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Effect of retrograde autologous priming based on miniaturized cardiopulmonary bypass in children undergoing open heart surgery

A STROBE compliant retrospective observational study

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Abstract

To evaluate the effectiveness of retrograde autologous priming (RAP) based on miniaturized cardiopulmonary bypass (CPB) circuit in children undergoing open heart surgery.

We performed a retrospective analysis of all patients (≤15 kg) who underwent open heart surgery with CPB in our center from January 1, 2017, to July 31, 2019. Propensity score matching was used to adjust for significant covariates, and multivariable stratified analysis was used to assess the association of the RAP technique with clinical outcomes.

A total of 1111 patients were analyzed. There were 355 (32.0%) children who underwent RAP, and 756 (68.0%) were in the non-RAP group. After propensity score matching, there were a total of 638 patients, with 319 patients in each group. The bloodless priming rate was significantly higher (P=.013), and the ultrafiltration rate was significantly lower (P=.003) in the RAP group than in the non-RAP group. Compared with patients in the non-RAP group, patients in the RAP group had a shorter postoperative mechanical ventilation time (P<.001) and shorter lengths of stay in the intensive care unit (ICU) (P<.001) and the hospital (P<.001). No differences were noted in postoperative hematocrit (P=.920), postoperative 24-hour blood loss (P=.435), and hospital mortality (P=.563). In the stratified analysis, the difference remained statistically significant (P<.05) when the patient weight was >4 kg or the Society of Thoracic Surgeons-European Association for Cardiothoracic Surgery (STAT) category was <3. However, when the patient weight was ≤ 4 kg or the STAT category was ≥ 3 , there was no significant difference between the 2 groups in terms of bloodless priming, ultrafiltration, postoperative mechanical ventilation time, or length of stay in the ICU or the hospital (P >.05).

The RAP technique based on miniaturized CPB system was safe and effective for children who underwent congenital heart surgery. The RAP technique can significantly reduce the priming volume, improve the rate of bloodless priming, and reduce blood product application. It was also associated with a shorter postoperative mechanical ventilation time and shorter lengths of stay in the ICU and the hospital.

Abbreviations: CPB = cardiopulmonary bypass, CUF = conventional ultrafiltration, HCT = hematocrit, ICU = intensive care unit, MUF = modified ultrafiltration, PLT = platelet, RAP = retrograde autologous priming, SD = standard deviation.

Keywords: retrograde autologous priming, congenital heart diseases, miniaturized cardiopulmonary bypass, propensity score matching

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1. Introduction

With the continuous development of anesthesia, extracorporeal circulation, and surgical and postoperative monitoring techniques, as well as the promotion of the concept of "early diagnosis and early treatment," the proportion of newborns and infants undergoing open heart surgery after cardiopulmonary bypass (CPB) has gradually increased.^[1] However, conventional CPB with a traditional circuit system has inherent disadvantages because of its large priming volumes and the large surface area of the circuit. It is known to cause a systemic inflammatory response, which results in multiple organ dysfunction and increased morbidity and mortality in children.^[2,3] Conventional CPB also causes hemodilution, which, at higher degrees, leads to decreased oxygen carrying capacity and decreased concentrations of major plasma proteins and coagulation factors.^[4,5]

Miniaturized CPB has progressively evolved over the past 20 years and is mainly aimed at decreasing priming volumes.^[6,7] Miniaturized CPB has been associated with decreased mortality, reduced systemic inflammatory responses and hemodilution, and reduced use of blood product.^[8,9] At present, miniaturized CPB has mainly been accomplished by using smaller and shorter circuit systems, having the system in closer proximity to the patient, using oxygenators with integrated arterial line filters and using the technique of retrograde autologous priming (RAP) based on a miniaturized circuit system.^[10–13] The method for RAP was first described and demonstrated by Rosengart et al in 1998, and the main difference is that the CPB circuit is partially primed with the patient's own blood.^[14] Studies have shown that RAP reduces hemodilution through passive exsanguination of the arterial and venous lines prior to the initiation of CPB and therefore reduces the need for blood transfusions.^[15]

In our previous article,^[5] we compared the effectiveness of the conventional circuit system and the miniaturized circuit system in children by analyzing the short-term outcomes after congenital heart surgery. A miniaturized circuit system with RAP was applied in our department to further reduce the priming volume and alleviate tissue edema. Therefore, in this study, we aimed to evaluate the effectiveness of the RAP technique based on miniaturized CPB in children undergoing open heart surgery.

