposure of blood to artificial surfaces and hemodilution of blood due to priming volume caused activation of platelets, coagulation, and fibrinolysis, leading to a decrease in platelet number and function, and a reduction in fibrinogen levels (1). Therefore, pediatric patients undergoing cardiac surgery are at high risk of excessive bleeding and needing blood transfusion, which may increase postoperative morbidity and mortality (2). Since the suspension of aprotinin in 2008, tranexamic acid (TXA) has become the main antifibrinolytic agent to prevent blood loss in cardiac surgery (3). However, as the guideline points out, clinical studies on the use of TXA in pediatric cardiac surgery have been limited by small sample sizes and marked heterogeneity in the data (4). Moreover, the effect of TXA in infants (age 31 days−1 year) weighing <10 kg and pediatric patients with cyanosis, who are at increased risk of bleeding due to the specific hemostatic characteristics, remained uncertain.

As a result of aprotinin story, TXA should be given more attention considering the adverse events. Pasquali et al suggested that TXA was associated with significantly reduced mortality compared with aprotinin in pediatric cardiac surgery (5). Unfortunately, however, recent clinical trials and meta-analyses have shown a dose-dependent association between TXA and the risk of seizures in adults who undergo cardiac surgery (6–8). Retrospective studies revealed that TXA use was associated with a significantly increased risk of seizures in pediatric cardiac surgery (9, 10). TXA associated seizures may worsen the prognosis in pediatric cardiac surgery (11). Therefore, safety evaluations of TXA remain sparse in pediatric patients undergoing cardiac surgery.

The aim of this study was to evaluate the efficacy and safety of TXA in pediatric patients undergoing cardiac surgery.

MATERIALS AND METHODS

Patients and Study Design

This study was a retrospective, single-center, cohort study. The study protocol was approved by the institutional review board of Fuwai Hospital. The requirement for written informed consent was waived by the board. Two thousand and twenty six consecutive pediatric patients aged 31 to 12 years who underwent primary surgical repair of acyanotic CHD, i.e., atrial or ventricular septal defect, or complete repair for cyanotic CHD, i.e., Tetralogy of Fallot, at Fuwai Hospital in Beijing, China, between January 1, 2009 and December 31, 2010, were eligible for inclusion. The patients were divided into a control group that did not receive an antifibrinolytic agent during surgery ($n = 1056$) and a TXA group that was intravenously administered TXA after induction with a pump by maintenance at 15 mg/kg/h until termination of CPB ($n = 970$).

Perioperative Management

The standard surgical and anesthetic management techniques used in patients with atrial or ventricular septal defect and Tetralogy of Fallot were followed. Systemic anticoagulation was achieved using heparin 400 U/kg, with additional doses administered to maintain an activated clotting time >480 s. Priming volumes in CPB circuit depended on pediatric patients' body weights. The CPB circuit was primed with crystalloid and colloid. Packed red blood cells were also added to the prime to achieve a hematocrit level of >25% if the body weight was <10 kg. CPB was used and modified ultrafiltration was performed after separation from CPB. Protamine was administered at a protamine to heparin ratio of 1–1.2 to 1. No additional hemostatic agents were administered intraoperatively.

Measurements

The primary outcome was postoperative blood loss. The nurse in the ICU recorded postoperative volume of pericardial and mediastinal fluid collected via a drainage tube per hour. Twelve-hour postoperative blood loss was measured as the accumulated volume of pericardial and mediastinal fluid collected via a drainage tube during the first 12 h after surgery and total postoperative blood loss was that measured before removal of the tubes. The secondary efficacy outcomes were the intraoperative blood loss, the volume and exposure of allogeneic transfusion after termination of CPB. The threshold for red blood cell transfusion was a hemoglobin concentration of $<100\ g\ l^{-1}$ after termination of CPB. The indication for fresh frozen plasma was a requirement for clotting factors based on the results of coagulation tests. Concentrated platelets were administered at the discretion of the attending surgeon. The secondary safety outcomes were postoperative morbidity and mortality. Morbidity parameters included stroke, seizure, renal failure, deep venous thrombosis, use of extracorporeal membrane oxygenation, reoperation for bleeding, and prolonged mechanical ventilation. Stroke was diagnosed as a new focal neurologic deficit lasting >24 h or leading to earlier death and was confirmed by computed tomography or magnetic resonance imaging showing cerebral infarction or hemorrhage (12). Seizure was identified as a new-onset neuropsychiatric disorder with increased motor activity or an agitated or hyperactive state (12). Renal failure was defined as a need for postoperative peritoneal dialysis (9). Deep venous thrombosis was confirmed by clinical symptoms and venous Doppler ultrasonography findings (9). Reoperation for bleeding was performed when massive bleeding occurred with a drainage rate >10% of total blood volume per hour for up to 2 h or if cardiac tamponade was detected. Prolonged mechanical ventilation was defined as postoperative mechanical ventilation lasting longer than 72 h (13). Additionally, other study variables were the maximum creatinine value in the first 48 h postoperatively, length of stay in the intensive care unit, and duration of hospital stay.

Statistical Analysis

Normally distributed continuous variables are shown as the mean and standard deviation and were compared using the Student's t-test. Non-normally distributed continuous variables

are shown as the median and interquartile range and were compared using the Wilcoxon-Mann-Whitney test. Categorical variables are presented as the frequency and percentage and were compared using the chi-square or Fisher's exact test. A general linear regression model was used to analyse the maximum hemoglobin level in the first 48 h postoperatively, blood loss, allogeneic transfusion volume. The mean difference (MD) and 95% confidence interval (CI) were calculated. A multivariate logistic regression model was used to analyse allogeneic transfusion exposure. The odds ratio and 95% CI were calculated. Covariates were included into the regression analysis if the baseline confounders showed significant difference between the two groups or exactly affected the pharmacokinetics of TXA (including age, weight, type of surgery, CPB time, aortic cross-clamp time and surgical time) (14). All tests were two-sided, and a probability value <0.05 was considered statistically significant. All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Perioperative Data

There were 1,056 patients in the control group and 970 in the TXA group. The patient characteristics, including patient gender, age, height, and weight, were comparable between the two groups. There were 584 infants aged <1 year and 884 pediatric patients weighing <10 kg. The preoperative data, including for hemoglobin, platelets, prothrombin time, prothrombin time activity, international normalized ratio, and creatinine, were comparable between the groups. An atrial septal defect was repaired in 161 patients (15.24%) in the control group and 118 (12.16%) in the TXA group and a ventricular septal defect in 735 (69.60%) and 626 (64.54%), respectively. One hundred and sixty one patients (15.24%) in the control group and 225 (23.20%) in the TXA group underwent complete repair for tetralogy of Fallot. Compared with the control group, the TXA group had a significantly longer CPB time (66.62 ± 32.137 vs. 75.64 ± 37.104 min, $P < 0.001$), aortic cross-clamp time (41.56 ± 24.731 vs. 48.31 ± 27.840 min, $P < 0.001$), and surgical time (147.87 ± 42.394 vs. 159.59 ± 48.668 min, $P < 0.001$). The perioperative data for cyanotic patients were comparable between the two groups (**Table 1**).

Blood Loss and Transfusion Outcomes

There was no significant difference in intraoperative blood loss between the two groups. Compared with that in the control group, there was a statistically significant reduction of the 12-h postoperative blood loss in the TXA group (6.573 ± 0.144 vs. 5.499 ± 0.133 ml kg^{-1}; MD, 1.074 ml kg^{-1}; 95% CI, 0.710 to 1.438 ml kg^{-1}; $p < 0.001$). Compared with that in the control group, there was a statistically significant reduction of the total postoperative blood loss in the TXA group (12.183 ± 0.298 vs. 9.973 ± 0.276 ml kg^{-1}; MD, 2.210 ml kg^{-1}; 95% CI, 1.456 to 2.964 ml kg^{-1}; $p < 0.001$). There was no significant difference in the maximum hemoglobin level in the first 48 h postoperatively or in the allogeneic transfusion volume or exposure after termination of CPB (**Table 2**).

TABLE 1 | Perioperative data.

	The control group (n = 1,056)	The TXA group (n = 970)	P-value
Patient characteristics			
Male gender(%)	588 (55.68)	581 (59.90)	0.059
Age (years)	2.553 ± 1.938	2.483 ± 2.039	0.423
Age in TOF (years)	1.626 ± 1.411	1.417 ± 1.135	0.122
Infants (<1 year, %)	289 (27.37)	295 (30.41)	0.131
Height (cm)	87.51 ± 17.138	86.33 ± 17.776	0.129
Weight (kg)	12.37 ± 4.834	12.14 ± 5.036	0.283
Weight in TOF (kg)	9.83 ± 3.103	9.67 ± 2.599	0.597
Weight (<10 kg, %)	430 (40.72)	454 (46.80)	0.006
Preoperative data			
Hemoglobin (g l^{-1})	120.22 ± 15.876	121.31 ± 17.540	0.286
Hemoglobin in TOF (g l^{-1})	133.53 ± 24.662	132.82 ± 25.168	0.840
Platelets (10^9 l^{-1})	304.77 ± 81.762	299.79 ± 90.578	0.373
PT (s)	14.08 ± 1.253	13.93 ± 1.133	0.171
PTA (%)	85.46 ± 12.099	85.16 ± 8.921	0.769
INR	1.09 ± 0.139	1.08 ± 0.118	0.505
Creatinine (umol l^{-1})	30.03 ± 12.000	31.63 ± 12.524	0.107
Surgical data			
Type of surgery (%)			<0.001
ASD	161 (15.24)	118 (12.16)	
VSD	735 (69.60)	626 (64.54)	
TOF	161 (15.25)	225 (23.20)	
CPB time (min)	66.62 ± 32.137	75.64 ± 37.104	<0.001
CPB time in TOF (min)	112.04 ± 37.364	115.60 ± 40.281	0.379
Aortic cross-clamp time (min)	41.56 ± 24.731	48.31 ± 27.840	<0.001
Aortic cross-clamp time in TOF (min)	76.35 ± 27.919	78.49 ± 28.587	0.466
Surgical time (min)	147.87 ± 42.394	159.59 ± 48.668	<0.001
Surgical time in TOF (min)	200.19 ± 48.463	203.29 ± 55.354	0.569

Data are presented as incidence (%) or mean ± SD. TXA, tranexamic acid; TOF, tetralogy of Fallot; PT, prothrombin time; PTA, prothrombin time activity; INR, international normalized ratio; ASD, atrial septal defect; VSD, ventricular septal defect; CPB, cardiopulmonary bypass.

Subgroup Analysis

The 12-h postoperative blood loss in patients aged <1 year was 7.309 ± 0.213 ml kg^{-1} in the control group and 5.765 ± 0.216 ml kg^{-1} in the TXA group and that for patients aged ≥1 year was 5.647 ± 0.126 ml kg^{-1} and 4.966 ± 0.130 ml kg^{-1}, respectively. There was a statistically significant reduction of the MD of 12-h postoperative blood loss due to TXA in patients aged <1 year compared with that in patients aged ≥1 year (MD, 1.544 vs. 0.681 ml kg^{-1}, $P = 0.007$). For total postoperative blood loss, there was no significant difference of the MD of total postoperative blood loss due to TXA between age and treatment with TXA (MD 1.764 vs. 1.367 ml kg^{-1}, $P = 0.547$).

The 12-h postoperative blood loss in patients weighing <10 kg was 7.692 ± 0.171 ml kg^{-1} in the control group and 6.150 ± 0.171 ml kg^{-1} in the TXA group, and that for patients weighing ≥10 kg was 4.932 ± 0.142 ml kg^{-1} and 4.476 ± 0.154 ml kg^{-1}, respectively. There was a statistically significant reduction of the MD of 12-h postoperative blood loss due to TXA in patients

TABLE 2 | Blood loss and transfusion outcomes.

	The control group (n = 1,056)	The TXA group (n = 970)	P-value	Mean difference (95%CI)	Odds ratio (95%CI)
BLOOD LOSS					
Intraoperative blood loss (ml kg^{-1})	5.316 ± 0.090	5.224 ± 0.083	0.428	0.092 (−0.135 to 0.319)	–
12-h postoperative blood loss (ml kg^{-1})	6.573 ± 0.144	5.499 ± 0.133	<0.001	1.074 (0.710–1.438)	–
Total postoperative blood loss (ml kg^{-1})	12.183 ± 0.298	9.973 ± 0.276	<0.001	2.210 (1.456–2.964)	–
Maximum hemoglobin (g l^{-1} 48 h^{-1})	127.042 ± 0.837	128.398 ± 0.652	0.180	−1.355 (−3.338 to 0.627)	–
ALLOGENEIC TRANSFUSION					
RBC (u)	0.185 ± 0.016	0.177 ± 0.016	0.715	0.008 (−0.036 to 0.053)	–
FFP (ml)	34.601 ± 1.897	32.578 ± 1.980	0.463	2.022 (−3.382 to 7.427)	–
Platelets (u)	0.08 ± 0.03	0.12 ± 0.03	0.329	−0.04 (−0.13 to 0.05)	–
PATIENTS EXPOSED (%)					
RBC	161 (15.25)	148 (15.26)	0.084	–	0.794 (0.611–1.031)
FFP	234 (22.16)	253 (26.08)	0.134	–	0.818 (0.629–1.064)
Platelets	6 (0.57)	14 (1.44)	0.364	–	1.592 (0.584–4.341)

Data are presented as mean ± SD or incidence (%). TXA, tranexamic acid; RBC, red blood cells; FFP, fresh frozen plasma.

weighing <10 kg compared with that in patients weighing ≥10 kg (MD, 1.542 vs. 0.456 ml kg^{-1}, $P < 0.001$). The total postoperative blood loss in patients weighing <10 kg was 13.173 ± 0.356 ml kg^{-1} in the control group and 10.978 ± 0.356 ml kg^{-1} in the TXA group, and that for patients weighing ≥10 kg was 9.354 ± 0.295 ml kg^{-1} and 8.425 ± 0.320 ml kg^{-1}, respectively. There was a statistically significant reduction of the MD of total postoperative blood loss due to TXA in patients weighing <10 kg compared with that in patients weighing ≥10 kg (MD, 2.195 vs. 0.929 ml kg^{-1}, $P = 0.036$).

There was no significant difference of the MD of 12-h postoperative blood loss due to TXA between cyanotic status and TXA treatment (MD 1.304 vs. 0.844 ml kg^{-1}, $P = 0.214$). The total postoperative blood loss in cyanotic patients was 14.013 ± 0.577 ml kg^{-1} in the control group and 10.632 ± 0.514 ml kg^{-1} in the TXA group, and that for acyanotic patients was 10.352 ± 0.235 and 9.314 ± 0.251 ml kg^{-1}, respectively. There was a statistically significant reduction of the MD of total postoperative blood loss due to TXA in cyanotic patients compared with that in acyanotic patients (MD, 3.381 vs. 1.038 ml kg^{-1}, $P = 0.002$) (**Table 3**).

TABLE 3 | Interactions of postoperative blood loss with age, weight, cyanotic status, and administration of tranexamic acid.

	The control group (n = 1,056)	The TXA group (n = 970)	Mean difference	P-value
12-H Postoperative Blood Loss (Ml Kg^{-1})				
Age (<1 year)	7.309 ± 0.213	5.765 ± 0.216	1.544	0.007
Age (≥1 year)	5.647 ± 0.126	4.966 ± 0.130	0.681	
Weight (<10 kg)	7.692 ± 0.171	6.150 ± 0.171	1.542	<0.001
Weight (≥10 kg)	4.932 ± 0.142	4.476 ± 0.154	0.456	
Cyanosis	7.289 ± 0.279	5.985 ± 0.248	1.304	0.214
Acyanosis	5.858 ± 0.114	5.014 ± 0.121	0.844	
TOTAL POSTOPERATIVE BLOOD LOSS (ML KG^{-1})				
Age (<1 year)	12.866 ± 0.443	11.102 ± 0.449	1.764	0.547
Age (≥1 year)	10.249 ± 0.261	8.882 ± 0.271	1.367	
Weight (<10 kg)	13.173 ± 0.356	10.978 ± 0.356	2.195	0.036
Weight (≥10 kg)	9.354 ± 0.295	8.425 ± 0.320	0.929	
Cyanosis	14.013 ± 0.577	10.632 ± 0.514	3.381	0.002
Acyanosis	10.352 ± 0.235	9.314 ± 0.251	1.038	

Data are presented as mean ± SD. TXA, tranexamic acid.

Postoperative Outcomes

There was no in-hospital mortality in the control group. One patient (0.10%) in the TXA group died of multiple organ failure ($P = 0.309$). There was no significant difference in in-hospital morbidity (including seizure, stroke, renal failure, deep venous thrombosis, use of extracorporeal membrane oxygenation, reoperation for bleeding, and prolonged mechanical ventilation) between the groups. There was also no significant difference in the maximum creatinine value in the first 48 h postoperatively between the two groups. The postoperative stays in the intensive care unit and in hospital were comparable between the two groups (**Table 4**).

DISCUSSION

The results of our study suggested that TXA took effects in reduction of postoperative blood loss but not the allogeneic transfusion requirement in pediatric patients undergoing cardiac surgery, particularly in infants weighing <10 kg and children with cyanosis. Moreover, the study suggested that the use of TXA was safe in pediatric cardiac surgery.

As we know, aprotinin was just withdrawn from the market in 2008, and the clinical blood protection was still in a transitional period during 2009-2010. The study on the effects of TXA in pediatric cardiac surgery was limited at that time. Therefore, the anesthesiologist decided whether to apply TXA for

TABLE 4 | Postoperative outcomes.

	The control group (n = 1,056)	The TXA group (n = 970)	P-value
Mortality at discharge (%)	0	1 (0.10)	0.297
MORBIDITY AT DISCHARGE (%)			
Seizure	0	0	>0.99
Stroke	1 (0.09)	0	0.338
Maximum creatinine (umol l^{-1} 48 h^{-1})	39.85 ± 12.939	40.97 ± 13.383	0.307
Renal failure	2 (0.19)	1 (0.10)	0.614
Deep venous thrombosis	1 (0.09)	0	0.338
ECMO	0	1 (0.10)	0.297
Reoperation for bleeding	3 (0.28)	2 (0.21)	0.724
Prolonged mechanical ventilation	16 (1.52)	15 (1.55)	0.954
POSTOPERATIVE TIME COURSE			
ICU stay (days)	1 (1–2)	1(1–2)	0.539
Hospital length of stay (days)	7.403 ± 3.698	7.640 ± 4.122	0.172

Data are presented as incidence (%), mean ± SD or median (IQR). TXA, tranexamic acid; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit.

blood protection in pediatric cardiac surgery according to their personal experience. The patients in our study were intravenously administered TXA after induction with a pump by maintenance at 15 mg/kg/h until termination of CPB as the method in US Children's Hospitals. At present, there is wide variation in the dosage of TXA recommended for use during pediatric cardiac surgery (Loading doses of TXA ranged from 10 to 100 mg/kg and maintenance doses ranged from 1 to 15 mg/kg.h.) (15). Most studies reported late postoperative drain losses to evaluate postoperative blood loss; however, some at this time post-surgery these might be a mixture of blood and serosanguinous due to the post-bypass inflammatory response (1). Therefore, we not only recorded late postoperative drain losses, but also recorded early postoperative drain losses to exclude the influence of serosanguinous. TXA took effects in reduction of the 12-h postoperative blood loss by 1.074 ml kg^{-1} and the total postoperative blood loss by 2.210 ml kg^{-1}, but had no effect on the requirement for allogeneic transfusion. These results are comparable with those of some meta-analyses published in recent years (16, 17). However, the meta-analysis published by Faraoni et al. (17) showed that TXA reduced both postoperative blood loss and the requirement for allogeneic transfusion in pediatric cardiac surgery. The inconsistencies in the findings between our study and their meta-analysis likely reflect the type of race selected and the dosage regimen of TXA used. Our results are consistent with those of a randomized trial showing that TXA took effects in reduction of blood loss in pediatric cardiac surgery but not the transfusion requirement (18).

In the present study, TXA took effects in reduction of both 12-h and total postoperative blood loss in pediatric patients undergoing cardiac surgery, especially in infants weighing <10 kg and in cyanotic children. Infants weighing <10 kg are at particularly high risk of postoperative blood loss because of their immature coagulation systems, lower levels of fibrinogen, and the mismatch between the CPB priming volume and the infants' blood volume, resulting in hemodilution of up to 50-100%, which activates the inflammatory cascade and increases fibrinolytic activity (1). Moreover, cyanotic children undergoing cardiac surgery reportedly have significant preoperative coagulation anomalies and require more fibrinogen supplementation postoperatively (19). Therefore, we considered that it might be related to the antifibrinolytic, anti-platelet activation, and anti-inflammatory effects of TXA (20, 21), which might be more beneficial in infants weighing <10 kg and pediatric patients with cyanosis undergoing cardiac surgery, who are at high risk of postoperative blood loss due to the specific hemostatic characteristics. As we all know, massive hemorrhage and allogeneic blood transfusion increased postoperative morbidity and mortality. If perioperative blood transfusion cannot be reduced by TXA, but only the postoperative thoracic mediastinal drainage fluid can be reduced, giving TXA in pediatric cardiac surgery at potential risk is worth considering. Based on the above results, it is not recommended to use TXA in children with simple CHD. TXA is more suitable for infants weighing <10 kg and pediatric patients with cyanosis undergoing cardiac surgery. Therefore, the specific antifibrinolytic regimens to these patients undergoing cardiac surgery require further study.

Actually, Maeda et al. (10) reported TXA use was associated with a significantly increased risk of seizures. However, accurate data on doses of TXA were not available in the database. They found 0.2% seizure in non-TXA group and 1.6% seizure in TXA group. According to their results, more than 710 patients per group are needed to evaluate side effects related to TXA administration. This retrospective, single-center, cohort study assessed the benefit of TXA in a very large number of consecutive pediatric patients (n = 2026) undergoing cardiac surgery, which was powerful to explain the safety of TXA. In our present study, no seizures occurred in either study group after pediatric cardiac surgery. This might be the dosage regimen in our center, which was intravenously administered TXA after induction with a pump by maintenance at 15 mg/kg/h until termination of CPB, without the central nervous system damaged due to excessive TXA concentration in the brain from a bolus injection or a high dose of TXA. Our present data showed that there was no correlation noted between TXA and postoperative morbidity and mortality, which is consistent with previous prospective and retrospective reports (10, 17, 22).

This study has several limitations. First, the present study is a retrospective single-center design. Therefore, the potential problems of a non-randomized study may remain despite multivariate adjustment being used to reduce overt bias. Second, the present study excluded some high-risk patients, such as complex CHD. Therefore, the sample population was not representative of all patients in our institution. The safety and efficacy of TXA during pediatric cardiac surgery for these high-risk patients remains unexplored. Third, although the data

presented in this study could be dated back to 2009 and 2010, the CHD in this study was relatively simple and the methods of surgery, anesthesia and CPB have not changed much. In addition, aprotinin was just withdrawn from the market at this stage, and there was no other hemostatic drugs except for TXA at that time, which reduced the heterogeneity of the study.

CONCLUSIONS

In this analysis of 2,026 consecutive pediatric patients undergoing primary cardiac surgery, TXA took effects in reduction of postoperative blood loss but not the allogeneic transfusion requirement, particularly in infants weighing <10 kg and children with cyanosis. Moreover, the study suggested that the use of TXA was safe in pediatric cardiac surgery. However, large, multicenter, prospective randomized controlled trials are needed to evaluate the benefits of TXA and the most appropriate way of administering it in infants weighing <10 kg and children with cyanosis undergoing cardiac surgery.

ETHICS STATEMENT

The study protocol was approved by the institutional review board of Fuwai Hospital. The requirement for written informed consent was waived by the board.

AUTHOR CONTRIBUTIONS

YZ responsible for interpretation of the data, statistical analysis, drafting of the manuscript, and approval of the final version to be published. XZ, YaW, JS, SY, FD, YuW, ZZ, YJ, JG, LL, and FY were responsible for study conception, data collection and interpretation, revision of the manuscript, and approval of the final version to be published. All authors read and approved the final manuscript.

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ORIGINAL RESEARCH
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Physiological Fontan Procedure

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Objective: The conventional Fontan circulation deviates the superior vena cava (SVC = 1/3 of the systemic venous return) toward the right lung (3/5 of total lung volume) and the inferior vena cava (IVC = 2/3 of the systemic venous return) toward the left lung (2/5 of total lung volume). A "physiological" Fontan deviating the SVC toward the left lung and the IVC toward the right lung was compared with the conventional setting by computational fluid dynamics, studying whether this setting achieves a more favorable hemodynamics than the conventional Fontan circulation.

Materials and Methods: An *in-silico* 3D parametric model of the Fontan procedure was developed using idealized vascular geometries with invariant sizes of SVC, IVC, right pulmonary artery (RPA), and left pulmonary artery (LPA), steady inflow velocities at IVC and SVC, and constant equal outflow pressures at RPA and LPA. These parameters were set to perform finite-volume incompressible steady flow simulations, assuming a single-phase, Newtonian, isothermal, laminar blood flow. Numerically converged finite-volume mass and momentum flow balances determined the inlet pressures and the outflow rates. Numerical closed-path integration of energy fluxes across domain boundaries determined the flow energy loss rate through the Fontan circulation. The comparison evaluated: (1) mean IVC pressure; (2) energy loss rate; (3) kinetic energy maximum value throughout the domain volume.

Results: The comparison of the physiological vs. conventional Fontan provided these results: (1) mean IVC pressure 13.9 vs. 14.1 mmHg (= 0.2 mmHg reduction); (2) energy loss rate 5.55 vs. 6.61 mW (= 16% reduction); (3) maximum kinetic energy 283 vs. 396 J/m^3 (= 29% reduction).

Conclusions: A more physiological flow distribution is accompanied by a reduction of mean IVC pressure and by substantial reductions of energy loss rate and of peak kinetic energy. The potential clinical impact of these hemodynamic changes in reducing the incidence and severity of the adverse long-term effects of the Fontan circulation, in particular liver failure and protein-losing enteropathy, still remains to be assessed and will be the subject of future work.

Keywords: congenital heart defects, congenital heart surgery, Fontan procedure, flow modeling, computational fluid dynamics, performance prediction, physiological design, univentricular heart

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INTRODUCTION

The principle of the Fontan circulation, successfully introduced by Frances Fontan for a patient with tricuspid atresia (1) in the early seventies, has been since applied to a huge variety of congenital heart defects, with various morphologies. All these complex congenital heart defects share the same characteristic of having "functionally" univentricular hearts, because of the presence of only one ventricle morphologically and/or functionally adequate to support the systemic circulation, pumping the oxygenated blood into the aorta (2–7). In the Fontan circulation, the low oxygen saturation blood returning from the superior vena cava (SVC) and from the inferior vena cava (IVC) is deviated directly through the lungs by various types of surgical connections with the pulmonary arteries (8).

A preparation is generally required in the neonatal period to either increase (9–11) or decrease (12–17) the pulmonary blood flow, or to use the right ventricle and the proximal pulmonary artery to provide blood flow to the systemic circulation (18–20), with the pulmonary blood flow obtained with either a modified Blalock-Taussig shunt (21, 22) or with a right ventricle to pulmonary artery conduit (23).

The vast majority of children with balanced forms of univentricular heart require a surgical palliation early in life (4, 24–26). After the neonatal palliation, the Fontan circulation is generally established in two stages: an end-to-side connection between the SVC and the right pulmonary artery (bi-directional Glenn) (27–31), followed later by the Fontan completion, with the connection of the IVC to the pulmonary artery. Early techniques of direct atrio-pulmonary connection have been virtually abandoned in subsequent years, so that the two Fontan completion techniques most frequently utilized nowadays are the lateral tunnel, or intra-cardiac Fontan (32–34), and the extra-cardiac Fontan, connecting the transected stump of the IVC to the pulmonary artery with the interposition of a tubular prosthesis (35–38).

The long-term results of the Fontan circulation are complicated by substantial morbidity. This includes: chronic venous hypertension with increased hydrostatic capillary pressure, recurrent pericardial and pleural effusions, ascites, generalized fluid retention, renal failure, hepatic failure, gastro-intestinal dysfunction, supra-ventricular and ventricular arrhythmias, pulmonary and systemic thromboembolism, protein-losing enteropathy, pulmonary arterio-venous malformations and veno-venous collaterals, persistent and/or progressive hypoxemia, progressive impairment of the ventricular function, exercise intolerance, plastic bronchitis (39–69).

Mathematical models and computational fluid dynamic (CFD) studies have been extensively applied to assess the various types of cavo-pulmonary connections (70–87).

All current surgical techniques for the completion of the Fontan circulation deviate the blood from the SVC, which constitutes approximately 1/3 of the systemic venous return, to the right lung, occupying 60% of the total lung volume, while the blood from the IVC, which constitutes ∼2/3 of the systemic venous return, is deviated to the left lung, with 40% of the total lung volume. Deviating the larger flow rate towards the lower volume lung and the converse suggests an unfavorable flow distribution, which may contribute to the poor long-term outcomes listed above.

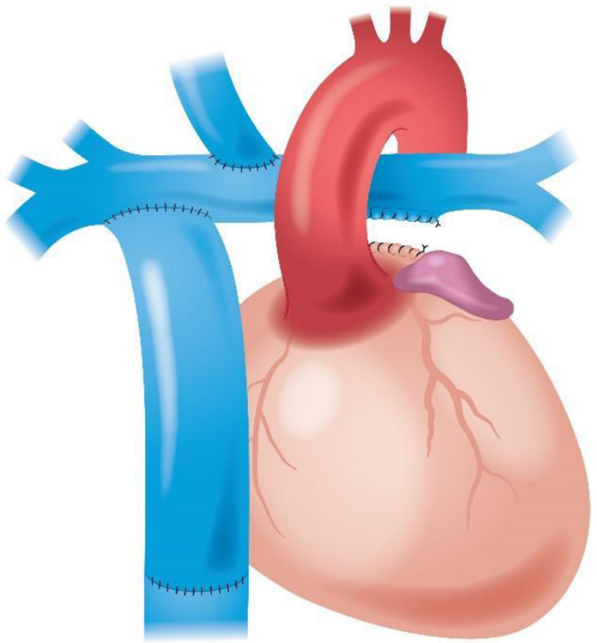

FIGURE 1 | Conceptual arrangement of the new physiological Fontan procedure.

The purpose of this study is to evaluate, using CFD models, a new "plumbing" for the completion of the Fontan circulation, with the SVC smaller venous return channeled toward the smaller left lung and the IVC larger venous return deviated toward the larger right lung, so that the ranking of the two blood flow rates matches the size ranking of the lungs. This study investigates whether this new proposal for the Fontan procedure, herein named "physiological" Fontan, can provide a better flow distribution than the "conventional" extra-cardiac Fontan.

MATERIALS AND METHODS

An *in-silico* three-dimensional (3D) parametric model of the physiological Fontan circulation was developed with the positions of the SVC and IVC connections varied along the pulmonary arteries (**Figure 1**). Idealized vascular geometries were constructed with constant dimensions of the SVC, the IVC, and the right and left pulmonary arteries. Steady velocity inflows for the IVC and SVC and constant equal outflow pressures for the right and left pulmonary arteries were set accordingly to established literature reports (70–84), to obtain finite-volume incompressible steady flow simulations, assuming a single-phase, Newtonian, isothermal, laminar blood flow.

The key reference data for dimensioning the vascular geometry were a 3.5 year old subject, body weight 16 kg, height 98 cm, body surface area 0.65 m^2, and total cardiac output

2.6 L/min, with an indexed cardiac output of 4 L/min/m^2. The pulmonary artery length ℓ, measured as the straight-line distance between the first branching of the right and left pulmonary arteries, was 7.3 cm. For comparison purposes, a baseline configuration was defined by modeling the conventional Fontan circulation, with the SVC confluence at 0.9 ℓ from the left pulmonary artery and the IVC confluence at 0.6 ℓ from the left pulmonary artery, with a right-angle (90°) connection for the SVC and a 75° connection facing toward the left pulmonary artery for the IVC. In the parametric model of the physiological Fontan, the IVC confluence was varied over the range $0.77\ \ell \leq x \leq 0.92\ \ell$ from the first branching of the left pulmonary artery. The IVC confluence angle with the pulmonary artery was varied with 15° increments between 60° and 90°. The SVC was connected to the pulmonary artery at a fixed angle of 60°. This connection was held at 0.50 ℓ from the first branching of the left pulmonary artery considering this as the maximum free length of transected SVC available after adequate surgical dissection when performing a bidirectional Glenn procedure.

These parametrized geometries defined the computational domain for finite-volume steady incompressible flow simulations, assuming a single-phase, Newtonian, isothermal, and laminar blood flow, of constant density $\rho = 1060\,\mathrm{kg\ m^{-3}}$ and molecular viscosity $3.5 \times 10^{-3}\,\mathrm{kg\,m^{-1}\,s^{-1}}$. A 3D solid model of the computational domain was obtained using the commercial Computational Aided Design (CAD) package Solidworks 2018 (Dassaut Systèmes, Vélizy-Villacoublay, France), in which all surfaces were rendered as non-uniform rational basis splines (NURBs) for dimensional accuracy. Two mm radius fillets were applied at the edges of all vascular intersections to reproduce the natural behavior of the tissue that rounds off at the anastomoses.

