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**THE IMPORTANCE OF THE NEW
HB/KG INDEX IN THE ASSESSMENT
OF PERIOPERATIVE BLOOD LOSS
IN CARDIAC SURGERY AND ITS
ASSOCIATION WITH COAGULATION
STATUS AND GENE POLYMORPHISMS**

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**NAUJOJO HB/KG INDEKSO SVARBA
VERTINANT PERIOPERACINĮ KRAUJO
NETEKIMĄ ŠIRDIES CHIRURGIJOJE IR
JO RYŠYS SU KREŠĖJIMO SISTEMOS
BŪKLE BEI GENŲ POLIMORFIZMAIS**

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LIST OF ABBREVIATIONS

ACT	–	activated clotting time
ADP	–	adenosine diphosphate
Adj. R²	–	adjusted coefficient of determination
AKI	–	acute kidney injury
ALT	–	alanine transaminase
ANCOVA	–	analysis of covariance
ANOVA	–	analysis of variance
AST	–	aspartate transaminase
AUC	–	area under the curve
aPTT	–	activated partial thromboplastin time
AV	–	atrioventricular
BMI	–	body mass index
BSA	–	body surface area
CABG	–	coronary artery bypass grafting
CBC	–	complete blood count
CI	–	confidence interval
CPB	–	cardiopulmonary bypass
CT	–	clotting time
CTD	–	chest tube drainage
DM	–	diabetes mellitus
DNN	–	deep neural network
DT	–	decision tree
EBV	–	estimated blood volume
EXTEM	–	extrinsic pathway test
FDR	–	false discovery rate
FFP	–	fresh frozen plasma
FIBTEM	–	fibrinogen-specific test
Hb	–	haemoglobin
Hb/kg Index	–	haemoglobin mass loss per kilogram of patient weight
Hct	–	haematocrit
HEPTEM	–	heparinase-modified thromboelastometry test
HR	–	hazard ratio
ICU	–	intensive care unit
INR	–	international normalized ratio
INTEM	–	intrinsic pathway test

IQR	– interquartile range
kNN	– k-nearest neighbours
LR	– linear regression
Lasso	– least absolute shrinkage and selection operator least squares
Lasso-OLS	– least absolute shrinkage and selection operator with ordinary
LTA	– light transmission aggregometry
LVEF	– left ventricular ejection fraction
MAPE	– mean absolute percentage error
MCF	– maximum clot firmness
ML	– machine learning
MSE	– mean squared error
NPV	– negative predictive value
NYHA	– New York Heart Association
OPCABG	– off-pump coronary artery bypass grafting
OR	– odds ratio
PA	– platelet aggregometry
PAI-1	– plasminogen activator inhibitor type 1
PLTEM	– platelet component of clot firmness (EXTEM – FIBTEM)
POC	– point-of-care
PPV	– positive predictive value
PRBC	– packed red blood cells
PT	– prothrombin time
RBC	– red blood cells
ROC	– receiver operating characteristic
ROTEM	– rotational thromboelastometry
SD	– standard deviation
SLT	– standard laboratory tests
SNP	– single nucleotide polymorphism
SVM	– support vector machine
VET	– viscoelastic testing
XGBoost	– extreme gradient boosting

INTRODUCTION

Cardiovascular diseases remain the leading cause of mortality in the European Union, which accounts for more than 1.6 million deaths annually. They comprise 33% of all deaths [1]. Although coagulation diagnostics and management have improved over the years, perioperative bleeding remains a significant problem in cardiac surgery [2, 3]. Excessive bleeding remains a significant clinical burden with a reported incidence ranging from 8% to 15% [4, 5]. The reoperation rate due to bleeding is between 2.3% and 8% [6]. Re-exploration is an independent predictor of adverse outcomes, including stroke, sternal wound infection and haemodialysis [7], which is associated with higher morbidity and mortality [8, 9]. A study of 798 surgical departments in the United States found that the percentage of patients requiring a blood transfusion ranged from 8% to 93% [10], significantly increasing costs and expenses from a financial perspective [11].

Despite its clinical relevance, there is no universally accepted method for quantifying and classification of blood loss [12]. Several classification systems have been developed based on chest tube volume, blood transfusions, or reoperations. For instance, the Kirklin and Barratt-Boyes criteria recommend immediate surgical re-exploration if the chest tube drainage (CTD) exceeds 5 mL/kg in any single hour or 3 mL/kg/hour for three consecutive hours [13]. Some authors define active bleeding as blood loss exceeding 1.5 mL/kg/hour for six consecutive hours [14], while others suggest blood loss of more than 200mL within 1 hour [9]. In contrast, the clinical protocol at Massachusetts General Hospital uses weight-adjusted volume guidelines (mL/kg) to trigger intervention [15]. Although these tools have prognostic value, they share significant limitations. Visual estimation of CTD is inaccurate, because the fluid in the CTD contains a mixture of blood and body liquids. Thus, it can be under- or overestimated [9, 14, 16, 17]. Moreover, these methods ignore patient weight. For instance, the same 1000-mL blood loss has markedly different haemodynamic implications for a 60 kg patient compared to 100 kg.

The etiology of bleeding in cardiac surgery is multifactorial: approximately one third of cases are related to surgical sources, while remainder are related to coagulation disorders [18]. CPB triggers significant haemostatic disturbances leading to coagulopathy [19]. This typically results from haemodilution due to bypass circuit priming, consumption of coagulation factors, tissue factor activation of the extrinsic system, and contact activation of the intrinsic system on circuit components [19–21]. In addition, residual unfractionated heparin contributes to haemostatic disorders [22, 23]. Together, these combined factors lead to complex and dynamic alterations in haemostasis [24].

Pathophysiologically, the balance between bleeding and normal haemostasis is determined by the interaction of thrombin generation, platelet activation and aggregation, fibrin clot formation, and the activity of the fibrinolytic system [25]. Increasing evidence suggests that fibrinolysis plays a crucial role, particularly in patients undergoing cardiac surgery with CPB [26]. Postoperative bleeding has been shown to correlate with fibrinogen concentration and platelet function, both of which are independent predictors of haemostasis and bleeding [27, 28].

While standard laboratory tests (SLT) have traditionally guided perioperative bleeding management, their clinical utility is limited by significant turnaround times required for plasma separation [29]. The Clauss method quantifies a fibrinogen, but does not capture the functional adequacy of clot formation [30]. Similarly, a standard platelet count provides only a quantitative measure and cannot detect qualitative dysfunction induced by antiplatelet therapy or extracorporeal circulation [31, 32]. Due to the limitations, interest in point-of-care (POC) tests has grown. The measurement of the viscoelastic properties of clot formation using viscoelastic testing (VET) reduces the need for blood transfusions by up to 50% [33]. VET provides the determination of the timing of clot formation, its quality, quantity and stability. It also assesses the overall level of hemostasis without the influence of heparin, which is extremely important after surgery under CPB [33]. In case of assessment of platelet reactivity, the light transmission aggregometry (LTA) remains the gold standard [34]. Some authors found that perioperative assessment of platelet aggregation (PA) predicts the risk of significant bleeding in cardiac surgery patients [35].

