

Optimization of Anticoagulation Strategy During Cardiopulmonary Bypass in Neonates with Congenital Heart Disease: A Randomized Controlled Trial

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Purpose: Neonatal hemostatic immaturity complicates anticoagulation during cardiopulmonary bypass (CPB). Current practice largely follows the strategy of 400 U/kg from adult protocols, as neonatal-specific guidelines and high-quality evidence remain lacking, which frequently fails to achieve adequate anticoagulation. This single-center randomized controlled trial evaluated the initial heparin dose of 600 U/kg to refine neonatal CPB anticoagulation.

Patients and Methods: In this prospective, randomized, controlled, single-blind trial, 76 neonates undergoing CPB were assigned to receive an initial heparin dose of either 600 U/kg ($n = 38$) or 400 U/kg ($n = 38$). The primary endpoint was the pre- to post-CPB change in prothrombin fragment 1+2 ($\Delta F1+2$). Secondary endpoints included the proportion of neonates achieving the ACT threshold after the initial heparin bolus, perioperative coagulation measures, postoperative blood loss, transfusion requirements, and adverse clinical outcomes.

Results: Mean $\Delta F1+2$ was 0.70 vs 4.08 nmol/L in the 600 and 400 U/kg groups, respectively (treatment-minus-control mean difference, -3.38 nmol/L; 95% confidence interval, -4.58 to -2.18 ; $P < 0.001$). ACT target achievement rate after the initial dose was higher in the 600 U/kg group (84.2% vs 44.7%, $P < 0.001$). Surgical preparation time was shorter (median 31.50 vs 36.50 mins, $P = 0.028$), and supplemental heparin use was lower, while total heparin dose did not differ significantly. Secondary endpoints showed lower 72-hour chest drainage and albumin use and shorter mechanical ventilation duration ($P = 0.036$, 0.028, and 0.031, respectively). Red-cell, plasma, and platelet transfusion requirements and most other clinical outcomes did not differ significantly. One in-hospital death occurred with 600 U/kg and none with 400 U/kg. Complete-case sensitivity analysis supported the primary finding.

Conclusion: An initial heparin dose of 600 U/kg in neonatal CPB was associated with a smaller F1+2 increase and greater initial ACT response. Larger studies are needed to establish clinical benefit and safety.

Clinical Trial Registry Number: ChiCTR2300069157.

Keywords: anticoagulation, cardiopulmonary bypass, congenital heart disease, heparin, neonates



Introduction

Cardiopulmonary bypass (CPB) is indispensable in neonatal open-heart surgery. During CPB, contact between circulating blood and the non-endothelialized surfaces of the extracorporeal circuit, together with surgical tissue injury, can activate coagulation and promote thrombin generation. In addition, CPB is associated with complex inflammatory and hemostatic disturbances. Effective anticoagulation is therefore a key component of neonatal CPB management, aiming to limit coagulation activation and circuit thrombosis while avoiding excessive anticoagulation and bleeding.^{1,2} Unfractionated heparin remains the most commonly used anticoagulant during CPB. Its anticoagulant effect depends on antithrombin; therefore, differences in patients' antithrombin activity may influence their response to heparin.^{1,2}

Neonates exhibit a developmentally distinct hemostatic system characterized by age-appropriate differences in coagulation factor and anticoagulant protein levels, including lower circulating concentrations of prothrombin, antithrombin III (AT-III), and other coagulation factors compared with adults.³ A pharmacokinetic study in preterm neonates also reported faster heparin clearance and a shorter plasma half-life than in adults.⁴ Another study involving infants found that lower baseline antithrombin activity was associated with lower anti-Xa activity after heparin administration and higher intraoperative heparin requirements.⁵ These findings suggest that evaluation of neonatal heparin dosing strategies should take developmental hemostasis and measured anticoagulant responses into account.

In this context, neonatal-specific anticoagulation strategies warrant further evaluation. The 2025 AmSECT Standards and Guidelines for Pediatric and Congenital Perfusion Practice emphasize patient-specific initial heparin dosing and anticoagulation testing to guide supplementation, without specifying a universal neonatal initial dose or activated clotting time (ACT) threshold.⁶ Before this trial, our center routinely administered an initial systemic dose of 400 U/kg of unfractionated heparin before CPB, with a target ACT >480 s before and throughout CPB and additional heparin if the target was not reached, in accordance with the 2019 NATA guidelines.⁷ However, this initial-dose strategy does not ensure that all neonates achieve the intended ACT target. In our published retrospective study, 61.5% of neonates failed to reach the target ACT threshold after an initial 400 U/kg bolus.⁸ Supplemental heparin may still be required during CPB even when the ACT target has been reached before CPB initiation.⁶ These observations support further evaluation of initial heparin dosing strategies in neonates.

In addition to the ACT response after the initial bolus, evaluation of anticoagulation strategies should consider their effects on thrombin generation. ACT remains the principal method of anticoagulation monitoring during CPB, but it measures whole-blood clotting time and does not directly quantify *in vivo* thrombin generation.¹ Besides, neonates had lower heparin anti-Xa activity during CPB and higher post-CPB markers of thrombin generation and activity than older children.⁹ These observations support assessment of *in vivo* thrombin generation alongside ACT monitoring. Prothrombin fragment 1+2 (F1+2) is a biochemical marker of prothrombin activation that reflects *in vivo* thrombin generation;¹ its pre- to post-CPB concentration change can provide complementary information for comparing anticoagulation strategies.¹

Our prior pilot study—a small, prospective, randomized trial in 30 neonates undergoing elective CPB—explored the effects of different initial heparin doses (400 U/kg, 500 U/kg and 600 U/kg). Results suggested that 600 U/kg achieved superior ACT target attainment, providing preliminary support for dose selection in the present study ([Supplementary Tables 1–4](#)). However, the pilot study was small and did not directly compare 600 U/kg with our center's conventional 400 U/kg initial dose. Whether a 600 U/kg initial dose produces a smaller CPB-related increase in F1+2 than a 400 U/kg initial dose therefore remains to be evaluated.

To address this question, the present single-center randomized controlled trial compared 600 U/kg and 400 U/kg initial heparin dosing strategies, both followed by ACT-guided supplementation. The primary effect measure was the between-group difference in the mean change in F1+2 concentration (post-CPB minus pre-CPB). We hypothesized that the 600 U/kg initial-dose strategy would result in a smaller mean increase in F1+2 than the 400 U/kg strategy. ACT response after the initial bolus, supplemental heparin requirements, bleeding, transfusion, and other clinical outcomes were evaluated as secondary outcomes.

