

## Review Article

# Are We Able to Dose Protamine Accurately Yet? A Review of the Protamine Conundrum

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**Abstract:** Without anticoagulation, cardiopulmonary bypass would not have developed over the last nearly 60 years into one of the most influential innovations in medicine; without the ability to reverse anticoagulation, cardiac surgery might not have become the common intervention, which is now practiced globally. Despite the recent breathtaking developments in extracorporeal technology, heparin and protamine remain the pillars of anticoagulation and its reversal until this day.

However, there is still much controversy in particular about protamine dosing regimens. A number of recent publications investigating various approaches to dosing protamine have rekindled this debate. This review is seeking to capture the current thinking about protamine dosing after cessation of cardiopulmonary bypass. **Keywords:** protamine, heparin, anticoagulation, cardiopulmonary bypass. *J Extra Corpor Technol. 2020;52:63–70*

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## INTRODUCTION

Anticoagulation remains fundamental in the treatment and prevention of thromboembolic phenomena. This is especially true of any procedures where blood is in contact with artificial surfaces, air, or where there is stasis. The discovery of heparin over a century ago provided a compound that facilitates such anticoagulation (1). The reversal with protamine was subsequently reported 20 years later in 1937 (2).

Despite recent advances with newer anticoagulants, heparin and protamine remain as the pillars of anticoagulation and reversal during surgical and interventional procedures involving extracorporeal circulation. Heparin remains unsurpassed because of its long track record of successful clinical use and its cheap cost. Other advantages include a titratable dosing profile, availability of point-of-care effect monitors, and protamine as a rapid-acting reversal agent. To this day, heparin is on the World Health Organization list of essential medications.

Protamine has received renewed attention in the last 5 years. It is widely accepted that underdosing of protamine leaves unbound heparin in the circulation and may increase blood loss post-operatively (3); there is increasing evidence that overdosing may cause a paradoxical coagulopathy due to protamine's inherent anticoagulant effects and has been shown to increase blood loss post-operatively (4–6). Because of complexities in the pharmacology and monitoring, we still do not have an effective means of identifying the ideal dose. It is recommended to reverse the heparin remaining at the time of discontinuation of extracorporeal

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support at a 1:1 ratio with protamine (7). The ideal protamine dose will antagonize the residual heparin only at the end of bypass, leaving no free molecules of either substance in circulation. However, the optimal method to find the amount of residual heparin remains elusive. This review aims to describe the use of heparin briefly and focusses on protamine and the different methods that have been used to arrive at a truly ideal dose of protamine at the discontinuation of cardiopulmonary bypass.

## MATERIALS AND METHODS

We searched key electronic databases (PubMed, Embase, and Cochrane) for review and original articles for articles published between 2009 and 2019. We used the search terms “protamine, protamine dosing, protamine algorithm, protamine titration, and protamine management”. All three authors evaluated the relatively small number of relevant articles. This paucity of evidence for protamine management confirms recent findings in a review of the anticoagulant properties of protamine (8).

### Heparin and Anticoagulation

Unfractionated heparins (UFHs) are a heterogeneous group of highly anionic polymers found and isolated from bovine or porcine lung tissue or gut. In the wake of the bovine spongiform encephalopathy outbreak of the mid 1990s, the vast majority of heparin in use today is of porcine origin. However, there is recent evidence in the literature that interest in obtaining heparin from bovine source is currently increasing as the global demand for heparin is threatening to outpace the supply made from porcine origin (9).

In October 2009, the United States Pharmacopeia (USP) changed the monograph for heparin to 1) get on top of a contamination issue that had led to more than 150 adverse reactions and 2) to bring USP in line with international units for heparin (10,11). This new heparin was shown to be about 10% less potent than what had previously been on the U.S. market. A post-introduction study showed that the activated clotting time (ACT) following the initial heparin bolus fell by 9.1% and that 12.8% fewer patients achieved the institutional target ACT for cardiopulmonary bypass (CPB) (12). Interestingly, neither FDA nor the literature mentions dose adjustments for protamine.

