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A Longitudinal Study on the Perioperative Period of Cardiac Surgery — Multiple Interactions and Effects of Heparin Pleiotropy and Immune Microenvironment

Qi Chen[1], Qianqian Zhang[1], Guanjun Li[2], Honglei Chen[1] & Ping Li[2]

[1] Wuhan University, China

[2] Union Hospital Tongji Medical College Huazhong University of Science and Technology, China

Correspondence: Honglei Chen, Wuhan University, China; Ping Li, Union Hospital Tongji Medical College Huazhong University of Science and Technology, China.

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Abstract

Objective: To investigate the differences and trends of multiple monitoring indicators during the peri-cardiopulmonary bypass (CPB) period in patients undergoing heart transplantation (HTx) and ventricular assist device (VAD) implantation, and to explore the interaction and impact of unfractionated heparin (UFH) on the immune microenvironment under short-term, high-dose UFH administration. ***Methods:*** This prospective study enrolled 15 HTx and 19 VAD recipients between 2023 and 2024. Monitoring was conducted at five observation points: pre-CPB, 30 min post-CPB, before shutdown, 10 min post-heparin neutralization, and 30 min post-heparin neutralization. Coagulation function (TAT, PIC, D-dimer, etc.), inflammatory markers (IL-6, etc.), and endothelial injury markers (TM, etc.) were assessed, with Anti-Xa not monitored pre-CPB. Biochemical indicators, including liver and kidney function (ALT, AST, Cr, etc.), blood glucose, and lipid profiles (TC, TG, etc.), were measured only at pre-CPB, before shutdown, and 30 min post-heparin neutralization. Intergroup differences and trend changes were analyzed. ***Results:*** Significant differences were observed between the two groups in age, BMI, CPB duration, heparin dose, and aortic cross-clamp time, whereas no significant differences were found in priming volume or protamine sulfate (PS) dosage. Pre-CPB, PIC ($P=0.019$) and CK ($P=0.044$) were significantly higher in the HTx group, while TP ($P=0.018$) and CHE ($P=0.023$) were significantly higher in the VAD group. No significant intergroup differences were observed 30 min post-CPB. At the before shutdown timepoint, TAT ($P=0.027$), ALT ($P=0.048$), AST ($P<0.001$), TP/ALB ($P<0.001$), TBA ($P=0.006$), CK ($P<0.001$), HBP ($P=0.038$), and IL-6 ($P<0.001$) were significantly higher in the HTx group. At 10 min post-UFH neutralization, PIC ($P=0.027$), IL-6 ($P=0.001$), and PT ($P=0.047$) were significantly higher in the HTx group. At 30 min post-UFH neutralization, AST ($P<0.001$), TBA ($P=0.027$), CK ($P=0.005$), and IL-6 ($P<0.001$) remained significantly elevated in the HTx group. Trend analysis revealed differing dynamic patterns between HTx and VAD groups for ALP, D-dimer (DD), HBP, LDL, PCT, TM, TBA, and tPAIC, while other indicators exhibited similar trends. UFH, APTT, and PT peaked during CPB and declined post-heparin neutralization, whereas DD (HTx group) peaked 10 min after heparin neutralization before declining. ALB, ATIII, FIB, and HDL reached their nadir during CPB. CK, AST, and IL-6 showed time-dependent increases, while CREA (HTx group) and HDL (HTx group) decreased over time. PCT (HTx group) displayed fluctuating changes. ***Conclusion:*** During the peri-CPB period in cardiac surgery, the pleiotropic effects and unique pharmacokinetics of UFH lead to complex and significant interactions with the immune microenvironment. High-dose UFH exerts a positive regulatory effect on immune homeostasis during CPB, while the immune microenvironment also influences UFH's anticoagulant efficacy through multiple pathways. Additionally, the rapid clearance of UFH by PS may trigger a "second hit" on the immune

microenvironment via inflammatory mechanisms.

Keywords: cardiopulmonary bypass (CPB), heart transplantation (HTx), ventricular assist device (VAD), unfractionated heparin (UFH), immune microenvironment, coagulation-inflammation interaction

1. Introduction

Cardiopulmonary bypass (CPB) serves as the cornerstone technology in cardiac surgery, with its management quality directly determining patient prognosis (Eltzschig, H.K., B. Zwissler, & T.W. Felbinger, 2003). CPB can induce multiple pathophysiological disorders, including abnormal shear stress caused by non-physiological blood flow (Jiang, Q., J. Sun, L. Xu, X. Chang, L. Sun, Y. Zhen, & Z. Guo, 2021), cascade activation of systemic inflammatory response syndrome (SIRS) (Evora, P.R., C. Bottura, L. Arcêncio, A.A. Albuquerque, P.M. Évora, & A.J. Rodrigues, 2016), imbalance in the coagulation-fibrinolysis system (Bartoszko, J., & K. Karkouti, 2021), oxidative stress (Zakkar, M., G. Guida, M.S. Suleiman, & G.D. Angelini, 2015), and ischemia-reperfusion injury (Salameh, A., & S. Dhein, 2015). These disorders intertwine to form a vicious cycle, ultimately leading to CPB-associated liver and kidney injuries during cardiac surgery (Kulthinee, S., M. Warhoover, L. Puis, L.G. Navar, & E.Y. Gohar, 2024; Wang, X.D., Z.Z. Zhao, X.Y. Yang, R. Bao, Y.Y. Wang, Y. Lan, Z.Y. Quan, J.F. Wang, & J.J. Bian, 2024).

Unfractionated heparin (UFH), as the fundamental anticoagulant for CPB, at present, it is still considered the "gold standard" for CPB anticoagulation (Chen, Y., P.H.Y. Phoon, & N.C. Hwang, 2022; Frederiksen, J.W., 2000; Erdoes, G., I. Birschmann, M. Nagler, & A. Koster, 2021), possesses mechanisms of action that extend far beyond traditional understanding. UFH not only enhances antithrombin (AT)-mediated inhibition of thrombin (IIa) and factor Xa through binding with AT, but also exhibits complex immunomodulatory effects. These include suppressing complement activation, modulating neutrophil-endothelial cell adhesion, and reducing von Willebrand factor (vWF) release, thereby significantly attenuating CPB-related inflammatory activation, protecting endothelial tissue, and regulating coagulation function (Beurskens, D.M.H., J.P. Huckriede, R. Schrijver, H.C. Hemker, C.P. Reutelingsperger, & G.A.F. Nicolaes, 2020; Weiler, J.M., R.E. Edens, R.J. Linhardt, & D.P. Kapelanski, 1992; Spiess, B.D., 2017). Conversely, the anticoagulant efficacy of UFH can be influenced by various factors in the immune microenvironment, including direct interactions between UFH and inflammatory mediators, as well as indirect effects resulting from insufficient anticoagulation due to cross-talk mechanisms between inflammation and coagulation networks (Maier, C.L., J.M. Connors, & J.H. Levy, 2024). Therefore, it warrants investigation to determine whether and to what extent changes in immune microenvironment homeostasis during the peri-CPB period of cardiac surgery affect UFH's anticoagulant efficacy.

In addition, in extracorporeal circulation applications, sulfate protamine (PS) achieves precise neutralization of UFH for controllable anticoagulant management and provides the possibility for individualized weaning time selection. This is the key reason why unfractionated heparin remains the most widely used anticoagulant in extracorporeal circulation (Boer, C., M.I. Meesters, D. Veerhoek, & A.B.A. Vonk, 2018; Foubert, R., G. Van Vaerenbergh, G. Cammu, S. Buys, N. De Mey, P. Lecomte, S. Bouchez, S. Rex, & L. Foubert, 2024). The use of PS further leads to the unique pharmacokinetic characteristics of UFH in CPB — short-term high-dose infusion and rapid neutralization, which results in a significantly different impact of UFH on the immune microenvironment of patients during CPB compared to other clinical applications of UFH. This may further increase the complexity of dynamic changes in the patient's microenvironment during extracorporeal circulation.

