Post-Neutralisation Activated Clotting Time and Postoperative Transfusions in Cardiac Surgery Outcome

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ABSTRACT

Objective: To assess the impact of post-protamine neutralisation activated clotting time (ACT) values on postoperative outcomes including chest drain output, transfusion requirements, and CICU stay, in patients undergoing cardiac surgery.

Study Design: Observational comparative study.

Place and Duration of the Study: Department of Anaesthesiology, The Aga Khan University Hospital, Karachi, Pakistan, from February to August 2023.

Methodology: Ethical approval was obtained to collect data from elective cardiac surgery patients' charts. A sequential sampling approach analysed the baseline and post-protamine neutralisation ACT values, categorising patients into two groups. Group A maintained ACT within 10% of baseline, while Group B deviated. The outcomes measured included transfusion needs, chest drain output, additional protamine, cardiac intensive care unit (CICU) stay, and postoperative reopening. Statistical analysis included mean, median, frequency, t-test / Mann-Whitney U test, and Chi-square test.

Results: The study comprised 101 patients (39 in Group A, 62 in Group B), with similar baseline health. No significant differences were found in tranexamic acid use, CICU stay, chest drain output, or transfusion rates between the groups ($p > 0.05$).

Conclusion: Maintaining ACT within 10% of baseline post-protamine neutralisation results in similar intraoperative and postoperative outcomes, suggesting potential benefits in avoiding the aggressive protamine therapy and ensuring haemostasis in cardiac surgery.

Key Words: *Coronary Artery bypass grafting, Cardiopulmonary bypass, Activated clotting time (ACT), Heparin, Postoperative bleeding, Blood transfusions.*

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INTRODUCTION

Each year, more than 200,000 people undergo cardiac surgeries, including Coronary Artery Bypass Grafting (CABG) in the United States of America, and 62 per 100,000 cases per annum undergo CABG in European countries.[1] On-pump CABG provides better survival and better revascularization at 3 months than off-pump CABG.[2] Severe or extensive bleeding after cardiac surgery is a critical issue.[3] End-organ damage, cardiovascular events, or death may occur if major bleeding is not treated timely.[4] Large-scale blood transfusions (defined as >10 units of RBC) and the rate of re-exploration have decreased from 4.6% and 5.6%, respectively, to 3.4%, specifically in patients undergoing on-pump cardiac surgeries, with no direct comparison to off-pump procedures.[5]

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Before the patient is prepared to go on the bypass machine, an activated clotting time (ACT) of 480 seconds, or 3 times the baseline with a minimum dose of 300IU/Kg of heparin, is recommended.[6] Protamine reverses heparin's blood-thinning effects during the cardiac surgery, which may include cardiopulmonary bypass (CPB). Protamine dosing should not exceed a protamine-to-heparin ratio of 1:1 or 1 mg for every 100 units of heparin. A larger dose of protamine is associated with a greater risk of transfusion and postoperative bleeding.[7] The ACT evaluates how long it takes for activated whole blood to coagulate after exposure to either celite or kaolin.[8] The average ACT range is 107 +/- 13 seconds.[9]

Current guidelines advise modest protamine dosage regimens, but these recommendations are based on low-quality evidence.[10] Further evaluation is warranted as it is a standard practice to provide additional doses of protamine as an artificial safety net.[11]

The purpose of this research is to evaluate how variations in post-protamine neutralisation ACT values affect postoperative results, particularly blood loss and blood transfusion requirement in on-pump cardiac surgery. This study identified that sticking to ACT values more than 10% from the initial surgery level contributes to less postoperative bleeding and transfu-

sions. The study aimed to determine the postoperative differences between the two groups according to post-neutralisation ACT values such as the additional requirement of protamine, length of cardiac intensive care unit (CICU) stay postoperations, and the incidence of re-opening of the sternum during CICU stay.