Heparin molecules exert their anticoagulant effect indirectly by first binding to antithrombin (AT) and potentiating its activity by up to 1,000 fold to inhibit thrombin and factor X. Dependent on the polysaccharide chain length, they may also bind to factors XI, IX, and XII (13). Only about 30% of UFH molecules in a commercial preparation will contain the active pentasaccharide sequence. The shortest polysaccharide chains (<18 saccharides) can no

longer link AT and other proteins involved in the clotting cascade. They only affect the tertiary structure of AT and will only bind factor Xa.

As the heparin dose increases, a secondary, AT-independent mechanism involving the longer polysaccharide chains' influence on thrombin via heparin cofactor II becomes increasingly important (14). This no longer requires the presence of the AT-activating pentasaccharides. If the heparin concentration increases further, low-affinity heparins with a minimum chain length of 24 saccharides inhibit factor IXa to reduce factor Xa generation independent of heparin cofactor II or AT. Long polysaccharides of UFH bind to endothelium and platelets, which may contribute to the clinical bleeding phenotype outside what can be quantified with plasmatic coagulation assays. Effects on endothelial growth and platelet aggregation have been detected *in vitro*; however, it is unclear over what amount of time this phenomenon can be observed *in vivo* (15).

The pharmacodynamics based on these mechanisms is highly variable between patients. The ACT is used to address this variability and is the most commonly used test to determine the anticoagulant effect after bolus heparin administration (16). The test takes a sample of whole blood and adds a contact activator that stimulates the intrinsic pathway. The test result is the time from addition of the reagents until the appearance of fibrin clot is detected by an optical or mechanical detector. The ACT is influenced by not only other anticoagulant medications but also by hypothermia, hemodilution, platelet count and function, factor deficiency, or low AT activity (13,17). High levels of protamine also prolong the ACT (18).

Heparin's pharmacokinetic properties are highly variable and complex. Removal of heparin occurs both via rapid renal elimination and uptake by the reticuloendothelial system (19). Elimination of heparin is context sensitive and follows non-linear kinetics with a combination of linear and saturable mechanisms (20). Heparin's half-life at a dose of 100 units/kg is approximately 61 minutes, increasing to 126 minutes at a dose of 400 units/kg (21). After small doses (10–5,000 units of heparin), heparin is found in urine as an inactivated and desulphated molecule, whereas after larger doses, it is eliminated intact, with its anticoagulant activity preserved (22).

### Physiology of Anticoagulation during CPB

The complex interaction of UFH, clotting factor consumption, bleeding, hemodilution, contact factor activation, and thrombin generation with excess fibrin monomers causes a transient coagulopathy at the end of CPB. Thrombin as the main effector of coagulation is activated in small quantities despite the presence of heparin. The accumulation of a relatively high concentration of thrombin goes hand-in-

hand with a reduction in its main substrate fibrinogen, some competitive inhibition of fibrin polymerization, and a high concentration of soluble fibrin monomers. Platelet activation, induced during cardiopulmonary bypass, leads to the release of platelet factor 4, which has an AT-neutralizing effect. The combination of these processes consumes AT and the level is markedly reduced at the end of CPB if compared with the beginning (23). Full recovery to baseline AT levels may take up to 48 hours (24–26).

CPB enhances the global heparin effect through a number of confounders such as hemodilution, consumption of thrombin, and other clotting factors as well as fibrinogen.

### Protamine

The benefit of giving protamine and reinstating hemostatic competence, as compared with omitting it and waiting for spontaneous elimination of heparin, has been known about for more than 50 years (27,28). Protamine is a mixture of polycationic basic peptides isolated from *Clupeidae* or *Salmonidae* fish sperm. Protamine inactivates unbound heparin only and is eliminated within 10 minutes in the absence of heparin (29). Its strong basic properties have coulombic interaction with the acidic heparin and form a 1:1 salt complex, which is unable to bind with AT (30). The resulting heparin/protamine complexes are cleared by the reticuloendothelial system with a half-life of 7.4 minutes (29). In addition, binding protamine to heparin breaks up the circulating heparin/AT complexes, thus recovering the AT activity, which is able to interact again with heparin, at least in vitro (8,31–33).

