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Activated Clotting Time (ACT) value as an independent predictor of postoperative bleeding and transfusion

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Resumo

A Cirurgia Cardíaca é a área cirúrgica dedicada ao tratamento das patologias do coração e grandes vasos torácicos. Uma característica fundamental que distingue a Cirurgia Cardíaca de todos os outros tipos de cirurgia é a utilização de circulação extra-corporal (CEC), mantendo a função cardíaca e pulmonar durante a cirurgia ao coração. Uma vez que os circuitos da CEC não são revestidos por endotélio, o contacto entre o sangue do paciente e as superfícies da máquina leva ao desencadeamento de uma resposta pró-trombótica e pró-inflamatória. Simultaneamente, durante a utilização de CEC são necessárias altas doses de heparina para contrariar a resposta pró-trombótica que ocorre no sangue do paciente, tentando prevenir a formação de trombos tanto dentro do circuito, como dentro dos próprios vasos sanguíneos do doente. Esta inflamação sustentada, juntamente com a ativação da cascata da coagulação e a utilização de altas doses de heparina, dificulta a manutenção de um equilíbrio adequado entre trombose e hemorragia. Desta forma, surge a necessidade de um parâmetro que monitorize rapidamente o grau de anticoagulação induzida pela heparina (e a sua reversão com protamina) e que possa ser realizado facilmente à beira do doente.

O Tempo de Coagulação Ativada (ACT) é um parâmetro comummente utilizado como teste para monitorizar a anticoagulação durante cirurgias cardíacas, tendo as vantagens de ser um teste rápido, feito à cabeceira do doente, e de utilizar o sangue na sua totalidade, incluindo a contribuição de outros elementos sanguíneos para a hemostase, além dos fatores de coagulação. Devido às alterações que ocorrem na hemostase aquando da realização de cirurgias cardíacas, a hemorragia e o consequente uso de transfusões é uma complicação frequente em pacientes submetidos a cirurgia cardíaca, aumentando a morbimortalidade dos doentes. Desta forma, a anticoagulação cuidadosa é um dos pilares da cirurgia cardíaca, especialmente aquando da utilização de CEC, sendo o controlo após a administração de protamina igualmente importante.

No entanto, até ao momento, não existe evidência suficiente para apoiar o valor ideal de ACT Final em Cirurgias Cardíacas com CEC. Tendo em conta o conteúdo supramencionado, o objetivo do nosso trabalho foi avaliar a utilidade do valor de ACT como preditor de hemorragia e do uso de transfusão no período pós-operatório.

De forma a atingir o nosso objetivo, realizámos um estudo observacional, retrospetivo, unicêntrico, baseado na recolha, tratamento e análise de dados de 722 doentes submetidos a cirurgia cardíaca com CEC entre julho de 2018 e outubro de 2021, no Hospital de Santa Maria, em Lisboa. Neste estudo foram incluídos pacientes com a idade mínima de 18 anos submetidos a cirurgia cardíaca com necessidade de CEC. Os critérios de exclusão foram os seguintes: (i) pacientes sob terapêutica anticoagulante; (ii) pacientes com complicação hemorrágica pós-operatória (requerendo, por exemplo, reexploração cirúrgica para controlo do foco hemorrágico); e (iii) pacientes sem registos clínicos pós-operatórios completos. A medição dos valores de ACT foi realizada recorrendo ao sistema *HMS Plus Hemostasis Management System* da *Medtronic*, utilizando cartuchos *High Range ACT* (HR-ACT) com caulim como ativador. Hemorragia significativa foi definida como ≥ 600 ml às 12 horas após cirurgia (Dyke et al., 2014).

Para caracterizar a relação entre ACT Final e hemorragia/necessidade de transfusão, dividimos os pacientes em: ACT Final < ACT basal e ACT Final ≥ ACT basal; bem como em ACT Final < 140 segundos e ACT Final ≥ 140 segundos. As variáveis contínuas foram analisadas recorrendo ao teste Wilcoxon Rank Sum, com correção de continuidade, e as variáveis categóricas foram analisadas usando o teste exato de Fisher ou o teste Qui-quadrado com a correção de continuidade de Yates. Um modelo de Regressão Linear Mista com Efeitos Fixos e Aleatórios foi aplicado para analisar a hemorragia durante as primeiras 24 horas pós-cirurgia em dois grupos de pacientes: ACT final < 140 segundos e ACT final ≥ 140 segundos. Várias outras variáveis independentes foram analisadas de forma a investigar a associação entre as ditas variáveis e a hemorragia e utilização de unidades de concentrado eritrocitário, recorrendo a modelos de Regressão Logística Binária.

Sumarizando os dados dos pacientes, a mediana de idades foi 71 anos e cerca de 55% dos pacientes eram homens. A grande maioria foi submetida a cirurgia eletiva (95.3%) e o tipo de cirurgia mais frequentemente realizada foi cirurgia valvular (92.2% de todos os pacientes). Relativamente a comorbilidades, 81.6% dos doentes tinham hipertensão arterial, 72.9% eram não-diabéticos e 65.2% tinham dislipidémia. Cerca de 9.1% dos pacientes tinha doença renal crónica pelo menos moderada e a fração de ejeção ventricular esquerda encontrava-se preservada em 86.2% dos doentes, com 2% da totalidade dos pacientes tendo uma fração de ejeção ventricular esquerda reduzida ou gravemente reduzida.

Relativamente aos resultados obtidos, pacientes com ACT final ≥ 140 segundos sangraram, em média, 99.62 ± 36.39 ml a mais nas primeiras 12 horas e 104.99 ± 36.39 ml a mais nas primeiras 24 horas após a cirurgia comparativamente a pacientes com ACT final < 140 segundos, havendo diferenças estatisticamente significativas tanto às 12h, como às 24h. Ademais, embora a taxa de hemorragia nas primeiras 12 horas tenha sido superior em pacientes que obtiveram um ACT final ≥ 140 segundos, a taxa de hemorragia entre as 12 e as 24 horas após a cirurgia é praticamente igual entre os dois grupos de pacientes, sugerindo que as diferenças hemorrágicas possivelmente decorrentes da diferença de ACT final são mais evidentes durante as primeiras 12 horas após a cirurgia. Adicionalmente, verificou-se que o tempo de CEC e o sexo masculino foram preditores de hemorragia significativa (OR 1.009 [1.002 – 1.015, IC 95%] e OR 2.842 [1.721 – 4.821, IC 95%], respetivamente). Além disso, a probabilidade de hemorragia significativa em ambos os sexos foi menor para valores mais elevados de hematócrito (pelo menos para valores dentro do intervalo fisiológico).

Relativamente ao uso de transfusão, verificámos que pacientes com ACT final ≥ 140 segundos têm maior risco de transfusão (OR 1.81 [1.13 – 2.89, IC 95%]; p = 0.0104) face a pacientes com ACT final < 140 segundos. O tempo de CEC e o ACT final foram preditores significativos do uso de transfusão (OR 1.019 [1.012 – 1.026, IC 95%] e OR 1.021 [1.010 – 1.032, IC 95%], respetivamente). Além disso, o sexo feminino foi um preditor do uso de transfusão, tendo sido obtida para as mulheres uma probabilidade prevista para o uso de unidades de concentrado eritrocitário de 27.23% (21.84 – 33.39%, IC 95%) no caso de cirurgia eletiva, e 60.38% (37.65 – 79.36%, IC 95%) em cirurgia urgente; para um paciente do sexo masculino com as mesmas variáveis foram obtidas probabilidades previstas para o uso de unidades de concentrado de eritrocitário de 10.92% (7.89 – 14.92%, IC 95%) e de 33.29% (16.89 – 55.08%, 95% CI), no caso de cirurgia eletiva e cirurgia urgente, respetivamente.

Em jeito de conclusão, embora os valores de ACT final não sejam um bom preditor de hemorragia significativa, eles têm um bom valor preditivo para o uso de transfusão, destacando-se o facto de pacientes com ACT final ≥ 140 segundos terem maior risco de

transfusão comparativamente a pacientes com valor de ACT final < 140 segundos. Além disso, verificámos diferenças significativas nos valores de hemorragia pós-operatória entre pacientes que têm um ACT final ≥ 140 segundos *versus* ACT final < 140 segundos. Os riscos de hemorragia e do uso de transfusão são maiores quanto maior o tempo de CEC e quanto menores os valores de hematócrito pré-operatório. Adicionalmente, indivíduos do sexo masculino têm maior risco pós-operatório de hemorragia significativa, mas indivíduos do sexo feminino têm maior risco do uso de transfusão. Por fim, a classificação da urgência da cirurgia influencia o uso de transfusão, sendo que pacientes submetidos a cirurgia urgente se encontram em maior risco para o uso de unidades de concentrado eritrocitário.

Palavras-chave: Cirurgia Cardíaca; Hemorragia; Pós-operatório; Tempo de Coagulação Ativada; Transfusão

Abstract

Introduction: Activated Clotting Time (ACT) is commonly used to monitor anticoagulation during cardiac surgeries. Bleeding and the consequent use of transfusion are some of the most frequent complications in patients submitted to cardiac surgery, increasing morbimortality. Final ACT values may be important to predict postoperative bleeding, with its management being relevant in the reduction of transfusions use. Yet, there is still no evidence to support the ideal ACT value after protamine administration. The aim of our work was to evaluate the utility of ACT value as a predictor of postoperative bleeding and transfusion use.

Methods: We have conducted an observational, retrospective, single center study, based on the collection, treatment and analysis of data from 722 patients undergoing surgery with cardiopulmonary bypass (CPB) between July 2018 and October 2021, at Hospital de Santa Maria (Lisbon, Portugal). To characterize the relationship between Final ACT and bleeding/the need for transfusion, we divided patients according to: Final ACT < basal ACT and Final ACT ≥ basal ACT; and Final ACT < 140 seconds and Final ACT ≥ 140 seconds. Continuous variables were analyzed using the Wilcoxon Rank Sum test, with continuity correction and categorical variables were analyzed using the Chi-square test with Yates' continuity correction or using Fisher's exact test. A Linear Mixed Regression model with Fixed and Random Effects was applied to analyze bleeding during the first 24 hours after surgery in two patient groups: Final ACT < 140 seconds and Final ACT ≥ 140 seconds. Several other independent variables were analyzed in order to investigate the association between said variables and bleeding/the use of packed red blood cells, resorting to Binary Logistic Regression models.

Results: Patients with a final ACT ≥ 140 seconds bled on average, 99.62 ± 36.39 ml more than patients with a final ACT < 140 seconds in the first 12 hours, and 104.99 ± 36.39 ml more in the first 24 hours after surgery, with this difference being statistically significant both at 12h and 24h. CPB time and masculine sex were significant predictors of significant bleeding (OR 1.009 [1.002 - 1.015, 95% CI] and OR 2.842 [1.721 – 4.821, 95%

CI], respectively). Furthermore, the probability of significant bleeding in both sexes was lower for higher values of hematocrit (at least within physiological range). Regarding transfusion use, we have found that patients with a Final ACT ≥ 140 seconds are at higher risk of transfusion (OR 1.81 [1.13 – 2.89, 95% CI]; $p = 0.0104$), when compared to patients with Final ACT < 140 seconds. CPB time and Final ACT were significant predictors of transfusion use (OR 1.019 [1.012 - 1.026, 95% CI] and OR 1.021 [1.010 - 1.032, 95% CI], respectively). Additionally, female sex was a predictor of the use of transfusion, with females having a predicted probability for the use of packed red blood cells of 27.23% (21.84 – 33.39%, 95% CI) in the case of elective surgery, and 60.38% (37.65 – 79.36%, 95% CI) in an urgent surgery; a male patient with the same variables had a predicted probability for the use of packed red blood cells of 10.92% (7.89 – 14.92%, 95% CI) in the case of elective surgery, and 33.29% (16.89 – 55.08%, 95% CI) in an urgent surgery.

Conclusions: Although Final ACT values are not a good predictor for significant bleeding, they have a good predictive value for the use of transfusion, with patients with Final ACT ≥ 140 seconds having a higher risk of transfusion when compared to those with Final ACT value < 140 seconds. Moreover, major differences in postoperative bleeding were found between patients who have a Final ACT ≥ 140 seconds *vs* Final ACT value < 140 seconds. The risk of bleeding and transfusion use is higher the longer the time under CPB and the lower the values of preoperative hematocrit. Males have a higher post-operative risk of bleeding, but females have a higher risk of transfusion use.

Keywords: Activated Clotting Time; Bleeding; Cardiac Surgery; Postoperative; Transfusion

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Abbreviations and acronyms

ACT	Activated clotting time
aPTT	Activated partial thromboplastin time
AUC	Area under the curve
CABG	Coronary artery bypass graft
CI	Confidence interval
CKD	Chronic kidney disease
CPB	Cardiopulmonary bypass
DDAVP	Desmopressin
eGFR	Estimated glomerular filtration rate
FFP	Fresh frozen plasma
IQR	Interquartile range
LVEF	Left ventricular ejection fraction
NIT	Non-insulin-treated
OR	Odds ratio
PCC	Prothrombin complex concentrate
PRBC	Packed red blood cells
PT	Prothrombin time
rFVIIa	Recombinant activated factor seven
ROC	Receiver operating characteristic
UFH	Unfractioned heparin
vWF	von Willebrand factor

Introduction

Cardiac surgery and extracorporeal circulation

Cardiac Surgery is the surgical field dedicated to the treatment of pathologies of the heart and proximal portion of large thoracic vessels, such as the aorta. Some of the most common surgeries in this field include heart valve replacement/repair, coronary artery bypass grafting (CABG) and the treatment of congenital heart diseases.

In a significant percentage of surgeries, the heart needs to be stopped, with a heart-lung machine connected to the arterial and venous circulation to allow the circulation of blood, while maintaining the adequate oxygenation and perfusion of the patient. This form of extracorporeal circulation is better known as cardiopulmonary bypass (CPB) and the procedures in which it is used are commonly known as "on-pump" procedures.

CPB machines and circuits are comprised of several components: pumps, venous and arterial cannulas, oxygenator, reservoir container, tubing, heat exchanger, cardiotomy suckers, cardiac vents and several adjuncts that allow the machine to monitor pressures, temperature, oxygen saturation, hemoglobin levels, blood gases, electrolytes, among other variables (Ismail et al., 2023; Sarkar & Prabhu, 2017).

CPB is a key feature that distinguishes cardiac surgery from all other types of surgery. As mentioned before, one of the main advantages of using CPB is the fact that it grants the possibility of stopping the heart. The heart can be isolated from patient corporeal circulation resorting to a cardioplegia solution (usually a high-concentration potassium chloride solution) and aortic cross-clamping. This allows the cardiac surgeon to operate on a non-beating heart in a field largely devoid of blood, presenting a clearer view and a more controlled environment for the highly precise techniques that cardiac surgery requires, while other end organs remain adequately oxygenated and perfused (Sarkar & Prabhu, 2017; T Cheung et al., 2022).

Anticoagulation and patient blood management

During CPB, high doses of heparin are needed to counteract the pro-thrombotic response in the patient's blood and prevent thrombosis both inside the circuit and inside the patient's own blood vessels (Prisco & Paniccia, 2003). Usually, unfractioned heparin (UFH) is the preferred type of heparin used due to its short half-life and easy reversibility. UFH binds to antithrombin III, potentiating the inactivation of thrombin and factor Xa (Boer et al., 2018; Horton & Augustin, 2013; Wahba et al., 2019).

The need for a parameter that rapidly monitors the degree of heparin-induced anticoagulation (and its reversal) demands an easy, quick and bedside solution. Besides, even if one wanted to use a laboratory test, such as activated Partial Thromboplastin Time (aPTT), the most commonly used test to monitor heparin-mediated anticoagulation, the elevated concentration of heparin in the patient's blood (heparin concentration > 1 IU/ml) does not allow aPTT to accurately monitor the anticoagulant's effects (Prisco & Paniccia, 2003; Bolliger & Tanaka, 2017). Additionally, the absence of clear threshold values for Prothrombin Time (PT) and aPTT during CPB and the fact that these parameters do not reflect the multifactorial changes in coagulation that occur during "on-pump" procedures compromise even further the use of these conventional tests (Bolliger & Tanaka, 2017).

