**The heparin-von Willebrand Factor interaction and conventional tests of haemostasis – the challenges in predicting bleeding in cardiopulmonary bypass**

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**Short title: Assessment of bleeding post-CPB**

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## Abstract

Bleeding is a significant complication of cardiopulmonary bypass (CPB) despite routine anticoagulation monitoring. This is likely to be multifactorial. In this prospective, single-

centre cohort study of thirty patients undergoing CPB surgery, our aim was to characterise the changes in VWF function, platelet interaction and the global coagulation changes during and after CPB surgery and to determine whether bleeding can be predicted. Samples were taken at 6 time points before, during and after CPB surgery. We observed a significant rise in VWF:Ag throughout surgery which continued postoperatively. The absolute VWF:CB and VWF:RCo rose significantly but the VWF:CB/VWF:Ag and VWF:Ag/VWF:RCo fell significantly (p=0.0015 and p=0.0143), suggesting loss of large multimers. We detected a non-significant trend to loss of VWF:RCo after heparinisation and a significant recovery after protamine reversal which could reflect a direct heparin effect. There was a significant increase in the R and K times with a fall in alpha angle and maximum amplitude after heparin administration, using heparinase-TEG. The parameters both significantly improved following protamine (p=0.007 and p=0.0054). The ACT and heparin anti-Xa level correlated poorly, neither predicted clinically significant bleeding. None of these parameters had a relationship with intraoperative blood loss or requirement for blood product replacement.

**Key words:** cardiopulmonary bypass; Unfractionated heparin; von Willebrand factor; bleeding; Thromboelastography; activated clotting time

## Introduction

Patients undergoing cardiopulmonary bypass (CPB) surgery are at risk of bleeding which is multifactorial. Most significant of these is the heparin anticoagulation used

to maintain patency of the bypass circuit. Unfractionated heparin (UFH) is a heterogeneous mix of sugar chains varying in molecular weight (MW) from 500 to 20000 Daltons. Heparin potentiates the effect of antithrombin (AT) in its inactivation of factor Xa and thrombin, by binding to AT via a specific pentasaccharide sequence (1). The anticoagulant effect of UFH during CPB is routinely monitored using coagulation tests, notably the Activated Clotting Time (ACT) or in some patients using heparin anti-Xa levels. It is known that heparin can also inhibit the von Willebrand factor – platelet glycoprotein Ib alpha (VWF-GPIbα) interaction (2-4) an anti-thrombotic effect distinct from its anticoagulant role via antithrombin. The physiological and clinical relevance of this effect is not known but it may, in part, explain why bleeding complications that occur during heparin therapy can be unpredictable, despite using anticoagulant monitoring techniques. This additional activity of heparin is likely to be of particular importance during CPB surgery when very high concentrations of heparin are used.

The structural aspects of heparin responsible for this interaction have been studied in detail (5) and found to depend on the GlcNS[6S]-IdoA2S disaccharide and the MW with a minimum chain length of 12 monosaccharides required for the inhibitory action (5). The AT-binding pentasaccharide sequence is not an essential requirement for the anti-VWF activity of heparins (6, 7).

UFH is the standard anticoagulant used for CPB due to its rapid onset of action, clinical efficacy, rapid neutralisation by protamine sulphate, safety and low cost. The ACT is a point of care test (POCT) used to assess the adequacy of heparinisation and is monitored every 30-40 minutes during bypass by the anaesthetic team. A typical dose of UFH is 300-400U/kg aiming for an ACT of greater than 480s (8). It is known that the ACT poorly correlates with the plasma heparin level and does not accurately predict the protamine dose needed for reversal (9). It may therefore be a poor predictor of bleeding risk. Although monitoring the inhibition of Factor Xa by heparin is the gold standard for measuring the anticoagulant effect of UFH, this is

not available as a POCT and many hospital laboratories are not able to provide the heparin activity, as measured by the anti-Xa level, in a timely manner for patients undergoing CPB. Therefore, the ACT remains the main measure of monitoring the anticoagulant effect of UFH during CPB. Viscoelastic assays such as Thromboelastography (TEG®) and rotational Thromboelastography (ROTEM®) provide global information on the dynamics of clot development, stability and dissolution in real-time and are now used to guide blood product replacement and haemostatic support during cardiac surgery.

Our hypothesis is that the unmonitored inhibitory effect of UFH on the VWF-GPIbα is interaction is important in patients receiving the highest doses of heparin in clinical use, that is CPB. The aims of this work were to characterise the changes in VWF concentration, function and platelet interaction that occur intra- and post-operatively in patients undergoing cardiac surgery receiving UFH and to determine whether there was a significant inhibitory effect of heparin on the VWF-GPIbα interaction that could be detected using the currently available laboratory assays of haemostasis. Viscoelastic methods are now widely used to guide replacement therapy and with the ACT being a poor measure of heparin activity, TEG parameters were measured in parallel for comparison of predictive value.

## Methods

### *Study Design and Participants*

This was a prospective, single centre observational study in a major cardiothoracic centre in the UK. Ethics approval was sought and granted by the Research Ethics Committee (REC) and Health Research Authority and Health and Care Research Wales (REC reference 18/LO/2158) and the study was conducted according to the Helsinki declaration. The study was granted inclusion on the National Institute for Health Research (NIHR) Clinical Research Network (CRN) portfolio and was also

registered on clinicaltrials.gov.uk (https://clinicaltrials.gov/ct2/show/NCT03861286). The study recruited 30 eligible adult patients (>18 years), undergoing first time cardiac surgery requiring CPB, for the correction of atrial septal defects (ASD) or tissue mitral valve replacement, and receiving IV UFH intra-operatively, over a 6-month period from February to August 2019. Exclusion Criteria were concomitant aortic stenosis, patients with a baseline platelet count of <100x109/L, patients with a known coagulation factor deficiency or platelet function disorder and patients receiving heparin or antiplatelet therapy prior to CPB. Written informed consent was obtained from each participant prior to surgery.