2. Methods

2.1. Patient population

We performed a retrospective analysis of all patients ($\leq 15 \text{ kg}$) who underwent open heart surgery with CPB in our center from January 1, 2017, to July 31, 2019. All children undergoing congenital cardiac surgical procedures were included in this retrospective analysis, with the exception of children who required unscheduled repeat operations after the primary surgery and emergency operations. Children with aortic valve-related disease, such as aortic valve stenosis, aortic valve insufficiency, and abnormalities of the aortic valve, were excluded. These patients may have decreased blood pressure in the aortic root when taking autologous blood, resulting in an insufficient blood supply to the coronary arteries and an elevated ST-segment of the electrocardiogram, thereby affecting myocardial function. Therefore, we did not use RAP for children with aortic valve-related disease. We also excluded children with preoperative tracheal intubation-associated long-term mechanical ventilation dependence and patients assisted by extracorporeal circulation after the operation, including extracorporeal membrane oxygenation. Finally, a total of 1111 children were included in the analysis. Because the data used for this study were collected for administrative purposes without any identification of individuals, the study was exempted from informed consent and ethics approval for the study was obtained from the Ethics Committee of Shenzhen Children's Hospital (SCHER-2018019).

2.2. Data collection and definitions

The analyzed data contained demographic characteristics, preoperative respiratory status, information on the cardiac surgery and CPB, postoperative records, and echocardiographic information. The cardiac surgery information included the preoperative diagnosis and type of surgical procedure, which were recorded immediately after the procedure. The intraoperative variables, including CPB time, aortic cross-clamp time, circuit type, priming volume, ultrafiltration type, urine volume, hematocrit (HCT) and lactate, were collected prospectively and documented in the CPB record sheet. The postoperative information primarily consisted of the extubation time in the intensive care unit (ICU), blood gas analysis, reintubation, the use of nasal continuous positive airway pressure, and survival status. The data we used were extracted by a nurse from the medical records of inpatients in our department.

The outcomes we compared in this study included the rate of bloodless priming and ultrafiltration, postoperative HCT, postoperative mechanical ventilation time, 24-hour blood loss, hospital mortality, and length of stay in the ICU, and hospital after surgery. All these data could be obtained from medical records directly. RAP group was defined as children with the use of RAP, and non-RAP group was defined as children with no use of RAP.

2.3. Anesthesia management

All surgeries were completed by the same team of anesthesiologists and surgeons. Our approach was to extubate every patient as soon as possible after surgery. Anesthesia was induced with intravenous midazolam, sufentanil, propofol, and rocuronium, and maintained with remifentanil, rocuronium, dexmedetomidine, and sevoflurane. After sternal closure, remifentanil, and rocuronium were discontinued, and sufentanil and tropisetron were administered once an intravenous analgesic pump was established. Sevoflurane was discontinued before skin closure and dexmedetomidine was continued until the child was transferred to the ICU.

2.4. CPB circuit

In our center, all patients used the Stokert-S5 CPB machine (Stockert GmbH, Germany), and we randomly selected the oxygenator from among a Terumo Capiox FX05 (Terumo Corp, Tokyo, Japan) and a Maquet safe Micro/VKMO 11000 (Maquet, Rastatt, Germany). The perfusion pressure of children (≤ 15 kg) was maintained at 30 to 50 mm Hg. If the perfusion pressure was consistently below 30 mm Hg under adequate perfusion flow, phenylephrine was given. After cross-clamping the aorta, cold-modified St Thomas' cardioplegic solution was given at a single dose of 20 mL/kg. If the cross-clamp time was longer than 100 minutes or perfusion was ineffective or severe myocardial hypertrophy occurred, the surgeon empirically increased the perfusion dose. For most children, the cardioplegic

solution flows back into the right atrium, where it will be sucked away by the suction device and not recycled into the venous blood reservoir.