Of all the coagulation monitoring tests available, Activated Clotting Time (ACT) is the preferred one (Horton & Augustin, 2013; Shore-Lesserson et al., 2018). It is commonly used to monitor the anticoagulant effects induced by heparin, as well as its reversal with the use of protamine during cardiac surgeries (Boer et al., 2018). This test was first described by Hattersley in 1966 and was the first bedside system to be employed to assess coagulation during CPB (Horton & Augustin, 2013; Prisco & Paniccia, 2003). It has some advantages over laboratory tests: 1) the need for a smaller blood sample; 2) short time between samples and results with consequent decrease in sample degradation with time; 3) reduced errors associated with sample mislabeling or mishandling; 4) no need for laboratory personnel to perform the test (Horton & Augustin, 2013). Furthermore, because it is a point-of-care test, the ACT can be measured at the patient's bedside prior to, during and after the surgery. Patient's blood is tested at the beginning of the surgery, then in relatively short intervals (usually every 30 minutes) to achieve and maintain a constant level of anticoagulation throughout the surgery (or to achieve the adequate ACT values with additional heparin administration, if needed) and, finally, at the end of the surgery (T Cheung et al., 2022).

ACT also has the advantage of using whole blood, including the contribution of platelets and phospholipids to hemostasis, in addition to coagulation factors (Bolliger & Tanaka, 2017; Horton & Augustin, 2013). The ACT test indicates the degree of inhibition of the intrinsic and common pathways, rather than the inhibition of the extrinsic pathway. In a very simplified manner, during the test, whole blood is added to a tube containing an activator (most commonly kaolin or celite), which stimulates contact activation of factor XII (from the intrinsic pathway of coagulation). The sample is then placed in a device that rewarms the blood and records the time it takes for a clot to form (Horton & Augustin, 2013; Levy & McKee, 2007).

Because the ACT test is nonspecific, there are many variables that can alter its results, such as: medication that impairs coagulation and platelet function, coagulation factor deficiencies, platelet dysfunction, hypothermia, glycoprotein IIb/IIIa antagonists, hemodilution, among others (Baumann Kreuziger et al., 2018; Bolliger & Tanaka, 2017; Horton & Augustin, 2013; Levy & McKee, 2007). Therefore, the amount of heparin needed to achieve therapeutic ACT levels depends on the patient´s status and his/her individual characteristics.

ACT is measured in seconds, and the higher the degree of anticoagulation, the longer the time to clot. Normal values without the use of heparin usually range from 70 to 150 seconds, depending on the used device (Levy & McKee, 2007). During cardiac surgery with CPB, these values must be several times higher to prevent clot formation. Therefore, the patient's ACT must be kept above a certain pre-determined threshold. Although there is no evidence to establish a cut-off value, the standard recommendation, based on limited data, is that patient's ACT should be higher than 480 seconds. (Baumann Kreuziger et al., 2018; Wahba et al., 2019). The problem with ACT assays is that they are not standardized and different machines use different methods for clot activation and detection, leading to considerable variability in ACT measurements and to the impossibility of values from different machines being used interchangeably (Baumann Kreuziger et al., 2018; Horton & Augustin, 2013).

As stated before, in addition to monitoring heparin-induced anticoagulation, ACT also allows the monitorization of heparin's effects reversal, which is achieved with protamine. Protamine binds to heparin, forming a complex that leads to the dissociation of heparin from antithrombin III, restoring normal hemostasis (and consequently lowering ACT values) to prevent post-operatory bleeding. The administered dose of protamine is usually based on the initial or total administered dose of heparin during surgery. It is advised not to exceed protamine dose in a 1:1 ratio to the initial heparin bolus due to increased risk of complications (described in the next section) (Boer et al., 2018).

The problem is that, so far, even though there is some data suggesting that a final post-protamine ACT value that is lower than its pre-heparin value might be safe and have the advantage of leading to lower surgery duration, bleeding, and post-operative transfusions (W. Wang et al., 2018), there is still no evidence to support the ideal ACT value after protamine administration (Baumann Kreuziger et al., 2018; Boer et al., 2018; Shore-Lesserson et al., 2018; Wahba et al., 2019). Thus, ACT monitoring is carried out empirically, trying to match ACT values prior to heparin administration.

Changes in coagulation during extracorporeal circulation

Given that CPB is a non-endothelial circuit, the contact between the patient's blood and the machine's surfaces results in a pro-thrombotic response by disturbing the interconnected coagulation and inflammatory systems, with the activation of both plasma proteases and cells, leading to several systemic effects (Baumann Kreuziger et al., 2018). Furthermore, the need for a priming solution for the CPB circuit (typically 1-2 liters of a balanced crystalloid solution) leads to hemodilution that may cause/aggravate anemia and changes in coagulation (T Cheung et al., 2022).

During CPB, blood contact with the circuit's non-endothelialized materials gradually activates factor XII from the intrinsic pathway of coagulation, setting off the rest of the coagulation cascade by the now activated factor XIIa. In this process, bradykinin is formed, further amplifying systemic inflammation. (Bartoszko & Karkouti, 2021)

In parallel, complement activation may occur via classical, alternative or lectin pathway, although during CPB complement activation occurs mainly via the alternative complement pathway due to the direct adhesion of C3 to the CPB circuit (Kefalogianni et al., 2022). Generally, this process leads to inflammatory cell activation by anaphylatoxin C5a, formation of the membrane attack complex and possible cell death.

Intravascular hemolysis is a common phenomenon during CPB, leading to higher levels of free hemoglobin which, in turn, interacts with von Willebrand factor (vWF), increasing platelet adhesion (Da et al., 2015). Furthermore, platelets and leucocytes can be activated and destroyed by the pump's shear forces, with platelet function and numbers declining after activation. CPB also induces the production of microvesicles from platelets which have an important role in the initiation and propagation of coagulation (Tempo et al., 2016).

Since CPB can activate neutrophils, monocytes, and endothelial cells: (i) inflammatory mediators such as oxygen free radicals and/or cytotoxic enzymes are released from intracellular granules, further propagating inflammation; (ii) tissue factor is expressed on the monocyte's cell surface in response to proinflammatory cytokines, enabling initiation of coagulation via the extrinsic pathway; (iii) due to the activation of endothelial cells by thrombin, which results in the formation of tissue plasminogen activator and other anticoagulant molecules in order to counterbalance the sustained coagulation activation, continuous fibrinolysis during CPB occurs within the circuit. (Baumann Kreuziger et al., 2018)

All these changes lead to a vigorous inflammatory response with resultant platelet activation, initiation of the coagulation cascade and decreased levels of circulating coagulation factors. The release of mediators by endothelial cells and leukocytes contribute to capillary leakage and tissue edema occurs. Furthermore, many of the difficulties experienced throughout the post-CPB period (such as myocardial dysfunction, vasodilation and bleeding) are believed to be partially attributable to this inflammatory sequence (T Cheung et al., 2022).

The sustained activation of the inflammatory and coagulation systems during CPB increases the difficulty to maintain a proper balance between thrombosis and hemorrhage. Careful anticoagulation is, therefore, one of the mainstays of cardiac surgery, especially when “on-pump”, to prevent both the formation of thrombi and excessive bleeding.

Although UFH is the anticoagulant of choice, it has important limitations that can contribute to post-surgery bleeding. UFH has both high and low-molecular-weight heparin components, with low-molecular-weight heparin components being less rapidly cleared from circulation and not as readily inactivated by protamine, contributing to post-CPB bleeding (Baumann Kreuziger et al., 2018). Moreover, there are various metabolism-related reasons to further contribute to post-operative bleeding. A considerable portion of heparin binds to plasma proteins and is internalized by endothelial cells and macrophages, not being available for protamine neutralization. Dissociation of heparin can occur several hours after the administration of protamine, reentering circulation and leading to an increased bleeding risk, a phenomenon known as heparin rebound (Baumann Kreuziger et al., 2018; Stone & Vespe, 2023). Another aspect to have into consideration and that might make anticoagulation control harder is that we might not always obtain the same response to a similar dose of heparin, since there are patient-specific characteristics that determine sensitivity to heparin, such as antithrombin III concentrations, for example.

Heparin-induced thrombocytopenia might also occur due to the formation of heparin/platelet factor-4 complexes, production of antibodies against these complexes, and binding of said antibodies on platelets’ surface with consequent activation, aggregation and consumption. Contributing to this thrombocytopenia is the phagocytosis of IgG antibody-heparin/platelet factor-4 immunocomplex-bound platelets by macrophages in the spleen, liver and bone marrow. (Ahmed et al., 2007)

As mentioned before, UFH is neutralized by protamine, a protein which has a half-life of 5 minutes and is undetectable within 20 minutes of administration. Because protamine induces the release of histamine from mast cells, its administration may be associated with inflammatory and immunological reactions such as anaphylaxis, pulmonary vasoconstriction and vasoplegia, in addition to possible platelet dysfunction and impaired thrombin generation (Boer et al., 2018; Shore-Lesserson et al., 2018). Therefore, appropriate protamine dosing is important because it might alter hemodynamics and hemostasis and paradoxically lead to bleeding and increased transfusion requirements (Boer et al., 2018; Wahba et al., 2019).

Even though many drugs have been studied for the reduction of coagulation disturbances during CPB, few have shown sufficient evidence to be used in clinical practice. Tranexamic acid might be the only one of them that is still in use today. It is an antifibrinolytic that inhibits the conversion of plasminogen to plasmin and is typically given prior to CPB, since it has been shown to reduce bleeding and the need for transfusions (Boer et al., 2018). Large-scale randomized placebo-controlled trials in cardiac surgery have disproved concerns about tranexamic acid's prothrombotic risks, although it modestly increases the risk of seizures. Optimal dosing strategies have not been defined, ranging from 30–100 mg/kg (lower doses should be used in patients with renal dysfunction) (Baumann Kreuziger et al., 2018).

Post-operative hemorrhage and blood transfusion

Cardiac surgery is associated with a high risk of perioperative bleeding, mainly due to the invasiveness of the procedure and the need for high-dose anticoagulation.

The need for surgical re-exploration due to significant bleeding is a strong risk factor for an increase in post-operative mortality and morbidity (Elassal et al., 2021). Up to 5% of all patients having elective cardiac surgery require emergent re-exploration in an attempt to correct ongoing bleeding and establish adequate hemostasis, a percentage that increases to 15% in the case of emergency operations (Elassal et al., 2021; Raphael et al., 2019).

Bleeding may lead to anemia and increase the need of transfusion of blood (either autologous or allogeneic) or other blood-derived procoagulant products. Just like reintervention for bleeding, high blood product transfusion requirements are associated with adverse clinical outcomes (Boer et al., 2018). Morbidity and mortality are directly proportional to the number of packed red blood cells (PRBC) transfused (Raphael et al., 2019).

An important predictor for the transfusion of allogeneic blood is pre-operative anemia (defined as an hemoglobin level <12g/dL in women and <13g/dL in men by the World Health Organization). Pre-operative anemia is quite prevalent in patients submitted to cardiac surgery, being present in 25-30% of the patients (Raphael et al., 2019). The high rate of pre-operative anemia justifies the screening and treatment of pre-operative anemia, whenever possible.

The use of CPB is associated with hemodilution, which is an additional risk factor for anemia and consequent allogeneic transfusions in the peri-operative period. Several strategies have been recommended to minimize this, such as the use of miniaturized circuits in CPB (with modified hemofiltration, reduced priming volume and retrograde autologous priming) and the use of acute normovolemic hemodilution, a technique in which a portion of the patient's blood is withdrawn and stored prior to the surgery, replaced by crystalloid (or colloid) fluids to maintain normovolemia during surgery, and then transfused back to the patient after CPB (Boer et al., 2018; Raphael et al., 2019). Even though this practice comes at a cost of lower hematocrit values during surgery, it is effective in reducing bleeding and allogeneic PRBC transfusions in the post-operative period (Boer et al., 2018; Raphael et al., 2019). Another strategy is the usage of cell-salvaged blood from operative blood loss and residual blood in the CPB circuit (Boer et al., 2018; Wahba et al., 2019). It has been demonstrated that this measure is better than the usage of the blood from the cardiotomy suction, since it was associated with less bleeding, reduced inflammation and a lower risk of post-operative complications, even though re-transfusion of larger volumes (> 1 L) may disrupt coagulation (Boer et al., 2018; Raphael et al., 2019).

When it comes to blood-specific variables, several factors can influence hemostasis. Coagulation factor deficiencies, although rare, can easily predispose to bleeding. Another more common situation, of iatrogenic nature, is dual antiplatelet therapy with aspirin and $P2Y_{12}$ inhibitors (such as ticagrelor or clopidogrel), a mainstay pharmacotherapy in patients with acute coronary syndromes. Given that perioperative bleeding after cardiac surgery is higher in these patients, it is recommended to stop all other drugs other than acetylsalycilic acid, whenever possible (Boer et al., 2018). It is also possible to use a point-of-care platelet function test before surgery, to consider if the surgery must be delayed due to the high risk of hemorrhagic complications (Boer et al., 2018).

Some patients may have altered responsiveness to heparin, being unable to reach the target ACT, despite administration of an adequate heparin dose. This situation is named heparin resistance and is most common in the context of previous heparin infusion before surgery, since antithrombin III concentrations may be lower (Baumann Kreuziger et al., 2018; Boer et al., 2018; Raphael et al., 2019; Wahba et al., 2019). These patients require higher doses of heparin and may need, consequently, higher doses of protamine to reverse anticoagulation, increasing the chances of possible adverse effects from the use of high doses from both drugs. To circumvent this situation, supplementation with antithrombin before CPB might improve heparin sensitivity, even though its prophylactic use is not recommended (Boer et al., 2018).

Another variable that influences bleeding is serum fibrinogen concentrations, since it is the first coagulation factor to be depleted during significant bleeding and hemodilution. Given that low fibrinogen levels detected in the peri-operative period have been linked to increased bleeding and transfusion requirements, when there is evidence of hypofibrinogenemia (levels <150 mg/dL) in patients with post-CPB bleeding, fibrinogen supplementation may be considered. (Boer et al., 2018)

Hence, if the patient continues to bleed after heparin reversal with protamine, blood parameters, such as fibrinogen, coagulation factors' levels and platelet's number and function, should be evaluated. It is also relevant to point out that anti-fibrinolytic agents play an important role in post-surgery hemostasis, since, as mentioned previously, CPB is associated with significant fibrinolysis. (Boer et al., 2018)

Antifibrinolytics, like tranexamic acid and ε-aminocaproic acid, are commonly used in the context of cardiac surgery with CPB to reduce bleeding, transfusions and reoperation for hemorrhage (Baumann Kreuziger et al., 2018; Boer et al., 2018). Additionally, antifibrinolytic therapy can be continued or restarted after surgery if there is severe postoperative bleeding (Raphael et al., 2019).

However, even with all these parameters in normal ranges, it is frequently necessary to resort to transfusions to correct anemia or hemostasis.

The evidence about transfusion strategies is conflicting, given that even though most guidelines recommend the use of a restrictive blood transfusion strategy (keeping hemoglobin levels between 7-8 g/dL) (Raphael et al., 2019), more recent studies seem to be in favor of liberal PRBC transfusions strategies (hemoglobin levels between 9-10 g/dL) (Boer et al., 2018). Even though there is a lack of consensus for the hemoglobin threshold for transfusion, recent studies support transfusions when hemoglobin is equal or inferior to 7,5 g/dL (Raphael et al., 2019). Nevertheless, the most recent guidelines on patient blood management for adult cardiac surgery by the European Association of Cardio-Thoracic Surgery and the European Association of Cardio-Thoracic Anesthesiology (Boer et al., 2018) mention that PRBC transfusion should be dictated by the patient's clinical condition in order to optimize the balance between oxygen delivery and extraction by the tissues and that it should be prioritized over a fixed hemoglobin threshold. This way, depending on the patient's risk profile and comorbidities, the acceptable hemoglobin levels during CPB might vary.

