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Clinical Investigation

CLINICAL INVESTIGATION

Optimising protamine dosing for heparin reversal after cardiopulmonary bypass: a population pharmacokinetic–pharmacodynamic study

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Abstract

Background: Protamine is administered to reverse unfractionated heparin (UFH) after cardiopulmonary bypass (CPB), but dosing strategies—typically based on protamine-to-heparin (P:H) ratios—vary, and the minimal effective dose remains unclear. Reversal is commonly assessed using activated clotting time (ACT), which may not reliably reflect residual heparin activity. We used pharmacometric modeling to determine a minimal effective P:H ratio and to characterise the anti-factor Xa (anti-Xa) activity–ACT relationship.

Methods: In this prospective, single-centre study, 68 adults undergoing CPB-assisted cardiac surgery were enrolled. A total of 757 blood samples were collected intraoperatively and after UFH reversal to measure anti-Xa activity and ACT. A population pharmacokinetic–pharmacodynamic model was developed using a nonlinear mixed-effects approach to describe UFH neutralisation by protamine. This model was then used to perform Monte Carlo simulations estimating the probability of complete reversal (anti-Xa <0.10 IU ml^{-1}) at various P:H ratios, based on cumulative intraoperative UFH dose.

Results: Patients received a mean total dose of 30 250 IU UFH and 200 mg protamine i.v. Measured anti-Xa activity decreased to <0.10 IU ml^{-1} in all patients within 10 min of protamine initiation, indicating rapid reversal. Model-based simulations predicted that a P:H ratio of 0.625:1 would achieve complete reversal in 95% of patients. Although ACT and anti-Xa activity were positively associated, ACT values varied widely at low anti-Xa concentrations.

Conclusions: A P:H ratio of 0.625:1 provided adequate UFH reversal. Given the imprecision of ACT, fixed low-ratio dosing without routine monitoring could be a practical alternative but requires prospective validation.

Clinical trial registration: EudraCT (2019-000859-14); www.ClinicalTrials.gov (NCT04092868).

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Keywords: anticoagulation reversal; cardiopulmonary bypass; heparin; Monte Carlo simulation; nonlinear mixed-effects modeling; pharmacokinetics; protamine

Editor's key points

- The optimal dosing of protamine for reversal of unfractionated heparin during cardiopulmonary bypass remains unclear.
- Pharmacometric modeling was developed to determine a minimal effective protamine-to-heparin (P:H) ratio and to characterise the anti-factor Xa activity–activated clotting time relationship.
- Model-based simulations predicted that a 0.625:1 P:H ratio would achieve complete reversal in 95% of patients.
- Fixed low-ratio dosing without routine monitoring could be a practical alternative but requires prospective validation.

Systemic anticoagulation with unfractionated heparin (UFH) is recommended in patients undergoing cardiopulmonary bypass (CPB)-assisted cardiac surgery.[1] During CPB, the UFH effect is monitored using activated clotting time (ACT) to guide additional dosing. At CPB weaning, protamine sulphate is administered to neutralise UFH. Protamine is a positively charged peptide that dissociates heparin–antithrombin complexes, forming inactive heparin–protamine complexes via electrostatic binding.[2] However, protamine also exhibits intrinsic anticoagulant properties through interactions with platelets, coagulation factors and fibrinolysis.[3] Thus, it should be dosed carefully to ensure effective reversal while avoiding paradoxical anticoagulant effects.[4–6]

Among strategies to estimate protamine dosing, the latest guideline recommends calculating a protamine-to-heparin (P:H) ratio based on the UFH dose administered during the procedure, which should be kept below 1:1 (1 mg protamine per 100 IU UFH).[1] However, recent studies suggest that ratios as low as 0.6:1 may suffice.[7,8] Despite this, the minimal effective ratio required for complete UFH neutralisation remains undefined.

Heparin reversal is often assessed using ACT. Although ACT may return to baseline despite residual heparin,[9] potentially increasing bleeding risk, it remains unclear whether, conversely, ACT might remain elevated even after full neutralisation, possibly leading to unnecessary protamine administration and side-effects. Additionally, anticoagulant activity may reappear after reversal, a phenomenon termed 'heparin rebound'. Whether this is because of heparin redistribution or protamine-induced effects on coagulation remains unclear.[10] Although its impact on postoperative bleeding is debated,[11] residual anti-factor Xa (anti-Xa) activity has been reported in up to 80% of patients after CPB.[9] As this phenomenon is unpredictable and may exacerbate coagulopathies in high-risk surgical settings,[9] its relationship with protamine dosing remains poorly understood.

Population pharmacokinetic/pharmacodynamic (PK/PD) modeling provides a quantitative framework to optimise drug dosing. Our group has previously developed models to individualise UFH administration during CPB.[12–14] However, no PK/PD model has yet characterised UFH reversal with protamine.