***Sample Processing and Laboratory Analyses***

Venous blood samples were taken at 6 time points [baseline, 30 minutes after administration of intravenous UFH, before reversal of UFH with protamine sulphate (PS), 30 minutes after reversal of UFH with PS, and 12hrs and 24hrs postoperatively] (Supplementary table 1) into 0.109M trisodium citrate at a ratio of 9:1 (Vacutainer Plus, Becton Dickinson, Franklyn Lakes USA), double centrifuged (2x 2000g for 10 min at room temperature) within 1 hour of collection and analysed immediately or stored at -80°C until analysed. Full blood count (FBC), coagulation screen including Clauss fibrinogen, viscoelastic analysis (TEG) and heparin anti-Xa levels were performed immediately. The ACT was performed in theatre. VWF antigen and VWF functional assays (ristocetin cofactor assay and collagen binding) were performed in batches once the study was completed.

The anti-Xa assay, using a chromogenic liquid anti-Xa assay (Werfen, Warrington, Cheshire, UK), and the activated partial thromboplastin time (APTT) using SynthASil (HemosIL®, Werfen, Warrington, Cheshire, UK) were performed on the same sample using an ACL TOP 500 (Werfen, Warrington, Cheshire, UK). The Clauss fibrinogen level was also performed in the same sample using FIB-C XL kit (Werfen, Warrington,

Cheshire, UK) on the ACL TOP 500. TEG was performed at each time point immediately after sample collection into 0.109M trisodium citrate using the TEG 6S Hemostasis Analyzer (Haemonetics Corp., Braintree, MA). Data generated in the heparinase cup were used for analysis. VWF antigen (VWF:Ag) and VWF ristocetin cofactor (VWF:RCo) analysis were performed on the ACL TOP 500 using reagents from Werfen, Warrington, Cheshire, UK. VWF collagen binding (VWF:CB) was performed in an enzyme-immuno-assay (ELISA) using ZYMUTEST vWF:CBA kits (HYPHEN BioMed,95000 Neuville-sur-Oise, France).

***Bleeding and use of Blood Products***

Blood loss from surgical drains in the first 24 hours post operatively was the parameter used to determine blood loss. Information on blood product administration, namely packed red cells (PRC), fresh frozen plasma, platelets and cryoprecipitate was collected.

***Statistical Analysis***

Results are presented as percentages for categorical data, and median and ranges for continuous data. Differences between study time points were assessed using the paired t-test. Correlation between the ACT and anti-Xa level was assessed using Spearman correlation as was correlation between bleeding and the heparin anti-Xa level and the VWF:RCo. Analyses were performed using SPSS version 24 software (IBM Corp., Armonk, NY, USA) and GraphPad Prism® version 8.3.1 (GraphPad Software, Inc. La Jolla, USA). Two-tailed $p<0.05$ were considered statistically significant.

**Results**

The baseline characteristics of the study participants have been summarised in Table 1.

### *Changes in VWF Parameters During Surgery*

There was a significant rise in the mean VWF:Ag throughout the operative period which continued postoperatively: from baseline to the Pre-Protamine ($p=0.0149$), Post-Protamine and the 12 and 24 hours post-operative points ($p<0.0001$ for latter time points) (Figure 1). The mean VWF collagen binding activity, which is highly sensitive to the presence of VWF high molecular weight multimers (HMWM), also rose from baseline at the end of surgery (Post Protamine), 12 and 24 hours postoperatively ($p=0.013$, $p<0.0001$ and $p<0.0001$ respectively) but to a lesser extent. Consequently there was a significant fall in the VWF:CB/VWF:Ag ratio which occurred primarily during surgery. The pattern of decreased VWF:CB/VWF:Ag over this period suggests that a reduction in VWF multimer size takes place during surgery and is still present 24 hours later (Figure 2B). There was a significant rise in absolute VWF:CB Post Protamine corresponding to heparin reversal ($p=0.0065$) (Figure 2A). However, the VWF:CB/VWF:Ag ratio did not change ($p=0.2376$) at this point.

The VWF Ristocetin Cofactor Assay (VWF:RCo) is a measure of the VWF-platelet GPIbα interaction. It is also dependent upon the presence of HMWM. As with VWF:CB, the rise in VWF:Ag level is accompanied by a rise in the absolute VWF:RCo beginning during surgery and persisting at 24 hours post-operative ($p<0.0001$) (Figure 3A). The VWF:RCo/VWF:Ag ratio fell through the operative period as would be expected from the previously noted fall in the VWF:CB/VWF:Ag ratio, presumed due to loss of larger VWF multimers (Figure 3B). There was a significant rise in the absolute VWF:RCo Post Protamine, $p=0.0003$ but the VWF:RCo/VWF:Ag ratio did not change ($p=0.5885$).

## Intraoperative Monitoring of Coagulation

### *TEG Parameters*

The changes in four TEG parameters; the R time, K time, alpha angle and maximum amplitude (MA) were monitored in this study. All parameters for analysis were

performed in heparinase containing cups. Despite the use of heparinase there was a significant increase in R and K times and fall in alpha angle and MA after heparin administration. There were significant differences in the R time and K time from baseline and when patients were fully anticoagulated during surgery (Pre-Protamine). Following reversal of anticoagulation (Post Protamine) the R time and K time were unchanged (p=0.45 and p=0.64) but the MA and alpha angle were significantly improved (higher) following administration of protamine (p=0.007 and p=0.0054 respectively (Figure 4 A-D).