The computational domains were meshed by a five-layers thick prism carpet mesh lining the walls that surrounds an unstructured tetrahedral mesh. A sample mesh of a conventional Fontan configuration is shown in **Figure 2**. The spatial discretization was obtained by the commercial CFD pre-processor ICEM CFD by ANSYS Fluent (Ansys Inc., Canonsburg, Pennsylvania, USA). Care was taken to produce meshes with adequate cell orthogonality and aspect ratio in light of current CFD practice. The cell orthogonality and aspect ratio were used as mesh quality parameters. In the conventional Fontan, 1% of the cells have orthogonality <0.4 and 1% of the cells have aspect ratio higher than 11.1. These values were also representative of the meshes obtained for the parametrized geometry for the physiological Fontan. A judicious selection of the spatial discretization level was used, based on textbook examples of laminar flows in pipes (88). The appropriateness of this selection is tested later on in this article by determining the variability of the predictions over six different computational meshes, with 1M, 2M, 4M, 8M, 12M, and 18M cells, respectively, for both the conventional and the physiological Fontan settings.

Boundary conditions were imposed over all cells outwards-facing the computational domain. The no-slip condition was applied on all walls. Parabolic velocity profiles at SVC and IVC inlets were used with a prescribed steady mass flow rate. Specifically, the SVC drained 1/3 and the IVC carried 2/3 of the total systemic venous return. The right and left pulmonary arteries were set at constant equal outflow pressures as previously reported in other CFD studies (87). This fully defined the boundary value problem in hand.

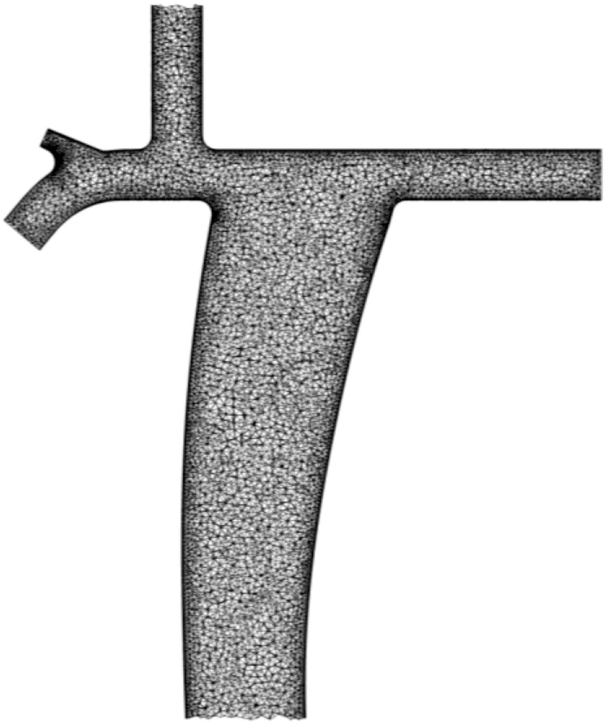

FIGURE 2 | Computational domain of the conventional Fontan configuration meshed by a five-layers thick prism carpet mesh lining the walls that surrounds an unstructured tetrahedral mesh.

Numerical solutions of the flow were obtained using the commercial CFD package ANSYS Fluent (Ansys Inc., Canonsburg, Pennsylvania, USA). Laminar flow simulations were obtained by application of the Semi-Implicit Method for Pressure Linked Equations (SIMPLE) (85), using a second order accurate upwind scheme for the velocity components (86). For numerical stability, under-relaxation factors of 0.7 and 0.4 were used for velocities and pressure, respectively. The convergence of the numerical solution was monitored by the residuals of the mass and of the momentum balances. Convergence was taken as a reduction to below 10^{-4} of the starting value of these residuals.

Numerically converged finite-volume mass and momentum flow balances determined the pressures and flow rates at the inlets and outlets from solving the model. Numerical closed-path integration of the energy fluxes across the domain boundaries determined the flow energy loss rate through the Fontan plumbing.

With invariant sizes of SVC (diameter = 7.5 mm), IVC (=18 mm conduit), right (diameter = 7.5 mm) and left (diameter = 7.5 mm) pulmonary arteries, invariant flows from SVC and IVC (output indexed/body surface area = 4 L/min/m^2 of body surface area), and invariant right and left pulmonary artery outflow pressures (=12 mm Hg), the comparison between the conventional extra-cardiac Fontan and the physiological Fontan plumbing evaluated: (1) mean IVC pressure; (2) energy loss rate; (3) kinetic energy maximum value throughout the domain volume.

TABLE 1 | Energy loss rate in mW from 21 configurations of "physiological" Fontan as predicted by CFD.

	$\theta = 60°$	$\theta = 75°$	$\theta = 90°$
0.77 ℓ	5.66	5.94	6.02
0.78 ℓ	5.70	5.91	6.12
0.81 ℓ	5.55	5.80	5.79
0.85 ℓ	5.57	5.75	5.96
0.88 ℓ	5.71	5.91	6.08
0.91 ℓ	5.84	5.98	6.10
0.92 ℓ	5.88	6.01	6.19

Twenty-one numerical experiments were conducted by systematically varying the IVC angle and location of confluence with the pulmonary artery (three angles and seven locations). The most promising configuration was identified based on the predicted lowest energy loss rate.

RESULTS

Energy loss rates for all the 21 tested configurations are reported in **Table 1**. Reported values are of the same order of magnitude as in previous CFD simulations of the Fontan circulation (75, 88, 89). The physiological Fontan with the IVC angle of confluence of 60° and centerline confluence $x = 0.81\ \ell$ is the configuration that has comparatively the lowest energy loss rate among the 21 variants considered in this study. Hence this is singled out as the preferred configuration.

A more detailed comparison between this configuration and the conventional Fontan was performed to identify flow features potentially responsible for the observed changes in the energy loss rate. **Figure 3A** shows the ribbons representing the predicted streamlines from the conventional Fontan model, using the 1M cells discretization. The ribbons were color coded by kinetic energy per unit volume. Most of the streamlines from the IVC were predicted to confluence toward the LPA, while the SVC was predicted to mainly supply the RPA (**Figure 3A**). The confluence from the SVC and IVC toward the RPA was characterized by localized flow acceleration that generated a kinetic energy peak shown in red in **Figure 3A**. This peak was about 350 J/m^3. In the modeled laminar flow, energy was lost by viscous stresses caused by the velocity gradients in the flow. The kinetic energy peak indicated local high values of velocity and, by inference, a high velocity gradient to the surrounding stationary walls.

Figure 3B shows the corresponding predictions from the physiological Fontan, using the same notation and color scale for kinetic energy per unit volume as **Figure 3A**. Approximately the same spatial discretization of 1M cells was used. In the physiological Fontan, most of the streamlines from the IVC were predicted to confluence toward the RPA. The IVC confluence angle of 60° appeared to yield a less tortuous flow path through the PA compared to **Figure 3A**, as suggested by a reduced twisting in the ribbons just above the IVC anastomosis. In this configuration, the kinetic energy peak per unit volume was reduced to about 250 J/m^3, as shown by the green iso-level, representing the peak iso-level in these ribbons. This reduced peak kinetic energy was expected to reduce the flow shear rate and thereby to reduce the viscous losses. This hypothesis was confirmed by the evaluation of the energy loss rate by integration over the inlets and outlets of the modeled vascular systems.

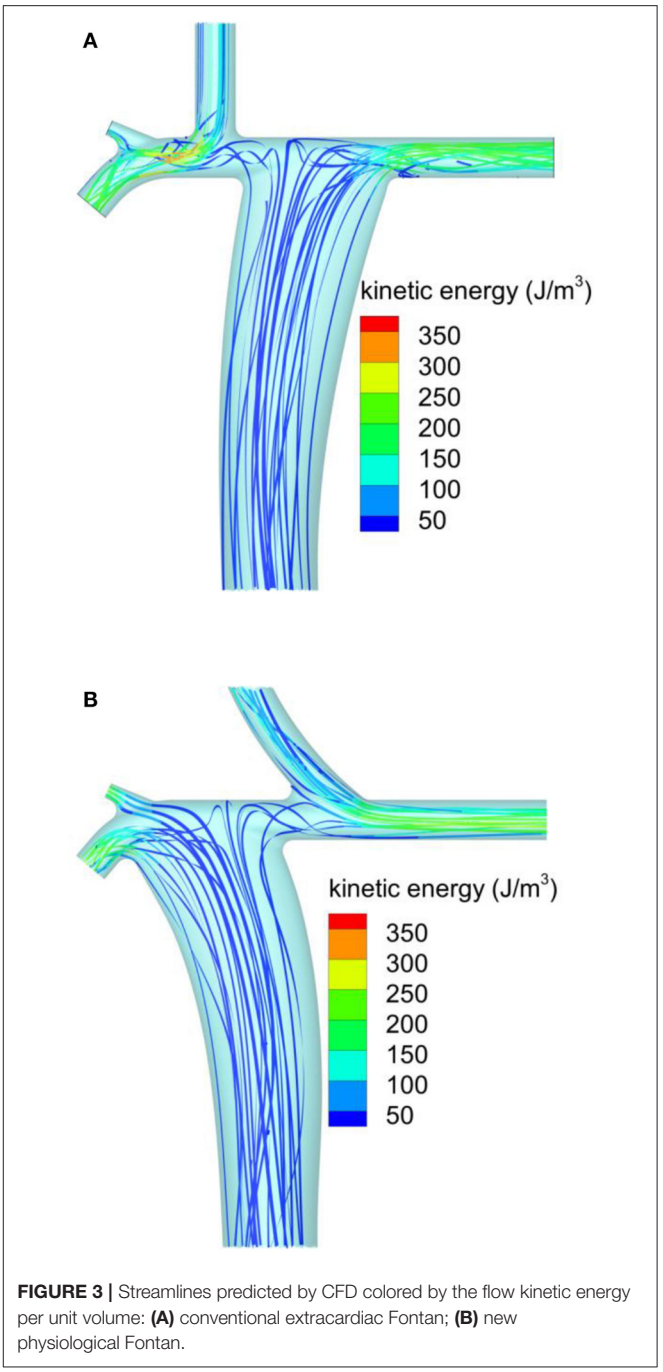

FIGURE 3 | Streamlines predicted by CFD colored by the flow kinetic energy per unit volume: **(A)** conventional extracardiac Fontan; **(B)** new physiological Fontan.

The comparison of the physiological vs. the conventional extra-cardiac Fontan provided the following results: (1) mean IVC pressure 13.9 vs. 14.1 mmHg (=0.2 mmHg reduction); (2) energy loss rate 5.55 mW vs. 6.61 mW (=16% reduction); (3) peak kinetic energy per unit volume 283 J/m^3 vs. 396 J/m^3 (=29% reduction).

TABLE 2 | Performance of six realizations of the "physiological" Fontan, predicted on different CFD meshes.

	Mean IVC pressure (mmHg)	Energy loss rate (mW)	Peak kinetic energy (J/m^3)
1M	13.92	5.55	284
2M	13.92	5.55	289
4M	13.95	5.66	290
8M	13.94	5.65	288
12M	13.96	5.67	292
18M	13.96	5.61	290
Mean	13.94 ± 0.017	5.62 ± 0.05	289 ± 3

TABLE 3 | Performance of six realizations of the conventional Fontan, predicted on different CFD meshes.

	Mean IVC pressure (mmHg)	Energy loss rate (mW)	Peak kinetic energy (J/m^3)
1M	14.05	6.61	396
2M	14.07	6.66	424
4M	14.05	6.54	421
8M	14.05	6.53	415
12M	14.05	6.53	423
18M	14.05	6.54	436
Mean	14.05 ± 0.008	6.57 ± 0.05	419 ± 13

At this point, we tested the sensitivity of the predictions to the spatial discretization used in the model, which, although judiciously selected based on the established CFD practice (88), represented an arbitrary model input. To this end, the best performing physiological Fontan geometry was modeled on six different computational meshes of approximate node numbers of 1M, 2M, 4M, 8M, 12M, and 18M. **Table 2** shows the predicted values from each computation, the ensemble mean, and the 95% confidence interval for the ensemble mean, based on the *t*-distribution of six samples. **Table 3** shows the corresponding values from the conventional Fontan simulations.

Table 2 shows that the ensemble mean and 95% confidence interval for energy loss rate for the physiological Fontan is 5.62 ± 0.05 mW against the corresponding result for the conventional Fontan of 6.57 ± 0.05 mW from **Table 3**. There was no overlap between the confidence interval bands, therefore the two groups of simulations were separable. As a result, the reduction in energy loss rate was statistically significant, to a 95% confidence, as determined by the *t*-test. The physiological Fontan was confirmed as having a lower energy loss rate than the conventional Fontan.

The corresponding result for the mean IVC pressure is 13.94 ± 0.017 mmHg for the physiological Fontan against 14.05 ± 0.008 mmHg for the conventional Fontan. There was no overlap between the confidence interval bands, therefore the two groups of simulations were separable and showed that the mean IVC pressure from the physiological Fontan is numerically lower than that of the conventional Fontan. Specifically, the *t*-distribution suggests that, with a 95% confidence, the physiological Fontan is reducing the mean IVC pressure compared to the conventional Fontan. As these simulations used an idealized geometry, the magnitude of the change in IVC pressure has lower significance than the sign of this change, since the magnitude of this change is likely to differ from subject to subject.

Finally, **Tables 2** and **3** report the maximum kinetic energy predicted by the two sets of simulations, as determined from the largest value of the scalar product of the velocity vectors in the computational domain interior. The kinetic energy values in **Tables 2** and **3** are reported per unit volume, in the form and units of the flow dynamic pressure. This permitted to evaluate and locate the maximum of this intensive property in the modeled flow domain. The conventional Fontan is predicted to produce a flow with peak kinetic energy of 419 ± 13 J/m^3, which is about one and a half the peak kinetic energy of 289 ± 3 J/m^3 from the physiological Fontan. The *t*-distribution analysis indicates that the two sets of simulations (conventional and physiological Fontan) are also separable based on peak kinetic energy, as their mean difference is 130 J/m^3 against a statistical uncertainty of 13.3 J/m^3, to a 95% statistical confidence.

DISCUSSION

The idea of deviating the systemic venous return from the SVC toward the left lung and the systemic venous return from the IVC toward the right lung was already a matter of discussion between one of the authors (AFC) and Dr. Hillel Laks, cardiac surgeon at University of California, Los Angeles. Dr. Laks realized in a series of patients a modified connection of the SVC to the left pulmonary artery and of the IVC to the right pulmonary artery by completely dividing the right and the left pulmonary artery (90). This surgical technique obtained the distribution of the systemic venous returns to the appropriate size lungs, but unfortunately the separation of right and left pulmonary artery resulted in the left lung receiving only the blood from the SVC, therefore without the hepatic factor contained in the blood drained from the IVC. This combination is documented to cause pulmonary arterio-venous fistulas within a few years, with the subsequent reduction of oxygen saturation, with very poor clinical tolerance (39, 42, 44, 45, 49, 54, 55, 61).

The new physiological Fontan proposed in this study can obtain the appropriate blood flow distribution, matching the ranking of the systemic venous returns to the lung sizes ranking, without separating the right and left pulmonary arteries, and therefore allowing the blood from the IVC, containing the hepatic factor, to reach both lungs and hence prevent the complications of pulmonary arterio-venous fistulas.

The reduction in energy loss rate in the physiological Fontan was positively correlated to the reduction in the peak kinetic energy in the flow, which, as reported in **Tables 2** and **3**, was also separable and statistically significant. Flow visualization located the kinetic energy peak of the conventional Fontan at the confluence between the SVC and the IVC, toward the LPA. This peak is detrimental to the circulation effort through the vascular

system, as kinetic energy is irreversibly lost by viscous stresses due to the velocity gradient between this fast moving flow and the stationary fluid wetting the walls of the Fontan vessels.

The reduction in energy loss rate associated to the physiological Fontan is enabled by the shallower confluence angles of the SVC and IVC with the PA, deviating these inflows respectively toward the LPA and RPA, thereby preserving the inflow momentum, compared to more right-angle confluences present in the conventional Fontan. In the clinical practice, the shallower angle confluence can be surgically accomplished only if the anastomoses of the SVC and IVC are transposed as in the physiological Fontan.

The physiological Fontan was also predicted, with 95% statistical confidence, to lower the IVC pressure compared to the conventional Fontan. The certainty in the direction of change of the IVC pressure is of prime importance, since high blood pressure has a strong causation link to liver and kidney failure in patients with Fontan circulation in the 20–30 years age group (43, 47, 50, 51, 60, 62, 64, 67, 69). The authors have used an idealized model for this comparative analysis, therefore the magnitude of the IVC pressure change is comparatively less significant, as this is dependent on the specific vascular geometry of the subject that is not rendered by the generalized geometry used in this work.

This surgical approach requires the first step of the Fontan circulation (bidirectional Glenn) with the end-to-side anastomosis of the superior vena cava obliquely moved toward the pulmonary artery bifurcation, instead that directly on the right pulmonary artery. This modification, deviating the flow toward the left lung, in principle should reduce the incidence of relative left pulmonary artery hypoplasia or small size frequently observed during the pre-operative investigations before Fontan completion, without interfering with the flow toward the right lung and therefore with the growth of the right pulmonary artery (91–93). By deviating the larger flow of the IVC towards the right lung, this new plumbing should also ensure that the right lung will not remain hypo-perfused after completion of the Fontan circulation.

As far as the pulmonary flow distribution is concerned, our results showed that, in addition to reducing the energy loss rate and the peak kinetic energy, the flow was uniformly divided between left and right pulmonary arteries in a very similar way as with the conventional Fontan.

Limits of the Study

This preliminary CFD study aimed at addressing the fundamental question of whether a more "physiological" Fontan could provide a viable alternative to the "conventional" Fontan. Obtaining a general indication required adopting a very idealized geometry with uniform anatomy, obtained from 3D reconstruction of investigations in a real patient, that could express the essence of the difference between the vascular layouts under consideration. It would be beneficial to perform a CFD evaluation on subject-specific vascular systems, acquired by medical imaging, pre-operation, to obtain more specific predictions about this new surgical option. Pressure and flow rates acquired from clinical practice can inform the boundary conditions used in the CFD model, using an approach similar to the one described in previous reports (70–74, 80–87).

The steady flow modeling approach could be improved by repeating the simulations in a time-dependent CFD framework, using prescribed waveforms of mass flow rate at the SVC and IVC inflows (75) and adding a lumped mass parameter model of the dependence of the LPA and RPA outflow pressures on the flow rate through these outlets (75, 91). Here, the challenge is to obtain a lumped-parameter model that renders the difference in resistance and blood flow capacitance between the left and the right lungs (also including the effects of the respiratory cycle), which has so far not been modeled in numerical studies of Fontan circulation.

The CFD model assumed rigid and non-compliant walls, without the interferences with the walls caused by the flow, in line with a previous CFD study of the Fontan circulation (75). The significance of the variation of the lumen cross-section during the cardiac cycle should be assessed, as this may have a significant impact on temporal effects not rendered by the current steady flow CFD model.

Finally, whilst the proposed anastomosis of the SVC would be expected to reduce the incidence of hypoplasia of the left pulmonary artery, the perfusion of the left and of the right lungs after Glenn should be investigated numerically, to make sure the right lung is not significantly hypo-perfused before the Fontan completion, which may lead to the opposite problem of hypoplasia of the right pulmonary artery.

Despite all the above limits, mathematical and CFD studies are still justified as methods for the first step in the "*in vitro*" evaluation of the cavopulmonary connections, even though currently they have to deal with all the variables present in the biological environment. Animals with univentricular hearts (such as frogs and turtles), are not suitable for experimental studies of cavopulmonary connections, and all animal models used to perform hemodynamic evaluation of cavopulmonary connections only allowed acute studies, as in our previous experience (66) and as confirmed by a recent systematic review (94).

CONCLUSIONS

This *in silico* study confirmed that the proposed Fontan configuration characterized by a more physiological flow distribution is accompanied by a little reduction of mean IVC pressure and by a substantial reduction of energy loss rate and of peak kinetic energy. The potential clinical impact of these hemodynamic changes is to improve the long-term outcomes of the Fontan circulation, in particular to reduce the adverse incidence of liver failure and protein-losing enteropathy. Further studies with *in vitro* mock circuits should provide more information about the potential advantages on fluid dynamics of the new physiological Fontan procedure. In particular, they could provide more information about the magnitude of the IVC pressure reduction that can be achieved by the physiological Fontan compared to the conventional Fontan, by regressing flow measurements obtained from different vascular geometries based on subject-specific measurements of the vascular system.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

AFC proposed the new procedure, collaborated to the preparation of the manuscript and the final revision. MO, AC, and EH produced the mathematical and CFD models used for the evaluations in the study, running all the required computations, and reviewed the manuscript. AR contributed and supervised the mathematical and CFD evaluations of the study, combining the requirements of the mathematical and clinical aspects, contributed to the preparation of the manuscript, and revised the final edition.

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Hypertension Prevalence Based on Three Separate Visits and Its Association With Obesity Among Chinese Children and Adolescents

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Background: Clinical practice guidelines recommended that hypertension in children and adolescents should be defined based on elevated blood pressure (BP) on at least three separate occasions. Therefore, in the present study, we aimed to estimate the prevalence of hypertension based on three separate visits among Chinese children and adolescents and to examine its relationship with obesity.

Methods: A school-based cross-sectional survey was performed in children and adolescents in Jinan, China between September 2012 and September 2014. A total of 7,832 children and adolescents aged 6–17 years were included. Anthropometric data and BP were measured by trained examiners. Elevated BP was defined as BP $\geq$ 95th percentile for age and sex based on the Chinese reference data. Participants with elevated BP at the first visit underwent a second visit 2 weeks later, and a third visit was conducted if BP was still high at the second visit. Hypertension was defined as having an elevated BP at all three visits. Obesity was defined in three ways by using body mass index, waist circumference, and waist-to-height ratio.

Results: The prevalence of elevated BP decreased substantially across three separate visits, with the prevalence of 17.2, 8.6, and 4.9%, respectively. Obesity was an independent risk factor for elevated BP during each visit. Based on the body mass index, obesity was associated with higher risk of elevated BP, with the adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of 8.6 (6.8–11.0), 12.5 (9.1–17.3), and 14.0 (8.9–22.2), respectively, at the first, second and third visit. The ORs of elevated BP were similar in association with obesity defined by waist circumference or waist-to-height ratio.

Conclusions: The prevalence of hypertension based on three visits was ~5% in Chinese children and adolescents. There was a dose-response relationship between obesity and elevated BP across three visits.

Keywords: blood pressure, hypertension, obesity, children, cross-sectional, repeated measurement

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INTRODUCTION

Hypertension in adults has been one of the major contributors to cardiovascular disease burden worldwide. It is estimated that nearly 10.7 million deaths and 211.8 million disability adjusted life-years were due to hypertension worldwide in 2015 (1). Notably, pediatric elevated blood pressure (BP) tends to track into adulthood (2) and it is associated with a series of early target organ damages in childhood (3), which can increase the risk of cardiovascular diseases later in life. Therefore, recognition and control of elevated BP at an early age may be an important strategy for reducing the hypertension-induced cardiovascular disease burden.

BP in children may vary a lot due to several potential reasons, such as the "white coat effects" and environmental factors (e.g., temperature and noise), which may cause transient elevated BP. Therefore, hypertension in children defined based on the BP measured at one single visit may overestimate the true prevalence. Indeed, clinical practice guidelines recommended that hypertension in children and adolescents should be defined based on elevated BP on at least three separate occasions (4–6). Up to now, several studies from western pediatric populations have assessed the prevalence of hypertension based on this recommendation (7, 8). However, few are from Chinese children and adolescents (9, 10). In addition, different populations may have different growth and development patterns and different exposures to lifestyle factors.

Adiposity has been shown to be an independent risk factor for elevated BP in children. However, most analyses were conducted using body mass index (BMI) as the indicator to assess weight status, which cannot distinguish fat distribution. Waist circumference (WC) and waist-to-height ratio (WHtR) are indicators of abdominal obesity that can better assess the distribution of the visceral adipose tissue (11).

Thus, based on a school-based, cross-sectional survey conducted in Jinan, China, we aimed to estimate the true prevalence of hypertension among Chinese children and adolescents aged 6–17 years. In addition, we examined the relationship between elevated BP and excess weight defined in three ways.

MATERIALS AND METHODS

Study Population

This study was conducted in four schools in urban region of Jinan, China between September 2012 and September 2014. The four public schools, including two primary schools, one junior high school and one senior high school, were chosen using convenient cluster sampling method. All students from the selected schools were invited to participate in the survey. A standard questionnaire, including demographic information, family history of hypertension, puberty status, and lifestyle factors, was finished by the students and/or their parents. Physical examinations (i.e., height, weight, WC, and BP) were conducted by trained research staff according to a standard protocol. A total of 7,832 students were included in the present study after excluding those with missing information on age, sex, height, weight, WC, and BP. Signed informed consent was obtained from all students and their guardians. Ethical approval was obtained from the Ethics Committee of the Capital Institute of Pediatrics in Beijing, China.

BP Measurements and Definitions

BP was measured by trained examiners with a clinically validated electronic device (OMRON HEM-7012) (12). After at least 10 min of rest, BP was measured on the right arm supported at the level of heart, in a sitting position with the back supported and feet flat on the floor. The mid-arm circumference was measured and appropriate cuff size was chosen (i.e. small, normal, or large cuff for a mid-arm circumference of 13.0–21.9, 22.0–31.9, or 32.0–42.0 cm, respectively). Three consecutive BP measurements were taken with at least 1 min apart. If the difference between any two of the three BP readings was more than 5 mmHg, then a fourth BP measurement was conducted. Mean value of the last two readings was used for data analysis.

Elevated BP was defined if systolic BP (SBP) and/or diastolic BP (DBP) ≥age- and sex-specific 95th percentiles of the Chinese BP references (13). Participants with elevated BP at the first visit underwent a second visit at least 2 weeks later. If elevated BP persisted at the second visit, a third visit was conducted at least another 2 weeks later, according to the same procedures. Those with elevated BP at all the three visits were identified as hypertensive (4–6). The flow chart of the BP screening procedures is shown in **Figure 1**. One hundred thirteen of 1,347 children and adolescents with elevated BP at the first visit and 97 of 662 children and adolescents with sustained elevated BP at the second visit were lost to follow-up for some reasons, such as refusal, absence from school, or having a disease. We compared the baseline characteristics between followed-up participants and those lost to follow-up and we found no significant differences (data not shown).

Adiposity Measurements and Definitions

Weight was measured twice in light clothes and without shoes using an electronic scale, and was approximated to 0.1 kg. Height was also measured twice and approximated to 0.1 cm. The mean of two measures was used for analysis. BMI was calculated as the weight divided by the height squared (kg/m^2). Normal weight, overweight, and obesity were defined based on the age- and sex-specific BMI cutoffs for Chinese children and adolescents (14). WC was also measured twice and approximated to 0.1 cm using a standard tape. Extend the tape around the waist in a horizontal plane at the level of 1 cm above the umbilicus. Central overweight and obesity were defined according to the age- and sex-specific WC cutoffs of Chinese children and adolescents (15). WHtR was calculated as the ratio of WC and height. WHtR ≥0.50 was used to define central obesity (16).

Abbreviations: BMI, Body Mass Index; BP, Blood Pressure; CIs, Confidence Intervals; DBP, Diastolic Blood Pressure; ER, Estrogen Receptors; ORs, Odds Ratios; SBP, Systolic Blood Pressure; SNS, Sympathetic Nervous System; WC, Waist Circumference; WHtR, Waist-to-height Ratio.

Covariates

The collected information includes age, sex, birth weight, puberty status, parental history of hypertension, and parental education levels. Puberty status was dichotomized based on whether they had menarche for girls or spermatorrhoea for boys. Parental history of hypertension (yes vs. no) was obtained from both father and mother using the following question: Have you ever been told by a physician to be hypertensive or taking anti-hypertensive drugs currently? Parental education levels were categorized into primary school, high school, college or university.

Statistical Analysis

Data were analyzed with SAS, version 9.3 (SAS Institute, Cary, NC, USA). Quantitative variables were presented as mean ± standard deviation (or standard error). The differences in quantitative variables between groups were compared by covariance analysis adjusted for age and sex (or BMI), and linear regression analysis was used to test trends. Categorical variables were expressed as percentages and the differences between groups were compared by chi-square test and Cochran-Armitage analysis was used for trend tests. Binary logistic regression analysis was used to assess the relationship between

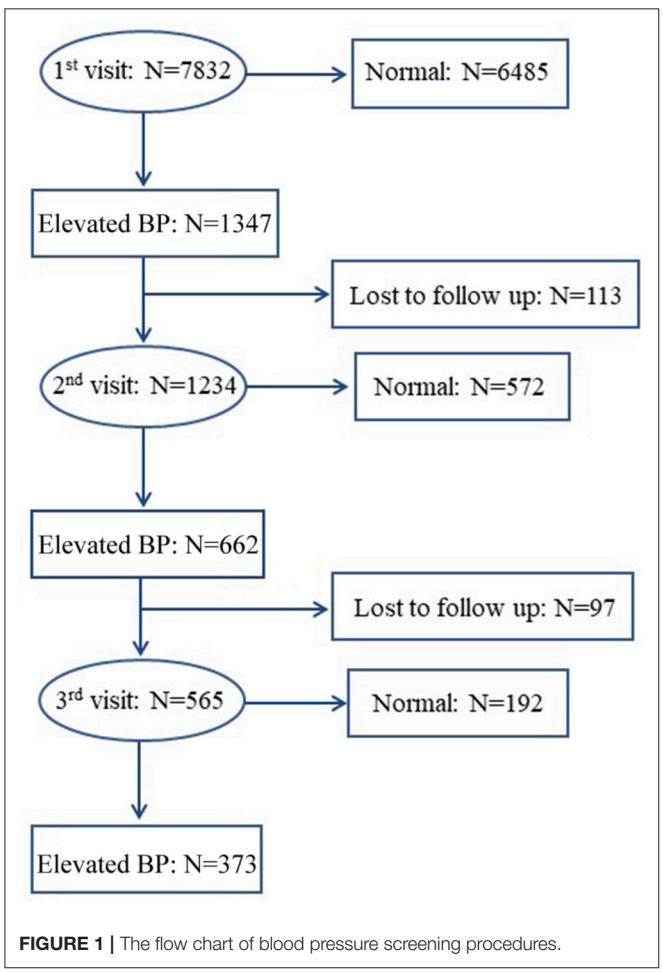

FIGURE 1 | The flow chart of blood pressure screening procedures.

TABLE 1 | Characteristics of the participants across three different visits.

	First visit				Second visit				Third visit			
	All	Boys	Girls	*p*	All	Boys	Girls	*p*	All	Boys	Girls	*p*
N	7,832	4,076	3,756		1,234	761	473		565	387	178	
Age (years)	11.3 ± 0.1	11.3 ± 0.1	11.3 ± 0.1	0.404	12.2 ± 0.1	12.4 ± 0.1	11.8 ± 0.2	0.012	12.5 ± 0.1	12.6 ± 0.2	12.0 ± 0.3	0.044
Height (cm)	151.0 ± 0.1	153.1 ± 0.1	148.8 ± 0.1	<0.001	155.7 ± 0.2	158.7 ± 0.3	152.7 ± 0.4	<0.001	156.5 ± 0.4	159.4 ± 0.4	153.6 ± 0.6	<0.001
Weight (kg)	47.6 ± 0.1	50.8 ± 0.2	44.3 ± 0.2	<0.001	59.1 ± 0.5	64.4 ± 0.6	53.7 ± 0.7	<0.001	62.9 ± 0.7	68.1 ± 0.8	57.7 ± 1.2	<0.001
BMI (kg/m^2)	20.1 ± 0.1	20.8 ± 0.1	19.4 ± 0.1	<0.001	23.5 ± 0.1	24.6 ± 0.2	22.3 ± 0.2	<0.001	24.8 ± 0.2	25.8 ± 0.3	23.7 ± 0.4	<0.001
WC (cm)	67.6 ± 0.1	70.7 ± 0.2	64.6 ± 0.2	<0.001	75.5 ± 0.4	80.0 ± 0.5	71.0 ± 0.6	<0.001	78.6 ± 0.6	83.1 ± 0.6	74.2 ± 0.9	<0.001
WHtR	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	<0.001	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	<0.001	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	<0.001
BMI categories,%												
Normal weight	59.1	53.3	65.3	<0.001	28.7	23.3	37.4	<0.001	17.5	12.9	27.5	<0.001
Overweight	21.0	21.7	20.3		24.6	23.9	25.8		22.8	23.3	21.9	
Obesity	19.9	25.1	14.4		46.7	52.8	36.8		59.7	63.8	50.6	
WC categories,%												
Normal WC	52.3	48.4	56.5	<0.001	25.0	20.9	31.5	<0.001	16.1	12.7	23.6	0.003
Central overweight	19.9	19.4	20.4		19.0	18.3	20.1		17.4	17.1	18.0	
Central obesity	27.9	32.2	23.1		56.1	60.8	48.4		66.6	70.3	58.4	
WHtR categories,%												
Normal WHtR	80.9	73.7	88.8	<0.001	57.0	48.5	70.6	<0.001	45.0	38.8	58.4	<0.001
Central obesity	19.1	26.3	11.2		43.0	51.5	29.4		55.0	61.2	41.6	

Data are shown as mean ± standard error.
Covariance analysis adjusted for sex and age if appropriate.