The treatment of end-stage heart failure (ESHF) continues to pose significant challenges in modern cardiovascular medicine. As two pivotal therapeutic approaches that overcome the limitations of pharmacological treatment, heart transplantation (HTx) and ventricular assist device (VAD) implantation significantly improve patient outcomes through fundamentally distinct mechanisms (Miller, L., E. Birks, M. Guglin, H. Lamba, & O.H. Frazier, 2019; Habal, M.V., & A.R. Garan, 2017). During the perioperative period, HTx causes more severe inflammatory activation, coagulation disorders, and tissue damage compared to VAD. This difference stems from the greater surgical trauma associated with HTx, which involves donor heart procurement, complete cardiac excision, and anastomosis — procedures that markedly activate systemic inflammatory responses (Napoli, F., R. Aleman, N. Zadneulitca, J. Navia, & N.A. Brozzi, 2024; Boeken, U., P. Feindt, M. Micek, T. Petzold, H.D. Schulte, & E. Gams, 2000). In addition, HTx relies on CPB as perioperative life support and involves a longer duration of CPB, which leads to prolonged blood contact time with artificial materials, more pronounced complement activation, platelet consumption, and fibrinolysis imbalance. VAD, on the other hand, can undergo minimally invasive surgery and use partially bypass CPB or not require CPB to complete the surgery, which significantly shortens the CPB bypass time of VAD surgery (Cheung, A., J.L. Soon, J. Bashir, A. Kaan, & A. Ignaszewski, 2014; Lewin, D., G. Nersesian, L. Roehrich, M. Mueller, J. Mulzer, J. Stein, M. Kukucka, C. Starck, F. Schoenrath, V. Falk, S. Ott, & E.V. Potapov, 2022).

Given that HTx and VAD recipients share similar underlying diseases but undergo surgical protocols with

significant differences in trauma extent, CPB duration, and bypass strategies, we aim to investigate the mutual interactions between UFH and the immune microenvironment during cardiac surgery CPB by observing multiple dimensions of biomarkers in both groups, including coagulation, inflammation, tissue injury, and organ function during the peri-CPB period, combined with monitoring of plasma UFH concentrations. This approach will yield more comprehensive conclusions regarding immune microenvironment-based heparin resistance and further expand research findings on CPB-associated UFH resistance. Moreover, we particularly focus on the unique pharmacokinetic characteristics of CPB-related UFH application — short-term high-dose administration and rapid clearance. By integrating UFH's pleiotropic drug effects, we further discuss the potential impact of rapid UFH clearance at CPB weaning on the shock-like effects to recipients' immune microenvironments.

2. Materials and Methods

2.1 Study Subjects

The study subjects consisted of patients who underwent heart transplantation (HTx) or ventricular assist device (VAD) implantation at our institution between 2023 and 2024. All HTx recipients required cardiopulmonary bypass (CPB) support, while the need for CPB in VAD recipients was determined based on preoperative evaluation criteria including severe cardiogenic shock (cardiac index [CI] <2.0 L/min/m² or systolic blood pressure <80 mmHg), right heart failure (tricuspid annular plane systolic excursion [TAPSE] <1.4 cm, pulmonary vascular resistance [PVR] >4 Wood units), or requirement for concomitant cardiac surgery. Additional factors considered in CPB decision-making included end-organ dysfunction (e.g., lactate >4 mmol/L or coagulation abnormalities), imaging findings of left ventricular thrombus or aortic pathology, and team emergency response capabilities (such as ECMO backup). The final decision was made through multidisciplinary discussion, weighing the benefits of CPB support against associated risks on an individualized basis. We recorded baseline information for all subjects including age, BMI, total CPB time (min), CPB flow time (min), aortic cross-clamp time (min), priming volume (ml/kg), heparin dose (mg/kg), and protamine sulfate (PS) dose (mg/kg).

2.2 CPB Management

For HTx, full-flow bypass was established via ascending aortic cannulation (2-3 cm from the aortic root) and bicaval venous cannulation (separate superior and inferior vena cava cannulation or single right atrial cannulation). After heparinization (ACT >480 seconds), the aorta was clamped and cardioplegia (either 4:1 blood cardioplegia or HTK solution) was administered, followed by recipient heart excision and donor heart anastomosis. For VAD implantation (e.g., left ventricular assist), partial bypass was employed using aortic cannulation for inflow and left atrial appendage or left atrial cannulation for drainage, preserving native heart function. During bypass, hemodynamics (MAP 50-70 mmHg, CVP, SvO_2 >65%), oxygenation (PaO_2, $PaCO_2$, lactate), temperature (28-32°C for HTx, normothermia or mild hypothermia for VAD), and coagulation (ACT, platelets) were monitored, with transesophageal echocardiography (TEE) used to assess cardiac filling and VAD positioning. For HTx weaning, rewarming to >36°C, stable sinus rhythm, CI >2.2 L/min/m², and absence of severe acidosis (pH >7.3) were ensured before gradual flow reduction and decannulation. VAD weaning required stable device flow (2.5-5 L/min) and absence of left ventricular over-decompression (confirmed by TEE) before gradual CPB separation.

The initial UFH dose was fixed at 3.5 mg/kg, with 40 mg UFH added to the CPB circuit prime (16 mg for patients under 14 years). If ACT failed to reach 480 seconds despite normal antithrombin (AT) activity, additional UFH (50-100 U/kg, with possible adjustments beyond this range based on individual variation and ACT results) was administered, followed by repeat ACT testing after 5 minutes to confirm target attainment. If ACT remained subtherapeutic with reduced AT activity, fresh frozen plasma (FFP) or ATIII concentrate was administered before further UFH dosing. PS neutralization was administered at a 1:1 to 1.3:1 ratio to total heparin dose after complete weaning and surgical hemostasis. Post-PS administration, ACT was measured at 5-10 minutes and subsequently every 30 minutes for 2 hours, with therapeutic targets of 120-150 seconds (consecutive measurements varying <10%) and no clinical bleeding. For ACT >150 seconds with active bleeding (after excluding surgical causes), additional PS (0.25-0.5 mg/kg) was given. For ACT >180 seconds without bleeding, repeat testing was performed within 30 minutes. Mild ACT elevation (150-180 seconds) without bleeding tendency required confirmation of persistent elevation before PS supplementation (0.25-0.5 mg/kg per 50 seconds above target, or 0.5-1 mg/kg empirically for active bleeding), with repeat ACT testing 5-10 minutes post-adjustment and total PS dose not exceeding 3 mg/kg.

All CPB procedures used the Stockert S5 system, with Medtronic BB541 oxygenators for adults and CAPIOX FX15 for patients under 18 years.

2.3 Observation Timepoints and Sampling Protocol

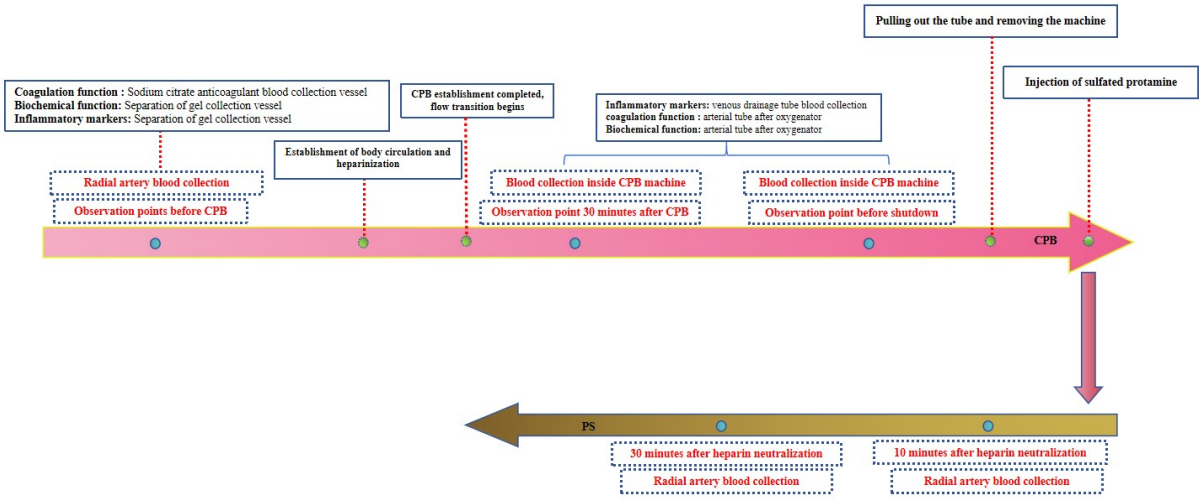

Figure 1. Experimental procedure. The specific location of each observation point during the CPB period, the sampling position and sampling method of each observation point.