Protamine has clinically relevant anticoagulant potential that can adversely affect both primary and secondary hemostatic pathways. It has been shown that it can inhibit platelet aggregation (34), impair their sensitivity to thrombin receptor agonist peptide (35), and, by inducing an immune response, once the antibodies have formed in sufficient quantities with a time of onset comparable with heparin-induced thrombocytopenia (HIT), they promote platelet activation, risking thrombosis, and a subsequent consumptive thrombocytopenia (36,37). This is clinically indistinguishable from HIT with onset in a similar time-frame, i.e., sometimes within hours but more typically between 5 and 10 days post heparin exposure. The inhibition of platelet aggregation is dose dependent, and the effect can be reduced by adjusting the protamine dose given after cessation of CPB (38). Protamine's potential to increase clot initiation time, slow clot propagation, decrease maximal clot strength, and promote clot breakdown by enhanced fibrinolysis has been demonstrated by thrombelastography (39). Similar to heparin, protamine may be able to sequester fibrinogen in vitro and reduce thrombin generation in vivo through inhibition of factor V activation (40–42). The combination of these factors has been shown to prolong the ACT in vivo and is not thought to be

attributable to analytical conditions (43). Antagonizing anticoagulation with a protamine/heparin ratio of 2:1 in dogs has been shown to decrease both platelet count and function (44); in patients after cardiac surgery, a ratio greater than 1.3:1 is associated with changes in ADP-induced platelet aggregation and prolongation of the ACT compared with lower protamine doses (18). Although still not fully understood, this is at least in part attributed to the rapid precipitation of fibrinogen before thrombin activation in the presence of protamine.

The ACT is far from ideal as a coagulation test. It is influenced by a variety of factors, such as hemodilution, hypothermia, reduced fibrinogen concentration, impaired platelet function, or reduced kidney function, all of which are seen regularly in the cardiac surgical population (45). Nevertheless, a post-protamine ACT prolonged by more than 10% compared with baseline on anesthetic induction should be interpreted with a suitable index of suspicion, as it may suggest either inadequate protamine with residual-free heparin or protamine excess.

Protamine can not only be potentially counterproductive in our efforts to restore normal hemostasis but also have significant cardiovascular and pulmonary sequelae. Hypotension is common during protamine administration and usually is the consequence of its interaction with mast cells, release of histamine, and a reduction in systemic vascular resistance, although additional mechanisms have also been implicated (46). Pulmonary hypertension and right ventricular failure have been described, although the mechanism is not completely clear and is currently thought to be a subtype of an anaphylactoid reaction with contributions from thromboxane A<sub>2</sub>, histamine, and serotonin (46). Anaphylaxis is also a well-documented cause of hemodynamic instability, especially in patients with a fish allergy, vasectomy, and previous exposure, e.g., through previous heparin reversal or long acting insulins containing protamine (47,48). These adverse reactions associated with protamine are not dose dependent and may occur even when giving small doses, such as antagonizing heparin at a ratio of .6:1. Anaphylaxis generally occurs while giving the first few milliliters of the drug.

A recent retrospective review of more than 800 patients demonstrated that high doses of protamine relative to heparin are associated with a greater risk of transfusion and bleeding than lower doses (6). Compared with a moderate protamine group (protamine:heparin ratio = .6–1.0) the low protamine group (ratio ≤.6) had a 56.6% reduced likelihood of red blood cell transfusion, whereas the high protamine group (ratio >1) had 241% increased association with red blood cell transfusion. In the absence of a viable alternative candidate, the question of finding the “correct” dose of protamine becomes ever more prevalent. Several groups have attempted different approaches.