METHODOLOGY

It was an observational study done retrospectively at the Aga Khan University Hospital between February and August 2023. The number of patients in the sample size was controlled by the available data, and 101 patients' data were recorded. To ensure all eligible patients were captured in the study period, sequential sampling was utilised. Patients aged between 18 and 80 years, undergoing elective cardiac surgeries, having haemoglobin baseline level greater than 10 mg/dl for maintaining haemodynamic balance, were included. Exclusion criteria were patients with known hypersensitivity to heparin, patients who required emergency surgery within the next 48 hours, patients who had used antiplatelet or fibrinolytic agents within 12 hours prior to the randomisation, patients with incomplete clinical records, patients with renal failure or liver disorder. Furthermore, any patient who had ACT more than 10% of baseline after protamine administration or those who required fresh frozen plasma or cryoprecipitate in the postoperative period or who received protamine in CICU within the first 24 hours, were also eliminated from the study. Patients were pre-screened for any history of protamine allergies before any surgery was performed and those who had protamine allergies were not included in the study since such reactions could be fatal.

This study was conducted after taking permission from the Aga Khan University's Ethical Review Committee (Reference Number: 2023-8427-23967). All data collected were kept confidential and anonymised so that only the principal investigator had access to it.

Preoperative ACT values were captured on registration of the patients in the operating room. ACT was monitored at three critical points: The measurements were taken before surgery (baseline), before protamine sulfate administration during surgery, and after the protamine neutralisation. After surgery, the patients were categorised into two groups based on their post-protamine neutralisation ACT values. The first group was Group A including patients with ACT levels that were close to the baseline within 10% above the baseline. The second group was Group B including patients with ACT levels exceeding 10% above the baseline. Monitoring was conducted in the CICU for 24 hours during which physiological and operational observations were recorded, including chest drain output, blood requisites, and additional protamine requirements. The protamine dose that was administered was based on a protamine / heparin ratio of 1mg protamine to 100 units of heparin. Regarding transfusion practice, the decisions were made by the clinical condition such as ongoing bleeding, hypotension, and low Hb levels and treated in a suitable way to avoid subsequent complications.

Data analysis was done using R Studio software version 4.1.2. To display the results of the quantitative variables, mean (standard deviation) or median (interquartile range) were computed and results of the categorical variables were presented in the form of frequencies (percentages). Comparisons of groups with transfusion requirement, CICU length of stay, and the need for extra protamine were made by t-tests or Mann-Whitney U test for parametric and non-parametric data, respectively, and Chi-square test or Fisher's exact test was used for categorical variables. A p-value of ≤ 0.05 was used as a level of significance for the present study.

RESULTS

In this study, patients were divided in two groups: Group A included 39 patients and Group B included 62 patients with a combined total of 101 patients. The mean age of the participants was 59.1 ± 9.61 years. The mean weight was 71.2 ± 11.6 kg, and the median height was 165 [IQR 170-160] cm. The mean body max index was 25.5 [IQR 28.5-23.5] kg/m^2. The number of female patients was 14 (13.9%) and male was 87 (86.1%). Age, the ASA classification, weight, height, BMI, and comorbidities such as diabetes, hypertension, and smoking showed no significant differences between the two groups (Table I).

In terms of intraoperative factors, the mean CPB values and median cross-clamp times were similar between the two groups. However, an important difference in the duration of surgical procedures was observed between the groups when categorised as ≤ 4 or >4 hours, with Group B undergoing longer procedures overall. This analysis demonstrated that apart from the duration of surgical procedures, there were no significant differences. Hence, confounding factors in outcomes observed between the two groups were less likely to be attributed to results.

The data showed that 59.0% of patients in Group A and 58.1% in Group B were administered tranexamic acid. The statistical analysis revealed no substantial disparity in the usage of tranexamic acid among the groups (p-value >0.900). When examining the length of stay in the CICU, both groups demonstrated a median duration of 1.00 day, accompanied by an interquartile range of 1.00. There is no statistically significant difference between the two groups in terms of their length of stay in the CICU (p-value = 0.166, Figure 1).

In addition, the output of chest drains had a mean value of 533 ± 225 ml in Group A, whereas Group B had a mean value of 551 ± 224 ml, showing no statistically significant distinction between the groups (p = 0.166, Figure 2). The overall mean for all groups combined was found to be 544 ± 223 ml.

Similarly, the examination of transfusion needs indicated no notable differences, as 64.1% of the individuals in Group A and 67.7% in Group B were administered transfusions (p = 0.829, Figure 3).

Table I: Patients' preoperative, intraoperative, and postoperative characteristics.