Genetic factors influence the variability of blood loss after cardiac surgery. Increasing evidence suggests that integrating genetic profiles with clinical data significantly improves the predictive power for bleeding risk compared to clinical data alone [36]. Most reactions in the coagulation process take place on the surface of platelets and fibrinogen receptors [37, 38]. In addition, plasminogen activator inhibitor 1 (PAI-1) plays a pivotal role by inhibiting fibrinolysis through the suppression of the conversion of plasminogen to plasmin [39, 40]. Therefore, genetic polymorphisms affecting platelet receptors and fibrinogen receptors, PAI-1 represent significant biomarkers potentially associated with increased bleeding susceptibility.

Despite significant advances in haemostatic monitoring, it remains difficult to predict who will experience significant blood loss and who will not. To address this gap, this work introduces a multimodal diagnostic framework that integrates the novel Hb/kg Index with machine learning-based stratification and comprehensive analysis of clinical, haemostatic, and genetic factors influencing blood loss in cardiac surgery.

1. THE AIM AND OBJECTIVES OF THE STUDY

1.1. The aim of the study

To develop and validate the Hb/kg Index as a novel method for quantifying perioperative blood loss in cardiac surgery and to evaluate its associations with haemostatic and genetic determinants of blood loss severity.

1.2. The objectives of the study

1. To develop and validate a new calculation method of actual blood loss in terms of haemoglobin mass loss per patient weight (Hb/kg Index);
2. To validate machine learning-derived clusters (Mild, Moderate and Severe) based on the Hb/kg Index.
3. To evaluate the perioperative dynamics of standard coagulation tests, platelet aggregometry (PA) and viscoelastic testing (VET) parameters across the Hb/kg Index-based clusters.
4. To analyze perioperative plasma concentrations of plasminogen activator inhibitor-1 (PAI-1) and evaluate their association with perioperative blood loss.
5. To investigate the associations of *ITGB3* (rs5918), *FGB* (rs1800790), and *SERPINE1* (rs2227631) single nucleotide polymorphisms (SNPs) with the severity of blood loss and to evaluate their clinical value in bleeding risk assessment in cardiac surgery.

1.3. Working hypothesis

The working hypothesis of this study is that perioperative blood loss in cardiac surgery can be quantified more precisely using the Hb/kg Index (haemoglobin mass loss per kilogram of body weight) compared to traditional visual estimations or volume-based methods. It was hypothesized that the Hb/kg Index offers an objective way to classify patients into blood loss clusters, where each of them is associated with distinct hemostatic profiles, perioperative dynamics, and clinical features. These groups are expected to be primarily associated with VET parameters rather than standard coagulation tests or PA. The severity of blood loss is affected by genetic factors related to fibrinogen synthesis (*FGB*), platelet function (*ITGB3*), and fibrinolysis regulation (*SERPINE1*), which determine a patient's unique risk profile for perioperative bleeding.

1.4. Scientific novelty of the study

The scientific novelty of this research based on the development of a novel approach for calculating actual blood loss – the Hb/kg index (haemoglobin mass loss per kilogram of patient weight) as a prognostic value.

First, the Hb/kg Index has been developed and validated as an objective and physiologically relevant metric for quantifying actual perioperative blood loss. The main components of the Index are patient weight, intraoperative haemoglobin mass loss, haemoglobin mass loss via chest tube drainage (CTD) and red blood cell (RBC) transfusion. It was demonstrated that the Hb/kg Index is highly predictable using multivariable modelling approaches. Among all evaluated models, Lasso OLS demonstrated the best overall performance, with the lowest mean squared error (0.08 ± 0.04) and mean absolute percentage error (0.18 ± 0.10), as well as the highest correlation (0.92 ± 0.06) and R^2 score (0.82 ± 0.13). These findings indicate that perioperative blood loss, quantified by the Hb/kg Index, is highly structured and explained by clinically relevant variables, confirming the robustness and physiological validity of the proposed model.

Second, this work presents a new approach to blood loss stratification using unsupervised machine learning (ML) algorithms (K-medoids). The analysis identified three distinct blood loss clusters (Mild, Moderate, and Severe), which are defined by objective, data-driven the Hb/kg Index thresholds of 1.72 and 2.10. Validation using ROC analysis showed that the model effectively distinguishes extreme phenotypes. The “Severe” cluster demonstrated the strongest discriminative performance (AUC = 0.790; 95% CI 0.721–0.859). Importantly, CTD volume did not differ significantly between clusters ($p = 0.097$), whereas haemoglobin mass loss via CTD showed a significant effect ($p = 0.011$), highlighting the limitations of visual or volume-based methods.

Third, VET demonstrated that fibrinogen-dependent clot firmness (FIBTEM A10 and MCF) is the main factor influencing blood loss severity. FIBTEM A10 (12mm) demonstrated excellent diagnostic performance for postoperative hypofibrinogenaemia (< 2.5 g/L) and is a key determinant of blood loss severity in cardiac surgery (AUC = 0.888). In contrast, SCTs and PA showed limited discriminatory value across the clusters.

Fourth, after developing and validating the Hb/kg Index, this study applied the Hb/kg Index-based blood loss clusters to evaluate perioperative plasminogen activator inhibitor-1 (PAI-1) plasma concentration as a marker of fibrinolytic response. This provided a novel opportunity to examine the relationship between fibrinolysis and objectively quantified blood loss severity.

Finally, this study provides an integrated evaluation of a targeted SNP panel representing key pathways of haemostasis: platelet aggregation (*ITGB3* rs5918), fibrinogen synthesis (*FGB* rs1800790), and fibrinolysis regulation (*SERPINE1* rs2227631) in relation to perioperative blood loss severity and predictive value in cardiac surgery. Although *FGB* (rs1800790) significantly influenced fibrinogen levels, the overall contribution of the investigated SNPs to actual blood loss severity was limited.