Materials and Methods

Study Design and Participants

This single-center, prospective, randomized, controlled, single-blind clinical trial evaluated the efficacy and safety of an initial heparin dose of 600 vs 400 U/kg, each followed by ACT-guided supplementation, during neonatal CPB. The study was conducted in accordance with the CONSORT (Consolidated Standards of Reporting Trials) guidelines to ensure methodological rigor, transparency, and reproducibility.¹⁰ Ethical approval was obtained from the institutional ethics committee of Fuwai Hospital (No. 2022–1830), and the trial was registered with the Chinese Clinical Trial Registry (ChiCTR2300069157). Written informed consent was obtained from the parents or legal guardians of all participants prior to study commencement, enrollment, randomization, and any study-related procedures. The study was conducted in accordance with the Declaration of Helsinki and its subsequent amendments.¹¹

This superiority trial was conducted between February 2023 and December 2024. 76 neonates were randomized 1:1 to receive either 600 U/kg heparin (treatment group) or 400 U/kg (control group). Inclusion criteria: Age ≤ 28 days with congenital heart disease (CHD); American Society of Anesthesiologists (ASA) class I–IV; written informed consent provided by the parents or legal guardians. Exclusion criteria: Pre-operative coagulopathy; severe hepatic, renal, or neurological disorders; pre-operative extracorporeal life support; unknown blood type or incomplete coagulation data. Elimination criteria: Patients requiring extracorporeal membrane oxygenation (ECMO) within 24 h post-operatively.

Randomization and Blinding

The random allocation sequence was generated by a researcher in the Statistics Department of the National Center for Cardiovascular Diseases using a computer-based randomization system developed by the department. Simple randomization was used without block or stratification. One of the authors assessed eligibility and obtained written informed consent from the parents or legal guardians. Only after eligibility had been confirmed and informed consent obtained did the researcher obtain the participant's treatment allocation through the randomization system. Personnel responsible for recruitment could not access the random allocation sequence or subsequent treatment assignments before allocation. Thus, allocation was concealed until the participant's group assignment was obtained. Patients together with their parents, surgeons, ICU physicians, and personnel responsible for recording and assessing study outcomes were unaware of treatment allocation. Laboratory personnel who measured F1+2 were also unaware of allocation, whereas anesthesiologists and perfusionists were unblinded due to their direct role in drug administration and anticoagulation management. The researcher who obtained treatment allocation was not involved in outcome assessment. The original statistical analyses were performed by researchers in the Department of Medical Statistics using coded treatment groups; the analysts did not know which code corresponded to 400 or 600 U/kg.

Intervention

The randomized intervention was the initial systemic heparin bolus dose, 600 or 400U/kg. In both groups, the ACT target for CPB initiation and maintenance was >480 s during CPB. If the target was not achieved after the initial bolus, supplemental heparin was administered after collection of the pre-CPB F1+2 blood sample, in 200 U/kg increments and ACT was reassessed until the clinical target was reached. Perioperative care and surgical protocols were otherwise standardized.

Endpoints

The primary endpoint was the change in F1+2 concentration, defined as post-CPB minus pre-CPB F1+2 (Δ F1+2, nmol/L). The pre-CPB F1+2 blood sample was collected within 5 mins after administration of the randomly assigned initial heparin dose (400 or 600 U/kg), at the time of the first post-dose ACT measurement and before any supplemental heparin. If the initial ACT did not exceed the clinical target of 480 s, supplemental heparin was administered only after this blood sample had been collected and before CPB initiation. The post-CPB sample was collected at CPB termination before protamine administration. Secondary endpoints included changes in coagulation parameters before and after CPB, the ACT target achievement rate following the initial heparin dose, post-operative blood loss within 72 h, and

perioperative blood product utilization (including red blood cells, platelets, fresh frozen plasma, and albumin), as well as the incidence of major bleeding, in-hospital mortality, length of hospital stay, and other adverse outcomes. ACT served as the procedural safety threshold, whereas F1+2 assessed thrombin generation.

Definitions

Target achievement rate for the initial heparin dose was defined as an ACT >480 s measured within 5 mins after administration of the randomized dose. Post-operative major bleeding was defined as meeting any of the following criteria: (1) blood loss >10 mL/kg/hour within the first 24 h post-operatively, sustained for ≥ 1 h; (2) total blood or intravascular volume loss $\geq 25\%$ within 24 h; (3) intracranial hemorrhage or other life-threatening bleeding in critical organs (eg, lung, liver, gastrointestinal tract). Surgical preparation time was defined as the interval from skin incision to initiation of CPB. The Risk Adjustment for Congenital Heart Surgery (RACHS-1) score was assigned according to the criteria proposed by Jenkins et al¹² The vasoactive-inotropic score (VIS) was calculated as defined by Gaies et al¹³ Post-operative infection was diagnosed based on positive bacterial/fungal cultures or viral nucleic acid tests within 30 days post-operatively. The neonatal sequential organ failure assessment (nSOFA) score was calculated according to the University of Florida criteria.¹⁴ Acute kidney injury (AKI) was diagnosed according to KDIGO criteria,¹⁵ based on elevated serum creatinine. Post-operative liver injury was defined as a simultaneous increase in both alanine aminotransferase and aspartate aminotransferase to more than twice the pre-operative baseline within 7 days post-operatively.

Anesthesia, CPB, and Surgical Protocol

All patients underwent median sternotomy under general anesthesia. Induction included sevoflurane inhalation, sufentanil, and cisatracurium, followed by tracheal intubation and mechanical ventilation. Anesthesia was maintained with sufentanil, dexmedetomidine infusion, and sevoflurane inhalation. Three senior pediatric cardiac surgeons performed all procedures.

CPB was conducted with a roller pump (Stockert S5, Sorin, Italy) using a neonatal circuit (1/4" tubing, FX05 oxygenator, ultrafiltration). Prime volume (250–350 mL) included packed red blood cells or colloid, heparin (1000 U), magnesium sulfate, sodium bicarbonate, albumin, and furosemide. Myocardial protection was achieved with HTK solution (40–60 mL/kg). Flow rates were weight- and temperature-adjusted to maintain mean arterial pressure at 30–50 mmHg and hemoglobin ≥ 70 g/L during CPB; before weaning, patients were rewarmed to 36 °C, hemoglobin optimized to ≥ 100 g/L, and modified ultrafiltration performed. Heparin (1000 IU) added to the CPB priming solution was not included in total heparin dose calculations (U/kg), as it was used solely for circuit anticoagulation and not weight-adjusted. Total systemic heparin dose was defined as the initial randomized bolus plus all supplemental systemic doses administered before and during CPB; it excluded circuit-priming heparin. Protamine was administered at the end of CPB for heparin reversal according to the total heparin dose, using a standard weight-based ratio (1:100).

