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Research Article

Post-heparinization autologous donation with autologous circuit priming versus acute normovolemic hemodilution in cardiac surgery: A retrospective cohort study

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ABSTRACT

Objective: To compare postoperative chest-tube drainage and cumulative allogeneic red blood cell (RBC) exposure between two integrated autologous blood-conservation strategies: post-heparinization pre-bypass acute autologous donation (PAAD), which incorporated autologous circuit priming, and pre-heparinization acute normovolemic hemodilution (ANH).

Methods: This single-center retrospective cohort included 220 adults undergoing first-time elective on-pump cardiac surgery with preoperative hemoglobin ≥ 12 g/dL; 117 received PAAD and 103 ANH as routine care. The primary outcome was cumulative chest-tube drainage during the first 24 hours after intensive care unit admission. The key secondary outcome was allogeneic RBC exposure from cardiopulmonary bypass-flow start through discharge or death. Crude and covariate-adjusted estimates were calculated.

Results: Mean (standard deviation) 24-hour drainage was 876.2 (692.2) mL with PAAD and 855.0 (657.8) mL with ANH. The crude PAAD-minus-ANH mean difference was 21.2 mL (95% confidence interval [CI], -159.1 to 201.6), and the adjusted difference was 44.3 mL (95% CI, -146.3 to 234.9). Cumulative RBC exposure occurred in 38/117 (32.5%) PAAD and 29/103 (28.2%) ANH patients (risk difference, 4.3 percentage points; 95% CI, -7.9 to 16.2; risk ratio, 1.15; 95% CI, 0.77 to 1.73).

Conclusion: In this selected elective cohort, neither outcome clearly differed between the integrated strategies, but the wide intervals were compatible with clinically relevant associations in either direction. These observational data do not establish equivalence, causation, or a preferred strategy.

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1. Introduction

Bleeding and allogeneic transfusion remain interrelated complications of cardiac surgery with cardiopulmonary bypass (CPB). Patient blood-management guidance emphasizes correction of preoperative anemia, antifibrinolytic therapy, careful anticoagulation and reversal, restrictive red blood cell (RBC) transfusion, and con-

servation of autologous blood [1–3]. Postoperative chest-tube drainage reflects surgical bleeding, CPB-associated coagulopathy, and hemostatic management and is therefore clinically relevant when comparing autologous whole-blood strategies.

Acute normovolemic hemodilution (ANH) withdraws whole blood before systemic heparinization, replaces intravascular volume, and returns the blood after CPB.

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Several studies suggested that larger withdrawal volumes could reduce allogeneic transfusion [4–6], but a 2010-patient multicenter randomized trial found no clinically important reduction in in-hospital RBC exposure compared with usual care [7].

Post-heparinization pre-bypass acute autologous donation (PAAD) withdraws blood after systemic heparinization but before CPB flow and before the venous reservoir and oxygenator. Venous-line collection after full heparinization and immediately before CPB has been described [8]. However, PAAD and ANH represent multi-component practices that differ not only in heparinization timing but also in collection route and speed, autologous circuit priming, collection-bag anticoagulant exposure, storage, and reinfusion relative to protamine. The direct comparative and related descriptive literature is small, old, and heterogeneous [9–13].

We evaluated the hypothesis that 24-hour postoperative chest-tube drainage would differ between these two integrated clinical strategies. Our primary aim was to estimate the PAAD-minus-ANH mean difference in 24-hour drainage; cumulative allogeneic RBC exposure was the key secondary outcome.

2. Methods

2.1. Study design, setting, and ethics

This single-center retrospective cohort included operations performed from February 1 through October 1, 2025, at a tertiary cardiac surgery center in Türkiye. Both PAAD and ANH were established routine clinical practices during this period, and no aspect of patient management was modified for research purposes. The study was approved by the institutional ethics committee after the clinical study period and before record review began. The study and its statistical analysis were not registered in advance. Reporting follows the STROBE statement; the RECORD extension informed the description of routinely collected clinical data [14,15].

2.2. Participants and strategy selection

All 624 adult cardiac surgical procedures in the fixed study period specified in the approved ethics protocol were screened. Eligibility required elective, first-time, on-pump surgery, a last preoperative hemoglobin of at least 12 g/dL, clinical suitability for autologous collection, and accessible records. Hierarchical exclusions were emergency surgery ($n = 77$), redo surgery ($n = 32$), off-pump surgery ($n = 11$), last preoperative hemoglobin below 12 g/dL ($n = 237$), another clinical contraindication to collection ($n = 45$), and inaccessible records ($n = 2$). The 45 other contraindications were classified by primary reason as marked cardiac functional limitation, hemodynamic instability, or a fixed-output condition such as severe aortic stenosis ($n = 35$); pulmonary disease or hypoxemia ($n = 8$); and marked renal dysfunction or oliguria ($n = 2$). Each patient was counted once, although multiple contraindications could coexist. The resulting unmatched cohort comprised 220 adults (Fig. 1).

The attending anesthesiologist selected PAAD or ANH

in routine care according to preoperative hemoglobin, body habitus, ventricular function, hemodynamic stability, overall clinical condition, and usual clinician practice. PAAD tended to be favored in patients with lower left ventricular ejection fraction. Five anonymized attending-anesthesiologist identifiers were available, and every anesthesiologist used both strategies. All eligible patients were included; there was no outcome-based sampling or matching.

2.3. Autologous blood collection and reinfusion

The strategies were evaluated as integrated clinical practices because they differed in heparinization timing, collection route and speed, autologous circuit priming, and collection-bag anticoagulant exposure. Because these components co-occurred, the comparison is best interpreted as one between the strategies as implemented and not as isolating the effect of heparinization timing.

For ANH, whole blood was collected by gravity from a central venous catheter before systemic heparinization, with concurrent crystalloid according to hemodynamic response. For PAAD, after the initial intravenous unfractionated-heparin bolus, confirmation of an activated clotting time (ACT) of at least 480 seconds, and cannulation, crystalloid in the arterial and venous limbs of the CPB circuit was displaced by the patient's own blood to complete autologous circuit priming. Immediately before full CPB flow, heparinized autologous whole blood was collected by gravity from a side arm of the venous drainage line, upstream of the venous reservoir and oxygenator, into sterile collection bags. PAAD did not include reservoir blood or collection during ongoing bypass. Concurrent crystalloid replacement was administered according to hemodynamic response, as in the ANH group.

Both strategies had an approximate center-level target of 650–700 mL of blood in total. Anesthesia records documented collection into two bags for all completed procedures (117 PAAD and 100 ANH); however, bag weights and calibrated fill volumes were not recorded. Because the degree of filling could vary, bag count could not be converted reliably into an achieved patient-level blood volume. Collection-replacement volumes and postcollection/pre-CPB hematocrit values were not systematically retained. Nominal 450-mL bags containing 63 mL of citrate-phosphate-dextrose-adenine solution 1 (CPDA-1) were used. For ANH, each bag retained its CPDA-1; for PAAD, the CPDA-1 solution was drained before collection because the blood was already systemically heparinized. Bags were stored at approximately 4°C in a medical blood-storage refrigerator, warmed, and reinfused through a peripheral intravenous line. Reinfusion began before protamine and usually ended afterward. No PAAD collection was clinically interrupted. Three ANH collections were interrupted after hypotension developed at approximate collected volumes of 250, 200, and 300 mL; crystalloid and ephedrine were administered, and the partially collected blood was returned. These patients remained in the primary cohort (Supplementary Fig. S1). The partial volumes do not estimate achieved volumes in completed collections.

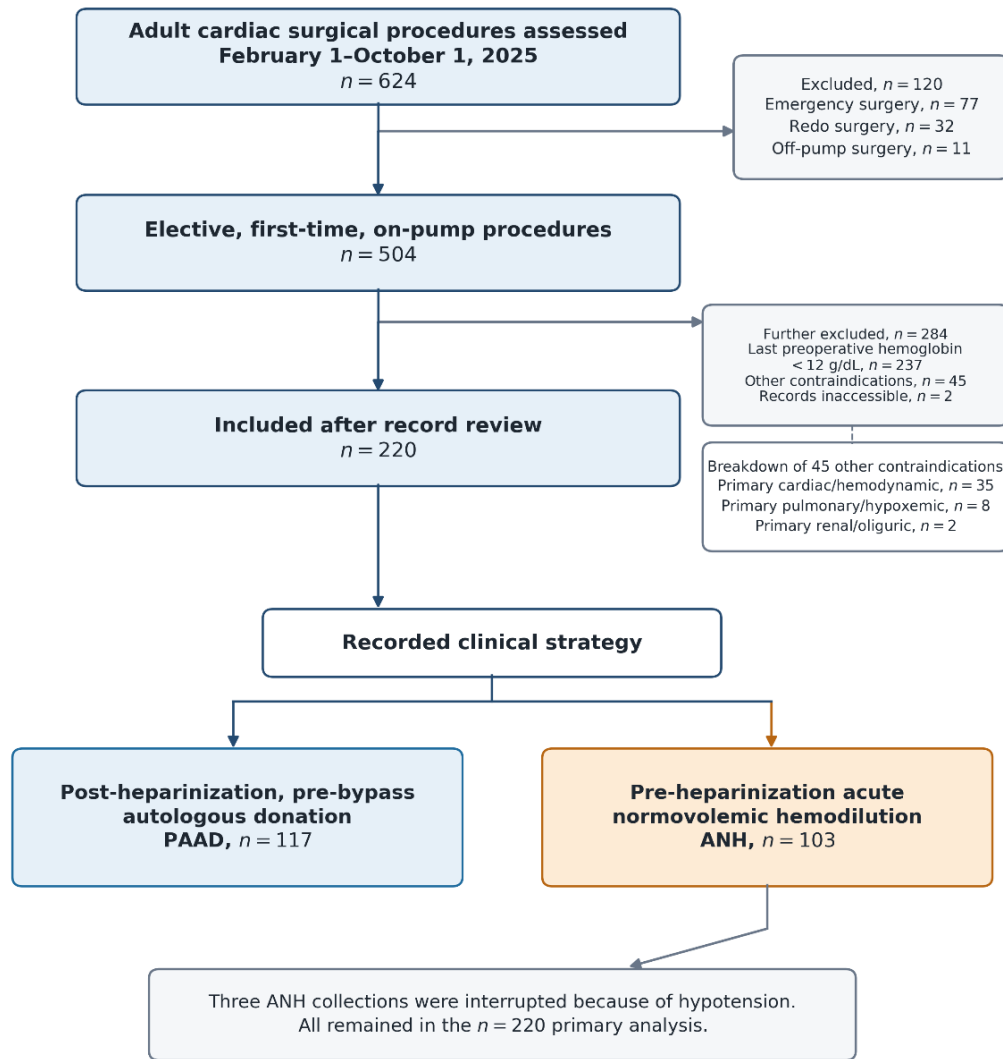


Fig. 1. Cohort assembly and collection strategies. Flow diagram showing all 624 adult cardiac surgical procedures screened during the fixed study period, hierarchical exclusions, and 220 analyzed patients divided into PAAD ($n = 117$) and ANH ($n = 103$). The three interrupted ANH collections remained in the primary cohort. ANH, acute normovolemic hemodilution; PAAD, post-heparinization pre-bypass acute autologous donation.