In this study, we applied population PK/PD modeling to quantify the relationship between protamine dose and UFH reversal kinetics (anti-Xa activity), aiming to identify a minimal effective protamine regimen. Secondary objectives included evaluating ACT as a surrogate for UFH neutralisation and investigating the occurrence of heparin rebound.

Methods

Study design and ethics

This prospective PK/PD study was conducted at the University Hospital of Saint-Étienne, France. The protocol was approved by the hospital's research committee (Ref: 19CH046; December 12, 2018), the French ethics committee (CPP Nord Ouest IV; Ref: 19.02.22.57019; September 26, 2019), and the French National Agency for the Safety of Medicines and Health Products (ANSM). The study was registered on EudraCT (Ref: 2019-000859-14; July 23, 2019) and ClinicalTrials.gov (Ref: NCT04092868; September 17, 2019). Written informed consent was obtained from all participants. The study adhered to the Declaration of Helsinki and Good Clinical Practice guidelines.

Study population and clinical procedure

Sixty-eight adult patients scheduled for CPB-assisted cardiac surgery (coronary artery bypass grafting, valve surgery, aortic dissection) were enrolled between November 2019 and March 2021. Exclusion criteria included contraindications to UFH, protamine, or tranexamic acid, early reintervention, pregnancy, and use of antithrombin or aprotinin.

All participants received a 1500 ml priming solution (500 ml hydroxyethyl starch and 1000 ml crystalloid). Heparin-free circuits (PVC; LivaNova Group, Clamart, France) and phosphorylcholine-coated oxygenators (Inspire 8F; LivaNova) were used. Tranexamic acid administration followed institutional protocols. Participants received the same porcine-sourced UFH (Heparin Choay®; Greifswald, Germany) and protamine sulphate (Protamine Choay®; 1 mg protamine neutralising 100 IU UFH); no other formulations were used. UFH was administered as a 300–400 IU kg^{-1} bolus, with additional doses to maintain ACT >400 s. Protamine sulphate was infused over 10 min at CPB weaning to target an ACT within 10% of baseline. Protamine dosing was left to clinician discretion. Chest tubes were removed on day 2. Postoperative bleeding was classified using the Universal Definition of Perioperative Bleeding (UDPB).[15] Venous thromboprophylaxis was initiated after final study sampling.

Biological sampling and analysis

The UFH pharmacokinetics were assessed using anti-Xa activity. Initial samples were collected at 5, 30, and 60 min after CPB priming, at the end of surgery, and at 5, 8, 11, 14, and 17 min after protamine initiation. To better capture early reversal, the protocol was later amended to include pre-protamine, and 2, 5, 8, 10, and 15 min post-protamine initiation. Additional ICU samples were collected at 1, 3, 5, and 7 h post-protamine to assess heparin rebound.

Anti-Xa activity during CPB and after protamine administration was measured using a STA R Max® 2 analyser (Diagnostica Stago, Asnière, France) with a one-stage chromogenic anti-Xa assay (Liquid anti-Xa®; Diagnostica Stago) that does not contain dextran sulphate or antithrombin. These measurements were performed after study completion and were not available to clinicians during surgery. The lower limit of quantification was 0.10 IU ml^{-1}. Samples exceeding 1.00 IU ml^{-1} were diluted (1:5 or 1:10) using CRYOcheck™ pooled normal plasma (Cryopep, Montpellier, France). ICU anti-Xa activity was measured using a BCS analyser (Siemens, Saint-Denis, France) and a chromogenic substrate assay (BIOPHEN™ Heparin; HYPHEN BioMed, Neuville-sur-Oise, France) containing dextran sulphate but no antithrombin. These measurements were part of routine care and were available to the treating clinicians. ACT was measured using Hemochron Signature Elite ACT+ (Werfen, Le Pré-Saint-Gervais, France) at clinician discretion.

Pharmacokinetic/pharmacodynamic modeling

A full description of the modeling process is provided in Supplementary Appendix 1. PK (anti-Xa activity) and PD (ACT) data of UFH before and after protamine reversal were analysed using nonlinear mixed-effects modeling (Monolix®, version 2021R1; Lixoft, Simulations Plus, Research Triangle Park, North Carolina, U.S.).