Sample Size

Based on prior data, the expected mean F1+2 difference was 0.09 ± 0.14 nmol/L in the high-dose group vs 0.17 ± 0.14 nmol/L in the control group. The calculation used an assumed between-group difference of 0.10 nmol/L, which incorporated clinical judgment and a SD of 0.14 nmol/L. With 80% power and $\alpha = 0.025$ (one-sided), 32 patients per group were required. The one-sided significance level addressed the superiority hypothesis that the 600 U/kg initial-dose strategy would produce a smaller mean F1+2 increase than the 400 U/kg strategy. Allowing for 10% attrition, 36 patients per group (total $n = 72$) were enrolled. 76 neonates were subsequently randomized.

Statistical Analysis

Continuous variables were expressed as mean $\pm$ SD or median (IQR) and compared using *t*-tests or Mann–Whitney *U*-tests. Categorical variables were expressed as counts and percentages and compared with χ^2 or Fisher's exact tests. The primary analysis followed the intention-to-treat (ITT) principle.¹⁶ Two randomized participants who required ECMO within 24 h postoperatively met the elimination criteria, therefore their F1+2 was not tested. Therefore, paired measurements were available for 74 participants, with one missing change value in each group. Under a missing-at-random

(MAR) assumption conditional on the observed predictors, missing $\Delta F1+2$ values were imputed separately within each randomized group using predictive mean matching.¹⁷ Pooled group means and the between-group mean difference were reported with two-sided 95% confidence intervals (CIs). The mean difference was retained to estimate the average $\Delta F1+2$ on the original concentration scale despite skewness. To examine specified departures from MAR, 5, 10, or 20 nmol/L was added to the missing treatment-group $\Delta F1+2$ value in each imputed dataset, while the control-group imputation was left unchanged, and the mean contrast was pooled again. Superiority was evaluated using the upper limit of the two-sided 95% CI for the treatment-minus-control mean difference in $F1+2$ change (post-CPB minus pre-CPB), with an upper limit below 0 nmol/L corresponding to the one-sided 0.025 criterion.¹⁸ And an unadjusted between-group mean contrast was used to estimate the average effect of the randomized initial-dose strategies on the original $F1+2$ concentration scale. Sensitivity to skewness was assessed in the 74 complete cases using Welch's *t*-test and a nonparametric bootstrap with 9999 resamples drawn separately within each group and percentile 95% CIs. Secondary analyses used available observations from the randomized cohort, without imputation or exclusion of an entire participant because another measurement was missing. No adjustment for multiple testing was applied to secondary outcomes or time-point comparisons. These findings were interpreted as exploratory. ACT readings of 999 s represented ACT ≥ 999 s. Between-group comparisons at individual time points used Log rank tests, treating ceiling readings as right-censored and lower readings as exact.¹⁹ To assess the robustness of the primary ITT result, a complete-case sensitivity analysis was performed in the 74 participants. Analyses were performed using SPSS 26.0; $P < 0.05$ was considered statistically significant.

Results

Baseline

From February 2023 to December 2024, 82 neonates who scheduled to undergo elective cardiac surgery were screened, 6 were excluded for meeting exclusion criteria. Therefore, 76 neonates were randomized, 38 to each initial-dose strategy; two patients were excluded from sensitivity analysis due to ECMO requirement within 24 h post-operatively, but both were retained in the primary intention-to-treat analysis (Figure 1). Therefore, paired $F1+2$ data were available for 37 patients per group; the remaining two $\Delta F1+2$ values were multiply imputed. Baseline demographics, pre-operative laboratory results, and CHD types showed no statistically significant differences between the two groups (Table 1 and Supplementary Table 5).

Intraoperative Data

CPB duration did not differ significantly; however, surgical preparation time was shorter in the trial group, 31.50 (24.00, 39.00) vs 36.50 (30.25, 44.75) min, respectively ($P = 0.028$). Intraoperative red blood cell (RBC) transfusion volumes did not show evidence of a group difference ($P = 0.799$) (Table 2).

The ACT attainment rate after the initial heparin bolus was significantly higher in the treatment group (84.2% vs 44.7%, $P < 0.001$). The risk ratio is 1.88 (95% CI 1.29 to 2.75), and the absolute risk difference is 39.5 percentage points (95% CI 18.1 to 56.3). The median total heparin dose before CPB was 608.59 (600.00, 722.87) vs 600.00 (424.43, 652.90) U/kg ($P = 0.081$). Supplemental heparin use was lower before CPB ($P < 0.001$) and after CPB initiation ($P = 0.0498$) in treatment group. Despite different assigned initial doses, total systemic heparin during the entire CPB was 663.62 (600.00, 800.00) vs 640.87 (600.00, 826.75) U/kg ($P = 0.723$), and protamine doses did not show evidence of a group difference ($P = 0.154$) (Table 2).

Coagulation Parameters

Pre-CPB $F1+2$ levels distributions did not show evidence of a group difference ($P = 0.875$). Post-CPB $F1+2$ was 3.53 (2.82, 4.23) nmol/L in the treatment group vs 5.77 (3.81, 9.46) nmol/L in controls ($P < 0.001$). Post-CPB APTT were 64.60 (57.70, 71.70) s in the treatment group and 53.60 (46.70, 68.40) s in controls ($P = 0.003$). TT distributions also differed, with medians of 19.40 (17.90, 20.70) vs 18.25 (17.25, 19.55) s ($P = 0.021$). D-dimer was 1.76 (1.30, 4.40) vs