Despite a significant number of patients only requiring transfusion of a single PRBC, the need for more than one transfusion component is not negligible. Even though blood transfusion decisions should be mainly made based on the patient's clinical condition and are usually safe, one must be aware of its risks and possible adverse reactions. These reactions might range in severity from mild (the majority, most commonly simply with fever and/or rash) to life-threatening (with possible respiratory distress, massive hemolysis or shock). Transfusion reactions can be divided in acute (<24h after transfusion) or delayed (>24h after transfusion). To prevent needless over-transfusion and its risks, clinical assessment of bleeding, hemoglobin evaluation, and coagulation system evaluation are required after each round of treatment. The practitioner must also treat other conditions that might predispose to coagulopathy, such as hypothermia and acidosis.

When it comes to correcting hemostasis, several components might be used to achieve it. Firstly, the transfusion of platelets is indicated in bleeding patients with thrombocytopenia or platelet dysfunction (for example, due to antiplatelet therapy). Recent guidelines indicate a platelet count threshold of 50 (10^9/L) or lower for transfusion, albeit platelets may be dysfunctional in the context of post-CPB hemorrhage, with patients requiring transfusion, even with a higher platelet count (Boer et al., 2018).

1-Deamino-8-d-arginine vasopressin (DDAVP), better known as desmopressin, stimulates the release of vWF from endothelial cells, improving platelet function and helping reduce bleeding in patients with mild to moderate A hemophilia. Hence, its administration should be considered when vWF deficiency (for example, due to aortic stenosis) is suspected/documented or when platelet dysfunction or mild to moderate A hemophilia exists in bleeding patients, although its prophylactic use is not recommended (especially in patients that don’t have these conditions) (Boer et al., 2018).

Fresh frozen plasma (FFP), obtained by separating plasma (with its coagulation factors and other protein and soluble constituents) from figurative elements in blood, might be effective in treating bleeding patients with laboratory evidence of coagulation factor deficiency. Therefore, FFP can be used in the case of prolonged perioperative bleeding or to reverse the effects of oral anticoagulation, but there is no evidence that prophylactic or therapeutic transfusions minimize blood loss during cardiac surgery. Additionally, prothrombin complex concentrates (PCC) were more successful than FFP for reversing vitamin K antagonists’ effects in patients who required urgent surgery, primarily because they had a quicker hemostatic impact. Other advantages of PCC over FFP are that a substantially lower volume is needed and there are fewer risks related to plasma transfusion. (Boer et al., 2018)

PCCs are human plasma-derived vitamin K-dependent coagulation factors (factors II, VII, IX and X). The concentration of these factors can vary depending on the product, but factor IX concentration is standardized. PCCs might contain antithrombin, proteins C and S and heparin to avoid excessive coagulation and eventual thrombosis. Thus, just like FFP, PCCs can be used in coagulation factor deficiency-related bleeding, but with the advantages stated above. (Boer et al., 2018)

Factor XIII is the final enzyme in the coagulation cascade, responsible for fibrin cross-linking and stabilization of the blood clot. Increased bleeding has been associated with low factor XIII levels, following cardiac surgery. It can be measured and administered in cases of severe postoperative bleeding with normal coagulation tests. However, in patients with normal concentrations of factor XIII (>70%), there is no evidence that administration of this coagulation factor might decrease postoperative bleeding volumes and transfusion rates. (Boer et al., 2018)

Recombinant factor VIIa (rFVIIa) is mainly used in the prevention and treatment of inherited bleeding disorders. Yet, due to its potent procoagulant effects, it can be used as last resort off-label drug in life-threatening bleeding. Its prophylactic use is not recommended and rFVIIa should only be used in patients with uncontrollable bleeding after other procoagulant interventions. It is not recommended after the use of PCC, since there is a very high risk of thrombotic events. (Boer et al., 2018)

In summary, even though all these tools are at our disposal, they must not be used carelessly. A tight control in hemostasis leads to fewer uses of transfusion products (and their possible side effects), contributing to a more successful surgery, favorable patient outcome and decreased costs (Boer et al., 2018). To achieve this, individualized therapy using point-of-care tests, such as Activated Clotting Time, or guided algorithms is recommended because they have been associated with improved patient outcomes when compared to standard laboratory-based or empiric transfusion therapy (Raphael et al., 2019).

The problem and main objectives

Considering that few evidence is available regarding the ideal ACT value after protamine administration, recommendations about using point-of-care guided algorithms to improve patient outcomes are more difficult to follow after cardiac surgery. Postoperative ACT values may be an important tool to predict and control postoperative bleeding, with its management being important in the reduction of transfusions use. Additionally, the existence of an ideal pre-determined value for surgery after protamine administration might contribute to the uniformization of cardiac surgery practices.

To achieve this goal, we conducted a retrospective, cross-sectional, observational and descriptive study based on the collection, treatment and analysis of data from patients undergoing “on-pump” surgery between July 2018 and October 2021, at Hospital de Santa Maria. The main objective was to characterize the relationship between ACT after protamine administration and the need for transfusion, and to investigate whether there is any ACT value associated with a lower risk of bleeding and/or transfusional events.

Methods

Population

This is an observational, retrospective, single center study, carried out in Hospital de Santa Maria, a tertiary hospital in Lisbon, Portugal. We have included patients with at least 18 years old submitted to cardiac surgery requiring CPB. Exclusion criteria were: (i) patients under anticoagulation therapy; (ii) patients with postoperative hemorrhagic complication (e.g. surgical re-exploration); and (iii) patients without complete postoperative clinical records. Data were collected from 722 patients submitted to cardiac surgery between January 2018 and October 2021 (figure 1).

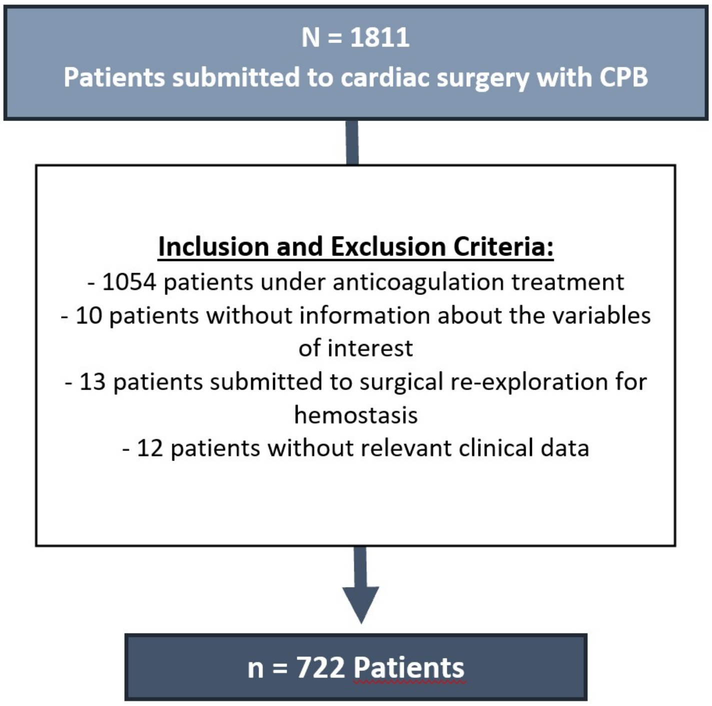

Figure 1. Flowchart of patient selection for the present study

Approval for the conduction of the study was obtained from the Institution Ethics Committee (Reference Nº 23/18, CHULN). Due to the retrospective nature of the study, using anonymous individual data, informed signed consent from patients was waived.

Surgical technique

The anesthesia protocol included fentanyl and propofol, with orotracheal intubation facilitated by rocuronium bromide. Patient maintenance under anesthesia was performed intravenously, with a propofol perfusion through a central venous catheter placed percutaneously via the right internal jugular vein. Invasive blood pressure monitoring was performed through a catheter in the left radial artery.

The CPB circuits that were used included two oxygenators [Inspire 6® (Sorin Group, Italy) or Quadrox-i® (Getinge, Sweden)] with a single rigid reservoir with cardiotomy filter, integrated arterial filter, and the respective tubes of the circuit in vinyl polychloride. Circuit priming was performed with approximately 700 milliliters of balanced electrolyte solution (polyelectrolyte) with or without the addition of 500 milliliters of Gelatin (4% Gelafundin). For CPB initiation, patients were anticoagulated with a 300 IU/Kg dose of sodium heparin, to obtain a target ACT greater than 480 seconds. CPB with non-pulsatile flow was used in all patients, with a flow indexed to the patient's body surface of 2.4 L/min/m².

Surgeries were performed with mild hypothermia or normothermia (32 to 36ºC, measured in the oropharynx). For myocardial protection, cold-blood cardioplegia, diluted with a hyperkalemic solution (Buckberg) at a ratio of 4:1, was used anterogradely for asystole induction/maintenance; warm-blood cardioplegia was used for reperfusion. The patients' perioperative blood glucose was maintained below 250 mg/dL. The allowed minimum hematocrit value during CPB was 24%.

In the case of hypotension during CPB, the first-line treatment was increasing the flow indexed to the patient’s body surface area from 2.4 to 2.6 L/min/m². If arterial hypotension was persistent, a single noradrenaline bolus was administered, followed by its intravenous infusion.

After completion of CPB, heparin was reversed with protamine sulfate at a 1:1 ratio. We have considered: (i) basal ACT as the ACT value before heparin administration and (ii) an ACT value with an upper limit of 140 seconds as an adequate postoperative ACT value (W. Wang et al., 2018). The ACT value obtained after protamine administration was also called Final ACT.

ACT values were assessed resorting to Medtronic's HMS Plus Hemostasis Management System® (Minneapolis, USA), using High Range ACT (HR-ACT) kaolin 12% cartridges.

Drainage of mediastinal blood in the postoperative period was performed using at least two drains, one in the anterior mediastinum and the other in the pericardium, with 24 or 28 French silicone drainage tubes. The chest tubes were both connected to a drainage bottle with saline solution, connected to a vacuum system for continuous siphon drainage. All patients were transferred to the intensive care unit after surgery and remained with mediastinal drains for, at least, 48 hours.

Data collection

Clinical information, such as cardiovascular risk factors, medication, comorbidities and demographic data was collected from patients' electronic process. Only the sex, the age (at the time of surgery), and the geographical origin are accessible regarding demographic data, noting that each patient has a unique electronic number attributed by the system and that the name and date of birth of each patient were kept confidential.

Additionally, peri-operative lab tests performed in the institution were also obtained, namely blood count and coagulation parameters (hemoglobin, hematocrit, platelets, fibrinogen, PT, aPTT and International Normalized Ratio [INR)]. Information related to the surgical procedure, such as that related to CPB, was collected from the database present in the data-recording computer system existing in the department (CardioBase®).

Postoperative variables recorded in the ICU were exported from the Anesthesia Manager® software (Picis Clinical Solutions, United States of America). Bleeding values from 12 and 24h after surgery were recorded in milliliters of drained blood. Patients were then categorized according to the severity of postoperative bleeding using the criteria defined by Dyke et al. (see table 1). Postoperative bleeding was classified as significant with at least 600mL in the first 12h. Information regarding the use of blood and blood products, such as PRBC, platelet transfusions, fibrinogen and FFP was also collected from the previously mentioned software. For the use of transfusion, our department follows the criteria described in the European Guidelines on patient blood management for adult cardiac surgery (Boer et al., 2018).

Bleeding definition	**Postoperative chest tube blood loss within 12 hours (mL)**	**PRBC (units)**	**FFP (units)**	**PLT (units)**	**Cryoprecipitate**	**PCCs**	**rFVIIa**	**Reexploration /tamponade**
Class 0 (insignificant)	<600	0*	0	0	No	No	No	No
Class 1 (mild)	601-800	1	0	0	No	No	No	No
Class 2 (moderate)	801-1000	2-4	2-4	Yes	Yes	Yes	No	No
Class 3 (severe)	1001-2000	5-10	5-10	N/A	N/A	N/A	No	Yes
Class 4 (massive)	>2000	>10	>10	N/A	N/A	N/A	Yes	N/A

PRBC, packed red blood cells; FFP, fresh frozen plasma; PLT, platelet concentrates; PCCs, prothrombin complex concentrates; rFVIIa, recombinant activated factor VII; N/A, not applicable. *Correction of preoperative anemia or hemodilution only.

Table 1. Bleeding categories according to the universal definition for perioperative bleeding in adult cardiac surgery (adapted from Dyke et al., 2014)

Statistical analysis

In the case of missing values relating to the different dependent quantitative variables, the absent values were replaced by the median of the respective variable. Since the amount of used blood units and blood products does not follow a normal distribution, they were categorized according to their need (needed/not needed).

No sample size or statistical power calculations were performed for this work.

Continuous variables were expressed as median and interquartile range (IQR) and analyzed using the Wilcoxon Rank Sum test, with continuity correction. Categorical variables were presented in the form of absolute frequency and percentages. The latter were analyzed using the Chi-square test with Yates' continuity correction or using Fisher's exact test. All tests were bilateral.

To explore the objectives of our work, a Linear Mixed Regression model with Fixed and Random Effects was applied to analyze bleeding during the first 24 hours after surgery in two patient groups: Final ACT < 140 seconds and Final ACT ≥ 140 seconds.

In addition, several Binary Logistic Regression models were applied in order to analyze the association between several independent variables and the following dependent variables: significant bleeding and the use of PRBC. To reduce bias, interactions between different types of priming and surgery were included.

The specificity and sensitivity of the statistical models used here were evaluated resorting to Receiver Operating Characteristic (ROC) curves and the calculation of the Area Under the Curve (AUC).

For all the data analyzes, the selected confidence interval was 95%, which means that the significance level (α) used was 5%. A p-value < 0.05 was considered statistically significant. All statistical analysis of this work were performed using the R Studio software[1].

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[1] *R Core Team (2022). R A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org*

Results

Patients

A total of 722 patients were enrolled in this study. Demographic data of the studied population is described in Table 2.

Table 2. Median and IQR demographic data of the studied population

	All Patients (722)	**Non-significant Bleeding (631)**	**Significant Bleeding (91)**	**p-value**	**Not Transfused (518)**	**Transfused (204)**	**p-value**
Age, years	71 (63 - 76)	70 (63 - 76)	73 (66 - 78)	0.07031	70 (62 - 75)	73 (65.75 - 78.25)	**<0.001**
Male, n (%)	397 (55)	331 (52.5)	66 (72.5)	**<0.001**	333 (64.3)	64 (31.4)	**<0.001**
Aorta Surgery, n (%)	127 (17.6)	106 (16.8)	21 (23.1)	0.1857	91 (17.6)	36 (17.6)	1
Valvular Surgery, n (%)	666 (92.2)	581 (92)	85 (93.4)	0.815	479 (83.5)	187 (91.7)	0.8342
Coronary Surgery, n (%)	141 (19.5)	114 (18.1)	27 (29.7)	**0.01355**	93 (17.9)	48 (23.5)	0.1102
Surgical Timing, n (%)							
Elective	688 (95.3)	605 (95.9)	83 (91.2)	0.06152	503 (97.1)	185 (90.7)	**<0.001**
Urgent	34 (4.7)	26 (4.1)	8 (8.8)		15 (2.9)	19 (9.3)	
Diabetes, n (%)							
Non-diabetic	526 (72.9)	458 (72.6)	68 (74.7)	0.5738	388 (74.9)	138 (67.6)	0.1095
NIT Type II Diabetes	179 (24.8)	159 (25.2)	20 (22)		120 (23.2)	59 (28.9)	
IT Type II Diabetes	17 (2.3)	14 (2.2)	3 (3.3)		10 (1.9)	7 (3.5)	
Dyslipidemia, n (%)	471 (65.2)	409 (64.8)	62 (68.1)	0.2529	344 (66.4)	127 (62.3)	0.3328
Arterial Hypertension, n (%)	589 (81.6)	511 (81)	78 (85.7)	0.3452	418 (80.7)	171 (83.8)	0.3844
Chronic Kidney Disease, n (%)							
No CKD (eGFR > 90 ml/min/ 1.73 m²)	602 (83.4)	532 (84.3)	70 (76.9)	**0.02598**	444 (85.7)	158 (77.5)	**0.004925**
Mild CKD (60-89 ml/min/1.73 m²)	54 (7.5)	42 (6.7)	12 (13.2)		39 (7.5)	15 (7.4)	
Moderate CKD (30-59 ml/min/1.73 m²)	46 (6.4)	42 (6.7)	4 (4.4)		27 (5.2)	19 (9.3)	
Severe CKD (15-29 ml/min/1.73 m²)	13 (1.8)	11 (1.7)	2 (2.2)		5 (1)	8 (3.9)	
Dialysis CKD (eGFR < 15 ml/min/ 1.73 m²)	7 (0.9)	4 (0.6)	3 (3.3)		3 (0.6)	4 (1.9)	
Left Ventricular Ejection Fraction, n (%)							
Normal (>50%)	622 (86.2)	551 (87.3)	71 (78)	0.07265	446 (86.1)	176 (86.3)	0.6586
Mildly reduced (41-50%)	54 (7.5)	45 (7.1)	9 (9.9)		39 (7.6)	15 (7.4)	
Moderately reduced (31-40%)	31 (4.3)	23 (3.7)	8 (8.8)		22 (4.2)	9 (4.4)	
Reduced (21-30%)	14 (1.9)	11 (1.7)	3 (3.3)		11 (2.1)	3 (1.5)	
Severely reduced (<21%)	1 (0.1)	1 (0.2)	0 ()		0 ()	1 (0.4)	

The median age of the patients was 71 years (IQR 63-76), with almost 55% of the patients being male.