A two-compartment model described UFH pharmacokinetics, with protamine represented by a latent kinetic compartment (Fig. 1). To describe neutralisation of UFH by protamine, a binding term was added into the model. The resulting structural model was as follows:

$$\begin{cases} \dfrac{dAcH}{dt} = INPUT_{UFH} - Cl \times \dfrac{AcH}{V_C} + Q \times \left(\dfrac{ApH}{Vp} - \dfrac{AcH}{V_C}\right) \\ \qquad\qquad -(k_{ant}{*}AcH{*}AcP) \\ \dfrac{dApH}{dt} = Q \times \left(\dfrac{AcH}{V_C} - \dfrac{ApH}{Vp}\right) \\ \dfrac{dAcP}{dt} = INPUT_{Protamine} - (k_e \times AcP) - (k_{ant}{*}AcH{*}AcP) \\ CcH = \dfrac{AcH}{V_C} \end{cases} \quad (1)$$

where AcH and ApH are the amount of UFH in the central and peripheral compartments, respectively, and AcP is the amount of protamine in the central compartment. PK parameters were assumed to follow log-normal distributions. The parameters Cl, V_C, Vp, and Q correspond to the elimination clearance, central and peripheral volumes of distribution, and intercompartmental clearance of UFH, respectively. The parameters k_e and k_{ant} represent the elimination rate constant of protamine and the UFH–protamine binding rate, respectively. Binding between UFH and protamine was assumed to be irreversible and modelled as a one-way second-order association process (rate constant k_{ant}), without dissociation. Elimination of both UFH and protamine was assumed to follow first-order kinetics. $INPUT_{UFH}$ and $INPUT_{Protamine}$ represent the administered dose of UFH and protamine, respectively, and CcH denotes the UFH concentration in the central compartment.

The ACT response was explored using linear, log-linear, and sigmoid Emax models. The sigmoid Emax describes ACT as increasing with anti-Xa activity in a saturable manner, according to equation (2):

$$ACT(t,\phi) = ACT_{Baseline} + \left(\frac{E_{MAX} \times C(t,\phi)^{\gamma}}{C_{50}{}^{\gamma} + C(t,\phi)^{\gamma}}\right) \quad (2)$$

Where $ACT(t,\phi)$ is the ACT value at time t for an individual with parameters ϕ. $ACT_{Baseline}$ is the ACT in the absence of heparin. E_{MAX}, C_{50}, and γ describe maximum effect, the concentration for half-maximal effect, and steepness, respectively. Model selection was based on goodness-of-fit and predictive performance.

Monte Carlo simulations of unfractionated heparin reversal

Simulations were performed using the final population PK/PD model. A total of 1000 virtual patients were generated, with UFH concentration and ACT profiles simulated during reversal and up to 24 h after surgery. Various P:H dose ratios (0.1:1 to 1:1), calculated by dividing the total protamine dose by the cumulative intraoperative UFH dose, were explored.[16] Reversal effectiveness was defined as achieving anti-Xa activity <0.10 IU ml^{-1} at the end of protamine infusion (10 min after initiation).

Heparin rebound and postoperative bleeding

Heparin rebound was defined as postoperative reappearance of anti-Xa >0.10 IU ml^{-1} and analysed both qualitatively and quantitatively. Postoperative bleeding was quantified via 24 h chest tube output and classified as per the UDPB. Associations with rebound, P:H ratio, CPB time, tranexamic acid dose, and surgery type were assessed using univariate logistic regression. Statistical significance was defined as $P<0.05$. All analyses were performed using R software (version 4.1.2; R Foundation for Statistical Computing, Vienna, Austria). Additional simulations were performed to estimate the protamine dosing regimen required to neutralise rebound after the initial 10-min protamine infusion (Supplementary Appendix 1).

Results

Clinical and biological data

Sixty-eight patients were enrolled between November 2019 and March 2021. Participant characteristics are summarised in Table 1. Mean CPB duration was 113 min. To maintain ACT >400 s during CPB, patients received a mean UFH dose of 30 250 IU. At CPB weaning, a mean protamine dose of 200 mg was administered, corresponding to a mean P:H ratio of