This longitudinal peri-CPB study employed a standardized multi-timepoint sampling protocol across five observation points: pre-CPB (radial artery sampling before incision), 30 minutes post-CPB initiation (circuit sampling), before shutdown (circuit sampling 5-10 minutes before decannulation), and 10/30 minutes post-heparin neutralization (radial artery sampling). Coagulation tests used citrate-anticoagulated tubes (2 mL whole blood), while inflammatory markers and biochemistry used serum separator tubes (2 mL pre-CPB and after heparin neutralization; 1 mL x 2 at 30 min post-CPB and before shutdown). To avoid artifact, coagulation samples were drawn from the oxygenator's arterial line (minimizing contact activation), inflammatory samples from venous drainage (reflecting tissue cytokine release), and biochemistry samples from the arterial line (representing end-organ perfusion). All samples were processed immediately (Figure 1).

2.4 Analytical Measurements

Thirty-four peri-CPB biomarkers were longitudinally monitored across coagulation (PT, APTT, FIB, D-dimer, FDP, AT, Anti-Xa, TAT, PIC, TM, tPAIC), inflammation (IL-6, PCT, HBP), liver function (ALT, AST, TBIL, DBIL, TP, ALB, TBA, CHE, GGT, ALP), renal function (UREA, CREA, UA), lipids (TG, TC, HDL, LDL), glucose (GLU), and others (AMY, CK). Select markers had dual clinical significance (e.g., TM/tPAIC/HBP for endothelial injury; AST for hepatic/myocardial damage). Coagulation/inflammatory markers were measured at all five timepoints; biochemistry only at pre-CPB, before shutdown, and 30 minutes after heparin neutralization.

2.5 Detection Technology and Instruments

Due to intraoperative space constraints, a centrifugal microfluidic platform was selected for its compact size, multi-analyte capacity (enabling simultaneous measurement of numerous parameters from minimal whole blood volumes without preprocessing), and methodological alignment with conventional laboratory techniques (optical detection for coagulation/biochemistry; magnetic particle chemiluminescence for immunoassays). Preloaded reagent discs limited test menu flexibility. All instruments (MC550 coagulation analyzer, MI600 chemiluminescence analyzer, MS200 biochemistry analyzer) and consumables were from Zhejiang Pustar Biotechnology.

2.6 Statistical Analysis

Data were analyzed using SPSS (v27.0). Normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) determined parametric (mean ± SD) or nonparametric (median [IQR]) descriptive statistics. Between-group comparisons used independent t-tests (normal distribution, equal variance), Welch's t-tests (normal, unequal variance), or Mann-Whitney U tests (non-normal). Timepoint-specific HTx-VAD comparisons were performed, with dynamic trends visualized via observation point vs. mean value line graphs.

3. Results

3.1 Comparison Between HTx and VAD Groups

This study included a total of 34 subjects, with 19 in the VAD group (15 LVAD and 4 BiVAD recipients) and 15 in the HTx group. We recorded basic information including age, BMI, total CPB time, CPB flow time, aortic cross-clamp time, and weight-adjusted doses of UFH, priming solution, and PS. Statistical analysis revealed

significant between-group differences in age (P=0.017) and BMI (P=0.005), with higher values in the VAD group. Regarding CPB parameters, the HTx group showed significantly longer total CPB time (P=0.012), CPB flow time (P<0.001), aortic cross-clamp time (P<0.001), and higher heparin dose (P=0.030), while PS dose (P=0.336) and priming volume (P=0.408) showed no significant differences (Table 1).

At the observation point before CPB, CHE (P=0.023) and TP (P=0.018) were significantly higher in the VAD group, whereas PIC (P=0.019) and CK (P=0.044) were significantly higher in the HTx group (Table2), with no other significant differences observed (STable1).

Subgroup analysis of BiVAD versus LVAD recipients revealed multiple significant differences in post-weaning biochemical markers but no differences in coagulation or inflammatory markers throughout the observation period. Due to limited sample size (The sample size of BiVAD group is too low), further analysis was not performed (STable 6).

At the observation point 30 minutes after CPB, coagulation and inflammatory markers showed no significant intergroup differences, indicating comparable coagulation/fibrinolysis and inflammatory status between groups during early CPB (STable 2). Biochemical markers were not assessed at this timepoint.

At the observation point before shutdown (5-10 minutes before decannulation), demonstrated significantly higher values in the HTx group for TAT (P=0.027), ALT (P=0.048), AST (P<0.001), TP/ALB ratio (P<0.001), TBA (P=0.006), CK (P<0.001), HBP (P=0.038), and IL-6 (P<0.001) (Table1). Other test results did not show significant differences (STable3).

Following heparin neutralization with PS, assessments at 10 minutes after heparin neutralization significantly higher values in the HTx group for PIC (P=0.027), IL-6 (P=0.001), and PT (P=0.047) (Table 1), other test results did not show significant differences (STable4). At 30 minutes after heparin neutralization, AST (P<0.001), TBA (P=0.027), CK (P=0.005), and IL-6 (P<0.001) remained significantly elevated in the HTx group (Table 1), other test results did not show significant differences (STable5).

3.2 Temporal Changes in Measured Parameters Between HTx and VAD Groups

To more intuitively observe and compare the dynamic changes between the HTx and VAD groups at each observation timepoint, we calculated the mean values of each measured parameter across subjects at respective timepoints and plotted them on coordinate axes. This generated dynamic trend curves with the y-axis representing the mean values of test results and the x-axis representing the chronological sequence of observation timepoints. However, considering the presence of extreme outliers in some parameters and non-normal distribution of data sets, we also created box plots within the same coordinate system to better reflect the distribution characteristics of test results between the two groups. In both the line graphs and box plots, the same parameters from HTx and VAD groups are presented in identical coordinate systems to facilitate direct comparison.