The most commonly performed surgery was valvular surgery (92.2% of the patients), followed by coronary surgery (19.5%) and aorta surgery (17.6%). Overall, 95.3% of patients were electively submitted to surgery.

Considering comorbidities, most of the patients were non-diabetic (72.9%). Within the group of patients who were diabetic, the majority had non-insulin-treated (NIT) type II diabetes (24.8%). More than half of the patients had dyslipidemia (65.2%), and the majority had arterial hypertension (81.6%). Around 9.1% of the patients (66) had at least moderate chronic kidney disease (CKD). Left ventricular ejection fraction (LVEF) was preserved in the majority of patients (86.2%), with 2% (15) of patients having reduced or severely reduced LVEF.

In the subgroup analysis, we verified that the statistically significant differences between patients with or without significant bleeding include sex (p-value < 0.001), coronary surgery (p-value = 0.01355) and presence of CKD (p-value = 0.02598).

Similarly, statistically significant differences in patients with or without the use of transfusion were found regarding patient sex (p-value < 0.001), surgery timing (p-value < 0.001), presence of CKD (p-value = 0.004925) and age variable (p-value < 0.001).

Table 3 presents median (IQR) of the preoperative blood parameters, ACT values, CPB time and clamping time of the studied population.

Table 3. Median preoperative lab values and surgery-depend variables and their respective IQR

	All Patients	**Non-significant Bleeding**	**Significant Bleeding**	**p-value**	**No Transfusion**	**Transfusion**	**p-value**
Hemoglobin, g/dL	13.5 (12.5 - 14.6)	13.5 (12.5 - 14.6)	13.4 (12.35 - 14.35)	0.1893	13.9 (13 - 14.8)	12.35 (11.3 - 13.3)	**<0.001**
Hematocrit, %	40.2 (37.2 - 42.8)	40.2 (37.2 - 42.9)	39.4 (36.85 - 42.3)	0.1651	41.3 (38.62 - 43.7)	36.9 (34 - 39.7)	**<0.001**
Fibrinogen, mg/dL	333 (280 - 394.8)	333 (283 - 395)	330 (260 - 388.5)	0.2413	333 (276 - 378.8)	347 (300.5 - 433.5)	**<0.001**
Platelets, x10^9/L	213 (177 - 255)	213 (177.5 - 257)	207 (173.5 - 234)	0.9659	206 (174.2 - 247.8)	220.5 (189 - 273.8)	**0.0013**
PT, seconds	12.1 (11.5 - 12.78)	12.1 (11.5 - 12.7)	12.1 (11.6 - 12.95)	0.3419	12.1 (11.6 - 12.7)	12.1 (11.5 - 12.9)	0.79
aPTT, seconds	29 (27 - 31)	29 (27 - 31.05)	29 (27.25 - 30.8)	0.9755	29 (27.1 - 30.98)	29 (26.6 - 31.3)	0.7509
Basal ACT, seconds	140 (126 - 150)	140 (126 - 150)	140 (130.5 - 151.5)	0.5786	139 (126 - 149)	142 (126 - 154)	**0.0381**
Post-heparin ACT, seconds	488 (428 - 558)	488 (425 - 558)	498 (452.5 - 575.5)	0.1667	488 (424 - 553)	493.5 (433 - 563.2)	0.3373
Post-protamine ACT, seconds	121 (110.2 - 132)	121 (110 - 132)	123 (112 - 135)	0.4023	120.5 (109 - 131)	125.5 (114.8 - 138)	**<0.001**
CPB time, minutes	62 (109 - 131)	62 (43 - 78)	74 (50.5 - 92.5)	0.00165	61 (42.25 - 79)	66 (49 - 84)	**0.0190**
Clamping Time, minutes	49 (33 - 64)	48 (32 - 62.5)	56 (39 - 75.5)	<0.001	48.5 (32 - 63)	49 (37 - 66)	0.0846

We have then assessed whether there were any differences regarding the variables present in table 3 between patients with or without significant bleeding and with or without the use of transfusion, similarly to what was done for the demographic variables.

We found that, even though there were no statistically significant differences between patients with or without significant bleeding, several significant differences were observed when comparing patients with or without the use of transfusion, specifically preoperative hemoglobin, hematocrit, fibrinogen, platelets, basal ACT and Final ACT and CPB time (see table 3). To better understand the impact of these differences in the use of transfusion, we will explore this further ahead in this work.

ACT and hemorrhage

To understand whether there was any relation between ACT and hemorrhage, we started by assessing blood drainage at 12h (figure 2) and 24h (figure 3) after surgery between patients with higher or lower Final ACT, when compared to their basal value.

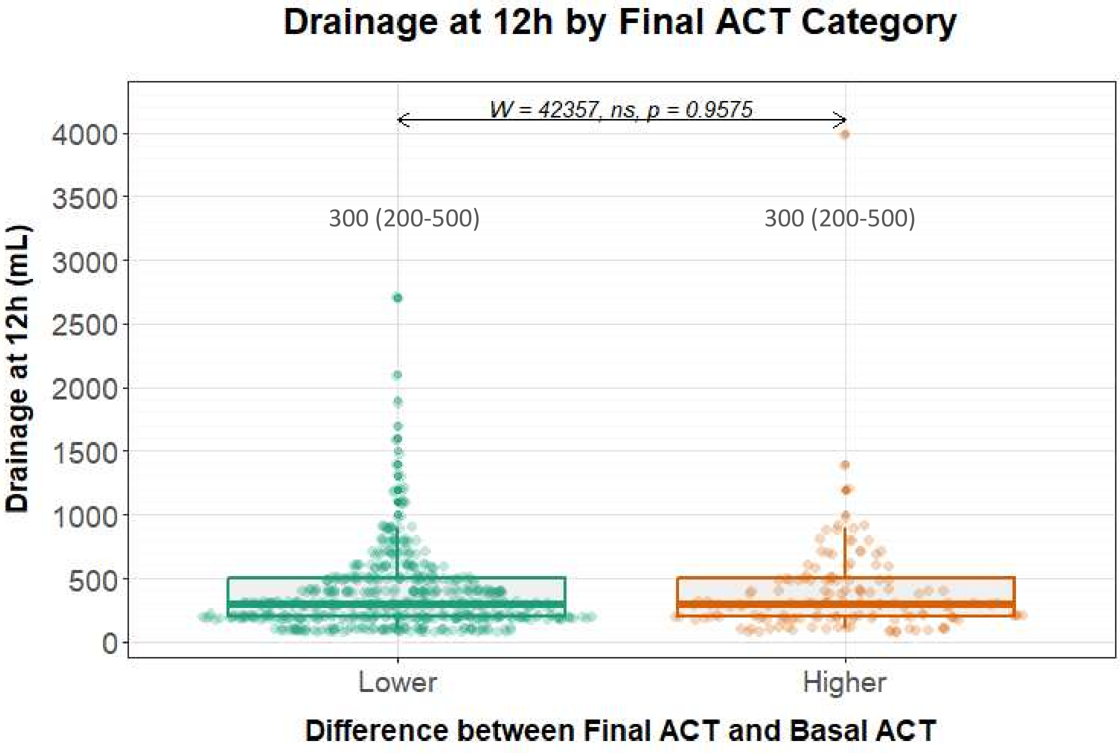

Figure 2. Diagram of extremes and quartiles relating drainage at 12 hours postoperatively (quantitative dependent variable) and the two categories of the difference between Final ACT and basal ACT

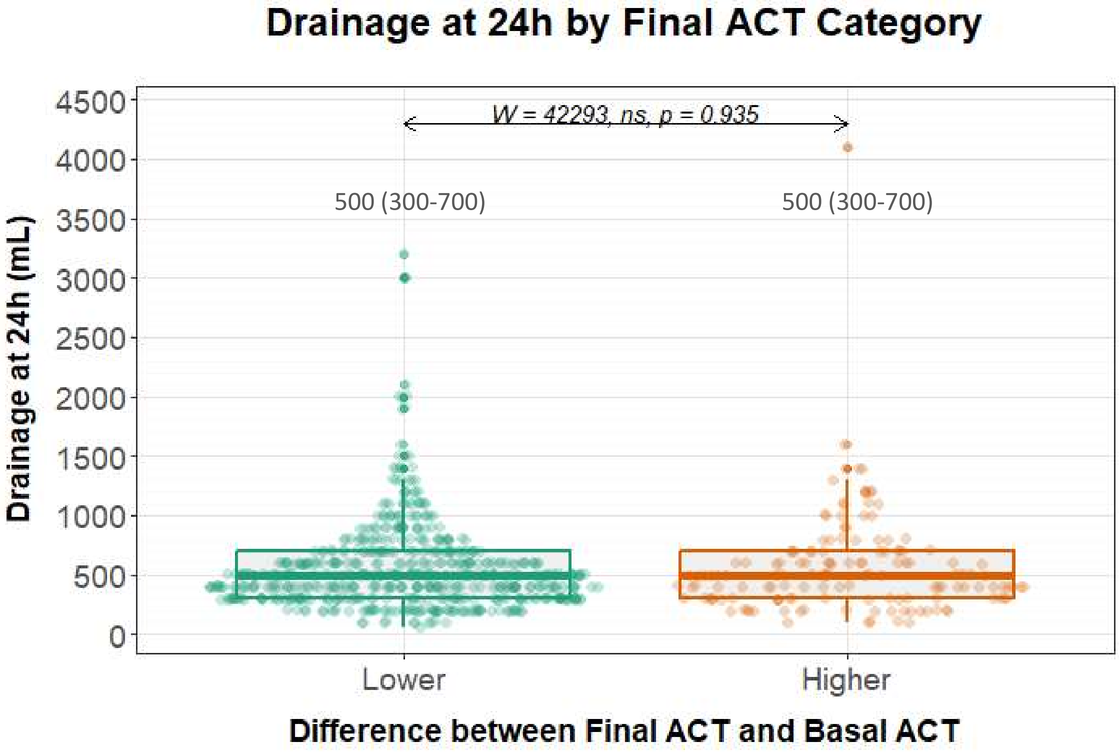

Figure 3. Diagram of extremes and quartiles relating drainage at 24 hours postoperatively (quantitative dependent variable) and the two categories of the difference between Final ACT and basal ACT

The comparison for both categories of Final ACT (higher *vs* lower than basal ACT) was made using the Wilcoxon Rank Sum test with continuity correction.

At 12 hours, no statistically significant difference was found between the two categories (*W = 42357, ns, p = 0,9575*), suggesting that postoperative bleeding at 12h might not have been influenced by Final ACT being either lower or higher than basal ACT (figure 2).

The same test was applied at 24h, with no statistically significant difference found between the two categories (*W = 42293, ns, p = 0,935*), suggesting that like we observed for 12h, postoperative bleeding at 24h might not have been influenced by an increase or decrease in each patient ACT value, compared to basal values (figure 3).

Afterwards, using the reference value of 140 seconds (W. Wang et al., 2018), we compared postoperative bleeding 12h (figure 4) and 24h (figure 5) after surgery in patients with a Final ACT lower or higher than 140 seconds, using the Wilcoxon Rank Sum test with continuity correction.

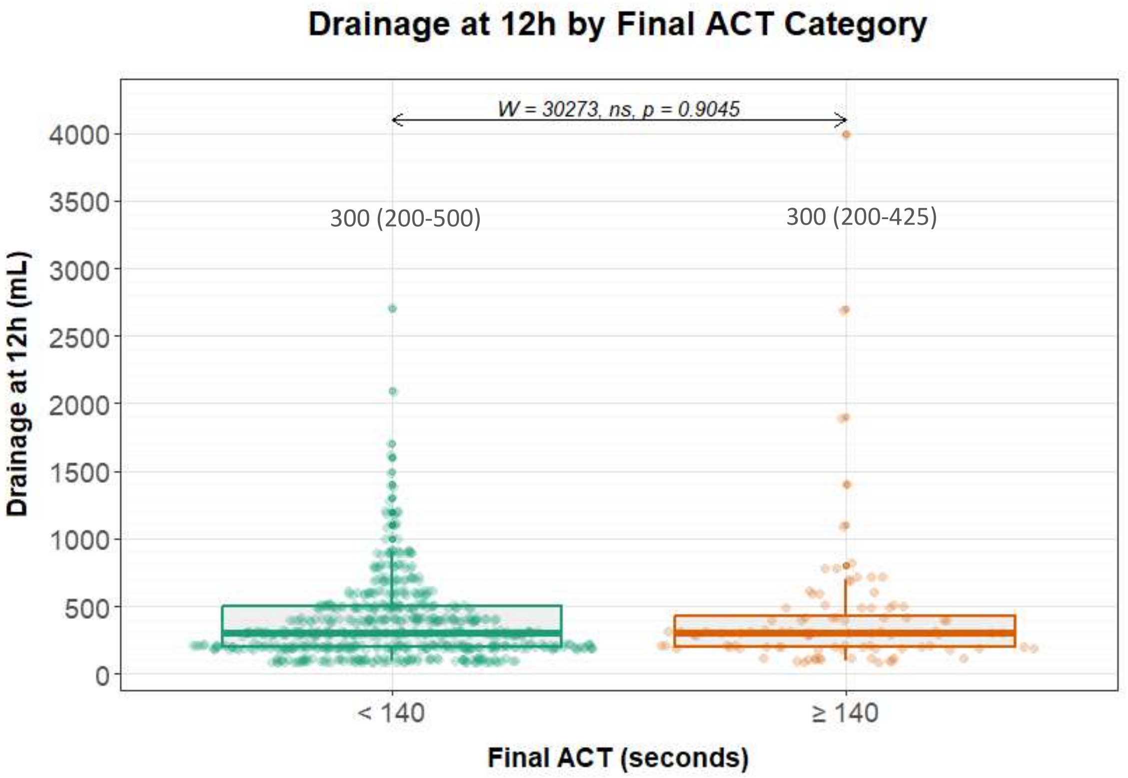

Figure 4. Diagram of extremes and quartiles relating drainage at 12 hours postoperatively (quantitative dependent variable) and the two categories of Final ACT (≥ 140 seconds vs < 140 seconds)