***Fibrinogen and Platelet Count***

There was a significant fall in fibrinogen from baseline to the end of the surgery (Post Protamine), (p=0.0008) which recovered 12hrs post-surgery and by 24hrs post-surgery some patients had higher levels than pre-surgery (Supplementary figure 1A). The platelet count followed a similar pattern from baseline to post protamine. However, unlike fibrinogen, which recovered within 12 hrs post-operatively, the platelet count remained significantly lower than baseline at 12- and 24-hours post-surgery (p<0.0001) (Supplementary figure 1B). However, neither fibrinogen nor platelet count fell to a level that is likely to promote bleeding (i.e fibrinogen <1.0g/L or platelet count $<50x10^9$/L)

***Monitoring of the Anticoagulant Effect of Unfractionated Heparin using the ACT and Heparin Anti-Xa Levels and Value in Predicting Bleeding***

The median intra-operative ACT was plotted against the anti-Xa level at the Pre-Protamine time point. The intra-operative ACT was maintained above 450s but there is high variability in the anti-Xa levels (range 4.29-12.2 IU/ml, mean 7.30±1.99 IU/ml) when the ACT is 500s. There was no correlation between the ACT and heparin anti-Xa levels; Spearman Correlation r=0.-03590, p=0.8618 (Supplementary Figure 2).

**Prediction of Bleeding**

We had hypothesised that higher levels of anticoagulation and/or lower levels of VWF:RCo would result in increased bleeding. The relationships between the drain volume and the effects of heparin, as assessed by the Pre-Protamine anti-Xa level and the pre protamine VWF:RCo were analysed. There is no correlation between the drain volume and VWF:RCo during full anticoagulation (Spearman correlation r=-0.1967, p=0.3255) (Figure 5A) or between the drain volume and anti-Xa level when fully anticoagulated (r=-0.2081, p=0.3077) (Figure 5B).

Seven patients in this study required blood product transfusion as summarised in Table 2. For these seven participants, the secondary effect of heparin, that is its inhibitory effect on the VWF-GPIbα interaction, could be identified from the VWF:RCo. The VWF:RCo, when fully anticoagulated (Pre-Protamine), for all seven patients who required transfusions, was within the normal range (NR 50-200 IU $dL^{-1}$). There was a fall from baseline in the absolute VWF:RCo 30 minutes after the administration of UFH in patients 20,24,26 and 29 although this was not sustained for patients 26 and 29 when measured at the end of surgery (Pre Protamine). The rise in VWF:Ag level could explain why there is recovery of the absolute VWF:RCo in these patients.

Participant 3 had a high intraoperative ACT of 614s and the highest anti-Xa level of the seven patients requiring transfusion. Total blood loss (TBL) (drain volume at 24 hours plus recorded loss into cell saver) was 3007ml but patients with lower ACT and anti-Xa levels had larger volume loss suggesting bleeding cannot be predicted from the ACT and anti-Xa levels only. Interestingly this patient's VWF:RCo actually rose from baseline after the administration of heparin and could have contributed to lower bleeding (Table 2). Furthermore, there were nine participants with a median intra-op ACT of >600s but of these, only participant 3 had bleeding requiring blood product replacement. The ACT alone is not a predictor of clinically significant bleeding. The

highest recorded median ACT was 1000s for participant 9 and despite a TBL of 3311ml this participant did not require blood product transfusion in the first 24 hours. This may be explained by the volume of blood returned to the patient from autologous cell salvage and it is possible that the patient received blood product transfusions later on in their post-operative recovery.

Participants 20, 24, 26 and 29 all had a drop in the VWF:RCo after the administration of heparin. Participant 29 is interesting in this respect because despite a fall in the VWF dependant platelet function, presumably induced by heparin, the heparin effect was the lowest as measured by heparin anti-Xa level 4.29 IU/ml) compared to others.

**Discussion:**

In this prospective single centre study with patients undergoing CPB for correction of ASD or tissue mitral valve replacement, we have identified that both UFH and protamine sulphate affect multiple components of haemostasis including platelet function and coagulation, all of which may contribute to bleeding. These effects may not be identifiable using a single test and may explain why some patients continue to bleed following CPB despite reversal of heparin with protamine sulphate and the administration of blood products based on monitoring of routine coagulation tests and the platelet count. Although TEG provides a global measure of coagulant activity and fibrinolysis, including some aspects of platelet function, it does not contain any element of shear and so will not detect the VWF–platelet interaction essential for primary haemostasis.

The changes in VWF during and after CPB are complex. The most striking effect in our study is the rise in VWF:Ag that takes place during the surgery and continues through the post-operative period. This is probably attributable to the well described effect of stress on endothelial cells resulting in VWF release from Weibel-Palade bodies (WPB) and increased synthesis of VWF as an acute phase reactant. Although this rise in

antigen is accompanied by a rise in VWF collagen binding and ristocetin activity, this is not as great as expected due to a fall in both VWF:CB and VWF:RCo specific activities, reflected in the ratios of these activities to antigen. The fact that both parameters fall throughout surgery,

strongly suggests that there is a common mechanism such as preferential reduction in VWF multimer size. This has been reported to arise from the shear stress in the extracorporeal circuit facilitating ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) cleavage of VWF.