### Fixed-Protamine:Heparin Ratio Dose

One of the most common strategies to calculate the protamine dose is based on the initial or total heparin dose given. The most commonly seen ratio is giving 1 mg protamine for every 100 international units of heparin administered before initiation of CPB. Ratios vary between institutions and practitioners. Advantages of this strategy include that it is simple, inexpensive, and it does not require any additional equipment. As previously described, heparin's half-life is context sensitive and is 126 minutes at a dose of 400 IU/kg. After an average CPB run of 90–120 minutes, a sizeable proportion of heparin will be decayed, making the limitation that heparin decay is not accounted for by the fixed-ratio dosing method immediately obvious. Although this might lead to protamine overdosing, the fact that any additional heparin given during bypass is not taken into the equation might lead to underdosing. As shown previously, there is good evidence that any under- or overdosing of protamine has the potential to cause bleeding because of residual heparin or to excess protamine. There is some evidence that a protamine to heparin ratio of less than .6 or greater than 2.6 is associated with increased bleeding (3,4,49). It is currently recommended, if this method is to be used, that the protamine dose is no more than a 1:1 ratio based on the initial heparin bolus (7).

### Titration Method

The Hemostasis Management System Plus (HMS Plus, Medtronic, Minneapolis, MN) was developed to provide an individualized heparin and protamine dosing regime and is based on an automated protamine titration technique. The individualized heparin level is calculated by mixing blood with stepwise increasing concentrations of protamine and the channel that clots first reflects the best estimate of the patient's neutralized heparin level.

Some authors report reduced blood use with this technology (50,51). It has been shown that the titration method reverses heparin activity reliably when correlating it with anti-Xa as a surrogate marker for heparin activity (52). The authors conclude that the HMS could lead to improved outcomes over ACT-based methods. However, it needs to be borne in mind that an analysis of agreement between ACT and anti-Xa is not possible because results are on different scales. A meta-analysis of four randomized controlled trials, including a total of 507 patients, showed both a reduction in total post-operative blood loss and in the incidence of transfusion in the HMS group, despite increased heparin and decreased protamine doses in that cohort (53). Based on the available evidence, current guidelines on blood management in adult cardiac surgery give the use of heparin-level guided management a class IIa level B recommendation (7).

Despite this recommendation, acceptance into clinical practice is far from complete. There are multiple studies which did not show any reduction in blood loss or transfusion rates (54–56). Significant criticisms of the publications showing decreased blood loss and incidence of transfusion include small sample size, outdated practice techniques, and liberal transfusion practices (57). Of note is also that most studies supporting the use of HMS only include low-risk cases and do not extend to prolonged cardiopulmonary bypass times, redo operations, or those requiring deep hypothermic circulatory arrest. The cost of the HMS plus has been estimated to be \$65 per case (55). Even if there was agreement throughout the literature, it may be difficult to justify the additional cost in the low-risk group of patients.

### ACT-Based Model

The heparin dose–response curve using the ACT test was originally described by Bull et al in 1975 (58). Cuenca et al (59) have recently refined the application of the heparin dose–response curve and developed a mathematical relationship to help determine the protamine dose required at the end of bypass. They propose that it is possible to calculate the appropriate protamine dose using ACT levels measured at the baseline, before CPB, and pre-protamine as well as the initial heparin dose and the patient's weight. They applied their formula in a retrospective observational trial that included 80 patients in the intervention arm and found a statistically relevant reduction of the protamine dose by 40 mg. However, no differences in total blood loss or transfusion requirements were described. The report also confessed that 9% of the data on bleeding and transfusion requirements were missing and not available for the analysis.