2. LITERATURE REVIEW

2.1. Epidemiology and impact of bleeding in cardiac surgery

Cardiovascular diseases remain the leading cause of death in the European Union, accounting for more than 1.6 million deaths annually, which represents approximately 33% of all mortality [1]. Given an optimal cardiac surgery rate of 1,000–1,200 open heart procedures per million population in high-income countries [41], the Department of Cardiac Surgery at Kauno Klinikos performs approximately 1,000 surgeries with cardiopulmonary bypass (CPB) each year [42]. Although the incidence of massive bleeding after cardiac surgery has decreased in recent decades, postoperative bleeding following CPB remains a common clinical challenge [3, 14]. Excessive bleeding significantly increases re-operation rates, the need for blood transfusions, the length of hospital stay and overall healthcare costs [7]. Furthermore, it has a negative impact on morbidity and mortality [8, 9, 43], with studies reporting up to an eight-fold increase in the risk of death [44]. While red blood cell transfusion is required in up to 93% of cases [10], it imposes a significant economic burden [11, 45]. While the average blood loss after cardiac surgery is approximately 300 ml, the impact of this volume loss varies significantly between patients weighing 80 kg and those weighing 120 kg. Excessive bleeding in cardiac surgery occurs in 8% to 15% of patients [4, 8, 46]. In addition, massive bleeding and major blood transfusion are independent risk factors for acute kidney injury (AKI) and liver dysfunction [47]. They are also associated with deep sternal wound infection and sepsis [48, 49]. The etiology of bleeding in cardiac surgery is multifactorial [19]. Bleeding may result from surgical sources, CPB-associated coagulopathy, or a combination of both. Surgical trauma, skin incision, cardiectomy suction, haemodilution, hypothermia, and anticoagulation contribute to haemostatic activation and impairment during CPB. In contrast, technical surgical sources include anastomotic rupture or incompetence and leakage from anastomosis or cannulation sites, which can be corrected surgically. Surgical sources account for approximately two-thirds of bleeding cases [18], and are identified in up to 67% of patients undergoing re-exploration [50–52]. CPB-associated coagulation disorders occur in approximately 11% of patients [53].

2.2 Definition of bleeding and re-exploration

While mild postoperative bleeding is clinically insignificant, severe bleeding after cardiac surgery is associated with catastrophic outcomes [43, 44, 54]. Despite this clinical relevance, there is no “gold standard” method for

quantifying actual blood loss [12]. In clinical routine, assessment of bleeding is commonly based on visual estimation of CTD volume [16, 55]. However, this method has a significant limitation. As CTD fluid is a heterogeneous mixture of whole blood, plasma, and serous fluid, it does not accurately reflect true haemoglobin loss [9, 14, 16, 17]. Moreover, visual estimation of CTD has been shown to systematically underestimate actual blood loss compared with more precise formula-based methods [56, 57].

The definition of active bleeding varies in the literature. Some authors define it as blood loss exceeding 1.5 mL/kg/hour over six consecutive hours [14], while others suggest blood loss of more than 200 mL within one hour [9]. Furthermore, the established Kirklin and Barratt-Boyes criteria recommend immediate surgical re-exploration if the CTD exceeds 5 mL/kg in any single hour or 3 mL/kg/hour for three consecutive hours [13]. These definitions often determine the decision to return to the operating theatre for re-sternotomy. Despite the significant burden of perioperative bleeding, the development of a universally accepted definition remains a major challenge [12, 16]. To address variation in individual study definitions, major international consensus groups have established standardized classification systems [58–60]. The Universal Definition of Perioperative Bleeding (UDPB) classifies bleeding severity into five classes based on delayed sternal closure, excessive chest tube output, and the use of haemostatic therapies [58]. Similarly, the Bleeding Academic Research Consortium (BARC) criteria provide a hierarchical grading system, where Type 4 relates to bleeding in the case of CABG, defined by severe complications, including intracranial haemorrhage, reoperation, or transfusion of five or more units of red blood cells within 48 hours [60]. Finally, The E-CABG classification provides a practical grading system (Grades 0–3) that emphasises clinically relevant outcomes, particularly transfusion requirements and the need for surgical re-exploration [59].

Re-exploration (re-sternotomy) for bleeding is a strong independent risk factor for adverse outcomes after cardiac surgery [61]. Systematic review and large meta-analyses ($n = 557,923$) report that a surgical source of bleeding is identified in 34% to 66% of patients requiring re-sternotomy [62, 63]. Re-exploration significantly increases the risk of post-operative mortality and morbidity (OR 3.27; 95% CI 2.44–4.37; $p < 0.00001$) [64]. In addition, a study of 16,793 adult cardiac surgery patients showed that re-exploration for bleeding is a lethal complication with a detrimental effect greater than any other known potentially modifiable risk factor [65]. However, despite its clinical relevance, re-exploration is a decision-dependent outcome influenced by institutional protocols and surgeon experience; therefore, it cannot be considered a fully objective or standardised measure of bleeding

severity. Taken together, current definitions of bleeding in cardiac surgery are heterogeneous, indirect, and based on visual estimation of chest tube drainage. None of the existing approaches provides a physiologically and scientifically grounded quantitative assessment of actual haemoglobin loss, highlighting a critical gap in perioperative blood loss evaluation.

2.3. Classical physiology of the coagulation (coagulation cascade)

Hemostasis is the normal process by which the coagulation cascade seals vascular damage to limit blood loss after injury [66]. It is a complex process that depends on the interaction of platelets, fibrinogen, plasma coagulation cascades, fibrinolytic proteins, blood vessels, and cytokine mediators [67], which form a clot with platelets and fibrin polymer (Figure 1). Once the injury begins to heal, the clot slowly remodels and dissolves with the restoration of normal tissue at the site of injury [66].

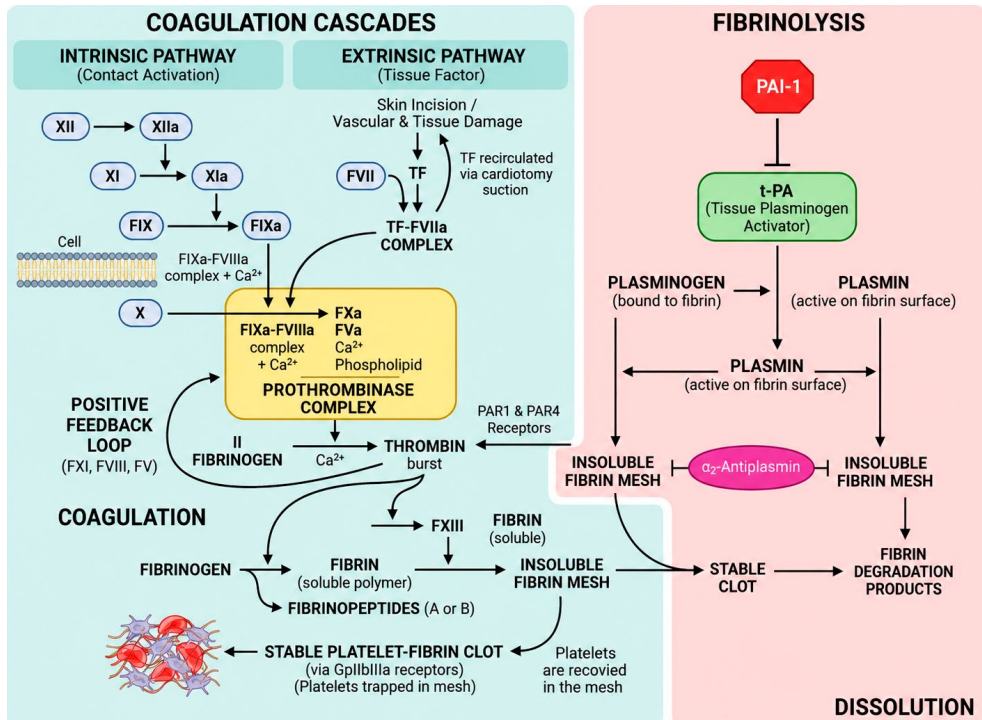


Fig. 1. Mechanisms of haemostasis: coagulation cascade, thrombin generation, and fibrinolysis pathways

There are two main components of hemostasis: primary and secondary. Primary hemostasis refers to platelet aggregation and the formation of platelet

plugs. The platelets are activated in a multi-sided process and as a result adhere to the injury site and to each other, sealing the injury.