2.4. Anesthesia, CPB, and transfusion practice

General anesthesia and monitoring followed routine cardiac surgery practice and included invasive arterial and central venous pressure, bispectral index, and bilateral cerebral oximetry. Anesthesia was induced with midazolam, thiopental, fentanyl, and rocuronium and maintained with sevoflurane plus intermittent fentanyl and rocuronium; volatile anesthesia continued during CPB.

The CPB circuit was started with a crystalloid prime containing 1,000 mL calcium-free ISOLYTE-S pH 7.4 (Koçak Farma, Tekirdağ, Türkiye), 60 mEq sodium bicarbonate, and 10,000 IU unfractionated heparin. The initial systemic heparin bolus was 300–350 IU/kg. ACT was maintained at 480 seconds or longer with additional 5,000-IU boluses when needed. Nonpulsatile pump flow was targeted to a cardiac index of 2.2–2.4 L/min/m², mean arterial pressure to 65–80 mm Hg, and hematocrit to 24%–32%; alpha-stat management was used. Conventional ultrafiltration was selective, and cell-salvaged blood was not used.

Protamine was administered over approximately 10 minutes after separation from CPB. ACT was rechecked

after completion of both protamine administration and autologous blood reinfusion. RBC guidance comprised hematocrit below 22% during CPB and hemoglobin below 8 g/dL afterward, interpreted with bleeding, hemodynamics, and oxygen delivery. Rapid bleeding could prompt transfusion before a new blood-gas result. Tranexamic acid administration was not assigned by study protocol; the attending anesthesiologist and surgeon made the decision jointly according to clinical judgment. When used, approximately 10 mg/kg was given as a single 500-, 750-, or 1000-mg intravenous bolus at the end of CPB. Fresh-frozen plasma was administered routinely to all study patients at CPB separation, with the dose individualized according to the patient's clinical condition. Postoperative fresh-frozen plasma was administered according to ongoing bleeding and clinical assessment. Platelets, cryoprecipitate, and fibrinogen concentrate were administered according to ongoing bleeding and clinical assessment. Neither rotational thromboelastometry (ROTEM) nor thromboelastography (TEG) was available at the hospital during the study period. No patient received aspirin on the morning of surgery.

2.5. Data sources, quality control, and outcomes

Data were collected retrospectively from operating-room, anesthesia, perfusion, ICU, ward, laboratory, and medication records. Patient records were divided among three members of the study team and abstracted using a standardized data-collection form. Records with missing or internally inconsistent information were reviewed jointly before finalization of the study dataset. General quality-control checks assessed completeness, plausible ranges, coding, and internal consistency. Exposure, primary and key secondary outcomes, and all primary-model covariates were complete. The additive European System for Cardiac Operative Risk Evaluation I (EuroSCORE I) value recorded in the preoperative clinical record was used as a baseline descriptor; EuroSCORE II was not retrospectively reconstructed. Outcome definitions and data sources are detailed in Supplementary Tables S1 and S2.

The primary outcome was the sum of recorded hourly chest-tube drainage during ICU hours 1–24 (Supplementary Fig. S2). In patients undergoing re-exploration, this remained the ICU-chart total over the same window; unavailable surgical-field blood loss was not included. The key secondary outcome was receipt of at least one allogeneic RBC unit from CPB-flow start through hospital discharge or in-hospital death. Exploratory windows were CPB-flow start to operating-room exit, operating-room exit through 24 hours afterward, and operating-room exit through discharge or death. No patient received RBCs before CPB flow.

Other exploratory outcomes included drainage above 1500 mL in 24 hours, re-exploration, RBC-unit burden, ICU and hospital length of stay, in-hospital death, and a post hoc 7-day acute kidney injury outcome based on Kidney Disease: Improving Global Outcomes (KDIGO) creatinine, urine-output, and renal replacement therapy (RRT) criteria [16]. Postoperative hemoglobin, hematocrit, platelet count, and creatinine were from the first postoperative-morning sample; lactate was from the ICU-admission blood gas.

2.6. Statistical analysis

The ethics protocol identified 24-hour drainage as the primary outcome and recorded a requirement of at least 85 patients per group. On rechecking, G*Power for a two-sided comparison of two independent means with equal allocation, an alpha of 0.05, 90% power, an anticipated between-group mean difference of 200 mL, and a standard deviation of 400 mL indicated an exact target of 172 patients (86 per group). The protocol's recorded target was therefore one patient per group below the exact integer requirement. All 220 eligible patients in the fixed period were analyzed. The protocol did not specify the final statistical estimands, covariate sets, sensitivity analyses, or analysis software; these were selected after record review and were not preregistered. Continuous variables are reported as mean (standard deviation) or median [interquartile range], and categorical variables as n/N (%). Absolute standardized mean differences describe baseline balance without significance testing.

The lead primary analysis estimated the crude PAAD-minus-ANH mean drainage difference by ordinary least squares with HC3 heteroskedasticity-consistent standard errors and Student t 95% confidence intervals (CIs). A supportive adjusted model included age, sex, body mass index, preoperative hemoglobin, platelets, fibrinogen, creatinine, ejection fraction below 40%, complex procedure, four indicators for the five anesthesiologists, continued aspirin, any P2Y12 inhibitor, and any oral anticoagulant. The adjusted model contained 18 parameters for 220 observations (residual degrees of freedom, 202). Complex procedures were aortic or ascending-aortic procedures, including Bentall surgery, coronary artery bypass grafting (CABG) plus valve, or CABG plus carotid surgery. Postexposure covariates and stepwise selection were not used. Sensitivity analyses used a 20,000-resample patient-level bootstrap and evaluated completed collections, exclusion of re-explorations, linear calendar time, and continuous ejection fraction. The completed-collection and re-exploration restrictions were post hoc, postexposure descriptive analyses.

For binary RBC outcomes, unadjusted risk differences used Newcombe hybrid-score intervals derived from separate Wilson score intervals, and risk ratios used log-scale intervals. Cumulative RBC receipt was supported by logistic standardization for age, sex, body mass index, preoperative hemoglobin, ejection fraction below 40%, procedure complexity, four indicators for the five anesthesiologists, continued aspirin, any P2Y12 inhibitor, and any oral anticoagulant. This model included 67 events and 15 parameters and was treated as supportive and exploratory. Percentile intervals came from 2,978 successful fits among 3,000 patient-level bootstrap resamples. The 22 unsuccessful unpenalized fits reached the 200-iteration limit in sparse resamples with complete or near-complete prediction and were omitted. As a sparse-data sensitivity analysis, the same model was refitted using Firth penalized logistic regression; all 3,000 Firth bootstrap fits converged. Secondary and sensitivity analyses were exploratory; no multiplicity adjustment or imputation was used. No subgroup or interaction analyses were conducted. All analyses were performed in Python 3.12.13 (Python Software Foundation, Wilmington, DE, USA) using NumPy 2.3.5 and pandas 3.0.1. HC3 regression, logistic standardization, Newcombe intervals, bootstrap analyses, and Firth regression were implemented directly in Python with NumPy; full computational details are provided in Supplementary Methods S5 and Table S15.

3. Results

3.1. Cohort and baseline characteristics

The cohort included 117 PAAD and 103 ANH patients (Fig. 1). Mean age was 59.8 versus 58.6 years, and women comprised 12.0% versus 11.7%, respectively. PAAD patients had lower preoperative hemoglobin (14.21 vs 14.46 g/dL; standardized mean difference [SMD], 0.20), and ejection fraction below 40% was more frequent (20/117 [17.1%] vs 7/103 [6.8%]; SMD, 0.32). Oral anticoagulant use was also more frequent with

PAAD (12.8% vs 6.8%; SMD, 0.20). All five anesthesiologists used both strategies; PAAD/ANH counts were 25/23, 22/24, 31/13, 27/16, and 12/27, showing

within-clinician overlap but differing preferences (Supplementary Table S3). Baseline and operative characteristics appear in Tables 1 and 2.

Table 1. Baseline characteristics by autologous collection strategy.

Characteristic	PAAD (<i>n</i> = 117)	ANH (<i>n</i> = 103)	Absolute SMD
Age (years)	59.76 (10.19)	58.55 (9.42)	0.12
Women	14 (12.0%)	12 (11.7%)	0.01
Body mass index (kg/m ²)	27.51 (3.97)	28.21 (4.34)	0.17
Hemoglobin (g/dL)	14.21 (1.24)	14.46 (1.21)	0.20
Platelet count (10 ⁹ /L)	259.4 (81.5)	249.9 (78.6)	0.12
Fibrinogen (mg/dL)	341.4 (66.5)	343.7 (70.5)	0.03
Creatinine (mg/dL)	0.89 (0.20)	0.88 (0.17)	0.08
Ejection fraction <40%	20 (17.1%)	7 (6.8%)	0.32
ASA physical status IV	8 (6.8%)	8 (7.8%)	0.04
Additive EuroSCORE I (points)	3 [2-4]	3 [2-4]	0.07
Hypertension	61 (52.1%)	48 (46.6%)	0.11
Diabetes mellitus	49 (41.9%)	39 (37.9%)	0.08
Coronary artery disease	97 (82.9%)	86 (83.5%)	0.02
Chronic obstructive pulmonary disease	6 (5.1%)	5 (4.9%)	0.01
Complex procedure	15 (12.8%)	15 (14.6%)	0.05
Aspirin continued to surgery	87 (74.4%)	83 (80.6%)	0.15
Any P2Y12 inhibitor use	20 (17.1%)	20 (19.4%)	0.06
Any oral anticoagulant use	15 (12.8%)	7 (6.8%)	0.20

Values are mean (standard deviation), median [interquartile range], or *n* (%). No listed variable was missing. Values were rounded for presentation; all calculations used unrounded data, and percentages may not total 100% because of rounding. SMDs for continuous variables were calculated from unrounded means and standard deviations, including variables presented as median (interquartile range). Complex procedure comprised aortic or ascending-aortic procedures, including Bentall surgery, coronary artery bypass grafting plus valve, and coronary artery bypass grafting plus carotid surgery. ANH, acute normovolemic hemodilution; ASA, American Society of Anesthesiologists; PAAD, post-heparinization pre-bypass acute autologous donation; SMD, standardized mean difference.