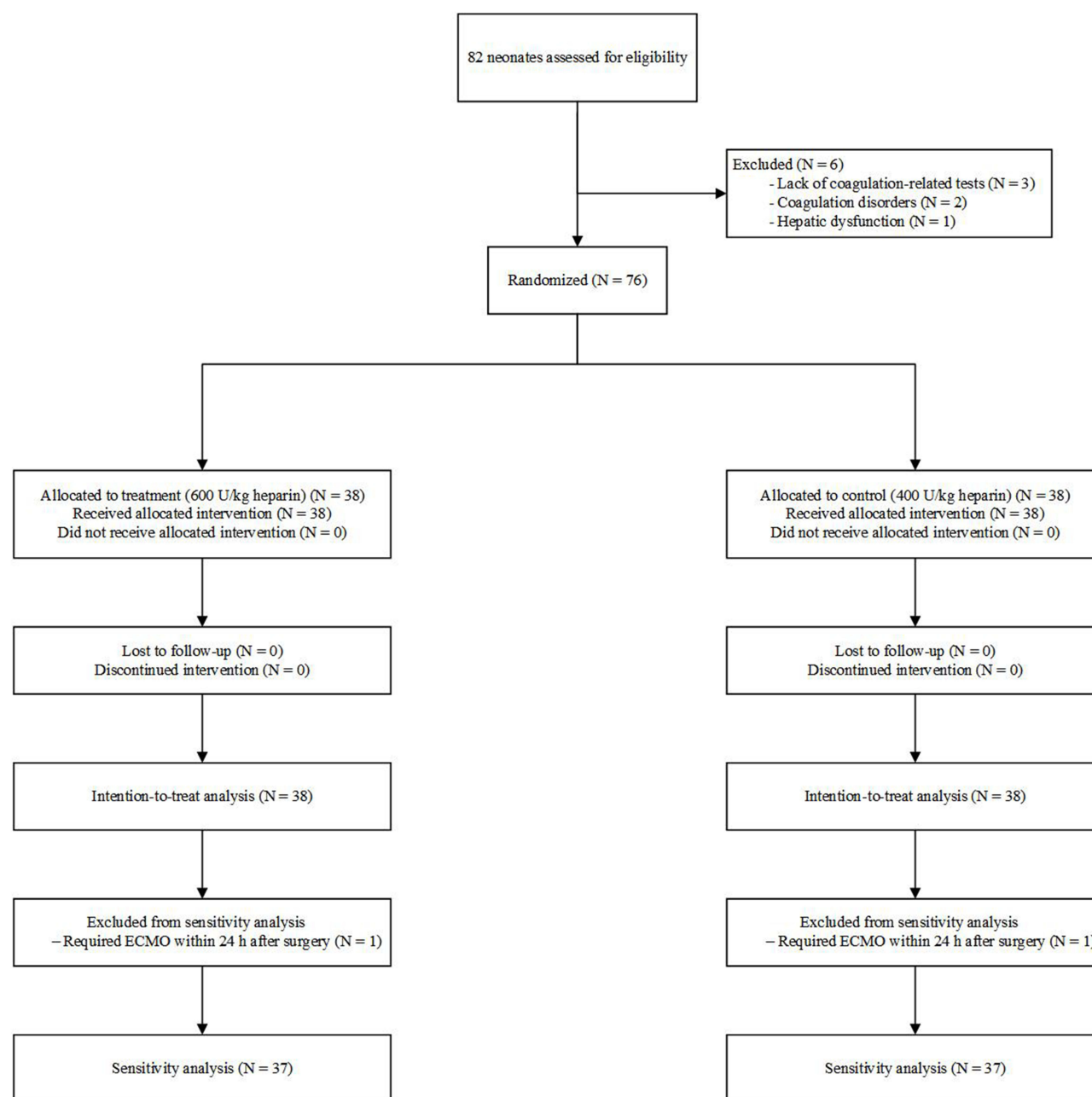


Figure 1 Flowchart of the study participants.

Abbreviation: ECMO: extracorporeal membrane oxygenation.

5.68 (2.73, 9.59) $\mu\text{g/mL}$ ($P = 0.002$), and FDP was 5.08 (3.27, 15.19) vs 10.29 (6.52, 22.10) $\mu\text{g/mL}$ ($P = 0.003$) (Figure 2). Other coagulation parameters were comparable between groups (Supplementary Table 6).

ACT differed between groups after the initial heparin dose ($P = 0.014$) and at the second measurement during CPB (CPB-T2; $P = 0.001$). There was no evidence of a between-group difference at the other four time points (all $P \geq 0.05$; Figure 2).

Post-Operative Bleeding, Transfusion, and Hemodynamics

The observed cumulative chest drainage over 72 h increased gradually in both groups and yielded a significant difference at 72 h ($P = 0.036$) (Supplementary Figure 1). Comparisons at 12, 24, and 48 h yielded $P = 0.299$, 0.173, and 0.076,

Table 1 Baseline Characteristics of Patients in Intention-to-Treat Analysis

Characteristic	Control 400 U/kg (N = 38)	Treatment 600 U/kg (N = 38)	P
Demographic characteristics			
Age (days)	11.50 (7.00, 16.50)	11.00 (7.00, 20.00)	0.913
Male sex, n/N (%)	22/38 (57.9%)	26/38 (68.4%)	0.476
Body surface area (m ²)	0.19 (0.18, 0.20)	0.19 (0.17, 0.21)	0.538
Gestational age (weeks)	38.00 (37.00, 39.00)	38.00 (37.00, 39.00)	0.879
RACHS-I score	4.00 (4.00, 4.00)	4.00 (4.00, 4.00)	0.152
Preoperative laboratory parameters			
RBC (10 ¹² /L)	4.06 (3.48, 4.49)	3.97 (3.60, 4.39)	0.795
WBC (10 ⁹ /L)	10.73 (8.09, 13.14)	9.81 (7.88, 11.64)	0.358
Albumin (g/L)	32.10 (30.90, 35.10)	32.85 (30.20, 34.27)	0.787
Total protein (g/L)	50.55 (44.05, 52.32)	47.95 (45.20, 53.00)	0.992
Creatinine (μmol/L)	42.72 (34.15, 55.92)	37.78 (27.97, 52.78)	0.201
ALT (U/L)	9.00 (7.00, 13.25)	12.50 (7.25, 18.75)	0.092
AST (U/L)	28.00 (22.25, 33.00)	30.00 (25.00, 46.25)	0.291
hs-CRP (mg/L)	0.24 (0.10, 1.22)	0.56 (0.12, 1.26)	0.279
Pre-CPB laboratory parameters			
Hb, pre-CPB blood gas (g/L)	120.50 (108.75, 135.00)	123.00 (114.50, 132.00)	0.669
Lactate, pre-CPB (mmol/L)	1.20 (1.00, 1.52)	1.10 (0.92, 1.30)	0.249
FI+2, pre-CPB (nmol/L)	2.82 (2.35, 3.15)	2.88 (2.34, 3.58)	0.875

Notes: Data are presented as median (IQR). Categorical variables are expressed as number (percentage).