All patients used the miniaturized CPB circuit system, which consists of smaller and shorter tubing components compared with the conventional circuit. The height of the venous reservoir was equal to that of the operating table, and vacuum-assisted venous drainage was used to maintain a negative pressure of less than -20 mm Hg. The automatic alarm level was set at 20 mL. Moreover, we did not use routine conventional ultrafiltration (CUF) or modified ultrafiltration (MUF) in the miniaturized CPB circuit; instead, we routinely gave diuretics to drain excess water through the kidneys, reducing the retention of sodium and water in the body. However, for children who required PLT and cryoprecipitate transfusion, MUF was conducted to provide volumetric space after separation from CPB. If the liquid level of the venous blood reservoir was high or the operation time was short and the urine level in the CPB was low, CUF was selectively initialized.

As for the priming liquid, in conventional CPB, we routinely used lactated Ringer solution, a liquid suspension of red blood cells (1–1.5 IU), 50 mL of fresh frozen plasma or 50 mL of human albumin, and priming drugs including ulinastatin (10,000 IU/kg), heparin (2500 IU), and furosemide (injection of 1 mg/kg, maximum 10 mg). In the miniaturized CPB, we used a multiple electrolyte solution, and priming drugs including ulinastatin (10,000 IU/kg) and heparin (1500–2000 IU). Furosemide (1 mg/ kg, maximum 10 mg/time) was intravenously injected before disinfection for surgery. Moreover, the perfusionist determined whether to prime red blood cells according to the preoperative HCT. The parameters of the custom-made circuit used in the operation, the corresponding priming volumes and preoperative HCT threshold of priming blood according to different body weights are shown in Supplemental Table 1, http://links.lww. com/MD/D611.

For children with satisfactory blood pressure and sufficient blood volume before CPB, we chose to use the RAP technique based on miniaturized CPB.^[13,14] When the activated clotting time reached 300 seconds, the arterial cannula was placed in the aorta, and the venous cannulas were placed in the superior and inferior vena cava. Then, the priming fluid for the CPB circuit (including the priming fluid in the oxygenator) was slowly

replaced with the patient's blood by retrograding the autologous arterial blood from the arterial line drainage and releasing the autologous venous blood from the venous line drainage. The retrograde priming procedure took approximately 2 minutes.

2.5. Statistical analysis

Continuous variables were described using the mean \pm standard deviation or median and interquartile range, and categorical variables were described using numbers and frequencies (%). Group differences for all categorical data were compared using a Chi-squared test or Fisher exact test, and all continuous data were compared between 2 groups using a *t* test or Wilcoxon rank-sum test.

To make outcomes comparable, we conducted propensity score matching. Patient sex, age at surgery, preoperative weight, preoperative pneumonia, STAT categories, CPB time, aortic cross-clamp time, and operation time were considered covariates for estimating the propensity score. The propensity score matching process was performed using the nearest method, with the number of patients in each group matched 1:1. The caliper was set to 0.005. The balance of risk factors was judged by comparing 2 groups using the matched data after propensity score matching.

We further conducted a multivariable stratified analysis after propensity score matching for the positive outcomes, according to the patient weight and STAT categories. Adjusted odds ratios (ORs) with 95% confidence intervals (CIs) were estimated using multivariable logistic regression models for categorical outcomes. Coefficients and t values were estimated using multivariable linear regression models for continuous outcomes.

A cut-off *P*-value of <.05 was considered statistically significant, and all *P*-values were 2-tailed. All data were analyzed using Stata software version 12.1 (StataCorp LP, College Station, TX).

3. Results

A total of 1111 patients were included in the analysis. The descriptive statistics and analysis of patients are shown in Table 1. Of these children, 355 (32.0%) underwent RAP, and

Table 1

Distribution of patient characteristics before and after matching in the RAP and non-RAP gro	ups.
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Characteristics	Before matching (n = 1111)			After matching (n=638)		
	RAP (n=355)	Non-RAP (n = 756)	P-value	RAP (n=319)	Non-RAP (n = 319)	P-value
Sex						
Male	191 (53.8)	387 (51.2)	.416	168 (52.7)	164 (51.4)	.751
Female	164 (46.2)	369 (48.8)		151 (47.3)	155 (48.6)	
Age, mo	9.3 (4.3-20.1)	5.4 (2.5-12.1)	<.001	8.3 (4.1-19.0)	7.5 (3.6–17.8)	.593
Preoperative weight, kg	6.8 (5.0-9.2)	6.1 (4.6-8.4)	<.001	7.2 (5.3–9.6)	7.0 (5.4–9.3)	.461
Preoperative pneumonia	26 (7.3)	122 (16.1)	<.001	26 (8.2)	24 (7.5)	.768
STAT categories						
Category 1	268 (75.5)	487 (64.4)	<.001	232 (72.7)	235 (73.7)	.267
Category 2	60 (16.9)	129 (17.1)		60 (18.8)	48 (15.1)	
Category ≥3	27 (7.6)	140 (18.5)		27 (8.5)	36 (11.3)	
CPB time, min	67 (54-87)	70 (55–91)	.116	69 (55-88)	66 (51-83)	.148
ACC time, min	33 (24–47)	37 (27-50)	<.001	34 (25–48)	34 (25-46)	.596
Operation time, min	130 (112–155)	135 (119–160)	.006	130 (113–158)	130 (115–155)	.981