Values are *n* (%), mean (standard deviation), or median [interquartile range]. Plasma means and medians were calculated across all patients in each strategy group; postoperative ranges were 0-6 units with PAAD and 0-9 units with ANH, and intraoperative ranges were 1-3 units in both groups. The two-bag entry applies to all completed procedures (117 PAAD and 100 ANH); bag weights and calibrated fill volumes were not recorded. Values were rounded for presentation; all calculations used unrounded data, and percentages may not total 100% because of rounding. *Center-level clinical target for blood volume; actual patient-level volume was not systematically measured. ACT, activated clotting time; ANH, acute normovolemic hemodilution; CABG, coronary artery bypass grafting; CPB, cardiopulmonary by-

pass; CPDA-1, citrate-phosphate-dextrose-adenine-1; PAAD, post-heparinization pre-bypass acute autologous donation.

Median CPB duration was 97 minutes in both groups. Lowest core temperature was 32 [30-32]°C with PAAD and 30 [30-32]°C with ANH. Additional CPB heparin was given to 66/117 (56.4%) and 54/103 (52.4%) patients. Tranexamic acid was given to 81/117 (69.2%; 62,500 mg total) and 72/103 (69.9%; 54,000 mg total), respectively. All patients received intraoperative fresh-frozen plasma: PAAD patients received 218 units in total (mean, 1.86 [standard deviation, 0.37]; median, 2 [range, 1-3]) and ANH patients received 186 units (mean, 1.81 [0.42]; median, 2 [range, 1-3]). Postoperative plasma was administered to 115/117 PAAD patients (269 units; mean per cohort patient, 2.30 [1.09]; median, 2 [range, 0-6]) and 101/103 ANH patients (228 units; mean, 2.21 [1.20]; median, 2 [range, 0-9]) (Supplementary Table S8).

3.2. Primary outcome

Mean (standard deviation) 24-hour drainage was 876.2 (692.2) mL after PAAD and 855.0 (657.8) mL after ANH; medians were 650 [550-860] and 680 [550-807.5] mL. The crude PAAD-minus-ANH mean difference was 21.2 mL (95% CI, -159.1 to 201.6; *p* = 0.817), and the adjusted difference was 44.3 mL (95% CI, -146.3 to 234.9; *p* = 0.647) (Table 3; Fig. 2). The adjusted bootstrap interval was -138.6 to 223.7 mL.

Table 2. Operative, anticoagulation, and collection characteristics.

Characteristic	PAAD (n = 117)	ANH (n = 103)
Isolated CABG	82 (70.1%)	77 (74.8%)
Valve only	16 (13.7%)	9 (8.7%)
CABG plus valve	5 (4.3%)	7 (6.8%)
CABG plus carotid	3 (2.6%)	2 (1.9%)
Aortic or ascending-aortic procedure, including Bentall surgery	7 (6.0%)	6 (5.8%)
Atrial septal defect	2 (1.7%)	0
Tumor/myxoma	2 (1.7%)	2 (1.9%)
Operation duration (min)	285 [255-325]	280 [247.5-325]
CPB duration (min)	97 [79-120]	97 [79.5-123.5]
Cross-clamp duration (min)	66 [54-85]	64 [49.5-87]
Lowest core temperature (°C)	32 [30-32]	30 [30-32]
Initial systemic heparin (IU)	25,000 [25,000-30,000]	25,000 [25,000-30,000]
Post-heparin, pre-CPB ACT (s)	577 [524-623]	572 [514.5-624]
Additional heparin during CPB	66 (56.4%)	54 (52.4%)
Total protamine (anti-heparin IU)	25,000 [25,000-25,000]	25,000 [25,000-25,000]
Post-protamine ACT (s)	136 [130-145]	136 [129-143]
Tranexamic acid, recipients/total dose (mg)	81/62,500	72/54,000
Intraoperative fresh-frozen plasma (units)	218 total; mean 1.86 (0.37); median 2 [2-2]	186 total; mean 1.81 (0.42); median 2 [2-2]
Postoperative fresh-frozen plasma (units)	115 recipients/269 total; mean 2.30 (1.09); median 2 [2-3]	101 recipients/228 total; mean 2.21 (1.20); median 2 [2-3]
Collection interrupted	0	3 (2.9%)
Documented bags in completed collections	2	2
Approximate collection target*	650-700 mL	650-700 mL
Concurrent crystalloid replacement	Used according to hemodynamics	Used according to hemodynamics
CPDA-1 in collection bags	Removed before collection	Retained

Table 3. Primary, transfusion, bleeding, and clinical outcomes.

Outcome	PAAD	ANH	PAAD-minus-ANH estimate (95% CI)	Analysis role
Chest-tube drainage, first 24 h (mL)	Mean 876.2 (SD 692.2); median 650 [550-860]	Mean 855.0 (SD 657.8); median 680 [550-807.5]	Crude mean difference 21.2 mL (-159.1 to 201.6); $p = 0.817$	Primary
Chest-tube drainage, adjusted	–	–	Mean difference 44.3 mL (-146.3 to 234.9); $p = 0.647$	Supportive primary model
Cumulative RBC recipient, CPB start to discharge/death	38/117 (32.5%)	29/103 (28.2%)	RD 4.3 pp (-7.9 to 16.2); RR 1.15 (0.77 to 1.73)	Key secondary
Cumulative RBC recipient, standardized	33.2%	27.4%	RD 5.7 pp (-5.9 to 17.7); RR 1.21 (0.82 to 1.82)	Supportive secondary
Intraoperative RBC recipient, CPB start to OR exit	26/117 (22.2%)	14/103 (13.6%)	RD 8.6 pp (-1.7 to 18.5); RR 1.63 (0.90 to 2.96)	Exploratory
Early postoperative RBC recipient, OR exit through 24 h after OR exit	18/117 (15.4%)	15/103 (14.6%)	RD 0.8 pp (-8.9 to 10.2); RR 1.06 (0.56 to 1.99)	Exploratory
Any postoperative RBC recipient, OR exit to discharge/death	24/117 (20.5%)	21/103 (20.4%)	RD 0.1 pp (-10.7 to 10.7); RR 1.01 (0.60 to 1.70)	Exploratory
Drainage >1500 mL in first 24 h	13/117 (11.1%)	12/103 (11.7%)	RD -0.5 pp (-9.4 to 8.0)	Exploratory
Re-exploration for bleeding	11/117 (9.4%)	5/103 (4.9%)	RD 4.5 pp (-2.7 to 11.8)	Exploratory
KDIGO acute kidney injury within 7 d	16/117 (13.7%)	14/103 (13.6%)	RD 0.1 pp (-9.3 to 9.2)	Post hoc exploratory
KDIGO stage 2 or 3 within 7 d	7/117 (6.0%)	3/103 (2.9%)	Descriptive	Post hoc exploratory
Renal replacement therapy within 7 d	3/117 (2.6%)	2/103 (1.9%)	RD 0.6 pp (-4.5 to 5.5)	Post hoc exploratory
ICU length of stay (d)	3 [3-4]	3 [3-4]	Descriptive	Exploratory
Hospital length of stay (d)	8 [7-10]	8 [7-10]	Descriptive	Exploratory
In-hospital death	3/117 (2.6%)	2/103 (1.9%)	RD 0.6 pp (-4.5 to 5.5)	Exploratory

Values were rounded for presentation; all calculations used unrounded data, and percentages may not total 100% because of rounding. Unadjusted binary-outcome confidence intervals are Newcombe score intervals for risk differences and log intervals for risk ratios. Adjusted primary estimates use ordinary least squares with HC3 standard errors and Student *t* confidence intervals. Adjusted cumulative red blood cell estimates use logistic standardization with a bootstrap confidence interval. ANH, acute normovolemic hemodilution; CI, confidence interval; CPB, cardiopulmonary bypass; ICU, intensive care unit; KDIGO, Kidney Disease: Improving Global Outcomes; OR, operating room; PAAD, post-hepa-

rinization pre-bypass acute autologous donation; pp, percentage points; RBC, red blood cell; RD, risk difference; RR, risk ratio; SD, standard deviation.

The completed-collection analysis included 217 patients and gave an adjusted difference of 25.4 mL (95% CI, -168.3 to 219.1; $p = 0.796$). Estimates were similar after adding calendar time (44.4 mL; 95% CI, -147.5 to 236.3) and modeling ejection fraction continuously (43.5 mL; 95% CI, -149.7 to 236.8). Excluding the 16 patients who underwent re-exploration gave a descriptive adjusted difference of -73.6 mL (95% CI, -202.0 to 54.7). Detailed drainage summaries and sensitivity analyses are provided in Supplementary Tables S4 and S5.

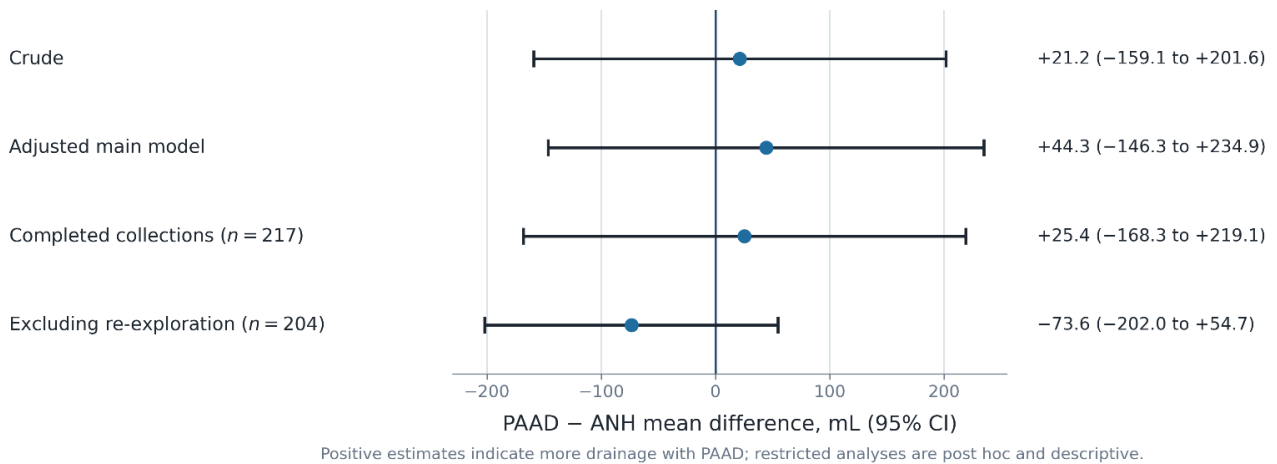


Fig. 2. Estimates for 24-hour chest-tube drainage. PAAD-minus-ANH mean differences with 95% confidence intervals for the crude and adjusted primary analyses and the post hoc completed-collection and re-exploration restrictions. Positive values indicate greater drainage with PAAD. Adjusted estimates are observational associations. ANH, acute normovolemic hemodilution; CI, confidence interval; PAAD, post-heparinization pre-bypass acute autologous donation.