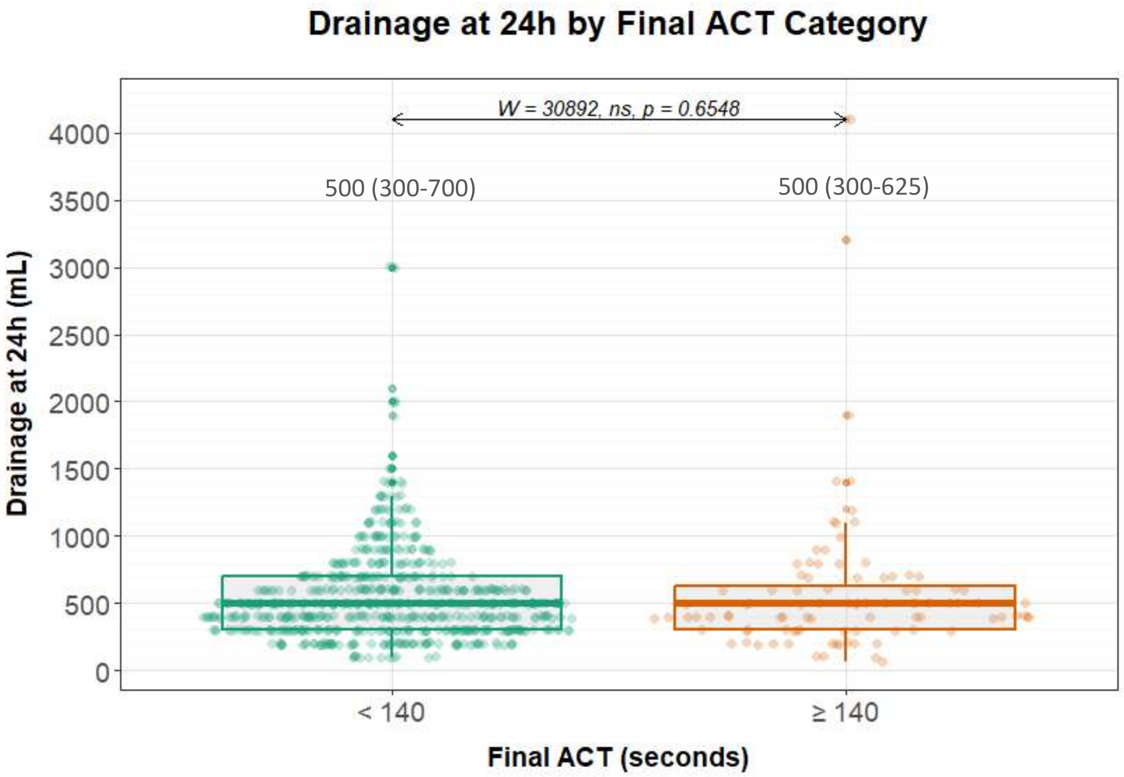

Figure 5. Diagram of extremes and quartiles relating drainage at 24 hours postoperatively (quantitative dependent variable) and the two categories of Final ACT (≥ 140 seconds vs < 140 seconds)

No statistically significant differences were found between the two groups at 12h (*W = 30273, ns, p = 0,9045*) and 24h (*W = 30892, ns, p = 0,6548*), suggesting that postoperative bleeding might not have been influenced by Final ACT being lower or higher than 140 seconds.

A chi-square test with Yates continuity correction was performed to assess the independence between the variables of interest: significant bleeding and categorical Final ACT (ACT higher *vs* lower than 140 seconds). The results indicate that there was no statistically significant evidence of independence between the variables ($X^2 = 0.0174$, df = 1, p = 0.8948) (figure 6).

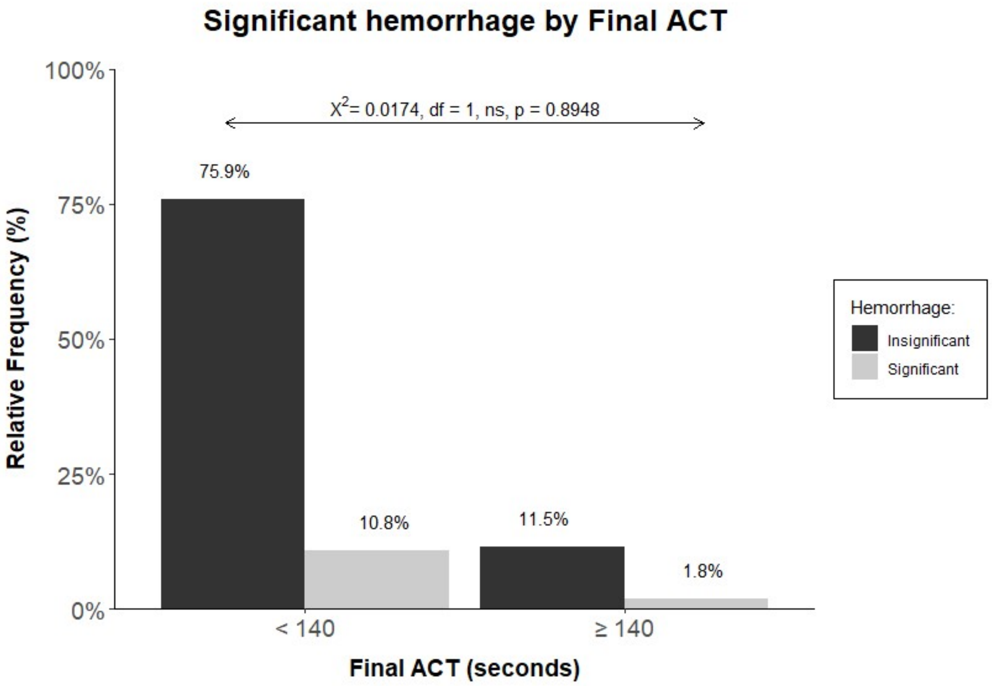

Figure 6. Graph comparing Significant Bleeding (categorical dependent variable) by categorical Final ACT (independent variable)

We have then assessed the relation between Final ACT and postoperative bleeding severity in the first 12 hours after surgery (figure 7).

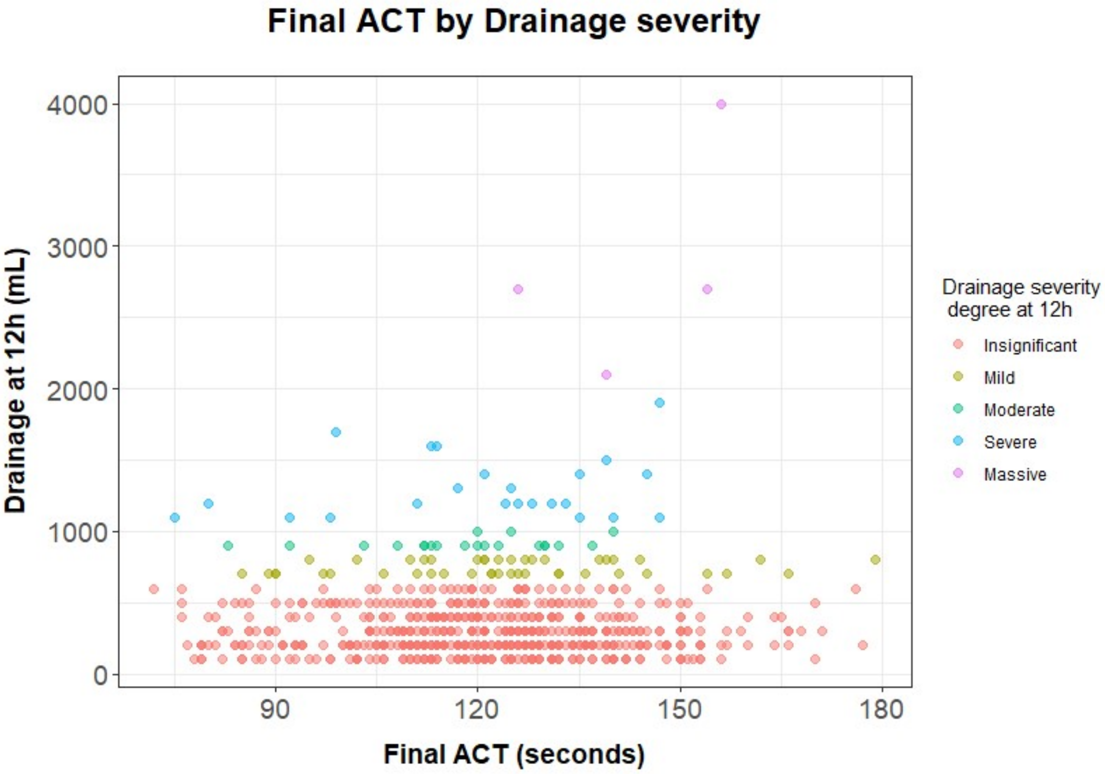

Figure 7. Scatter chart relating drainage at 12 hours postoperatively (quantitative dependent variable) and Final ACT (independent quantitative variable) highlighting the different categories of hemorrhage severity based on the classification of Dyke et al.

A linear mixed regression analysis model with fixed and random effects was made, adjusted by the restricted maximum likelihood method.

The dependent variable was bleeding volume (in milliliters), and the independent variables were "Group" (individuals with Final ACT < 140 seconds or Final ACT ≥ 140 seconds) and "Time" (baseline, at 12 hours postoperatively, and at 24 hours postoperatively), as well as the interaction between the latter two. In addition, a specified random effect for the identification variable of each individual ("ID"), was included, thus assuming that each individual has its intercept, that is, it has its ordinate at the origin point.

What stood out from this analysis was that "Time" has a strong positive effect, with the effect of "Time 12h post-op" estimated at 379,384 ± 14,187 (***, $p < 0.001$) and "Time 24h post-op" estimated at 560,088 ± 14,188 (***, $p < 0.001$).

Furthermore, there are significant interactions between "Group" and "Time". Specifically, the interaction effect between "ACT ≥ 140 seconds" and "Time 12h post-op" is estimated to be 99,616 ± 36,286 (**, $p = 0.00626$), and the interaction effect between "ACT ≥ 140 seconds" and "Time 24h post-op" is estimated at 104.999 ± 36.387 (**, $p = 0.00396$).

Table 4. Estimated effects of the independent variables "Time" and "Group" on bleeding volume (dependent variable) using a linear mixed regression analysis

	Estimate	**Std. Error**	**df**	**t value**	**Pr(>|t|)**	
(Intercept)	1.828	13.865	1559.680	0.132	0.89512	
Time12h post-op	379.384	14.187	1510.837	26.742	< 0.001	***
Time24h post-op	560.088	14.188	1510.208	39.476	< 0.001	***
Group≥140s:Time12h post-op	99.616	36.386	1510.125	2.738	0.00626	**
Group≥140s:Time24h post-op	104.999	36.387	1510.029	2.886	0.00396	**

According to the used model, 379.38 ± 14.19 mL (354.04 – 408.38) is the estimated bleeding at 12 hours postoperatively for a patient with Final ACT < 140 seconds. The same patient has an estimated bleeding, 24 hours after surgery, of 560.09 ± 14.19 mL (534.74 – 589.09).

If we consider a patient with a Final ACT ≥ 140 seconds, his estimated postoperative bleeding at 12 hours after surgery is 479 ± 36.39 mL (415.79 – 544.21) and 665.09 ± 36.39 mL (601.88 – 730.30) at 24 hours after surgery.

It has also been verified that, on average, a patient with a Final ACT ≥ 140 seconds has an increase of postoperative bleeding of 99.62 ± 36.39 mL in the first 12h, and 104.99 ± 36.39 mL 24h after surgery, when compared to a patient with a Final ACT < 140 seconds, being statistically significant.

Table 5. Estimated bleeding volume at 12 hours and 24 hours for patients with Final ACT ≥ 140 seconds and Final ACT < 140 seconds

Group	Predicted (mL)	Std.error	Conf.low	Conf.high
12h post-op <140s	379.38	14.19	354.04	408.38
12h post-op ≥140s	479.00	36.39	415.79	544.21
24h post-op <140s	560.09	14.19	534.74	589.09
24h post-op ≥140s	665.09	36.39	601.88	730.30

In summary, this model suggests that time after surgery is a strong predictor of the dependent variable "Bleeding". Furthermore, the interaction between the two analyzed groups and time seems to be significant, suggesting that the effect of time on the dependent variable may vary depending on which group the patient had been inserted.

In other words, the results of this model show that there is evidence that patients with a Final ACT ≥ 140 seconds have higher postoperative bleeding than patients who achieved a Final ACT < 140 seconds.

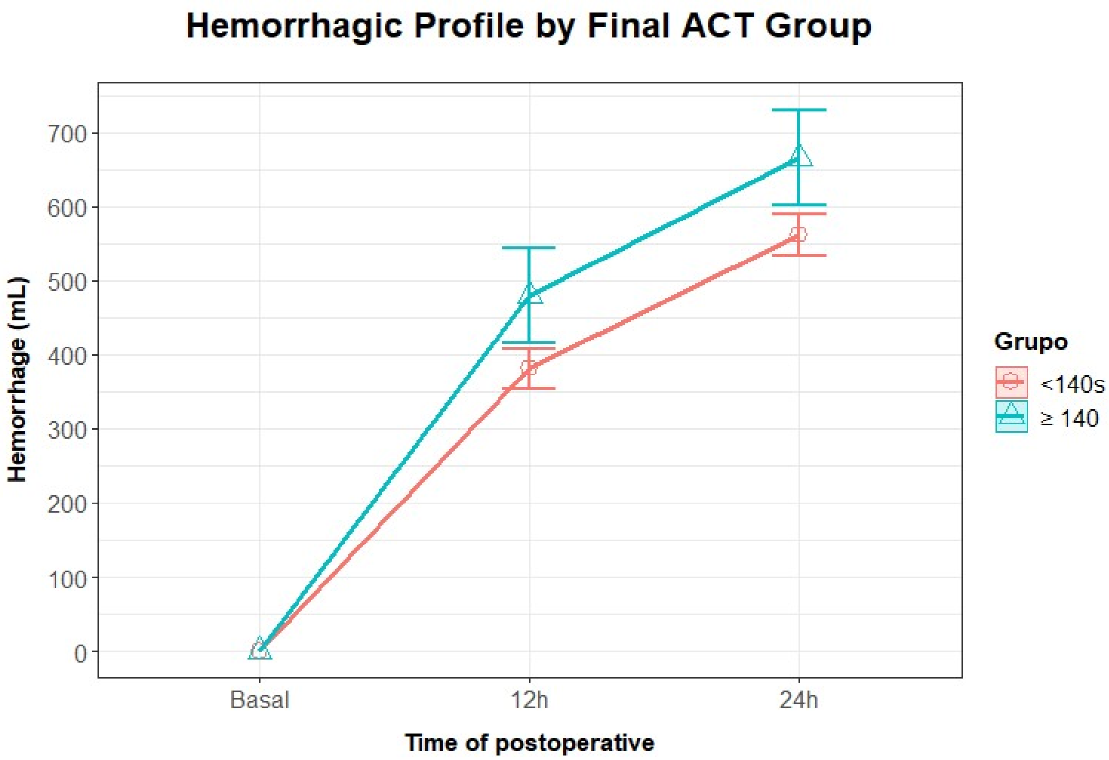

Figure 8. Median postoperative bleeding of patients with Final ACT ≥ 140 vs < 140 seconds in the first 12 and 24 hours after surgery

By visually interpreting the graph above (figure 8), we can see that the bleeding rate is higher in the first 12 hours after surgery, with a slower bleeding rate in the following 12 hours (between 12 and 24 hours after surgery). Indeed, after 12h and up to 24h the rate of postoperative bleeding is similar between both groups (parallel regression lines). The difference of postoperative bleeding between both groups is statistically significant both at 12h and 24h. Therefore, when compared to the group of patients who had a Final ACT < 140 seconds, bleeding appears to be higher in those who had a Final ACT ≥ 140 seconds.

All model assumptions were verified, namely the absence of multicollinearity of all variables involved in the model. The normality of the model's residuals was another of the verified assumptions. In terms of model suitability tests, the R-square was calculated, having obtained a value of 0.65 (65%), from which 0.32 came from fixed effects and 0.32 came from random effects.

The RMSE (Root Mean Squared Error) and the MAE (Mean Absolute Error) were used to assess the quality of data adjustment. The present model was the one in which the lowest values of RMSE (220.63 ml) and MAE (133.78 ml) were presented, indicating that it was the best model adjustment to the data.

The analysis resulting from the comparison of the residual values' dispersion versus the adjusted values' allowed to verify that there were no trend patterns or non-linearities that could have meant problems in model adequacy. In general, the residuals are randomly scattered around zero, with no clear trend that could indicate a problem in model specification or variable selection.