TABLE 2 | Mean blood pressure values across three different visits.

	Visit	SBP, mmHg	DBP, mmHg
Total	First	109.5 ± 0.1	64.3 ± 0.1
	Second	122.3 ± 0.3	69.6 ± 0.2
	Third	126.4 ± 0.4	70.7 ± 0.4
	p for trend	<0.001	<0.001
Boys	First	111.6 ± 0.1	63.9 ± 0.1
	Second	125.1 ± 0.4	68.5 ± 0.3
	Third	129.3 ± 0.5	69.2 ± 0.4
	p for trend	<0.001	<0.001
Girls	First	107.5 ± 0.2	64.7 ± 0.1
	Second	119.6 ± 0.5	70.6 ± 0.4
	Third	123.6 ± 0.8	72.1 ± 0.6
	p for trend	<0.001	<0.001
6–11 years	First	107.2 ± 0.2	63.7 ± 0.1
	Second	118.7 ± 0.5	69.0 ± 0.4
	Third	119.9 ± 0.8	69.9 ± 0.6
	p for trend	<0.001	<0.001
12–17 years	First	111.8 ± 0.2	64.8 ± 0.1
	Second	125.7 ± 0.4	69.2 ± 0.3
	Third	130.4 ± 0.6	71.1 ± 0.5
	p for trend	<0.001	<0.001

Data are shown as mean ± standard error.
Covariance analysis adjusted for sex, age, and BMI.

TABLE 3 | Prevalence of elevated blood pressure across three different visits by sex and age.

	Visit	Elevated SBP	Elevated DBP	Elevated BP
Total	First	15.3	6.0	17.2
	Second	8.2	2.5	8.6
	Third	4.8	1.2	4.9
	p for trend	<0.001	<0.001	<0.001
Boys	First	19.1	5.8	20.6
	Second	10.9	2.5	11.2
	Third	6.6	1.2	6.7
	p for trend	<0.001	<0.001	<0.001
Girls	First	11.2	6.1	13.6
	Second	5.2	2.4	5.8
	Third	2.8	1.3	2.9
	p for trend	<0.001	<0.001	<0.001
6-11 years	First	13.1	5.4	14.7
	Second	6.7	2.3	7.0
	Third	3.7	1.0	3.9
	p for trend	<0.001	<0.001	<0.001
12-17 years	First	17.4	6.5	19.6
	Second	9.5	2.6	10.1
	Third	5.8	1.4	5.9
	p for trend	<0.001	<0.001	<0.001

Data are shown as percentages (%).

adiposity measures and hypertension, with adjustment of potential covariates. Odds ratios (ORs) with the corresponding 95% confidence intervals (CIs) were calculated. Two-sided $p < 0.05$ was considered as statistically significant.

RESULTS

Characteristics of the Participants Across Three Different Visits

A total of 7,832 students (boys: 52.0%), aged 6–17 years, were included at the first visit, 1,234 (boys: 61.7%) at the second visit, and 565 (boys: 68.5%) at the third visit. Characteristics of the participants across the three separate visits are presented in **Table 1**. Boys had higher height, weight, BMI, WC, WHtR, and higher prevalence of general and central obesity than girls at each visit (all $p < 0.001$). Of note, the prevalence of obesity was particularly high in both genders at the third visit, with the estimates reaching 59.7, 66.6, and 55.0%, respectively, based on different adiposity measures including BMI, WC and WHtR.

Prevalence of Elevated BP Across Three Different Visits

The levels of SBP and DBP increased greatly across the three separate visits, and the similar trends were found in the subgroups by sex and age (**Table 2**). **Table 3** shows the prevalence of elevated BP across three separate occasions among Chinese children and adolescents by age and sex. The prevalence of elevated BP was 17.2, 8.6, and 4.9% at the first, second and third visit, respectively. There was a downward trend in the prevalence of elevated BP over three repeated visits (p for trend<0.001). There were similar trends in elevated SBP and DBP across three visits, with the prevalence being 15.3, 8.2, and 4.8%, respectively, for elevated SBP and 6.0, 2.5, and 1.2%, respectively, for elevated DBP.

Elevated BP was significantly more prevalent in boys than girls during each visit (first: 20.6 vs. 13.6%, second: 11.2 vs. 5.8% and third: 6.7 vs. 2.9%). Elevated BP was also more prevalent in adolescents aged 12–17 years compared with children aged 6–11 years, with the prevalence of 19.6 vs.14.7%, 10.1 vs. 7.0%, and 5.9 vs. 3.9% at the first, second and third visit, respectively (**Table 3**).

Associations Between Obesity and Elevated BP

Table 4 shows the associations of general and central obesity with elevated BP across three different visits. Obesity was an independent risk factor for elevated BP at each visit, irrespective of anthropometric indices used to define weight status. Based on the BMI definition, obesity was associated with higher risk of elevated BP, with the adjusted ORs (95% CIs) being 8.6 (6.8–11.0), 12.5 (9.1–17.3), and 14.0 (8.9–22.2), respectively, across the three visits. Corresponding values were 5.7 (4.6–7.2), 7.7 (5.6–10.6), and 10.9 (6.7–17.9), respectively, for the WC definition, and 4.9 (3.9–6.1), 6.3 (4.8–8.3), and 7.7 (5.3–11.1), respectively, for the WHtR definition.

TABLE 4 | Associations of different adiposity measures with elevated blood pressure across three different visits.

		First visit		Second visit		Third visit	
		OR (95%CI)	***p***	**OR (95%CI)**	***p***	**OR (95%CI)**	***p***
BMI categories	Normal	1		1		1	
	Overweight	2.5 (1.9–3.2)	<0.001	2.5 (1.7–3.6)	<0.001	2.9 (1.7–5.1)	<0.001
	Obesity	8.6 (6.8–11.0)	<0.001	12.5 (9.1–17.3)	<0.001	14.0 (8.9–22.2)	<0.001
WC categories	Normal WC	1		1		1	
	Central overweight	1.8 (1.4–2.4)	<0.001	1.8 (1.2–2.8)	<0.001	2.6 (1.4–4.9)	<0.001
	Central obesity	5.7 (4.6–7.2)	<0.001	7.7 (5.6–10.6)	<0.001	10.9 (6.7–17.9)	<0.001
WHtR categories	Normal WHtR	1		1		1	
	Central obesity	4.9 (3.9–6.1)	<0.001	6.3 (4.8–8.3)	<0.001	7.7 (5.3–11.1)	<0.001

Adjusted for age, sex, birth weight, puberty, family history of hypertension (father, mother), and parental education levels.

DISCUSSION

Our study shows that the prevalence of pediatric elevated BP decreases substantially across three visits, from 17.2% at the first visit to 4.9% at the third visit. This trend might be explained by the "white-coat" effects (17, 18), or anxiety (19) that can result in increased BP levels. As children became more familiar with BP measurements from the first visit to the third visit, the BP measurements became more reliable. Thus, BP measured at the third visit might better reflect the true BP status of the children.

Previous studies have also reported substantial decreases in the prevalence of elevated BP in children across different visits. A study conducted in Switzerland showed that the prevalence of elevated BP was 11.4, 3.8, and 2.2%, respectively, at the first, second and third visit (8). Another study in Chinese pediatric population reported that the prevalence of elevated BP was more than halved between the first and third visit (18.2 vs. 3.1%) (9). A recent meta-analysis demonstrated that the prevalence of pediatric elevated BP decreased from 12.1% at the first visit to 2.7% at the third visit (7). The findings above are similar with our study. BP screening based on one occasional visit may mislabel a substantial number of children as hypertensive ones, which may lead to unnecessary stress for children/parents. Additionally, the Cardiovascular Risk in Young Study reported that repeated observations of elevated BP among children and adolescents enhanced the prediction of hypertension in adults (20). Altogether, these findings emphasized the necessity of BP measurements on at least three different occasions to estimate the true prevalence of hypertension in the pediatric population or to diagnose hypertension in clinical practice.

Boys had higher prevalence of elevated BP than girls in our study, which was similar to several previous studies (21, 22). Data from the Victorian Family Heart Study showed that estrogen receptors (ER)-alpha and ER-beta might play more important roles in the genetic regulation of BP in men, and the sex steroid-related genes may contribute to the observed sex differences in BP (23). In addition, boys were more likely to have adverse healthy behaviors [e.g., tobacco smoking (24), alcohol use (25) and sedentary behaviors (26)] than girls. Moreover, the prevalence of elevated BP was much higher in adolescents than in children, which was consistent with several previous studies (27, 28). Possible explanations for the age differences included intense hormonal changes and elevated insulin resistance during pubertal period, and a much higher prevalence of overweight and obesity in adolescents vs. children in the present study.

The study also presented that adiposity was an independent risk factor for elevated BP in children, similar with previous publications (8, 9, 29). The novelty of our study includes the use of different adiposity measures to define obesity. BMI is a widely used indicator of obesity, but it cannot distinguish fat distribution. WC and WHtR are indicators of abdominal obesity that can better assess the distribution of the visceral adipose tissue. The link between obesity and hypertension may be mediated partly by the hyperactivity of the sympathetic nervous system (SNS) (30, 31). In children, SNS can cause a hyperdynamic hemodynamic state (32), contributing to elevated BP. Obese children with elevated BP often have increased heart rate and BP variability, which also suggests a primary role of heightened SNS activity in the association between obesity and hypertension (33).

The major strengths of our study include the large sample size, a fair participation rate (first visit: 99.8%; second visit: 91.6%; third visit: 85.3%), and the well-standardized BP measurements. However, our study also has some limitations. First, BP re-assessments were only made in children who had elevated BP readings at the initial visit. Consequently, this may cause some underestimation in hypertension, especially when some children have normal BP at first visit, but may actually have an elevated BP (i.e., masked hypertension) at the subsequent visits. Second, we used the Chinese children and adolescents BP references (13) to define elevated BP across three visits, which made our results incomparable with others. Third, our data were collected using a convenient cluster sampling method in urban area of Jinan, China, thus, the generalizability of our results to the whole Chinese children might be limited. Finally, based on a cross-sectional study design, our study is unable to provide causal associations between adiposity and pediatric elevated BP, but reverse causation is unlikely.

CONCLUSIONS

The current study demonstrates that the prevalence of elevated BP decreases substantially across three different visits, and the true prevalence of hypertension is about ∼5% in Chinese children and adolescents. Our findings highlight the importance of repeated BP assessments over at least three visits before making a final diagnosis of pediatric hypertension. In addition, early accurate detection and effective control of hypertension in children should be emphasized, especially among those with excess body weight.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

All subjects gave written informed consent in accordance with the Declaration of Helsinki. Signed informed consent was obtained from all students and their guardians. Ethical approval was obtained from the Ethics Committee of the Capital Institute of Pediatrics in Beijing, China.

AUTHOR CONTRIBUTIONS

QZ, LY, and BX conceptualized the study and drafted the article. QZ, LY, and YZ analyzed the data. YZ, MZ, YL, and BX revised the manuscript. All authors approved the final submitted and published versions.

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Device Closure of Perimembranous Ventricular Septal Defect: Choosing Between Amplatzer Occluders

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Background: Off-label device closure of perimembranous ventricular septal defect (pmVSD) is well reported in the literature with encouraging results. However, technical challenges may be encountered.

Objectives: To evaluate and compare feasibility, technical aspects, procedural outcomes, and mid-term follow-up of pmVSD closure using Amplatzer™ occluders.

Patients and Methods: From July 2015 to July 2018, patients in whom pmVSD closure was attempted using an Amplatzer occluder were retrospectively identified from our institution's database. Device selection was made according to the defect anatomy that was obtained via ventriculography and trans-esophageal echocardiography. Follow-up evaluations were done at discharge, then at 1, 3, 6, and 12 months and yearly thereafter with transthoracic echocardiography and electrocardiogram.

Results: In total, 8 Amplatzer Duct Occluder (ADO), 27 ADO II, and 17 Amplatzer Muscular VSD Occluder (AMO) were used in 51 patients with a mean age of 7.4 ± 6.9 years and a mean weight of 25.4 ± 19.8 kg. Implantation was successful in 50/51 patients (98.0%). There was no procedure related mortality. One ADO accidentally embolized to the aorta after release and was surgically recaptured from the iliac artery. All ADO II were delivered retrogradely with the least amount of time ($p = 0.002$) and the lowest radiation exposure ($p < 0.001$). Minor valvular disturbances occurred in 8/49 patients (16.3%), including five tricuspid regurgitation (three with ADOII and two with AMO) and three trivial aortic regurgitations (two with ADO and one with ADOII). On a median follow-up of 194 days (range, 60–895 days), no surgical device removal was necessary. At 6 months of follow-up, trivial residual shunt was present in 5/49 patients (10.2%), among which none occurred with ADO. One complete atrioventricular block was detected 18 months after ADO implantation and required permanent pacing.

Conclusions: Transcatheter closure of PmVSD using Amplatzer occluders is feasible, safe and efficacious in properly selected patients. The major key factor behind high procedural success rate is proper device selection. ADOII is remarkably superior in terms of device softness, flexibility and faster implantation process. Yet, its use is limited to small defects with particular anatomy.

Keywords: ventricular septal defect, perimembranous, amplatzer, device closure, complete heart block

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INTRODUCTION

Ventricular septal defect (VSD) is the most common congenital heart disease (CHD) with the perimembranous VSD (pmVSD) being the most common subtype (1–3). While spontaneous closure rates are high, surgical repair may be indicated during early infancy in case of severe pulmonary hypertension, or failure to thrive despite optimal medical management (3). Later in life, an unknown percentage of patients with small residual defect develop cardiac problems, and then become candidates for closure. Due to advances in cardiac imaging modalities and techniques, interventional pmVSD closure has become increasingly acceptable with the availability of different occlusion systems but it remains technically challenging (4, 5). When compared to surgery, percutaneous approach avoids sternotomy and has the potential advantages of lower morbidity, faster recovery, shorter hospital stay, and reduced costs (6–8). Ideally, device closure would be easily handled with low rates of residual shunt (RS) and a special attention to the aortic and tricuspid valves while avoiding the conducting tissues. The first serious device designed for pmVSD closure was conceived by Amplatzer in the late 90's and had an asymmetrical design (9). However, high incidences of complete atrioventricular block (CAVB) led to abundance of this device with a continuous search for a better substitute (10–12). Therefore, published literature reported successful use of devices that were originally conceived for other defects (4, 5). The aim of this study is to review our transcatheter pmVSD closure experience using different Amplatzer™ devices (Abbott, USA) and to evaluate the midterm follow-up outcomes.

PATIENTS AND METHODS

Study Population

This is a retrospective monocentric study. The records of all patients with a hemodynamically significant pmVSD and scheduled for attempted closure using an Amplatzer™ occluder between July 2015 and July 2018 at the Saint Joseph university teaching hospital, Hotel Dieu de France, were reviewed and included in this study. Permission was obtained from the company to use and mention their product in this submission. Data were collected from first admission until last available follow-up. Patients' demographic information, cardiac diagnosis, procedural data, complications, size and type of the duct occluder devices, re-interventions needed, and procedural outcomes were collected from the medical records. Study protocol was reviewed and approved by the institutional review board.

Prior to cardiac catheterization, 2D transthoracic echocardiography (TTE) was performed to all patients by an experienced operator, with a GE Vivid 3 machine including M mode, two-dimensional and Doppler examination. Size and shape of VSD were determined by standard four-chamber view. Sub-aortic rim (SAR) was defined by the distance between the upper margin of the defect and the aortic valve (AoV) and was evaluated using the five-chamber view and parasternal long axis view (PLAV). Parasternal short axis view was used to identify the defect position on an analog clock, number and diameters of the right ventricle (RV) exit(s) as well as LV entry diameter. These echocardiographic measurements guided the selection of the device size which was later reassessed, intraoperatively, by angiography & transesophageal echocardiography (TEE).

Inclusion Criteria

For the purpose of this study, pmVSD with indication for transcatheter closure was defined by clinical or TTE evidence of a significant left to right shunt due to isolated pmVSD, with the presence of at least one of the following criteria: (1) estimated pulmonary-to-systemic blood flow ratio (Qp/Qs) $>$ 1.5; (2) prominent cardiomegaly, defined as cardiothoracic ratio $>$0.55 on standard chest X-ray (CXR); (3) left atrial (LA) enlargement, defined as a LA-to-aortic diameter ratio $>$1.5 on the PALV examination; (4) left ventricle (LV) overload and enlargement, defined as LV end-diastolic z-score on echocardiogram, indexed to body surface area $\geq$2.0; (5) history of infective endocarditis related to the pmVSD; and (6) symptoms, including recurrent respiratory infections (defined as $\geq$6 events in the preceding 12 months) and/or failure to thrive.

Exclusion Criteria

Patients considered not eligible for the procedure had one or more of the following anatomical or clinical criteria: (1) pmVSD with a prolapse aortic cusp, aortic regurgitation (AR) or aortic valve stenosis, infundibular defect, septal mal-alignment, SAR $\leq$ 1 mm (in non-aneurysmal anatomy); (2) severe pulmonary artery (PA) hypertension and a right-to-left shunt (unless PA banding or congenital pulmonary valve stenosis) or pulmonary vascular resistance $>$ 8 Wood units or documented irreversible pulmonary vascular disease; (3) presence of any other associated CHD unrepairable percutaneously; (4) active bacterial infections or endocarditis or sepsis (local/generalized); (5) contraindication to antiplatelet or anticoagulation therapy or agents; (6) and a body weight $<$8 kg. Preoperative routine examination including standard 12 leads electrocardiogram (EKG), CXR, TTE, and blood test were performed on all patients.

Interventional Procedural Technique

Written informed consent was signed by the patients or parents of the children after they were provided with a comprehensive explanation about the procedural details, the advantages and possible complications. All procedures were performed by the same operators, in the catheterization laboratory, under general anesthesia, TEE, and fluoroscopic control. Special attention was given to minimize hypothermia. One femoral vein (FV) and one 5F contralateral arterial line were obtained. After that, intravenous (IV) heparin (100 IU/kg, 5,000 IU maximum) was administered to all patients and was regularly monitored to maintain activated clotting time longer than 200 seconds. Prophylactic antibiotic therapy using IV cefazolin (30 mg/kg, 2,000 mg maximum) was also given at the beginning of the procedure and two subsequent doses (every 8 hours during the following 24 hours). Standard right and left cardiac catheterization were performed and data was gathered. Left ventriculography with a marked pigtail catheter was performed at 55–60° left anterior oblique to 20° cranial projection in order

to profile the defect and was combined to intraoperative TEE to accurately determine the pmVSD location, shape, depth, size and its relationship with adjacent aortic and tricuspid valves. The RV defect exit was more clearly evaluated on TEE especially in aneurysmal anatomy. In case of multiple RV exits, the largest one was chosen as a target measurement. The defect entry diameter was measured on angiography at the largest diastolic phase on LV side.

Device Selection

The three available Amplatzer™ occluders (Abbott, USA) used in this procedure were Amplatzer Duct Occluder (ADO), Amplatzer Duct Occluder II (ADO II), and Amplatzer Muscular VSD Occluder (AMO). Due to its soft and flexible nature design and its fast retrograde deliverability, ADO II was our first choice of selection and it was installed when the following criteria were met: defect diameter <5 mm with an SAR larger than 3 mm in non-aneurysmal type defects. In aneurysmal type, the RV exit should be <5.5 mm with a LV entry diameter <12–12.5 mm but large enough to accept the left disk (LD) in the aneurysm especially when the SAR is <3 mm. The diameter of the device waist in ADO II was chosen to be 1 mm (±0.5 mm) greater than the smallest VSD diameter.

When ADO II device was not applicable, ADO device was favored over the AMO in patients with big aneurysms. The device size was selected so that the right disk (RD) diameter (the pulmonary end) would be 2 mm (±0.5 mm) greater than the smallest VSD diameter, in order for the subsequent left retention skirt diameter (aortic end of the device) to be totally accommodated inside the aneurysm, especially when the defect depth allowed the RD to reach the RV exist. On the other hand, when LD diameter was greater than LV entry diameter, ADO was installed when SAR was longer than 3–3.5 mm with a 7mm maximal VSD depth to allow RD squeezing in the RV exist.

Finally, the AMO device was chosen according to the following protocol: when the SAR was >4.5 mm, the device waist was chosen according to the LV entry diameter without any need to oversize. When the SAR was <4.5 mm, the device waist was chosen equally to the RV diameter of the aneurysm, and was implanted when the subsequent LD fitted entirely in the aneurysmal LV opening. It is noteworthy that in low budget countries' catheterization labs, it is not feasible to keep all range on shelves. For that, device selection was sometimes influenced by device price and availability.

Delivery

Venous Approach

This antegrade approach was used to implant all ADO and AMO. The VSD was crossed retrogradely from the LV side, using a 4 or 5 F Judkins right (JR) coronary catheter (Cordis Corporation, Florida, USA) and 0.035 inch J tip Terumo glide wire (Terumo Corp. Japan) combination. Once across the VSD, the catheter was advanced into either branch of the PA, or preferably into the superior or inferior vena cava. The Terumo wire was replaced with a 300 cm noodle wire (Abbott, USA) that was then snared and exteriorized through FV, using an Amplatz Gooseneck Snare (ev3 Inc.,; Minnesota, USA) to create an arteriovenous circuit (AVC). Over this wire, an appropriate 6, 7, or 8 F Amplatzer 45 or 180° delivery system was advanced from the FV across the VSD all the way until the tip of the sheath arrived to the ascending aorta. The dilator was then removed from the vein line while the guide wire and the end-hole catheter were removed from the arterial line. After flushing the long sheath, the chosen device was loaded under a saline solution and was advanced, without rotation, to the tip of the delivery sheath under fluoroscopy. The distal disk was partially opened in the ascending aorta and then gently pulled back through the AoV into the LV. After that, delivery sheath was slowly retracted until the distal disc was completely deployed at the LV side of the VSD. The entire assembly (delivery cable and delivery sheath) was then pulled back as one unit into the defect and the sheath was retracted to deploy the waist of the device in the VSD. Once the position was confirmed by angiography and TEE, sheath was retracted to deploy the proximal disc. After full deployment of the occluder, TEE combined with LV angiography were performed again to verify the position and the shape of the device, RS and the absence of interference with the AoV cusps. At this point, the delivery cable passing across the TV generated some regurgitation and it was difficult to predict its evolution after device release. The device was then released by turning the cable counterclockwise after confirmation of good device position and absence of AoV disturbances. Final result was only assessed by TEE to avoid angiography, catheter manipulation in the LV and accidental mobilization of the device.

Arterial Approach

This retrograde approach was used to implant all ADOII. After crossing the defect from the LV side using the same technique as above, a 5F delivery catheter TorqVue™ LP was introduced from the FA and advanced over an exchange wire, through the VSD. The selected device was loaded in the delivery sheath and advanced to its tip into the RV. The delivery catheter was pulled back slowly under TEE guidance into the RV, near the defect. Once the position was confirmed, the distal disk was slowly advanced out the catheter and all system was pulled back as one unit against the septum. At this stage, the absence of tricuspid regurgitation (TR) related to the RD was confirmed by TEE. The catheter was then retracted to allow the waist and proximal disk to open against the left side of the septum with gentle tension. The device position was then assessed using TEE in multiple views to evaluate the position and stability of the device, its proximity to the AoV and the presence of significant TR. Before detaching the device, a hand injection in the ascending aorta through the guiding catheter was mandatory to document absence of LD interference with the AoV. Device was then released by a contra-clockwise cable rotation and the final result was assessed by TEE.

After achieving femoral hemostasis, IV heparin (starting dose of 25 units/kg/h) was administrated until the next morning, to maintain activated partial thromboplastin time 2–3 times greater than the reference value. Patients stayed in the hospital for overnight observation with vital signs monitoring. Platelet anti-aggregation therapy with oral aspirin 3–5 mg/kg/day (children) or 100 mg/day (adults) was prescribed for 6 months. The

following day, all patients underwent clinical examination, and CXR to detect early complication such as occult hemorrhage and pulmonary complication. Twelve-lead EKG was performed to ensure sinus rhythm. Echocardiograms were also performed to detect pericardial effusion, aortic insufficiency, tricuspid valve stenosis, or insufficiency, LV outflow tract obstruction, LV function, and degree of shunting through the device. Urine analysis was done to rule out hemolysis in case of important RS or dark-colored urine. All patients in whom the procedure was uncomplicated were discharged from hospital 24 h after procedure. Endocarditis prophylaxis was done for the first six months in all patients but prolonged thereafter when persisted RS was documented on TTE. Patients were also instructed to avoid strenuous activity for one month.

Follow-Up Protocol

Routine follow-up clinic visits were scheduled for 1 week then 1, 3, 6, and 12 months post-procedure and thereafter annually. New onset adverse events were monitored in each visit on the basis of basic clinical evaluation, TTE and EKG. The TTE included an assessment of changes in AR, TR, and RS. Holter monitoring (24 h) was performed only when clinically indicated.

Statistical Analysis

Discrete variables were summarized as percentages and continuous variables as mean with standard deviation or median with range as appropriate. Statistical analysis of the categorical variables was conducted using Fisher's exact test and by ANOVA test for continuous variables. Statistical analyses were computed using the Statistical Package for the Social Sciences (SPSS Statistics), version 21 for Macintosh (IBM, Armonk, NY), with a $P < 0.05$ considered statistically significant. All reported P values are two-sided.

RESULTS

Patient Characteristics (Table 1)

During the period of the study and following inclusion criteria, 51 patients (45.1% male) were identified. The mean age at the time of procedure was 7.4 ± 6.9 (range 0.3–33) years and the mean body weight was 25.4 ± 19.8 (range, 8–95) kg. There were 5 adults patients (age ≥ 18 years) (9.8%; female 80.0%). All 51 patients showed echocardiographic LV enlargement. Forty-nine patients showed aneurysmal type defect. Three patients (5.9%) had previously documented endocarditis and all of them were treated with antibiotics several months prior to attempted device closure. There was no residual vegetation along the margins of the defect at the time of the procedure. Three patients had minor associated CHD and were managed percutaneously in a different setting.

Procedural Characteristics, Outcomes, and Complications (Tables 1–3)

Device closure of the pmVSD was successful in 98% of the cases (50/51 patients) with the use of 52 Amplatzer devices. The only failure was attributed to inaccurate angiographic measurements misleading appropriate device size selection. In this patient, the initially chosen AMO device (size 6) pulled through the defect due to device's small size. The device was retrieved and the procedure was then aborted. In another patient's case, an (8 × 6) ADO device was judged suitable to close a pmVSD with no SAR, a 12 mm LV entry diameter and a 4 mm narrowest Doppler RV exit diameter. However, the unreleased device was small and unstable upon deployment which led to its replacement by an (10 × 8) ADO with a subsequent successful implantation.

A total of 50 Amplatzer devices were implanted as follows: 27 ADOII devices (54.0%), 16 AMO devices (32.0%), and 7 ADO devices (14.0%). The most commonly used ADOII device size was 6 × 4 (in 14 patients; 51.8%) followed by 5 × 4 and 4 × 4 (each one used in 6 patients; 22.2%), and 3 × 4 (in 1 patients; 3.8%). The most commonly used AMO device size was 6 (in 5 patients; 31.2%), and 8 (in 6 patients; 37.5%) followed by device size 10 (in 4 patients; 25.0%), and 14 (in one patient; 6.3%). The most commonly used ADO device size was 12 × 10 (in 4 patients; 57.1%), followed by device size 10 × 8 (in one patient; 14.3%), and 8 × 6 (in one patient; 14.3%), and 6 × 4 (in one patient; 14.3%). The mean total procedural time was 68.8 (± 33.6) min while the mean fluoroscopy time (FT) was 18.1 (± 12.84) min. All ADO II devices were delivered with the least amount of time

TABLE 1 | Demographic and procedural characteristics.

	***n* = 51**
Age (years), *M ± SD (range)*	7.4 ± 6.9 (0.3–33)
Weight (kg), *M ± SD (range)*	25.4 ± 19.8 (8–95)
BSA (m^2), *M ± SD (range)*	0.9 ± 0.4 (0.4–2.1)
Male, *N (%)*	23 (45.1)
Sub-aortic rim (mm), *M ± SD (range)*	4.7 ± 3.4 (0–14)
LV entry (mm), *M ± SD (range)*	10.5 ± 4.0 (4–20)
RV exit (mm), *M ± SD (range)*	4.7 ± 1.7 (2–8)
Indication for Closure*	
Left chamber enlargement	51 (100)
Endocarditis	3 (5.9)
Associated CHD	
Patent ductus arteriosus	1 (2.0)
Atrial septal defect	1 (2.0)
Muscular ventricular septal defect	1 (2.0)
Device type, *N* (%)	
Amplatzer Muscular VSD Occluder (AMO)	17 (33.3)
Amplatzer Duct Occluder (ADO)	7 (13.7)
Amplatzer Duct Occluder II (ADO II)	27 (52.9)
Device delivery approach, *N* (%)	
Venous	24 (47.1)
Arterial	27 (52.9)
Total procedural time, sheath in-out (min), *M ± SD (range)*	68.8 ± 33.6 (30–225)
Fluoroscopy time (min), *M ± SD (range)*	18.1 ± 12.9 (3.6–65.4)
Total dose area product (Gy.cm²), *M ± SD (range)*	34.0 ± 44.6 (1.1–244.8)
K_{ar} (mGy), *M ± SD (range)*	377.2 ± 365.5 (19–1,878)

M ± SD = Mean ± Standard deviation.
BSA, body surface area; LV, left ventricle; RV, right ventricle; CHD, congenital heart defects; K_{ar}, Cumulative air kerma at the patient entrance reference point.
**More than one choice applied.*

($p = 0.002$) and the lowest radiation exposure ($p < 0.001$) when compared to AMO and ADOI.

There was no procedure related mortality nor major vascular access complications. In one patient, a defect with 10 mm LV opening diameter, 7.5 mm depth and a 4.5 mm RV exit diameter was successfully closed with an ADO (8 × 6). However, following the completion of left ventriculography to check to check device position, we did not notice that the pigtail catheter was trapped between the device and the interventricular septum (IVS) leading upon its retrieval from the LV to device accidental embolization into the thoracic aorta. The device was surgically recaptured from the left iliac artery after multiple failed attempts to retrieve it transvenously (**Figures 1A–D**). In 2 other patients, AVC misconstruction led to transient severe bradycardia that was bailed out with circuit re-establishment leading to prolonged procedural duration.

On a median follow up period of 194 days (range, 60–895 days), 8/49 (16.3%) had persistent new-onset valvular disturbances, including 5 (10.2%) insignificant TR (AMO group, $n = 2$ and ADOII group, $n = 3$) and 3 (6.1%) trivial AR (ADOII group, $n = 1$ and ADO group, $n = 2$) (**Table 3**). All valvular lesions were considered as minor complications since none progressed in severity on follow-up nor needed to be cured until this manuscript was drafted. Complete occlusion rate was 32% (16/50) immediately upon completion of the procedure, rising up to 89.8% (44/49) at 6 months of follow-up. Persistent RS were trivial in all five cases (ADOII group, $n = 2$ and AMO group, $n = 3$) and presented benign courses with no hemodynamic significance and no incidence of mechanical hemolysis. Progression of new onset complications during follow-up (based on echocardiography) is summarized in **Chart 1**.

The most serious complication was CAVB and occurred in one 15 years old patient (2%) immediately upon release of an (12 × 10) ADO device. The implanted device was not retrieved since sinus rhythm was restored 5 min after IV atropine and steroids therapy, and the complication was classified as transient. After hospital discharge, this same patient was rediagnosed, on the 18 months routine follow-up EKG, with a persistent CAVB. She was then treated with permanent pacemaker and there was no sign of sinus rhythm recovery during follow-up visits. No future complication was encountered.

During the study period, no new other cases of CAVB or device surgical retrieval occurred and no major adverse event such as device embolization or malposition, thrombus or clot formation and thromboembolism were detected. None of the patients with a RS developed hemolysis. To this date, there has been no incidence of device related infectious endocarditis. We have seen that enlarged LV and LA decreased to normal size during the follow-up in all the patients even those with RS.

TABLE 2 | Procedural outcomes and complications.

Successful implantation, *N (%), n = 51*	50 (98.0)
Transient CLBBB, *N (%), n = 51*	2 (3.9)
Device embolization, *N (%), n = 50*	1 (2.0)
Follow-up duration (days), *Median (range), n = 49*	194 (60–895)
Persistent complications at the latest follow-up, *n = 49*	
CAVB, *N (%)*	1 (2.0)
Trivial residual shunt, *N (%)*	5 (10.2)
Valvular disturbances, *N (%)*	
Mild tricuspid regurgitation	5 (10.2)
Trivial aortic regurgitation	3 (6.1)

CLBBB, complete left bundle branch block; CAVB, complete atrioventricular conduction block.