3.3. Red blood cell transfusion outcomes

Cumulative RBC exposure occurred in 38/117 (32.5%) PAAD and 29/103 (28.2%) ANH patients. The crude risk difference was 4.3 percentage points (95% CI, -7.9 to 16.2), and the risk ratio was 1.15 (95% CI, 0.77 to

1.73). Standardized risks were 33.2% and 27.4%, giving an adjusted risk difference of 5.7 percentage points (95% bootstrap CI, -5.9 to 17.7) and risk ratio of 1.21 (95% bootstrap CI, 0.82 to 1.82) (Table 3; Fig. 3). Firth-penalized estimates were similar to the primary adjusted analysis (Supplementary Table S6).

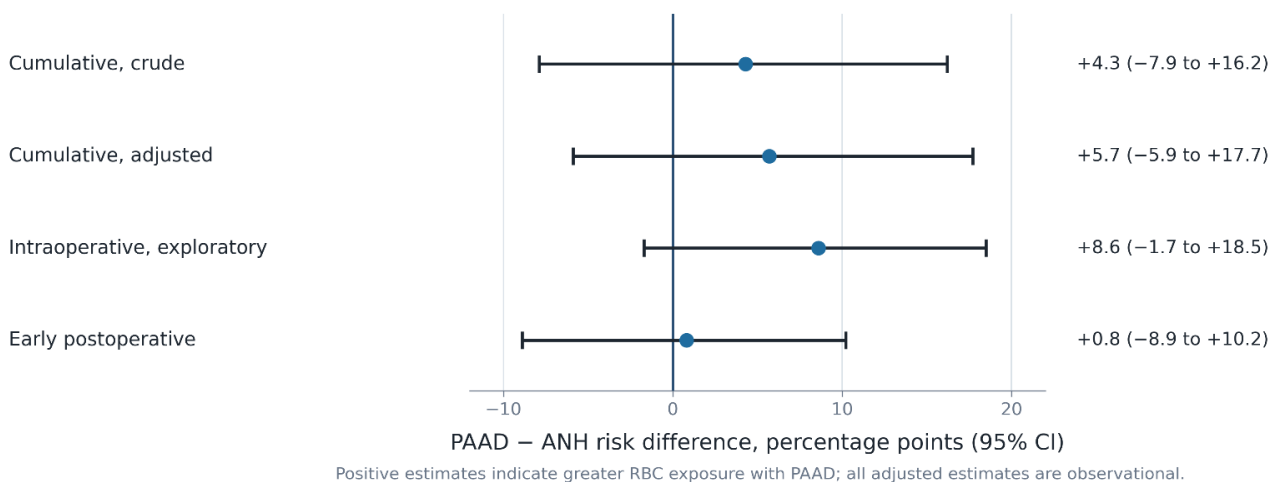


Fig. 3. Estimates for red blood cell exposure. PAAD-minus-ANH risk differences with 95% confidence intervals for cumulative, intraoperative, and early postoperative RBC exposure. Positive values indicate greater risk with PAAD. Crude intervals use Newcombe score methods; the adjusted cumulative-RBC interval is a percentile-bootstrap interval. Adjusted estimates are observational associations. ANH, acute normovolemic hemodilution; CI, confidence interval; PAAD, post-heparinization pre-bypass acute autologous donation; RBC, red blood cell.

Intraoperative RBC exposure occurred in 26/117 (22.2%) and 14/103 (13.6%) patients (risk difference, 8.6 percentage points; 95% CI, -1.7 to 18.5; risk ratio, 1.63; 95% CI, 0.90 to 2.96). Early postoperative exposure occurred in 18/117 (15.4%) and 15/103 (14.6%), while any postoperative exposure occurred in 24/117 (20.5%) and 21/103 (20.4%). Cumulative RBC-unit totals were 109 and 85. Two patients, one in each strategy group, had cumulative totals of 17 RBC units. Complete recipient-window and unit-distribution results are provided in Supplementary Tables S6 and S7.

3.4. Other clinical outcomes

Drainage above 1500 mL occurred in 13/117 (11.1%) PAAD and 12/103 (11.7%) ANH patients. Re-exploration occurred in 11/117 (9.4%) and 5/103 (4.9%). Post hoc 7-day acute kidney injury occurred in 16/117 (13.7%) and 14/103 (13.6%), and RRT was initiated within seven days in 3/117 (2.6%) and 2/103 (1.9%) patients, respectively. Median ICU stay was 3 [3–4] days and hospital stay 8 [7–10] days in both groups. In-hospital death occurred in three and two patients, respectively; all five deaths were associated with low cardiac output or cardiogenic shock (Supplementary Tables S8–S10). General data-quality checks, anticoagulation, antithrombotic treatment, and postoperative laboratory details are provided in Supplementary Tables S11–S14. Hourly urine-output trajectories are shown in Supplementary Fig. S3.

4. Discussion

In this comparison of two integrated clinical strategies, the estimated difference in 24-hour chest-tube drainage was small relative to its uncertainty. The crude PAAD-minus-ANH difference was 21 mL and the adjusted difference 44 mL; both confidence intervals included lower and higher drainage with PAAD. The drainage distribution was markedly right-skewed, and medians differed in the opposite direction from means (650 vs 680 mL). Excluding the 16 patients who underwent re-exploration changed the adjusted point estimate from +44.3 mL to -73.6 mL, although both confidence intervals included no difference. This finding indicates sensitivity of the mean-based estimate to extreme bleeding events without changing the overall conclusion of uncertainty.

Cumulative in-hospital RBC exposure was also imprecisely estimated. The crude absolute difference was 4.3 percentage points and the adjusted difference 5.7 percentage points, with both intervals spanning no difference. The larger intraoperative point estimate was exploratory, and postoperative RBC exposure was nearly identical. The findings should not be interpreted as evidence that either strategy reduces bleeding or transfusion.

Most evidence compares ANH with usual care rather than another autologous collection strategy. Several studies associated larger ANH volumes with lower transfusion [4–6], whereas a multicenter trial found no clinically important reduction in its primary in-hospital RBC outcome [7]. One 2026 meta-analysis found no reduction in in-hospital RBC exposure [17], whereas another re-

ported lower transfusion and total blood loss but no significant difference in chest-tube drainage [18]. Neither directly compared ANH with PAAD.

A 2026 administrative-database cohort associated ANH with lower 7-day RRT use than no ANH, but defined acute kidney injury by RRT alone and remained vulnerable to residual confounding [19]. The KDIGO analysis used here was post hoc and contained few severe events. A separate propensity-score-matched cohort using low-volume ANH (median withdrawal, 450 mL) found no significant reduction in allogeneic RBC transfusion, reinforcing the likely importance of collection volume and clinical context [20].

In this cohort, the PAAD strategy combined autologous priming of the arterial and venous circuit limbs with venous-line whole-blood collection after full heparinization and immediately before CPB; the latter technique has been described [8]. It remained distinct from reservoir sequestration and collection during ongoing bypass. Older studies of pre- and post-heparinization collection, including direct comparisons, differed in anticoagulants, storage, volume, and reinfusion and reported inconsistent platelet, bleeding, and transfusion findings [9–13].

ANH may protect platelets and coagulation factors from heparin and CPB but produces pre-bypass hemodilution through crystalloid replacement. The PAAD strategy combined autologous circuit priming and concurrent crystalloid replacement with venous-line collection; its product was heparinized and was not exposed to CPDA-1. ANH blood retained CPDA-1, whereas CPDA-1 was removed from PAAD collection bags. Because actual blood volume and bag-specific filling were not measured, the precise anticoagulant-to-blood ratio for ANH was unavailable. Cold storage and reinfusion spanning protamine provide additional mechanisms, but platelet function, peri-reinfusion ionized calcium, and actual collected volume were unavailable.

Although anesthesia records documented two bags for every completed procedure, bag weights and calibrated fill volumes were unavailable. Because the degree of filling could vary, we could not determine whether individual patients received comparable collection volumes or evaluate a volume-response relation. Collection-replacement volume and postcollection/pre-CPB hematocrit were also unavailable. The three interrupted ANH volumes document partial collections but do not inform achieved volumes in completed procedures. Differences in achieved collection volume could therefore have influenced the observed comparison in either direction.

Clinician-dependent strategy selection is another plausible explanation. Lower preoperative hemoglobin and ejection fraction below 40% were more common with PAAD. All five anesthesiologists used both strategies, but the proportion managed with PAAD ranged from 30.8% to 70.5%. This overlap allowed within-clinician comparisons, but adjustment for measured differences and anesthesiologist indicators cannot eliminate residual confounding or confounding by indication. Procedural factors occurring after strategy selection were not treated as baseline confounders.

Tranexamic acid and plasma were post-selection co-interventions that could affect drainage and transfusion. Tranexamic acid use was similar by group, but treatment

was based on clinical judgment rather than study assignment. Routine plasma at CPB separation and postoperative plasma in 216/220 patients may have reduced the ability to observe between-strategy differences. These center-specific practices also limit generalizability to contemporary algorithm-guided patient blood-management pathways.

The study has several strengths. Every procedure in the period specified in the approved retrospective ethics protocol was screened, the complete eligible cohort was retained, and the primary outcome stated in that protocol was used. Drainage was reconstructed from hourly ICU records, a common CPB-flow landmark was applied to RBC outcomes, and general data-quality checks were performed. Primary and key secondary outcomes and all primary-model fields were complete.

This study has important limitations. It was retrospective, single-center, nonrandomized, and vulnerable to unmeasured confounding; surgeon or team identity and operative hemostatic technique were unavailable. Statistical methods were selected after record review and were not registered in advance, creating potential analysis-selection bias. The observed drainage standard deviations of 692.2 mL with PAAD and 657.8 mL with ANH were substantially greater than the 400-mL value assumed during sample-size planning, resulting in less precise estimates than anticipated. Accordingly, interpretation is based on the effect estimates and their confidence intervals; no post hoc power calculation was performed. Drainage was strongly right-skewed, and excluding re-explorations changed the direction of the point estimate. The ICU-based outcome omitted unrecorded operating-room and re-exploration surgical-field blood loss and may therefore incompletely characterize total bleeding in the most severe cases. Actual collected volume, bag-specific fill, replacement volume, platelet function, ionized calcium, and systematically timed postoperative coagulation tests were unavailable. Transfusion thresholds guided but did not determine care, and neither ROTEM nor TEG was available. The absence of cell salvage and the center-specific use of plasma limit transportability. The groups also differed descriptively in lowest core temperature; because temperature was a postexposure intraoperative variable, its contribution could not be separated from other procedural factors. Only 220 of 624 screened procedures (35.3%) entered the cohort, which therefore represents elective, first-time, on-pump cardiac surgical patients with preoperative hemoglobin ≥ 12 g/dL who were considered clinically suitable for autologous blood collection. Generalization to patients with anemia, hemodynamic vulnerability, emergency or redo surgery, or off-pump procedures should therefore be made cautiously. Because PAAD and ANH differed in several procedural components in addition to the timing of heparinization, the findings are best interpreted as a comparison of the two clinical strategies as implemented at our center, rather than as an isolated comparison of pre- versus post-heparinization blood collection. Few women were included.