Abbreviations: RACHS-I score, risk adjustment for congenital heart surgery-I Hb, hemoglobin; RBC, red blood cell count; WBC, white blood cell count; Alb, albumin; TP, total protein; Cr, creatinine; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Lac, lactate; hs-CRP, high-sensitivity C-reactive protein.

respectively. Albumin administered within 72 h was 2.62 (1.78, 3.48) vs 3.33 (2.34, 4.73) g/kg in the treatment and control groups, respectively ($P = 0.028$). Other blood product use within 72 h and during hospitalization was comparable ([Supplementary Table 7](#)). Platelet counts at PICU admission differed in the exploratory comparison ($P = 0.007$), with higher values in the treatment group ($P = 0.007$). No RBC or hemoglobin time-point comparison yielded $P < 0.05$ ([Figure 3](#)), while perioperative vasoactive–inotropic score (VIS) values were similar ([Supplementary Figure 2](#)).

Clinical Outcomes and Complications

One in-hospital death occurred in the treatment group (1/38, 2.6%), compared with none in controls (0/38; $P = 1.000$). Major bleeding and reoperation for bleeding each occurred in 0/38 treatment participants vs 1/38 controls ($P = 1.000$ for each). Recorded ventilation duration was 45.00 (26.00, 74.25) h in the treatment group vs 52.50 (42.75, 142.00) h in controls ($P = 0.031$). PICU stay and hospital stay had $P = 0.093$ and 0.116, respectively. AKI occurred in 5/38 (13.2%) vs 10/38 (26.3%) participants ($P = 0.249$). Liver injury occurred in 12/38 (31.6%) vs 15/38 (39.5%) ($P = 0.632$). Other observed clinical outcomes are reported in [Table 3](#).

Primary Endpoint

In the primary intention-to-treat analysis, the pooled mean Δ FI+2 was 0.70 nmol/L (95% CI 0.30–1.11) in the treatment group and 4.08 nmol/L (95% CI 2.94–5.22) in controls ([Table 2](#)). The treatment-minus-control mean difference was -3.38 nmol/L (SE 0.60; 95% CI -4.58 to -2.18 ; two-sided $P < 0.001$). The upper CI limit was below zero, favoring the 600 U/kg initial-dose strategy for this biochemical endpoint under the MAR assumption. When the missing treatment-group Δ FI+2 was shifted upward by 5, 10, or 20 nmol/L relative to its imputed value, the pooled mean differences were -3.25 (95% CI -4.47 to -2.03), -3.12 (-4.41 to -1.82), and -2.85 (-4.43 to -1.28) nmol/L, respectively; all three P values were < 0.001 . The primary direction persisted in these specified scenarios.

Table 2 Intraoperative Parameters in Intention-to-Treat Analysis

Characteristic	Control 400 U/kg (N = 38)	Treatment 600 U/kg (N = 38)	P
Surgical and CPB parameters			
CPB duration (min)	153.50 (133.25, 176.00)	137.00 (110.50, 166.00)	0.164
Aortic cross-clamp duration (min)	90.50 (74.50, 108.50)	82.50 (65.25, 100.75)	0.136
Operative duration (min)	262.50 (227.00, 310.50)	237.00 (195.25, 279.00)	0.133
Preparation duration (min)	36.50 (30.25, 44.75)	31.50 (24.00, 39.00)	0.028
Lowest NP temperature (°C)	28.00 (26.72, 28.87)	28.90 (27.02, 29.73)	0.158
Intraoperative fluid management			
CPB input (mL/kg)	180.15 (142.96, 251.78)	173.43 (128.78, 218.01)	0.228
Ultrafiltration volume (mL/kg)	173.11 (129.86, 264.22)	161.73 (114.68, 255.49)	0.763
Intraoperative input (mL/kg)	190.95 (157.33, 265.80)	182.14 (136.05, 226.67)	0.116
Intraoperative output (mL/kg)	33.01 (14.89, 56.19)	22.41 (11.29, 45.31)	0.250
RBC transfusion during CPB (mL/kg)	108.19 (73.63, 128.02)	98.39 (74.07, 128.02)	0.799
Anticoagulation and primary efficacy			
ACT target achievement rate, n/N (%)	17/38 (44.7%)	32/38 (84.2%)	<0.001
Systemic heparin before CPB (U/kg)	600.00 (424.43, 652.90)	608.59 (600.00, 722.87)	0.081
Heparin added before CPB (U/kg)	200.00 (0.00, 217.91)	0.00 (0.00, 0.00)	<0.001
Heparin added during CPB (U/kg)	0.00 (0.00, 200.00)	0.00 (0.00, 0.00)	0.0498
Total systemic heparin (U/kg)	640.87 (600.00, 826.75)	663.62 (600.00, 800.00)	0.723
Protamine dose (mg)	30.00 (25.00, 35.00)	30.00 (30.00, 40.00)	0.154
ΔF1+2 (nmol/L), mean (95% CI)	4.08 (2.94, 5.22)	0.70 (0.30, 1.11)	<0.001

Notes: Data are presented as median (IQR). Categorical variables are expressed as number (percentage).

Abbreviations: CPB, cardiopulmonary bypass; NP, nasopharyngeal; ACT, activated clotting time; ΔF1+2, change in prothrombin fragment 1 +2 concentration before and after CPB.

Sensitivity Analyses

In the sensitivity analysis excluding the two neonates who required ECMO within 24 h postoperatively (37 per group), the ΔF1+2 mean difference was -3.35 nmol/L (95% CI -4.56 to -2.14 ; $P < 0.001$), with a bootstrap 95% CI of -4.54 to -2.22 nmol/L. Initial ACT target rate was more frequent with 600 than 400 U/kg (86.5% vs 45.9%; $P < 0.001$). Pre-CPB preparation time and supplemental heparin before and during CPB were lower with 600 U/kg ($P = 0.028$, <0.001 , and 0.0484 , respectively). Mechanical ventilation duration was numerically shorter (median 45 vs 51 h), with $P = 0.064$. Other results were summarized in [Supplementary Tables 8–14](#) and [Supplementary Figures 3–6](#).

Discussion

In this single-center randomized controlled trial, an initial heparin dose of 600 U/kg was associated with a smaller mean increase in F1+2 from pre- to post-CPB than the 400 U/kg. Besides, a higher proportion of neonates also achieved ACT target after the initial bolus. Exploratory analyses showed lower 72-hour chest drainage and albumin use, as well as a shorter duration of mechanical ventilation, with no statistically increasing bleeding or adverse outcomes. These findings suggest that a higher initial heparin dose, followed by ACT-guided supplementation, may better limit prothrombin activation during CPB. However, the exploratory clinical findings require confirmation in larger studies.