To further explore our results, a binary logistic regression was used to investigate the relation between significant bleeding, ≥ 600 mL in the first 12 hours postoperatively, (dependent variable), and several other possibly explanatory variables.

A wide range of potentially explanatory variables were used: CPB time; basal ACT; post-heparin ACT; Final ACT; difference between Final ACT and basal ACT; final ACT relative to 140 seconds; preoperative fibrinogen; preoperative hematocrit; preoperative platelets; preoperative PT; preoperative aPTT; hypertension; surgery timing; surgery type (valvular, aorta or coronary) and their interactions; age; sex; diabetes; dyslipidemia; and smoking. We started with a simple model and then proceeded to automatically select variables using the Akaike Information Criterion method.

The final model only included the following explanatory variables: CPB time ($\chi^2 = 7.6850$, df = 1, **, $p = 0.005568$), preoperative hematocrit ($\chi^2 = 8.7867$, df = 1, **, $p = 0.003034$) and patient sex ($\chi^2 = 15.9175$, df = 1, ***, $p < 0.001$).

The explanatory variable relating to CPB time generated a positive coefficient (see table 6). Therefore, it can be concluded that an increase in CPB time is associated with an increased risk of significant bleeding.

Preoperative hematocrit generated a negative coefficient (see table 6), which demonstrates that this variable is inversely related to the dependent variable, meaning that an increase in preoperative hematocrit is associated with a decrease in hemorrhagic risk.

Finally, for the patient sex variable, a positive coefficient was obtained for the reference category (male) (see table 6), which shows that males have a higher bleeding risk when compared to females.

All other variables were not significant in the final model. The interest variable of the problem raised by this work (Final ACT) did not prove to explain differences in postoperative bleeding ($\chi^2 = 0.3423$, df = 1, ns, p = 0.558487). Furthermore, the inclusion of variables conditioned by Final ACT, such as the absolute difference between Final ACT and initial ACT (difference between ACT) and the binary categorization of the Final ACT (< 140 seconds or ≥ 140 seconds), were also considered not significant, not being able to explain differences in postoperative bleeding ($\chi^2 = 0.1066$, df = 1, ns, p = 0.744042 and $\chi^2 = 0.2206$, df = 1, ns, p = 0.638568, respectively) . Overall, the results suggest that being male and a longer duration of CPB may increase the risk of postoperative hemorrhage, while higher values of preoperative hematocrit may reduce this risk.

Table 6. Coefficients of the statistically significant explanatory variables, using a binary logistic regression to explore the relation between significant bleeding and several possible relevant variables

	Coefficients				
	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**	
(Intercept)	-0.213	0.975	-0.218	0.82707	*ns*
CPB_Time	0.009	0.003	2.772	0.00557	**
Pre_Htc	-0.076	0.025	-2.964	0.00303	**
Sex_Masculine	1.044	0.262	3.990	<0.001	***

Table 7. Odds Ratio of the statistically significant explanatory variables, using a binary logistic regression to explore the relation between significant bleeding and several possible relevant variables

	OR	**95% CI**
(Intercepto)	0.808	0.117 - 5.399
CPB_Time	1.009	1.002 - 1.015
Pre_Htc	0.927	0.882 - 0.975
Sex_Masculine	2.842	1.721 - 4.821

The odds ratio (OR) for the variable "CPB Time" is equal to 1.009 (1.002 - 1.015, 95% CI), representing that with each minute of CPB Time, the OR for the presence of significant bleeding increases 1.009 times. The graph below shows the progressive increase in OR for the CPB time variable, while maintain the remaining variables constant. As we can see, the OR for significant postoperative bleeding almost increases exponentially with time. For a CPB time of 1 hour, the OR is 1.83, but almost doubles with another hour of CPB (2h, OR 3.34), and triples with 3 hours (OR 6.11). The OR for 6 hours of CPB is 37.36.

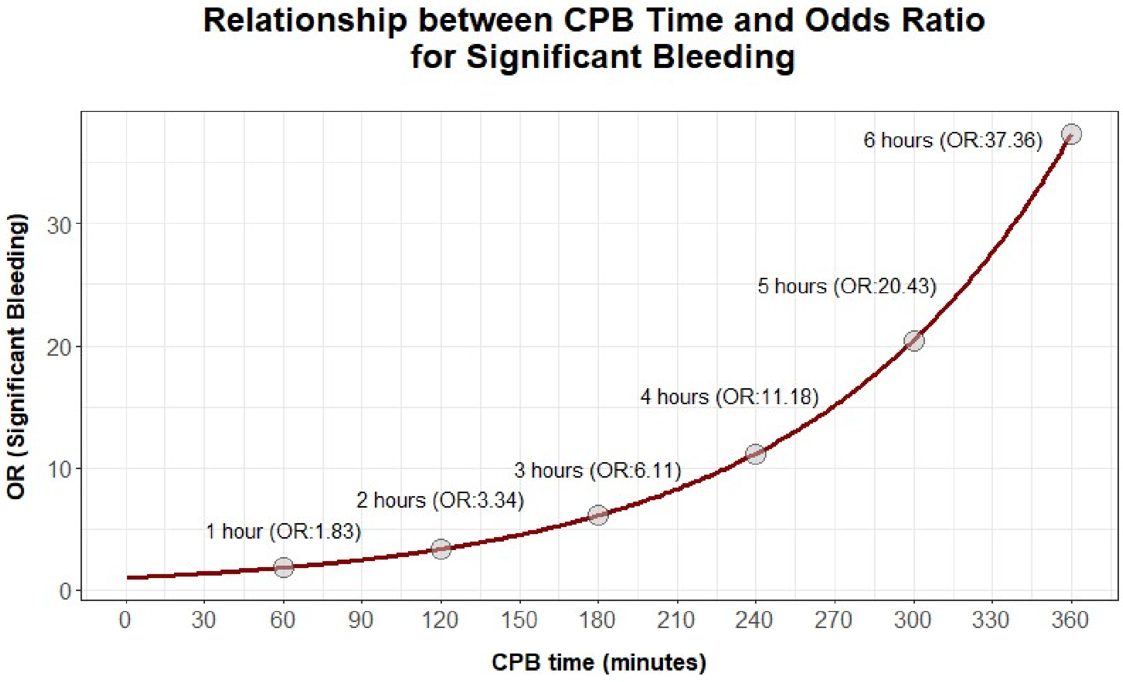

Figure 9. Visual representation of relation between the OR for Significant Bleeding and the time under CPB

Predicted probabilities for the model's dependent variable (significant bleeding) by patient sex were also estimated for patients with a CPB time and preoperative hematocrit that correspond to their respective median value (62 minutes and 40.2%, respectively). If we consider a female patient, the predicted probability of significant bleeding is, according to this model and with 95% confidence, 6.23% (4.10 – 9.36%). If a patient with the same variables is male, then he has a predicted probability of significant bleeding, with 95% confidence, of 15.89% (12.47 – 20.02%).

The following graph (figure 10) represents the predicted probability of significant bleeding by CPB time and patient sex.

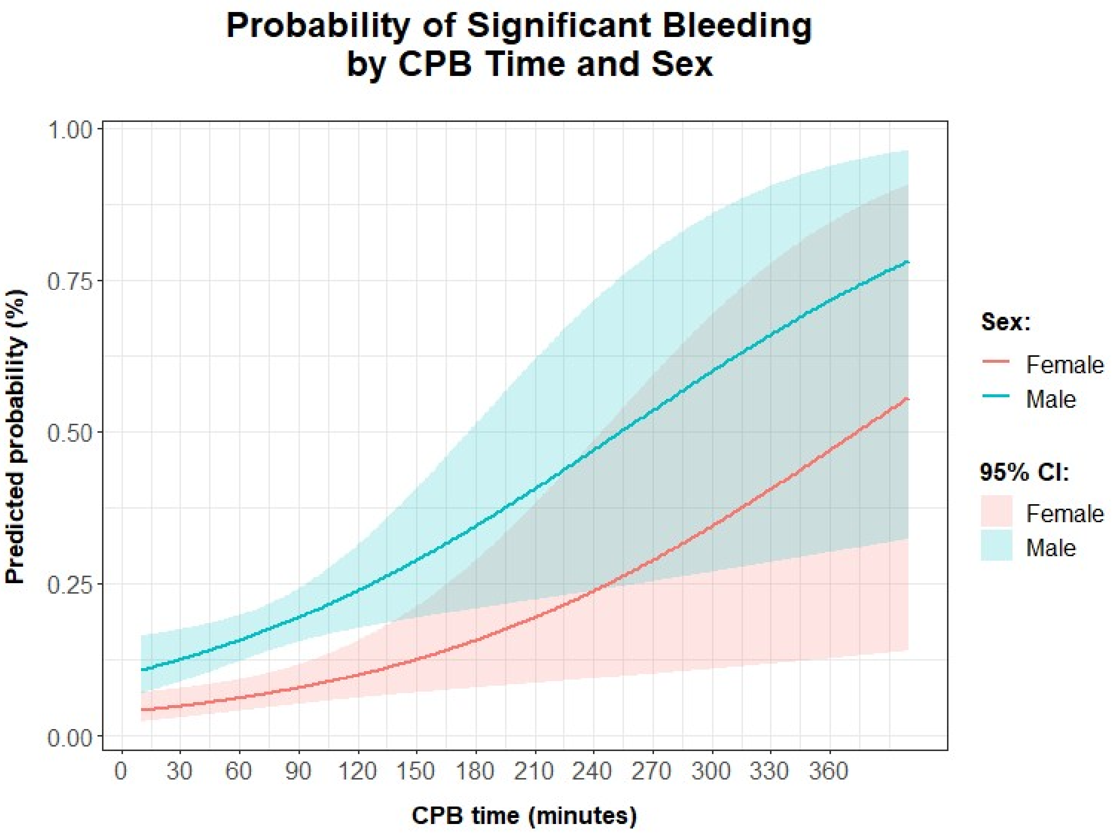

Figure 10. Line chart with 95% confidence interval bands relating significant bleeding probability and time under CPB for males (blue) and females (pink)

According to the interpretation of the coefficients and OR explained previously, males are at a higher risk of having significant bleeding after cardiac surgery. This risk, in comparison with females and keeping the remaining variables constant, is on average 2.842 (1.721 – 4.821, 95% CI) times higher.

It also appears that, between 15 and 135 minutes, the predicted probability of significant bleeding is significantly higher in males when compared to females, with no intersection between the bands of the respective confidence intervals. Moreover, we can also verify that, regardless of sex, as CPB time increases so does the probability of significant bleeding.

Figure 11 represents the predicted probability of significant bleeding based on preoperative hematocrit and patient sex. Again, males are at a higher risk of significant bleeding after cardiac surgery. Furthermore, it appears that, with a preoperative hematocrit between approximately 32% and 46% there is a significantly higher predicted probability for bleeding in males when compared to females, with no intersection between the bands of the respective confidence intervals.

We also found that, while statistically different, the probability of significant bleeding in men and women is lower for higher values of hematocrit. There is an approximation of the predicted probability curves between the two sexes for higher values of hematocrit.

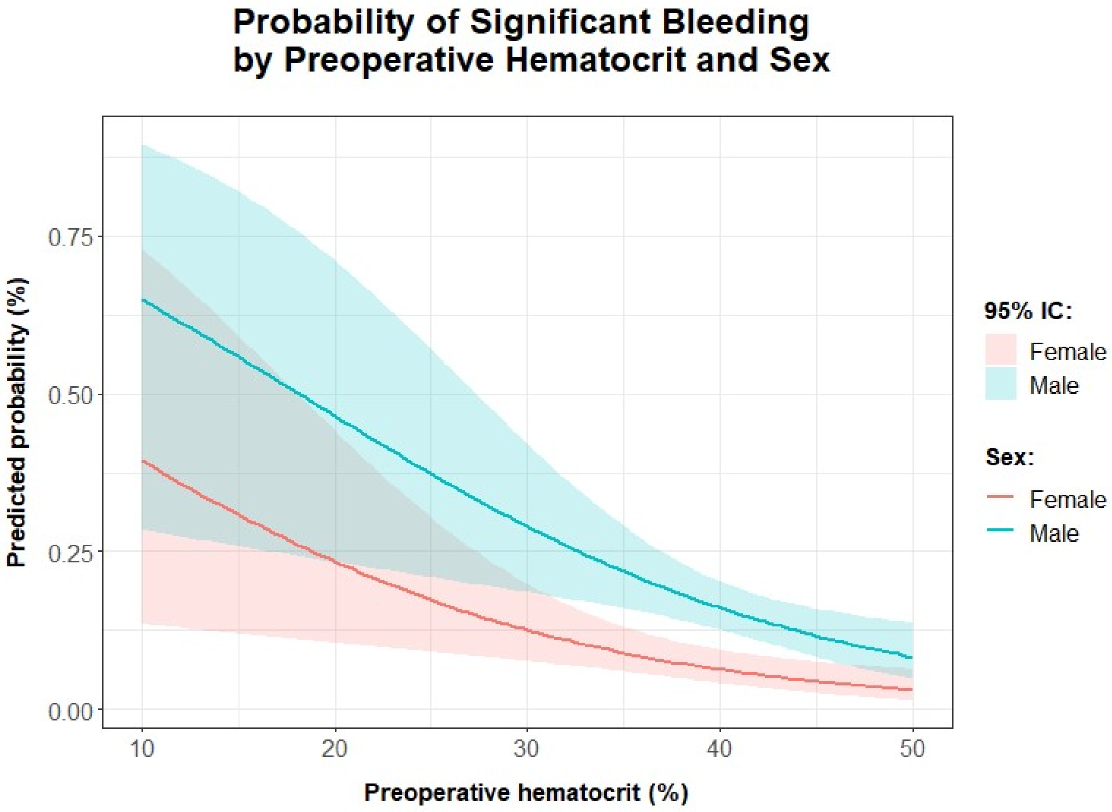

Figure 11. Line chart with 95% confidence interval bands relating significant bleeding probability and preoperative hematocrit for males (blue) and females (pink)

A ROC curve was elaborated for the binary logistic regression model (figure 12). The AUC represents the performance of the binary classification model, such as the one presented here, where 1 indicates a perfect model and 0.5 indicates a model that is no better than randomness.

Therefore, an AUC value of 0.702 indicates that the model had a moderate performance in distinguishing between the two categories used here (significant bleeding vs. non-significant bleeding). In other words, the model was able to predict 70.2% of the cases of significant bleeding.