5. Conclusions

Among selected adults undergoing elective, first-time, on-pump cardiac surgery with preoperative hemoglobin

of at least 12 g/dL, 24-hour chest-tube drainage and cumulative RBC exposure were imprecisely estimated and did not clearly differ between the two integrated clinical strategies. The confidence intervals remained compatible with clinically relevant associations in either direction and do not establish equivalence, causation, or a preferred strategy. These findings apply to the two strategies as implemented and should not be attributed solely to the timing of blood collection relative to heparinization. Actual collection volume, clinician-dependent selection, and center-specific hemostatic co-interventions limit interpretation and generalizability. Prospective comparisons with measured collection volumes and standardized co-interventions are needed.

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Conflict of Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this manuscript.

Data Availability

Deidentified participant-level data, the data dictionary, quality-control documentation, and machine-readable analysis results are available from the corresponding author on reasonable request, subject to institutional ethics and data-governance approval.

AI Assistance

OpenAI Codex (OpenAI) was used for English-language editing and statistical assistance. All outputs were reviewed and verified by the authors, who take full responsibility for the manuscript.

Ethics Approval and Consent to Participate

The study was approved by the Medical Sciences Ethics Committee of the University of Health Sciences, Bursa Yüksek İhtisas Training and Research Hospital, on November 5, 2025 (Approval No. 2024-TBEK 2025/11-15), after the clinical study period and before retrospective record review began. Individual informed consent was not obtained in this retrospective study. The study and its statistical analysis were not registered in advance.

Consent for Publication

Not applicable; no identifiable individual participant information is reported.

Trial Registration

Not applicable; this retrospective observational study was not registered.

Author Contributions

Anıl Onur: conceptualization; methodology; supervision; investigation; data curation; writing – original draft.

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Gürcan Güler: resources; formal analysis; writing – review and editing.

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Supplementary Material

Post-heparinization autologous donation with autologous circuit priming versus acute normovolemic hemodilution in cardiac surgery: A retrospective cohort study

This supplement documents cohort assembly, clinical practice, outcome definitions, data sources, quality control, and supporting analyses for the accompanying manuscript. The retrospective record review was approved by the institutional ethics committee. The primary outcome stated in the approved retrospective ethics protocol was first-24-hour postoperative drainage. The statistical methods reported here were selected after retrospective review of the records and are not presented as prospectively specified analyses.

CONTENTS

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- Methods S2. Clinical practice and exposure definitions
- Methods S3. Outcomes and time origins
- Methods S4. Data sources and quality control
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- Tables S1-S14
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Methods S1. Cohort construction and analysis populations

Study period and screening

The source population comprised 624 adult cardiac surgical procedures recorded between February 1 and October 1, 2025. Exclusions were applied hierarchically: emergency surgery (n=77), redo surgery (n=32), off-pump surgery (n=11), last preoperative hemoglobin <12 g/dL (n=237), another contraindication to autologous collection (n=45), and inaccessible records (n=2). The 45 other contraindications were classified by primary reason as marked cardiac functional limitation, hemodynamic instability, or a fixed-output condition such as severe aortic stenosis (n=35); pulmonary disease or hypoxemia (n=8); and marked renal dysfunction or oliguria (n=2). More than one contraindication could be present, but each patient was counted once according to the primary reason. The remaining 220 patients underwent elective, first-time, on-pump cardiac surgery with one of the two autologous blood-collection strategies. This was an unmatched cohort; no matching was performed.

Analysis populations

The all-records population comprised 117 patients managed with post-heparinization pre-bypass acute autologous donation (PAAD) and 103 managed with pre-heparinization acute normovolemic hemodilution (ANH). This population was used for the primary analysis.

No PAAD collection was clinically interrupted. Collection was interrupted because of hypotension in three ANH patients. Their approximate partial collection volumes were 250, 200, and 300 mL. Rapid fluid administration and ephedrine were used, and the partially collected blood was returned. These patients remained in the all-records analysis according to the strategy selected for their care. A completed-collection sensitivity analysis excluded only these three patients (n=217). Because strategy allocation was not randomized, neither population is described as intention-to-treat.

Methods S2. Clinical practice and exposure definitions

The exposure was the multicomponent autologous whole-blood collection strategy used in routine care. The strategies differed in heparinization timing, collection route and speed, autologous circuit priming, and collection-bag anticoagulant exposure and were therefore evaluated as integrated clinical strategies rather than as an isolated contrast in timing.

Strategy selection

The attending anesthesiologist selected the collection strategy according to usual clinical practice, considering preoperative hemoglobin, body habitus, ventricular function, hemodynamic stability, and overall clinical status.

Choice also reflected clinician preference and familiarity. Patients with an ejection fraction <40% tended to be managed with PAAD, but no deterministic allocation rule was used. Five anonymized attending-anesthesiologist identifiers were available, and each clinician used both strategies (Table S3).

ANH

ANH began after central venous catheter placement and before systemic heparinization. Whole blood was collected by gravity from the central venous catheter while crystalloid was administered according to hemodynamic response. Collection generally required approximately 15–20 minutes.

PAAD

PAAD began after the initial systemic unfractionated-heparin bolus, confirmation of an activated clotting time (ACT) of at least 480 seconds, and arterial and venous cannulation, but before cardiopulmonary-bypass (CPB) flow. Crystalloid in the arterial and venous limbs of the CPB circuit was first displaced by the patient's own blood to complete autologous circuit priming. Immediately before full CPB flow, heparinized autologous whole blood was collected by gravity from a side arm of the venous drainage line, upstream of the venous reservoir and oxygenator, directly into sterile collection bags. It therefore had not entered the venous reservoir or oxygenator and was not collected during ongoing CPB flow. Concurrent crystalloid replacement was administered according to hemodynamic response, as in the ANH group. No roller pump was used. Collection was rapid, but patient-level collection duration was not retained.

Collection bags, storage, and reinfusion

Both strategies had an approximate clinical target of 650–700 mL of blood in total. The target was an estimate; completed-collection volumes were not systematically weighed or recorded. Nominal 450-mL collection bags prefilled with 63 mL citrate-phosphate-dextrose-adenine solution 1 (CPDA-1) were used. For ANH, CPDA-1 was retained in the bags. For PAAD, CPDA-1 was drained before collection because the blood was already systemically heparinized. Bags were kept at approximately 4°C in a medical blood-storage refrigerator, warmed before use, and reinfused through a peripheral intravenous line. Reinfusion began before protamine and usually ended after protamine. Patient-level reinfusion duration, bag order, peri-reinfusion ionized calcium, and ex vivo platelet function were not recorded systematically. The two protocol timelines are summarized in Fig. S1.

Anesthesia, CPB, anticoagulation, and transfusion practice

No premedication was administered. Monitoring included standard monitors, invasive radial arterial pressure, central venous pressure, bispectral index (target 40–60), and bilateral cerebral oximetry. Anesthesia was induced with midazolam 0.02–0.03 mg/kg, thiopental 3–5 mg/kg, fentanyl 2 µg/kg, and rocuronium 0.8 mg/kg, and was maintained with sevoflurane 0.8–1.0 minimum alveolar concentration plus intermittent fentanyl and rocuronium. Volatile anesthesia continued during CPB. Initial ventilation used an inspired oxygen fraction of 0.50, tidal volume 6 mL/kg, positive end-expiratory pressure 5 cmH₂O, and respiratory rate 12/min, with subsequent adjustment to gas exchange.

The CPB circuit was primed with 1,000 mL calcium-free ISOLYTE-S pH 7.4 (Koçak Farma, Tekirdağ, Türkiye), 60 mEq sodium bicarbonate in 60 mL, and 10,000 IU unfractionated heparin. Nonpulsatile flow was targeted to a cardiac index of 2.2–2.4 L/min/m², mean arterial pressure to 65–80 mmHg, and hematocrit to 24%–32%; alpha-stat acid-base management was used. Conventional ultrafiltration was used according to the patient-level judgment of the perfusionist and clinical team.

The initial systemic heparin bolus was 300–350 IU/kg and was separate from heparin in the prime. ACT was measured at approximately 30-minute intervals and maintained at 480 seconds or longer; additional 5,000-IU boluses were administered when required. After CPB, protamine was administered slowly over approximately 10 minutes. The initial protamine dose was calculated from the initial systemic heparin bolus; any additional protamine was included in the recorded total. ACT was rechecked after completion of both protamine administration and autologous blood reinfusion.

During CPB, an RBC transfusion threshold of hematocrit <22% was used. After CPB, a hemoglobin threshold of <8 g/dL was interpreted with active bleeding, hemodynamics, oxygen delivery, and clinician judgment; in rapidly evolving bleeding, transfusion could begin before a new blood-gas result was available. The decision to administer tranexamic acid was made jointly by the attending anesthesiologist and surgeon. When used, approximately 10 mg/kg was given as a single 500-, 750-, or 1000-mg intravenous bolus at the end of CPB. No cell-salvaged blood was used. Fresh-frozen plasma was administered routinely to all study patients as a bolus at separation from CPB; the dose was individualized according to bleeding up to that point and the patient's clinical condition. Postoperative fresh-frozen plasma was administered according to the treating clinician's judgment based on ongoing bleeding and overall clinical assessment; no fixed laboratory or viscoelastic threshold was used. Platelets, cryoprecipitate, and fibrinogen concentrate were administered for ongoing diffuse bleeding after exclusion of a surgical source. Viscoelastic testing was not used. No patient received aspirin on the morning of surgery.

Methods S3. Outcomes and time origins

Primary outcome

The primary outcome was cumulative chest-tube drainage during the first 24 hours after admission to the intensive care unit (ICU), in milliliters. Hourly interval volumes were transcribed from ICU charts, and the primary value was calculated as the sum of hours 1–24. For patients who underwent re-exploration, the primary outcome remained the recorded ICU-chart total over the same 24-hour window; surgical-field blood loss during re-exploration was unavailable and was not included. Cumulative 2-, 4-, 6-, 12-, and 48-hour values were supporting postoperative drainage summaries. The 24- to 48-hour record was available as an interval total. Recorded hourly drainage and urine-output trajectories are shown separately in Figures S2 and S3.