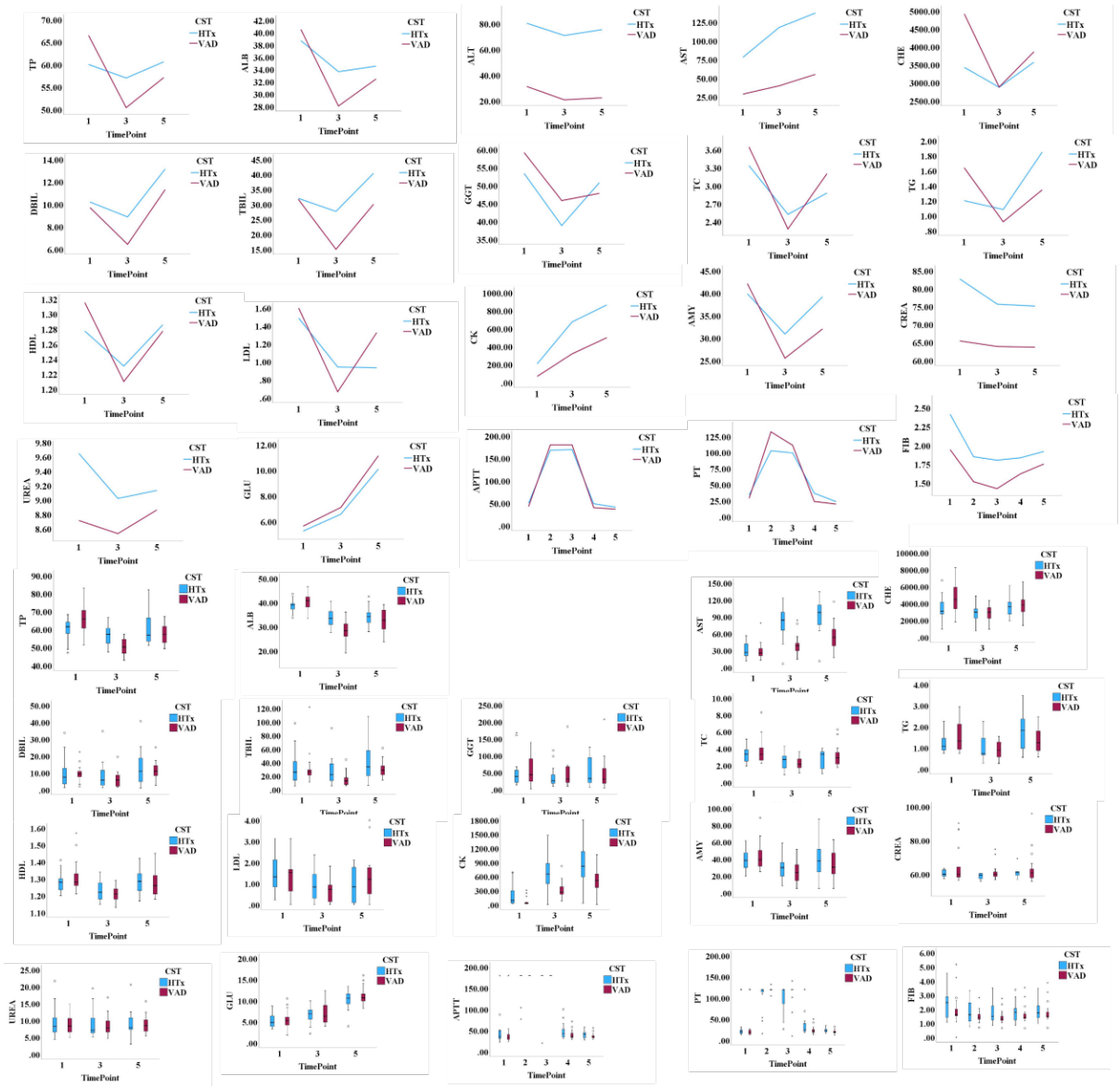

Figure 2.

Line chart and box chart of detection items with differences in observation point trends. Each point in the line chart is the average data of the detection results for the same item (HTx and VAD) in the group, while the box plot shows the overall distribution of the data. CST represents the type of cardiac surgery. TimePioneer is the time point corresponding to each observation point. On the left side of the coordinate axis are the corresponding detection items.

Parameters including TP, ALB, ALT, AST, CHE, DBIL, TBIL, GGT, TC, TG, HDL, LDL, CK, AMY, CREA, UREA, GLU, APTT, PT, and FIB demonstrated similar trends between groups. Most biochemical markers (TP, ALB, CHE, DBIL, TBIL, GGT, TC, TG, HDL, LDL, AMY, UREA) reached nadir values at the before shutdown timepoint (except ALT which showed minimal change). AST, GLU, and CK exhibited progressive increases across three timepoints, while CREA progressively decreased. Coagulation parameters APTT and PT peaked during CPB (30min post-CPB and before shutdown), whereas FIB reached its nadir during CPB (Figure 2).

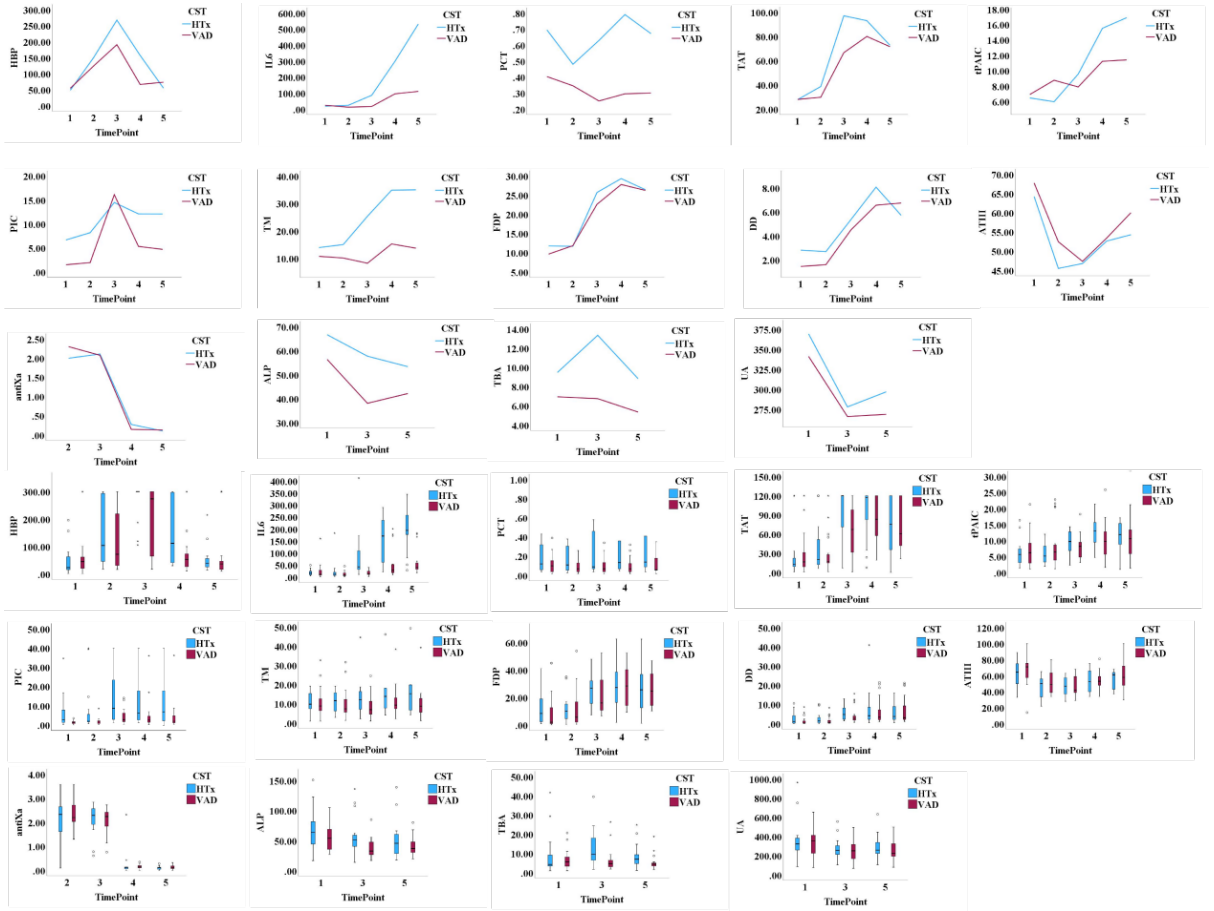

Figure 3. Line and box plots of detection items with relatively consistent trends at each observation point.

IL-6 dynamics differed markedly: VAD recipients showed CPB-associated suppression (levels below baseline during CPB), while HTx recipients demonstrated progressive elevation. Both groups showed after heparin neutralization increases, with more pronounced elevation in HTx (Figure 3).