We hypothesised that the high doses of heparin administered at the beginning of bypass would significantly impede platelet-VWF interaction. Although this has been demonstrated clearly in vitro and in flow-based platelet capture assays, the fall in VWF:RCo/VWF:Ag from baseline to Post-heparin did not quite reach significance in our study ($p=0.0556$) although it may have done so if the sample size was larger. We note that previous studies have detected the expected fall (6). The VWF assay uses ristocetin to simulate the effect of shear on the VWF molecule and our results suggest that this gives an incomplete measure of platelet-VWF interaction, perhaps particularly in modern automated versions of the assay. It is possible that a platelet-based form of the assay may be able to detect the effect of heparin binding more sensitively. There is also a non-significant fall in VWF:CB/VWF:Ag ratio after heparin administration and so a direct effect of UFH on both CB and RCo is not excluded. This is plausible because in the resting state A1A2A3 are folded together and heparin binding could impede interactions of both the A1 and A3 domains containing the GPIbα and collagen binding sites.

Although reversal of heparin's anticoagulant effect with protamine might be expected also to reverse any effect on VWF, this is complicated by a direct interaction of protamine with VWF. It has been shown that protamine sulphate inhibits platelet GPIbα-VWF interaction (10) and addition of protamine to platelet rich plasma (PRP) significantly reduced VWF-dependent platelet agglutination in the presence of

ristocetin by up to 30%. Ristocetin and protamine are of opposing charge, raising the possibility that these effects are in vitro artefacts.

Our work highlights that the standard measures of coagulant activity used in CPB are incomplete and misleading. Notably the ACT, used routinely to monitor and adjust heparin anticoagulation, did not correlate with the heparin activity as measured by the anti-Xa assay.

Despite using TEG data from the heparinase cup for analysis, where the effect of heparin has been eliminated, the R time was significantly prolonged after the administration of heparin and this was not reversed with the administration of protamine ($p=0.45$) suggesting that excess heparin is not the only reason for the prolongation of the R time. A dilutional coagulopathy is a known complication of CPB and would also prolong the R time and reduce the recovery of the R time after the reversal of heparin. The most common practice is to prime the cardiopulmonary circuit with crystalloid (11), a volume of up to 1.7L (12) which is contributory to this dilutional coagulopathy. Prolongation of the R time has been reported in other studies looking at thromboelastography results in CPB (13) and it has been demonstrated that protamine itself affects the R time in a dose-dependent manner (13, 14). Indeed, whilst protamine primarily neutralises heparin, it is well established to have an anticoagulant effect (15), and thus the dosing of protamine is crucial when reversing heparin. Protamine neutralises the effect of heparin through electrostatic binding between the cationic arginine groups of protamine and the anionic residues in heparin in a 1:1 ratio. A higher protamine to heparin ratio of 3:1 significantly affected the R time in the study by Khan et al (14). Effects of protamine that may reduce thrombin generation (16, 17) include reduction in FV and FVII activation (18) and FVIII activity (19). However, in our study there was no evidence that protamine added additional anticoagulant effect as the R time and K time remained unchanged or in some patients the R time reduced. There was also a significant improvement in the MA and alpha angle following the

administration of protamine, possibly aided by the administration of a near perfect protamine to heparin ratio at the end of CPB.

Protamine also has other effects not measured in this study. It impairs platelet function as a result of reduced thrombin-induced platelet aggregation (20), (21) and impairs the VWF-GPIbα interaction. However, there was no evidence to suggest that protamine affected the VWF:RCo in this study, possibly due to same reason described above (near perfect protamine:heparin ratio). Protamine also enhances fibrinolysis by reducing clot strength (22).

It is also well known that heparin can bind to platelets. Previous studies have shown that when rapidly administered intravenously, UFH reduces platelet counts and platelet function causing prolongation of the bleeding time (23). We noted a significant fall in MA and alpha angle following heparin administration that rose again after neutralisation with protamine. The MA depends largely on both platelet number and fibrin cross-linking between platelets whereas the alpha angle provides information on fibrin formation and cross-linking. These effects will not be captured by conventional tests..

The recovery of the MA and the alpha angle after protamine administration is not explained by reversal of heparin anticoagulant activity. These changes and the delayed recovery of the K time may therefore result from the effects of protamine on thrombin generation as described above. We also note that the heparin degradation fragments may retain some biological activity, such as altering $Ca^{2+}$ flux, which could theoretically affect platelet function (24).

The MA or clot firmness is most affected by a qualitative dysfunction or quantitative deficiency of platelets. There is a statistically significant fall in the MA following the administration of UFH with a statistically significant rise after the reversal of heparin. This is most likely due to the effect of UFH on platelet aggregation and fibrin cross-linking. This conclusion is further supported by that fact that although platelet counts

fell from baseline to intraoperatively and remained reduced at 24 hours post-op, the MA improved following the administration of protamine, indicating that the fall in platelet count alone is not responsible for the change in the MA. This suggests that heparin exerts a direct effect on platelet function or inhibits the binding of platelets to the fibrin mesh. The significant rise in both the alpha angle and MA following the reversal of heparin could be due to high dose UFH interfering with fibrin cross-linking and or the ability of platelets to bind to fibrin.

It seems likely that the fall in platelet count observed through the time points as well as the initial fall in fibrinogen levels are due to a consumptive coagulopathy, a well-recognised complication of CPB surgery. There were no common drugs these patients received that affected the platelet count. Three patients resumed anti-platelet therapy post-op and 2 restarted their anti-Xa inhibitors, neither class of drug is known to affect the platelet count.

Perhaps the most striking finding in this study is that none of these measures had a significant relationship with intraoperative blood loss or requirement for blood product replacement. There were nine participants with a median intra-op ACT of >600s but of these, only participant 3 had bleeding requiring blood product replacement. All 7 patients requiring blood products had VWF:RCo measurements within the normal range. The ACT and VWF:RCo alone are therefore not predictors of clinically significant bleeding. The highest recorded median ACT was 1000s for participant 9 and interestingly they did not receive any blood products.