Secondary hemostasis refers to the deposition of insoluble fibrin formed by the proteolytic coagulation cascade. This insoluble fibrin forms a mesh that is incorporated into and around the platelet plug. This network serves to strengthen and stabilize the blood clot [25]. The coagulation cascade is represented by various intrinsic and extrinsic pathways [67].

The intrinsic pathway (contact activation) begins after contact activation of the blood by contact of the blood with the foreign surface of the extracorporeal circuit [68]. This pathway includes Hageman factor (FXII), factor XI, factor IX and factor VIII. The process is initiated when factor XII comes into contact with a foreign surface of the cardiopulmonary bypass system and is activated to factor XIIa [25]. Subsequently, factor XIIa activates factor XI to factor XIa, and factor XIa activates factor IX to factor IXa. Factor IXa works together with activated factor VIIIa to activate factor X. Once the FIXa-FVIIIa complex and calcium ions activate factor X, the cascade continues along the common pathway [69].

The extrinsic pathway (tissue factor) corresponds to the initiation phase of coagulation. It is triggered when damaged vascular system and tissue (starting from a skin incision) increases the release of TF into the bloodstream [69]. Factor VIIa binds to tissue factor, and this complex activates factor X. Usually, the body limits this process through tissue factor pathway inhibitor (TFPI) and antithrombin (AT), which neutralise activated factors Xa and thrombin. However, when a high concentration of tissue factor is released, the process overcomes inhibition [70]. Moreover, the blood in the wound is exposed to air and TF and is recirculated via the cardiotomy suction during surgery under CPB [68]. The complex of TF and factor VIIa activates factor X and factor IX. Factor IXa also activates factor X, in the presence of its cofactor factor VIIIa. Factor Xa, also in the presence of its cofactor factor Va, then activates prothrombin to generate thrombin [25]. Both pathways converge to a common pathway at the FXa/Fva (prothrombinase) complex level with calcium ions that split prothrombin to thrombin. This initiates the Promotion (or Propagation) phase. Thrombin generated by the extrinsic pathway activates nearby adherent platelets via PAR1 and PAR4 protease receptors. These thrombin-activated platelets release partially activated factor V and bind factors XI and VIII, creating a positive feedback loop that significantly amplifies thrombin generation [70]. In addition, the combination of enzyme, cofactor, calcium, and phospholipid surface increases the speed of these reactions several thousand-fold [71]. This leads to an explosive increase in thrombin production, which is sufficient to form fibrin from fibrinogen [72]. Finally, the fibrin monomers polymerize and trap the blood cells, forming the

clot. The platelets are bound together via their fibrin receptor glycoprotein IIb/IIIa (GpIIb/IIIa) in this platelet-fibrin clot. Fibrinolysis is the process that leads to the dissolution of the clot. This process is catalysed by plasmin, formed from plasminogen through the activation of tissue plasminogen activator (tPA) [73]. As with thrombin formation, either the intrinsic or extrinsic pathway can trigger fibrinolysis. The fibrinolysis pathway also plays an important role in hemostasis. Pathological thrombus formation, also known as thrombosis, or pathological bleeding can occur if this process is disrupted [25]. In the context of cardiac surgery, especially during CPB, the balance of whole coagulation system is profoundly disrupted. In addition, surgical trauma triggers further release of TF into the bloodstream, activating the extrinsic coagulation pathway. As a result, perioperative bleeding in cardiac surgery is caused by multiple factors and cannot be explained solely by abnormalities in individual components of the coagulation cascade.

2.4. Key components of haemostasis

The balance between normal haemostasis and bleeding is strongly influenced by platelet function and fibrinolytic activity [26]. Therefore, platelet dysfunction and excessive fibrinolysis play a pivotal role in the development of coagulopathy, a major cause of postoperative bleeding [21].

2.4.1 Fibrinogen and fibrin formation

The major protein of the coagulation system is fibrinogen [74]. Fibrinogen (coagulation factor I) is a key soluble protein synthesised in the liver, with a plasma half-life of 3 to 5 days [75]. In healthy individuals, plasma concentrations typically range from 2.0 to 4.5 g/l [76]. Fibrinogen has a dual role in haemostasis: it serves as a ligand for platelet aggregation and as a substrate for fibrin formation [77]. In primary haemostasis, fibrinogen promotes platelet aggregation by binding to glycoprotein GPIIb/IIIa receptors on the platelet surface (Figure 2). This bridging interaction leads to the formation of the initial platelet plug at the site of vascular injury [78]. In secondary haemostasis, fibrinogen acts as the final substrate of the coagulation cascade. Thrombin cleaves fibrinogen into fibrin monomers, which polymerise into fibrin fibres and are subsequently stabilised by activated factor XIII (FXIIIa), forming an insoluble fibrin meshwork. This mesh provides the structural backbone of the clot, ensuring mechanical strength and resistance to premature fibrinolysis [75, 79]. Because it is consumed in both platelets plug formation and fibrin mesh generation, fibrinogen is rapidly depleted during surgical procedures, making it a critical and often rate-limiting factor in perioperative haemostasis [80]. Alterations in plasma fibrinogen levels

may result in impaired coagulation, increased blood viscosity, reduced blood velocity, and stimulation of platelet aggregation and adhesion [81, 82]. During CPB, fibrinogen concentration decreases by approximately 30–36%, primarily due to haemodilution, with additional contributions from consumption and degradation. This reduction typically reaches its nadir immediately after protamine administration [24, 83, 84]. As fibrinogen levels fall, clot structure weakens and becomes more susceptible to fibrinolysis, significantly increasing the risk of bleeding. Clinically, low fibrinogen levels are independently associated with severe postoperative bleeding [85, 86]. Both preoperative and, more importantly, post-CPB fibrinogen concentrations have been shown to predict bleeding risk and the need for surgical re-exploration. Notably, post-CPB fibrinogen levels show stronger associations with bleeding outcomes than baseline values, highlighting the dynamic nature of haemostatic impairment during cardiac surgery [87].