HBP peaked during CPB in both groups (maximum assay limit: 300 ng/ml), but after heparin neutralization patterns diverged - HTx showed continuous decline while VAD exhibited mild rebound at 30 minutes (Figure 3).

PCT dynamics varied: HTx reached nadir at 30min post-CPB then rose before declining after heparin neutralization; VAD nadired before shutdown with partial recovery after heparin neutralization (Figure 3).

TAT trajectories differed: HTx peaked before shutdown then declined after heparin neutralization; VAD peaked at 10min after heparin neutralization before declining (Figure 3).

tPAIC patterns diverged: HTx nadired at 30min post-CPB then rose continuously; VAD rose initially before nadiring before shutdown then rising again (Figure 3).

While PIC trends were generally similar, VAD showed abrupt before shutdown elevation exceeding HTx levels (Figure 3).

TM dynamics differed: HTx rose continuously until stabilizing after heparin neutralization; VAD declined before shutdown before peaking at 10min after heparin neutralization (Figure 3).

FDP patterns were generally concordant except for HTx showing CPB-associated suppression at 30min (Figure 3).

DD trajectories diverged: HTx nadired at 30min post-CPB then rose until neutralization; VAD rose continuously (Figure 3).

AT activity declined during CPB in both groups but reached nadir earlier in HTx (30min post-CPB) versus VAD (before shutdown) (Figure 3).

Anti-Xa (reflecting UFH concentration) peaked earlier in VAD (30min post-CPB) versus HTx (before shutdown), with faster after heparin neutralization decline in VAD (Figure 3).

ALP decreased continuously in HTx versus VAD which nadired before shutdown then recovered (Figure 3).

TBA peaked before shutdown in both groups but with more pronounced elevation in HTx (Figure 3).

UA nadired before shutdown in both groups, with VAD showing stable after heparin neutralization levels (Figure 3).

These temporal analyses comprehensively demonstrate the dynamic changes of various parameters during HTx and VAD procedures, reflecting the real-time alterations in subjects' internal environment and organ function. By superimposing the curves, we can further examine the differences in trend patterns between the two groups of subjects.

4. Discussion

Based on the baseline characteristics of the subjects, although significant differences existed in age and BMI between the two groups, among all parameters measured at the pre-CPB (preoperative) timepoint, only CHE and TP showed significantly higher values in the VAD group compared to HTx, while PIC and CK were significantly higher in the HTx group. All other parameters showed no significant differences, indicating relatively comparable coagulation function, inflammatory status, and tissue damage levels between the two groups before CPB. The absence of significant difference in weight-adjusted priming volume further suggests similar degrees of hemodilution effects. Data analysis revealed that the HTx group maintained consistently higher TAT levels throughout the peri-CPB period, with significantly higher values at before shutdown compared to VAD, and IL-6 levels were persistently higher in HTx, becoming significantly elevated after the before shutdown timepoint. These findings suggest stronger inflammatory and coagulation activation in HTx recipients. Interestingly, while the fibrinolytic system appeared more activated in HTx, the formation of fibrinolytic products (DD, FDP) was not markedly evident during CPB due to UFH administration (no significant intergroup differences), and PIC generation also showed no significant difference during CPB.

Our initial observation was that the HTx group received significantly more UFH than VAD, yet both groups required nearly equivalent PS doses for neutralization. Anti-Xa measurements revealed comparable plasma UFH concentrations between groups throughout CPB, with VAD even showing slightly higher levels at 30 minutes post-CPB initiation. This suggests immediate UFH loss in HTx recipients upon initial administration. Therefore, a thought-provoking question arises, which is why the HTx group used more UFH compared to the VAD group, while the two groups of subjects used almost equal amounts of PS to neutralize UFH, and within 30 minutes after UFH neutralization, the plasma UFH levels of the two groups of subjects were almost the same, indicating that UFH was also effectively cleared in the HTx group. Has this additional UFH really been effectively cleared?

Assuming that the UFH of both groups of subjects is effectively cleared, we believe that there may be three factors leading to an increase in UFH consumption in the HTx group: prolonged CPB duration, accelerated heparin metabolism due to hepatic injury, and inflammatory mediator-mediated UFH "sequestration" in the immune microenvironment. While HTx did involve significantly longer CPB times, the negative correlation between UFH metabolism and dosage, combined with continuous infusion during CPB, ensures relatively stable circulating UFH levels. Therefore, time difference alone cannot fully account for the observed UFH depletion.

Biochemical analysis showed no marked differences in lipid profiles, glucose, or renal function between groups. However, the HTx group demonstrated significantly higher ALT, AST, TP/ALB ratio, TBA, and CK at before shutdown. ALT, AST, and CK are sensitive markers for parenchymal injury in liver and heart, while TP, ALB, and TBA reflect hepatic synthetic function. The progressive rise in CK and AST aligns with expected cardiac injury patterns during surgery, whereas the more stable ALT trend suggests hepatic injury may reach its peak earlier during CPB. Notably, HTx showed both greater hepatic injury and better preserved synthetic function compared to VAD — an apparent paradox. We propose that while HTx's longer CPB duration and procedural complexity cause more severe hepatic and cardiac injury, the liver's strong compensatory capacity enables it to enter an acute-phase response under prolonged stress. This fundamentally indicates more significant organ dysfunction in HTx despite apparent synthetic function preservation.

As UFH clearance primarily occurs via heparinase in the reticuloendothelial system rather than hepatic enzymes, acute-phase liver changes may variably affect UFH metabolism through alterations in hepatic hemodynamics and Kupffer cell activation by inflammatory mechanisms. While enhanced protein synthesis in HTx might suggest faster UFH clearance, the nonlinear pharmacokinetics of heparin metabolism in acute liver injury precludes definitive conclusions about its predominant role in UFH depletion.

We posit that heightened inflammatory activation and endothelial injury in HTx represent the primary mechanisms for UFH "disappearance." Although UFH's sulfate groups exhibit higher affinity for AT ($Kd \approx 10^{-9}$ M) than inflammatory mediators (e.g., HMGB1 with $Kd \approx 10^{-6}$ M), acute inflammation dramatically increases cytokine concentrations. According to mass action principles, this "quantity advantage" enables competitive occupation of UFH binding sites, reducing AT binding efficiency and anticoagulant efficacy. Simultaneously, endothelial heparan sulfate (HS) can electrostatically bind circulating UFH ($Kd \approx 10^{-7}$–10^{-6} M). While normally

forming a “heparin reservoir,” injured endothelium sheds HS, accelerating heparin clearance through three mechanisms: direct removal, ATIII binding interference, and procoagulant microenvironment formation. Our data shows significantly higher IL-6 in HTx, while endothelial injury markers (HBP, TM, tPAIC) demonstrated consistently (though not always significantly) higher trends in HTx — all evidence supporting UFH sequestration by inflammation and endothelial damage.

There appears to be a remaining gap in the logical chain that needs to be addressed. Assuming that the UFH in the HTx group was indeed sequestered by inflammatory mediators, this portion of UFH did not vanish — rather, after neutralization by protamine sulfate (PS), it remained in circulation. However, why was this residual UFH not detected via Anti-Xa assay? We hypothesize that this fraction of UFH may predominantly exert non-anticoagulant pharmacological effects, such as modulation of inflammatory pathways. Since the Anti-Xa assay is based on the anticoagulant properties of UFH, the assay’s inability to detect this fraction could be attributed to the suppression of UFH’s anticoagulant activity by abundant inflammatory mediators. Consequently, this additional UFH may continue to exert limited non-anticoagulant effects and is gradually metabolized until elimination. Subsequent observations revealed no heparin rebound-like symptoms in the HTx group, suggesting that the UFH in this cohort was effectively cleared. The excess UFH appears to have been masked by various antagonistic mechanisms against its anticoagulant function. Furthermore, dynamic changes in inflammatory mediators seem to support this hypothesis: following PS-mediated UFH clearance, a surge of inflammatory mediators re-entered systemic circulation (In the following discussion, there will be further elaboration). However, if we further assume that this situation does exist, we may need to consider whether using zero balance ultrafiltration (ZBUF) and Modified Ultrafiltration (MUF) before CPB shutdown to clear a large amount of inflammatory factors, while still using PS at a dose lower than the total UFH dosage, will result in insufficient heparin clearance, especially in pediatric cardiac surgery where higher CPB flow rates lead to more thorough clearance of inflammatory mediators.