This is a well-designed study exploring many aspects of complex haemostasis including Thromboelastography, in patients undergoing CPB. The limitations of the study are the relatively small number of patients and the lack of measuring ADAMTS13 activity in the complex haemostatic process occurring during CPB. However, other studies have demonstrated a fall in ADAMTS13 activity intraoperatively(25, 26) suggesting an alternative mechanism for loss of HMWM, such as shear-induced proteolysis by the bypass circuit. The selection criteria for participants were designed

to avoid confounding factors such as aortic stenosis and antiplatelet therapy resulting a relatively young and sex balanced cohort. Although, we did not perform the assessment of the multimeric composition of VWF which is important in understanding changes in VWF functional activity, in comparative studies of VWD, collagen binding has proved a useful and valid surrogate for multimer size(27, 28). We have therefore used VWF: CB and the ratio of collagen binding and RCo activity to draw our conclusions.

In conclusion this study demonstrates a complex relationship between haemostatic markers and drug effect during CPB. The off-target effects of both heparin and protamine sulphate make it difficult to confidently isolate the individual effects of each drug, as well as the biological variables in the stress response induced by surgery, which affect the haemostatic system, such as the release of inflammatory cytokines. This is further compounded by the dilutional and consumptive coagulopathy of CPB and the effects of shear in the extracorporeal circuit. Therefore, it is not possible to use these data to create a predictor of bleeding due to the heparin-induced inhibition of the platelet GPIbα-VWF interaction. There was no relationship between the haemostatic parameters analysed in this study and intraoperative blood loss or requirement for blood product replacement.

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**Authorship**

AR designed the study, performed sample assays, analysed and interpreted the data and wrote the manuscript. MG, TJ, LV, JC were involved in sample collection and processing and performing Thromboelastography. AE and AG were involved in consenting and recruiting participants and sample collection. SF performed some of the assays. ML was involved in the study concept, data interpretation, writing and

revision of the manuscript. DJA was involved in the study design, conducting the study, patient selection, analysis and interpretation of the data, writing and revision of the manuscript. All authors reviewed and approved the final manuscript

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**Disclosure of conflict of interest**

Authors have no conflict of intertest to declare

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**Table 1:** Baseline Characteristics of Study Participants

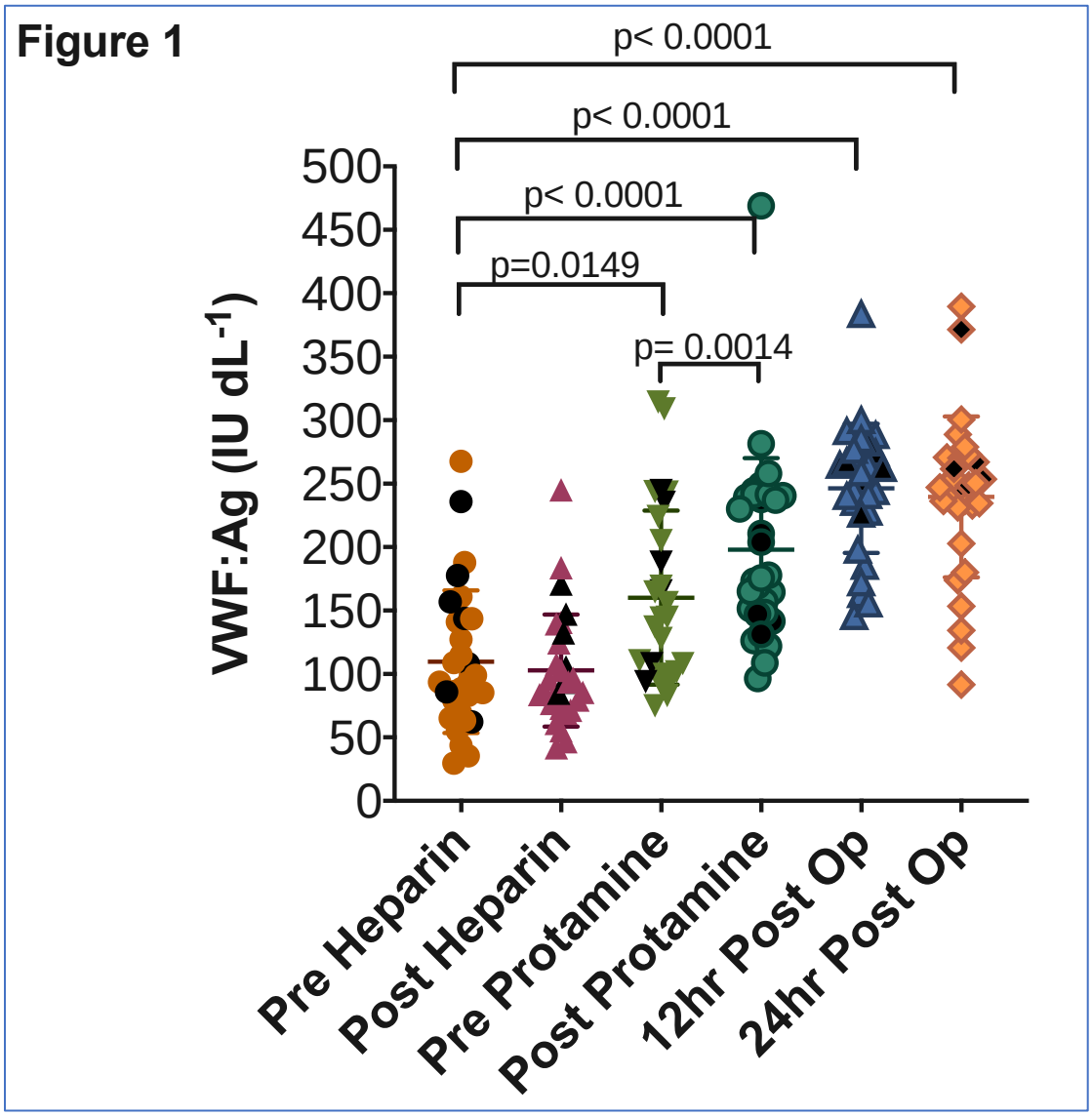
| Variable | Median | Interquartile Range | Normal Range |
|---|---|---|---|
| **Gender** | | | |
| Male | n=17 (56.7%) | | |
| Female | n=13 (43.3%) | | |
| **Age (years)** | 62 | 25.5 | |
| **Height (cm)** | 177 | 18 | |
| **Weight (Kg)** | 84 | 27.15 | |
| **BMI (kg/m$^2$)** | 26.3 | 6.9 | |
| **Baseline Haemoglobin (g/L)** | 136 | 18.5 | 121-172 |
| **Baseline Platelet Count (x10$^9$/L)** | 192 | 92 | 150-450 |
| **Baseline APTT (seconds)** | 28.2 | 3.45 | 26-36 |
| **Baseline PT (seconds)** | 13.6 | 2.1 | 10.2-13.2 |
| **Baseline INR** | 1.1 | 0.2 | |
| **Baseline Fibrinogen (g/L)** | 2.6 | 1.25 | 1.5-4.5 |
| **Baseline Creatinine Clearance (ml/min)** (using Cockcroft-Gault Equation) | 100.9 | 54.89 | 88-137 |