Neonates with CHD possess unique physiological characteristics and cannot be regarded as “small adults”.³ Physiologically lower antithrombin levels in neonates are part of developmental hemostasis and do not, by themselves, indicate a pathological deficiency.³ However, during CPB, this developmental physiology is profoundly altered by hemodilution and circuit-related factors, complicating heparin pharmacodynamics and anticoagulation monitoring.^{1,5} Exposure of blood to the artificial circuit may contribute to coagulation activation, fibrinogen degradation, platelet activation, fibrinolysis, and the release of vasoactive and inflammatory mediators.^{1,20} Inadequate thrombin inhibition

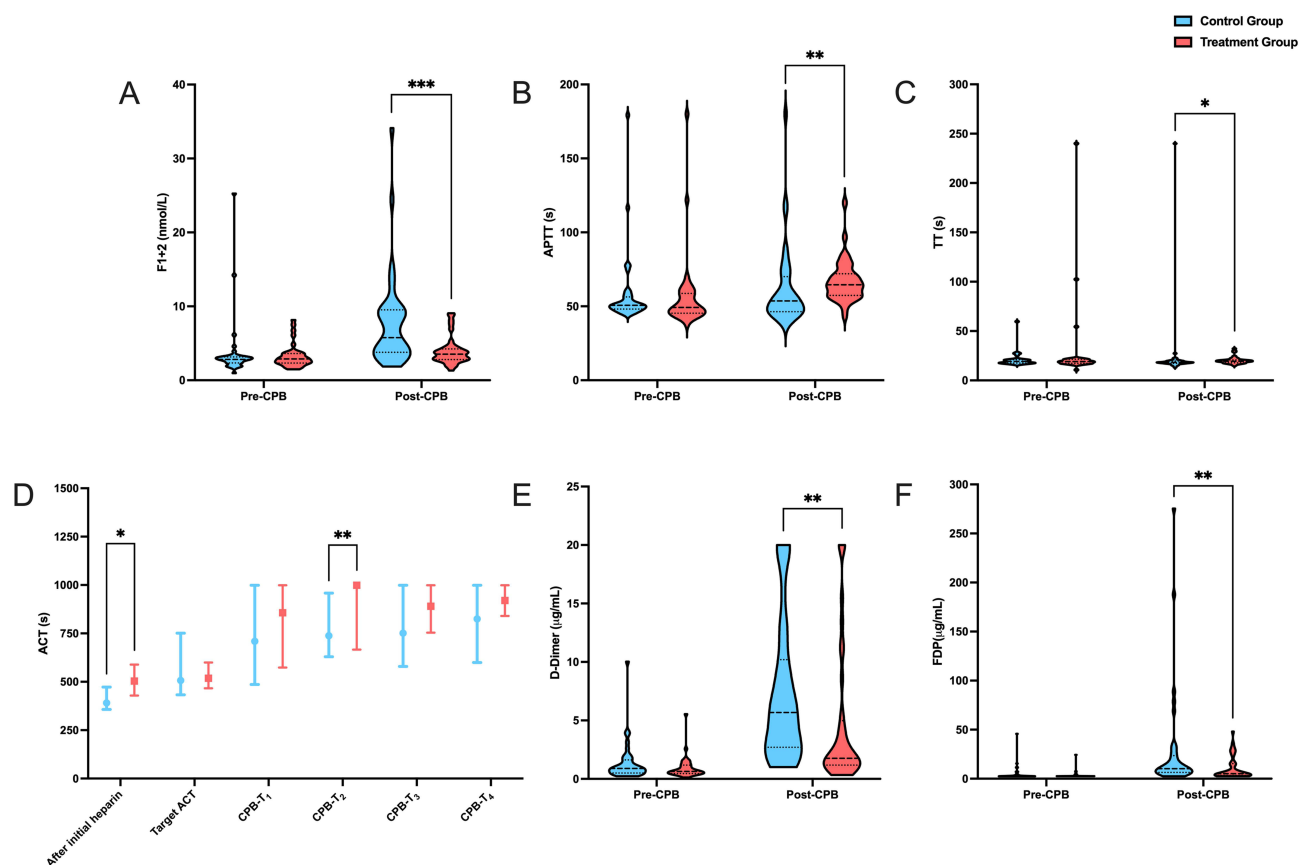


Figure 2 Comparison of coagulation and anticoagulation indicators between control and treatment groups before and after CPB. (A) Plasma levels of prothrombin fragment F1+2 (nmol/L). (B) Activated partial thromboplastin time (APTT, s). (C) Thrombin time (TT, s). (D) Activated clotting time (ACT, s) measured at six timepoints: after initial heparinization, at target ACT, and four intraoperative timepoints during CPB (CPB-T1 to CPB-T4). (E) D-dimer levels ($\mu\text{g/mL}$). (F) Fibrin degradation products (FDP, $\mu\text{g/mL}$). Data are presented as median (IQR). $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***)

during CPB has been linked to poor surgical outcomes.^{2,9} Therefore, anticoagulation management in neonatal CPB is both critically important and technically challenging.

Clinical researches and guidelines for anticoagulation in neonatal CPB remain scarce. The neonatal hemostatic balance is fragile and changes dynamically with maturation.³ Baseline AT-III activity is markedly lower in neonates—approximately 40% in our cohort—compared with adults, which may contribute to variability in heparin responsiveness. Low AT-III levels are associated with reduced heparin efficacy and higher intraoperative heparin requirements in infants undergoing CPB.⁵ Although perioperative AT-III supplementation has been explored, studies such as Panigada et al reported no consistent benefit in reducing heparin requirements or post-operative bleeding or thrombosis, and high costs limit widespread neonatal use.^{21,22}

Developmental differences may influence heparin response and dosing requirements in neonates.¹ In addition, many present with a prothrombotic state due to complex CHD,²³ compounded by circuit-induced coagulation activation.¹ These features provide a rationale for evaluating initial-dose strategies in neonatal CPB.