Based on this consideration, it is possible to further evaluate the optimal dose of PS for UFH clearance by combining the degree of ultrafiltration with the concentration of inflammatory mediators in plasma. This may be a valuable research direction for optimizing PS dosage. This may be a valuable research direction for optimizing PS dosage.

Given UFH’s unique peri-CPB pharmacokinetics (bolus + continuous infusion with rapid neutralization), we specifically analyzed IL-6 dynamics. From 30min post-CPB to before shutdown, IL-6 elevation was minimal, with VAD even showing slight decline. However, post-PS neutralization triggered dramatic IL-6 surges, particularly in HTx. This suggests UFH’s direct cytokine binding reduces plasma inflammatory mediator concentrations, exerting anti-inflammatory effects that protect endothelial integrity and modulate coagulation. Although UFH suppresses IL-6 during CPB, ongoing production leads to cytokine accumulation, which floods circulation upon UFH clearance by PS.

HBP, released by neutrophils during tissue injury/inflammation, showed characteristic peri-CPB elevation followed by after heparin neutralization decline to pre-UFH levels. While previous studies attributed this pattern to UFH-neutrophil interactions, we propose an endothelial mechanism: At resting state, HBP binds activated endothelium via high-affinity β-integrins (Kd≈nM). During CPB, bolus UFH (reaching 30-60 μM) competitively displaces endothelial-bound HBP through sheer concentration advantage despite lower affinity (Kd≈μM). This explains both the dramatic HBP surge during UFH administration and rapid after heparin neutralization decline as PS clears UFH, allowing HBP re-binding to endothelium. While HBP-endothelial reattachment may exacerbate barrier damage, our TM/tPAIC data showed no significant after heparin neutralization spikes, though delayed endothelial injury markers cannot be excluded.

Collectively, surgical trauma and CPB constitute the “first hit” to immune homeostasis, while PS-mediated UFH clearance and consequent inflammatory mediator redistribution may represent a “second hit” to the immune microenvironment — a phenomenon warranting further investigation.

5. Conclusions

It is evident that during CPB, the pleiotropic effects of UFH and its unique pharmacokinetic characteristics (short-term high-dose infusion + rapid neutralization) interact with the patient’s immune microenvironment in a complex and significant manner. First, high-dose UFH in circulation can regulate immune microenvironment homeostasis by modulating coagulation function, inflammatory responses, and mitigating tissue damage. On the other hand, inflammatory mediators and endothelial cell injury within the immune microenvironment, under the influence of UFH’s non-anticoagulant effects, further impact UFH’s anticoagulant efficacy. This reminds us that when addressing UFH resistance during the peri-CPB period, it is essential to consider not only factors such as AT deficiency but also the broader immune microenvironment, including the degree of inflammation, endothelial injury, and circulatory dysfunction, to comprehensively understand the causes of UFH resistance. Furthermore, the clearance dose of PS on UFH may also need to be comprehensively evaluated in conjunction with the plasma

levels of inflammatory mediators. Additionally, the rapid clearance of UFH by PS may lead to the redistribution of inflammatory mediators in circulation. Furthermore, the loss of UFH's protective effects on endothelial tissue raises the question of whether PS-mediated neutralization of UFH could result in a "second hit" to the immune microenvironment — a topic highly worthy of further investigation.

6. Limitations

The primary limitation of this study lies in its small sample size, which may introduce uncertainty to the findings. Due to experimental constraints, our selection of biomarkers was limited, thereby restricting the comprehensiveness and sensitivity of our results. For instance, in studying inflammatory mechanisms, we initially aimed to assess multiple levels, including inflammatory initiators and inhibitors, rather than relying solely on IL-6 measurements. Additionally, CK and AST lack specificity for myocardial injury. Incorporating markers such as cTnI, BNP, and others could have provided a clearer distinction in myocardial damage between the two surgical approaches. Therefore, in future research, we hope to conduct more longitudinal studies with larger sample sizes and a broader range of detection indicators to enhance the rigor of our findings.

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Appendix

Table 1. Comparison of Basic information characteristics results of subjects

Basic information	**VAD**	**HTx**	**P**
Number	19 (L 15, B 4)	15	-
Age (year)	58 (48,62)	45 (14,54)	0.017*
BMI	85 (74,105)	19.39±4.59	0.005**
Total extracorporeal circulation time (min)	85 (74,105)	111 (99,118)	0.012*
Heparin dosage (mg/kg)	4.7 (4.25,5.21)	5.24 (4.64,6.17)	0.030*
Pre flushing solution (ml/kg)	18.38 (15.00,19.35)	20.75 (18.93, 29.61)	0.408
Ascending aorta occlusion time (min)	37 (30,50)	24 (21,31)	<0.001***
Extracorporeal circulation time (min)	49.32±30.24	80.47±14.88	<0.001***
Dosage of sulfated fish protein (mg/kg)	4.44 (4.23, 4.67)	4.46 (4.29, 5.38)	0.336

In the VAD group, L represents LVAD and B represents BiVAD; Data columns that follow a normal distribution are expressed as Mean ± SD, while data that do not follow a normal distribution are expressed as median (lower quartile, upper quartile); For P-values, they are annotated based on the degree of statistical significance*, p<0.05, **p<0.01, ***p<0.001.

Table 2. The detection results show significant differences between two sets of data at all observation points

Observation Point	**Item**	**VAD**	**HTx**	**P**
Before CPB	PIC (μg/ml)	1.52±0.97	2.83 (1.41,9.45)	0.019*
	TP (g/L)	66.49±8.04	60.03±6.30	0.018*
	CK (U/L)	29 (25.55,50.70)	94.00 (29.40,387.00)	0.044*
	CHE(U/L)	4916.53±1949.39	3430.87±1492.00	0.023*
Before shutdown	TAT (ng/ml)	67.31 (29.27,110.78)	120.00 (65.84,120.00)	0.027*
	ALT (U/L)	14.95 (10.25,28.38)	25.8 (15.00,44.10)	0.048*
	AST (U/L)	38.90 (28.96,43.68)	84 (63.7,101.00)	<0.001***
	TP (g/L)	50.25 (46.65,54.65)	57.02±5.67	<0.001***
	ALB (g/L)	28.07±4.29	33.65±3.83	<0.001***
	TBA (μmol/L)	4.78 (3.08,6.59)	9.61 (6.26,21.8)	0.006**
	CK (U/L)	273.50 (222.25,389.00)	674.17±360.80	<0.001***
	IL-6 (pg/ml)	17.32±9.95	42.57 (29.90,152.92)	<0.001***

10 mins after heparin neutralization	PIC (μg/ml)	2.65 (1.49,4.86)	7.11 (2.74,19.90)	0.027*
	IL-6 (pg/ml)	28.36 (16.76,54.35)	174.75 (61.74,254.75)	0.001**
	PT (s)	21.30 (18.60,27.60)	27.00 (21.40,39.90)	0.047*
30 mins after heparin neutralization	PIC (μg/ml)	2.06 (0.85,5.95)	8.71 (2.23,17.91)	0.036*
	AST (U/L)	53.65 (37.95,68.93)	102 (76.40,125.00)	<0.001***
	TBA (μmol/L)	4.45 (3.31,5.22)	7.53 (4.77,14.80)	0.027*
	CK (U/L)	499.81±247.66	865.68±450.99	0.005**
	IL-6 (pg/ml)	39.98 (31.77,66.45)	196.89 (177.27,290.18)	<0.001***

STable 1.