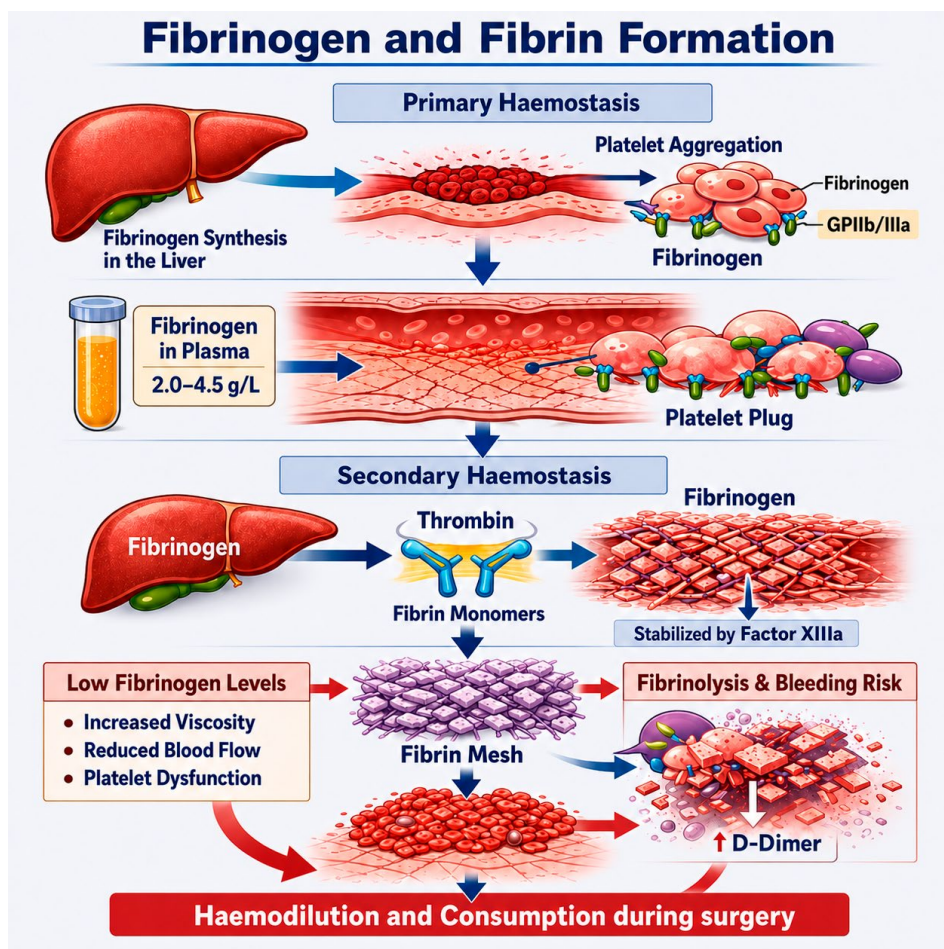


Fig. 2. Fibrinogen dynamics: synthesis, haemostatic function, and mechanisms of consumption

2.4.2. Platelets

Platelets are essential cellular mediators of haemostasis that act synergistically with fibrinogen; the formation of the platelet plug is closely integrated with the coagulation cascade [88]. In primary haemostasis, platelets adhere to the site of injury, become activated and aggregate. Clot consolidation relies on platelet surface receptors, as fibrinogen binds to GPIIb/IIIa receptors on activated platelets, bridging them together. This interaction integrates the platelet plug with the developing fibrin mesh, creating a stabilised seal at the site of vascular injury [89]. However, during CPB, not only haemodilution, but both platelet activation and consumption on the extracorporeal circuit surface

significantly alter platelet function. This leads to quantitative thrombocytopenia and qualitative platelet dysfunction, contributing to coagulopathy [90, 91]. Platelet count decreases by approximately 40–50% during CPB, primarily due to haemodilution, with additional contributions from consumption and sequestration [83, 92, 93]. Although reduced platelet count is associated with increased postoperative blood loss, quantitative changes alone do not fully explain bleeding risk, underscoring the limited predictive value of platelet count in isolation [94, 95]. More importantly, CPB induces significant platelet dysfunction through multiple mechanisms. Contact with artificial surfaces and shear stress within the extracorporeal circuit lead to platelet activation, degranulation (e.g., β -thromboglobulin release), and subsequent functional exhaustion [90, 96, 97]. In parallel, activation of plasma protease systems, including coagulation, fibrinolytic, and inflammatory pathways, results in thrombin and plasmin generation, further modulating platelet activity [98, 99]. These alterations are compounded by hypothermia, acidosis, and preoperative use of antiplatelet agents [100, 101]. Functionally, platelets exhibit reduced responsiveness to agonists and impaired aggregation capacity during and immediately after CPB, with reductions in ADP-induced aggregation of up to 40–55% reported [102, 103]. Importantly, bleeding time is often prolonged beyond what would be expected based on platelet count alone, underscoring the predominance of qualitative defects. The severity of platelet dysfunction correlates with the duration of cardiopulmonary bypass. Platelet function typically recovers within hours after CPB; however, this transient dysfunction is clinically critical, as effective haemostasis in the early postoperative period depends heavily on platelet activity [102]. Despite widespread platelet dysfunction, excessive bleeding occurs only in some patients, reflecting the multifactorial nature of CPB-associated coagulopathy and the interaction between platelet abnormalities, fibrinogen depletion, and fibrinolysis [91]. However, the pathogenesis of CPB-induced platelet dysfunction remains incompletely understood. Available evidence suggests that only a minority of platelets become activated, while the majority continue to circulate with reduced function [104]. Thus, the mechanisms by which haemostasis is partially maintained despite CPB-induced platelet injury are still unclear and may involve compensatory mobilisation of platelets from spleen and other endogenous reserves or preservation of functionally competent circulating platelets.

2.4.3. Fibrinolysis and D-dimer

While fibrinogen is the substrate for clot formation, D-dimer is generated as a direct result of fibrinolytic degradation of a stable fibrin clot and represents

the final measurable endpoint of fibrin clot breakdown. Thrombin converts fibrinogen into fibrin monomers, which are then cross-linked by activated Factor XIII (FXIIIa) to form a stable meshwork. When the fibrinolytic system is activated, plasmin cleaves this cross-linked fibrin network [105]. D-dimer is a specific degradation product produced exclusively from the breakdown of FXIIIa-cross-linked fibrin. Unlike general fibrin degradation products, the presence of D-dimer specifically indicates that both thrombin generation (clot formation) and plasmin activation (fibrinolysis) have occurred [106]. Thus, D-dimer is a specific degradation product of cross-linked fibrin and serves as a key biomarker for assessing fibrinolytic activity [107]. Overall, fibrinogen depletion, platelet dysfunction, and activation of fibrinolysis represent interdependent processes that collectively determine haemostatic competence during cardiac surgery with CPB.