At our center, heparin is administered after vascular dissection and cannulation preparation, with CPB initiation contingent upon achieving the target ACT (>480 s). If the threshold is not reached, additional heparin is administered and ACT is rechecked. In this trial, the ACT attainment rate after the initial dose was 84.2% in the 600 U/kg group, which was accompanied by a shorter pre-CPB preparation interval compared with the conventional group. The shorter preparation time observed in the 600 U/kg group may attribute to improved procedural efficiency due to reduced need for repeated heparin administration and ACT testing. Notably, systemic heparin doses before CPB were 608.59 and 600.00 U/kg in the treatment and control groups, respectively. Total systemic heparin doses also did not differ significantly. Accordingly, these findings suggest a possible procedural advantage of the higher initial heparin dose.

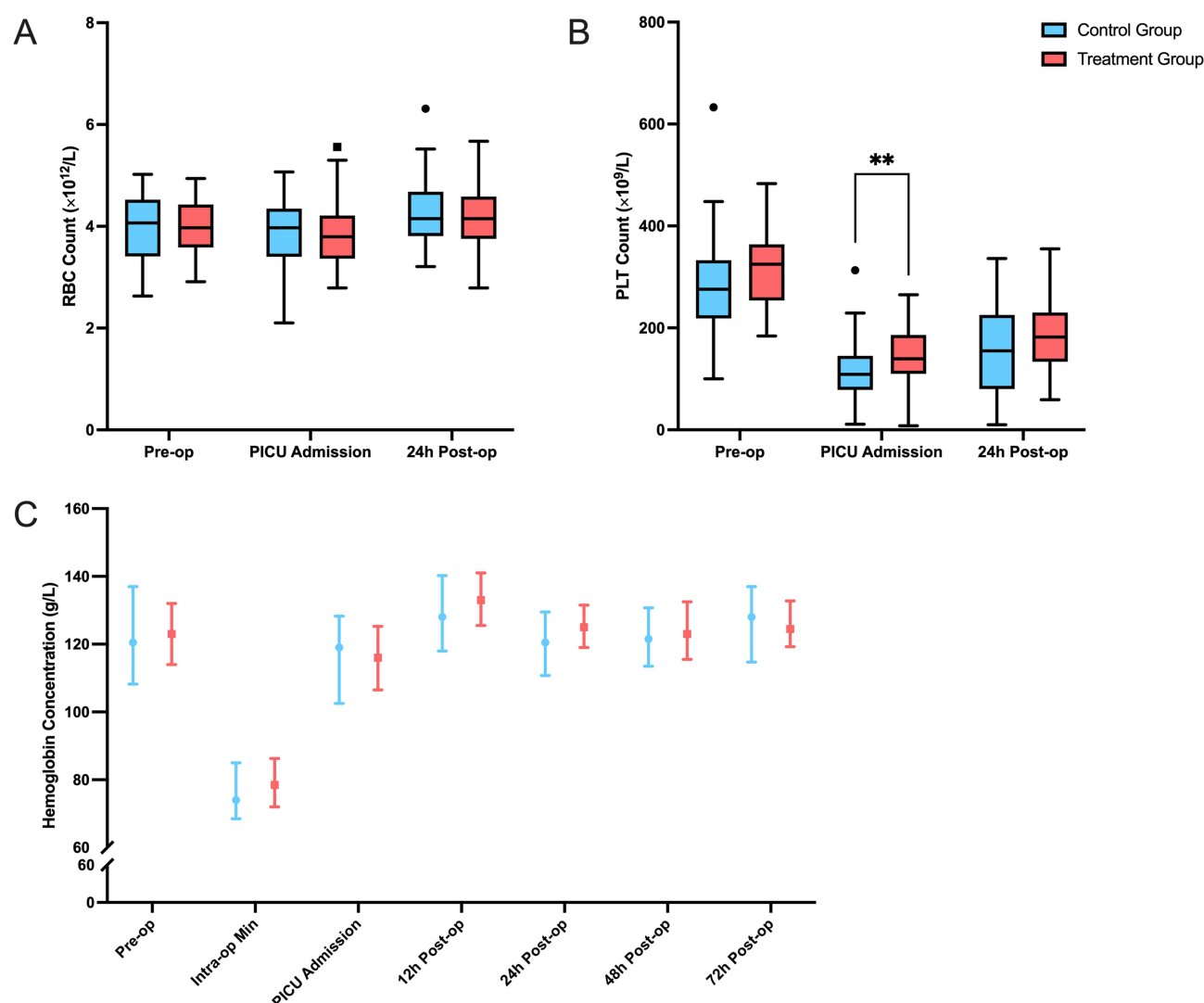


Figure 3 Changes in hematologic parameters between the control and treatment groups during the perioperative period. **(A)** RBC counts measured preoperatively, upon PICU admission, and at 24 h postoperatively showed no significant differences between groups. **(B)** Platelet counts at the same time points, with the treatment group demonstrating significantly higher counts at PICU admission. **(C)** Hemoglobin concentrations measured preoperatively, at the intraoperative minimum, upon PICU admission, and at 12 h, 24 h, 48 h, and 72 h postoperatively, showing similar trends between groups. Data are presented as median (IQR). $P < 0.01$ (**).

Because anticoagulation monitoring during CPB is intermittent, inadequate anticoagulation may go undetected until the next measurement. In our study, the 600 U/kg initial-dose strategy was associated with lower supplemental heparin use during CPB. Higher ACT values were also observed after the initial bolus and at the second recorded intra-CPB measurement. This pattern is consistent with a more pronounced ACT response to the higher initial dose and may partly explain the lower supplemental heparin use under the ACT-guided protocol.

Prothrombin fragment 1+2 (F1+2) was selected as the primary endpoint because it is a biochemical marker of in vivo prothrombin activation and thrombin generation, a key process that anticoagulation during CPB is intended to suppress. In contrast to ACT, which is influenced by hypothermia, hemodilution, platelet count, and device-specific measurement limitations, F1+2 provides complementary information on coagulation activation.²⁴ This distinction is particularly important in neonates undergoing CPB, whose developmental hemostasis and circuit-related changes complicate the interpretation of ACT, and previous studies have used F1+2 to assess coagulation activation during CPB.^{2,9,20} In the present trial, the 600 U/kg initial-dose strategy was associated with lower post-CPB F1+2 levels and a smaller mean pre- to post-CPB increase compared with the control group at the end of CPB. These findings are consistent with reduced prothrombin activation under the higher initial-dose strategy followed by ACT-guided supplementation. Exploratory

Table 3 Postoperative Clinical Outcomes and Complications in Intention-to-Treat Analysis