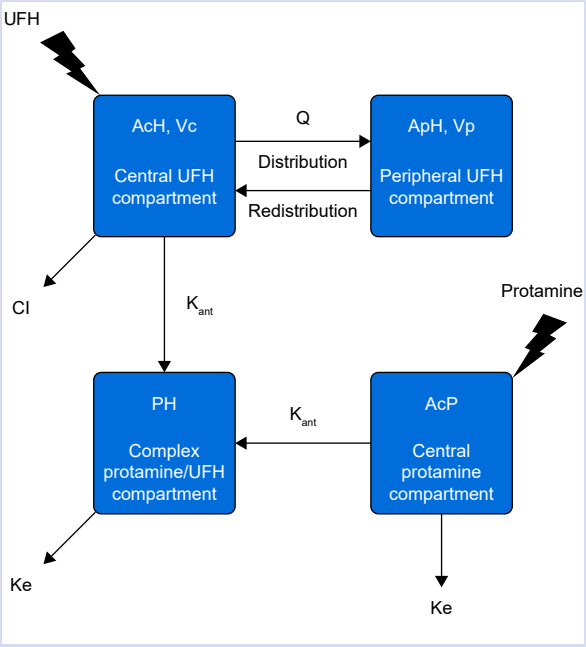

Fig 1. Schematic representation of the joint pharmacokinetic model for unfractionated heparin (UFH) and protamine. The model comprises four compartments: (1) a central UFH compartment, (2) a peripheral UFH compartment, (3) a central protamine compartment, and (4) a compartment for the UFH–protamine complex, representing UFH neutralisation. The amounts of UFH in the central and peripheral compartments are denoted as *AcH* and *ApH*, respectively, whereas *AcP* represents the amount of protamine in the central compartment. *PH* denotes the amount of UFH–protamine complex formed. The parameters *Cl*, V_C, *Vp*, and *Q* correspond to the elimination clearance, central and peripheral volumes of distribution, and intercompartmental clearance of UFH, respectively. The formation of the UFH–protamine complex is governed by a second-order binding process with an association rate constant k_{ant}. The UFH–protamine complex was assumed to bind irreversibly and to be eliminated at the same rate (k_e) as unbound protamine.

Table 1 Participant characteristics, surgical procedures, and outcomes. Continuous variables are reported as mean and range (min–max). anti-Xa, anti-factor Xa activity; CABG, coronary artery bypass grafting; CPB, cardiopulmonary bypass; UDPB, Universal Definition of Perioperative Bleeding; UFH, unfractionated heparin.

Participant characteristics	
Patients, *n*	68
Age (yr)	67.74 (22–84)
Body weight (kg)	76.47 (48–110)
Male, *n* (%)	51 (75)
ASA physical status, *n* (%)	
2	35 (51)
3	32 (47)
4	1 (2)
Previous cardiac surgery, *n* (%)	0 (0)
Minimally invasive, *n* (%)	8 (12)
Surgery type, *n* (%)	
CABG	35 (51)
Valve	22 (32)
CABG + valve	11 (16)
CPB time (min)	113 (47–251)
Cross-clamp time (min)	84 (30–197)
Treatment characteristics	
First dose of UFH (IU)	25 000 (15 000–35 000)
Total dose of UFH (IU)	30 250 (20 000–57 000)
Total UFH boluses, *n*	3 (1–8)
Initial dose of protamine (mg)	200 (100–350)
Protamine-to-heparin ratio	0.64 (0.43–1.07)
Total tranexamic acid dose (mg)	2334 (802–5037)
Biological and postoperative outcomes	
Pre-reversal anti-Xa concentration (IU ml^{-1})	3.96 (1.65–5.89)
Post-reversal anti-Xa concentration (IU ml^{-1})	<0.10 (<0.10–<0.10)
Heparin rebound, *n* (%)	41 (60)
Maximum anti-Xa rebound (IU ml^{-1})	0.17 (0.1–0.43)
Chest tube output at 24 h (ml)	584 (62–2124)
Bleeding UDPB class, *n* (%)	
0 (insignificant)	58 (85)
1 (mild)	2 (3)
2 (moderate)	4 (6)
3 (severe)	4 (6)
4 (massive)	0 (0)

0.64:1. In two patients, a second protamine dose was administered because ACT values did not return within 10% of baseline.

A total of 757 blood samples were analysed. Figure 2 shows the 15-min time course of anti-Xa activity and ACT after initiation of the 10-min protamine infusion. Anti-Xa activity rapidly decreased to <0.10 IU ml^{-1} in all samples by 10 min after protamine initiation, whereas ACT ranged from 100 to 146 s.

Optimal protamine-to-heparin ratio

A population PK model was developed to describe UFH neutralisation by protamine. Random effects were estimated for UFH clearance (Cl), central (Vc) and peripheral (Vp) volumes, and intercompartmental clearance (Q). A proportional residual error model was applied. No covariates significantly influenced neutralisation (k_{ant}), elimination (k_e), or distribution (Q). Parameters estimates of the final model are provided in Supplementary Table 1. In this model, UFH clearance was 1.5 L h^{-1} with an intercompartmental clearance of 0.29 L h^{-1} (redistribution half-life ~3.5 h), whereas protamine exhibited very rapid elimination (Ke=12.8 h^{-1}; half-life ~3 min). Model diagnostics, including visual predictive checks, goodness-of-fit plots, and normalised prediction distribution errors (Supplementary Figs 1–3), demonstrated good agreement with observed data.

Monte Carlo simulations were performed using the final PK model to evaluate P:H ratios, defined as the total protamine dose divided by the cumulative intraoperative UFH dose, across the 0.1:1 to 1:1 range. Anti-Xa activity was simulated at the end of the 10-min protamine infusion. A P:H ratio of 0.625:1 yielded a 95% probability of complete neutralisation, defined as anti-Xa <0.10 IU ml^{-1} (Fig. 3a). Higher P:H ratios improved the likelihood of complete reversal (Fig. 3b).