DISCUSSION

Perimembranous VSD is one of the most common type of CHD with recent growing interest in whether interventional

TABLE 3 | Groups comparison.

	AMO, *n* = 17	ADO, *n* = 7	ADO II, *n* = 27		
		***N* (%)**		**F[a]**	***p*-value**
Trivial residual shunt	3 (60.0)	–	2 (40.0)	1.680	0.389
Valvular disturbances	2 (25.0)	2 (25.0)	4 (50.0)	1.654	0.420
Mild tricuspid regurgitation	2 (40.0)	–	3 (60.0)	3.811	0.286
Trivial aortic regurgitation	–	2 (66.7)	1 (33.3)	–	–
		M ± SD		**F[b]**	***p*-value**
Total procedural time, sheath in-out (min)	74.4 ± 30.8	102.9 ± 55.0	56.0 ± 18.9	7.191	**0.002**
Fluoroscopy time (min)	21.7 ± 9.0	30.7 ± 17.8	12.1 ± 10.1	9.170	**<0.001**
Total dose area product (Gy.cm²)	37.6 ± 21.2	125.9 ± 104.4	18.6 ± 17.8	13.911	**<0.001**
K_{ar} (mGy)	450.5 ± 258.9	1131.3 ± 659.4	231.0 ± 170.7	16.145	**<0.001**

[a]*Fisher Test;* [b]*Anova.*
M ± SD = Mean ± Standard deviation.
AMO, Amplatzer Muscular VSD Occluder; ADO, Amplatzer Duct Occluder; ADO II, Amplatzer Duct Occluder II.
K_{ar}, Cumulative air kerma at the patient entrance reference point.
Bold values are significant p-values.

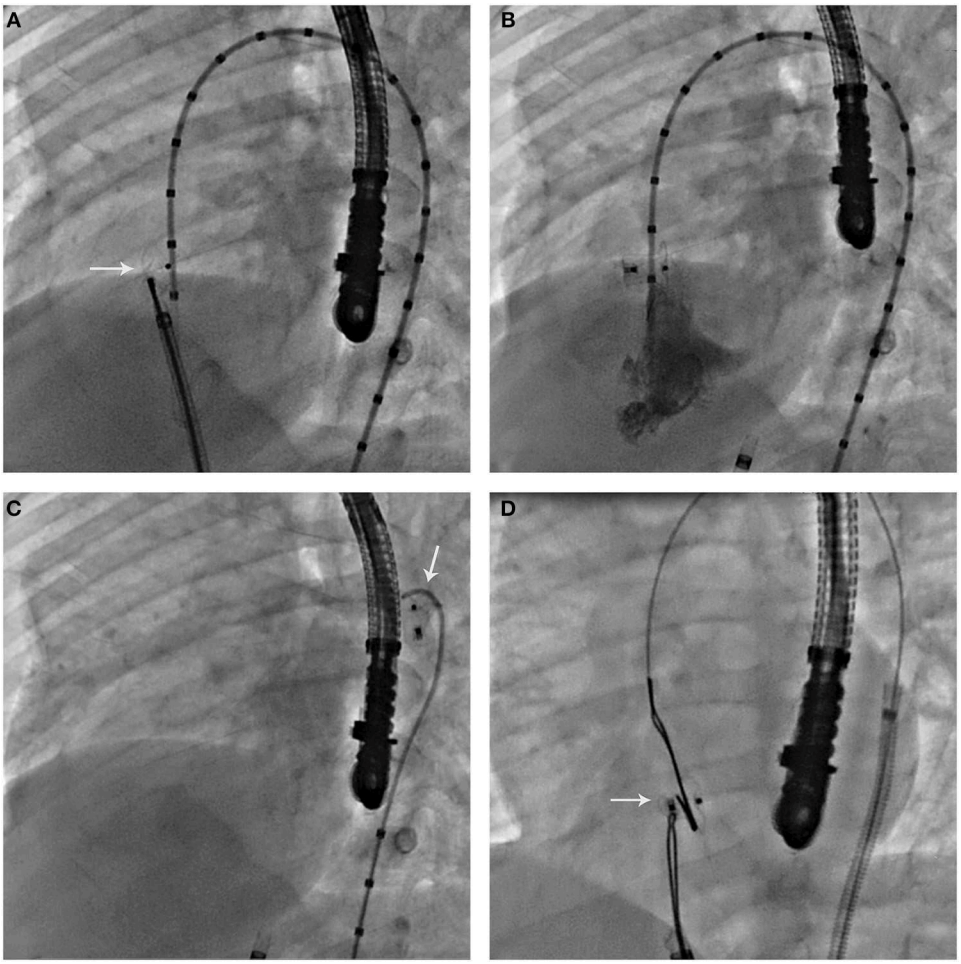

FIGURE 1 | (A) Left ventricular angiography in 55–60° left anterior oblique to 20° cranial projection incidence, showing the unreleased ADO in good position within the defect. Note the misdiagnosed entrapment of the pigtail catheter between the device retention disk and the interventricular septum. **(B)** After releasing the device, left ventricular angiography in in 55–60° left anterior oblique to 20° cranial projection incidence, showing pigtail catheter entrapment with a satisfactory device position. **(C)** Pigtail catheter retrieval leading to accidental device displacement. Note the embolized device in the thoracic aorta. **(D)** Bilateral snaring of the embolized device after re-establishment of arteriovenous circuit for transvenous retrieval. Note the entrapped device across the defect with a satisfying position. Failure to unsnare it in this position or to pull it back across the defect for transvenous retrieval lead to surgical recapture from the iliac artery.

approach can replace traditional open-heart surgical closure as the contemporary standard therapy for pmVSD (6–8). This new alternative has been wildly used in developing countries. Previously cited reports showed a variety of devices that have been used to treat pmVSD with promising results (4, 5). Despite that, percutaneous VSD closure is still not currently approved in the United States because of unacceptably high rates of post-procedural and late-onset heart block (HB) (10, 13–15).

In fact, CAVB is the huge cornerstone that limits the widespread use of transcatheter pmVSD closure with high reported incidence in young patients (13, 16) and no available clear data on the precise mechanisms involved in its occurrence (11, 17). Compared to surgery, in which CAVB usually appears immediately after the operation (10), reports showed that CAVB can occur at any time from a few minutes to months and years even after successful and uncomplicated procedures (12, 14, 18–20), may be reversible with medication or may become persistent, requiring permanent pacing (19, 21) when sudden death is escaped. Previous reports also showed that the Hiss bundle passes at the postero-inferior margin of pmVSD and is vulnerable to HB during device closure, especially in oversized device cases (22, 23). With this in mind, we believe that ADO II has an advantage as it may keep CAVB incidence low. Due to its flexible profile and small delivery sheath, ADOII can be deployed with easier manipulation through angulation and faster implantation process. Besides, ADO II is made of soft, fabric-free, multi-layered Nitinol wire mesh with low-profile retention disks, minimizing clamp force to the IVS and radial stress on the conduction system (24). This device property was previously

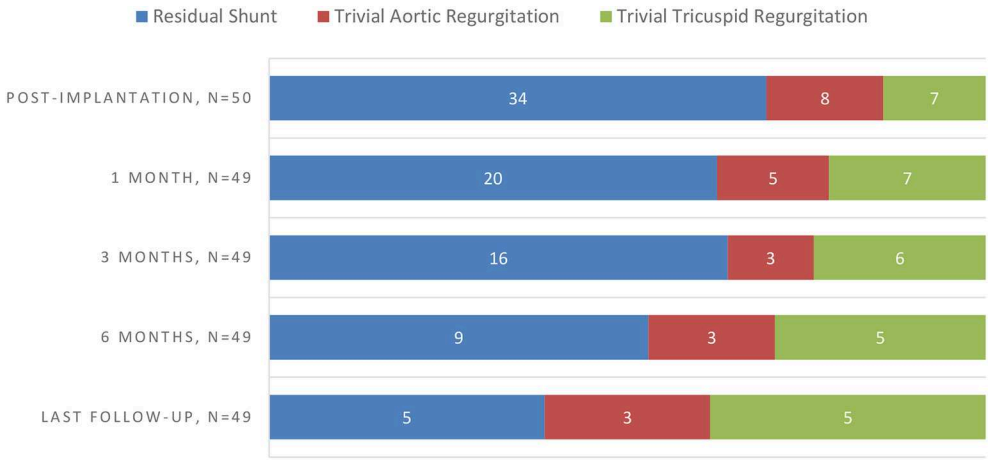

CHART 1 | Progression of new-onset complications on follow-up (based on echocardiography).

emphasized by Vijayalakshmi et al. where none of the patients had HB (25).

In our series, only one 15 year old patient developed transient CAVB immediately after the release of an ADO device. On the 18 months routine follow-up, this same patient was re-diagnosed with persistent CAVB requiring permanent pacing. This incidence confirms that HB can appear as a late unpredictable complication that requires high vigilance for appropriate diagnosis and treatment (26). Moreover, CAVB rate in our study was comparable with the one of surgical closure (27–33). In other words, device PmVSD closure using Amplatzer occluders may be a good alternative therapy to surgical closure in suitable patients. Ghaderian et al. reported that ADO with it shorter distal rim and no proximal disc reduces CAVB rates with less squeezing on the His bundle (34). For that, we retrospectively investigated this complication and found out that it could have been prevented. In fact, the chosen (12 × 10) ADO device for the closure of a tubular shaped defect (14 mm LV entry diameter, 4 mm exist diameter) was oversized and led to IVS compression (**Figures 2A–C**). We also believe that the immediate manifestation of our HB case was the consequence of a significant direct mechanical damage caused by the delivery system or by device deployment, while its late manifestation was highly due to fibrosis, compression or inflammation of the conduction system.

Aortic insufficiency is another serious complication to be aware of upon procedure completion and during follow-up. Upon full device deployment and before device release, TEE as well as dye injections were regularly performed to document LD non-interference with the AoV cusps. Despite this, we did face three cases of trivial AR that appeared upon device release, with no requiring therapy. For that, we believe that all our cases of AR were related to complex manipulation processes and difficult AVC establishment. This theory was supported by Zhao et al. who emphasized ADOII little effect on the AoV (33) and by other authors who discussed the ability of this device to adapt to different shapes and to fit into the defect without disturbing the AoV (24, 35–37).

Another important factor that greatly impacted the success of the intervention was the SAR length, with various studies suggesting a minimum length for safe deployment (35, 36). We carefully selected our devices, in defects with insufficient SAR, so that the LD could be safely deployed inside the defect left entry, aiming for less AR and more stability. On top of this, device selection was influenced by the aneurysmal anatomy, found in 96.1% of cases. In accordance with other authors, we found that implantation of patent arterial duct occluders would be more convenient in aneurysmal defects, since the retention disc can be set entirely within the aneurysm and the cylindrical portion of the device secures in an opening of the aneurysm on the RV side (14, 38, 39). Therefore, device will not get in contact with the AoV and will create minimal pressure on the IVS.

We did face five cases of insignificant TR of which none was documented after ADO implantation, as expected. With the absence of RD, septal leaflet will not be caught within the device, but given that aneurysm is often adjacent to or even part of the TV apparatus, caution should be always taken when the operator passes the wire and catheter through the valve to establish the AVC (6). Besides, a recent study reported late ADO II-related TR (40). However, we noticed that ADO II (with its short waist), when compared to AMO (with its larger lateral disk), has less chance to interfere with TV or to obstruct RV outflow tract.

In our experience, results of transcatheter VSD closure with Amplatzer occluders were satisfactory: the procedure was successfully performed in 98% of cases, confirming the results reported in other published studies (4, 5). However, closure rate was only 89.8% at six months of follow-up when compared to higher previous reports (36, 37, 41). In fact, some procedure related complications are reduced with precise defect sizing and proper device selection (42, 43). While oversized devices cause more damage to the adjacent structures, undersized devices may increase the rate of device embolization and RS. Respect to our device selection protocol helped us to control the risk of

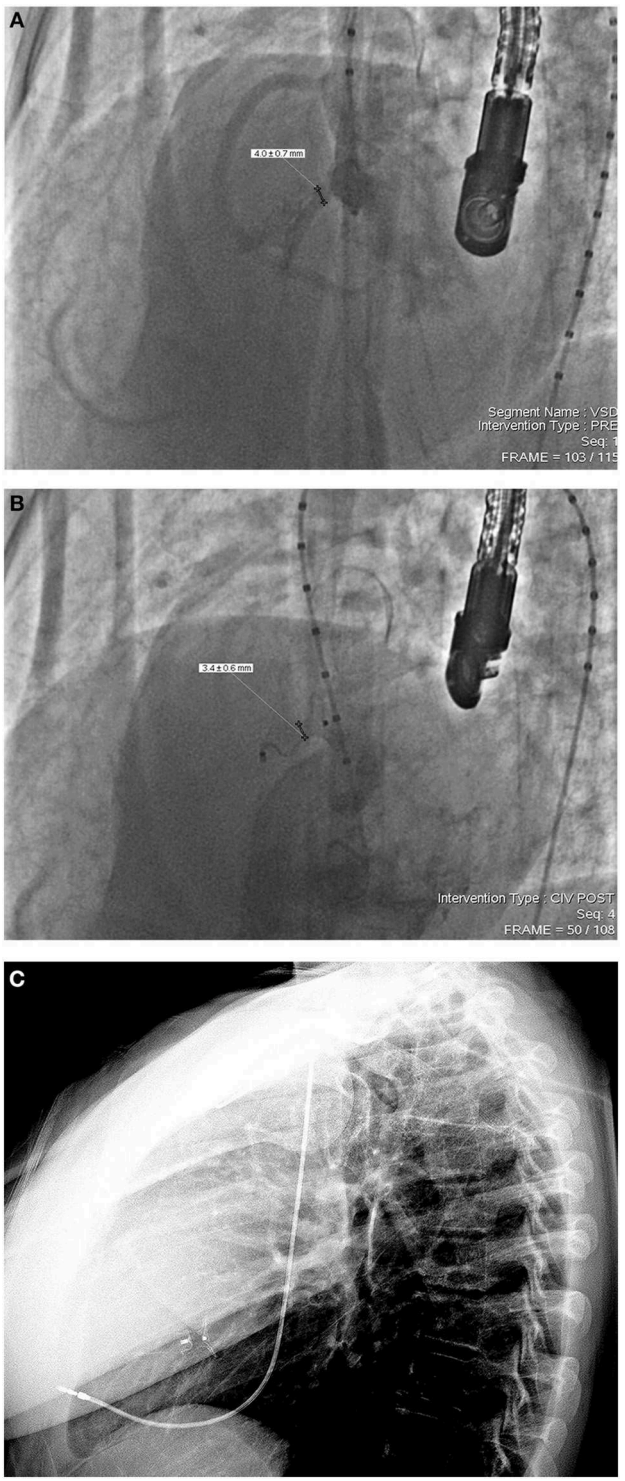

FIGURE 2 | (A) Left ventricular angiography in in 55–60° left anterior oblique to 20° cranial projection incidence, showing an aneurysmal type pmVSD with a 4 mm right ventricular exist, 14 mm left ventricular entry and a 4 mm sub-aortic rim. **(B)** Note the compressed 12 × 10 ADO device inside the defect with a "bone-shaped" deformation immediately after release. **(C)** Lateral chest X-ray done the day following permanent pacing (18 months after device implantation). Note the device regaining its original conical shape despite tissue compression.

incomplete closure, yet 5 cases of RS where still encountered. In these patients with large aneurysm and multiple exists, we believe that incomplete occlusion occurred since the chosen device was unable to cover the defect LV entry and the aneurysm together, leading to para-prosthetic RS (5, 35). For that, further clinical experience will help us develop better algorithms using a combination of ultrasound and angiographic measurements of defect size and in choosing the correct device.

One case of device embolization occurred with ADO and was strictly accidental (**Figures 1A–D**). The incidence was transient and the patient had no sequelae. Retrospectively, this complication affected our device selection priority since we had the tendency to prioritize AMO over ADO, when ADOII was not applicable. The double disk design was more reassuring against the risk of embolization. This same accidental embolization was described by Muthusamy, who switched from using ADO to AMO for pmVSD closure, while emphasizing on retrograde approach advantages (44). Among few reporting off label-use of AMO for pmVSD closure with promising results (10, 14, 15), Muthusamy was also the only one recently discussing AMO limited profile (44). Although we do support his findings (45, 46) and believe that AMO large and stiff lateral disks presents high radial and clamping force tension on the IVS, 16 AMO devices were implanted in our series since we thought that the 7 mm relatively long waist might reduce clamping force, thereby minimizing injury to the conducting system.

Finally, an ideal device would perfectly occlude the defect and the aneurysm together, without damaging the surrounding valve and conduction tissues. Among available Amplatzer devices, ADO II (whenever applicable) can satisfy these conditions when high operator's experience and implantation techniques are met. Besides its soft and flexible design, the efficient retrograde deliverability is by far its biggest advantage (35, 36, 47). In fact, we found out that excessive shearing force on the defect margins and surrounding valves during AVC establishment may be higher than the ones in the retrograde approach, with significantly longer fluoroscopic time. Besides, while El Sisi et al emphasized on deploying LD at first (48), we noticed that RD deployment before proceeding with the rest of the device, allows its complete positional adjustment and lowers TR incidences.

STUDY LIMITATIONS AND STRENGTHS

First and foremost, this was a single-center retrospective study with a limited number of participants and a wide age range. However, the strict protocol of percutaneous PmVSD closure in our institute before, during and after the procedure made the collected data comprehensive and accurate. In addition, all procedures were performed by the same operator, offering to a higher representation of routine practice. Besides the limited follow-up duration, this series of patients may not be representative of those encountered in developed countries. Well-designed prospective cohort studies that stratify patients based on age and device type are definitely needed to

establish clinical guidelines, recommending routine pmVSD transcatheter closure.

CONCLUSION

Percutaneous closure of pmVSD is a challenging and risky procedure, owing to variable anatomical morphology, proximity to valves and conduction tissues as well as complex manipulation process. The major key to improve the results of this treatment, while minimizing complications, consists in careful case and device selections as well as accurate defect sizing strategy. We showed that the mid-term results of our interventional approach for pmVSD closure using different Amplatzer occluders are equally promising with zero mortality and tolerable rate of morbidity. The procedure is relatively safe and effective. It appears that ADOII is the best available device to close defects with a diameter up to 5.5 mm, especially in aneurysmal type and in small children, because of its better profile and avoidance of a continuous AVC. Finally, CAVB remains the most potential serious complication that can occur during the procedure or any time later. For that, long-term follow-up in a large number of patients is mandatory to confirm safety of this intervention while monitoring unknown late onset complications.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation to any qualified researcher.

ETHICS STATEMENT

This study was reviewed and approved by Saint Joseph university research ethics committee.

AUTHOR CONTRIBUTIONS

RH performed calculations, analyzed the data, interpreted the results, and took the lead in writing the manuscript. ZS conceived of the presented idea and supervised the project. All authors discussed the results, read, and approved the final manuscript.

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Echocardiographic Prediction of Left Ventricular Dysfunction After Transcatheter Patent Ductus Arteriosus Closure in Children

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Objectives: To evaluate the change of left ventricular (LV) systolic function after transcatheter patent ductus arteriosus (PDA) closure in children, and to identify whether echocardiography parameters could be the predictors of LV dysfunction post-PDA closure if present.

Methods: This study enrolled 191 pediatric PDA patients, and all of them underwent successful transcatheter PDA closure between January 2016 and December 2018. The patent ductus arteriosus diameter (PDAd), aortic root diameter (AOd), left atrial diameter (LAd), right ventricular outflow tract dimension (RVOT), LV end-diastolic dimension (LVEDD), and LV end-systolic dimension (LVESD) were all measured by echocardiography at pre-closure, post-closure (within 24 h after the procedure), and follow-up (3 months after the procedure). The ratio of PDAd to AOd (PDAd/AOd), the ratio of LAd to AOd (LAd/AOd), the left ventricular ejection fraction (LVEF), and the fractional shortening (FS) were calculated.

Results: The LAd, LVESD, LVEDD, FS, and LVEF decreased significantly in the 24 h after closure, compared to pre-closure levels. However, all echocardiography parameters recovered to pre-closure levels at 3 months after PDA closure in all patients. Moreover, the pre-closure LAd, LVEF, PDAd/AOd, and LAd/AOd were higher in the patients with post-closure LV systolic dysfunction than in those without post-closure LV systolic dysfunction. Furthermore, the pre-closure LVEF, PDAd/AOd, and LAd/AOd were correlated with the post-closure LVEF, and pre-closure LVEF $\leq$ 66.5%, PDAd/AOd $\geq$ 0.28, and LAd/AOd $\geq$ 1.54 predict the post-closure LV systolic dysfunction.

Conclusion: Transcatheter closure of PDA causes a significant deterioration in LV systolic function early after PDA closure, which recovered completely within 3 months of post-closure in children. Pre-closure LVEF, PDAd/AOd, and LAd/AOd can be the predictors of post-closure left ventricular systolic dysfunction.

Keywords: patent ductus arteriosus, transcatheter closure, left ventricular dysfunction, echocardiography, children

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INTRODUCTION

Patent ductus arteriosus (PDA) is a common form of congenital heart disease with a left-to-right shunt (1). It has a broad spectrum of clinical manifestations, and the natural history of PDA mainly depends upon its size. Hemodynamically significant PDA leads to a left ventricle (LV) volume overload and remodeling and ultimately leads to severe complications, such as congestive heart failure, Eisenmenger's syndrome, atrial arrhythmias, endarteritis, and ductus aneurysm (2–4).

Transcatheter closure of PDA has been in development for nearly 30 years. Now, transcatheter PDA closure has been proven to be safe and effective with short- and long-term results comparable to surgical closure, and it has become the leading approach to the closure of most instances of PDA (5). Recently, several reports demonstrated that left ventricular systolic properties altered in adult PDA patients (6, 7), and PDA closure also led to an immediate deterioration of LV systolic function in children (8, 9). However, pre-closure predictors of the LV systolic dysfunction after PDA closure have not yet been clearly demonstrated.

Echocardiography is the most common diagnostic method that provides information regarding PDA size and hemodynamics, and it is also the most frequently used method for the evaluation of cardiac chamber size and LV systolic performance (10). Moreover, these parameters obtained by echocardiography correlate well with those measured during cardiac catheterization and radionuclide angiography (11, 12), and the possibility of the use of echocardiographic parameters in risk assessment for adverse cardiac events has been proven in recent studies (13–15). Therefore, the present study aimed to investigate the changes of LV systolic function after PDA closure, and to identify whether echocardiography indicators, if present, could be the predictors of LV dysfunction after PDA closure.

METHODS

Study Population

The study was approved by the institutional ethics committee at the Children's Hospital of Soochow University, and written informed consent was obtained from the patients' parents in all cases.

This study enrolled 191 children. All of them were diagnosed with isolated PDA and underwent successful transcatheter PDA closure between January 2016 and December 2018 in the cardiology department of the Children's Hospital of Soochow University. Patients of unsuitable PDA size for interventional closure were excluded.

Echocardiography

All echocardiographic examinations were carried out using the General Electric (GE) VIVID 7 ultrasound (Horten, Norway) with M4S and 5S probe and the GE EchoPAC workstation (BT 09, Horten, Norway). Some PDA children required sedation for echocardiography examination. For each PDA patient, echocardiography examinations were conducted at pre-closure, post-closure (within 24 h after PDA closure), and follow-up (3 months after PDA closure), respectively.

The patent ductus arteriosus diameter (PDAd) was measured in the high left parasternal short-axis view. The aortic root diameter (AOd), left atrial diameter (LAd), right ventricular outflow tract dimension (RVOT), LV end-diastolic dimension (LVEDD), and LV end-systolic dimension (LVESD) were obtained from the parasternal long-axis view. From these measurements, the following LV parameters were calculated: Fractional shortening (FS) $= \frac{\text{LVEDD}-\text{LVESD}}{\text{LVESD}} \times 100$, and LV ejection fraction (EF) $= \frac{\text{LVEDD volume}-\text{LVESD volume}}{\text{LVEDD volume}} \times 100$, where LVEDD volume $= \frac{7 \times LVEDD^3}{2.4 + \text{LVEDD}}$ and LVESD volume $= \frac{7 \times LVESD^3}{2.4 + \text{LVESD}}$ (16). To normalized echocardiographic indicators, the ratio of LAd to AOd (LAd/AOd) as well as the ratio of PDAd to AOd (PDAd/AOd) was calculated. LV systolic dysfunction was defined as a post-PDA closure LVEF of <55% (8).

Cardiac Catheterization

Midazolam, ketamine, or propofol were used for sedation in all patients, and a dose of 100 unit/kg of heparin was given after vein and artery puncture. The lateral aortogram was performed at the distal aortic arch before PDA closure. Transcatheter closure of PDA was performed using an antegrade or retrograde technique. An additional aortogram was performed to confirm complete shutdown after the procedure.

Statistical Analysis

Data are expressed as mean ± standard deviation. Changes in echocardiographic parameters were analyzed with paired t-test. The correlation between two continuous variables was determined using linear regression analysis. Multiple stepwise linear regression analyses were used to identify pre-closure echocardiography indicators of post-closure LV systolic dysfunction. Firstly, several statistically significant risk factors were screened out with univariate analysis, and $P < 0.05$ was considered statistically significant. Afterwards, multivariate analysis was performed using variables that were significant on univariate analysis, and $P < 0.1$ was considered significant. Receiver operating characteristic (ROC) analysis was used to find optimal cut-offs for each parameter for when the post-closure LVEF was below 55%. All of the statistical analyses used the Statistical Package for the Social Sciences (SPSS), version 19.0 for Windows (SPSS, Chicago, IL, USA).

RESULT

Clinical Characteristics of Patients

The clinical features of these PDA patients are described in **Table 1**. There were 60 boys and 131 girls in this study, and the median age was 23 months (range 3–184 months) at the time of the procedure. The PDA pulmonic end size was 3.06 ± 1.25 mm. The closure was achieved in all 191 patients who underwent transcatheter, and Amplazter duct occluders were used in all cases. The heart rate, systolic blood pressure, and diastolic blood pressure levels of PDA patients were similar between pre- and post-closure (**Supplemental Table 1**).

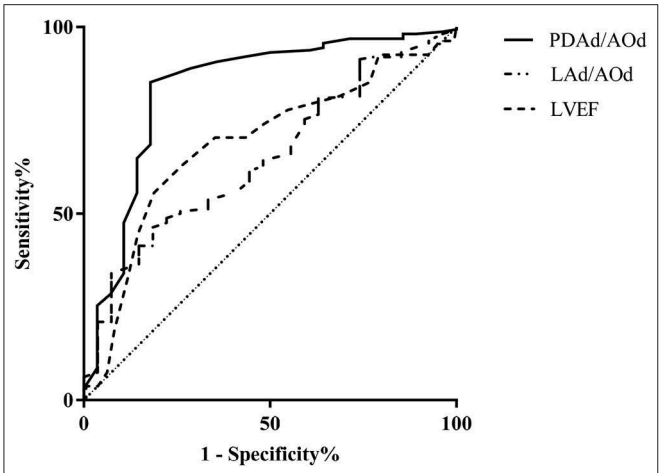

FIGURE 1 | Receiver operating characteristic curve of pre-closure LVEF, PDAd/AOd, and LAd/AOd echocardiography parameters for predicting LV systolic dysfunction.

TABLE 1 | Clinical data of 191 children undergoing PDA occlusion.

	PDA patients (n = 191)
Age (month)	33.71 ± 32.68
Gender (Boys/Girls)	60/131
Weight (kg)	8.10 ± 2.34
PDA diameter (mm)	3.06 ± 1.25

The Comparison of Echocardiography Parameters Between Pre-closure and Post-closure

The LAd, LVESD, LVEDD, FS, and LVEF significantly decreased within 24 h after closure compared to pre-closure levels, while there was no difference in AOd and RVOT values between pre- and post-closure.

Of the 191 children, 27 of them showed LV systolic dysfunction within 24 h of PDA closure. The pre-closure LVEF, LAd, PDAd/AOd, and LAd/AOd were higher in the patients with post-closure LV systolic dysfunction than those without post-closure LV systolic dysfunction (**Table 2**).

At 3 months after PDA closure, the LAd, LVESD, and LVEDD were no different in comparison to the pre-closure baseline. Also, the LVEF and FS values recovered to pre-closure levels in all patients (**Table 2**).

The Correlation of Echocardiography Parameters With LVEF Post-closure

To identify the factors associated with post-closure LV systolic function, stepwise multiple linear regression analysis was conducted. Univariate linear regression analysis showed that pre-closure PDAd ($r = -0.48$, $P < 0.01$), PDAd/AOd ($r = -0.50$, $P < 0.01$), and LAd/AOd ($r = -0.55$, $P < 0.01$) negatively correlated with post-closure LVEF, and pre-closure LVEF ($r = 0.66$, $P < 0.01$) positively correlated with post-closure LVEF (**Table 3**).

TABLE 2 | Comparison of echocardiography parameters at pre-closure, post-PDA closure and follow up (n = 191).

	Pre-closure	Post-closure 24 h	Follow up post-closure 3 months
AOd (mm)	14.24 ± 3.45	14.26 ± 2.77	14.14 ± 3.57
Lad (mm)	19.86 ± 3.95	18.63 ± 3.57*	19.54 ± 5.39
LVESD (mm)	21.19 ± 7.68	20.28 ± 5.37*	21.81 ± 8.62
LVEDD (mm)	35.61 ± 6.46	34.48 ± 7.17*	35.37 ± 8.31
RVOT (mm)	18.46 ± 3.63	18.03 ± 4.14	18.37 ± 4.11
FS (%)	38.81 ± 4.32	34.55 ± 4.76*	38.38 ± 4.78
LVEF (%)	70.03 ± 5.07	65.55 ± 6.61*	70.29 ± 5.36

*$P < 0.05$ vs. pre-closure.
AOd, Aortic root diameter; Lad, Left atrial diameter; LVEDD, Left ventricular end-diastolic dimension; LVESD, Left ventricular end-systolic dimension; RVOT, Right ventricular outflow tract dimension; FS, Fractional shortening; LVEF, Left ventricle ejection fraction.

TABLE 3 | Comparison of pre-closure echocardiography parameters in patients with or without post-closure LV systolic dysfunction.

	No LV dysfunction (n = 164)	LV dysfunction (n = 27)
PDAd (mm)	2.87 ± 0.92	4.21 ± 1.37*
AOd (mm)	14.24 ± 3.23	13.94 ± 4.73
Lad (mm)	19.89 ± 3.86	19.69 ± 4.73
LVESD (mm)	21.09 ± 3.83	21.53 ± 5.84
LVEDD (mm)	35.60 ± 6.02	35.20 ± 9.35
RVOT (mm)	17.99 ± 4.40	18.53 ± 3.50
FS (%)	38.70 ± 4.20	39.55 ± 4.89
LVEF (%)	70.53 ± 4.73	67.30 ± 6.12*
PDAd/AOd	0.21 ± 0.08	0.33 ± 0.09*
LAd/AOd	1.41 ± 0.26	1.54 ± 0.24*

*$P < 0.05$ vs. No LV dysfunction.
PDAd, Patent ductus arteriosus diameter; AOd, Aortic root diameter; Lad, Left atrial diameter; LVEDD, Left ventricular end-diastolic dimension; LVESD, Left ventricular end-systolic dimension; RVOT, Right ventricular outflow tract dimension; FS, Fractional shortening; LVEF, Left ventricle ejection fraction; PDAd/AOd, the ratio of PDA diameter to aortic root diameter; LAd/AOd, the ratio of left atrial diameter to aortic root diameter.

Among these parameters, pre-closure LVEF ($\beta = 0.443$, $P < 0.01$), PDAd/AOd ($\beta = -0.216$, $P < 0.01$), and LAd/AOd ($\beta = -0.211$, $P < 0.01$) were statistically significant factors on multivariate stepwise linear regression analyses for the deterioration of post-closure LV (**Table 4**).

The Cutoff Value of Echocardiography Parameters Associated With Left Ventricular Dysfunction

By ROC analysis, the area under the curve (AUC) for pre-closure PDAd/AOd was 0.833, and pre-closure PDAd/AOd $\geq$ 0.28 showed a sensitivity of 81.5% and specificity of 98.5%. Moreover, pre-closure LAd/PDAd $\geq$ 1.54 showed a sensitivity of 81.5% and specificity of 68.5%. Its AUC was 0.692, whereas pre-closure LVEF $\leq$ 66.5% showed a sensitivity of 81.3% and specificity of 57.5% in predicting the immediate post-closure LV systolic dysfunction, and its AUC was 0.693 (**Figure 1**).

TABLE 4 | Multiple linear regression analysis for post-closure LV dysfunction.