### Statistical Models

Davidsson et al. (60) recently attempted to use a multivariate regression analysis to identify clinical variables that could generate a statistical model, allowing clinicians to predict protamine dosing with the same accuracy as the HMS method. Those variables that gave the strongest coefficient of determination were used to generate a formula, which was eventually compared with the HMS results using Bland–Altman plots. Body surface area, total heparin dose per kilogram, heparin clearance, and pre-operative platelet count were included in the model. When applied in 30 patients undergoing elective isolated coronary artery bypass grafting, there was no statistical difference between the protamine dose calculated using the formula and the HMS method. The proposed advantages of the statistical model include reduced cost, ease of use, and no need for additional blood sampling. Although an encouraging result, it was achieved in a small group of patients in an elective setting exposed to a single type of

surgical intervention. It is not known if this agreement between methods would still apply in a larger population in a wider context of cardiac surgical interventions or cardiovascular pathologies. Even if there is consistent agreement between these two methods, as previously discussed, the HMS method has mixed evidence for its use and might possibly not be the ideal benchmark for comparison.

When compared with the 1:1 fixed-dose regime, using the statistical model significantly reduced the amount of protamine given. Thrombelastometry parameters of coagulation were significantly improved in the statistical model group. There were no significant clinical outcome differences, which the authors attribute to the small sample size ( $n = 30$  in each group) (61).

### Pharmacokinetic Model

Recent work with both one- and two-compartment models has shown great promise to help guide protamine dosing. A pilot study using a single compartment model calculating the heparin concentration over time has shown reduced protamine doses and reduced blood loss in the study group when compared with a 1:1 fixed-ratio protamine dose in the control group (62). Meesters et al. (63) applied the same principle in a two-compartment model. Both algorithms are used to calculate the heparin concentration based on the formula in Figure 1.

This equation can be applied multiple times for each additional dose of heparin, and the sum of the results represents the final heparin concentration at a given time.

This method was applied in a retrospective case-control study in a single center and included 62 patients who were compared with 56 patients treated with a conventional fixed-dose regime. The heparin concentration calculated using the algorithm decreased the protamine dose given by more than 50% (186 vs. 416 mg). The authors also reported improvements in multiple thrombelastogram parameters and a reduction in allogenic transfusion in the algorithm group (63).

There are multiple limitations to both the development of the model and clinical evidence in support of its use. The pharmacokinetic coefficients used in the calculations are derived from rodent models and may not be representative of human pharmacology (64). A small number of patients undergoing elective cardiac surgical procedures were investigated. Exclusion criteria including end-stage renal disease, hematologic disorders, and extremes of BMI are

understandable to help determine the feasibility of this method but limit its generalizability. Further prospective, randomized work on a broader range of patients, and cardiac surgical interventions would help determine if this pharmacokinetic model could be adopted as a routine strategy to dose protamine.

### DISCUSSION

Blood loss, transfusion, and return to the operating theater for unacceptable chest tube output or tamponade remain some of the most important end points studied in patients requiring cardiopulmonary bypass. Although failure of surgical hemostasis can be implicated on occasions, this does not absolve coagulopathy from its significant contribution to these undesirable outcomes. Much important work into appropriate blood component therapy to correct this has been done and has been recently summarized in the EACTS/EACTA guidelines in blood management for adults undergoing cardiac surgery (7). Despite these advances, it is likely that blood component therapy is used at times when the interaction between heparin and protamine has not been optimally managed. Till date, only randomized controlled trial comparing a high protamine dose (protamine:heparin ratio = 1.3:1) with a low dose (ratio .8:1), Meesters et al. (5) showed that patients in the higher dose group received more blood products than patients in the low-dose group (fresh frozen plasma 11% vs. 0%, platelets 21% vs. 6%). The ideal management of heparin and protamine remains challenging despite passionate work in this area.