Relationship between activated clotting time and anti-factor Xa activity

The PK/PD relationship between anti-Xa activity and ACT was best described by a sigmoid E_{Max} model including random

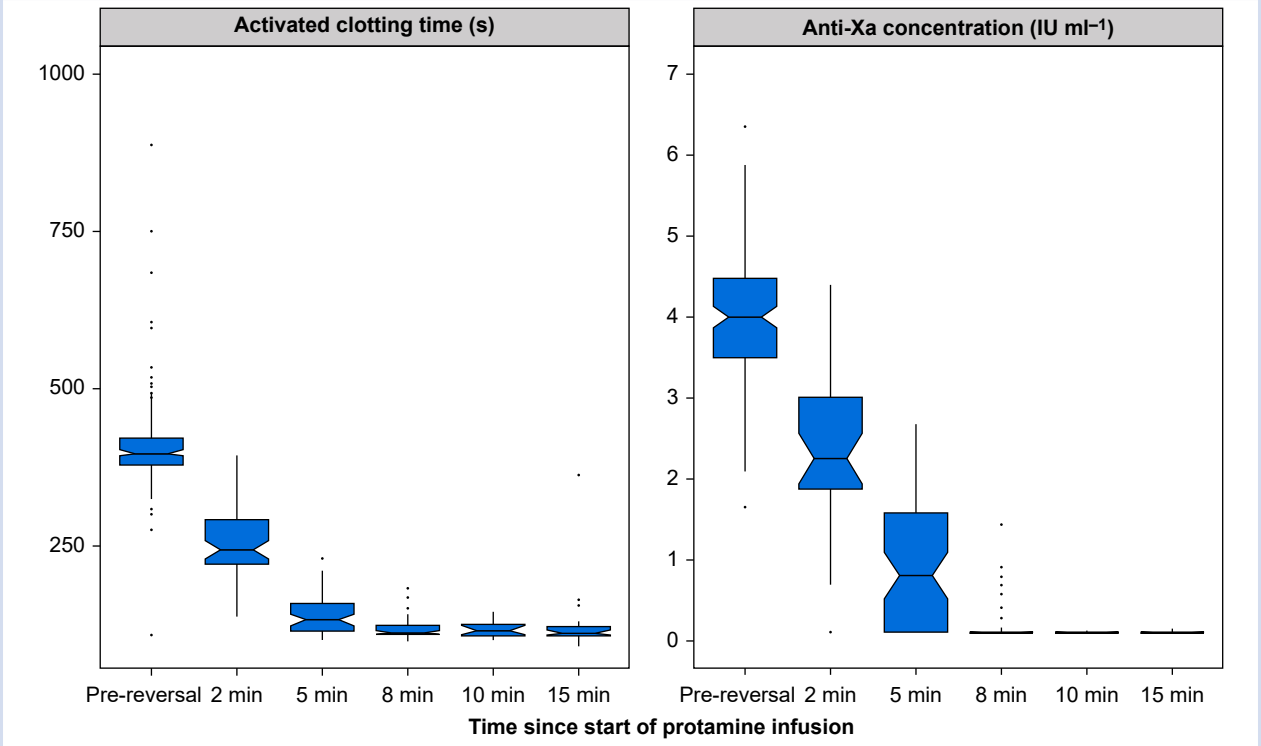

Fig 2. Observed activated clotting time and anti-factor Xa activity before and after protamine administration. Boxplots depict the 15-min time course of activated clotting time (a) and anti-factor Xa activity (b) after the initiation of the 10-min protamine infusion. The 'Pre-reversal' time point corresponds to the last observation measured just before protamine administration. Both biomarkers declined rapidly after protamine infusion, consistent with the expected neutralisation of unfractionated heparin.

effects to account for inter-individual variability on $ACT_{Baseline}$ and E_{Max}. A proportional error model was used to describe residual variability in ACT. Final PK/PD parameter estimates are provided in Supplementary Table 1. Model evaluation showed no evidence of systematic bias (Supplementary Figs 1–3).

As shown in Figure 4 (a), a strong but nonlinear association was observed between anti-Xa and ACT. However, ACT displayed high variability at any given anti-Xa concentration. At the threshold used to define reversal (ACT within +10% of baseline), 20% of simulations had anti-Xa >0.30 IU ml^{-1}, and 10% exceeded 0.50 IU ml^{-1}. Conversely, anti-Xa <0.10 IU ml^{-1} was at times associated with ACT increases >50% from baseline.

Heparin rebound

Heparin rebound, defined as an anti-Xa >0.10 IU ml^{-1} at 1, 3, 5, or 7 h post-protamine, was observed in 41 patients (60%). Peak rebound occurred at a mean of 3.8 (range 1.0–7.0) h, with mean anti-Xa activity of 0.17 (range 0.01–0.43) IU ml^{-1} (Supplementary Fig. 4).