ACC=aortic cross-clamp, CPB=cardiopulmonary bypass, RAP=retrograde autologous priming, STAT=The Society of Thoracic Surgeons-European Association for Cardiothoracic Surgery. *Data are presented as n (%) for categorical variables and mean ± standard deviation or median (interquartile range) for continuous variables.

 Table 2

 Outcomes after propensity score matching in the RAP and non-RAP groups.

Outcomes	RAP	Non-RAP	P-value
Bloodless priming rate, n (%)	195 (61.1)	164 (51.4)	.013
Ultrafiltration rate, n (%)	12 (3.8)	31 (9.7)	.003
Postoperative HCT, %	35.2±16.8	35.3±5.0	.920
Postoperative MV time, min	4.3±16.8	9.0±27.6	<.001
24 h blood loss, mL	57.6±36.5	61.2 <u>+</u> 36.4	.435
LOS in the ICU, d	0.9 (0.9–1.9)	1.7 (0.9–1.9)	<.001
LOS in hospital, d	5 (5-7)	6 (5-8)	<.001
Hospital mortality, n (%)	1 (0.3)	2 (0.6)	.563

HCT=hematocrit, ICU=intensive care unit, LOS=length of stay, MV=mechanical ventilation, RAP=retrograde autologous priming.

756 (68.0%) were in the non-RAP group. There were significant differences in patient age, preoperative weight, preoperative pneumonia, STAT categories, aortic cross-clamp time and operation time (P < .05) between the RAP and non-RAP groups. After propensity score matching, there were a total of 638 patients, with 319 patients in each group. The median age was 7.9 (3.7–18.1) months, and the median preoperative weight was 7.1 (5.4–9.5) kg. There were no significant differences in the compared characteristics (P > .05).

The outcomes in the 2 groups after matching are shown in Table 2. We found that the bloodless priming rate in the RAP group was significantly higher than that in the non-RAP group (61.1% vs 51.4%, P = .013). However, the ultrafiltration rate in the RAP group was significantly lower than that in the non-RAP group (3.8% vs 9.7%, P = .003). Compared with patients in the non-RAP group, patients in the RAP group had a shorter postoperative mechanical ventilation time (4.3 minutes vs 9.0 minutes, P < .001), a shorter length of stay in the ICU (0.9 days vs 1.7 days, P < .001) and the hospital (5 days vs 6 days, P < .001). No differences were noted in postoperative HCT (P = .920), postoperative 24-hour blood loss (P = .435) and hospital mortality (P = .563).

Table 3

Multivariable stratified analysis for categorical positive outcomes after propensity score matching using multivariable logistic regression models.

Categorical outcomes	OR	95% CI	P-value
Weight $\leq 4 \text{ kg}^*$			
Bloodless priming	3.29	0.85-12.74	.085
Ultrafiltration	0.41	0.06-2.81	.362
Weight >4 kg*			
Bloodless priming	1.60	1.07-2.39	.023
Ultrafiltration	0.31	0.13-0.71	.006
STAT category $<3^{\dagger}$			
Bloodless priming	1.67	1.09-2.58	.019
Ultrafiltration	0.26	0.10-0.69	.007
STAT category $\geq 3^{\dagger}$			
Bloodless priming	3.00	0.77-11.69	.114
Ultrafiltration	0.55	0.14-2.22	.403

 $\mbox{Cl=confidence}$ interval, $\mbox{OR=odds}$ ratio, $\mbox{STAT=The}$ Society of Thoracic Surgeons-European Association for Cardiothoracic Surgery.

* Adjusted for patient sex, age at surgery, preoperative pneumonia, STAT categories, cardiopulmonary bypass time, aortic cross-clamp time, and operation time.