Key secondary outcome

The key secondary outcome was receipt of at least one allogeneic RBC unit from initiation of CPB flow through hospital discharge or in-hospital death. No patient received an allogeneic RBC unit before CPB flow. Recorded RBC units were divided into CPB-flow start to operating-room exit, operating-room exit through 24 hours after operating-room exit, and more than 24 hours after operating-room exit through discharge/death. Cumulative RBC units were the sum of these mutually exclusive intervals.

Other exploratory outcomes

Exploratory RBC recipient outcomes were intraoperative receipt (CPB-flow start to operating-room exit), early postoperative receipt (operating-room exit through 24 hours after operating-room exit), and any postoperative receipt (operating-room exit to discharge/death). Other exploratory outcomes included RBC-unit counts; plasma, platelet, cryoprecipitate, and fibrinogen-concentrate administration; drainage >1500 mL in 24 hours; any first-24-hour interval with drainage >400 mL; a study-defined post hoc exploratory bleeding composite (either drainage criterion or re-exploration); re-exploration; first postoperative-morning laboratory results; ICU and hospital length of stay; recorded complication categories; RRT within seven days (post hoc exploratory); and in-hospital mortality. These bleeding definitions are study operational definitions and are not labeled as BARC, UDPB, or E-CABG bleeding.

Postoperative hemoglobin, hematocrit, platelet count, and creatinine refer to the first postoperative-morning laboratory panel; lactate refers to the ICU-admission value. Preoperative INR, activated partial thromboplastin time, and fibrinogen were recorded. Postoperative INR, activated partial thromboplastin time, and fibrinogen were not analyzed because they were not measured systematically.

Post hoc 7-day KDIGO outcome

A 7-day Kidney Disease: Improving Global Outcomes (KDIGO) acute-kidney-injury analysis was performed after the primary dataset review and was exploratory [16]. Baseline creatinine, the highest postoperative creatinine within seven days, their recorded sampling times relative to ICU admission, hourly urine output for hours 1–24, the 24- to 48-hour urine interval total, recorded body weight, and RRT start time were used.

The creatinine component applied the KDIGO time windows: an increase of at least 0.3 mg/dL within 48 hours or to at least 1.5 times baseline within seven days defined stage 1; at least 2.0 times baseline within seven days defined stage 2; and at least 3.0 times baseline within seven days, or a peak of at least 4.0 mg/dL after the acute-kidney-injury definition had been met, defined stage 3. No patient reached a peak creatinine of 4.0 mg/dL. Urine-output stages used actual recorded body weight: <0.5 mL/kg/h for a rolling 6-hour window defined stage 1; <0.5 mL/kg/h for a rolling 12-hour window defined stage 2; and <0.3 mL/kg/h across a complete 24-hour interval or anuria for a rolling 12-hour interval defined stage 3. Rolling 6- and 12-hour windows were available only during the first 24 hours; the 24- to 48-hour record could support only a complete 24-hour rate. RRT starting within seven days defined stage 3. The combined stage was the maximum of the creatinine, urine-output, and RRT components.

Methods S4. Data sources and quality control

Data sources

Patient-level values were abstracted from anesthesia, perfusion, operating-room, ICU, ward, laboratory, and medication records and reconciled into one study record per patient; no external or probabilistic database linkage was performed. General quality-control checks assessed completeness, plausible ranges, group and procedure coding, and internal consistency among drainage, transfusion, anticoagulation, and kidney-outcome variables. Exposure, primary and key secondary outcomes, and all primary-model fields were complete. A summary of the data-quality checks is provided in Table S11. Reporting was guided by STROBE and the RECORD extension [14,15].

Methods S5. Statistical analysis

Analysis status

The primary outcome stated in the approved retrospective ethics protocol was first-24-hour postoperative drainage. The statistical estimands, covariate sets, and sensitivity analyses below were selected after retrospective review of the records. They are reported as the analyses actually implemented and are not a prospectively registered or prespecified statistical plan. No model was selected by stepwise testing or by choosing the most favorable result.

Descriptive analysis

Continuous variables are summarized as mean (standard deviation) and median [interquartile range] where useful; categorical variables are summarized as n/N (%). Baseline balance is described with standardized mean differences rather than baseline significance tests. All confidence intervals are conventional 95% intervals. P values are two-sided and descriptive.

Primary analysis: 24-hour drainage

The lead estimand was the unadjusted PAAD-minus-ANH difference in mean 24-hour chest-tube drainage. It was estimated by ordinary least squares with an intercept and strategy indicator. Heteroskedasticity-consistent HC3 standard errors were used, and the confidence interval and P value used the Student t reference distribution with residual degrees of freedom.

The supportive adjusted analysis used ordinary least squares with the same HC3 and Student t inference. The fixed, clinically chosen, pre-exposure covariates were age, sex, body mass index, preoperative hemoglobin, platelet count, fibrinogen, creatinine, ejection fraction <40%, complex procedure, four indicators for the five anesthesiologists, aspirin continued to surgery, any P2Y12 inhibitor use, and any oral anticoagulant use. Complex procedures comprised aortic or ascending-aortic procedures, including Bentall surgery, CABG plus valve surgery, and CABG plus carotid surgery. Post-exposure variables were excluded. The model included 220 observations and 18 parameters (residual df=202).

Supporting sensitivity analyses used 20,000 patient-level bootstrap resamples for the adjusted coefficient, excluded the three interrupted ANH collections, excluded 16 patients who underwent re-exploration, added linear calendar time, or replaced ejection fraction <40% with continuous ejection fraction. The completed-collection and re-exploration restrictions were post hoc, postexposure descriptive analyses and were not interpreted as causal estimands.

Key secondary analysis: cumulative RBC receipt

For each RBC window, absolute risks were calculated from integer counts. Risk differences used Newcombe hybrid-score confidence intervals; risk ratios and odds ratios used log-Wald intervals. In this context, “hybrid-score” is the name of the confidence-interval method.

The cumulative-RBC adjusted analysis was supportive and exploratory. An unpenalized logistic model included strategy, age, sex, preoperative hemoglobin, body mass index, ejection fraction <40%, complex procedure, four anesthesiologist indicators, aspirin continued to surgery, any P2Y12 inhibitor use, and any oral anticoagulant use. Risks were marginally standardized by predicting every patient once under PAAD and once under ANH and averaging over the same 220-person covariate distribution. Percentile intervals used 3,000 patient-level bootstrap resamples; 2,978 fits were successful and 22 were excluded because the fit did not converge. Because 67 cumulative events supported a 15-parameter model, this adjusted analysis is not treated as confirmatory. As a post hoc supportive sparse-data sensitivity analysis, the same 15-parameter model was re-estimated with Firth penalized logistic regression. Standardized risks, risk differences, and risk ratios used the same marginal covariate distribution; percentile intervals used 3,000 patient-level bootstrap resamples, all of which converged. The conditional odds-ratio interval used penalized profile likelihood.

Multiplicity, missing data, and software

Secondary and sensitivity analyses are exploratory; no multiplicity adjustment was applied and no confidence interval is a simultaneous interval. The analysis variables used in the reported models were complete, so no imputation was used. Analyses were run in Python 3.12.13 (Python Software Foundation, Wilmington, DE, USA), with NumPy 2.3.5 and pandas 3.0.1. Pseudorandom-number generation was used only for bootstrap resampling.

Supplementary Tables

Values were rounded for presentation; all calculations used unrounded data, and percentages may not total 100% because of rounding.

Tables S1 and S2 define the outcome hierarchy and data sources; Table S3 describes anesthesiologist distribution; Tables S4 and S5 report drainage summaries and analyses; Tables S6 and S7 report RBC outcomes; Table S8 reports hemostatic products; Table S9 reports the post hoc KDIGO analysis; Table S10 reports exploratory clinical outcomes; Table S11 summarizes general data-quality checks; Tables S12 and S13 describe anticoagulation and antithrombotic treatment; and Table S14 reports first postoperative-morning laboratory values and ICU-admission lactate.

Table S1. Outcome hierarchy and operational definitions

Role	Outcome	Window or rule	Data source
Primary	Cumulative chest-tube drainage	Sum of ICU hours 1–24, mL	Derived from 24 recorded hourly intervals
Key secondary	Cumulative allogeneic RBC recipient	At least 1 unit from CPB-flow start to discharge/death	Derived from three recorded unit windows
Exploratory	Intraoperative RBC recipient	At least 1 unit from CPB-flow start to OR exit	Derived from recorded units
Exploratory	Early postoperative RBC recipient	At least 1 unit from OR exit through 24 hours after OR exit	Derived from recorded units
Exploratory	Any postoperative RBC recipient	At least 1 unit from OR exit to discharge/death	Derived from recorded units
Exploratory	High 24-hour drainage	>1500 mL in ICU hours 1–24	Derived
Exploratory	High hourly drainage	Any ICU hour 1–24 >400 mL	Derived
Post hoc exploratory	Study-defined bleeding composite	Either drainage rule or re-exploration	Derived
Post hoc exploratory	7-day KDIGO stage	Maximum creatinine, urine-output, and RRT stage	Derived from recorded values and times

ANH, acute normovolemic hemodilution; CPB, cardiopulmonary bypass; ICU, intensive care unit; OR, operating room; PAAD, post-heparinization pre-bypass acute autologous donation; RBC, red blood cell; RRT, renal-replacement therapy.

Table S2. Key data sources and analysis roles

Domain	Recorded source	Derived use
Exposure	Recorded strategy code; interruption status; approximate volume only for three interrupted collections	PAAD/ANH indicator; collection-completed indicator
Primary drainage	ICU chest-tube intervals for hours 1–24 and interval total for hours 24–48	Cumulative 2-, 4-, 6-, 12-, 24-, and 48-hour drainage; binary drainage rules
RBC transfusion	Units from CPB start to OR exit, during the first 24 hours after OR exit, and >24 hours after OR exit through discharge/death	Recipient indicators; postoperative and cumulative units
Kidney	Baseline and peak creatinine with sample times; hourly urine for 24 hours; 24–48-hour urine total; weight; RRT and start time	Creatinine, urine, and combined 7-day KDIGO stages
Anticoagulation	Initial systemic heparin, baseline/pre-CPB/post-protamine ACT, additional CPB heparin, total protamine	Heparin dose per kilogram; additional-heparin indicators
Antithrombotics	Aspirin, P2Y12 inhibitor, warfarin, and direct oral anticoagulant use and last-dose dates	Aspirin continued, any P2Y12 inhibitor, any oral anticoagulant, stop-day variables
Patient factors	Age, sex, height, weight, hemoglobin, platelets, fibrinogen, creatinine, ejection fraction	Body-mass index, ejection fraction <40%, model covariates

Table S3. Distribution of cases across attending anesthesiologists

Identifier	PAAD	ANH	Total
ANE-01	25	23	48
ANE-02	22	24	46
ANE-03	31	13	44
ANE-04	27	16	43
ANE-05	12	27	39
Total	117	103	220

Table S4. Cumulative postoperative chest-tube drainage

Landmark after ICU admission	PAAD mean (SD), mL	PAAD median [IQR], mL	ANH mean (SD), mL	ANH median [IQR], mL
2 hours	211.2 (167.7)	160 [130–200]	204.0 (141.4)	165 [125–200]
4 hours	378.5 (311.9)	280 [235–365]	363.3 (269.0)	290 [225–360]
6 hours	501.5 (410.2)	365 [310–500]	484.8 (369.4)	380 [307.5–465]
12 hours	727.8 (578.4)	545 [460–715]	708.5 (546.5)	560 [450–670]
24 hours (primary)	876.2 (692.2)	650 [550–860]	855.0 (657.8)	680 [550–807.5]
48 hours	1038.5 (824.7)	780 [645–1055]	1025.3 (803.9)	800 [622.5–940]

n = 117 for PAAD and n = 103 for ANH at every landmark; no drainage value was missing. IQR, interquartile range; SD, standard deviation.