**Table 2:** The VWF:RCo parameters (the baseline absolute VWF:RCo and the absolute VWF:RCo when fully (Pre Protamine), summary of the blood products transfused within the first 24 hours for the seven patients requiring blood product transfusion (Participants 3,6,13,20,24,26 & 29) with the surgical drain output in the first 24 hours, median intra-operative ACT and Pre Protamine anti-Xa level are given.

| ID | Absolute VWF: RCo Baseline | Absolute VWF: RCo Pre- Protamine | PRC (ml) | Units of Platelets | FFP (ml) | Cryoprecipitate (pools) | Drain Vol (ml) | Intra Op ACT (s) | Pre- Protamine anti-Xa level (IU/ml) |
|---|---|---|---|---|---|---|---|---|---|
| **3** | 81.1 | 114 | 629 | 1 | 515 | 1 | 1025 | 614 | 9.02 |
| **6** | 118 | 118.2 | 1242 | 0 | 0 | 0 | 925 | 493 | 4.73 |
| **13** | 53.4 | 126.2 | 542 | 1 | 0 | 0 | 1995 | 503 | 5.07 |
| **20** | 112.3 | 80.8 | 0 | 0 | 0 | 2 | 510 | 537 | 8.32 |
| **24** | 211.1 | 114.6 | 807 | 2 | 550 | 0 | 790 | 520 | 6.62 |
| **26** | 115.2 | 151.7 | 0 | 1 | 0 | 0 | 975 | 485 | 8.99 |
| **29** | 95.1 | 58 | 762 | 2 | 0 | 3 | 1990 | 504 | 4.29 |

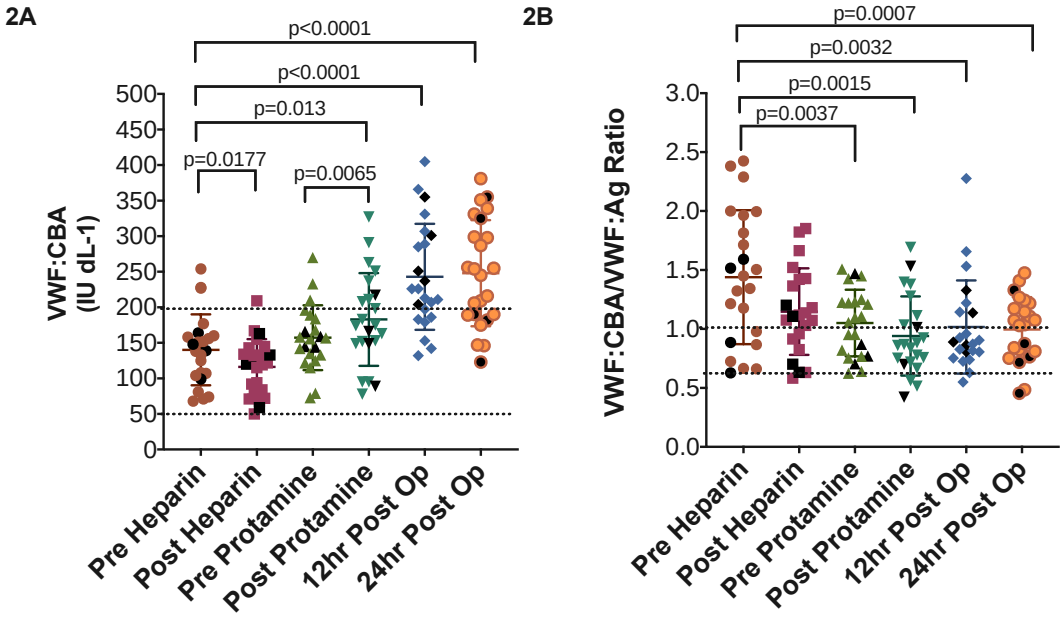
**Legends to figures**

**Figure 1: Changes in the VWF Antigen Level Pre, Intra- and Post Operatively in Patients Receiving IV UFH for Cardiopulmonary Bypass Surgery**




Blood samples were taken before the administration of IV UFH and after its reversal by Protamine Sulphate. Samples were analysed for the VWF antigen level and demonstrate a statistically significant rise of the mean VWF:Ag from baseline to the Pre-Protamine (p=0.014), Post Protamine, 12 and 24 hours post operatively (p<0.0001 for latter time points). Symbols in black represent participants requiring blood product transfusion within the first 24 hours.