[†] Adjusted for patient sex, age at surgery, preoperative weight, preoperative pneumonia, cardiopulmonary bypass time, aortic cross-clamp time, and operation time.

To determine whether the RAP technique was an independent factor of surgical outcomes and the effect of RAP in different populations, we performed a multivariable stratified analysis using multivariable regression models.

Interestingly, as shown in Tables 3 and 4, in the stratified analysis according to patient weight, the RAP technique was an independent factor of bloodless priming (OR, 95% CI: 1.60, 1.07–2.39; P=.023) and ultrafiltration (OR, 95% CI: 0.31, 0.13–0.71; P=.006) when the patient weight was >4. Compared with patients in the non-RAP group, patients in the RAP group had a significantly shorter postoperative mechanical ventilation time (P=.005) and a shorter length of stay in the ICU (P=.002) and the hospital (P=.003) when the patient weight was >4. However, when the patient weight was ≤ 4 , there was no significant difference between the 2 groups in terms of bloodless priming, ultrafiltration, postoperative mechanical ventilation time, or length of stay in the ICU or the hospital (P>.05).

Moreover, in the stratified analysis according to STAT categories, similar interesting results were found. The RAP technique was an independent factor of bloodless priming (OR, 95% CI: 1.67, 1.09–2.58; P = .019) and ultrafiltration (OR, 95% CI: 0.26, 0.10–0.69; P = .007) when the STAT category was <3. Compared with patients in the non-RAP group, patients in the RAP group had a shorter postoperative mechanical ventilation time (P < .001) and a shorter length of stay in the ICU (P < .001) and the hospital (P = .002) when the STAT category was <3. However, when the STAT category was ≥3, no significant difference was noted in terms of the categorical outcomes or continuous outcomes between the 2 groups (P > .05).

4. Discussion

In conventional CPB, it is usually necessary to use stock red blood cells, whole blood or other blood products to maintain a safe HCT and colloid osmotic pressure to ensure adequate oxygen

Table 4

Multivariable stratified analysis for continuous positive outcomes after propensity score matching using multivariable linear regression models.

Continuous outcomes	β	t	P-value
Weight \leq 4 kg *			
Postoperative MV time, min	-5.00	-0.79	.436
LOS in the ICU, d	-0.79	-1.54	.130
LOS in the hospital, d	-1.03	-0.76	.449
Weight >4 kg $*$			
Postoperative MV time, min	-4.49	-2.79	.005
LOS in the ICU, d	-0.55	-3.05	.002
LOS in the hospital, d	-2.14	-2.94	.003
STAT category $<3^{\dagger}$			
Postoperative MV time, min	-4.41	-3.52	<.001
LOS in the ICU, d	-0.58	-3.50	<.001
LOS in the hospital, d	-2.27	-3.12	.002
STAT category $\geq 3^{\dagger}$			
Postoperative MV time, min	-2.14	-0.18	.861
LOS in the ICU, d	0.12	0.15	.881
LOS in the hospital, d	1.28	0.86	.394

ICU=intensive care unit, LOS=length of stay, MV=mechanical ventilation, STAT=The Society of Thoracic Surgeons-European Association for Cardiothoracic Surgery.

* Adjusted for patient sex, age at surgery, preoperative pneumonia, STAT categories, cardiopulmonary bypass time, aortic cross-clamp time, and operation time.

[†]Adjusted for patient sex, age at surgery, preoperative weight, preoperative pneumonia, cardiopulmonary bypass time, aortic cross-clamp time, and operation time.

delivery capacity and reduce edema of the organs and tissues. However, blood transfusion will trigger a series of inflammatory reactions, increasing the risk of organ dysfunction, especially for lung and right ventricular function.^[16] Blood transfusion also brings the risk of early postoperative hyperthermia and allergies, which affect the prognosis of children.^[17,18] Therefore, achieving bloodless priming and reducing priming volume has always been the goal of CPB. In this study, we used RAP based on the miniaturized CPB to further reduce priming volume and increase bloodless priming. Our results demonstrated that patients underwent RAP had a higher bloodless priming rate, a lower ultrafiltration rate, a shorter postoperative mechanical ventilation time, and a shorter length of stay in the ICU and the hospital compared with patients who did not undergo RAP for children with simple congenital heart surgery or whose weight was more than 4 kg.