Variable	Group A (n = 39)	Group B (n = 62)	Overall (n = 101)	p-value
Age (years)	58.6 ± 10.0	59.3 ± 9.39	59.1 ± 9.61	0.738[a]
Weight (kg)	69.5 ± 9.99	72.3 ± 12.4	71.2 ± 11.6	0.203[a]
Height (cm)	167 [170 - 161]	165 [169 - 160]	165 [170 - 160]	0.345[b]
BMI (kg/m²)	25.7 [28.1 - 22.6]	25.1 [29.0 - 23.6]	25.5 [28.5 - 23.5]	0.643[b]
BSA (m²)	1.78 ± 0.158	1.80 ± 0.174	1.79 ± 0.168	0.450[a]
Gender - Female	6 (15.4%)	8 (12.9%)	14 (13.9%)	0.772[c]
Gender - Male	33 (84.6%)	54 (87.1%)	87 (86.1%)	
With diabetes mellitus	24 (61.5%)	38 (61.3%)	62 (61.4%)	>0.900[c]
Having hypertension	24 (61.5%)	46 (74.2%)	70 (69.3%)	0.192[c]
Smokers	6 (15.4%)	20 (32.3%)	26 (25.7%)	0.066[c]
Other comorbidities	6 (15.4%)	9 (14.5%)	15 (14.9%)	>0.900[c]
CPB Time (hours)	1.67 [135 - 87.5]	1.67 [129 - 90.0]	1.67 [130 - 90.0]	0.801[b]
CC Time (hours)	1.0 [82.5 - 47.5]	1.0 [70.0 - 47.0]	1.0 [80.0 - 47.0]	0.609[b]
Procedure (hours)	5.50 [6.00 - 4.50]	5.50 [5.50 - 4.50]	5.50 [6.00 - 4.50]	0.918[b]
Temp at induction (°C)	36.7 [36.5 - 36.8]	36.6 [36.8 - 36.5]	36.7 [36.8 - 36.5]	0.773[b]
Min. Temp (°C)	32.0 [32.0 - 32.0]	32.0 [32.0 - 32.0]	32.0 [32.0 - 32.0]	0.838[b]
Total Heparin (Units)	25000 [25000 - 24000]	25000 [25000 - 25000]	25000 [25000 - 24000]	0.278[b]
Temp CICU (°C)	34.5 (0.66)	34.6 (0.77)	34.5 (0.73)	0.604[a]
Protamine (mg)	262 (29.5)	263 (35.2)	262 (32.9)	0.867[a]
Tranexamic acid used intraoperatively (mg)	23 (59.0%)	36 (58.1%)	59 (58.4%)	0.900[c]
Stay in CICU (days)	1.0 [1.0 - 1.0]	1.0 [2.0 - 1.0]	1.0 [2.0 - 1.0]	0.166[b]
Chest drain (ml)	495 [628 - 395]	500 [620 - 410]	495 [620 - 405]	0.837[b]
ACT baseline (seconds)	120 ± 10	125 ± 15	123 ± 13	0.05[a]
ACT post-protamine (seconds)	130 ± 10	140 ± 12	135 ± 11	0.03[a]
Blood units transfused (median)	2	3	2.5	0.04[b]
Number of patients reopened	3	5	8	0.02[b]
Transfusion performed	25 (64.1%)	42 (67.7%)	67 (66.3%)	0.829[c]

BMI = Body mass index, BSA= Body surface area, DM = Diabetes mellitus, HTN = Hypertension, CPB = Cardiopulmonary bypass, CC = Cross clamp, Temp = Temperature, Min = Minimum. a: Independent test; b: Mann-Whitney U test; c: Chi-square or Fisher's exact test.

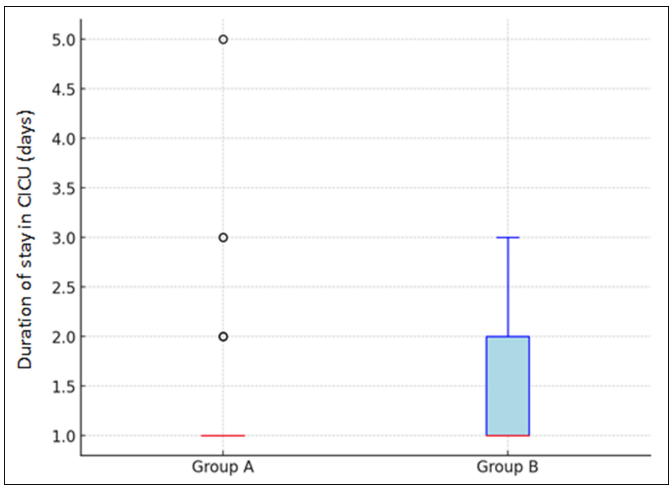

Figure 1: Box plot showing median duration of stay in CICU in Group A and Group B.