The gold standard of heparin monitoring is considered to be heparin protamine titration based on the aPTT in platelet poor plasma (65). Producing platelet-poor plasma requires a 5–10-minute centrifugation step and is not practical for intra-operative use. Whole blood assays that rely on protamine titration are, therefore, attractive, but mean additional analytical variables in the form of platelet count and platelet dysfunction associated with CPB and variable hematocrit. The relative simplicity of the fixed-protamine:heparin ratio is the reason for its long history and relatively successful use. This fixed method does not accommodate the variability in pharmacokinetics of heparin, which may lead to excess of heparin in one patient and excess of protamine in the next. The ACT method, whereby a heparin dose-response curve is plotted for every individual, addresses the variability in heparin response throughout a prolonged period of anticoagulation. This does, however, add the additional task of manually plotting the dose-response relationship that may take attention away from other critical tasks during a cardiac case. There are commercially available devices (Hemochron, Werfen, Barcelona, Spain; Hemotec, Medtronic, Dublin, and Ireland) that

$$C_t = C_0 \cdot A \cdot e^{\alpha t} + C_0 \cdot B \cdot e^{-\beta t}$$

**Figure 1.**  $C_t$ : heparin concentration time  $t$ ,  $C_0$ : initial heparin dose,  $A$  and  $B$ : distribution coefficients into respective compartments,  $\alpha$  and  $\beta$ : time constant of compartments.

automate this process but come with quite significant additional cost. Regardless of the method used, determining the amount of remaining heparin after cessation of CPB is an indirect process. As previously described, the ACT may be affected by hemodilution, hypothermia, hypofibrinogenemia, thrombocytopenia, and qualitative platelet insults in addition to residual heparin (45). To use the heparin dose–response curve generated pre-bypass and apply it in a significantly different physiological milieu post-bypass is a major drawback to this technique. Although this method may help guide protamine dosing, it has not shown any advantage to weight-based dosing of heparin or fixed-protamine dosing.

A direct measurement of heparin concentration, as done by the HMS, would seem to address one of the major shortcomings of the heparin dose–response curve method. The use of the heparin assay cartridge allows direct measurement of the heparin concentration enabling clinicians to maintain a target heparin concentration during bypass and calculating the required protamine dose when the time for reversal has come. This, however, is not a functional test of heparin activity. It assumes that the distribution of heparin is equal to the blood volume and that it only interacts with AT and no other plasma proteins. The difficult task of estimating patients' blood volume remains and could limit its ultimate clinical utility. Finally, the HMS lacks quality prospective evidence, such as improving clinical outcomes or decreasing the incidence of transfusion, to justify its routine clinical use and its cost (55).

Statistical and pharmacological models seem to be promising. They have the benefit of being quick and inexpensive, and early results are promising when compared with contemporary methods of protamine dosing. However, they are both in their early development phase and will require substantial validation in randomized, controlled trials before their recommendation in guidelines and routine clinical care. There is, however, an emerging trend: although mostly small in patient numbers or retrospective and non-randomized in design, all studies comparing transfusion rates between higher and lower protamine doses or heparin/protamine ratios from the mid-1990s until now favor the group given a lower protamine dose (5,6,62,66).

Further research in this field is warranted and should include more work on the statistical and pharmacologic approaches to protamine dosing (8). Standard fixed–heparin: protamine dosing and early heparin dose–response curve methods will likely start to see less clinical use as these newer methods take advantage of our current and future understanding of anticoagulation, hemostasis, and pharmacology. The ultimate goal has to be applying these methods in our sickest patients and those undergoing longer, complex surgeries. These patients, so often excluded from prospective evaluation, have the greatest need for reduction in transfusion of blood products and for avoiding the side

effects associated with protamine. Cost containment provides another meaningful end point.

## CONCLUSION

Hemostasis still remains one of the most important variables to the outcomes of patients post cardiac surgery. Despite the long and successful history of protamine use, its narrow therapeutic index, intrinsic anticoagulant properties, and adverse reactions still challenge the perioperative team. Residual heparin and excessive protamine are key players in developing coagulopathy. Excellent work has recently been carried out to produce a variety of strategies to address the problem of protamine dosing. Unfortunately, a clinically superior and also cost-effective method, which may be applied to all cardiac surgical patients in the myriad contexts they present in, remains elusive, although there might be a trend toward favoring patients receiving lower protamine doses. Further work with the interaction of heparin and protamine dosing as well as developing alternative anticoagulant medications and their reversal agents will be vital to improving patient outcomes.

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