Before CPB	**VAD**	**HTx**	**P**
TM (U/ml)	8.86 (6.26,12.84)	9.81 (6.85,17.23)	0.471
TAT (ng/ml)	16.76 (8.79,40.11)	13.17 (9.41,24.79)	0.811
PIC (μg/ml)	1.52±0.97	2.83 (1.41,9.45)	0.019*
tPAIC (ng/ml)	6.23 (2.69,9.55)	5.68 (2.97,7.67)	0.864
PT (s)	18.6 (14.6,25.1)	20.80 (15.85,26.43)	0.377
APTT (s)	33.8 (28.6,43.9)	36.1 (30.98,50.70)	0.397
FIB (g/L)	1.6 (1.53,2.15)	2.42±1.09	0.304
D-Dmier (μg/ml)	0.33 (0.24,2.24)	1.20 (0.28,4.56)	0.212
ATIII (%)	67.74±19.78	64.21±16.33	0.553
FDP (μg/ml)	2.01 (0.91,20.55)	8.35 (2.15,19.98)	0.226
ALT (U/L)	31.16±20.46	13.50 (11.70,41.50)	0.433
AST (U/L)	25.9 (19.1,35.05)	27.00 (20.80,42.20)	0.455
ALP (U/L)	56.37±23.84	66.65±36.82	0.455
GGT (U/L)	59.15±41.19	38.80 (19.60,59.60)	0.526
TP (g/L)	66.49±8.04	60.03±6.30	0.018*
ALB (g/L)	40.51±3.50	38.68±2.62	0.109
DBIL (μmol/L)	9.5 (6.55,11.4)	7.62 (2.99,13.50)	0.576
TBIL (μmol/L)	26.2 (19.85,32.65)	25.70 (9.99,41.90)	0.941
TBA (μmol/L)	5.68 (3.0,8.18)	4.31 (3.09,9.38)	0.970
GLU (mmol/L)	5.66±2.21	5.27±1.58	0.580
CK (U/L)	29 (25.55,50.70)	94.00 (29.40,387.00)	0.044*
TC (mmol/L)	3.3 (2.56,4.17)	3.34±1.02	0.823
TG (mmol/L)	1.64±0.75	1.08 (0.90,1.49)	0.132
HDL (mmol/L)	1.28 (1.25,1.35)	1.28±0.62	0.350
LDL (mmol/L)	1.54 (0.65,1.87)	1.49±0.90	0.941
CHE(U/L)	4916.53±1949.39	3430.87±1492.00	0.023*
AMY (U/L)	39.3 (30.56,50.55)	39.87±13.77	0.710
UA (μmol/L)	341.25±155.95	323 (258,397)	0.882
UREA (mmol/L)	8.71±2.56	8.25 (6.10,11.70)	0.970
CREA (μmol/L)	60 (58.15, 70.05)	60.10 (58.80,63.50)	0.628
HBP (ng/ml)	45.6 (19.49,63.01)	23.51 (15.51,75.35)	0.560
PCT (ng/ml)	0.1 (0.04,0.16)	0.12 (0.06,0.43)	0.256
IL-6 (pg/ml)	11.84 (7.23,33.61)	16.16 (8.91,29.94)	0.811

The comparison results of all detection data at the observation points before CPB are described by the mean ± SD for data columns that conform to normal distribution, and the median (lower quartile, upper quartile) for data columns that do not conform to normal distribution; The results of the significant difference analysis are represented by the P-value. For data that follows a normal distribution and has homogeneous variance, independent sample t-test is used for analysis; If the data follows a normal distribution but with uneven variance, Welch's t-test is used; For data that does not follow a normal distribution, Mann Whitney U test is uniformly used. If the P value is less than 0.05, it indicates a significant difference and is marked with an asterisk (*). The marking rule is p<0.05**, p<0.01, ***p<0.001. (The following tables all adopt this standard)

STable 2. Comparison of data at observation points 30 minutes after body circulation

30 minutes after CPB	VAD	HTx	P
TM (U/ml)	7.4 (5.36,12.32)	11.69 (6.06,16.20)	0.336
tPAIC (ng/ml)	6.4 (3.88,8.99)	5.16 (3.19,8.40)	0.451
TAT (ng/ml)	17.44 (14.32,30.25)	20.56 (12.16,60.39)	0.681
PIC (μg/ml)	1.58 (0.82,2.37)	2.12 (1.71,8.38)	0.071
PCT (ng/ml)	0.07 (0.04,0.13)	0.11 (0.04,0.38)	0.190
IL-6 (pg/ml)	7.49 (5.35,17.69)	11.48 (6.13,22.31)	0.391
PT (s)	120.00	120.00	-
APTT (s)	180.00	180.00	-
FIB (g/L)	1.42 (1.28,1.75)	1.85±0.82	0.141
ATIII (%)	52.47±14.66	50 (33,56)	0.256
DD (μg/ml)	0.70 (0.29,1.78)	1.45 (0.5,3.42)	0.242
FDP (μg/ml)	5.65 (1.98,20.8)	10.05 (4.51,15.57)	0.471
Anti-Xa (U/ml)	2.30±0.61	2.00±0.96	0.173
HBP (ng/ml)	124.44±112.58	104.00 (42.16,300)	0.451

STable 3. Comparison of data from observation points before shutdown

Before shutdown	VAD	HTx	P
TM (U/ml)	6.95 (4.49,12.46)	12.17 (6.95,8.02)	0.051
TAT (ng/ml)	67.31 (29.27,110.78)	120.00 (65.84,120.00)	0.027*
PIC (μg/ml)	3.01 (1.86,6.84)	14.49±14.06	0.060
tPAIC (ng/ml)	8.21 (4.83,9.68)	9.58±3.68	0.214
PT (s)	120	120	-
APTT (s)	180	180	-
FIB (g/L)	1.32 (1.17,1.54)	1.80±0.78	0.202
D-Dmier (μg/ml)	2.79 (1.77, 4.82)	5.37±3.74	0.354
ATIII (%)	42.00 (39.00,60.00)	47.00 (36.00,58.00)	0.811
FDP (μg/ml)	22.63±14.78	25.71±12.49	0.524
ALT (U/L)	14.95 (10.25,28.38)	25.8 (15.00,44.10)	0.048*
AST (U/L)	38.90 (28.96,43.68)	84 (63.7,101.00)	<0.001***
ALP (U/L)	32.70 (26.48,48.15)	51.50 (39.10,62.80)	0.051
GGT (U/L)	30.70 (20.65,68.00)	26.80 (14.9,48.1)	0.532
TP (g/L)	50.25 (46.65,54.65)	57.02±5.67	<0.001***
ALB (g/L)	28.07±4.29	33.65±3.83	<0.001***
DBIL (μmol/L)	5.94 (2.20,8.91)	5.81 (1.91,12.6)	0.789
TBIL (μmol/L)	12.95 (7.46,18.28)	22.5 (9.76,39.1)	0.052

TBA (μmol/L)	4.78 (3.08,6.59)	9.61 (6.26,21.8)	0.006**
GLU (mmol/L)	6.37 (4.91,9.09)	6.58±2.19	0.530
CK (U/L)	273.50 (222.25,389.00)	674.17±360.80	<0.001***
TC (mmol/L)	2.28±0.74	2.52±1.02	0.434
TG (mmol/L)	0.90 (0.56,1.28)	1.08±0.61	0.355
HDL (mmol/L)	1.21 (1.18,1.25)	1.23±0.06	0.298
LDL (mmol/L)	0.67±0.60	0.94±0.77	0.256
CHE(U/L)	2972.00 (2121.75,3548.00)	2876.87±980.73	0.987
AMY(U/L)	24.15 (14.48,34.20)	30.91±14.38	0.268
UA (μmol/L)	266.27±115.04	256.00 (207.00,307.00)	0.766
UREA (mmol/L)	7.83 (6.48,10.14)	7.13 (6.23,11.2)	0.762
CREA (μmol/L)	60.00 (58.63,61.95)	59.70 (56.50,60.60)	0.486
HBP (ng/ml)	273.89 (40.78,300.00)	300	0.038
PCT (ng/ml)	0.70 (0.04,0.14)	0.90 (0.07,0.58)	0.066
IL-6 (pg/ml)	17.32±9.95	42.57 (29.90,152.92)	<0.001***
Anti-Xa (U/ml)	2.24 (1.83,2.42)	2.29 (1.76,2.57)	0.632