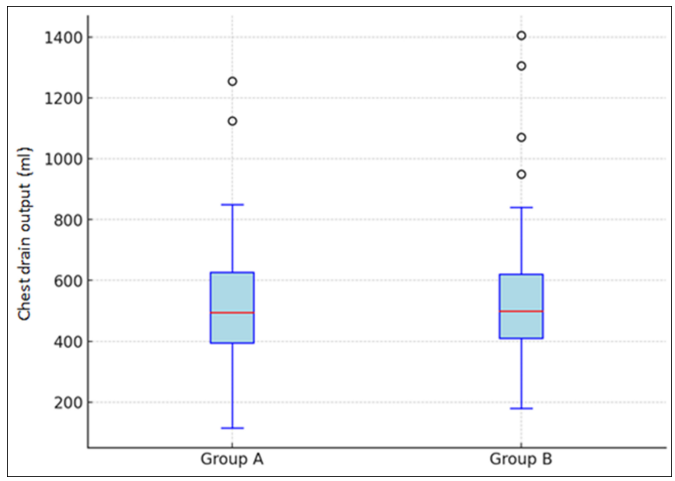

Figure 2: Box plot showing chest drain output in Group A and Group B.

The subgroup analysis of the data indicated no statistically significant disparities in the median duration of hospitalisation in the CICU, occurrences of chest drain placement, and rates of transfusion between Group A and B among the cohort of patients aged 60 years or younger. It is important to note that chest drains are typically necessary in such procedures to manage excess fluid and prevent complications. Likewise, there were no statistically significant variations in patient outcomes within each age group when comparing BMI categories, specifically normal and overweight.

Furthermore, the outcomes were not significantly influenced by additional characteristics such as body surface area (BSA), gender, diabetes mellitus (DM) status, smoking behaviours, and the existence of other comorbidities. Although there was an observed tendency indicating a longer stay in the CICU for patients without hypertension in Group B, this trend did not retain its statistical significance after adjusting for multiple tests. There were no significant connections observed between patient outcomes within each age group and factors such as CPB time, creatinine clearance, procedural complexity, temperature in the CICU, and the intraoperative use of tranexamic acid.

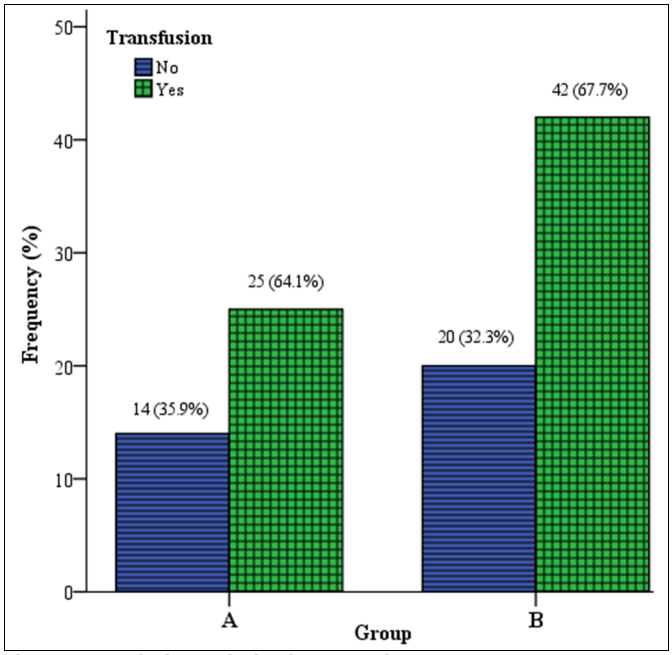

Figure 3: Need of transfusion between the two groups.