Postoperative bleeding, classified as per the UDPB, occurred in 10 patients (15%): two mild, four moderate, and four severe. No association was found between P:H ratio and either rebound or bleeding. Similarly, no relationship was found between rebound extent—whether assessed qualitatively or quantitatively—and either 24-h chest tube output or bleeding according to the UDPB.

To explore strategies for rebound prevention, simulations evaluated extended protamine dosing after the initial 10-min reversal infusion (Supplementary Appendix 1 and Supplementary Fig. 5). Among the regimens tested, a continuous infusion of 7.5 mg h^{-1} for 7 h maintained anti-Xa activity <0.1 IU ml^{-1} in 95% of patients.

Discussion

This study investigated UFH reversal after CPB using a pharmacometric approach based on anti-Xa activity and ACT, with the primary aim of identifying a minimal effective protamine dosing regimen. Our analysis showed that a P:H ratio of 0.625:1 ensured, with 95% probability, that anti-Xa activity decreased below 0.10 IU ml^{-1} at the end of a 10-min protamine infusion.

These findings are consistent with the guideline recommendations advocating a P:H ratio below 1:1 to minimise protamine overdose. Two randomised trials reported reduced postoperative blood loss using a 0.8:1 ratio compared with higher ratios (1.3:1 or 2:1) calculated from total UFH or protamine titration devices.[17,18] Even lower ratios have shown benefit. In a before–after study, a 0.6:1 ratio reduced transfusion requirements compared with 0.8:1.[7] However, a narrow safety margin may limit such low ratios. In a randomised trial comparing individualised haemostasis management with conventional strategies, a 0.55:1 ratio increased postoperative bleeding compared

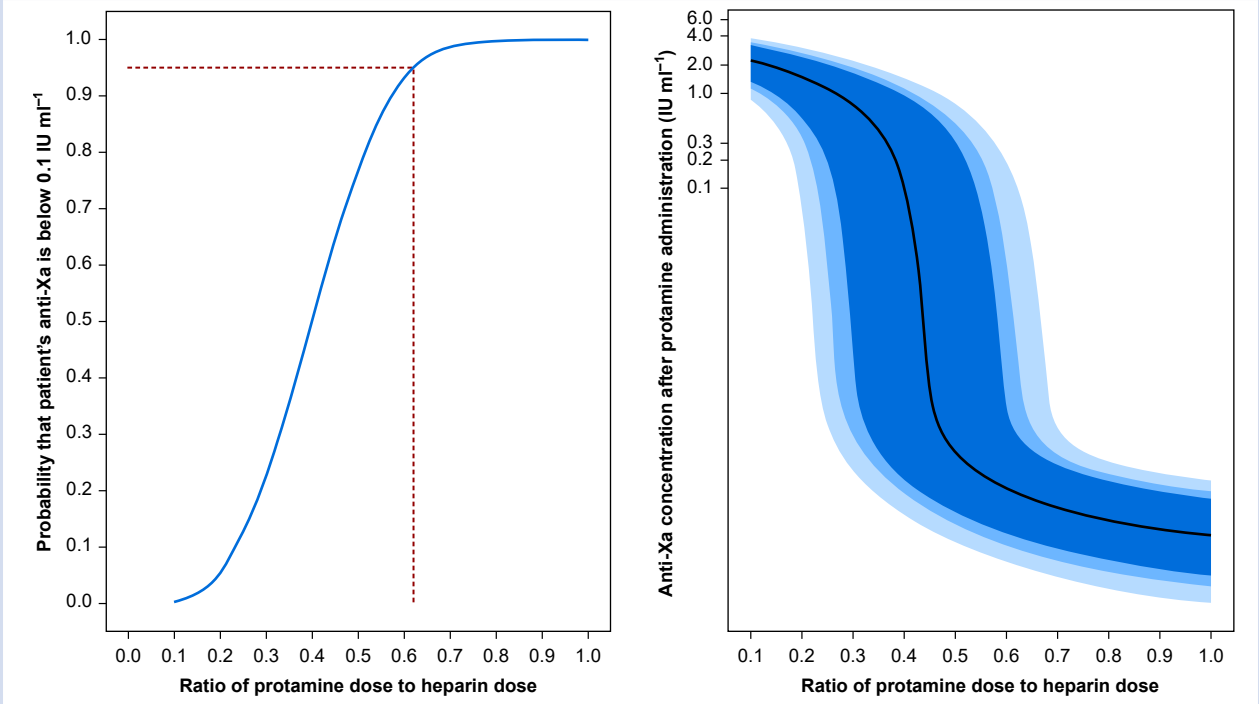

Fig 3. Relationship between the protamine-to-heparin (P:H) dose ratio and residual anti-factor Xa (anti-Xa) activity 10 min post-reversal. (a) Probability that a patient's anti-Xa concentration decreases below 0.1 IU ml^{-1} as a function of the P:H dose ratio. The red dashed lines indicate that a ratio of 0.625:1 corresponds to a 95% probability of complete neutralisation. (b) Predicted distribution of anti-Xa concentrations by P:H dose ratio. The solid black curve shows the median predicted anti-Xa concentration. The dark, standard and light shaded bands represent the 90%, 80%, and 70% prediction intervals, respectively. The horizontal grey dotted line denotes the lower limit of quantification for anti-Xa (0.1 IU ml^{-1}). The P:H ratio was defined as the total protamine dose divided by the cumulative intraoperative UFH dose.