STable 4. Comparison of data at the observation point 10 minutes after heparin neutralization

10 minutes after heparin neutralization	**VAD**	**HTx**	**P**
TM (U/ml)	9.33 (7.24,13.17)	14.59 (7.83,18.51)	0.167
tPAIC (ng/ml)	9.75 (5.45,13.12)	13.45 (9.53,16.20)	0.096
TAT (ng/ml)	83.28 (54.04,120)	120 (82.68,120)	0.286
PIC (μg/ml)	2.65 (1.49,4.86)	7.11 (2.74,19.90)	0.027*
PCT (ng/ml)	0.07 (0.04,0.13)	0.13 (0.07,0.36)	0.051
IL-6 (pg/ml)	28.36 (16.76,54.35)	174.75 (61.74,254.75)	0.001**
PT (s)	21.30 (18.60,27.60)	27.00 (21.40,39.90)	0.047*
APTT (s)	35.70 (33.00,45.40)	41.40 (34.90,53.00)	0.157
FIB (g/L)	1.43 (1.32,1.68)	1.83±0.72	0.380
ATIII (%)	53.00 (47.00,60.00)	46.00 (40.00,66.00)	0.656
DD (μg/ml)	3.39 (2.02,9.14)	4.65 (2.34,8.64)	0.681
FDP (μg/ml)	28.42 (13.24,40.69)	28.41±16.90	0.905
Anti-Xa (U/ml)	0.15±0.10	0.12 (0.07,0.14)	0.319
HBP (ng/ml)	53.36 (17.27,74.88)	108.47 (38.89,297.37)	0.137

STable 5. Comparison of data at the observation point 30 minutes after heparin neutralization

30 minutes after heparin neutralization	**MCS**	**HTx**	**P**
TM (U/ml)	8.76 (5.06,14.17)	15.56 (6.72,19.84)	0.089
TAT (ng/ml)	60.74 (40.99,120)	79.50 (35.80,120.00)	0.732
PIC (μg/ml)	2.06 (0.85,5.95)	8.71 (2.23,17.91)	0.036*
tPAIC (ng/ml)	10.57 (5.52,13.96)	12.00 (8.84,16.37)	0.945
PT (s)	19.00 (17.40,22.60)	23.40 (20.20,16.10)	0.056
APTT (s)	34.90 (32.60,38.20)	41.13±9.29	0.167
FIB (g/L)	1.50 (1.42,1.87)	1.93±0.77	0.391
D-Dmier (μg/ml)	3.17 (2.19,14.18)	4.12 (2.25,8.99)	0.837

ATIII (%)	60±16.93	60.00 (43.00,64.00)	0.220
FDP (μg/ml)	24.67 (13.51,37.81)	26.11±16.42	0.979
ALT (U/L)	15.2 (8.03,34.78)	32.10 (14.40,44.40)	0.135
AST (U/L)	53.65 (37.95,68.93)	102 (76.40,125.00)	<0.001***
ALP (U/L)	42.17±17.33	44.80 (25.40,60.60)	0.708
GGT (U/L)	30.60 (17.33,66.78)	48.86±39.53	0.735
TP (g/L)	57.12±5.30	57.70 (53.50,66.40)	0.464
ALB (g/L)	32.46±4.66	34.45±4.12	0.206
DBIL (μmol/L)	11.15 (7.81,14.43)	8.74 (4.98,18.80)	0.901
TBIL (μmol/L)	28.80 (21.30,35.70)	31.80 (20.70,57.80)	0.442
TBA (μmol/L)	4.45 (3.31,5.22)	7.53 (4.77,14.80)	0.027*
GLU (mmol/L)	10.70 (9.79,11.98)	10.70 (9.28,11.60)	0.735
CK (U/L)	499.81±247.66	865.68±450.99	0.005**
TC (mmol/L)	2.92 (2.21,3.63)	3.31 (1.66,3.64)	0.682
TG (mmol/L)	1.35±0.57	1.82±1.00	0.087
HDL (mmol/L)	1.28±0.08	1.28±0.07	0.796
LDL (mmol/L)	1.21 (0.52,1.77)	0.87 (0.10,1.77)	0.464
CHE (U/L)	3863.39±1453.18	3570.60±1085.22	0.524
AMY (U/L)	30.35 (21.88,47.13)	39.1 (24.90,53.30)	0.155
UA (μmol/L)	269.02±114.55	273.00 (224.00,339.00)	0.325
UREA (mmol/L)	8.50 (6.73,10.40)	7.81 (6.60,10.80)	0.762
CREA (μmol/L)	60.90 (58.08,64.03)	61.00 (59.50,61.70)	0.901
HBP (ng/ml)	36.9 (16.19,46.92)	38.45 (21.27,56.30)	0.945
PCT (ng/ml)	0.06 (0.05,0.21)	0.13 (0.08,0.41)	0.056
IL-6 (pg/ml)	39.98 (31.77,66.45)	196.89 (177.27,290.18)	<0.001***
Anti-Xa (U/ml)	0.13±0.10	0.11 (0.05,0.13)	0.384

STable 6. Comparison of Bivad and LVAD surgeries. The numbers in the table represent the P-values of the differential analysis

BiVAD vs LVAD	**Before CPB**	**30 min after CPB**	**Before shutdown**	**10 min after PS**	**30 min after PS**
TAT	0.885	0.665	0.736	0.124	0.124
TM	0.100	0.530	0.530	0.596	0.596
PIC	0.979	1.000	0.596	0.185	0.152
tPAIC	0.307	0.053	0.530	0.411	0.736
IL6	0.062	0.080	0.515	0.885	0.411
HBP	0.885	0.530	0.185	0.469	0.262
PCT	0.665	0.185	0.262	0.469	0.469
PT	0.961	0.885	0.665	0.411	0.411
APTT	0.469	1.000	1.000	0.262	0.530
FIB	1.000	0.596	0.307	0.307	0.411
DD	0.596	0.885	0.530	0.810	0.736
FDP	0.596	0.596	0.549	0.596	0.596
ATIII	0.810	0.886	0.357	0.665	0.957

Anti-Xa	-	0.654	0.810	0.542	0.431
ALT	0.358	-	0.192	-	0.192
AST	0.045*	-	0.505	-	0.158
TBIL	0.477	-	0.001***	-	1.000
DBIL	0.549	-	0.001***	-	0.505
TP	0.085	-	0.025*	-	0.673
ALB	0.711	-	0.001***	-	0.782
GGT	0.221	-	0.001***	-	0.327
ALP	0.572	-	0.046*	-	0.654
TBA	0.045*	-	0.001***	-	0.192
CREA	0.202	-	0.001***	-	0.959
UREA	0.409	-	0.019***	-	0.721
UA	0.984	-	0.001***	-	0.586
GLU	0.822	-	0.101	-	0.878
CHE	0.429	-	0.001***	-	0.702
CK	0.296	-	0.001***	-	0.065
AMY	0.060	-	0.001***	-	0.195
TC	0.130	-	0.001***	-	0.721
TG	0.785	-	0.001***	-	0.495
HDL	0.477	-	0.001***	-	0.310
LDL	0.060	-	0.194	-	0.442