Overall, intraoperative factors, such as the use of tranexamic acid, CPB time or cross-clamp duration, and postoperative outcomes including the length of stay in the CICU, chest drain output, and transfusion rates, showed no significant differences between the groups.

DISCUSSION

The study aimed to assess the impact of post-protamine neutralisation ACT values on postoperative outcomes in on-pump cardiac surgeries that includes bleeding, transfusions, and CICU stay. The findings showed that there were no statistically significant distinctions observed between Group A (consisting of patients whose ACT values returned to within 10% of baseline levels) and Group B (comprising the patients with deviations from baseline ACT values) in terms of utilising tranexamic acid, length of stay in the CICU, chest drain output, and transfusion requirements. This indicates that these factors did not exert a meaningful influence on outcomes observed between the two groups.

These findings of this study are divergent from prior researches suggesting a potential impact of these variables on intraoperative and postoperative outcomes. For instance, the results of Wang *et al.*'s study showed that lower postoperative transfusions and bleeding are associated with the group having their final ACT values lesser than pre-heparinisation ACT values.[12] A randomised controlled trial revealed a potential association between the dosing ratio of protamine and heparin and coagulation status after the cardiac surgery.[5] The group with a higher protamine-to-heparin dosing exhibited greater blood loss and transfusion rates compared to their counterparts in the low-dosing group. In addition, differences were observed in intrinsic clotting times and maximum post-protamine thrombin generation between these two groups.[5] This present study did not yield significant differences in outcomes based on ACT values used for the stratification into high and low-dosing groups. This may indicate that the dosing ratio alone may not be a decisive factor in coagulation status post-surgery. It should also be mentioned that the current study did not consider the dosing ratio of the applied medications, and the possible effects of this difference need to be investigated in the future.

The present study's results also imply that ACT can be maintained close to baseline and aggressive protamine therapy is not required. Hence, patients can be protected from the potential side effects of high doses of protamine. Protamine administration observed to result in immunological and inflammatory changes, potentially leading to anaphylactic reactions presenting with hypotension, bradycardia, bronchoconstriction, and allergic responses.[13,14] A systematic review found that protamine exposure led to adverse events in about 0.06 - 10.6% of cases.[15] Other possible risk factors include known abnormal pulmonary haemodynamics, severe left ventricular dysfunction, and sensitivity to fish.[16]

Initially, Berger *et al.* proposed a modification of protamine in view of heparin's half-life dynamics and intraoperative utilisation patterns.[17] When administered through sequential dosages, the initial protamine dose was often adequate for promoting coagulation, while subsequent doses led to continuous bleeding episodes.[18,19] Contrary to the findings in this study, two studies have demonstrated a positive correlation between higher protamine dosing ratios and improved clinical outcomes as compared to lower protamine doses.[20,21]

This study found no significant differences in postoperative outcomes such as chest drain output, transfusion requirements, and CICU stay between patients categorised based on their ACT values after protamine neutralisation, despite concerns about the heparin rebound effect, and potential bleeding following CBP procedures. However, it is important to note that thromboelastographic and heparin assays exhibit greater sensitivity in detecting residual effects of heparin anticoagulation compared to the ACT.[22,23]

Despite the fact that the total operating time was similar in both groups, data from other studies indicate that surgery procedures lasting longer than 4 hours have worse results accompanied by complications.[24] This current study also indicated that age, BMI, BSA, gender, and presence of comorbidities did not yield significant differences in outcomes between Group A and B within each subgroup. Overall, this research contributes to a comprehensive understanding of the correlation between post-protamine ACT values and postoperative outcomes in on-pump cardiac surgeries.

The results of this study are subjected to potential selection bias due to its retrospective design. In addition, a single centre cohort design restricts the generalisability of results due to the possibility of varying surgical practices across different institutions affecting the external validity.[25] The rela-

tively moderate sample size also serves as another notable limitation. The present study utilised ACT values as a means of categorising patients. However, this approach may not possess the same level of sensitivity as alternative tests in detecting the residual heparin anticoagulation.[21] Lastly, the brief follow-up period of only 24 hours may prevent a comprehensive assessment of longer-term coagulation-related outcomes and bleeding complications.