Characteristic	Control 400 U/kg (N = 38)	Treatment 600 U/kg (N = 38)	P
Clinical outcomes			
In-hospital mortality, n/N (%)	0/38 (0.0%)	1/38 (2.6%)	1.000
PICU stay (days)	17.50 (12.00, 27.00)	14.00 (11.00, 19.25)	0.093
Hospital stay (days)	23.00 (19.75, 30.25)	22.00 (15.75, 26.00)	0.116
Mechanical ventilation duration (h)	52.50 (42.75, 142.00)	45.00 (26.00, 74.25)	0.031
Delayed sternal closure, n/N (%)	9/38 (23.7%)	7/38 (18.4%)	0.779
Postoperative complications			
Major bleeding, n/N (%)	1/38 (2.6%)	0/38 (0.0%)	1.000
Reoperation for bleeding, n/N (%)	1/38 (2.6%)	0/38 (0.0%)	1.000
Chest drainage duration (days)	5.00 (4.00, 6.00)	5.00 (4.00, 6.00)	0.528
Reintubation, n/N (%)	4/38 (10.5%)	0/38 (0.0%)	0.115
Cardiac arrest, n/N (%)	2/38 (5.3%)	1/38 (2.6%)	1.000
Peritoneal dialysis, n/N (%)	21/38 (55.3%)	13/38 (34.2%)	0.106
Peritoneal dialysis duration (days)	3.00 (0.00, 6.50)	0.00 (0.00, 4.75)	0.095
Postoperative infection, n/N (%)	27/38 (71.1%)	21/38 (55.3%)	0.234
nSOFA at 24 h	8.00 (6.00, 9.00)	6.00 (5.00, 8.00)	0.083
AKI, any stage, n/N (%)	10/38 (26.3%)	5/38 (13.2%)	0.249
AKI stage I, n/N (%)	6/38 (15.8%)	4/38 (10.5%)	0.736
AKI stage II, n/N (%)	3/38 (7.9%)	1/38 (2.6%)	0.615
AKI stage III, n/N (%)	1/38 (2.6%)	0/38 (0.0%)	1.000
Postoperative liver injury, n/N (%)	15/38 (39.5%)	12/38 (31.6%)	0.632

Notes: Data are presented as median (IQR). Categorical variables are expressed as number (percentage).

Abbreviations: PICU, pediatric intensive care unit; nSOFA score, neonatal sequential organ failure assessment; AKI, acute kidney injury.

analyses also showed lower 72-hour chest drainage and albumin use and shorter mechanical ventilation duration, whereas red-cell, plasma, and platelet transfusion requirements and most other clinical outcomes did not differ significantly. These findings highlight the gap between biochemical markers and clinical outcomes and require confirmation in larger trials.

APTT and TT were measured at the end of CPB prior to protamine reversal, while patients remained heparinized. Higher values in the 600 U/kg group may reflect a stronger heparin effect at this stage. Higher platelet counts upon PICU admission may also be consistent with reduced perioperative platelet consumption. In addition, lower post-operative D-dimer and FDP levels further suggest less fibrin turnover. Taken together, these laboratory findings complement the F1+2 results and suggest less perioperative coagulation activation with the 600 U/kg initial-dose strategy.

Previous studies have reported that decreased AT-III levels in cardiac surgery patients are associated with adverse post-operative outcomes.²⁵ Although, in theory, higher heparin doses may lead to greater AT-III consumption and consequently lower post-operative AT-III levels, our study found no significant difference between the groups. This finding may be explained by the fact that heparin exerts its anticoagulant effect primarily by potentiating AT-III activity rather than directly reducing its concentration.² Moreover, AT-III activity is influenced by multiple factors, including hepatic synthesis, hemodilution, and coagulation-related consumption,^{2,21} which may partly explain the absence of significant postoperative differences between groups.

Although the initial heparin dose was higher, the results showed lower 72-hour chest drainage and albumin use in the 600 U/kg group, with no significant differences in transfusion requirements and adverse outcomes. Protamine dose also did not differ significantly between groups. These observations provide preliminary support for the feasibility of the higher initial-dose strategy within the study's ACT-guided supplementation protocol.

Several limitations affect interpretation. This single-center trial randomized only 76 neonates. As the study was powered for F1+2 rather than clinical endpoints such as bleeding, transfusion, or mortality, these results should be

interpreted with caution. Second, the anesthesiologists and perfusionists administering and monitoring anticoagulation were necessarily unblinded, which could have influenced intraoperative management despite standardized protocols and blinded outcome assessment. Third, the ACT analyzer has an upper measurement limit of 999 s, and values reaching this ceiling were analyzed as right-censored at 999 s, which may have attenuated the observed between-group differences in intraoperative ACT. Besides, single-center study may be influenced by local surgical protocols, patient characteristics, and institutional resources, limiting generalizability. Another limitation is that thrombin generation markers were not measured at multiple intra-CPB time points, limiting detailed assessment of coagulation kinetics. Additionally, follow-up was limited to the early post-operative period; long-term effects on coagulation function and clinical outcomes remain unknown. Finally, secondary outcome comparisons were not adjusted for multiplicity. Accordingly, the present findings should be regarded as confirmatory, mechanistic evidence from a single center rather than definitive practice-changing evidence, and they require validation in larger, preferably multicenter, trials powered for clinical outcomes.

Conclusion

An initial heparin dose of 600 U/kg in neonatal CPB improved first-pass ACT target attainment and reduced thrombin generation (lower $\Delta F1+2$). However, these effects did not translate into differences in transfusion, or clinical outcomes, and larger studies are needed to assess their clinical significance.

Abbreviations

ACT, activated clotting time; AKI, acute kidney injury; APTT, activated partial thromboplastin time; AT-III, antithrombin III; CHD, congenital heart disease; CPB, cardiopulmonary bypass; ECMO, extracorporeal membrane oxygenation; F1+2, prothrombin fragment 1+2; FDP, fibrin degradation products; KDIGO, Kidney Disease: Improving Global Outcomes; nSOFA, neonatal sequential organ failure assessment score; PICU, pediatric intensive care unit; RACHS-1, Risk Adjustment for Congenital Heart Surgery score; TT, thrombin time; VIS, vasoactive-inotropic score.

Data Sharing Statement

Our hospital information center is very strict about data confidentiality and does not recommend sharing data. De-identified participant data may be shared upon reasonable request, after the potential benefits and risks of each request have been carefully weighed, and with the permission of the institutional ethics committee of Fuwai Hospital. Requests for data access should be addressed to Jinping Liu, Email: liujinping@fuwai.com.

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Jinping Liu is the first corresponding author.

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Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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