Pre-closure variables	Univariate analysis *P*	Multivariate analysis *P*
AOd (mm)	0.570	
LAd (mm)	0.360	
PDAd (mm)	0.003	
LVESD (mm)	0.105	
LVEDD (mm)	0.192	
ROVT (mm)	0.293	
LVEF (%)	0.003	0.001
PDAd/AOd	0.001	0.001
LAd/AOd	0.001	0.001

AOd, Aortic root diameter; Lad, Left atrial diameter; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end systolic dimension; ROVT, Right ventricular outflow tract dimension; FS, fractional shortening; LVEF, Left ventricle ejection fraction; PDAd/AOd, the ratio of PDA size to aortic root diameter; LAd/AOd, the ratio of left atrial diameter to aortic root diameter.

DISCUSSION

PDA is a relatively common congenital heart defect. The incidence of PDA is ∼1 per 2,000 live births in full-term newborns, and accounts for 5–10% of all congenital heart diseases (17). Now, the prevalence rate of PDA is 0.78 per 1,000 in China according to a recent report (18). Since the first transcatheter PDA closure was conducted by Porstmann et al. in 1967 (19), there have been many significant developments in PDA closure. Now, transcatheter PDA occlusion has become the priority choice to treat most PDA in both children and adults (5). The current study demonstrated an early deterioration of LV function following successful transcatheter ductal closure, which recovered completely within 3 months post-closure. Moreover, pre-closure PDAd/AOd, LAd/AOd, and LVEF were the predictors of post-closure LV dysfunction.

As is known, PDA frequently results in left ventricular volume overload, which is required to increases left ventricular output by Frank-Starling response, and it can therefore overcome the left-to-right shunt and maintain systemic circulation (20, 21). Because the left ventricular remodeling is caused by a significant left-to-right shunt through PDA, it is conceivable that left ventricular reverse remodeling occurs after ductal closure. In the current study, the LAd, LVESD, LVEDD, FS, and LVEF significantly decreased in the 24 h after PDA closure. Consistently, Gupta et al. also found there was a significant reduction in LVEDD and LVESD in immediate post-closure as compared to pre-closure baseline (22); these results confirmed that early PDA closure in childhood may benefit the remodeling of LV.

In 2005, Eerola et al. demonstrated that changes in LV systolic function were caused by PDA closure in children (9). Similarly, Galal et al. found that the closure of relatively large PDA led to a significant immediate deterioration of LV systolic performance in children (23). Consistent with these previous reports, the current study demonstrated that LVEF reduced immediately 24 h after PDA closure, which indicated impaired LV systolic function. The possible explanation for this is that when a hemodynamically significant PDA is closed it abolishes the left-to-right shunt, thereby reducing the preload of the LV. However, it also increases the afterload by eliminating the low-resistance pulmonary circulation from LV outflow circulation. Due to a phenomenon called "afterload mismatch," this simultaneous reduction in the LV preload and increase in the afterload may lead to LV systolic dysfunction (24).

Furthermore, all of the LV dysfunction recovered to baseline in the follow-up periods in the current study, which is consistent with previous children studies (9, 23). However, about 11% of transcatheter PDA adult patients showed the persistent long-term deterioration of LV systolic function, according to Jeong's study (6). This discrepancy is probably due to the longer duration of volume overload and consequently more extensive and irreversible changes in LV in adults compared to children.

In a study conducted by Agha et al., PDAd was proved to be the predictor of post-closure LV systolic function (25). However, the current study found that PDAd/AOd is a predictor of post-closure LV systolic dysfunction, rather than the absolute diameter of PDA. This inconsistency may be due to age, which can affect the PDA size; "PDA size" is also a relative term with no standardization attached to it, which may limit its practical value somewhat. "PDAd/AOd," therefore, is a better indicator in predicting post-closure LV systolic function, and these results suggest that the larger the PDAd/AOd value the more likely post-closure LV dysfunction would be. Interestingly, the LAd/AOd ratio also predicted the post-closure LV dysfunction in our study. As is known, a significant hemodynamic shunt via a PDA leads to the enlargement of the left heart and a decreased LV ejection fraction (26). LAd/AOd may thus reflect the severity of ductal shunting in these PDA patients; Iyer et al. also reported that a LAd/AOd of >1.4:1 was associated with a hemodynamically significant ductal flow in a preterm infant (27). Our results suggest that PDA closure should be conducted before LAd/AOd reaches more than 1.54 to achieve a normal LVEF after PDA closure.

The current study found that the incidence of LV systolic dysfunction was 14.1% in this cohort ($n = 27/191$). This is comparable with the studies conducted by Jeong et al. (6) (11.1%), Kim et al. (28) (18.6%) on the Korean population, and Eerola et al. (9) (15.2%) on the Finland cohort. However, it is much lower than a study conducted by Kiran et al., which reported the incidence of LV dysfunction is 22.8% in Indian PDA patients (29). These studies suggest the influencing factors of LV systolic dysfunction may also involve ethnicity and social factors in addition to the heart hemodynamics parameters.

The present study had some limitations. Firstly, this study was retrospective and in a single center despite having a relatively large sample size. Secondly, the study only involved the assessment of the systolic function of the LV by two-dimension echocardiography method.

In conclusion, transcatheter closure of PDA is associated with reversible LV systolic dysfunction in children patients. Pre-closure PDAd/AOd $\geq$ 0.28, LAd/AOd $\geq$ 1.54, and LVEF $\leq$ 66.5%, measured by echocardiography, were the cutoff values used to predict post-PDA closure LV systolic dysfunction. The results of the present study can provide a useful and convenient strategy to predict who (of the patients undergoing PDA device closure) is likely to have LV dysfunction after PDA closure in clinical practice.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of the Children's Hospital of Soochow University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR'S NOTE

The authors declare that neither this manuscript nor any similar paper, in whole or in part, have been or will be submitted to or published in any other scientific journal.

AUTHOR CONTRIBUTIONS

MH, WQ, and LS conceived and designed the study and analyzed data and wrote the manuscript. MH, WQ, BW, WZ, JZ, YD, QX, JH, JS, LC, HL, and LS performed this study. BW, WZ, JZ, YD, QX, JH, JS, LC, and HL reviewed and edited the manuscript. All authors read and approved the manuscript.

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SUPPLEMENTARY MATERIAL

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Physical Activity Among Children With Congenital Heart Defects in Germany: A Nationwide Survey

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Objective: In children with congenital heart defects (CHD), a sedentary lifestyle should be avoided and usually WHO recommendations on physical activity (PA) are supposed to be followed. In order to obtain representative data of the actual amount of PA (and potential influencing factors) in children with CHD we performed a nationwide online survey.

Methods: All patients aged 6–17 years registered in the German National Register for CHD were contacted by email and asked to participate in the survey using the comprehensive questionnaire of the "Motorik-Modul" from the German Health Interview and Examination Survey for Children and Adolescents (KiGGS), thus allowing the comparison with a representative age-matched subset of 3.385 participants of the KiGGS study. The questionnaire for CHD-patients was amended by specific questions regarding medical care, sports recommendations and PA restrictions.

Results: Complete datasets of 1.198 patients (mean age of 11.6 ± 3.1 years) were available for evaluation. Compared to the reference group, CHD patients significantly less frequently reached the WHO recommended level of 60 min of daily PA (8.8 vs. 12%; $p < 0.001$). Enjoyment in sports was almost equally distributed across CHD and reference groups, and strongly correlated with the level of PA ($r = 0.41$; $p < 0.001$). Remarkably, 49.2% of children with complex CHD, 31.7% with moderate, and even 13.1% with simple CHD were advised by their physician to restrict PA.

Conclusions: According to this nationwide survey, PA is markedly reduced in children with CHD. An important reason for this might be an unexpected high rate of physician-recommended restrictions on levels of PA.

Keywords: congenital heart defect (CHD), physical activities and sports, survey, pediatric cardiology, exercise limitation

INTRODUCTION

Occurring at a rate of 1.1% out of all newborns, congenital heart defects (CHD) are the most frequent congenital malformation diagnosed in children (1). Due to recent improvements in surgical and interventional techniques as well as perioperative intensive care management, survival of children with CHD has markedly improved during the last decades, resulting in a growing number to survive to adulthood (2). As CHD patients get older, their cardiac health can additionally be affected by acquired cardiovascular risk factors (i.e., arterial hypertension, obesity, diabetes) commonly seen in the general population, thus increasing the risk of metabolic disease, stroke and coronary artery disease (3, 4). In fact, a recent study has suggested that myocardial infarction will become the leading cause of death in CHD patients with simple cardiac defects (5). It is known, that development of arteriosclerotic and metabolic disease manifesting in adulthood usually starts already in early childhood. Childhood obesity and sedentary lifestyle are known to represent major contributing factors (5). This highlights the need for primary prevention, hence lifestyle interventions are required to promote physical activity (PA) of pediatric CHD-patients (6, 7). Not to mention, that PA is indispensable for physical, emotional, and psychosocial development of children (8–10).

Thus, there is emerging consensus that children and adolescents with CHD should be encouraged to adopt a physically active lifestyle, and consequently, current sports recommendations for the majority of patients with simple and moderate CHD include participation in competitive sports, leisure sports and PA unrestricted following the World Health Organisation (WHO) recommendations for healthy children, i.e., daily participation of 60 min in moderate-to-vigorous PA that is developmentally appropriate and enjoyable (11). Complex CHD often requires more specific recommendations yet still with the aim to enable a physically active lifestyle.

Whether these recommendations on PA in children with CHD are adequately considered and generally implemented remained unanswered to this day. Previous studies investigating PA in children with CHD showed rather conflicting results. While some authors reported of reduced PA, especially in complex CHD, others revealed similar PA-levels compared to healthy controls (12–20). Discrepancies of results might be explained by differences in study designs and PA assessment tools, and furthermore, common to all of these studies are relatively small patient numbers included.

Therefore, we conducted a nationwide survey in collaboration with the German National Register for CHD (NRCHD) in order (I) to obtain representative data regarding the real world situation of the amount of PA and sports participation and its impact on physical self-perception in children with CHD living in Germany, (II) to detect differences compared to children without CHD using an appropriate reference cohort, and (III) to study factors potentially influencing PA and sports participation in CHD patients.

Abbreviations: CHD, congenital heart defects; KiGGS, German Health Interview and Examination Survey for Children and Adolescents; MoMo, Motorik-Modul; NRCHD, National Register for congenital heart defects; PA, Physical Activity; PACES, Physical Activity Enjoyment Scale for children and adolescents; PAQ, Physical Activity Questionnaire; WHO, World Health Organization.

MATERIALS AND METHODS

Study Design

This cross-sectional online survey was conducted from January to March 2018. Participants were recruited via the patient database of the NRCHD, the largest European registry for CHD patients (21). For patient recruitment, the database was searched for patients aged between 6 and 17 years at the time of the survey. Respective individuals and their parents were contacted and invited to take part in the survey via email. Ethical approval was obtained by the institutional ethical committee. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

Survey Instruments

PA and sports participation were assessed using the validated comprehensive questionnaire of the "Motorik-Modul" (MoMo) from the German Health Interview and Examination Survey for Children and Adolescents (KiGGS), thus allowing the comparison of obtained data with a representative age-matched subset of 3.385 same-aged participants of the MoMo wave 2 study (2015–2017) (22). Design and results of the MoMo Baseline and Longitudinal Study and details on the structure and content of the MoMo Physical Activity Questionnaire (MoMo-PAQ) have been published previously (22–25). Briefly, it consists of 28 items regarding frequency, duration and intensity of PA to capture habitual PA in different domains (PA in sports clubs, leisure time PA outside of sports clubs, extra-curricular PA, outdoor play, active commuting to school). Furthermore, the MoMo-PAQ consists of 36 items, which assess physical self-description based on the German version of the Physical Self-Description Questionnaire with answer categories to a 4-point Likert Scale (26). These 36 items represent the basic functions of physical performance: strength, endurance, speed, skills, coordination, and flexibility.

In addition, MoMo-PAQ included the German version of the Physical Activity Enjoyment Scale for children and adolescents (PACES) (27). The scale consists of 16 items (nine positive poled items, seven negative poled items) with answer categories to a 5-point Likert Scale.

The questionnaire for CHD-patients was amended by eight specific questions capturing the medical background of sports recommendations, sports restrictions and access to sports.

Statistical Analysis

Values of continuous variables are reported as mean ± standard deviation. The Pearson's chi-square test was used for group comparisons including nominal data (e.g., gender and age). In order to assess the impact of potential contributing factors on PA, analysis of variance and analysis of covariance, Pearson's correlation as well as multiple and linear regression analysis was used, as appropriate. IBM SPSS statistics version 25.0 (IBM

TABLE 1 | Classification of CHD severity according to Warnes et al. (28).

Simple CHD	Moderate CHD	Complex CHD
Isolated congenital aortic valve disease	Aorto–left ventricular fistulas	Conduits, valved, or nonvalved
Isolated congenital mitral valve disease (e.g., except parachute valve, cleft leaflet)	Anomalous pulmonary venous drainage, partial or total	Cyanotic congenital heart (all forms)
Small atrial septal defect	Atrioventricular septal defects (partial or complete)	Double-outlet ventricle
Isolated small ventricular septal defect (no associated lesions)	Coarctation of the aorta	Eisenmenger syndrome
Mild pulmonary stenosis	Ebstein's anomaly	Fontan procedure
Small patent ductus arteriosus	Infundibular right ventricular outflow obstruction of significance	Mitral atresia
Previously ligated or occluded ductus arteriosus	Ostium primum atrial septal defect	Single ventricle (also called double inlet or outlet, common, or primitive)
Repaired secundum or sinus venosus atrial septal defect without residua	Patent ductus arteriosus (not closed)	Pulmonary atresia (all forms)
Repaired ventricular septal defect without residua	Pulmonary valve regurgitation (moderate to severe)	Pulmonary vascular obstructive disease
	Pulmonary valve stenosis (moderate to severe)	Transposition of the great arteries
	Sinus of Valsalva fistula/aneurysm	Tricuspid atresia
	Sinus venosus atrial septal defect	Truncus arteriosus/hemitruncus
	Subvalvular AS or SupraAS (except HOCM)	Other abnormalities of atrioventricular or ventriculoarterial connection not included above (i.e., crisscross heart, isomerism, heterotaxy syndromes, ventricular inversion)
	Tetralogy of Fallot	
	Ventricular septal defect with: Absent valve or valves, Aortic regurgitation, Coarctation of the aorta, Mitral disease, Right ventricular outflow tract obstruction, Straddling tricuspid/mitral valve, Subaortic stenosis	

CHDs were divided into simple, moderate and complex CHD according to the classification system by Warnes et al. (28). It is possible to be classified from a lighter group to a heavier group due to interventions and/or operations. Conversely, this is not possible. Therefore, this table shows only a rough schema of the severity classification. Taking all factors into account would be too extensive.

Inc., Armonk, NY, USA) was used for statistical analyses. A significance level of $p \leq 0.05$ was applied.

RESULTS

Patient Characteristics

Of 21.354 eligible patients, invitation was successfully delivered to 14.496 patients. 1.718 patients decided to participate in the study. Only complete datasets were considered for evaluation and were available from 1.198 CHD patients with a mean age of 11.6 ± 3.1 years. Of those 53.8% were male, and 46.2% female. The study participants were allocated into simple, moderate and complex CHD classification according to Warnes et al. (28) (**Table 1**). Thus, 411 (34.3%) were classified as simple CHD, 423 (35.3%) moderate, and 364 (30.4%) as complex CHD. Included patients had untreated CHD in 49.3%, 1–3 operations/interventions in 30.2%, and more than three operations/interventions in 20.5%. Genetic syndromes and chromosomal disorders were present in 70 patients (5.8%), most frequently trisomy 21 in 37 patients (3.1%) and Di-George-Syndrome in 15 patients (1.3%). Of all patients, 57.2% stated to live in an urban or suburban environment, whereas 42.8% live in rural areas. Patient characteristics of the study group did not significantly differ to the entire cohort of eligible patients.

Physical and Sports Activity

Compared to MoMo participants, CHD patients reached significantly less frequently the WHO recommended level of 60 min of daily PA (8.8 vs. 12%; $p < 0.001$), simple CHD 9.2%, moderate 9.2%, and complex CHD 8.0%. Children with CHD were 0.62 days per week less active than those of the reference group ($p < 0.001$) (**Figure 1**).

Participation in sports clubs was significantly reduced in children with complex CHD compared to MoMo participants (53.6 vs. 67.6%; $p < 0.001$), whereas patients with simple and moderate CHD showed similar frequent participation as the reference group (74.9 and 66.2%, respectively). 61.4% of children with simple, 56.8% with moderate and 47.7% with complex CHD stated to participate even in competitive sports, compared to 53.1% of the reference group.

Enjoyment in Sports

Enjoyment in sports was almost equally distributed across CHD groups and MoMo participants. Using the positive dimension of the PACES Scale ranging from 9 to 45 points we found 35.9 points for patients with simple CHD, 34.6 points for moderate and 33.2 points for patients with complex CHD, compared to 35.2 points for the reference group. Enjoyment in sports correlated with the level of PA ($r = 0.41$; $p < 0.001$).

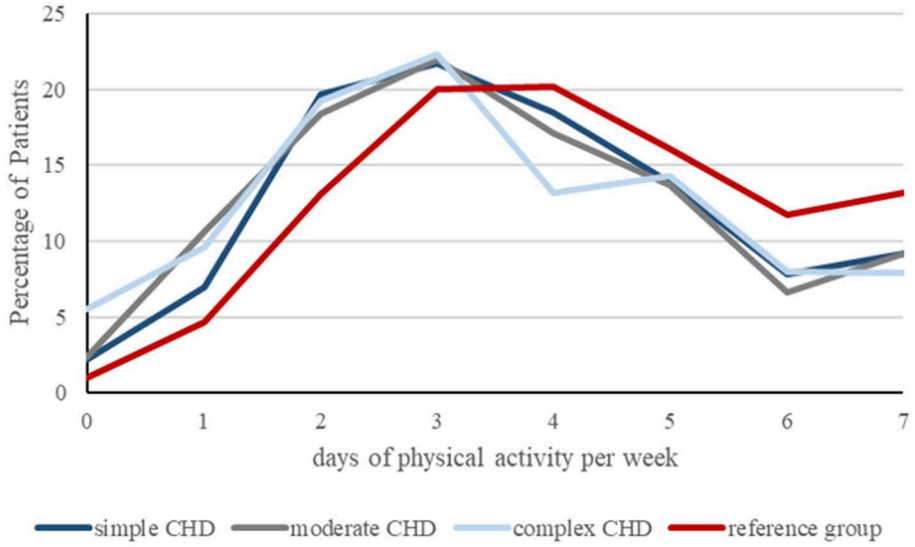

FIGURE 1 | Graphical presentation of days of physical activity per week, achieved by patients with simple ($n = 411$), moderate ($n = 423$) and complex CHD ($n = 364$) compared to the reference group ($n = 3.338$) in percent. Children with CHD were 0.62 days per week less active than those of the reference group ($p < 0.001$).

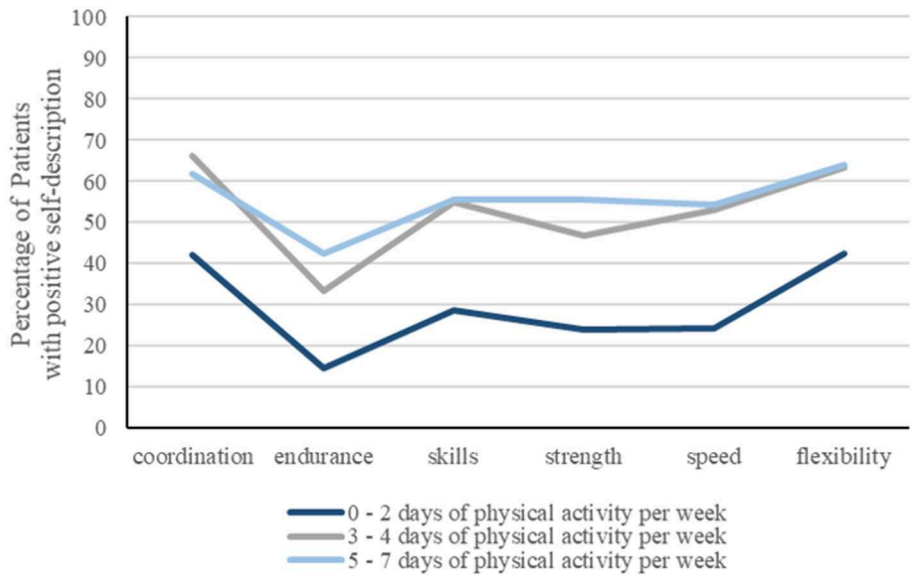

FIGURE 2 | Relationship between positive physical self-description of basic functions of physical performance (strength, endurance, speed, skills, coordination, flexibility) with the level of physical activity (PA), expressed as days per week. Percentage of patients revealing a positive self-description is significantly higher when physically active on more than 2 days per week.

Physical Self-Description of Basic Functions of Physical Performance

Physical self-description of all basic functions of physical performance (strength, endurance, speed, skills, coordination, flexibility) appeared to be related to the level of PA (**Figure 2**). More physically active patients showed higher percentage of positive self-description of basic functions of physical performance, most consistent for the dimension "endurance."

Further analyzing the physical self-description of the dimension "endurance" (ranging from 6 to 24 points) showed significant reduction in CHD patients compared to the reference cohort ($p < 0.001$), most noticeable in complex CHD (11.9 ± 4.9 points) compared to 16.6 ± 4.5 points in the reference group (**Figure 3**). The dimension "endurance" correlated with the level of PA in all CHD groups ($p < 0.001$).

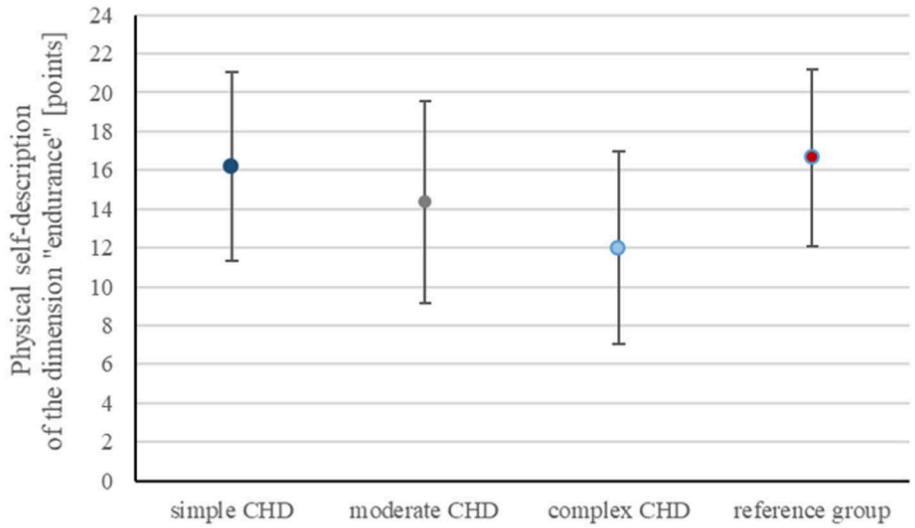

FIGURE 3 | Graphical presentation of physical self-description of the dimension "endurance" (ranging from 6 to 24 points) provided as mean ± standard deviation showing reduction in CHD patients compared to the control cohort, most noticeable in complex CHD (11.9 ± 4.9 points) compared to 16.6 ± 4.5 points in the reference group.

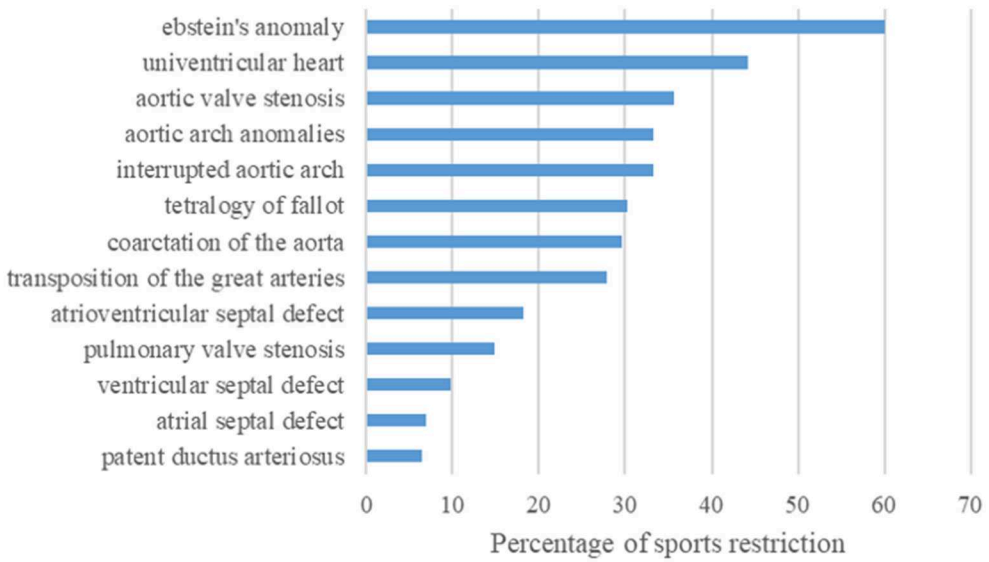

FIGURE 4 | Bar graph showing the frequency of physician-recommended sports restriction in percent dependent on the corresponding CHD diagnosis. Of the 271 of 1.198 children with CHD receiving a sports restriction, most frequent restrictions have been advised in patients with Ebstein's anomaly, followed by single ventricle hemodynamics, and aortic valve stenosis.

Physician-Recommended Sports Restrictions

49.2% of the children with complex CHD, 31.7% with moderate and even 13.1% with simple CHD were advised by their physician to restrict PA. 2.3% of all CHD patients received even a complete dispensation. Most frequently, sports restrictions have been advised in patients with Ebstein's anomaly, followed by single ventricle hemodynamics, and aortic valve stenosis (**Figure 4**). Interestingly, also patients with more simple CHD (i.e., ventricular septal defect or atrial septal defect) received sports restrictions. Advisory physicians in terms of sports were predominantly pediatric cardiologists (55.4%), followed by pediatricians (24.9%), family physicians (9.2%), orthopedists (2%), and sports medicine specialists (1.1%). In 7.4% the participant was not aware of the exact professional title of the advisory physician.

TABLE 2 | Potential factors influencing physical and sports activity.

Variable	Regression coefficient	Significance *p*
Age	−0.077[a]	<0.001
Gender	0.210[d]	0.050
Number of interventions	−0.056[b]	0.005
Residence in rural areas	−0.011	0.707
Sports activity father	0.216	0.047
Sports activity mother	0.017	0.571
Enjoyment in sports	0.103[c]	<0.001
Recommended restrictions	−0.294	0.012

[a] *Reduction of physical activity per year.*
[b] *Reduction of physical activity per intervention.*
[c] *Increase of physical activity per reached point in the PACES scale.*
[d] *Increase of physical activity of male children.*

Factors Influencing the Amount of Physical Activity

Regression analysis demonstrated a significant impact of age and gender on PA, whereas girls with CHD demonstrated lower levels of PA. Both, number of interventions and enjoyment in sports play a relevant role on the level of sports activity. Multiple regression analysis revealed physician-recommended PA restrictions having significant impact on the amount of PA, followed by sports activity of the father, whereas sports activity of the mother and residence in rural areas obviously had no significant impact on patient's PA (**Table 2**).

DISCUSSION

To the best of our knowledge, the present study is the largest cohort of children with CHD that has been investigated for their physical and sports activity behavior. According to this nationwide survey, PA is markedly reduced in children with CHD living in Germany, and as expected, children with complex CHD showed the most relevant PA reduction. In addition, this study provides information on possible contributing factors that may influence the amount of PA beyond the burden of the heart defect itself, including role model function (regarding sports activity) of the father and an unexpected high rate of physician-recommended restrictions on PA levels.

Misjudgment in regard of risks vs. benefits of sports participation, as well as overprotection of parents or caregivers, as well as teachers and sport trainers have been previously reported as potential contributing factors (11, 29, 30). Our results suggest, that also physicians and health care professionals might tend to overprotect, when treating children with CHD, and this obviously often neither in accordance to current recommendations nor supported by scientific evidence, as it has previously been demonstrated that sudden death during exercise is extremely rare in CHD patients (31). Misperception is most notably when considering the relatively high rate of PA restrictions in simple CHD, i.e., patients who usually were allowed to perform unrestrictive leisure and competitive sports.

Recommendations for most patients with CHD include participation in competitive sport, leisure sport and PA unrestricted like healthy children, allowing participation in sports clubs and thus integration in normal social life, except for children with complex CHD or other risk factors (for example pacemaker, cardioverter-defibrillator, channelopathies) who frequently need specific individualized solutions. The latter can be covered by preventive cardiac rehabilitation groups organized by local pediatric heart centers or alternatively by individualized training programs. However, in Germany there are currently only eleven active regional cardiac rehabilitation groups for children with CHD compared to ∼6,000 active cardiac rehabilitation groups for adults (32). Undoubtedly, this availability appears inadequate and is suggested to be influenced by differences in age, gender, interests, severity of CHD and exercise capacity, and additional logistical issues due to living in rural areas with long distances to the regional pediatric heart center. Indeed, our results were able to demonstrate that age, gender, enjoyment in sports and severity of CHD had significant impact on PA levels, however living in rural areas appears not to be a limiting factor, at least in a relatively small country as Germany.

Remarkably, our results show that children with CHD, even with complex CHD had well preserved enjoyment in sports on a similar level as the control group. This has to be considered and implicates that in children the motivation regarding PA and sports appears to be high, underlining the social implications of sports participation in childhood and supports the feasibility of individual exercise training in the pediatric age group. To maintain or even increase motivation, training programs designed for children should usually prefer varied activity tasks focusing mainly on skills, coordination, and speed, and avoid monotonous endurance training, as frequently used in adults (11, 33). The fact that reduced PA correlated with impaired physical self-perception demonstrates the negative psychological consequences of a sedentary lifestyle. This is alarming and might result in a downwards spiral of inactivity. Physicians should therefore regularly advice on patients' PA level and sports activity in every clinical consultation.

Study Limitations

Data of this survey are based on self- or proxy-reports and might therefore be prone to bias, including recall bias and social desirability, as children with CHD may overestimate their PA level (34). Furthermore, the absence of direct contact with patients may leave the chance for misunderstanding of questions or incorrect answers. To prevent such occurrences each study participant was offered to contact the study management if having any questions. In addition, at the end of the survey there was the possibility to provide ambiguities and questions directly as free text. Clearly, measuring PA by objective methods (i.e., accelerometry, pedometers, activity trackers) might be more precise (14). However, the objective assessment of PA in different domains can be challenging and requires complex strategies, which can hardly be applied in large patient populations. The strength of the current study is the consideration of different

domains of PA, the large sample size and the inclusion of school-aged children with a wide age range, as well as the representative reference group.

Since this is a cross-sectional study, we provide descriptive information and report on associations rather than claiming to report causal relationships between PA reduction and potential contributing factors (2). Moreover, the results reflect respondents' subjective statements. The results may not be applicable to CHD patients outside Germany, since they are affected by the life situation of the patients, as well as the organization of the health care and educational system (2).

Since the patients have been invited by emails to participate in the survey, the response rate of 11.9% was relatively low. Nevertheless, the sample of patients participating in the online survey did not differ significantly to the entire CHD cohort of NRCHD and therefore seems to be representative for the German CHD community at large.

Theoretically, by considering the prevalence of CHD in the German population of 1.1%, it cannot be ruled out that a minority of participants in the KiGGS study may also be affected by CHD. Due to inaccurate recording of CHD diagnoses in the KiGGS dataset a removal of these participants was not feasible. However, from a statistical point of view, having these children included in the reference group should not result in a relevant bias.

CONCLUSIONS

According to this nationwide survey, PA is markedly reduced in children with CHD, at least in part due to an unexpected high number of physician-recommended PA restrictions. As sedentary lifestyle may have negative implications on cardiovascular risk profile and prognosis, future efforts should be directed toward facilitating the access to PA for all CHD patients.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Review board of the Charité, Berlin (Approval number 2/034/17). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

JS, CN, and CA conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript. UB, HA-K, ES, MU, MF, and AJ designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the manuscript. PH conceptualized and designed the study, coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Immediate Post-operative Enterocyte Injury, as Determined by Increased Circulating Intestinal Fatty Acid Binding Protein, Is Associated With Subsequent Development of Necrotizing Enterocolitis After Infant Cardiothoracic Surgery

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Objectives: 1 Measure serial serum intestinal fatty acid binding protein levels in infants undergoing cardiac surgery with cardiopulmonary bypass to evaluate for evidence of early post-operative enterocyte injury. 2 Determine the association between immediate post-operative circulating intestinal fatty acid binding protein levels and subsequent development of necrotizing enterocolitis.

Design: Observational cohort study. Intestinal fatty acid binding protein was measured pre-operatively, at rewarming, and at 6 and 24 h post-operatively. Percent of goal enteral kilocalories on post-operative day 5 and episodes of necrotizing enterocolitis were determined. Multivariable analysis assessed for factors independently associated with clinical feeding outcomes and suspected/definite necrotizing enterocolitis.