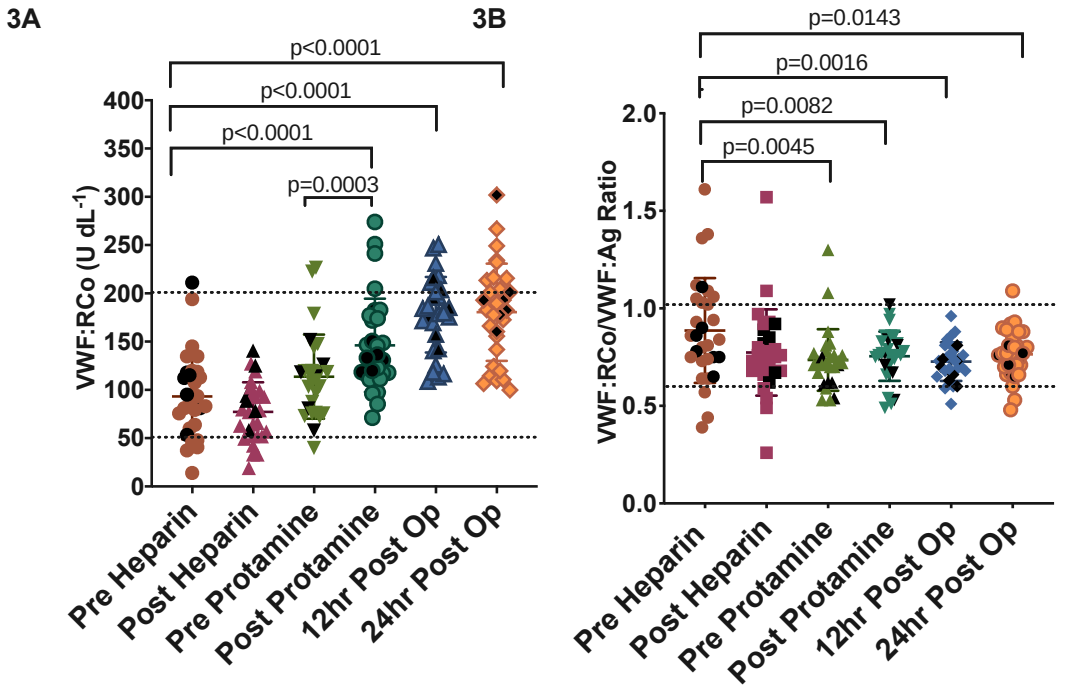
**Figure 2: Changes in the VWF Collagen Binding Activity (A) and VWF:CB/VWF:Ag ratio (B) Pre, Intra- and Post Operatively in Patients Receiving IV UFH for Cardiopulmonary Bypass Surgery**




The VWF:CB (2A) was measured at 6 time points pre-, intra- and post- operatively as described. There was a statistically significant rise in the mean VWF: CB from baseline at the end of surgery (Post Protamine), 12 and 24 hours post-op (p=0.013, p<0.0001 and p<0.0001 respectively).

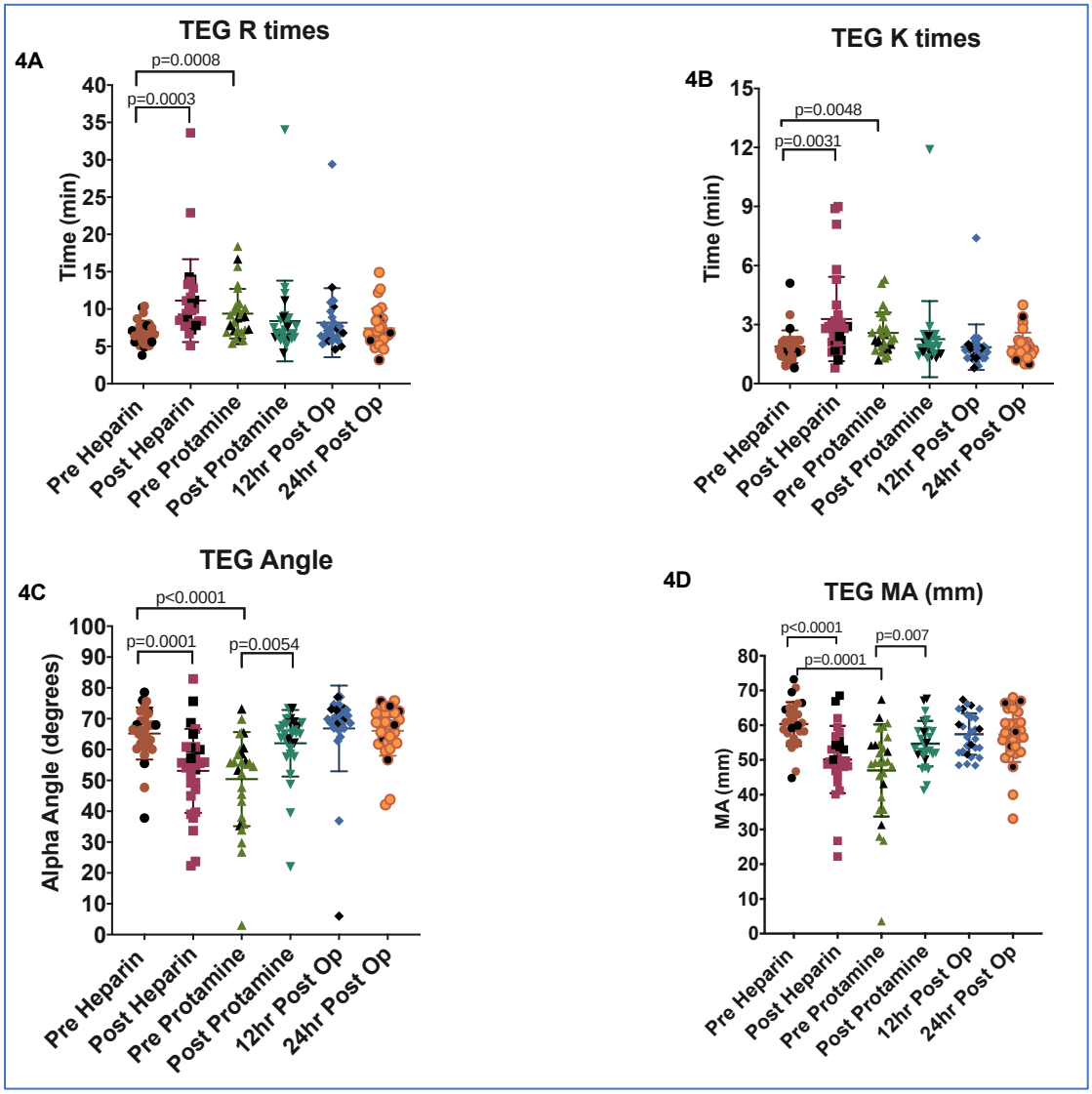
VWF:CB/VWF:Ag ratio (2B). There was no statistically significant difference in the ratio Pre and Post Protamine. The VWF:CB/VWF:Ag ratio fell significantly during surgery and remained reduced from baseline at the end of surgery and at 12 and 24 hours post operatively (p=0.0015, p=0.0032 and p=0.0007 respectively). Symbols in black represent participants requiring blood product transfusion within the first 24 hours. Values within the dotted lines are within normal range.