Miniaturized CPB systems demonstrated significant benefits vs conventional CPB systems in the reduction of the transfusion requirements, and the resultant intact microcirculation.^[8,19] Pereira et al suggested that mini CPB was beneficial in reducing the transfusion of packed red blood cells and achieving higher levels of HCT.^[11] Moreover, it has been proposed that the RAP technique could further reduce the priming volume by using autologous blood instead of stock blood.^[14] Boettcher et al demonstrated that asanguineous priming of miniaturized pediatric CPB circuits for congenital heart surgery may help to avoid blood transfusions in patients on CPB and reduce the transfusion requirements and transfusion-related morbidity.^[20]

Clinically, red blood cells are generally infused when hemoglobin is <70 g/L or the HCT is <22, and albumin (instead of plasma) is infused when the colloid osmotic pressure is <13mm Hg. The immediate HCT of children with bloodless priming in the ICU was 34% (31-37%), and the minimum value was 28%. This means that children with bloodless priming can still reach a satisfactory HCT. Moreover, in our experience, different sizes of circuit systems are used according to the different perfusion flow needs of children with different body weights, so that the priming volume during CPB is significantly reduced, especially for children with low body weights or for newborns. Our priming volume was drastically reduced from the original 300 mL to only 65 mL for a newborn, and the bloodless priming rates were significantly improved in children treated with miniaturized CPB and RAP.

Conventional CPB often requires ultrafiltration to remove excess priming liquid, and MUF has been widely used in infant CPB since 1991.^[21] It is generally believed that MUF can concentrate blood, increase HCT and platelet count, reduce postoperative blood product infusion, improve postoperative lung function, and shorten the time of ventilator ventilation and the ICU stay.^[22-24] According to Harvey et al, in the United States, approximately 71% of children's heart centers used MUF in 2011.^[25] However, side effects of UF have gradually been recognized, mainly because the aortic cannula obstructs the aortic lumen, resulting in unstable hemodynamics in the systemic circulation and brain stealing, hypothermia, prolonged blood and noncellular endothelial contact time, and additional surgery time, as well as some rare serious complications such as ventricular fibrillation and air embolism.^[26] An important part of our CPB strategy is the selective use of ultrafilters. We reduce the priming volume via the miniaturized circuit and RAP technique to relieve blood dilution and provide furosemide intravenously before surgery to maintain the fluid balance through renal filtration. Moreover, the surgeon uses an aspirator to draw the crystal cardioplegia from the coronary sinus when the cardioplegia is infused during the operation and does not return it to the CPB cycle, reducing the dynamic priming of the CPB and further blood dilution.

The ultrafast track has been proven to be effective in reducing ventilator-associated pneumonia, stays in the ICU and hospital, and hospitalization costs.^[27,28] Shorter mechanical ventilation times not only require excellent anesthesia management but also require good hemodynamics and an internal environment. Therefore, the total mechanical ventilation time after surgery is regarded as one of the important indicators for the evaluation of postoperative respiratory function. In our study, patients who underwent RAP had a significantly shorter mechanical ventilation time compared with patients who did not undergo RAP. Thus, we believe that the choice of RAP based on miniaturized CPB can even promote the recovery of postoperative respiratory function for children with simple congenital heart surgery or whose weight was more than 4 kg.

There were some limitations that should be considered. First, our study was limited to a single-center database, the grouping was not random and the retrospective analysis had its inherent limitations in design. Second, our study lacked data of other complications and outcomes for renal function, inflammatory response and long-term outcomes; perhaps these factors can be examined in further studies.

5. Conclusion

The RAP technique based on miniaturized CPB system was safe and effective for children who underwent congenital heart surgery. The RAP technique can significantly reduce the priming volume, improve the rate of bloodless priming and reduce blood product application. It was also associated with a shorter postoperative mechanical ventilation time and shorter length of stay in the ICU and the hospital, especially for children with simple congenital heart diseases or heavy children. We hope that more large prospective studies focusing on long outcomes of the RAP technique will be published in the future and that the RAP technique can be widely promoted in children with congenital heart diseases.

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Author contributions

Baoying Meng and Keye Wu made substantial contributions to the study design, analysis, and drafting of the manuscript. Yuanxiang Wang and Yiqun Ding made substantial contributions to the study design and were involved in revising the manuscript. Sheshe Zhang and Xing Zhou participated in field investigation and data collection. All authors read and approved the final manuscript.

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