Setting: Quaternary free-standing children's hospital pediatric cardiac intensive care unit.

Patients: 103 infants <120 days of age undergoing cardiothoracic surgery with cardiopulmonary bypass.

Interventions: None.

Results: Median pre-operative intestinal fatty acid binding protein level was 3.93 ng/ml (range 0.24–51.32). Intestinal fatty acid binding protein levels rose significantly at rewarming (6.35 ng/ml; range 0.54–56.97; $p = 0.008$), continued to rise slightly by 6 h (6.57 ng/ml; range 0.75–112.04; $p = 0.016$), then decreased by 24 h (2.79 ng/ml; range 0.03–81.74; $p < 0.0001$). Sixteen subjects (15.7%) developed modified Bell criteria Stage 1 necrotizing enterocolitis and 9 subjects (8.8%) developed Stage 2 necrotizing

enterocolitis. Infants who developed necrotizing enterocolitis demonstrated a significantly higher distribution of intestinal fatty acid binding protein levels at both 6 h ($p = 0.005$) and 24 h ($p = 0.005$) post-operatively. On multivariable analysis, intestinal fatty acid binding protein was not associated with percentage of goal enteral kilocalories delivered on post-operative day 5. Higher intestinal fatty acid binding protein was independently associated with subsequent development of suspected/definite necrotizing enterocolitis (4% increase in odds of developing necrotizing enterocolitis for each unit increase in intestinal fatty acid binding protein; $p = 0.0015$).

Conclusions: Intestinal fatty acid binding protein levels rise following infant cardiopulmonary bypass, indicating early post-operative enterocyte injury. Intestinal fatty acid binding protein was not associated with percent of goal enteral nutrition achieved on post-operative day 5, likely due to protocolized feeding advancement based on clinically observable factors. Higher intestinal fatty acid binding protein at 6 h post-operatively was independently associated with subsequent development of necrotizing enterocolitis and may help identify patients at risk for this important complication.

Keywords: biomarker, pediatric, congenital heart disease, IFABP, post-operative care, nutrition, NEC, cardiopulmonary bypass

INTRODUCTION

Infants undergoing surgery for congenital heart disease (CHD) with cardiopulmonary bypass (CPB) are at high risk for intestinal injury, both during the surgery itself and subsequently from ongoing post-operative low cardiac output (1, 2). This intestinal injury can lead to major postoperative complications including intestinal barrier dysfunction, dysmotility, post-operative feeding intolerance, and post-operative necrotizing enterocolitis (NEC) (3–13). NEC, in particular, is a dangerous complication. Comorbid CHD and NEC have been shown to have high overall mortality (50–57%) (14, 15) and significantly increased length of hospitalization compared to CHD alone (36 vs. 19 days) (16). At this time, no clinically available biomarker exists to help identify specific intestinal damage following infant cardiac surgery.

Intestinal epithelial injury can be assessed by minimally invasive testing of intestinal fatty acid-binding protein (IFABP). IFABP is a 15-kD cytosolic protein localized mainly to mature enterocytes of the small intestinal villi with normally low circulating levels (17–20). IFABP plasma concentration has been shown to correlate to intestinal epithelial injury in animal models (21, 22). Much of the recent research involving IFABP has been as a predictor of NEC in the neonatal population (23–25), but it has also been utilized as an indicator of intestinal injury across many different contexts including cardiac surgery in both adults and children (3, 26–30). Previous studies performed in mixed age cohorts of children undergoing surgery for CHD indicate that IFABP levels rise immediately following surgery and then fall to below preoperative levels in the subsequent recovery period (3, 26). However, no previous studies have looked at IFABP levels in specifically the *infant* CHD population, which is a unique and important age group given the complexity of the surgeries performed at this age and the risk for diffuse organ injury. Additionally, associations between IFABP levels and feeding tolerance or clinical suspicion of NEC have not been assessed in this high-risk population.

In this study, we sought to describe the pattern of serum IFABP in infants undergoing CPB. In addition, we aimed to determine the clinical risk factors associated with elevated preoperative and postoperative IFABP levels, hypothesizing that higher IFABP levels would be associated with worse clinical severity of disease. Finally, we investigated whether there was an association between IFABP and early enteral feeding outcomes/development of NEC, hypothesizing that greater elevation of IFABP levels in the immediate post-operative period would predict worse enteral feeding outcomes and greater odds of developing NEC.

MATERIALS AND METHODS

This study was a pre-specified secondary aim of a prospective cohort study evaluating alkaline phosphatase activity after infant CPB (31), with prospective collection of samples for IFABP analysis and *post-hoc* analysis of infant feeding outcomes. Over a period of two-and-a-half years from September 2013 to February 2016, infants $\leq$120 days at time of cardiothoracic surgery with use of CPB were enrolled. Exclusion criteria were weight $<$2 kg (as limited blood volume could increase risk of anemia with research blood draws), adjusted gestation age $<$34 weeks (given concerns for altered biomarker production), and prior participation in this protocol. The protocol was approved by the Colorado Multiple Institution Review Board, and informed consent was obtained from the subjects' parents prior to enrollment. The primary aims of the current study were to measure pre- and post-op IFABP levels in an infant cohort undergoing CPB and determine the association between IFABP

levels and early enteral feeding outcomes and development of NEC.

Operative and Post-operative Management

As previously published (32), CPB was performed using a neonatal circuit consisting of a roller head pump (S5, LivaNova, Arvada, CO, USA) and a Terumo FX05 oxygenator with a blood prime. The blood prime routinely underwent pre-bypass hemofiltration using a Minntech Hemocor HPH Junior hemoconcentrator (Medivators Inc., Minneapolis, MN, USA) with a polysulfone membrane prior to initiating bypass, allowing for partial filtration of molecules up to 65,000 Daltons. Anticoagulation was achieved prior to CPB by administering 500 units/kg of heparin systemically to the patient. Initial target flow rate was ∼200 ml/kg/minute. Cardioplegia was accomplished using del Nido formula cardioplegia solution at an initial dose of 30 ml/kg and subsequent dosing was considered after 60 min of aortic cross-clamp time. Per our clinical protocol, all neonates (age <1 month) received high dose methylprednisolone (10 mg/kg) at 10 and 4 h prior to surgery to attempt to reduce post-CPB systemic inflammation.

Sample Collection and Analysis

IFABP levels were obtained pre-operation, immediately prior to withdrawal of CPB, and 6 and 24-h post-operation. Serum concentration of IFABP was analyzed at the University of Colorado, Denver using Meso Scale Discovery (MSD) multiplex immunoassay system, Meso Scale Diagnostics, LLC, Gaithersburg, MD.

Clinical Variables

Pre-operative, intraoperative, and post-operative variables were collected to evaluate for potential risk factors for pre- and post-operative intestinal damage. Pre-operative variables included age, weight, sex, Aristotle score (comprehensive and basic) (33), pre-operative mechanical ventilation, pre-operative inotropic support, pre-operative initiation of enteral feeding, prostaglandin use, single ventricle physiology, and prematurity. Intraoperative and post-operative variables included CPB time, cross-clamp time, deep hypothermic circulatory arrest time, selective cerebral perfusion time, duration of mechanical ventilation, vasoactive inotropic score (VIS) (34, 35) at 6-h post-operative, and clinically obtained peak creatinine and peak lactate levels.

Post-operative NEC

Diagnosis and staging of NEC was made based on the modified Bell's Staging Criteria via retrospective chart review (36). Although the Bell Staging Criteria are designed primarily for NEC associated with prematurity, they remain the primary diagnostic criteria for cardiac-associated NEC (16, 37–39). Only post-operative cases of NEC during the same hospitalization as the operation were included.

Infant Feeding Outcomes

A *post-hoc* data collection from the electronic medical record was utilized to gather nutrition and feeding data. We examined the primary feeding outcome of percent of goal feeds achieved enterally on post-op day 5. Additionally, we examined secondary outcomes of percent of goal feeds achieved on post-op day 3. To

TABLE 1 | Diagnoses and operations performed.

Diagnoses	*n*	%	Operation	*n*	%
Hypoplastic left heart syndrome	20	19.6	Norwood procedure	19	18.6
VSD/aortic arch hypoplasia	13	12.7	VSD repair	14	13.7
Tetralogy of fallot	12	11.8	Tetralogy of fallot repair	12	11.8
Ventricular septal defect	11	10.8	Arterial switch	9	8.8
Atrioventricular septal defect	9	8.8	Aortic arch repair	8	7.8
Double outlet right ventricle	8	7.8	Atrioventricular septal defect repair	6	5.9
PA/VSD	5	4.9	TAPVR repair	5	4.9
TAPVR	5	4.9	Truncus arteriosus repair	5	4.9
Transposition of great arteries	5	4.9	Systemic-pulmonary shunt	4	3.9
Truncus arteriosus	4	3.9	VSD/aortic arch repair	4	3.9
Aortic arch hypoplasia	1	1.0	Double outlet right ventricle repair	3	2.9
Double inlet left ventricle	1	1.0	Glenn, pulmonary arterioplasty	2	2.0
PA/IVS	1	1.0	Mitral valve repair	2	2.0
Other	7	6.9	Right ventricle-pulmonary artery conduit	2	2.0
			Right ventricular outlet tract repair	2	2.0
			Cardiac tumor resection	1	1.0
			Left ventricular assist device	1	1.0
			Reimplantation of LPA from Ao to MPA	1	1.0
			Ross procedure	1	1.0
			Yasui procedure	1	1.0

VSD, ventricular septal defect; PA, pulmonary atresia; TAPVR, total anomalous pulmonary venous return; IVS, intact ventricular septum; LPA, left pulmonary artery; Ao, aorta; MPA, main pulmonary artery.

TABLE 2 | Baseline clinical characteristics (described in full cohort column) and comparison of clinical characteristics between infants with lower (<50th percentile) and higher (>50th percentile) pre-operative IFABP levels.

Baseline characteristics	Full cohort	Pre-operative IFABP level ≤50%	Pre-operative IFABP level >50%	*p*-value ≤50 vs. >50
Pre-operative IFABP level (ng/ml), median (range)	3.9 (0.2, 51.3)	1.9 (0.2, 3.9)	7.8 (4.3, 51.3)	**<0.0001**
Age at surgery, days; median (range)	21.5 (1, 120)	6.0 (1, 119)	55 (2, 120)	**0.0004**
Weight; median (range)	3.6 (2.1, 7.4)	3.3 (2.2, 7.4)	3.8 (2.1, 7.2)	**0.0474**
Birthweight; median (range)	3.1 (1.3, 4.6)	3.1 (2.3, 4.0)	3.0 (1.3, 4.6)	0.1598
Male (%)	57 (55.3%)	31 (60.8%)	25 (50%)	0.2756
Aristotle Score-Comprehensive; median (range)	9.0 (3.0, 19.5)	10 (3.0, 19.5)	9 (3.0, 15.0)	0.3177
Aristotle Score-Basic; Median (range)	9.0 (3.0, 15.0)	9.0 (3.0, 14.5)	8.5 (3.0, 15.0)	0.4033
Pre-operative mechanical ventilation (%)	32 (31.4%)	15 (29.4%)	16 (32%)	0.7780
Pre-operative inotropic support (%)	13 (12.6%)	9 (17.7%)	4 (8%)	0.2343
Single ventricle physiology (%)	29 (28.7%)	16 (32%)	12 (24%)	0.3730
Pre-operative enteral nutrition initiated (%)	95 (92.2%)	44 (86.3%)	50 (100%)	**0.0125**
Preterm (%)	15 (14.7%)	4 (7.8%)	11 (22.5%)	0.0518
Prostaglandins (%)	51 (50%)	35 (68.6%)	15 (30%)	**0.0001**

Bold values represent statistically significant values ($p < 0.05$).

calculate the percent of goal feeds achieved enterally, we took the patient's achieved enteral feeds (in kcals) as recorded in the patient's electronic medical record flowsheet and divided this by the goal kcals as documented daily by the cardiac intensive care unit dieticians. The feeding protocol utilized in our CICU is included as **Supplemental Figure 1.**

Statistical Analysis

Patients' demographics and clinical characteristics are summarized using descriptive statistics. The comparison groups were divided by IFABP level below and equal or above 50th percentile at the given time point. The distributions of continuous variables were inspected before data analysis, all the continuous variables were summarized with median and range (min and max). Wilcoxon rank sum test was conducted to determine the significant difference in distributions between the comparison groups. The categorical data was summarized as count and percentage for the "Yes" category for a giving measurement. To model the association between percentage of goal enteral kilocalories achieved and IFABP, multivariable general linear model was used with pre-selected clinical variables as covariates. Backwards model selection strategy was used, and R-square and type III *P*-values were used to evaluate the most harmonious model. Multivariable logistic regression model was performed to assess the association between NEC and IFABP, demographic and clinical covariates were selected through univariate logistic regression with area under curve (AUC) >0.6, then added to the multivariable model. Akaike information criterion *(*AIC*)* was used to evaluate the best fitting model. *P*-values < 0.05 were considered as statistically significant. All statistical analysis was performed using SAS version 9.4 (SAS Institute, Cary, NC) and all plots were produced with GraphPad Prism version 8.

Data Availability Statement

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

RESULTS

Subjects

The study consecutively enrolled 100 and two subjects, plus one screen failure (converted to non-bypass surgery after enrollment). Of these subjects, one patient did not have a preoperative IFABP level, one patient did not have an IFABP level at rewarming, three patients did not have a 6 h IFABP level, and two patients did not have a 24 h IFABP level due to insufficient sample volume, leaving a total of 101 preoperative and rewarming samples, 99 six hour samples, and 100 twenty-four hour samples (99% overall collection rate). **Table 1** lists the cardiac diagnoses and the operations performed. Clinical characteristics for this cohort are shown in **Table 2**.

IFABP Measurements and Association With Clinical Variables

The distribution of IFABP levels initially rose at rewarming, increased slightly at 6 h, and by 24 h had trended down to near preoperative levels as shown in **Figure 1**. The association between clinical characteristics and IFABP levels pre-operatively are shown in **Table 2**. Pre-operative clinical characteristics found to be significantly associated with higher IFABP include older age, higher weight, prior initiation of pre-operative enteral nutrition, and lack of prostaglandin use. The association of operative and post-operative variables and IFABP levels at 6 h and at 24 h are shown in **Table 3.** The only variable found to be significantly associated with higher IFABP at 6-h post-operative was higher vasopressor-inotropic score (VIS). At 24 h, single ventricle physiology, longer CPB time, longer duration of mechanical ventilation, higher VIS, and higher peak creatinine were all found to be significantly associated with higher IFABP.

Post-operative NEC and Association With Clinical Variables

The median number of days from surgery to NEC onset was 10 days (IQR of 4–14.5; range 1–52). The association between clinical characteristics and post-operative NEC are

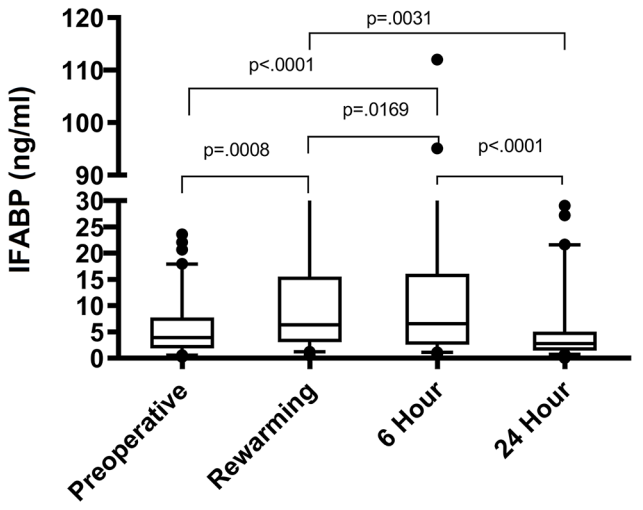

FIGURE 1 | IFABP levels (ng/ml) shown over time at 4 different time points: pre-operative, rewarming, 6 h post-operative, and 24 h post-operative.

shown in **Table 4**. Clinical characteristics found to be significantly associated with post-operative NEC include lower weight, longer deep hypothermic circulatory arrest time, longer duration of mechanical ventilation, higher VIS, higher peak lactate, and higher peak creatinine.

Early Enteral Feeding and Association With IFABP

Infants steadily progressed in terms of amount of enteral feeds achieved over time, with mean percent of goals kcals achieved by enteral nutrition advancing from 26% on post-op day 1, to 48% on post op day 3, to 71% on post-op day 5, to 82% on post-op day 7. On multivariable analysis, IFABP was not associated with percentage of goal enteral kcal achieved. Rather, selective cerebral perfusion time, higher Aristotle score, longer intubation time, and the need for pre-operative intubation were all independently associated with lower percentage of goal Kcals given enterally on post-op day 5, as shown in **Table 5.** Higher Aristotle score, longer length of intubation, need for pre-operative intubation, lower weight, and higher VIS at 24 h were independently associated with a lower percentage of goal Kcals given enterally on post-operative day 3 (**Table 5**).

Post-operative NEC and Association With IFABP

Of our patients, by modified Bell Staging Criteria, 75.5% ($n = 77$) were categorized as Stage 0, 15.7% ($n = 16$) were categorized as Stage 1 (suspected NEC), and 8.8% ($n = 9$) were categorized as Stage 2 (definite NEC). We did not have any patients categorized as Stage 3 (advanced NEC). None of the patients in our study had previous clinical NEC, though prior subclinical intestinal stress cannot be completely ruled out by this study design**.** A comparison of the distribution of IFABP levels at 6 and 24 h demonstrates a significantly higher distribution of IFABP levels at both time points in patients who subsequently developed suspected or definite NEC vs. patients who did not develop post-operative NEC (**Figure 2).** On multivariable analysis, IFABP and peak creatinine were independently associated with development of suspected or definite NEC, as shown in **Table 6.** Of note, both age and weight were initially included in our multivariable model for predicting IFABP, but they were removed in backwards selection, as they did not demonstrate a significant association with NEC when controlling for other independent variables in the model. While not significantly independently associated with development of suspected or definite NEC, VIS at 24 h improved the overall fit of the model and was therefore included.

DISCUSSION

Key Findings

In this study, we present the first evaluation of IFABP levels following cardiothoracic surgery with CPB in a specifically infant population. Additionally, we present the associations between clinical factors and pre-operative and post-operative IFABP levels. Counter to our initial hypothesis, immediate post-operative IFABP levels were not found to be associated with the percentage of goal kcals achieved enterally on post-operative day 3 or 5; rather, clinician-observable factors indicating illness severity were associated with achieved enteral kcals. We present the novel finding of an independent association in infants undergoing CPB between early enterocyte injury, as identified by circulating IFABP levels, and subsequent post-operative NEC.

Pattern of IFABP Rise and Fall After CPB

We show that IFABP levels rise immediately after CPB, continue to rise at 6 h, and then return back to pre-operative levels by 24 h after surgery. A physiologic understanding of IFABP offers an explanation for this pattern of early rise and rapid fall. IFABP is located predominantly in the mature enterocytes in the villi as opposed to younger enterocytes in the crypts (20). These mature enterocytes are at the distal extreme of mucosal blood flow and therefore susceptible to early ischemic damage in the setting of compromised intestinal blood flow, consequently leading to a spike in systemic IFABP (20). IFABP is eliminated renally (40), resulting in a downtrend to pre-operative levels once adequate intestinal perfusion is restored. Prior studies in mixed-age pediatric cardiac surgery cohorts demonstrated a similar pattern of rise and fall in IFABP with comparable magnitudes of IFABP elevation (3, 26). Adult cardiac surgery cohorts have also shown this pattern of rise and fall in IFABP, but with smaller magnitudes of IFABP elevation (27–30, 41). This discrepancy in magnitude of IFABP elevation would be potentially explained by the differences in GI complication rates in pediatric patients vs. adult patients after cardiac surgery, with pediatric rates of just NEC ranging from 3.3 to 13% (42) and adult rates of any GI complication ranging from 0.3 to 2% (43). The early rise in IFABP is particularly notable, as early rise in IFABP and the associated intestinal barrier injury has been associated with a post-bypass inflammatory cascade (26). Normal IFABP levels are not well-defined in the infant population, and future studies would ideally include age-matched controls.

TABLE 3 | Comparison of baseline, intra-operative, and post-operative characteristics between infants with lower (<50th percentile) and higher (>50th percentile) post-operative IFABP levels at 6 and 24 h.

Baseline, intraoperative, and post-operative characteristics	IFABP level 6 h ≤50%	IFABP level 6 h >50%	*p*-value ≤50 vs. >50	IFABP level 24 h ≤50%	IFABP level 24 h >50%	*p*-value ≤50 vs. >50
Pre-operative IFABP level (ng/ml); median (range)	2.7 (0.2, 16.1)	5.1 (0.6, 51.3)	**0.0261**	3.2 (0.4, 11.5)	5.1 (0.2, 51.3)	**0.0245**
Age at surgery, days; median (range)	14.0 (2, 119)	22 (1, 120)	0.5812	19.5 (2.0, 120.0)	28 (1, 115)	0.1975
Weight; median (range)	3.6 (2.2, 6.0)	3.5 (2.1, 7.4)	0.3542	3.6 (2.2, 7.4)	3.6 (2.1, 6.4)	0.5409
Birthweight; median (range)	3.2 (1.9, 4.3)	2.9 (1.3, 4.6)	0.1294	3.0 (1.7, 4.0)	3.1 (1.3, 4.6)	0.2061
Male (%)	28 (56%)	29 (59%)	0.7486	26 (52%)	29 (58%)	0.5465
Preterm (%)	5 (10%)	10 (20.8%)	0.1670	7 (14%)	8 (16.3%)	0.7469
Aristotle Score-Comprehensive; median (range)	10 (3,16)	9(3, 19.5)	0.4859	9 (3,16)	10 (3, 19.5)	0.3520
Aristotle Score-Basic; Median (range)	9 (3, 14.5)	8 (3,15)	0.5954	9 (3, 14.5)	9 (3, 15)	0.2234
Single ventricle physiology (%)	12 (24%)	17 (35%)	0.2158	8 (16%)	20 (40.8%)	**0.0061**
Pre-operative enteral nutrition initiated (%)	46 (92%)	46 (94%)	1.0000	47 (94%)	46 (92%)	0.6951
CPB time, minutes; median (range)	123.5 (54, 399)	131.0 (55, 372)	0.4726	119 (54, 399)	147.5 (55, 298)	**0.0259**
Cross-clamp time, minutes; median (range)	71 (0, 205)	69 (0, 241)	0.8642	66.5 (0, 241)	75 (0, 205)	0.2637
Deep hypothermic circulatory arrest, minutes; median (range)	0 (0, 77)	0 (0, 59)	0.8675	0 (0, 77)	0 (0, 76)	0.9145
Selective cerebral perfusion, minutes; median (range)	0 (0, 82)	0 (0, 115)	0.2516	0 (0, 82)	0 (0, 115)	0.0736
Duration of mechanical ventilation, hours; median (range)	32.5 (0.1, 213.7)	45.3 (0.5, 762.6)	0.1812	26.6 (0.1, 762.6)	51.7 (7.5, 237.8)	**0.0116**
VIS at 6h; median (range)	8 (2.5, 17)	10 (0, 27)	**0.0326**	5 (0, 18)	8.8 (0, 30)	**0.0055**
Lactate peak; median (range)	3.2 (1.1, 11.4)	3.4 (0.9, 12.6)	0.6806	3.2 (0.9, 12.6)	3.6 (1.1, 11.4)	0.2003
Peak Creatinine; median (range)	0.5 (1.3, 1.7)	0.5 (0.3, 1.9)	0.8972	0.5 (0.3, 1.1)	0.5 (0.3, 1.9)	**0.0347**
IL-6 at 6 h; median (range)	48.6 (10.2, 524.8)	57.0 (1.3, 296.9)	0.6327	43.5 (11.0, 351.0)	60.7 (12.3, 908.7)	0.1040
TNFα at 6 h; median (range)	7.6 (2.7, 24.7)	7.4 (3.1, 72.3)	0.8587	6.5 (3.4, 11.6)	6.8 (2.7, 29.2)	0.6183

Bold values represent statistically significant values ($p < 0.05$).

Clinical Factors Associated With Higher Pre-operative IFABP

Higher pre-operative IFABP levels were associated with increased patient age, increased patient weight, prior initiation of pre-operative nutrition, and no prior prostaglandin use. The association of IFABP with age is both a pronounced and an interesting finding, as previously Pathan et al. (3) found no association between age or weight and IFABP levels. A potential explanation for this discrepancy is that during gestation and early infancy, IFABP levels rise as gut mass increases and IFABP accumulates. Pathan et al. (3) examined an older population (median age was 11.2 months as opposed to our median of 21.5 days) and more heterogeneous age range, and therefore potentially did not capture the initial rise in IFABP during infancy. In support of this explanation, Guthman et al. (44) has demonstrated an association between birth weight and IFABP in premature infants <33 weeks gestation age, and our data suggests that this trend may continue during early infancy as well. Examining normal IFABP levels during the infancy period would be helpful to further clarify the natural trajectory of IFABP. Likely the finding of pre-operative nutrition being associated with higher IFABP levels is collinear with the age and weight, as older patients are more likely to already have nutrition started. Another interesting finding was that prostaglandin use was associated with lower levels of IFABP, which is in contrast to a previous finding in Pathan et al. (3), who demonstrated increased IFABP in patients with ductal dependent lesions. Again, this discrepancy is most likely explained by the difference in populations: In our younger population, patients requiring prostaglandins were younger than the rest of our population and therefore the effect of their age on IFABP likely outweighed the effect of increased disease severity and decreased gut perfusion due to their ductal dependent lesions. Similarly, other indications of pre-operative disease severity (pre-operative mechanical ventilation, pre-operative inotropic support, single ventricle physiology) were also likely outweighed by the effect of age, as the more severe patients were also likely to go for their surgery at a younger age. Knowing which factors are associated with higher pre-operative IFABP is helpful in further understanding IFABP and how to properly interpret its levels.

TABLE 4 | Comparison of baseline, intra-operative, and post-operative characteristics between infants without NEC or with NEC as defined by bell stage 1 or Stage 2.

Baseline, intraoperative, and post-operative characteristics	No NEC ($n = 75$)	NEC (Bell stage 1 or Stage 2) ($n = 25$)	*p*-value
Pre-operative IFABP level (ng/ml); median (range)	4.3 (0.2, 22.1)	3.4 (0.5, 51.3)	0.3844
Age at surgery, days; median (range)	36 (1, 120)	9 (2, 115)	0.1178
Weight; median (range)	3.7 (2.1, 7.4)	3.2 (2.3, 4.7)	**0.0040**
Birthweight; median (range)	3.2 (1.9, 4.6)	2.9 (1.3, 3.9)	0.1327
Male (%)	38 (50.7%)	18 (72%)	0.0627
Preterm (%)	9 (12%)	5 (20.8%)	0.3176
Aristotle score-comprehensive; median (range)	9 (3,16)	10 (3, 19.5)	0.4295
Aristotle score-basic; median (range)	9 (3, 14.5)	9 (3,15)	0.3370
Single ventricle physiology (%)	19 (25.3)	11 (45.8%)	0.0572
Pre-operative enteral nutrition initiated (%)	72 (96%)	22 (88%)	0.1447
CPB time, minutes; median (range)	122 (55, 399)	131 (54, 277)	0.6595
Cross-clamp time, minutes; median (range)	69 (0, 241)	72 (0, 136)	0.7298
Deep hypothermic circulatory arrest, minutes; median (range)	0 (0, 77)	4 (0, 76)	**0.0457**
Selective cerebral perfusion, minutes; median (range)	0 (0, 115)	0 (0, 65)	0.2430
Duration of mechanical ventilation, hours; median (range)	27.8(0.1, 726.6)	82.6 (12.4, 237.8)	**0.0005**
VIS at 6 h; median (range)	8 (0, 25)	12.5 (2.5, 27)	**0.0083**
Lactate peak; median (range)	3.1 (0.9, 10.8)	5.0 (1.1, 12.6)	**0.0115**
Peak CREATININE; median (range)	0.5 (0.3, 1.1)	0.6 (0.4, 1.9)	**0.0011**
IL-6 at 6 h; median (range)	50.2 (1.3, 524.8)	61.1 (13, 296.9)	0.8819
TNFα at 6 h; median (range)	7.6 (2.7, 24.7)	7.5 (3.1, 72.3)	0.8096

Bold values represent statistically significant values ($p < 0.05$).

TABLE 5 | Multivariable model for predicting percent goal kcals achieved enterally.

Independent variable	Goal kcals achieved enterally on POD5			Goal kcals achieved enterally on POD3		
	% Decrease per unit increase in independent variable	Standard error	*p*-value	% Decrease per unit increase in independent variable	Standard error	*p*-value
Aristotle score	3.2	1	0.002	3	1.0	0.002
Selective cerebral perfusion (min)	0.4	0.1	0.01	–	–	–
Pre-operative intubation (yes)	14.1	6.1	0.02	15.9	6.7	0.02
Intubation time (hours)	0.1	0	0.0004	0.1	0.0	0.0007
VIS at 6 h	1.2	0.7	0.09	–	–	–
VIS at 24 h	–	–	–	1.8	0.6	0.006
Weight (kg)	–	–	–	–9.4	3.4	0.008

Clinical Factors Associated With Higher Post-operative IFABP

Higher post-operative IFABP levels were associated with longer CPB time, higher VIS, higher peak creatinine, single ventricle physiology, and duration of mechanical ventilation. Increased VIS has been prospectively shown to be associated with longer length of intubation, ICU stay, and hospital stay (35), and its correlation with higher IFABP could be due to its association with disease severity. Additionally, the physiologic state driving high VIS score (hypotension, decreased cardiac output, poor perfusion) would also likely lead to poor mesenteric blood flow, which would then be expected to cause more enterocyte injury and higher IFABP levels. A direct effect of vasoactive inotropes on mesenteric blood flow could be contributing as well, as there is evidence that vasopressin and epinephrine decrease mesenteric blood flow in both humans and (more extensively) in animal models of critical illness (45–50). Creatinine reflects perfusion of another abdominal organ, and so a physiologic state of hypoperfusion would intuitively affect both similarly. Additionally, since IFABP is renally cleared, acute kidney injury would be expected to potentiate elevated serum IFABP levels (40). Patient with single ventricle physiology have been shown to have low postprandial blood flow velocities (51), and therefore it is not surprising that higher levels of IFABP are seen in these patients. Longer CPB time's association with higher IFABP levels could potentially be due to insufficient flow during CPB, the continuous nature of flow during CPB, or it could be another surrogate for disease severity. This association has previously been documented in in adult and pediatric populations undergoing CPB (26, 41). Finally, duration of mechanical ventilation would

indicate worsening disease severity and also lead to increased central pressures, venous congestion, and subsequent intestinal edema and injury. Alternatively, the intestinal injury and subsequent endotoxin leak could cause direct lung injury and cause longer duration of mechanical ventilation (52, 53). Our data confirm the previous finding in Typpo et al. (26) that CPB time and vasopressor use are associated with higher post-operative IFABP, but the remainder of the associations are novel in a pediatric population. It is of further note that none of our clinical factors found to be significantly associated with pre-operative IFABP were significantly associated with post-operative IFABP. This difference indicates that the previously discussed operative and post-operative variables outweigh preoperative variables and exemplifies the high degree of stress that cardiac surgery with CPB places on the intestine. Knowing which clinical factors are associated with higher IFABP levels can potentially help predict which infants are at higher risk for intestinal damage and its clinical sequelae.

IFABP and Early Enteral Feeding Practices

We did not find a significant association between IFABP levels and early enteral feeding initiation/advancement, as measured by percentage of goal enteral kcal achieved by post-operative days 3 or 5. Our lack of a significant association is in contrast to the study by Typpo et al. (26) which does show an association with clinical feeding outcomes, though interestingly demonstrating that lower IFABP levels were associated with more feeding dysfunction. This discrepancy could potentially be explained by the fact that younger infants based on our data would have lower levels of IFABP based purely on age and decreased intestinal mass compared to older pediatric patients, confounding the association with feeding dysfunction.

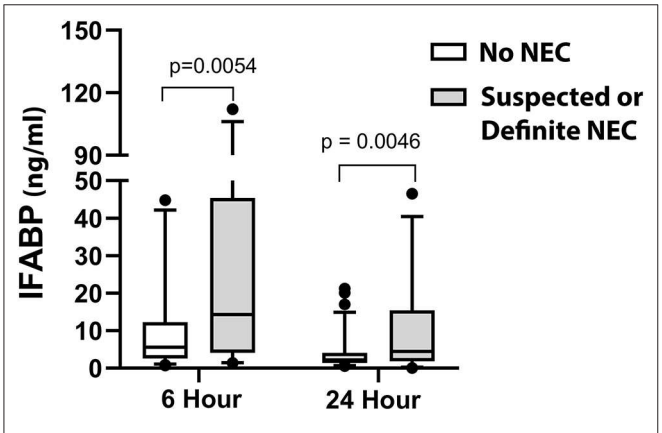

FIGURE 2 | Comparison of early IFABP levels (6 and 24 h) in patients without NEC vs. with suspected or definite NEC (Modified Bell Criteria scores 1 or 2).