2.5. The influence of cardiopulmonary bypass (CPB) on coagulation

The invention of the heart-lung machine (CPB) was one of the greatest achievements of the 20th century, making it possible to develop new methods for treating all heart diseases [108]. During cardiac surgery, CPB maintains systemic perfusion and gas exchange via an extracorporeal circuit, providing a motionless and bloodless operative field. Venous blood is diverted, oxygenated, and returned to the arterial system, allowing controlled support of organ function [109]. While the safety of CPB continues to improve over time, the risk of coagulopathy and bleeding remains a clinical burden [21]. CPB-induced coagulopathy develops from the combined effects of haemodilution, hypothermia, consumption of coagulation factors, and activation of both intrinsic and extrinsic coagulation pathways. In addition, surgical trauma and cardiotomy suction further amplify coagulation cascade activation through tissue factor release [20, 21].

The haemostasis system faces a “double hit” from two simultaneous physiological stressors: haemodilution and hypothermia [110, 111]. Haemodilution significantly reduces the concentration of coagulation factors, impairing thrombin generation and clot formation. Hypothermia further exacerbates haemostatic dysfunction by impairing both the enzymatic activity of coagulation factors and platelet function. While mild hypothermia primarily affects platelet adhesion and aggregation, deeper temperature reduction significantly slows enzymatic reactions [89], [92]. CPB is typically performed under systemic hypothermia (25–32°C) or normothermic conditions to provide neuroprotection [112]. The impact of temperature is specific: while mild hypothermia (33–37°C) primarily impairs platelet adhesion and aggregation, temperatures below 33°C significantly slow the

enzymatic activity of coagulation factors. This deceleration prolongs the initiation phase and reduces the rate of thrombin generation [111, 113]. Clinically, this creates a “diagnostic trap”: standard laboratory assays (aPTT, PT) are typically performed at a standardised 37°C, which often masks the true severity of the coagulopathy at the patient’s actual systemic temperature [114].

After discontinuation of CPB, on average, fibrinogen concentration decreases by 36%, absolute platelet count by 45%, and platelet function by 50% to 70%, while PT and INR increase by 33% and PTT by around 20% [24]. The activity of coagulation factors is reduced by 24–47% [83]. Among all haemostatic components, fibrinogen is the most vulnerable factor, often reaching critically low levels (<1.5–2.0 g/L). This depletion leads to the formation of structurally weak and unstable clots that are highly susceptible to fibrinolysis [24].

2.6. Heparin rebound

Heparin rebound, defined as the recurrence of anticoagulant activity after adequate neutralisation with protamine sulphate, has been a recognised phenomenon in cardiac surgery for over half a century. The term was first introduced by Hyun et al., who described the reappearance of hypocoagulability in the early postoperative period [115]. Later, Gollub et al. confirmed these findings in “Heparin rebound in open heart surgery”, demonstrating that postoperative prolongation of clotting times could be corrected by administering additional protamine, thus linking the phenomenon directly to residual heparin activity [116]. Despite its long-standing recognition, the reported incidence of heparin rebound varies widely, from as low as 4% to as high as 100%, depending on the sensitivity of the detection method and the specific anticoagulation protocols used [117, 118]. The mechanisms driving heparin rebound are complex. Heparin binds not only to antithrombin (AT) but also non-specifically to plasma proteins, endothelial cells, and extracellular matrix proteins [23]. Research indicates that this non-specific binding creates a physiological “reservoir”. During the initial reversal, protamine effectively neutralises the circulating free heparin; however, over time, the sequestered heparin dissociates from proteins and tissues, re-enters the circulation, and causes a delayed anticoagulant effect [23, 119]. Furthermore, endothelial cells actively bind and internalise heparin, subsequently resecretory it into the circulation. This reservoir effect is exacerbated by CPB-induced inflammation, which releases acute-phase proteins that also bind heparin. While protamine’s shorter half-life was implicated in rebound, modern data suggest this is secondary to tissue redistribution [120, 121]. Reliably

detecting this rebound remains a significant clinical challenge. The traditional “gold standard” method (ACT) lacks sensitivity at low concentrations. Studies have highlighted its high variability and poor correlation with post-CPB plasma heparin levels [122, 123]. Hardy et al. and Ichikawa et al. demonstrated this discrepancy by detecting circulating heparin in 100% of patients despite complete neutralization [22, 124]. In contrast, viscoelastic testing appears superior for detection of residual anticoagulation. Mittermayr et al. demonstrated high sensitivity using the thromboelastometry (ROTEM) HEPTM/INTEM ratio [125, 126], a finding validated by Ichikawa et al., who reported a strong correlation ($r = 0.72$) with plasma heparin, significantly outperforming ACT and aPTT [22]. The clinical impact of this biochemical rebound remains debated. Teoh et al. showed that although continuous postoperative protamine infusion almost eliminated rebound, it reduced chest tube drainage by only 13% and did not decrease transfusion requirements [23]. These results are consistent with findings by Despotis et al. and Karkouti et al., who suggest that bleeding is multifactorial, primarily caused by platelet dysfunction and hyperfibrinolysis rather than residual heparin alone [127, 128]. Given the ongoing debate about the clinical significance of heparin rebound, further investigation using sensitive viscoelastic methods is warranted to clarify its role in postoperative blood loss.

2.7. Assessments of haemostasis in cardiac surgery

2.7.1. Standard laboratory tests (SLTs)

To manage perioperative bleeding, standard laboratory tests (SLTs) including PT, aPTT, fibrinogen level measured by Clauss method, and platelet count are commonly used. Except for the platelet count, these tests require plasma separation, resulting in a turnaround time of 30 to 90 minutes [29]. However, the limitations of conventional assays extend beyond turnaround time. The assessment of primary haemostasis remains a critical challenge, as SLTs do not fully reflect platelet function [129]. While the Clauss method provides a quantitative assessment of fibrinogen, it fails to capture the functional quality of clot formation [30]. Similarly, a standard platelet count offers only a quantitative measure and cannot detect qualitative dysfunction induced by antiplatelet therapy or extracorporeal circulation [31, 32]. In patients undergoing cardiac surgery, a delicate balance must be maintained between anticoagulation and stable haemostasis after weaning from CPB. Various disorders, including platelet dysfunction and hyperfibrinolysis, may lead to uncontrolled bleeding and poor outcomes [130]. According to the literature, there is a growing tendency to show that standard coagulation tests

have failed to monitor hemostasis and to predict increased bleeding risk [131, 132].

2.7.2. Point-of-care testing: Platelet aggregometry

Today, clinicians can choose from various POC methods designed to rapidly identify specific coagulation disorders. Unlike SLTs, POC methods are used directly at the bedside, in the operating theatre, intensive care unit, eliminating transport and processing delays. This significantly shortens the time from blood sampling to result interpretation, enabling immediate evaluation of treatment efficacy and the precise need for individual blood components. Due to their speed and accessibility, POC-guided targeted therapy is now widely recommended. According to the literature, implementing POC-based transfusion protocols in clinical practice has reduced the use of allogeneic blood products by up to 50% [133–135].