This study's results demonstrate several practical implications in a clinical setting. The identified correlation between maintaining post-protamine ACT values within 10% of baseline and similar outcomes suggest the possibility of keeping the ACT values near the baseline and avoid aggressive protamine therapy. Furthermore, similar transfusion requirements among groups imply that adherence to certain ACT thresholds may not compromise haemostasis, providing valuable insights for reducing blood product usage after cardiac surgery. The study's limitations include a retrospective design, single-centred setting, selection bias, ACT values' sensitivity, and a short 24-hour follow-up period, potentially limiting generalisability and long-term outcomes evaluation.

CONCLUSION

ACT values remain within 10% of baseline after protamine neutralisation are similar to intraoperative and postoperative outcomes, such as chest drain output and CICU stay, as compared to patients with ACT values exceeding 10% above the baseline. The patients with higher ACT values require more blood transfusions and more frequently underwent postoperative re-opening, underlining the necessity of an appropriate and strict control of ACT. This study has significant implications for highlighting practical aspects to avoid overuse of protamine and yet achieving adequate haemostasis. It offers important information to fine-tune the guidelines addressing ACT management and transfusion in cardiac surgical settings.

ETHICAL APPROVAL:
This study was conducted after taking permission from the Aga Khan University's Ethical Review Committee (Reference Number: 2023-8427-23967).

PATIENTS' CONSENT:
Informed consent was obtained from all patients.

COMPETING INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
MAZ: Concept and design of work and analysis or interpretation of data.
MSY, SAW: Drafting of the work and critical revision for important intellectual content.
MIA, SSA: Concept and design of work, analysis or interpretation of data.
MH: Final approval.
All authors approved the final version of the manuscript to be published.

REFERENCES

1. Melly L, Torregrossa G, Lee T, Jansens JL, Puskas JD. Fifty years of coronary artery bypass grafting. *J Thorac Dis* 2018; **10(3)**:1960-67. doi: 10.21037/jtd.2018.02.43.
2. Thakur U, Nerlekar N, Muthalaly RG, Comella A, Wong NC, Cameron JD, *et al.* Off- *vs.* on-pump coronary artery bypass grafting long-term survival is driven by incompleteness of revascularisation. *Heart Lung Circ* 2020; **29(1)**:149-55. doi: 10.1016/j.hlc.2018.11.019.
3. Zeroual N, Blin C, Saour M, David H, Aouinti S, Picot MC, *et al.* Restrictive transfusion strategy after cardiac surgery. *Anesthesiology* 2021; **134(3)**:370-80. doi: 10.1097/ALN.0000000000003682.
4. Despotis GJ, Filos KS, Zoys TN, Hogue CW Jr, Spitznagel E, Lappas DG. Factors associated with excessive postoperative blood loss and hemostatic transfusion requirements: A multivariate analysis in cardiac surgical patients. *Anesth Analg* 1996; **82(1)**:13-21. doi: 10.1097/00000539-199601000-00004.
5. Meesters MI, Veerhoek D, de Lange F, de Vries JW, de Jong JR, Romijn JW, *et al.* Effect of high or low protamine dosing on postoperative bleeding following heparin anticoagulation in cardiac surgery. A randomised clinical trial. *Thromb Haemost* 2016; **116(2)**:251-61. doi: 10.1160/TH16-02-0117.
6. O'Carroll-Kuehn BU, Meeran H. Management of coagulation during cardiopulmonary bypass. *Cont Edu Anaesth Crit Care Pain* 2007; **7(6)**:195-8. doi: 10.1093/bjaceaccp/mkm036.
7. Hecht P, Besser M, Falter F. Are we able to dose protamine accurately yet? a review of the protamine conundrum. *J Extra Corpor Technol* 2020; **52(1)**:63-70. doi: 10.1182/ject-1900038.
8. Horton S, Augustin S. Activated clotting time (ACT). *Methods Mol Biol* 2013; **992**:155-67. doi: 10.1007/978-1-62703-339-8_12.
9. Laffan M, Manning R. Laboratory control of anticoagulant, thrombolytic, and antiplatelet therapy. In: Lewis SM, Bain BJ, Bates I, Eds. Dacie and Lewis Practical Haematology. ed. 10th, Philadelphia: Churchill Livingstone; 2006: p. 465-79.
10. Rajsic S, Breitkopf R, Jadzic D, PopovicKrneta M, Tauber H, Treml B. Anticoagulation strategies during extracorporeal membrane oxygenation: A narrative review. *J Clin Med* 2022; **11(17)**:5147. doi: 10.3390/jcm11175147.
11. Foubert R, Van Vaerenbergh G, Cammu G, Buys S, De Mey N, Lecomte P, *et al.* Protamine titration to optimize heparin antagonization after cardiopulmonary bypass. *Perfusion* 2022; **39(6)**:1062-9. doi: 10.1177/02676591221144702.
12. Wang W, Wang Y, Wang J, Xu R, Chai J, Zhou W, *et al.* ACT values after neutralization lower than pre-heparinization act leads to lower operative times, bleeding, and post-operative transfusions in cabg patients: An observational study. *Braz J Cardiovasc Surg* 2018; **33(6)**:588-96. doi: 10.21470/1678-9741-2018-0116.
13. Chooriyil N, Jom D, Panattil P, Sreedharan S. Protamine-induced hypotension and pulmonary arterial hypertension. *J Pharmacol Pharmacother* 2021; **12(1)**:23-5. doi: 10.4103/jpp.JPP_157_20.
14. De Simone F, Nardelli P, Licheri M, Frau G, Redaelli MB, Monaco F, *et al.* Less is more: We are administering too much protamine in cardiac surgery. *Ann Card Anaesth* 2021; **24(2)**:178-82. doi: 10.4103/aca.ACA_26_19.