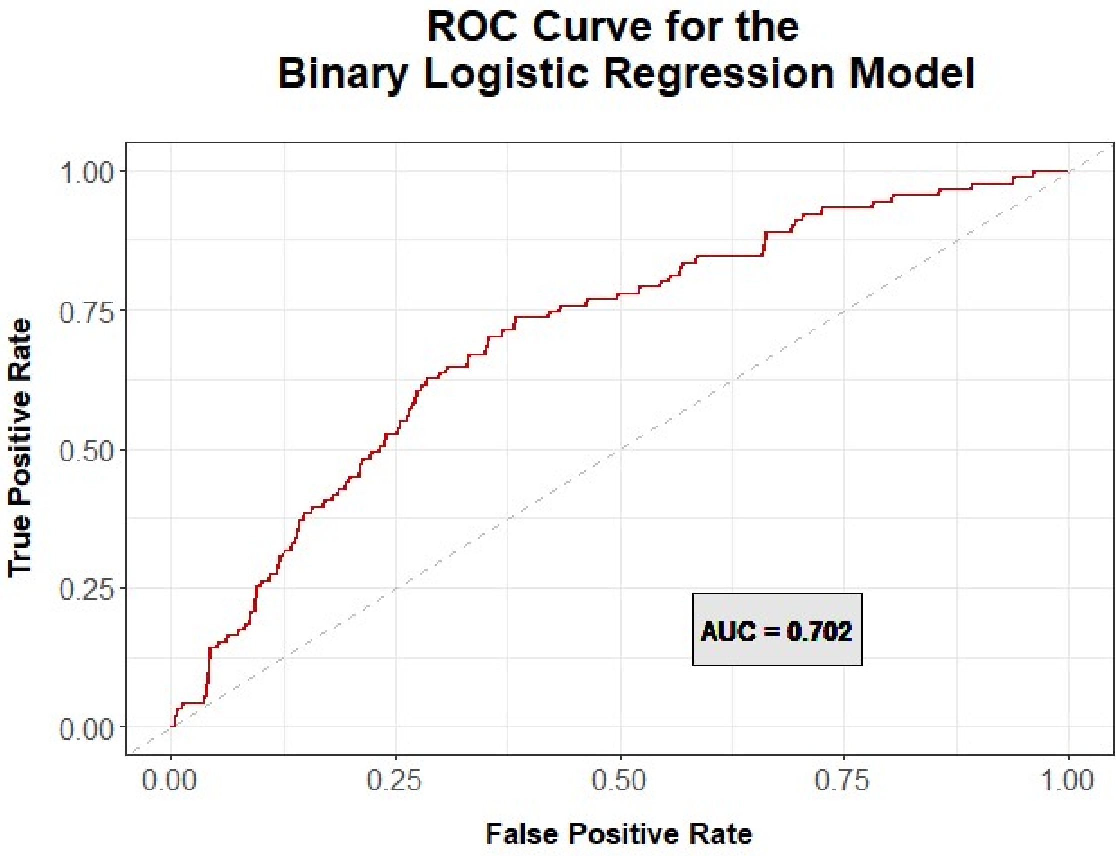

Figure 12. ROC curve for the binary logistic regression model used to explore variables associated with significant bleeding

The assumptions for the binary logistic regression were verified, including the absence of multicollinearity of the explanatory variables. The observations that stood out were analyzed and the existence of potentially influential and effectively influential observations was assessed using the Cook distance, with which we verified the absence of distances greater than 0.5. In addition, the Hosmer & Lemeshow test was performed to evaluate model suitability, having found the model adequate (p = 0.9910).

ACT and use of transfusion

We have then tested the association between the use of at least one PRBC and Final ACT, dividing patients into two groups (< 140 seconds or ≥ 140 seconds), with a Fisher's test.

With this analysis, a significant effect was observed (p = 0.0104) and the estimated OR was 1.81 (1.13 – 2.89, 95% CI), suggesting a positive association between the variables. Therefore, a Final ACT ≥ 140 seconds is associated with a higher need for transfusion of PRBC (figure 13).

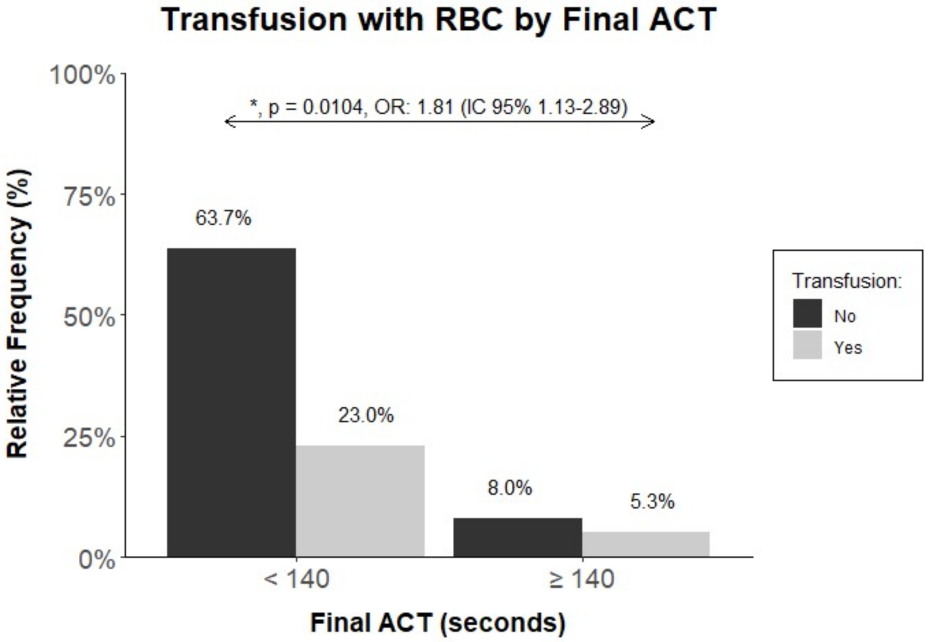

Figure 13. Graph comparing PRBC Use (categorical dependent variable) vs. categorical Final ACT (independent variable)

To further explore variables associated with the use of at least one PRBC, a binary logistic regression was used.

The same wide range of potential explanatory variables was used: CPB time; basal ACT; post-heparin ACT; final ACT; difference between final ACT and basal ACT; Final ACT relative to 140 seconds; preoperative fibrinogen; preoperative hematocrit; preoperative platelets; preoperative PT; preoperative aPTT; hypertension; surgery timing; surgery type (valvular, aorta or coronary) and their interactions; age; sex; diabetes; dyslipidemia; and smoking. We also started with a simple model and then proceeded to automatically select variables using the Akaike Information Criterion method.

The final model only included the following explanatory variables: CPB time ($\chi^2 = 29.5481$, df = 1, ***, $p < 0.001$), Final ACT ($\chi^2 = 12.8903$, df = 1, ***, p = < 0.001), preoperative hematocrit ($\chi^2 = 93.0216$, df = 1, ***, $p < 0.001$), surgery timing ($\chi^2 = 8.5668$, df = 1, **, $p = 0.0034$), age ($\chi^2 = 6.6213$, df = 1, *, $p = 0.01$) and patient sex ($\chi^2 = 24.5074$, df = 1, ***, $p < 0.001$).

Quantitative explanatory variables relating to CPB time, Final ACT and patient age generated positive coefficients (see table 8). Hence, it can be concluded that an increase in CPB time, an increase in Final ACT and an increase in age are associated with a higher risk of using at least one PRBC unit postoperatively.

On the other hand, preoperative hematocrit generated a negative coefficient (see table 8), which demonstrates that this variable is inversely related to the use of PRBC, meaning that an increase in preoperative hematocrit is associated with a decrease in the use of PRBC.

Considering surgery timing, a positive coefficient was obtained for the reference category (urgent surgery) (see table 8), which shows that urgent surgeries are associated with a higher risk of using PRBC in the postoperative period.

Finally, regarding the patient sex variable, a negative coefficient was obtained for the reference category (male sex) (see table 8), which shows that males have a lower risk of using PRBC when compared to females.

All other variables were not significant in the final model. Other variables including ACT values, such as the absolute difference between Final ACT and initial ACT (difference between ACT) and the binary categorization of the Final ACT (< 140 seconds or ≥ 140 seconds), were considered not significant enough to explain differences in postoperative use of PRBC ($\chi^2 = 2.1480$, df = 1, ns, $p = 0.14275$ and $\chi^2 = 1.5268$, df = 1, ns, $p = 0.21659$, respectively).

Table 8. Coefficients of the statistically significant explanatory variables, using a binary logistic regression to explore the relation between the use of at least one PRBC and several possible relevant variables

	Coefficients				
	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**	
(Intercept)	4.012	1.684	2.382	0.01722	*
Time_CPB	0.019	0.003	5.436	<0.001	***
Final_ACT	0.021	0.006	3.590	<0.001	***
Pre_Htc	-0.290	0.030	-9.645	<0.001	***
Urgent (Non Elective)	1.382	0.472	2.927	0.00342	**
Age	0.025	0.010	2.573	0.01008	*
Sex (Masculine)	-1.088	0.220	-4.950	<0.001	***

Table 9. Odds Ration of the statistically significant explanatory variables, using a binary logistic regression to explore the relation between the use of at least one PRBC and several possible relevant variables

	OR	**IC a 95%**
(Intercept)	55.230	2.075 - 1546.331
Time_CPB	1.019	1.012 - 1.026
Final_ACT	1.021	1.010 - 1.032
Pre_Htc	0.748	0.704 – 0.792
Urgent (Non Elective)	3.982	1.589 – 10.195
Age	1.026	1.006 - 1.046
Sex (Masculine)	0.337	0.218 – 0.516

The OR for the variable “CPB Time” is 1.019 (1.012 - 1.026, 95% CI), meaning that with each minute of CPB Time, the OR for the use of PRBC in the postoperative period increases 1.019 times. The graph below (figure 14) shows the progressive increase in OR for the CPB time variable, while maintain remaining variables constant. With one hour of CPB, the OR increases to 3.04, and to 9.25 with just 2 hours, rising exponentially up to an OR of 85.5, 259.98 and 790.56 with 4, 5 and 6 hours of CPB, respectively.

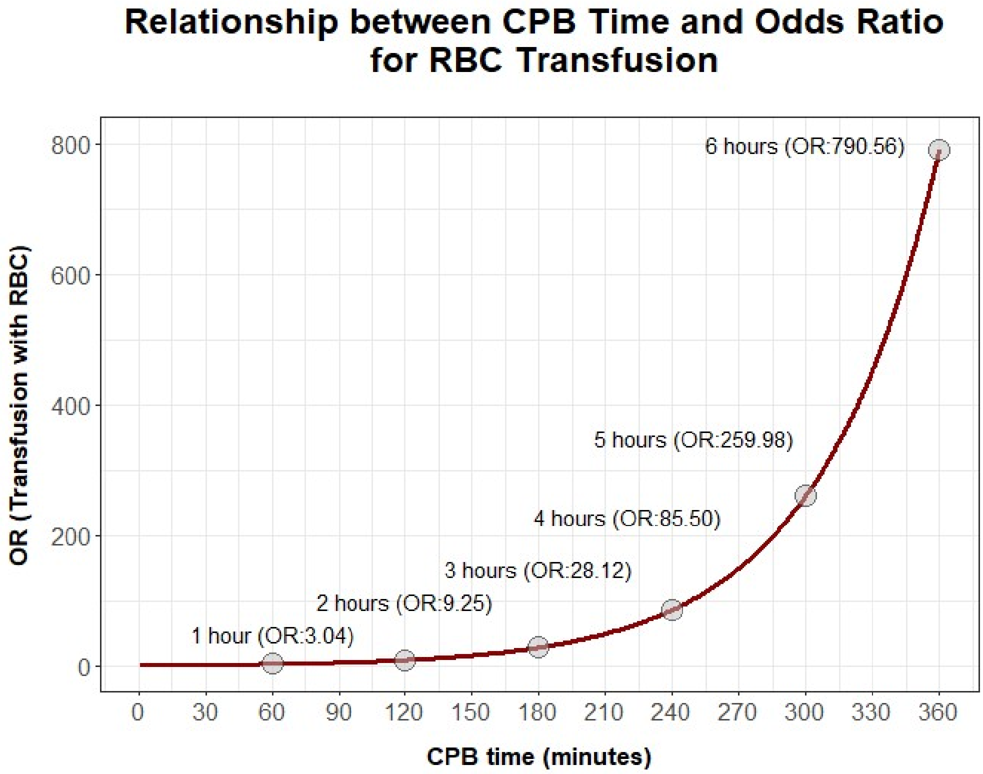

Figure 14. Visual representation of relation between the OR for PRBC use and the time under CPB

Considering the variable "Age", the OR was 1.026 (1.006 - 1.046, 95% CI). Once again, there is an interesting and significant increase in the OR with an increase in age. If we consider a patient with 40 years-old, the OR for a single RBC is 2.75, raising to an OR of 4.55 with 60 years-old and OR 7.54 with 80 years-old.

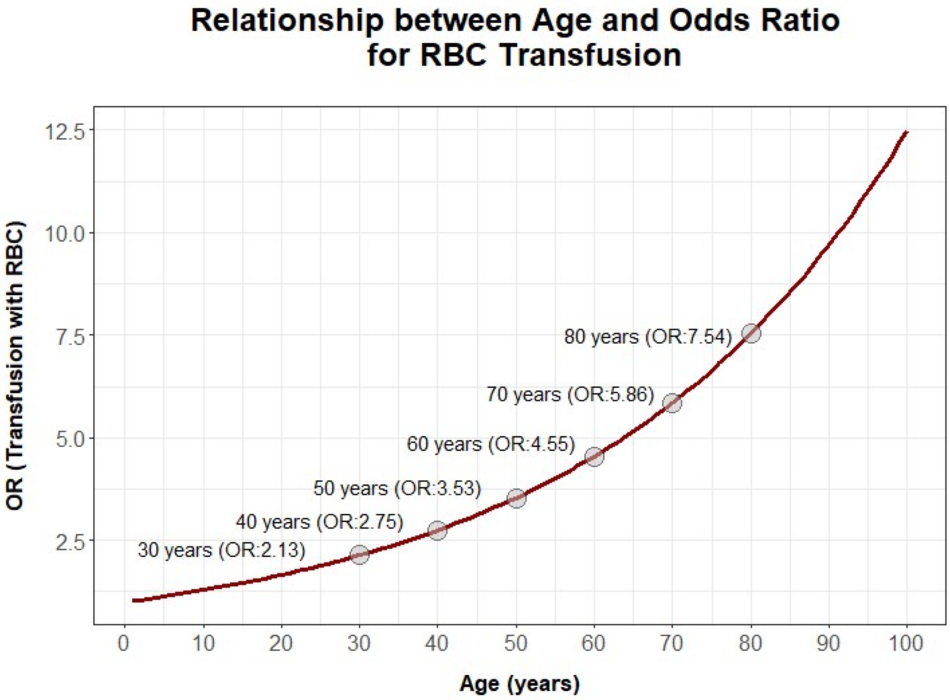

Figure 15. Visual representation of relation between the OR for PRBC use and patient age

The OR for the variable "Final ACT" was 1.021 (1.010 - 1.032, 95% CI). This means that with each increasing second in ACT after reversal with Protamine, the OR for using PRBC postoperatively increases 1.021 times.

We have then estimated the predicted probabilities for the model's dependent variable (PRBC use) by patient sex and surgery timing (see table below).

Table 10. Predicted probabilities and their respective 95% CI for PRBC use based on patient sex and surgery timing

Time_CPB	FinalACT	Pre Htc	Age	Urgency	Sex	Predicted Prob.	95% CI
62	121	40.2	71	Elective	Masculine	10.92	7.89 - 14.92
62	121	40.2	71	Elective	Feminine	27.23	21.84 - 33.39
62	121	40.2	71	Urgent	Masculine	33.29	16.89 - 55.08
62	121	40.2	71	Urgent	Feminine	60.38	37.65 - 79.36

We have calculated these probabilities considering the hypothetical scenario in which CPB time, Final ACT, preoperative hematocrit and age correspond to the median value of our population (62 minutes, 121 seconds, 40.2% and 71 years, respectively).

In the case of females, the predicted probability for the use of PRBC is, according to this model and with 95% confidence, 27.23 % (21.84 – 33.39%) in the case of elective surgery, and 60.38% (37.65 – 79.36%) in an urgent surgery.

If a patient with the same variables is male, then he has a predicted probability for the use of PRBC, with 95% confidence, of 10.92% (7.89 – 14.92%) in the case of elective surgery, and 33.29% (16.89 – 55.08%) in an urgent surgery.

Figure 16 represents the predicted probability of PRBC use by Final ACT time, patient sex and surgery timing.