Platelet aggregation plays a central role in cardiac surgery: it is essential for haemostasis and wound healing, yet CPB induces significant platelet dysfunction [136]. CPB induces both quantitative and qualitative platelet abnormalities, including reduced aggregation, impaired granule secretion, and altered platelet receptor function [102]. Platelet aggregometry serves critical roles in cardiac surgery, including the prediction of postoperative bleeding risk, assessment of residual antiplatelet drug effect and monitoring of haemostatic interventions [137]. Light transmission aggregometry (LTA) is widely used and considered the gold standard for assessing platelet function [34]. LTA is also recommended as a first-step test for evaluating inherited and acquired platelet function disorders [138, 139]. LTA is based on the principle that platelet-rich plasma (PRP) is initially turbid and limits light transmission; as platelets aggregate in response to a specific agonist, the plasma becomes clearer and light transmission increases proportionally [140, 141]. The aggregation response is induced by agonists, including adenosine diphosphate (ADP), collagen, arachidonic acid (AA), epinephrine, or thrombin receptor-activating peptide (TRAP) and is expressed according to the degree of light transmission [142]. In this study, platelet function was assessed using the 8-channel TA-8V Thrombo-aggregometer (SD Medical/STAGO), a light transmission aggregometry system that allows simultaneous evaluation of platelet aggregation in response to multiple agonists.

Point-of-care (POC) methods have been developed to simplify platelet function testing in whole blood. Commonly used systems include Multiplate (Roche, Germany), VerifyNow (Accumetrics, USA), and Plateletworks (Helena Laboratories, USA) [143, 144]. These systems use specific reagents to activate platelets, with aggregation measured by impedance, optical

detection, or comparison with control samples [179]. Platelet activation is induced by agonists such as ADP, arachidonic acid, collagen, epinephrine, TRAP, and ristocetin, each reflecting different aspects of platelet function [142]. Studies demonstrated that ADP-induced PA can decline by up to 55% and arachidonic acid-induced PA by up to 78% during CPB [103, 145]. The PLATFORM study of 490 patients found that post-CPB ADP-induced PA was the strongest predictor of severe bleeding (OR 0.962 per unit increase, $p = 0.005$), with an AUC of 0.712. A cut-off value of ≤ 8 U had the best positive predictive value (42%) for severe bleeding [146]. Although predictive thresholds vary between platforms (e.g., the ≤ 8 U cut-offs identified in the Multiplate-based PLATFORM study), the overall clinical significance of reduced ADP-induced aggregation remains consistent across methodologies. Preoperative PA also predicts outcomes. Reduced ADP-induced PA at baseline is associated with increased postoperative blood loss, particularly in patients receiving antiplatelet therapy [103]. In patients undergoing CABG, preoperative aggregometry provides critical prognostic value for those treated with P2Y₁₂ inhibitors. For instance, in a cohort of 226 patients receiving ticagrelor, ADP-induced aggregation results showed a strong inverse correlation with postoperative blood loss ($r = -0.6$, $p < 0.001$) [147]. In addition, a comparative study of 220 cardiac surgery patients found that replacing standard platelet counts with the Multiplate ADP test as a transfusion trigger reduced the incidence of platelet transfusions from 41.82% to 13.64% ($p < 0.001$), while also decreasing mean 12-hour postoperative blood loss from 440 mL to 300 mL ($p < 0.001$) [148]. Furthermore, PA offers superior accuracy in guiding secondary transfusion decisions. In CABG patients receiving early haemostatic support PA demonstrated a positive predictive value of 86% for requiring further transfusions, compared with 33% for a standard platelet count [149]. Clinical guidelines support the use of platelet aggregometry to optimise the timing of cardiac surgery in patients exposed to P2Y₁₂ inhibitors. The Society of Thoracic Surgeons (STS) recommends laboratory or point-of-care assessment of antiplatelet drug effects to evaluate bleeding risk and guide surgical scheduling (Class IIa, Level B-R) [150]. Similarly, the American Heart Association (AHA) suggests that such testing is a reasonable tool for clinical decision-making in the perioperative period (Class IIb, Level C) [151]. However, platelet aggregation reflects only one aspect of haemostasis and does not fully account for interactions with coagulation factors or fibrin formation. Consequently, its predictive value for postoperative bleeding is limited and should be interpreted within the broader context of haemostatic assessment.

2.7.3. Point-of-care testing: Viscoelastic testing

Bleeding usually becomes evident upon separation from CPB, as blood is redirected to the pulmonary circulation and heart, making it visible in the surgical field. At this point, rapid and accurate assessment of the patient's coagulation status is essential to guide targeted transfusion therapy and haemostatic management [152]. Conventional SLTs (aPTT, PT/INR) assess only isolated components of coagulation cascade and fail to reflect the complex interactions between coagulation factors, platelets, and fibrinolysis [153]. Given the limitations, interest in point-of-care (POC) tests has increased. Viscoelastic POC testing have been shown to reduce transfusion requirements by up to 50% and improve patient outcomes compared to conventional management strategies [154]. VET evaluate the entire coagulation process in whole blood, from clot initiation through propagation, stabilisation, and lysis, with results available within 10–20 minutes [155, 156]. Importantly, these tests can be performed intraoperatively, including during CPB under heparin anticoagulation, allowing results to be available at the time of CPB discontinuation. Current guidelines recommend incorporating VET into transfusion algorithms in cardiac surgery (Class I, Level B-R) [150, 157].

The most commonly used viscoelastic systems are thromboelastography (TEG) and rotational thromboelastometry (ROTEM) [158]. Previous ROTEM systems (Gamma and Delta) are routinely used to manage perioperative bleeding; however, they require trained staff to manually pipette blood samples and liquid reagents through multiple steps [155]. In contrast, ROTEM *Sigma* uses fully automated, cartridge-based technology, minimizing operator error and allows testing directly at the patient's bedside [159]. The system contains four different assays: EXTEM C, FIBTEM C, INTEM C, and APTEM C (type 1 or complete cartridge) or EXTEM C, FIBTEM C, INTEM C, and HEPTTEM C (type 2 or complete + hep cartridge). EXTEM, activated by tissue factor, assesses the extrinsic pathway, including clot formation, clot strength (factors VII, X, V, II, I), platelet contribution, and fibrinolysis [159]. INTEM is activated via the contact phase and reflects the intrinsic pathway (factors XII, XI, IX, VIII, II, I) as well as sensitivity to heparin. FIBTEM is based on EXTEM but includes cytochalasin D to inhibit platelets, thereby allowing isolated assessment of fibrinogen contribution to clot firmness. APTEM corresponds to EXTEM with added aprotinin and is used to detect hyperfibrinolysis by comparison with the EXTEM baseline. HEPTTEM is an INTEM-based assay containing heparinase, which neutralises up to 7 IU/mL of heparin in the ROTEM *Sigma* system, enabling analysis of blood samples with high heparin concentrations [19], [160], [161]. In addition, PLTEM, calculated as EXTEM minus FIBTEM, is commonly used to estimate the

platelet contribution to clot firmness, although its accuracy in reflecting platelet function after CPB remains under investigation [162].