Table S5. Primary 24-hour drainage analyses

Analysis	N	PAAD-minus-ANH estimate, mL	95% CI, mL	P value	Role/method
Crude mean difference	220	21.2	–159.1 to 201.6	.817	Lead; OLS, HC3, Student t
Adjusted mean difference	220	44.3	–146.3 to 234.9	.647	Supportive; OLS, HC3, Student t
Adjusted bootstrap interval	220	44.3	–138.6 to 223.7	—	Sensitivity; 20,000 resamples
Completed-collection analysis	217	25.4	–168.3 to 219.1	.796	Post hoc, postexposure descriptive analysis
Excluding re-exploration	204	–73.6	–202.0 to 54.7	.259	Post hoc, postexposure descriptive analysis
Adding linear calendar time	220	44.4	–147.5 to 236.3	.649	Sensitivity; same adjusted model plus time
Continuous ejection fraction	220	43.5	–149.7 to 236.8	.657	Sensitivity; replaces EF <40%

The adjusted model included age, sex, body mass index, preoperative hemoglobin, platelets, fibrinogen, creatinine, ejection fraction <40%, complex procedure, anesthesiologist fixed effects, aspirin continued, any P2Y12 inhibitor, and any oral anticoagulant. CI, confidence interval; EF, ejection fraction; OLS, ordinary least squares.

Table S6. RBC recipient outcomes

Analysis window	PAAD	ANH	Risk difference, percentage points (95% CI)	Risk ratio (95% CI)	Odds ratio (95% CI)	Role
Cumulative, CPB start to discharge/death	38/117 (32.5%)	29/103 (28.2%)	4.3 (–7.9 to 16.2)	1.15 (0.77–1.73)	1.23 (0.69–2.19)	Key secondary, crude
Cumulative, adjusted standardized risks	33.2%	27.4%	5.7 (–5.9 to 17.7)	1.21 (0.82–1.82)	—	Supportive secondary
Cumulative, Firth standardized risks	34.0%	28.4%	5.6 (–5.6 to 17.2)	1.20 (0.84–1.76)	1.40 (0.71–2.83)	Post hoc supportive sensitivity
Intraoperative, CPB start to OR exit	26/117 (22.2%)	14/103 (13.6%)	8.6 (–1.7 to 18.5)	1.63 (0.90–2.96)	1.82 (0.89–3.70)	Exploratory
Early postoperative, OR exit through 24 hours after OR exit	18/117 (15.4%)	15/103 (14.6%)	0.8 (–8.9 to 10.2)	1.06 (0.56–1.99)	1.07 (0.51–2.24)	Exploratory
Any postoperative, OR exit to discharge/death	24/117 (20.5%)	21/103 (20.4%)	0.1 (–10.7 to 10.7)	1.01 (0.60–1.70)	1.01 (0.52–1.94)	Exploratory

Crude risk-difference intervals are Newcombe hybrid-score intervals. The adjusted cumulative row is based on unpenalized logistic standardization; 2,978 of 3,000 bootstrap fits were successful. The Firth row uses the same covariates and standardization distribution; 3,000 of 3,000 bootstrap fits were successful, and its odds-ratio interval is a penalized profile-likelihood interval. CI, confidence interval.

Table S7. RBC-unit distributions

Window	PAAD recipients/total units	PAAD recipient median [IQR], range	ANH recipients/total units	ANH recipient median [IQR], range
Intraoperative	26/33	1 [1–1.75], 1–2	14/17	1 [1–1], 1–2
Early postoperative, OR exit through 24 hours after OR exit	18/60	2.5 [2–3], 2–12	15/54	3 [2–3.5], 2–12
Late postoperative, >24 hours after OR exit	12/16	1 [1–1], 1–5	9/14	1 [1–1], 1–5
Any postoperative	24/76	2 [1.75–3.25], 1–17	21/68	2 [2–3], 1–17
Cumulative	38/109	1 [1–4], 1–17	29/85	2 [1–3], 1–17

Two patients, one in each strategy group, had cumulative totals of 17 units.

Table S8. Hemostatic products and tranexamic acid

Product/phase	PAAD	ANH
Intraoperative fresh-frozen plasma, recipients/total units	117/218	103/186
Postoperative fresh-frozen plasma, recipients/total units	115/269	101/228
Intraoperative apheresis platelets, recipients/total adult doses	6/6	2/2
Postoperative apheresis platelets, recipients/total adult doses	34/48	24/42
Postoperative cryoprecipitate, recipients/total bags	15/107	12/87
Fibrinogen concentrate, recipients/total grams	11/21	12/22
Tranexamic acid, recipients/total dose, mg	81/62,500	72/54,000

All recipient and total-unit counts represent products actually administered, rather than products ordered or issued. Fresh-frozen plasma at CPB separation was routine center practice in all 220 patients; postoperative plasma was administered in 216/220 patients. The plasma dose and any additional intraoperative or postoperative product administration were individualized according to bleeding and clinical judgment. Product administration is descriptive and was not included as a covariate because it occurred after strategy selection. Platelet units represent adult single-donor apheresis therapeutic doses.

Table S9. Post hoc 7-day KDIGO stage distributions

Component	Stage	PAAD (n=117)	ANH (n=103)	Total (N=220)
Urine output	0	109	97	206
Urine output	1	5	4	9
Urine output	2	1	0	1
Urine output	3	2	2	4
Creatinine	0	101	89	190
Creatinine	1	11	12	23
Creatinine	2	5	2	7
Creatinine	3	0	0	0
Combined	0	101	89	190
Combined	1	9	11	20
Combined	2	4	1	5
Combined	3	3	2	5

The combined stage is the maximum of the creatinine, urine-output, and RRT components. All five RRT episodes began within seven days. The analysis was developed after dataset review and is exploratory.

Table S10. Exploratory clinical outcomes

Outcome	PAAD	ANH	PAAD-minus-ANH risk difference, percentage points (95% CI)
Drainage >1500 mL/24 hours	13/117 (11.1%)	12/103 (11.7%)	-0.5 (-9.4 to 8.0)
Any hourly drainage >400 mL	2/117 (1.7%)	1/103 (1.0%)	0.7 (-3.8 to 5.1)
Study-defined post hoc exploratory bleeding composite	14/117 (12.0%)	12/103 (11.7%)	0.3 (-8.6 to 8.9)
Re-exploration	11/117 (9.4%)	5/103 (4.9%)	4.5 (-2.7 to 11.8)
Any 7-day KDIGO acute kidney injury	16/117 (13.7%)	14/103 (13.6%)	0.1 (-9.3 to 9.2)
RRT within seven days	3/117 (2.6%)	2/103 (1.9%)	0.6 (-4.5 to 5.5)
Cardiac complication	3/117 (2.6%)	2/103 (1.9%)	0.6 (-4.5 to 5.5)
Neurologic complication	3/117 (2.6%)	2/103 (1.9%)	0.6 (-4.5 to 5.5)
Pulmonary complication	17/117 (14.5%)	11/103 (10.7%)	3.9 (-5.3 to 12.7)
Infection complication	19/117 (16.2%)	14/103 (13.6%)	2.6 (-7.1 to 12.0)
In-hospital death	3/117 (2.6%)	2/103 (1.9%)	0.6 (-4.5 to 5.5)

Risk-difference intervals are Newcombe hybrid-score intervals. Complication categories may overlap. Cardiac complications comprised low-cardiac-output syndrome or cardiogenic shock requiring mechanical circulatory support (intra-aortic balloon pump, n=4; extracorporeal membrane oxygenation, n=1). Neurologic complications comprised stroke (n=2), transient ischemic attack (n=1), and CAM-ICU-defined delirium (n=2). Pulmonary complications comprised mechanical ventilation >24 hours (n=16), reintubation (n=5), pleural effusion (n=5), and pneumothorax (n=2); pneumonia was documented in 14 of these patients. Infectious complications comprised surgical-site infection (n=15), pneumonia (n=14), sepsis (n=2), and urinary tract infection (n=2); pneumonia therefore contributed to both pulmonary and infectious categories. All five deaths were attributed in the records to low-cardiac-output syndrome/cardiogenic shock.

ICU length of stay was 3 [3–4] days in each group; hospital length of stay was 8 [7–10] days in each group. Values are medians [IQR].

Table S11. General data-quality summary

Domain	Final result	Status
Analysis records	220	Pass
PAAD/ANH counts	117/103	Pass
Missing primary outcome	0	Pass
Missing key secondary outcome	0	Pass
Missing primary-model fields	0	Pass
Procedure and group coding checks	Completed	Pass
Range, logic, and arithmetic checks	Completed	Pass
Internal outcome-consistency checks	Completed	Pass

Table S12. Recorded anticoagulation and ACT values

Measure	PAAD (n=117)	ANH (n=103)
Initial systemic heparin, IU/kg	322.6 [300.1–350.1]	337.5 [300.1–350.1]
Baseline ACT, seconds	140 [134–145]	139 [132.5–143.5]
Post-heparin, pre-CPB ACT, seconds	577 [524–623]	572 [514.5–624]
Additional heparin during CPB, n (%)	66 (56.4%)	54 (52.4%)
Additional heparin during CPB, IU	5000 [0–10,000]	5000 [0–10,000]
Total protamine, anti-heparin IU	25,000 [25,000–25,000]	25,000 [25,000–25,000]
Post-protamine ACT, seconds	136 [130–145]	136 [129–143]

Values are median [IQR] unless otherwise indicated. Initial systemic heparin excludes heparin in the prime. Additional heparin excludes the initial systemic bolus and heparin in the prime. Total protamine includes any additional dose. ACT, activated clotting time; CPB, cardiopulmonary bypass.