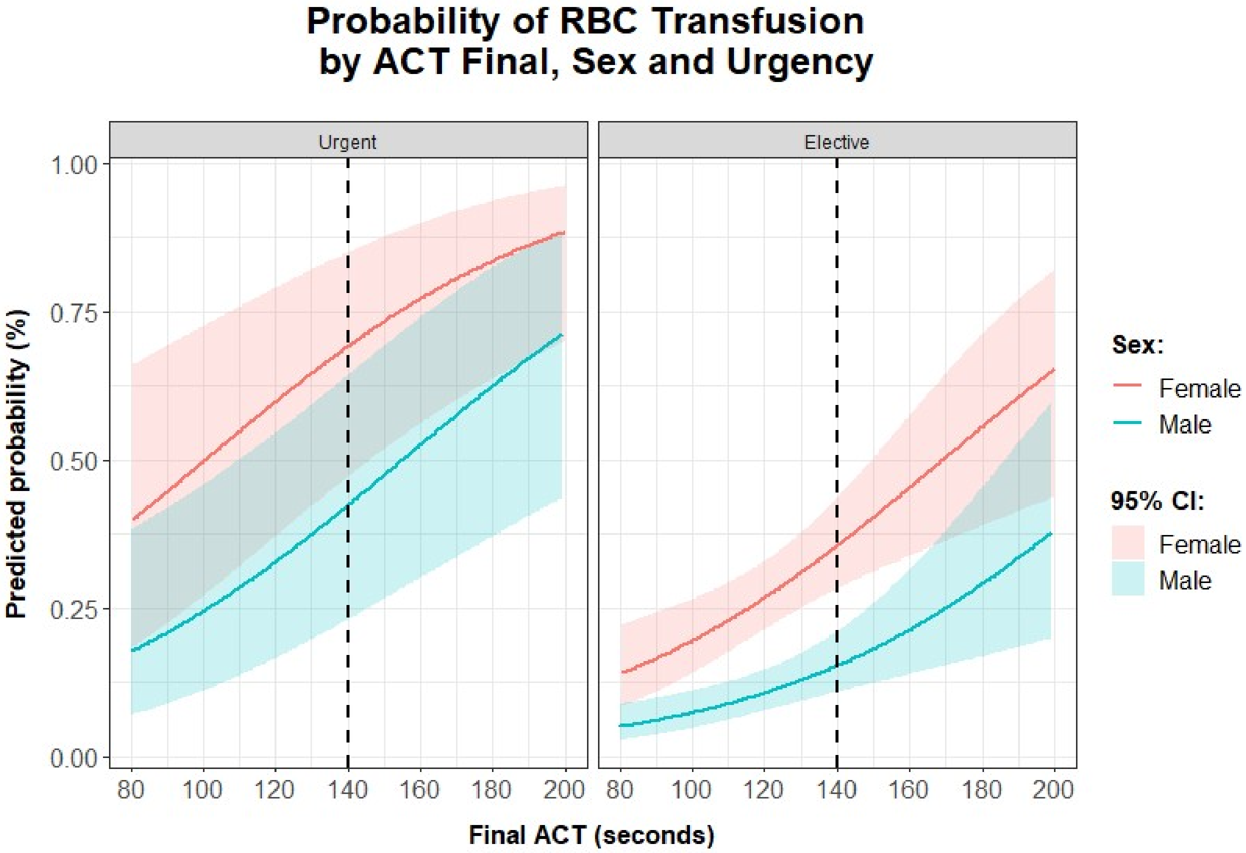

Figure 16. Line charts with 95% confidence interval bands relating PRBC use probability and Final ACT for males (blue) and females (pink) in the case of urgent (left) and elective (right) surgeries

A ROC curve was elaborated for the binary logistic regression model used (figure 17), with an AUC value of 0.854. The value indicates that the model performed well in distinguishing between the two categories used here (use of PRBC vs. non-use of PRBC). In other words, the model was able to predict 85.4% of the cases regarding the use of PRBC.

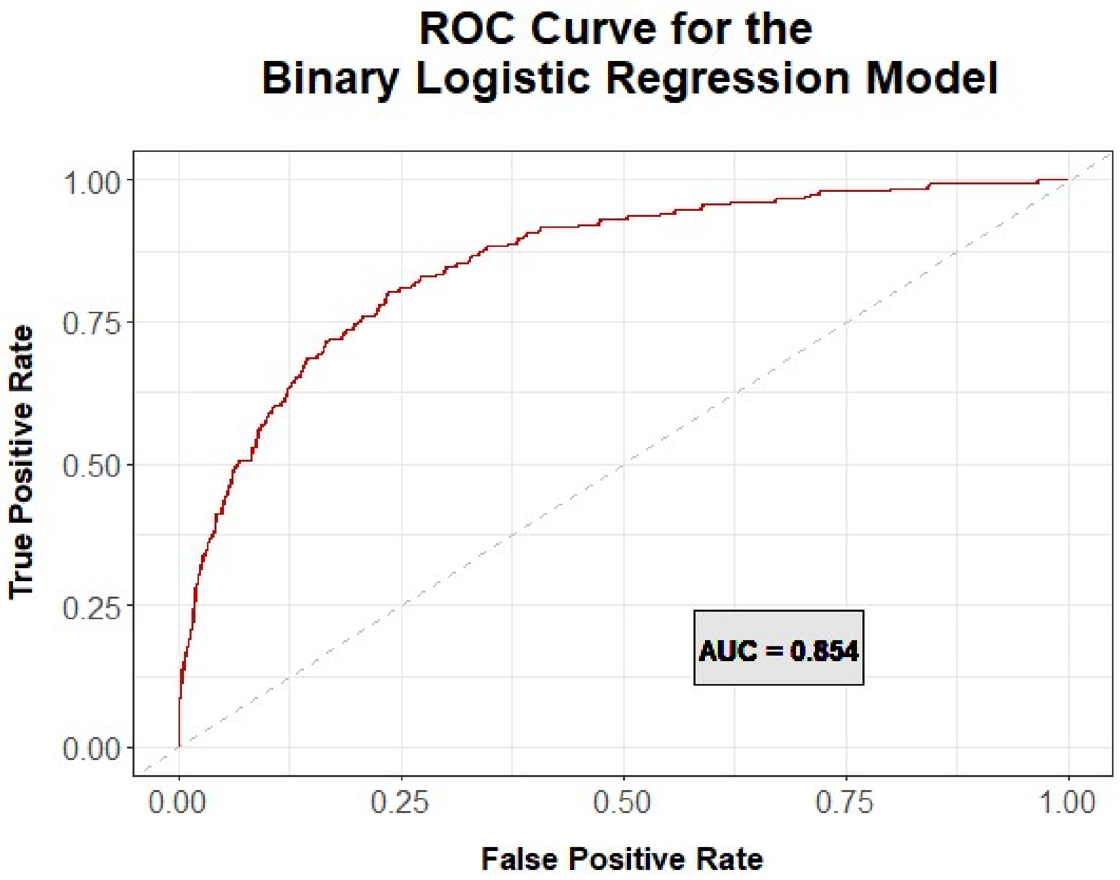

Figure 17. ROC curve for the binary logistic regression model used to explore variables associated with the use of at least one PRBC

The assumptions for the binary logistic regression were verified, including the absence of multicollinearity of the explanatory variables. The observations that stood out were analyzed and the existence of potentially influential and effectively influential observations were evaluated using the Cook distance, with which we verified the absence of distances greater than 0.5. In addition, the Hosmer & Lemeshow test was performed to evaluate model suitability, and it was found that the model is adequate (p = 0.9999).

Discussion

Despite recent advances in surgical technique, specifically in cardiopulmonary bypass, anesthesia management and postoperative care, postoperative bleeding remains one of the most common complications after cardiac surgery. Approximately 10% of all patients submitted to cardiac surgery suffer from severe or massive blood loss (Raphael et al., 2019).

PRBC transfusion is commonly associated with cardiac surgeries, with patients having among the highest overall rate of red blood cell transfusion use, accounting for 10-15% of all PRBC transfusions in the United Kingdom and United States of America (Raphael et al., 2019). Even with current improvements in technology, drugs, and guidelines recommendations, only a modest decline over the last decade has been observed, and more than 50% of high-risk patients still require transfusions (Raphael et al., 2019).

However, although transfusions are essential to maintain patient's clinical and hemodynamic stability, they are far from being innocuous. For this reason, it is important to control patient's hemostasis, to reduce bleeding and, consequently, the use of transfusions; this may improve surgical outcomes, and may decreased hospital-associated costs (Boer et al., 2018).

In our work, after analyzing several possible contributing variables, we found that CPB time is a predictor of significant bleeding, with the risk of significant bleeding increasing 1.009 times with each minute of CPB time. It is known that the use CPB is associated with changes in various cellular and molecular components that influence coagulation and inflammation (Baumann Kreuziger et al., 2018). Moreover, the use of high concentrations of heparin needed for CPB leads to a greater predisposition for imbalances in hemostasis. Therefore, as time under CPB is prolonged and additional heparin is needed, changes in hemostasis may be aggravated and patients are more prone to bleeding. CPB time is also a predictor of PRBC use, with the risk of transfusion increasing 1.019 times with each minute of CPB time. At first glance, these values might not seem very high, but with the average time of cardiac surgery being 3-5 hours (with a considerable part using CPB), the risk of significant bleeding increases exponentially with surgery progression (see figure 9); the same is valid for the use of transfusion (see figure 14).

Another important variable with impact in postoperative bleeding and the use of transfusion which was analyzed was preoperative hematocrit, frequently reduced in cardiac surgery patients. In fact, around 25-30% of patients submitted to cardiac surgery have pre-operative anemia (Raphael et al., 2019). Patients might suffer from different types of anemia, with several possible etiologies, such as: chronic inflammatory processes (e.g. infective endocarditis); iron-deficiency, from indolent blood loss (e.g. Heyde syndrome in patients with aortic stenosis); hemolysis, as a complication of previous mechanical valve implantation; among others. According to our model, preoperative hematocrit is also a predictor of significant bleeding, with patients with higher hematocrit values (at least within physiological range) having a lower risk of bleeding, regardless of patient sex. Similarly to our study, in patients submitted to CABG, low preoperative hematocrit levels were found to be associated with increased blood drainage during and after surgery, as well as with increased need for blood transfusion and prolonged intensive care unit stay (Kumar et al., 2021). These findings open the discussion of whether we should transfuse a patient before surgery or have a conservative approach and accept preoperative anemia. Exploring this question, a recent study by LaPar et al. involving 33,411 patients from 19 cardiac surgery centers, found that PRBC transfusion was a stronger risk factor for morbidity and mortality than preoperative hematocrit level, supporting efforts to reduce unnecessary PRBC transfusions.

In this study, we also observed that men have a higher risk of significant postoperative bleeding that is on average 2.842 times higher than in females, with males having a 15.89% probability of significant bleeding, which is more than twice of the female's 6.23%. On the contrary, females have a higher chance of needing transfusions in the postoperative time, when compared to males, despite surgery timing. In the case of elective surgery, the probability for the use of transfusion was 27.23% for females, a number almost 3 times higher than the males' 10.92%. The same situation applies in the urgent surgery setting, with an almost 2 times higher probability of females needing transfusion when compared to males (60.38% *vs* 33.29%, respectively). These results are supported by the findings from the study conducted by E. Wang et al., where it was found that regardless of tranexamic acid use, females had less blood loss after CABG, but female sex was the risk factor for PRBC transfusion after surgery, with morbidities in women also more frequent than that in men.

A recent meta-analysis, although only including patients undergoing isolated CABG, pointed out that females are at higher risk for operative and late mortality when compared to males, with also higher risk of non-fatal occurrences, including major adverse cardiac events, myocardial infarction and stroke (Bryce Robinson et al., 2021). Since transfusions are not innocuous, with several studies finding that the transfusion of a single PRBC increases morbidity (higher rate of stroke, respiratory complications, acute kidney injury, etc.) and mortality in patients undergoing cardiac surgery (Boer et al., 2018; Ivascu Girardi et al., 2023; Raphael et al., 2019), the female's higher risk of postoperative transfusion may explain, at least in part, the aforementioned consequences in morbimortality found in this sex group. Further studies might be able to explore if these findings are observed in all types of cardiac surgery and what biological mechanisms explain these differences.

Regarding other qualitative variables, we found that, not surprisingly, surgery timing influences transfusion use, regardless of patient sex. In females, the probability of transfusion use was more than 2 times higher in an urgent setting when compared to an elective setting (60.38% *vs* 27.23%, respectively). In the case of males, the probability for the use of transfusions was around 3 times higher in urgent surgeries, compared to elective surgeries (33.29% *vs* 10.92%, respectively). These differences in transfusion use might be due to several factors. Patients that need to be submitted to urgent surgery require loading doses of antiplatelet and anticoagulant drugs, often without having into account underlying diseases (such as CKD), which might lead to an increased predisposition to bleeding. Moreover, in the context of acute disease, compensatory mechanisms or attempted correction by physicians may not yet have occurred, leading to an increased need for transfusions to maintain the patient's clinical stability.

Throughout this work, we have highlighted the importance and utility of ACT as a parameter for assessing the degree of anticoagulation, reflecting changes in hemostasis. It has the advantages of considering the various elements in blood that contribute to thrombus formation and being a point-of-care test, unlike most laboratory test. It has a recommended value for anticoagulation during “on-pump” cardiac surgery, albeit based on limited data.

However, as mentioned earlier, there is still few evidence to support the ideal ACT value after protamine administration, and which should be the final ACT value at the end of cardiac surgery. So far, matching of final ACT values to those before heparin administration has been the most widely used strategy. Wang et al. have already found some data suggesting that a Final ACT value that is lower than the basal ACT might lead to less bleeding and post-operative transfusions. With this in mind, we used patient’s basal and Final ACT values to test whether there was any relation between Final ACT being higher or lower than basal ACT and the risk of bleeding. Similarly, we have compared postoperative bleeding and the use of transfusions between patients with a final ACT lower or higher than the used reference value of 140 seconds (W. Wang et al., 2018).

We found that the final ACT value is not a good predictor for significant bleeding (defined as bleeding ≥ 600 ml at 12 hours postoperatively [Dyke et al., 2014]), regardless of comparing final ACT to the patient’s basal ACT or to the fixed 140 seconds reference value. Nevertheless, there was a major difference in bleeding between patients who had final ACT values ≥ 140 seconds and those that had final ACT values < 140 seconds, being statistically significant. On average, a patient with a final ACT ≥ 140 seconds bled 99.62 ± 36.39 ml more than a patient with a final ACT < 140 seconds in the first 12 hours, and 104.99 ± 36.39 ml more in the first 24 hours after surgery. We also found that, even though the bleeding rate in the first 12 hours is higher in patients that had a final ACT ≥ 140 seconds, bleeding rate between 12-24 hours after surgery is almost the same between both groups of patients. This suggests that differences in bleeding that are possibly related to final ACT are most evident during the first 12 hours after surgery.

Even though final ACT value does not predict the occurrence of significant bleeding, we found that, according to our model, it is a predictor of the need for transfusion, with the risk of transfusion use increasing 1.021 times with each second in Final ACT. Furthermore, we have found that patients with a final ACT ≥ 140 seconds have a risk of transfusion 1.81 times higher than patients with a final ACT < 140 seconds.

Therefore, having into account the fact that bleeding and transfusions have a strong impact on patient morbimortality, we believe that a tight monitoring on final ACT should be the standard of care, with evidence supporting that values under 140 seconds might be safer and lead to less post-surgical bleeding and lower PRBC use. Additional prospective multi-center studies with larger cohorts of patients might help determine the ideal final ACT. Moreover, the fact that the final ACT is a good predictor of the need for transfusion, but not a good predictor for significant bleeding was surprising. This might be explained due to our chosen cut-off of 140 seconds for the ACT value; further studies with different cut-off values might lead to different conclusions.

This study has several limitations. This is a retrospective, observational, single-center study, which may limit the generalization of our findings. Observational studies are open to confounders and bias. The results from our study may not be extrapolated to other different populations. The evidence found on ACT might vary depending on the machines used, since different machines use different methods for clot activation and detection, possibly leading to considerable variability in ACT measurements and to the impossibility of values from different machines being used interchangeably. The number of patients included may be another limitation, however more than seven hundred were included. Independently of the absolute or relative numerical values of ACT in different machines and settings, we had results to support the conclusions that follow herein.

Conclusion

In conclusion, although Final ACT values are not a good predictor for significant bleeding, they have a good predictive value for the use of transfusions, with patients with Final ACT ≥ 140 seconds having a higher risk of transfusion when compared to those with Final ACT value < 140 seconds. Moreover, major differences in postoperative bleeding were found between patients who have a Final ACT ≥ 140 seconds *vs* Final ACT value < 140 seconds, with notable distinction in bleeding rate in the first 12h after surgery. The risk of bleeding and transfusion use is higher the longer the time under CPB and the lower the values of preoperative hematocrit. Males have a higher post-operative risk of bleeding, but females have a higher risk of transfusion use. Surgery timing influences transfusion use, with patients undergoing urgent surgery at higher risk for the use of PRBC.

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