with 0.85:1.[19] These discrepancies likely reflect interindividual variability in protamine responsiveness. One observational study found that a low 0.3:1 ratio achieved neutralisation in approximately one-third of patients, whereas higher ratios were needed in the remainder.[20] Thus, although fixed ratios remain clinically pragmatic, they must ensure a high probability of complete reversal. Our results support a 0.625:1 ratio as a minimal effective regimen, potentially limiting protamine overdose and related adverse effects.

Alternative strategies for guiding protamine administration include point-of-care titration devices, which estimate the heparin dose–response curve and may reduce bleeding,[21] but these are costly and not widely available. Mathematical models estimating real-time heparin concentrations have not shown consistent clinical benefit,[22–25] and disagree on optimal protamine dosing.[26] A fixed-dose approach may offer a pragmatic alternative, as a recent randomised trial showed that a protamine dose of 250 mg achieved blood loss comparable with a 1:1 ratio based on the initial UFH dose,[27] though in patients at weight extremes, fixed dosing may lead to substantial variation in P:H ratios and increased risk of underdosing or overdosing.

We also examined the PK/PD relationship between UFH and ACT. Although ACT is commonly used to guide reversal, it is influenced by postoperative factors such as hypothermia, haemodilution, medications, thrombocytopenia, and coagulopathy, limiting its reliability.[28] Our simulations indicate that ACT can both underestimate and overestimate residual anticoagulation after reversal. These findings support previous studies that ACT alone is insufficient for monitoring UFH reversal.[9] Current guidelines recommend protamine titration assays or thromboelastography[1]; however, as noted above, these are costly and not universally available. In such settings, a fixed 0.625:1 ratio may offer a pragmatic alternative when advanced monitoring is unavailable.

Heparin rebound, of uncertain incidence and mechanism, can occur hours after protamine administration.[10] During CPB, UFH binds plasma proteins, forming complexes incompletely neutralised by protamine.[29] After protamine is cleared, slowly dissociating heparin may cause delayed anticoagulant effects, typically seen as a secondary anti-Xa increase in up to 80% of patients.[9,30,31] Rebound occurred in 60% of our cohort but, consistent with prior findings,[11] was not associated with greater blood loss. This may partly reflect our use of dextran sulphate-containing reagents for postoperative anti-Xa assays, which dissociate heparin–protamine complexes *in vitro*, overestimating the level of biologically active heparin and limiting reliability.[32,33] Postoperative anti-Xa results should therefore be interpreted cautiously, especially with dextran sulphate-containing reagents, alongside clinical outcomes such as bleeding. However, a randomised study showed that a postoperative protamine infusion (25 mg h^{-1} for 6 h) abolished