**Figure 3: Changes in the VWF Ristocetin Cofactor Activity Pre, Intra- and Post Operatively in Patients Receiving IV UFH for Cardiopulmonary Bypass Surgery**



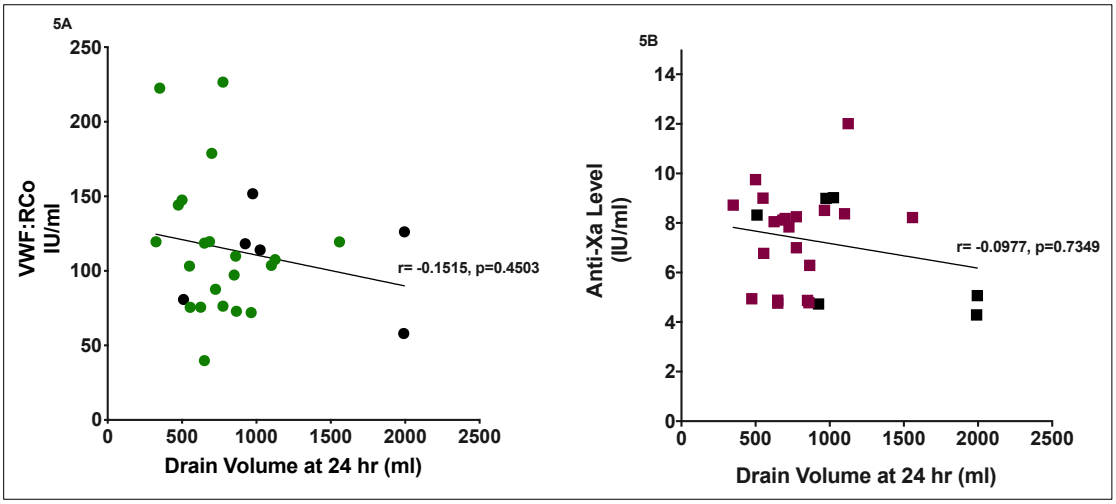

The VWF:RCo (3A) was measured at 6 time points pre-, intra and post operatively as described. There was a statistically significant rise in the mean VWF:RCo from baseline to the Post Protamine, 12 hours and 24 hours post-operative time points ($p<0.0001$ for all). Given there was rise in the VWF:Ag levels, the VWF:RCo/VWF:Ag ratio was calculated (3B). There was no statistically significant difference in the ratio Pre and Post Protamine. Symbols in black represent participants requiring blood product transfusion within the first 24 hours. Values within the dotted lines are within normal range.

**Figure 4: Thromboelastography Data**




TEG data were obtained at 6 pre-, intra- and post-operative time points from patients undergoing cardiopulmonary bypass surgery, receiving IV UFH heparin. Heparinase lined cuvettes were used to determine the true R time (Figure 4A), K time (Figure 4B), alpha angle (Figure 4C) and MA (Figure 4D). Pre- and Post- reversal of heparin anticoagulation there was a statistically significant rise in both the alpha angle and MA (p=0.0054 and p=0.007 respectively). Symbols in black represent participants requiring blood product transfusion within the first 24 hours.

**Figure 5: The relationship between the VWF:RCo (5A), the anti-Xa level (5B) when fully anticoagulated and drain volume at 24 hours**




There is no statistically significant correlation between the surgical drain volume at 24 hours and the VWF:RCo when fully anticoagulated (Spearman correlation coefficient r= -0.1515, p=0.4503). There is also no correlation between the drain volume and anti-Xa level when fully anticoagulated (r= -0.0977, p=0.7349). Symbols in black represent participants requiring blood product transfusion within the first 24 hours.