We did show that higher Aristotle score, longer selective cerebral perfusion time, the need for pre-operative intubation, longer post-operative intubation time, and higher VIS were all associated with slower enteral feeding advances. A prior study by Alten et al., using the Pediatric Cardiac Critical Care Consortium registry to examine perioperative feeding management showed a wide variety of clinical practice surrounding feeding advancements (8), highlighting the uncertainty of perioperative feeding. The authors demonstrated a delay in initiation of post-operative feeds in hypoplastic left heart syndrome patients, who typically require longer duration of mechanical ventilation and vasoactive medications, although only univariate analysis was performed (8). Our findings on multivariable analysis are consistent with Alten's study, identifying independent associations between both surgical complexity (Aristotle scores) and post-operative clinical illness (mechanical ventilation and VIS) and delayed provision of post-operative enteral nutrition.

In both our study and the study by Alten et al., the variables found to be associated with enteral feeding initiation/advancement were known to the clinicians making the decisions regarding the speed of enteral feed advances. This finding is not surprising, as decisions to initiate and advance enteral nutrition in the early post-operative period generally hinge on observable physiologic markers and clinically visible risk factors. Whether an infant will tolerate advancing nutrition is more difficult to assess, as evidenced by the findings of Lannucci et al., who elegantly demonstrated similar clinical characteristics among infants who were initiated on post-operative enteral nutrition and did or did not subsequently proceed to develop NEC (38). Therefore development of novel biomarkers could be useful as a supplement to clinical risk factors in the assessment of readiness to feed and tolerance of enteral nutrition, particularly with the current movement toward early enteral feeding after congenital heart surgery and protocolized feeding advances (54, 55). Ideally, candidate biomarkers would provide an early signal that could be detected prior to initiation of post-operative enteral nutrition and be predictive of clinically significant feeding intolerance, especially development of NEC.

IFABP and Post-operative NEC

In this study, we demonstrate for the first time in CHD patients an independent association between early IFABP levels and

TABLE 6 | Multivariable model for predicting suspected or definite NEC (modified bell criteria scores 1 or 2).

Independent variable	Percent increase in odds of NEC per unit increase in independent variable (point estimate)	95% wald confidence intervals	*p*-value
IFABP (6 h)	4% (1.04)	1.0, 1.1	0.0015
VIS (24 h)	9% (1.09)	1.0, 1.2	0.06
Creatinine (peak)	1500% (15.0)	1.4, 162.0	0.03

subsequent development of suspected or definite post-operative NEC. This association is consistent with prior studies in the premature neonate population (23–25, 56). Specifically, our data suggest that infants with immediate post-operative enterocyte injury, as evidenced by higher circulating IFABP at 6 h post-operatively, are at increased risk of subsequent development of suspected or definite NEC. The early appearance of IFABP in the blood (prior to the time enteral nutrition would be initiated in our post-operative infants) increases its potential value for risk stratification for timing of enteral nutrition initiation as well as speed of enteral nutrition advancement. The mechanism linking early enterocyte injury with subsequent development of NEC remains unclear. It is possible that early enterocyte injury is simply a marker for patients with an underlying predisposition for recurrent injury based on their cardiovascular or intestinal physiology, but who then have an intervening period of healing. Alternatively, it is possible that the early enterocyte injury results in an ongoing local process that does not lead to the persistent presence of circulating IFABP but remains incompletely healed. Translational animal modeling would be helpful in differentiating these two potential mechanisms. Finally, while our study was not designed to evaluate the longitudinal use of IFABP in early detection of NEC, there is growing body of evidence supporting IFABP use for this purpose in premature infants (23–25, 57), making this a promising and worthwhile target for future investigations in the CHD population.

Limitations

Our study has several limitations. The subjects were recruited from a single institution, limiting generalizability to other centers. Additionally, while limited to infants, this study incorporated a range of congenital defects and subsequently a range of clinical severity. A large multi-institutional study would better be able to account for variances in disease process and clinical severity. The study was designed to only look at pre- and immediate post-operative IFABP, and did not look at ongoing IFABP levels, which would help better establish the temporal relationship between serum IFABP and NEC, as well as its potential utility as a point-of-care biomarker. Finally, the modified Bell Staging Criteria for NEC is imperfect in its application in the cardiac population, as it was designed primarily for the neonatal population. In particular, it is very challenging to differentiate post-operative ileus from NEC, as there is significant overlap between the radiographic and gastrointestinal findings in these two disease processes.

CONCLUSION

IFABP levels rise initially following surgery with CPB in infants with CHD, then begin to fall by 24 h. IFABP levels were not associated with the clinical feeding outcome of percent of goal kcals achieved enterally on post-operative day 5. Instead, the primary predictors of clinical feeding outcomes were clinically observable factors. IFABP at 6 h was independently associated with subsequent development of suspected or definite NEC and may be useful for identifying patients at risk for development of post-operative NEC.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Colorado Multiple Institution Review Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

JW, PW, and JD conceived the design of the study. TU and JD performed patient recruitment. JW and TU performed data extraction. LK and JD performed IFABP analysis. JW, ST, JZ, and JD performed the data analysis and interpretation. JW and JD wrote the primary manuscript. All authors were responsible for reviewing and approving the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2020.00267/full#supplementary-material

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Child Excess Weight Status, Adult Excess Weight Status, and Cardiometabolic Risk Profile

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Background: The potential effects of excess weight status in childhood on later adult cardiometabolic risk factors have been undetermined in a Chinese population. Additionally, the potential mitigation of these effects if adult weight status returns to normalcy has been unresolved. Accordingly, we aimed to assess the association of childhood excess weight status and its long-term change with adult cardiometabolic risk factors.

Methods: A cohort study from the China Health and Nutrition Survey 1991–2009 consisted of 541 participants who were measured in childhood (≥6 and <18 years) and underwent laboratory assessment in adulthood (≥18 years). In childhood, the participants were classified into four groups as age-sex-specific body mass index (BMI) z-score quartiles. The adult cardiometabolic risk factors included overweight and obesity, hypertension, high total cholesterol, high triglyceride, low high-density lipoprotein cholesterol, high low-density lipoprotein cholesterol, and high hemoglobin A1c.

Results: The prevalence was 61.0, 36.2, and 19.0% for ≥1, 2, and 3 cardiometabolic risk factors, respectively, with a mean 14.9-year follow-up. There was a significant trend in the progression of the number of adult cardiometabolic risk factors across childhood BMI quartiles ($P < 0.001$). Additionally, participants with childhood BMI z-scores ≥ 75th percentile and adult BMI z-scores < 75th percentile did not have increased cardiometabolic risks compared with those with both childhood and adulthood BMI z-scores < 75th percentile.

Conclusions: Our findings revealed that child excess weight status increased adult cardiometabolic risks. However, the effects of excess weight status in childhood on adult cardiometabolic risk factors were mitigated if adult weight status returned to normalcy.

Keywords: weight status, cardiometabolic risk factors, childhood, adulthood, cohort

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INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of mortality across the globe (1). Cardiometabolic risk factors increase the risk of CVD, which results in a future burden of CVD (2). Controlling the epidemic of cardiometabolic risk factors has proven to be one effective strategy for the prevention of CVD.

Cardiometabolic risk factors tend to cluster and include excess weight status, high blood pressure, dyslipidemia, and hyperglycemia (3–5). Excess weight status causes inflammation and insulin resistance, which play a key role in the onset and clustering of cardiometabolic risk factors (6). Excess weight status is the easiest cardiometabolic risk factor to identify, and often is attained in early life (3–6).

The worldwide prevalence of childhood excess weight status has increased at an alarming rate in recent years (7). Observational studies have shown that children with excess weight status have an increased risk of cardiometabolic risk factors in adulthood (8, 9). Furthermore, evidence regarding the impact of the change in weight status from childhood to adulthood on health consequences has revealed that the adverse health consequences can be reversed if children with excess weight status attain normal weight as adults (10–13). However, few similar studies have been conducted in China. As industrialization and urbanization have accelerated and the population has aged, CVD has become a major public health challenge in China. Reliable information is essential for the development of national health policies for the prevention and control of CVD (14). Consequently, we aimed to assess the association of childhood weight status and its long-term change with cardiometabolic risk factors in early adulthood based on the China Health and Nutrition Survey (CHNS).

MATERIALS AND METHODS

Study Population

Launched in 1989, the CHNS is an ongoing, open, and population-based longitudinal cohort study that is designed to examine health and nutrition status in the Chinese population (15). The survey sample was drawn with the use of a stratified multistage cluster method. As part of the CHNS, a follow-up survey is conducted every 2–4 years. Participants are asked to complete an interview questionnaire and a physical examination at each survey instance (15). Notably, fasting blood was collected for the first time in the 2009 survey (16, 17). The survey procedures used for the CHNS are described in detail elsewhere (15). This study was approved by the Institutional Review Board at the University of North Carolina at Chapel Hill, Institute of Nutrition and Food Safety, China Centers for Disease Control, and the China-Japan Friendship Hospital, Ministry of Health and China. All participants or their guardians provided written informed consent.

We established a cohort study from childhood (≥6 and <18 years) to adulthood (≥18 years) based on the CHNS. To ensure adequate follow-up length, individuals with a full record of key information (sex, age, blood pressure, weight, and height) on the first measurement during childhood, collected before the 2009 survey, were included in the present study. The follow-up survey was conducted among adult participants in the 2009 survey. In total, 660 participants were eligible. However, 119 participants did not undergo laboratory assessment in 2009. As a result, the cohort consisted of 541 individuals with a full record of key information during childhood before 2009, and had a full record of key information and had undergone laboratory assessment during adulthood in 2009.

Abbreviations: BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides.

General Examinations and Laboratory Assessment

Sex, age, and adult risk factors (smoking and alcohol consumption) were collected by self-administered questionnaire. Weight and height were measured by trained workers, respectively. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Blood pressure (BP) was measured using certified mercury sphygmomanometers. Three consecutive BP measurements were obtained, and the average of the latter two measurements was used for further analyses.

Fasting blood was collected in the 2009 survey. The methods of blood sample collection and preservation, the measurement procedures, the measurement equipment, and the test method are described in detail in several previous publications (16, 17). Total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and hemoglobin A1c (HbA1c) were measured.

Definitions

BMI was used to evaluate childhood weight status (18). Childhood overweight and obesity were defined as BMI ≥ corresponding sex- and age-specific overweight cutoffs presented in the national reference for Chinese children (19). Adulthood overweight and obesity were defined as BMI ≥ 24 kg/m^2 (20). Childhood elevated BP was defined as BP ≥ 90th percentile for sex, age, and height or 120/80 mm Hg as per the BP reference for Chinese children (21, 22). Adulthood hypertension was defined as BP ≥ 140/90 mm Hg or taking anti-hypertension medications according to the 2018 Chinese Guidelines for Prevention and Treatment of Hypertension (21). Adulthood high TC was defined as TC ≥ 200 mg/dL, high TG as ≥150 mg/dL, high LDL-C as ≥130 mg/dL, and low HDL-C as <40 mg/dL in terms of the 2016 Chinese Guidelines for the Prevention and Treatment of Dyslipidemia in adults (23). High HbA1c was defined as ≥5.6% (16). The number of individual cardiovascular risk factors (overweight and obesity, hypertension, high TC, high TG, high LDL-C, low HDL-C, and high HbA1c) in adulthood was calculated to assess the cardiovascular risk profile.

Statistical Analysis

After adjusting for sex and age by regression residual analyses, childhood BMI was standardized using the Z-transformation (mean = 0, SD = 1). The participants were categorized into four groups as quartiles of childhood BMI z-scores. The data between groups were presented as means (SDs), medians (interquartile ranges), or frequencies (%) as appropriate. The differences between groups were tested using either analysis

of variance, the chi-square test, Fish's exact probabilities, or the nonparametric test. Poisson models with robust standard errors were used to calculate relative risks (RRs) and 95% confidence intervals (CIs) with adjustment for covariates, and to investigate the relation of childhood weight status to cardiovascular risk factors in adulthood (24). The trend across the quartiles was tested using quartiles as a continuous ordinal variable.

TABLE 1 | Characteristics of the participants by childhood BMI quartiles.

	All participants (n = 541)	Childhood BMI quartiles				
		Q1 (n = 135)	Q2 (n = 136)	Q3 (n = 135)	Q4 (n = 135)	*P*
Male (%)	71.9	71.9	72.1	71.9	71.9	0.999
Childhood						
Age (years)	11.4 (3.2)	12.0 (2.9)	10.8 (2.9)	11.1 (3.6)	11.7 (3.4)	0.011
BMI (kg/m^2)	17.1 (2.7)	15.0 (1.5)	16.0 (1.4)	17.2 (1.7)	20.3 (2.7)	<0.001
Overweight and obesity (%)	9.1	0.0	0.0	0.0	36.3	<0.001
SBP (mm Hg)	95.6 (13.2)	94.6 (12.2)	93.8 (11.7)	94.6 (12.6)	99.4 (15.3)	0.001
DBP (mm Hg)	62.4 (10.1)	61.1 (9.4)	60.4 (9.2)	62.6 (9.4)	65.3 (11.6)	<0.001
Elevated BP (%)	17.9	13.3	14.7	17.0	26.7	0.019
Adulthood						
Age (years)	26.2 (5.0)	26.7 (4.9)	26.0 (4.6)	26.1 (5.0)	26.3 (5.4)	0.696
BMI (kg/m^2)	21.9 (3.4)	19.9 (2.6)	21.6 (3.0)	22.5 (2.9)	23.7 (3.6)	<0.001
Overweight and obesity (%)	24.0	5.9	15.4	30.4	44.4	<0.001
SBP (mm Hg)	113.5 (11.7)	110.0 (11.1)	113.0 (11.1)	114.6 (12.2)	116.3 (11.4)	<0.001
DBP (mm Hg)	75.1 (9.3)	71.8 (8.4)	74.6 (9.0)	75.9 (8.8)	77.9 (9.9)	<0.001
Hypertension (%)	7.8	4.4	6.6	9.6	10.4	0.234
Smoking (%)						0.269
Never	60.3	60.7	59.6	54.1	66.7	
Former	2.2	1.5	1.5	2.2	3.7	
Current	37.5	37.8	39.0	43.7	29.6	
Drinking (%)						0.814
Never	52.7	48.9	52.9	53.3	55.6	
Almost every day	4.1	3.0	5.9	5.2	2.2	
3–4 times/week	4.8	5.2	7.4	3.7	3.0	
1–2 times/week	11.8	11.1	9.6	12.6	14.1	
1–2 times/month	15.3	17.8	14.0	14.8	14.8	
<1 time/month	11.3	14.1	10.3	10.4	10.4	
TC (mg/dl)	170.2 (36.4)	167.6 (34.1)	173.1 (38.2)	171.2 (37.6)	168.8 (35.8)	0.600
High TC (%)	16.8	14.8	18.4	14.8	19.3	0.662
TG (mg/dl)	99.2 (66.4–147.9)	92.1 (66.4–127.6)	99.2 (63.8–139.3)	103.6 (68.2–172.7)	106.3 (67.3–155.9)	0.180
High TG (%)	23.7	17.8	18.4	32.6	25.9	0.012
LDL-C (mg/dl)	101.3 (33.4)	96.6 (29.6)	103.9 (37.0)	103.0 (32.8)	101.5 (33.7)	0.279
High LDL-C (%)	15.7	11.9	17.6	14.8	18.5	0.425
HDL-C (mg/dl)	53.0 (13.2)	55.1 (12.0)	55.0 (14.4)	51.8 (13.7)	50.2 (12.1)	0.003
Low HDL-C (%)	14.8	9.6	13.2	18.5	17.8	0.135
HbA1c (%)	5.3 (0.6)	5.2 (0.6)	5.2 (0.4)	5.3 (0.4)	5.4 (0.7)	0.008
High HbA1c (%)	25.7	20.0	22.8	28.9	31.1	0.128
≥1 cardiovascular risk factors (%)	61.0	44.4	56.6	68.9	74.1	<0.001
≥2 cardiovascular risk factors (%)	36.2	22.2	31.6	43.7	47.4	<0.001
≥3 cardiovascular risk factors (%)	19.0	10.4	16.2	23.7	25.9	0.004

BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; HbA1c, hemoglobin A1c.

Data are presented as means (SDs), medians (interquartile ranges), or frequencies (%) as appropriate.

Differences between continuous variables were compared using the analysis of variance or nonparametric test.

Differences between categorical variables were compared using the χ^2 test or Fish's exact probabilities.

Individuals were classified into 4 groups based on combinations of childhood and adulthood weight status: childhood BMI z-scores < 75th percentile and adult BMI z-scores < 75th percentile (Group 1), childhood BMI z-scores < 75th percentile and adult BMI z-scores ≥ 75th percentile (Group 2), childhood BMI z-scores ≥ 75th percentile and adult BMI z-scores < 75th percentile (Group 3), childhood BMI z-scores ≥ 75th percentile and adult BMI z-scores ≥ 75th percentile (Group 4). Differences in the number of cardiovascular risk factors between groups were tested using the chi-square test. Covariate-adjusted Poisson models were used to assess the association between weight status change from childhood to adulthood and the number of cardiovascular risk factors in adulthood.

We used SAS 9.4 (SAS Institute Inc., Cary, NC, USA) to conduct the analyses and considered a two-tailed $P < 0.05$ to be statistically significant.

RESULTS

The present study included 541 participants (males, 71.9%) from the CHNS 1991–2009. The participants' age ranged from 6 to 17 years (mean age, 11.4 years) in childhood and from 18 to 35 years (mean age, 26.2 years) in adulthood. The mean follow-up duration was 14.9 years (median, 16.0 years; range, 3–19 years).

Table 1 summarizes the characteristics of all participants. The prevalence of overweight and obesity in childhood and adulthood was 9.1 and 24.0%, respectively. Adult hypertension, high TC, high TG, high LDL-C, low HDL-C, and high HbA1c prevalences were 7.8, 16.8, 23.7, 15.7, 14.8, and 25.7%, respectively. 61.0, 36.2, and 19.0% of all individuals had ≥1, ≥2, and ≥3 cardiovascular risk factors in adulthood, respectively. Additionally, compared with individuals in the first quartile of childhood BMI, those in the fourth quartile tended to have more cardiovascular risk factors in adulthood.

Table 2 presents the association between childhood BMI and adult cardiometabolic risk profile. Participants in the fourth quartile of childhood BMI had an increased risk of cardiovascular risk factors in comparison with those in the first quartile after adjusting for sex, childhood age, and elevated BP (Model 1). RR did not vary significantly after adjusting further for the follow-up duration, adult smoking, and drinking (Model 2). There was a significant trend in the progression of the number of adult cardiometabolic risk factors across the quartiles of childhood BMI in the fully adjusted model (*P* for trend <0.001).

Figure 1 describes the prevalence of cardiometabolic risk factors among the four groups as defined in the combinations of childhood and adulthood weight status. Interestingly, the difference in the prevalence of cardiometabolic risk factors between the participants with both child and adult BMI z-scores < 75th percentile and those with child BMI z-scores ≥ 75th percentile and adult BMI z-scores < 75th percentile was not significant ($P > 0.05$).

Table 3 shows the impact of weight status change from childhood to early adulthood on cardiometabolic risk factors. In comparison with the participants who had both child and adult BMI z-scores < 75th percentile, those with child BMI z-scores ≥ 75th percentile and adult BMI z-scores < 75th percentile had no increased risk of adult cardiometabolic risk factors (all $P_s > 0.05$).

We excluded 134 participants with child elevated BP or overweight (including obesity) to perform a sensitivity analysis. The sensitivity analysis results were similar (**Tables S1**, **S2**). In addition, we used the national reference for Chinese children and Asia adult reference (BMI ≥ 24 kg/m^2) to define child and adult overweight (including obesity), respectively (19, 20). We performed a sensitivity analysis and obtained similar results (**Table S3**).

TABLE 2 | Association of childhood BMI with adult cardiometabolic risk profile.

	Model 1: RR (95%CI)	Model 2: RR (95%CI)
Outcome: ≥1 cardiovascular risk factors		
Continuous childhood BMI	1.15 (1.08–1.23)***	1.16 (1.09–1.24)***
Childhood BMI quartiles		
First quartile	Ref	Ref
Second quartile	1.30 (1.03–1.64)*	1.27 (1.01–1.59)*
Third quartile	1.56 (1.26–1.94)***	1.54 (1.24–1.90)***
Fourth quartile	1.64 (1.33–2.02)***	1.64 (1.33–2.01)***
P for trend	<0.001	<0.001
Outcome: ≥2 cardiovascular risk factors		
Continuous childhood BMI	1.25 (1.13–1.38)***	1.28 (1.16–1.42)***
Childhood BMI quartiles		
First quartile	Ref	Ref
Second quartile	1.43 (0.96–2.12)	1.37 (0.93–2.02)
Third quartile	1.97 (1.38–2.82)***	1.94 (1.35–2.77)***
Fourth quartile	2.11 (1.47–3.02)***	2.11 (1.47–3.01)***
P for trend	<0.001	<0.001
Outcome: ≥3 cardiovascular risk factors		
Continuous childhood BMI	1.32 (1.15–1.51)***	1.38 (1.19–1.61)***
Childhood BMI quartiles		
First quartile	Ref	Ref
Second quartile	1.60 (0.86–2.97)	1.52 (0.82–2.84)
Third quartile	2.30 (1.31–4.04)**	2.24 (1.27–3.98)**
Fourth quartile	2.34 (1.33–4.13)**	2.38 (1.34–4.24)**
P for trend	<0.001	<0.001

BMI, body mass index; CI, confidence interval; RR, relative risk.
Continuous childhood BMI was transformed into age- and sex-specific Z-scores.
Model 1: adjusted for sex, childhood age and elevated blood pressure; Model 2: additionally adjusted for the length of follow-up and adult risk factors (smoking and drinking).
**P<0.05; **P<0.01; ***P<0.001.*

DISCUSSION

We revealed that childhood excess weight status increased adult cardiometabolic risks based on a cohort study with a mean 14.9-year follow-up from the CHNS 1991–2009. Moreover, our results suggested that the effects of excess weight status in childhood on adult cardiometabolic risk factors were mitigated if adult weight status returned to normalcy. Our findings have important

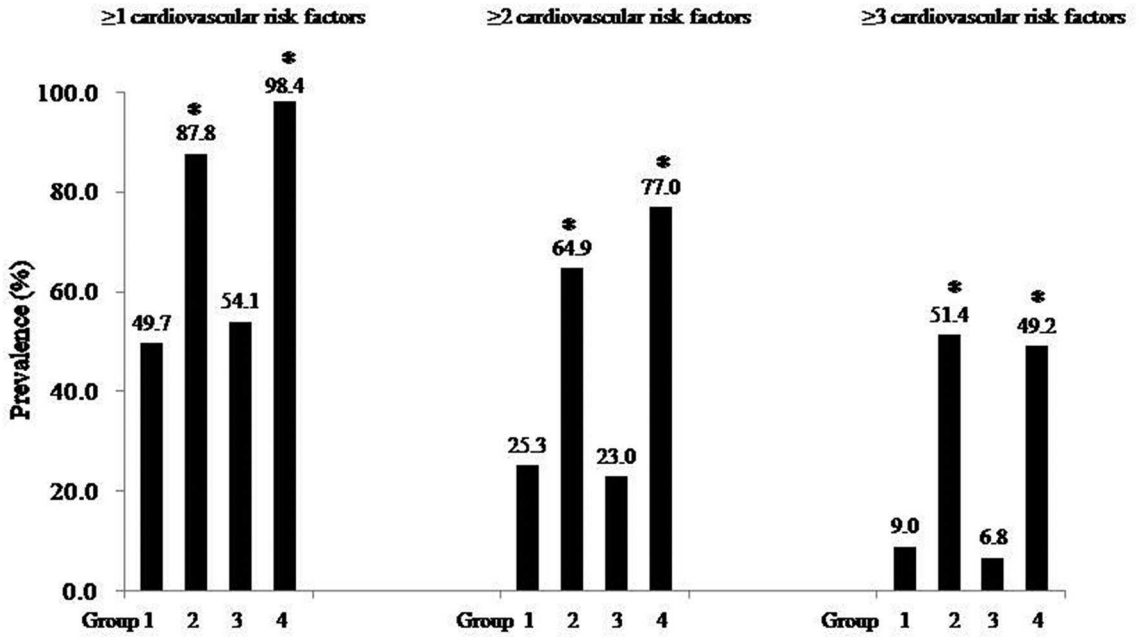

FIGURE 1 | Prevalence of cardiometabolic risk factors among four groups. Group 1: childhood BMI z-scores < 75th percentile and adult BMI z-scores < 75th percentile; Group 2: childhood BMI z-scores < 75th percentile and adult BMI z-scores ≥ 75th percentile; Group 3: childhood BMI z-scores ≥ 75th percentile and adult BMI z-scores < 75th percentile; Group 4: childhood BMI z-scores ≥ 75th percentile and adult BMI z-scores ≥ 75th percentile. *P < 0.001 (Compared with group 1).

TABLE 3 | Weight status change from childhood to adulthood and adult cardiometabolic risk factors.

	Outcome: ≥1 cardiovascular risk factors		Outcome: ≥2 cardiovascular risk factors		Outcome: ≥3 cardiovascular risk factors	
	RR (95%CI)[†]	*P*	RR (95%CI)[†]	*P*	RR (95%CI)[†]	*P*
Group 1 (n = 332)	Ref		Ref		Ref	
Group 2 (n = 74)	1.80 (1.58–2.06)	<0.001	2.57 (2.03–3.25)	<0.001	6.17 (4.12–9.23)	<0.001
Group 3 (n = 74)	1.10 (0.87–1.38)	0.440	0.94 (0.60–1.48)	0.801	0.77 (0.32–1.86)	0.567
Group 4 (n = 61)	1.94 (1.71–2.19)	<0.001	3.00 (2.36–3.80)	<0.001	5.31 (3.48–8.08)	<0.001

CI, confidence interval; RR, relative risk.

[†] *Adjusted for sex, childhood age, and elevated blood pressure, follow-up duration, adult smoking, and drinking.*

Group 1: childhood BMI z-scores < 75th percentile and adult BMI z-scores < 75th percentile; Group 2: childhood BMI z-scores < 75th percentile and adult BMI z-scores ≥ 75th percentile; Group 3: childhood BMI z-scores ≥ 75th percentile and adult BMI z-scores < 75th percentile; Group 4: childhood BMI z-scores ≥ 75th percentile and adult BMI z-scores ≥ 75th percentile.

implications for the prevention and control of CVD in the Chinese population.

Child excess weight status increased the risk of early-onset and clustering of future cardiometabolic risk factors (10). Previous studies reported that the number of cardiometabolic risk factors increased with the extent of childhood excess weight status (8). Our results were consistent with the previous findings, indicating that childhood excess weight status was an important determinant in the development of cardiometabolic risk factors. In addition, several prospective cohort studies began in childhood presented BMI trajectory and incremental area under the growth curve to obtain similar findings (25, 26). Moreover, childhood excess weight status predicted significantly future CVD and both cardiovascular and all-cause mortality across the life span (9, 27–29).

Maintaining an ideal weight in early life is an effective means for the prevention and control of future CVD. The International Childhood Cardiovascular Cohort (i3C) Consortium pooled data from four prospective cohort studies in the Western population, and revealed that children with excess weight status who achieved an ideal weight in adulthood did not have an increased risk of cardiovascular risk factors (10). Moreover, ample evidence demonstrated that the effect of childhood adiposity on cardiometabolic profile was mediated by current BMI (11–13). Consistent with previous publications, the present study showed that the long-term adverse effects of childhood excess weight status can be reversed if weight status returned to normalcy in adulthood in the Chinese population. Our findings were also supported by existing evidence from the Chinese population, which showed that a decrease in excess weight status

from childhood to adulthood was related to significant reductions in the risks of hypertension, metabolic syndrome, nonalcoholic fatty liver disease, subclinical atherosclerosis, arterial stiffness, and left ventricular hypertrophy (30–33).

There were several limitations in our study. First, data regarding blood glucose and lipid levels in childhood were not collected, which may affect our results. However, the sensitivity analysis results did not vary significantly after excluding 134 participants with childhood elevated BP or overweight (including obesity). In addition, pediatric overweight and obesity were equivalent to metabolic syndrome in terms of the power to predict adult adverse health consequence (9). Second, this observational study was not able to assess the causality between weight status change from childhood to adulthood and cardiometabolic risk factors. Third, 18% (119/660) of participants were lost to follow-up. However, no significant difference in childhood characteristics, except systolic BP, was observed between those who were included in the present study and those who were lost to follow-up (**Table S4**). Fourth, the sample size was insufficient to perform stratification analysis. Fifth, the number of female participants was small, which may affect our findings.

In conclusion, the current study demonstrated that childhood excess weight status is positively associated with adult cardiometabolic risk factors. Given that China is facing an excess weight epidemic in childhood (34), our results underscore the importance for weight management in early life in regard to the prevention and control of adult cardiometabolic risk factors and CVD.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board at the University of North Carolina at Chapel Hill, Institute of Nutrition and Food Safety, China Centers for Disease Control and the China-Japan Friendship Hospital, Ministry of Health and China. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

HF conceptualized and designed the study, carried out the initial analyses, drafted the initial manuscript, and reviewed and revised the manuscript. QZ and XZ critically reviewed and revised the manuscript. All authors approved the final manuscript for submission.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2020.00301/full#supplementary-material

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Paracetamol vs. Ibuprofen in Preterm Infants With Hemodynamically Significant Patent Ductus Arteriosus: A Non-inferiority Randomized Clinical Trial Protocol

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Background: Currently, the first line treatment of persistent ductus arteriosus (PDA) is either indomethacin or ibuprofen. However, the potentially life-threatening side effects associated to their use have prompted physicians to look for alternative options. The incorporation of paracetamol as an alternative to ibuprofen in the management of PDA is still based on insufficient clinical evidence. Hence, more clinical trials are needed to establish a therapeutic role for paracetamol in the management of PDA that take into consideration short- and long-term safety and efficacy outcomes.

Study Design: This is a non-inferiority, randomized, multicenter, double-blinded study to evaluate the efficacy, and safety of intravenous (IV) paracetamol vs. IV ibuprofen (standard treatment) for PDA in preterm patients with a gestational age ≤30 weeks. At baseline, patients will be randomized (1:1) to treatment with paracetamol or ibuprofen. The primary endpoint is closure of the ductus after the first treatment course. Secondary endpoints are related to effectiveness (need for a second treatment course, rescue treatment, reopening rate, time to definitive closure, need for surgical ligation), safety (early and long-term complications), pharmacokinetics, and pharmacodynamics, pharmacogenetics, pharmacoeconomics, and genotoxicity. Long-term follow-up to 24 months of corrected postnatal age will be performed using Bayley III neurodevelopmental scale.

Trial Registration: ClinicalTrials.gov Identifier: NCT04037514. EudraCT: 2015-003177-14.

Keywords: ductus, paracetamol, efficacy, safety, pharmacokinetics, pharmacogenetics

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BACKGROUND

Ductus arteriosus (DA) is an essential vascular shunt during fetal life that connects the pulmonary artery with the aorta (1). Under physiologic conditions, the DA closes spontaneously a few hours after birth, leading to the complete independence of the systemic and pulmonary circulations. Incidence of failure of the DA to close after birth is inversely proportional to gestational age (GA), with an incidence ranging from 10 to 20% in preterm neonates >32 weeks to 60% in those <28 weeks of gestation (2–4). When the ductus remains open, a portion of the circulating blood volume is redirected from the systemic to the pulmonary circulation. Depending on the size of the ductus, the diverted flow may cause pulmonary overflow and impaired end-organ perfusion. This hemodynamic situation is known as hemodynamically significant patent ductus arteriosus (hsPDA). HsPDA is associated with an increased risk of potentially severe clinical complications such as necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH), bronchopulmonary dysplasia (BPD), periventricular leukomalacia (PVL), acute renal failure, and death (5, 6). Therefore, recognizing and effectively treating hsPDA is a key point in the management of premature infants.

The first line therapy for hsDPA is a non-steroidal anti-inflammatory drug (NSAID), either indomethacin or ibuprofen. For patients who don't respond to NSAIDs or for whom pharmacologic treatment is contraindicated, surgical ligation is performed (7–9). The adverse effects of surgical management, although not frequent, are potentially severe. They may include reversible complications such as pneumothorax, infection, hemorrhage, or chylothorax, and/or irreversible complications such as vocal cord or diaphragmatic paralysis (1).

Indomethacin, a potent prostaglandin inhibitor, has traditionally been the drug of choice in the treatment of hsPDA. However, despite its established efficacy, its use has been linked to complications related to decreased cerebral, renal, and mesenteric perfusion (1). Ibuprofen has shown similar efficacy rates of up to 80% and lower hemodynamic effects compared to indomethacin. However, renal and mainly gastrointestinal complications are still present, such as NEC or intestinal perforation (10, 11).