Table S13. Recorded preoperative antithrombotic treatment

Measure	PAAD (n=117)	ANH (n=103)
Aspirin use	96 (82.1%)	89 (86.4%)
Aspirin continued to surgery	87 (74.4%)	83 (80.6%)
Any P2Y12 inhibitor use	20 (17.1%)	20 (19.4%)
Any oral anticoagulant use	15 (12.8%)	7 (6.8%)
No recorded antithrombotic	18 (15.4%)	13 (12.6%)
Aspirin alone	64 (54.7%)	63 (61.2%)
Dual antiplatelet treatment	20 (17.1%)	20 (19.4%)
Oral anticoagulant with or without aspirin	15 (12.8%)	7 (6.8%)
Aspirin stop interval among users, days	1 [1–1]	1 [1–1]
P2Y12 stop interval among users, days	5 [5–6]	5 [5–5]
Warfarin stop interval among users, days	5 [5–5]	5 [4.5–5]
Direct oral anticoagulant stop interval among users, days	2 [2–2]	2 [2–2]

Values are n (%) or median [IQR]. Stop intervals were derived from recorded last-dose and surgery dates. No patient received aspirin on the morning of surgery. Oral anticoagulant use was the union of warfarin and direct oral anticoagulant use.

Table S14. First postoperative-morning laboratory measurements and ICU-admission lactate

Measure	PAAD (n=117)	ANH (n=103)
Hemoglobin, g/dL	9.07 (1.42)	9.19 (1.25)
Hematocrit, %	26.79 (4.39)	27.09 (3.63)
Platelet count, 10 ⁹ /L	203.8 (72.7)	195.1 (65.9)
Creatinine, mg/dL	0.88 [0.74–1.04]	0.85 [0.72–1.04]
Lactate, mmol/L	2.10 [1.90–2.40]	2.10 [1.80–2.55]

Hemoglobin, hematocrit, platelet count, and creatinine were obtained from the first postoperative-morning laboratory panel; lactate was obtained from the ICU-admission blood gas. Values are mean (standard deviation) for hemoglobin, hematocrit, and platelet count and median [interquartile range] for creatinine and lactate. All listed measurements were available for all 220 patients. No between-group hypothesis test was performed. ANH, acute normovolemic hemodilution; ICU, intensive care unit; PAAD, post-heparinization pre-bypass acute autologous donation.

Supplementary Figure Legends

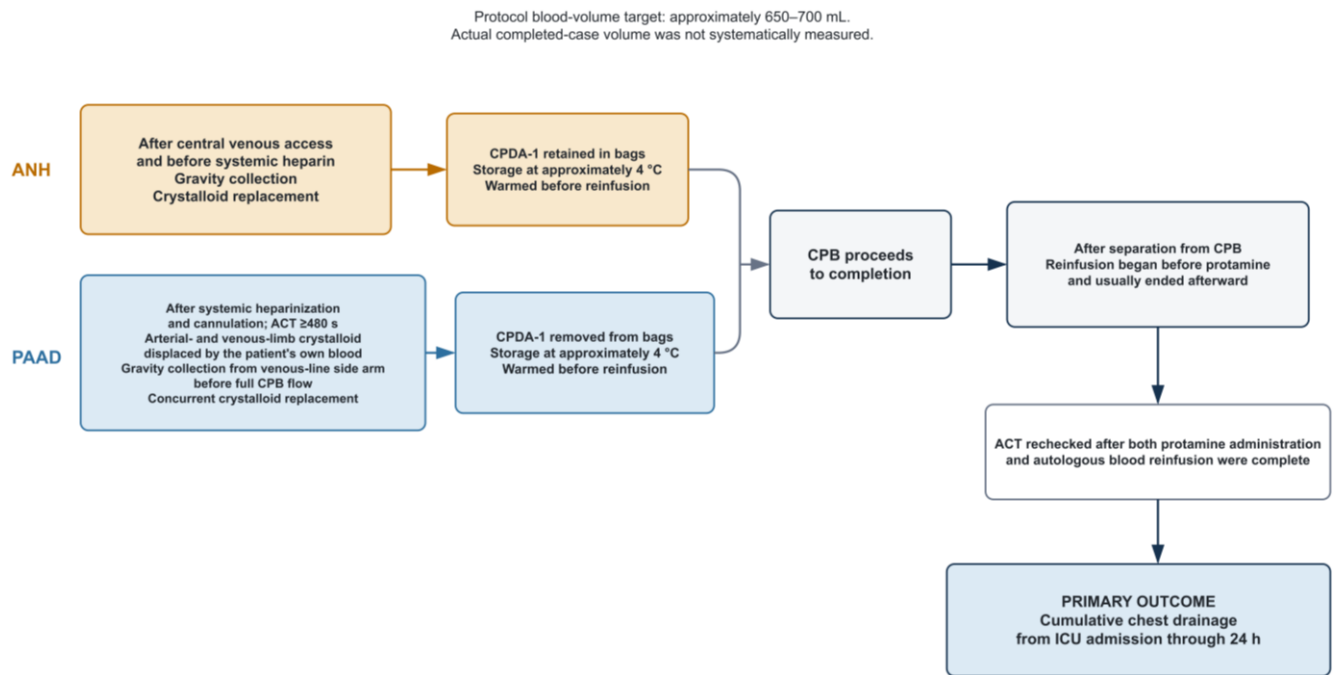
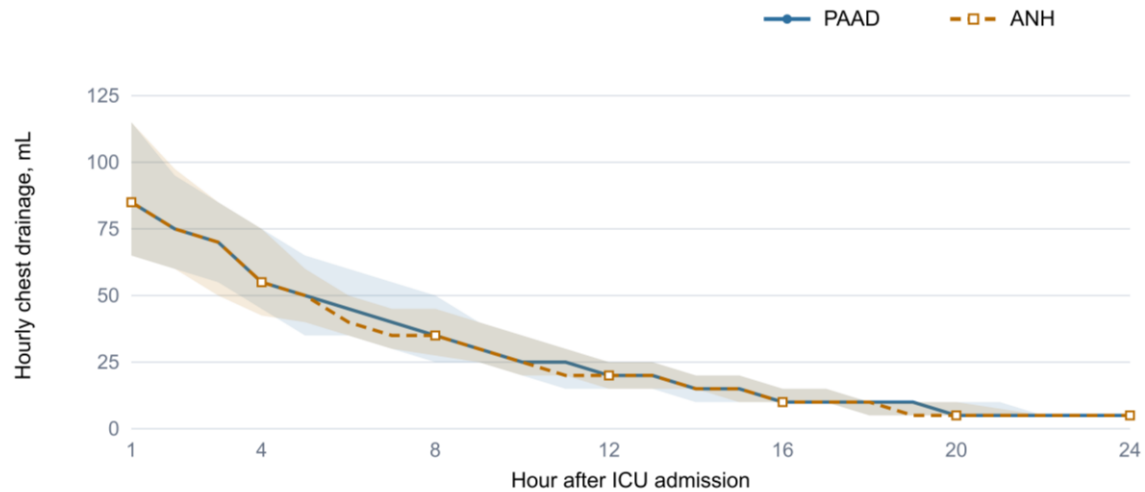


Fig. S1. Protocol timelines and outcome windows

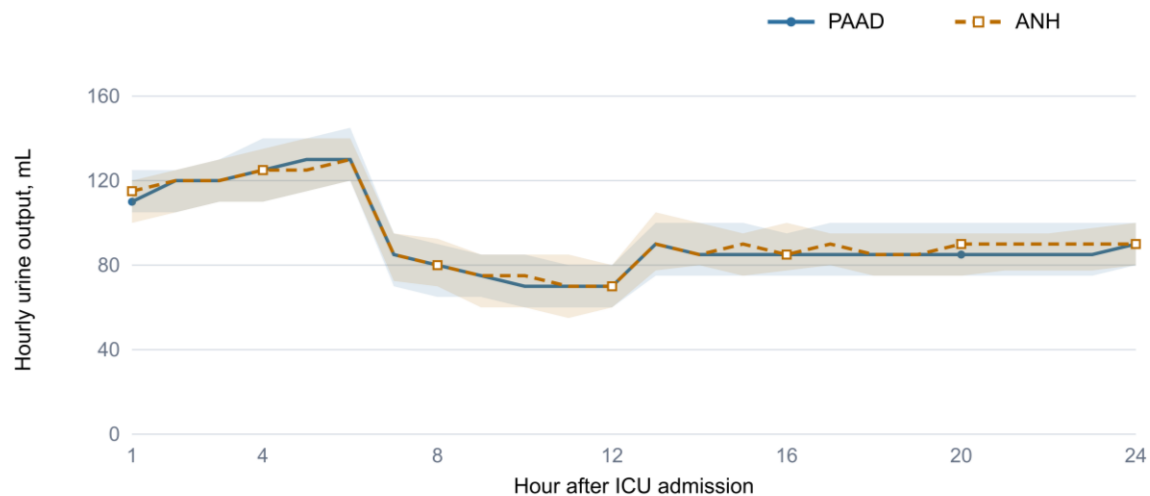
Schematic, not-to-scale comparison of ANH before systemic heparinization and the multicomponent PAAD strategy after systemic heparinization and cannulation but before full CPB flow. In PAAD, autologous circuit priming preceded gravity collection of heparinized whole blood from a side arm of the venous drainage line; CPDA-1 had been removed from the collection bags, and crystalloid replacement was administered concurrently. Both products were stored at approximately 4°C, warmed before reinfusion, and returned after separation from CPB, with reinfusion beginning before protamine and usually ending afterward. ACT was rechecked after both protamine administration and autologous-blood reinfusion were complete. The approximate 650–700-mL collection target and timing annotations summarize clinical practice rather than measured patient-level collection volumes. ANH, acute normovolemic hemodilution; ACT, activated clotting time; CPB, cardiopulmonary bypass; PAAD, post-heparinization pre-bypass acute autologous donation.



Lines show medians; shaded bands show interquartile ranges.

Fig. S2. Hourly postoperative chest-drainage trajectories

Group medians and interquartile ranges for recorded chest-drainage volumes during ICU hours 1-24. PAAD is shown with a solid blue line and filled circles; ANH is shown with a dashed orange line and open squares. The plot is descriptive, and no hour-specific inferential comparison was performed. ANH, acute normovolemic hemodilution; ICU, intensive care unit; PAAD, post-heparinization pre-bypass acute autologous donation.



Lines show medians; shaded bands show interquartile ranges.

Fig. S3. Hourly postoperative urine-output trajectories

Group medians and interquartile ranges for recorded urine-output volumes during ICU hours 1-24. PAAD is shown with a solid blue line and filled circles; ANH is shown with a dashed orange line and open squares. The plot is descriptive, and no hour-specific inferential comparison was performed. ANH, acute normovolemic hemodilution; ICU, intensive care unit; PAAD, post-heparinization pre-bypass acute autologous donation.