15. Nybo M, Madsen JS. Serious anaphylactic reactions due to protamine sulfate: A systematic literature review. *Basic Clin Pharmacol Toxicol* 2008; **103(2)**:192-6. doi: 10.1111/j.1742-7843.2008.00274.x.

16. Levy JH, Ghadimi K, Kizhakkedathu JN, Iba T. What's fishy about protamine? Clinical use, adverse reactions, and potential alternatives. *J ThrombHaemost* 2023; **21(7)**:1714-23. doi: 10.1016/j.jtha.2023.04.005.

17. Berger RL, Ramaswamy K, Ryan TJ. Reduced protamine dosage for heparin neutralization in open-heart operations. *Circulation* 1968; **37(4Suppl)**:II154-7. doi: 10.1161/01.cir.37.4s2.ii-154.

18. Downey LA, Faraoni D. Coagulation, cardiopulmonary bypass, and bleeding. In: Andropoulos DB, Mossab EB, Gottlieb EA, Eds. Anesthesia for congenital heart disease. ed. 4th, John Wiley & Sons; 2023: p. 377-408.

19. Bartoszko J, Karkouti K. Managing the coagulopathy associated with cardiopulmonary bypass. *J ThrombHaemost* 2021; **19(3)**:617-32. doi: 10.1111/jth.15195.

20. Svenarud P, Ovrum E. Clinical effects of different protamine doses after cardiopulmonary bypass. *Asian Cardiovasc Thorac Ann* 2002; **10(2)**:119-23. doi: 10.1177/02184923021000206.

21. Guo Y, Tang J, Du L, Liu J, Liu RC, Liu X, *et al.* Protamine dosage based on two titrations reduces blood loss after valve replacement surgery: A prospective, double-blinded, randomized study. *Can J Cardiol* 2012; **28(5)**:547-52. doi: 10.1016/j.cjca.2012.03.012.

22. Liu Y, Yuan Z, Han X, Song K, Xing J. A Comparison of activated partial thromboplastin time and activated coagulation time for anticoagulation monitoring during extracorporeal membrane oxygenation therapy. *Hamostaseologie* 2023; **43(3)**:171-8. doi: 10.1055/a-1796-8652.

23. Stone ME, Vespe MW. Heparin rebound: An in-depth review. *J Cardiothorac Vasc Anesth* 2023; **37(4)**:601-12. doi: 10.1053/j.jvca.2022.12.019.

24. Cornella N, Sancho J, Sitges-Serra A. Short and long-term outcomes after surgical procedures lasting for more than six hours. *Sci Rep* 2017; **7(1)**:9221. doi: 10.1038/s41598-017-09833-7.

25. Das MK. Multicenter Studies: Relevance, design and implementation. *Indian Pediatr* 2022; **59(7)**:571-9.