Paracetamol, or acetaminophen, has recently emerged as an alternative to ibuprofen. This approach was first published in 2011, when Hammerman et al. (12) reported a case series of use of paracetamol as treatment of hsPDA in five neonates who had either failed or had contraindications to ibuprofen therapy. Rate of ductus closure was 100%, with no adverse events reported. In subsequent years, additional case series and clinical trials evaluating this new treatment option have been published (7, 12–30).

Table 1 summarizes the existing randomized clinical trials (RCTs) comparing paracetamol to standard treatment. Based on the data provided by these studies, paracetamol appears to have promising clinical results with a low rate of side effects.

The systematic review published in 2016 by Terrin et al. included two RCTs and 14 uncontrolled studies (32). It found no difference in the rate of ductal closure when paracetamol was used in place of ibuprofen (risk ratio [RR] 1.07, 95% CI 0.87–1.33 after 3 days of treatment, RR 1.03, 95% CI 0.92–1.16 after 6 days of treatment). In addition, safety profiles of paracetamol and ibuprofen were similar. The results are limited, however, by the poor quality of the included studies.

In 2018, Huang et al. (33), published a systematic review of five RCTs including a total of 677 neonates treated with either paracetamol or ibuprofen. The rates of primary and overall PDA closure were similar between treatments (RR 1.03, $p = 0.56$ and RR 1.02, $p = 0.62$ for paracetamol and ibuprofen, respectively). No differences were observed in the incidence of

TABLE 1 | Randomized clinical trial: paracetamol vs. active drug (ibuprofen or indomethacin).

References	Drug	Administration	Dosage	*N*	% total closure rate	Serum level	Hepatotoxicity
Dang et al. (13)	Paracetamol	Oral	15 mg/kg/6 h	80	81.2	No	No
	Ibuprofen	Oral	10–5–5 mg/kg	80	78.8	No	No
Oncel et al. (14)	Paracetamol	Oral	15 mg/kg/6 h	40	96.6	No	No
	Ibuprofen	Oral	10–5–5 mg/kg	40	93.6	No	No
El-Mashad et al. (30)	Paracetamol	IV	15 mg/kg/6 h	100	88	No	No
	Ibuprofen	IV	10–5–5 mg/kg	100	83	No	No
	Indomethacin	IV	10–5–5 mg/kg	100	87	No	No
Bagheri et al. (7)	Paracetamol	Oral	15 mg/kg/6 h	67	91	No	No
	Ibuprofen	oral	20–10–10 mg/kg	62	90.3	No	No
Yang et al. (27)	Paracetamol	Oral	15 mg/kg/6 h	44	70.5	No	No
	Ibuprofen	Oral	10–5–5 mg/kg	43	76.7	No	No
Dash et al. (28)	Paracetamol	Oral	15 mg/kg/6 h	38	100	No	No
	Indomethacin	IV	0.2 mg/kg/ 24 h	39	94.6	No	No
Al-lawama et al. (29)	Paracetamol	Oral	10 mg/kg/6 h	13	92	No	No
	Ibuprofen	Oral	10 mg/kg/24 h	9	89	No	No
Kumar et al. (31)	Paracetamol	Oral	15 mg/kg/6 h	81	78	No	No
	Ibuprofen	Oral	10–5–5 mg/kg	80	81	No	No

PDA complications: NEC (RR 0.86, $p = 0.70$), IVH (RR 0.84, $p = 0.55$), BPD (RR 0.69, $p = 0.16$), ROP (RR 0.58, $p = 0.15$), sepsis (RR 0.88, $p = 0.48$), or death (RR 1.45, $p = 0.45$). However, paracetamol showed a trend toward a reduced risk of renal failure (RR 0.20, $p = 0.07$), and a significantly reduced risk of gastrointestinal bleeding (RR 0.28, $p = 0.009$).

In 2018, Jasani et al. (34) performed a meta-analysis including RCTs comparing paracetamol to any cyclooxygenase (COX) inhibitor. Six RCTs were identified, involving 688 neonates treated with either paracetamol or ibuprofen. No differences in PDA closure were observed after the first course of treatment [RR 0.90, 95% confidence interval (CI) 0.71–1.13]. However, neonates treated with paracetamol had a lower incidence of gastrointestinal hemorrhage (RR 0.28; 0.12–0.69), acute renal impairment or increased serum bilirubin. No significant differences in alanine aminotransferase (ALT) or clinical outcomes such as NEC, BPD, IVH, ROP, pulmonary hemorrhage, surgical ligation, or mortality were assessed. The same meta-analysis examined two RCTs comparing paracetamol to indomethacin among 273 enrolled neonates. No differences in PDA closure were observed after the first course of treatment (RR 0.96; 0.55–1.65). In a pooled analysis of seven RCTs comparing paracetamol to any COX inhibitor no differences in PDA closure rate were observed among 861 neonates after the first treatment course (RR 0.90; 0.72–1.13), and paracetamol treatment was associated with a lower rate of gastrointestinal hemorrhage (RR 0.51; 0.28–0.91). No differences were observed in rates of NEC, ROP, BDP, IVH, pulmonary hemorrhage, surgical ligation, or mortality (34).

The Cochrane Systematic Review performed in 2018 by Ohlsson et al. (35) included eight studies that reported data collected on 916 infants. Studies that achieved at least moderate-quality evidence according to the GRADE classification suggested that paracetamol is as effective as ibuprofen; the group of low-quality evidence studies suggested that paracetamol is more effective than placebo or no intervention and also that paracetamol is as effective as indomethacin in PDA closure. In view of these results, Ohlsson et al. (35) concluded that paracetamol appears to be a promising alternative to indomethacin or ibuprofen for PDA closure, potentially with fewer adverse effects. However, further research regarding the effectiveness and safety of paracetamol is needed before the evidence is definitively established or rejected.

Moreover, there are no data published regarding neurodevelopment follow-up in patients receiving paracetamol.

In summary, published systematic reviews and meta-analyses conclude that the existing evidence is still not sufficient to establish a therapeutic role for paracetamol in the treatment of hsPDA and additional larger trials are required, with special focus on developmental consequences associated with the use of this drug.

In addition to the lack of definitive clinical evidence supporting its use in hsPDA, there is also insufficient knowledge about the pharmacokinetics and pharmacodynamics of paracetamol in the neonatal period, especially in patients with hsPDA.

The aim of this study is to demonstrate the non-inferiority of paracetamol compared with ibuprofen and to address the safety and cost-effectiveness of this treatment in premature infants.

METHODS

Study Design and Population

Study Design

This is a randomized, multicenter, double-blinded non-inferiority study to evaluate the efficacy, and safety of IV paracetamol (intervention) vs. IV ibuprofen (standard treatment) for the treatment of hsPDA in preterm neonates. Patients will be randomized (1:1 ratio) to the paracetamol or ibuprofen group. The study will be conducted at four hospitals: University and Polytechnic Hospital La Fe (Valencia, Spain), Regional University Hospital of Malaga (Málaga, Spain), University Hospital Reina Sofía (Córdoba, Spain), and Cabueñes University Hospital (Gijón, Spain).

Study Population

Preterm infants with GA $\leq$30 weeks with diagnosis of hsPDA based on clinical suspicion and confirmed by echocardiogram performed by a pediatric Cardiologist will be eligible for the study.

The definition of "Hemodynamically significant PDA" has been selected from the most common and reliable echocardiographic parameters widely used to consider the treatment of the PDA (36). It is defined as a ductal diameter >1.5–2.0 mm and at least one of the following:

- Continuous flow through DA.
- Retrograde diastolic flow in the descending aorta.
- Dilation of the left atrium, defined as left atrial/aortic ratio (LA/AO) > 1.5 mm (measured on M-mode echocardiogram)
- Ductus size/descending aorta diameter ratio > 0.5 mm.

Inclusion and exclusion criteria are described in **Table 2** and comprise mainly the contraindications of ibuprofen in this population.

Subjects will be screened to determine whether they meet all the inclusion criteria and have none of the exclusion criteria.

The study will only include hsPDA requiring treatment in the first 2 weeks of life, as the odds of closure decrease with time (4).

Objectives

The primary objective of the study is to evaluate the efficacy of IV paracetamol vs. standard IV ibuprofen treatment for PDA closure.

Secondary objectives are: (i) to compare the safety of both treatments; (ii) to improve the knowledge of pharmacokinetics, pharmacodynamics, and pharmacogenetics of paracetamol and ibuprofen in the neonatal period; (iii) to make a pharmacoeconomic evaluation of the use of both drugs; and finally (iv) to perform a genotoxicity study of administered drugs.

Primary Outcome

The primary outcome is the rate of hsPDA closure after one round of treatment with paracetamol (experimental drug) vs.

TABLE 2 | Inclusion and exclusion criteria of the study.

Inclusion criteria	Exclusion criteria
Written informed consent of parents/guardians	Major congenital malformations or chromosomopathies
GA ≤ 30 weeks	Imminent death
Postnatal age ≤ 2 weeks	Impossible or erroneous randomization
Need for ventilatory support	Participation in another clinical trial with medication
Birth or arrival in participating hospital within the period of application of the treatment	Diuresis <1 mL/kg/h in the 8 h prior to treatment or creatinine >1.8 mg/dL
First episode of hsPDA	Platelets <50,000/μL or active hemorrhage (tracheal, digestive, or renal)
	Recent (past 48 h) IVH (grades 3–4)
	Septic shock
	Severe hyperbilirubinemia or severe coagulopathy or liver failure
	Active NEC or intestinal perforation

ibuprofen (control drug). A ductus will be considered to be closed when the diameter measures <1 mm on echocardiography performed by a pediatric cardiologist.

Secondary Outcomes

Outcomes related to effectiveness

- Need for a second treatment course
- Closure rate after two treatment courses
- Need for rescue treatment after two courses of treatment
- Rate of ductus reopening after closure
- Closure rate after reopening
- Time to ductus closure
- Need for surgical ligation.

Outcomes related to safety

- Incidence of early complications (occurring during the course of treatment): renal failure, NEC, IVH, hyperbilirubinemia, bleeding, gastrointestinal perforation
- Incidence of late complications (over the course of the admission): BPD, PVL, NEC, neonatal retinopathy, sepsis, death.

Outcomes related to pharmacokinetics and pharmacodynamics

- Determination of serum levels of paracetamol achieved with standard doses
- Pharmacodynamic model of paracetamol in the context of hsPDA
- Relationship of effectiveness/adverse reactions to serum levels
- Quantification of metabolites in urine and their relationship with drug elimination.

Outcomes related to pharmacogenetics

- Determination of genetic polymorphisms in TFAP2B, TGFBR2, EPAS1, MD-2, and GM2A genes in dry blood spots (DBS) and their relationship to efficacy or incidence of adverse reactions.

Outcomes related to pharmaco-economics

- Price-effectiveness ratio, assessed via a cost-effectiveness analysis accounting for observed efficacy.

Outcome related to genotoxicity

- Percent DNA damage.

Sample Size Calculation and Power

For the estimation of the sample size, data from previous studies (13, 14) were used to establish a Gaussian with mean 0.15 and standard deviation 0.3 for the coefficient that determines the log-odds of closure of the paracetamol group with respect to the ibuprofen group. Assuming this previous distribution of the log-odds, that the ibuprofen group produces closure in 80% of the cases, and establishing the inferiority limit for the paracetamol group in 70% of closures (−10%), it has been estimated that 150 patients per group would be needed to establish the non-inferiority of the paracetamol treatment compared to the ibuprofen treatment with a statistical power of 80 and 95% credibility.

La Fe and Carlos Haya University Hospitals are a reference centers in their areas having 6,000–6,500 births per year and patients referred from other centers. They have ∼100–150 preterm admissions <1,500 gr per year. In addition, there is other two recruiting centers (Reina Sofía Hospital and Cabueñes University Hospital) included in the study, so we estimate that the sample size could be accomplished in the 3 years period of the study duration.

The study is also designed to perform a pharmacoeconomic analysis of the treatment. In the case that paracetamol arises as non-inferior to ibuprofen, that is, they are assumed to have the same therapeutic effectiveness, we will perform a cost-minimization analysis, calculating, and comparing the costs associated with each therapeutic strategy based in the cost per unit (laboratory sale price), preparation cost and administration cost. If both drugs are not equivalent in efficacy, we will perform a cost-effectiveness analysis using the closure rate after the first round of treatment and the rate of occurrence of clinically relevant adverse reactions related to treatment and score in the Bayley III test. The incremental cost and the incremental cost-effectiveness ratio will be calculated.

Treatment of Subjects

Intervention

The paracetamol group will receive IV doses of 15 mg/kg IV every 6 h for 3 days. The ibuprofen group will receive an initial dose of 10 mg/kg IV followed by 5 mg/kg IV at 24 and 48 h (three doses are considered a treatment course). Given that the treatments have different dosing schedules, to maintain blinding, patients in the ibuprofen group will receive an equivalent volume of placebo (glucose 5%, normal saline or according to the center's usual practice) at 6-h intervals to correspond with the dosing times of paracetamol. If the duct remains open >1 mm after the

full treatment course has been completed, another course of the same drug will be administered (maximum two courses).

If the medical treatment fails (defined as the ductus measuring >1 mm after completion of two rounds of treatment), a course of ibuprofen (not blinded) at usual doses will be considered in both groups with the intention of offering at least one course of the standard treatment to all patients before considering surgery. If this rescue course fails, surgical closure will be pursued if deemed appropriate. **Figure 1** described diagram of study.

The paracetamol dose of 15 mg/kg every 6 h was chosen based on previously-reported data for paracetamol in the treatment of hsPDA in neonates (12–14, 16, 17, 26, 37).

Assessments During Study Treatment

Related to efficacy

If possible, daily echocardiography will be performed in order to monitor ductus diameter and timing of closure. Compulsory echocardiography will be performed at diagnosis and at the end of each treatment course.

Safety assurance

Daily blood tests including creatinine, platelet count, bilirubin, and liver enzymes will be performed in conjunction with routine samples to rule outside effects due to the medication.

Pharmacokinetic study

In hemodynamically stable patients, blood samples will be obtained to determine the serum levels of drugs during treatment to develop a population pharmacokinetic model for IV paracetamol in premature infants with hsPDA. On the 1st day, samples will be taken at 30 min and at 1, 3, 6, and 12 h. In the subsequent days, samples will be taken at 24, 48, and 72 h following the first dose. The blood volume per sample will be 100–200 μL. On the 1st day of the study, sampling will be limited to patients with a central catheter, from whom blood can be obtained without venipuncture.

Non-linear mixed-effects models will be constructed from paracetamol serum concentration–time data in NONMEM v7.3. Covariates will include the number of doses administered, hour of sample extraction, body weight, gestational age, postnatal age, sex, creatinine, total bilirubin, and estimated glomerular filtration rate.

Urine will be collected once the first course of treatment begins and within 24 h of completion of the last dose to measure drug metabolites.

Pharmacogenetics analysis

A blood spot will be deposited in a WhatmanTM 903 card or similar once during the study and left to dry at room temperature. The collector cards will be stored at room temperature until analysis. The genomic DNA will be extracted by the method validated by Ramos et al. (38), based on an alkaline lysis to obtain the genetic information, then subjected to PCR amplification with primers responsible for amplifying the polymorphism to be determined. The genetic polymorphisms in the TFAP2B, TGFBR2, EPAS1, MD-2, and GM2A genes will be determined. The collector card and the leftover pellets of the plasmatic samples of the pharmacokinetic analysis will be saved for further determinations of other polymorphisms that may be useful, such as AGTR1, TRAF1, etc. or others that may add to our knowledge of relevant pathology.

Genotoxicity of drugs

The genomic damage caused by the treatments with paracetamol and ibuprofen will be determined using a modification of the alkaline electrophoresis of individual cells, or "comet assay," with repair enzymes for the detection of specific lesions in the DNA in the pellet (polymorphonuclear cells). The repair enzyme that we will use in our study is formamido-pyrimidine-DNA glycosylase

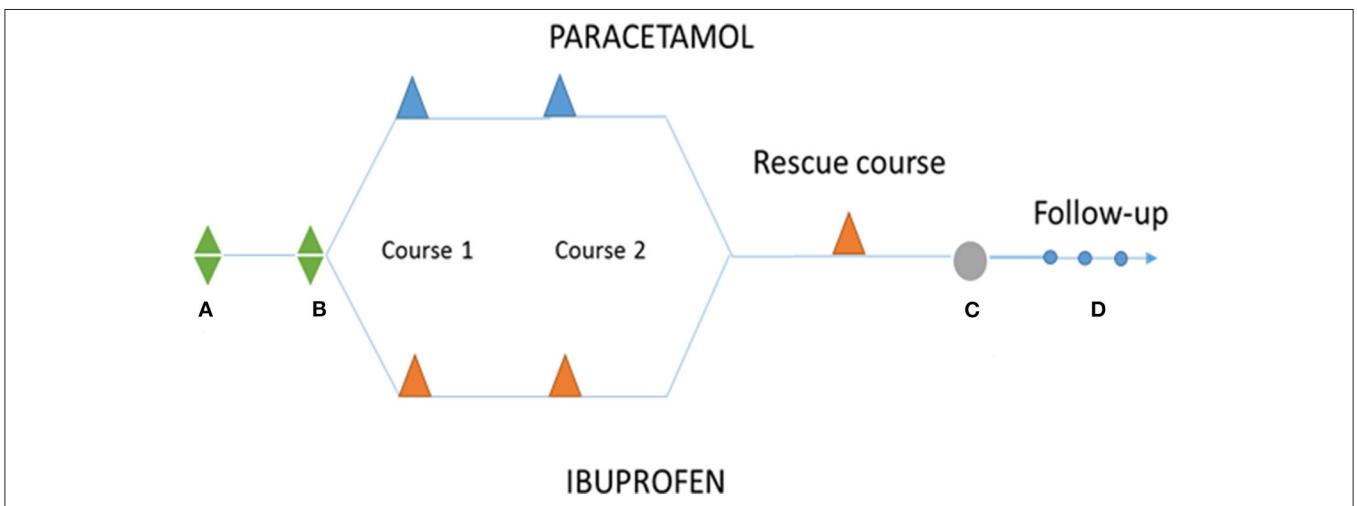

FIGURE 1 | Diagram of study. **(A)** Birth; **(B)** diagnostic and randomization; The PARACETAMOL group will receive IV doses of 15 mg/kg administered every 6 h for 3 days (maximum two courses = 6 days of treatment). The IBUPROFEN group (control group) will receive an initial dose of IV 10 mg/kg followed by 5 mg/kg at 24 and 48 h (the three doses are considered a treatment course, maximum two courses). **(C)** Surgical close. **(D)** FOLLOW-UP: 40 weeks, 12 months, and 24 months.

(FPG), which recognizes oxidized purines. The comet assay is a well-established approach for detecting genotoxicity from drugs.

Follow-Up Evaluations

The study will incorporate follow-up visits at 40 weeks, 12 months, and 24 months of corrected age. At each visit, weight, height, and head circumference will be measured and a complete structured physical evaluation of the child will be performed. In the third follow-up visit, a comprehensive neurodevelopmental assessment using Bayley III-ES, GMFCS, and sensorial visual and auditory acuity will be performed.

Procedures

Recruitment and Consent

Once a potential study participant has been identified, investigators will explain the nature of the study to the infant's parents or legal guardians and answer any questions they may have regarding the study. Written informed consent will be obtained before the start of any study-related procedures and will be reviewed, signed and dated by investigator and parents/legal guardians. The information sheet will include all the complementary tests that will be carried out during the study. A separate consent will be signed to obtain pharmacogenetics samples.

Randomization, Blinding, Treatment Allocation, and Administration

Randomization will be carried out by the biostatistician of Health Research Institute La Fe using the R® 3.5.1 software (R Development Core Team, Auckland, New Zealand). The biostatistician will provide the randomization list to the Pharmacy Department. Patient numbers will be assigned sequentially in order of entry into the study. Patients will be randomly assigned to either the paracetamol or ibuprofen group using randomization by blocks and stratified by GA (24^{+0}–26^{+6} and 27^{+0}–30^{+6}).

The drug solutions will be prepared in a daily basis including weekends by the Pharmacy Department in indistinguishable syringes to keep the study blind. Four numbered syringes with the daily treatment for each patient will be prepared every day. Administration will proceed sequentially, beginning with syringe 1. In the ibuprofen group, syringe 1 will contain ibuprofen, while syringes 2–4 contain a placebo.

Due to the incompatibility of ibuprofen with other medications and parenteral nutrition, no other medications or parenteral nutrition should be administered within 15 min of syringe 1. There are no similar compatibility concerns with either paracetamol or the placebo, so syringes 2–4 can be administered either alone or via Y-site as a short infusion over 15 min, preferably undiluted.

Withdrawal of subjects

Parents/legal guardians can withdraw consent at any time and for any reason if they wish to do so without any consequences.

The researcher may also withdraw a patient from the study at any time if he believes study procedures have not been followed, for the benefit of the patient, or in the event of unacceptable levels of toxicity.

The reasons for withdrawal will be registered in the electronic Case Report Form (eCRF). All infants who leave the study will receive treatment as per standard unit practice.

Unblinding

The blinding can be opened in emergency cases in which the knowledge of the assigned treatment is essential for the medical care and well-being of the patient. In this case, the date and reason for the unmasking must be noted in the eCRF by the researcher.

The researcher or neonatologist in charge will communicate with the Pharmacy Department regarding treatment provided to the patient.

Once the final patient recruited into the study has been discharged from the hospital, the blinding will be lifted to allow for statistical analysis of the results. The neurodevelopmental specialist who will provide long-term follow-up will remain blinded until all follow-up assessments have been completed.

Adverse Events (AEs)

An AE is any harmful and unintended reaction to a drug under investigation.

AE will be monitored until 28 days after the end of the treatment period and/or until all AE-related consultations for the patients have been resolved. Data of all study participants will be included in the safety analyses. We will not consider those conditions commonly related with prematurity, such as jaundice, apneic-bradycardic syndrome, anemia, or electrolyte, and glucose abnormalities, to be AEs.

A serious adverse event (SAE) is any untoward medical occurrence that results in death, is life-threatening, requires inpatient hospitalization, or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect. In the event of SAE occurs, the researcher will inform the promoter (La Fe Research Institute) within a maximum period of 24 h from the moment the event is identified, completing, and signing the SAE notification form.

During the study, the promoter will prepare yearly safety reports following the recommendations indicated in the International Conference on Harmonization (ICH) E2F guide and will present them to the regulatory authorities and to the relevant Institutional Review Board (IRB) following the schedule established in the current legislation.

Statistical Analysis

The analytic strategy has been based on the intention-to-treat principle. Categorical variables are described as the numerical count (percentage) of each category, and are compared with Pearson's chi-squared test or Fisher's exact test. Continuous variables are represented by a box-and-whisker plot. If the continuous variables are normally distributed ($p > 0.05$ in the Shapiro-Wilk test), they are described as the mean ± standard deviation, and are compared using Student's *t*-test, first testing the hypothesis of equality of variances using the test of Levene.

If they are not normally distributed, they are described as the median (p25, p75) and compared using the Mann-Whitney *U*-test. Comparison of repeated measures between the two groups is performed using two-way analysis of variance (ANOVA), first testing the Mauchly sphericity hypothesis. Survival times are expressed as the median time (95% CI), and the comparison between groups is performed with the log-rank test. Throughout the study, $p < 0.05$ is accepted as the limit of statistical significance. The magnitude of the effect is quantified with the risk difference (expressed as a percentage), and its accuracy is indicated with a 95% CI.

Data Handling and Study Monitoring

A record in an eCRF must be completed for each patient included in the study. Data will be anonymized using a unique code for each participant. The key document that will contain the name of each patient related to the code number will be stored in the folder of the principal investigator (PI) at each center. Data will be kept in an institutional research location of the PI, secured with a password or key for the period specified by legislation.

Periodic monitoring visits will be carried out during the trial by an external monitor independent from the research team to ensure that the protocol and good clinical practices are being followed. The monitors will be able to review the source documents to confirm that the data collected in the eCRFs are accurate. The researcher and the institution guarantee the monitors direct access to the source documents and to the relevant regulatory authorities for verification.

Ethical Considerations

All the procedures have been reviewed and approved by the IRB of the PI's hospital (Comité de Ética e Investigación Médica (CEIm); University and Polytechnic Hospital, Valencia, Spain) and the Approval Number is: 5/27-06-2018/439) also by the local IRBs of the participating hospitals. It has been also approved by the Spanish Drug Agency as per legal requirement.

According to good-practice guidelines, blood samples for pharmacokinetics will be only obtained if a central catheter in place is available and the total amount of blood per day will be limited to 1 mL/kg/day.

The study will be conducted in accordance with the protocol, ICH guidelines, the applicable regulations, and guidelines governing the conducting of clinical studies in Spain, and the ethical principles originating from the Declaration of Helsinki.

DISCUSSION

Many Neonatal intensive care units (NICUs) consider off-label use of paracetamol in hsPDA cases where ibuprofen (the current first line option) is contraindicated or has proven inefficient. This therapeutic approach has yielded promising results, with high rates of ductus closure and a good safety profile. However, in our own experience, efficacy rates of paracetamol have been much lower than those reported (non-published data). This discrepancy may be related to many factors, including the low publication index of studies with negative results compared with studies with positive results [publication bias (39)], or data from studies that report on several individuals with unclear selection approach [possible selection bias (40)].

We choose a non-inferiority study because is the best way to demonstrate that the experimental treatment is not unacceptably less effective than an existing treatment. With this design, we are able to collect data regarding potential advantages over the established treatment, such as a lower incidence of adverse reactions or a more favorable cost-effectiveness profile. It is known that the efficacy of ibuprofen in PDA closure is 70–85% (41), but it is associated with significant renal and mainly gastrointestinal, such as NEC and intestinal perforation (10, 11). Paracetamol safety is well-documented, as it is a common drug used for treating fever and pain in infants and children. If non-inferiority is demonstrated, the lower incidence of side-effects and the lower cost would make paracetamol an ideal drug for neonates with hsPDA.

Of published clinical trials, only study of Kumar et al. (31, 42) has a non-inferiority design but administration of treatment was oral and patients included were <32 weeks of gestation. One of the main inconveniences of this design is the large population size needed to demonstrate non-inferiority. To date, our sample size is the biggest of reported trials (35).

Failure of DA closure after birth is inversely proportional to GA, with incidence ranging from 10 to 20% in preterm neonates >32 weeks to 60% in those <28 weeks of gestation (2–4). We chose GA ≤30 weeks because it includes the majority of newborns requiring treatment for this condition, thus increasing the external validity and allowing our results to be generalized to this population at large. A more narrow GA range, as chosen in other trials, limits external generalization. Using randomization by blocks and stratifying by GA limits bias and further enhances the applicability of our results.

Pharmacologic management of PDA is not without risk, and limiting treatment to only those neonates with hsPDA is the most appropriate strategy for balancing the benefits of treatment with the risks of potential adverse effects (43–45). Therefore, the diagnosis of hsPDA should rely on objective parameters. There is no consensus for what constitutes a hemodynamically significant PDA and therefore when is most appropriate to treat a PDA in preterm infants. We have chosen published criteria with high sensitivity and specificity to define when a PDA should be classified as hemodynamically significant (46, 47). These include a ductal diameter >1.5–2.0 mm and at least one of the following: continuous flow through the DA, retrograde diastolic flow in the descending aorta, LA/AO ratio > 1.5, or ductus size/descending aorta diameter ratio >0.5.

The primary outcome of our study is the rate of closure of the hsPDA after a single course of treatment. For the purpose of our study, closure is defined as a ductus diameter <1 mm on echocardiography, as defects off this size are typically not hemodynamically significant and in most cases proceed to complete closure. In some published studies (28), it is not clear how many treatment courses were required for ductal closure, or if the rate of the ductus reopening differs between treatments, so potential bias can be found in the results. The appropriate duration of treatment of PDA with paracetamol has not yet been established, and in our study echocardiogram will be

performed every day of treatment in order to help to resolve this question. We believe that this information will improve future PDA management and will give us useful information to avoid unnecessary or excessive treatment.

Paracetamol, a non-classical NSAID, is an analgesic and antipyretic agent that has weak antiplatelet and anti-inflammatory activities. It reduces the synthesis of prostaglandins by inhibiting prostaglandin synthetase (PGHS), an enzyme in the peroxidase (POX) region instead of the COX region, as is the case of ibuprofen or indomethacin (12).

Paracetamol is metabolized almost exclusively by the liver (90–95%) and eliminated mainly as paracetamol-glucuronide (47–62%), paracetamol-sulfate (47–62%), and, to a lesser extent, cysteine conjugates (48).

Age-related changes in bioavailability, metabolism, and the rate of elimination of paracetamol take place during childhood. These changes are particularly evident during infancy. Neonates have lower metabolic and elimination capacities than older infants, and varying rates among different subjects is explained by covariates, such as size or weight, as well as different disease characteristics. Preterm neonates have a higher distribution volume, lower elimination rate, and higher half-life values for plasma concentration of paracetamol than older infants (48). Neonates, infants and children up to 10 years old eliminate a significantly lower amount of glucuronide conjugates and more sulfate conjugates than adults (49).

At present, there is scarce information available regarding the paracetamol plasma concentration required for PDA closure. Plasma levels of paracetamol for analgesic and antipyretic effects range from 10 to 30 μg/mL (15, 37, 48, 50). However, only three studies have addressed paracetamol plasma concentrations in the management of hsPDA (15, 37, 50). The small number of patients enrolled in these studies doesn't allow for conclusions regarding the efficacy of paracetamol related to its plasma concentration. Recently, Bin-Nun et al. (51) reported the association between serum paracetamol concentration measured at steady-state (they chose 4 h after the 8th dose) and ductal closure in 10 neonates treated with oral paracetamol (15 mg/kg/q6h). A paracetamol concentration >20 mg/L had 100% sensitivity and specificity for ductal closure. The El-Khuffash's study (16) showed that the clinical efficacy of paracetamol in PDA closure may depend on the duration of treatment, the dose and the mode of administration. This would suggest that a critical serum concentration of paracetamol is needed in order to achieve a maximum therapeutic effect.

Because there is no established critical serum concentration of paracetamol, our study is designed to confirm a therapeutic threshold of serum concentration required for ductal closure, identify optimal timing for evaluation of serum concentration and relate concentration to gestational age, treatment duration, and paracetamol metabolism (assessed via urine metabolites) to optimize treatment success. Moreover, although paracetamol dosage was chosen according to previous reported studies (12–14, 16, 17, 26, 37), it was adopted without appropriate pharmacokinetic or pharmacodynamic studies to establish safety and efficacy, and our study will add this information regarding the paracetamol dosage most used for treatment of hsPDA.

Regarding the route of administration, the protocol followed in our NICU is administered via IV, since the oral route is not always an option in neonates. The data in favor of orally- vs. IV- administered paracetamol have not yet been fully confirmed. We believe that route of administration may be very relevant, as the IV route is likely more suitable than in this population due to the frequency of feeding intolerance and intestinal complications and in whom enteral absorption is uncertain. Moreover, the oral preparation is hyperosmolar thus should be used with caution when infants are NPO or allowed only low-volume intakes (52). Some authors suggest that the slower rate of absorption of oral paracetamol relative to IV paracetamol could lead to a longer exposure of the ductus to the drug and a greater response rate (53). Singla et al. (54) administered a single dose of intravenous, oral, or rectal paracetamol to adults, and intravenous paracetamol achieved faster and higher plasma concentrations.

One of the greatest concerns when administering paracetamol to neonates is the possible hepatotoxicity due to the toxic metabolite N-acetyl-p-benzoquinone-imine (NAPQI) (53). In general, toxicity is lower in neonates. They have relatively low levels of CYP2E1 enzymatic activity so their oxidation of paracetamol is slower, forming smaller amounts of toxic metabolites. In addition, neonates have higher rates of glutathione synthesis (53). However, extremely preterm infants (<28 weeks) have a limited capacity for glutathione synthesis due to the lack of expression of the enzyme gamma-cystathionase in the trans-sulfuration pathway and therefore limited or no ability to synthesize L-cysteine, a component of the tripeptide glutathione (55). According to published literature, no signs of hepatotoxicity have been reported during PDA treatment (34).

In conclusion, between the available drugs for PDA treatment, paracetamol seems to be a promising alternative to NSAIDs. Most authors agree that there is a need of better designed trials to establish its efficacy, short- and long-term safety and neurodevelopmental outcomes.

Our study is an adequately-powered RCT that will allow for the establishment of paracetamol as standard therapy for the management of PDA and to definitively establish its safety and efficacy. The ultimate aim would be to achieve an individualized therapeutic approach, selecting the best treatment according to the patient's characteristics and including pharmacologic aspects aiming to reduce toxicity.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University and Polytechnic Hospital, Valencia, Spain. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

AG-R, AG, MS, MP, AP, JP-A, MV, and MA were all involved in development of the study protocol. AG-R prepared the initial draft of the manuscript. AG-R, AG, and MA set up the database

infrastructure for the intervention. All authors read, contributed to editing, and approved the final manuscript.

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