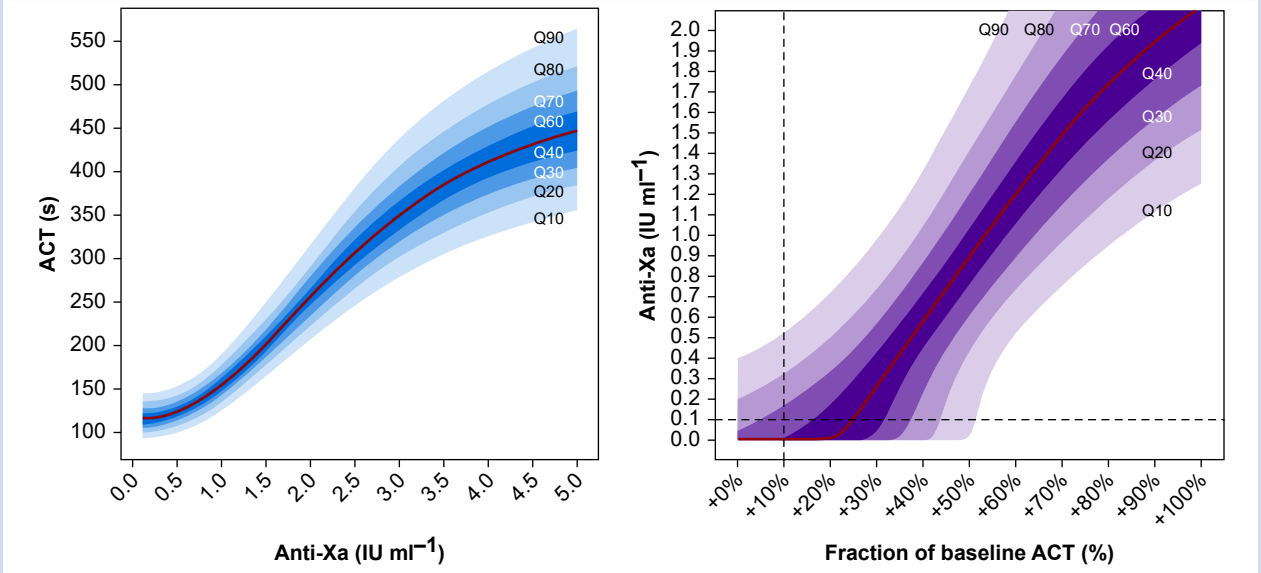

Fig 4. Predicted relationship between activated clotting time (ACT) and anti-factor Xa (anti-Xa) activity. (a) Pharmacokinetic–pharmacodynamic relationship between ACT and anti-Xa concentration. The red curve shows the median prediction; shaded blue areas represent the 10th to 90th percentile prediction intervals. (b) Predicted anti-Xa concentration *vs* percentage increase in ACT from baseline. The red curve represents the median prediction; shaded purple areas denote the 10th to 90th percentiles. Dashed lines indicate thresholds defining heparin reversal: +10% increase in ACT from baseline (vertical) and 0.10 IU ml^{-1} anti-Xa concentration (horizontal).

rebound and reduced blood loss *vs* placebo,[34] and some guidelines now include this approach.[35] Our simulations suggest that a lower-dose infusion (7.5 mg h^{-1} for 7 h) may suffice. The pronounced mismatch in half-lives (protamine ~3 min *vs* UFH redistribution ~3.5 h) makes it unlikely that a single bolus at CPB termination, even if larger than our proposed ratio, would prevent heparin rebound and may transiently increase adverse-effect risk.

This study has several limitations. Firstly, anti-Xa activity was used as a surrogate for UFH exposure, and successful reversal was defined as an anti-Xa <0.10 IU ml^{-1}. These measures are limited by the PD nature of anti-Xa and the absence of a universal threshold, respectively, yet both are regarded as valid and appropriate for research and routine clinical care.[36] Secondly, given the single-centre design and use of one type of heparin, protamine, and ACT device, our findings might lack generalisability. The strategy might also be unsuitable for subgroups not represented here (e.g. patients with extreme body weight, heparin resistance,[37] or procedures without CPB). Thus, our results should be interpreted with caution pending external validation of the model and dosing strategy. In addition, our P:H ratio was derived from the total intraoperative heparin dose to capture cumulative exposure during CPB. This differs from other commonly used approaches,[16] such as ratios based on the initial pre-CPB bolus or titration-based estimates of circulating heparin,[27,38] which were not assessed here. Our method did not account for time-dependent elimination of UFH, which would require PK modeling and dedicated point-of-care tools, adding complexity to routine practice. Accordingly, our ratio applies only when calculated from the total intraoperative UFH dose and should not be extrapolated to substantially longer procedures. Thirdly, our joint PK/PD model was constrained by data availability. Because protamine concentrations were not measured, a latent compartment was used; peripheral distribution was not identifiable and would risk overfitting.[30] More complex models incorporating additional covariate or physiological effects, such as heparin resistance or CPB-related changes (haemodilution and reduced plasma protein concentrations, which can lower total drug concentrations, increase the unbound fraction and modify pharmacologic activity), were beyond this study's scope. Despite the model's parsimony, diagnostic plots showed no systematic bias, confirming adequate description of the data and robustness of the protamine dosing recommendations. Finally, the clinical impact of the 0.625:1 ratio and the exploratory postoperative protamine infusion, both derived from simulations, was not evaluated. Randomised trials are needed to confirm whether this strategy reduces bleeding and improves other patient-centred outcomes.

In conclusion, this pharmacometric study supports a P:H ratio of 0.625:1 as a minimal effective regimen for UFH reversal after CPB. The findings also reinforce the limitations of ACT in assessing anticoagulation reversal and suggest that fixed low-ratio dosing could provide a pragmatic alternative when advanced monitoring is unavailable. Randomised trials are warranted to confirm the clinical benefits of this strategy.

Authors' contributions

Study design: JL, XD, EO
Data collection: JL, IGT, AP, CM, PN, XD
Data analysis: AG, EO
Data interpretation: JL, AG, AM, MG, SM, JM, KA, AP, PJZ, EO
Writing up the first draft of the manuscript: JL, AG, MG, PJZ, EO
Critical review for important intellectual content of the first draft of the manuscript: IGT, AM, AP, CM, SM, JM, PN, KA, AP, XD

Declaration of interest

The authors declare that they have no conflicts of interest.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT (OpenAI) to improve readability and language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bja.2025.11.057.

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