The ROTEM Sigma system conducts four distinct tests. Clot formation is shown graphically, and mechanical changes are quantified as numerical parameters and graphical outputs for analysis [163, 164]. The ROTEM generates a graphical curve representing the entire clotting process: Clotting Time (CT), Clot Formation Time (CF), Clot amplitude at 5 or 10 minutes (A5/A10), Maximum Clot Firmness (MCF), and Maximum Lysis (ML). These parameters provide a comprehensive, dynamic assessment of haemostasis, enabling rapid identification of specific coagulation abnormalities [160]. In case of hypocoagulation, ROTEM analysis might reveal prolonged CT or CFT, explaining slower and weaker clot formation. Conversely, in hypercoagulation these findings can be opposite, reflecting faster and stronger clot formation [165]. The clinical utility of ROTEM in cardiac surgery is well established, particularly for guiding transfusion therapy and reducing postoperative bleeding. Randomised trials and studies have shown significant reductions in bleeding and transfusion requirements with ROTEM-guided management [166, 167]. In paediatric cardiac surgery, ROTEM-guided therapy reduced postoperative blood loss (9 vs 16 mL/kg, $p<0.001$) and RBC transfusion (11 vs 23 mL/kg, $p=0.005$) [166]. Similarly, implementation studies in adult populations have demonstrated reductions in allogeneic transfusion (41.4% vs 31.9%, $p=0.026$), PRBC (31.3% vs 19.8%, $p=0.002$), and FFP (9.8% vs 3.8%, $p=0.008$), along with a decrease in postoperative bleeding incidence (9.5% vs 5.3%, $p=0.037$) [167]. A comprehensive meta-analysis of 35 randomised trials ($n=3,096$) demonstrated significant reductions in FFP transfusion (RR 0.52, 95% CI 0.35–0.76), platelet transfusion (RR 0.69, 95% CI 0.55–0.87), surgical re-exploration (RR 0.63, 95% CI 0.45–0.88), and bleeding volume (SMD -0.31 , 95% CI -0.51 to -0.11) [168]. In addition, ROTEM-guided strategies have been associated with reductions in blood loss and lower rates of surgical re-exploration (RR 0.63, 95% CI 0.45–0.88) [169], [167]. Beyond global haemostatic assessment, specific ROTEM parameters offer clinically relevant insights. FIBTEM A10 shows a moderate to strong correlation with plasma fibrinogen concentration ($R = 0.67$ – 0.87), supporting its use as a rapid marker for fibrinogen status. In case of PLTEM, which represents the platelet contribution to clot firmness, correlates more strongly with platelet count ($R = 0.85$) than EXTEM alone ($R = 0.74$), and PLTEM A5 has demonstrated superior diagnostic accuracy in predicting thrombocytopenia [162, 170]. Currently, there is insufficient evidence to support the prognostic effectiveness of preoperative thromboelastometry in this patient population. Addressing this gap in objective perioperative risk stratification is a key rationale for the present study.

2.8. The influence of gene variants on blood loss

Bleeding in cardiac surgery is a complex, multifactorial event influenced by surgical factors, perioperative management, and the patient's genetic profile. Genetic determinants may affect haemostasis via multiple pathways, including coagulation factor activity, platelet function, fibrinolysis, and drug metabolism [171]. Better identification of bleeding risk is becoming possible with the discovery of numerous variants in the human genome. While the role of gene variants has been extensively investigated in thrombosis and cardiovascular diseases, their contribution to bleeding remains less clearly defined and often inconsistent across studies [172]. Donahue et al. investigated the factor V Leiden (FVL) polymorphism (506G>A) in 517 cardiac surgery patients and demonstrated reduced postoperative blood loss among carriers of this variant [173]. However, this finding was not confirmed by Boehm et al., who reported no significant association between FVL and bleeding [174]. Later, Welsby et al. analyzed 19 polymorphisms of 13 genes in 780 patients undergoing cardiac surgery. They identified seven gene variants significantly correlated with postoperative bleeding, including polymorphisms in platelet receptors (GPIa/IIa, GPIba), tissue factor, prothrombin, and TFPI [36]. Similarly, Motovska et al. demonstrated a significant association between bleeding risk and specific COX-1 gene variants in patients undergoing elective coronary angiography ($p = 0.013$) [175]. In a large prospective cohort ($n = 1,036$), Greiff et al. identified SNPs in the PROC (Protein C), Factor IX, ABO, and ITGA2 genes as significant predictors of excessive bleeding. Although including genetic variables improved predictive models, their overall discriminative performance remained moderate ($AUC < 0.74$) [176]. This suggests that, while genetic factors contribute to bleeding risk, their predictive value is limited when used alone. Further evidence suggests that not all haemostatic gene variants translate into bleeding risk. Over an 11-month follow-up period, Grinshtein et al. found that ITGB3, ITGA2, and CYP2C19*2 polymorphisms were associated with adverse cardiovascular outcomes (mortality, myocardial infarction, and stroke), but not with bleeding events in CABG patients [177]. More recent studies have focused on pharmacogenetic determinants. Wang et al. reported that the ABCB1 C3435T polymorphism significantly affected perioperative bleeding in OPCABG patients, with the wild-type genotype associated with increased bleeding risk (OR 2.941). Importantly, the combination of genetic predisposition and clinical factors (clopidogrel discontinuation) yielded the highest predictive accuracy ($AUC = 0.834$) [178]. In contrast, Cha et al. in a large PCI cohort ($n = 4,489$), found no association between bleeding and ABCB1, PON1, or

CYP2C19 variants, but identified the P2Y₁₂ G52T polymorphism as an independent predictor of major bleeding (adjusted HR 2.51) [179].

Taken together, these polymorphisms represent key components of haemostasis, including platelet function, fibrin formation, and fibrinolysis. Given the crucial role of fibrin stability and platelet–fibrinogen interactions in haemostasis, the following sections provide a detailed analysis of three key genetic markers: *ITGB3* (rs5918), *FGB* (rs1800790), and *SERPINE1* (rs2227631).

2.8.1. *ITGB3* (rs5918)

Most studies investigating platelet glycoprotein receptor polymorphisms in surgical patients have focused primarily on thrombotic rather than bleeding outcomes. Nevertheless, these receptors are biologically relevant to perioperative haemostasis because they regulate platelet adhesion, activation, and aggregation [180]. Among them, the *ITGB3* gene, which encodes the $\beta 3$ subunit of the platelet glycoprotein IIb/IIIa receptor (integrin α IIb β 3), has attracted considerable attention due to its central role in platelet aggregation and fibrinogen binding [181]. The *ITGB3* (integrin beta 3) gene, also known as platelet glycoprotein IIIa, is located on chromosome 17q21.32 and encodes the $\beta 3$ subunit of the integrin receptor complex, a key mediator of platelet aggregation and clot formation [182]. The most clinically relevant polymorphism within this gene is the nonsynonymous single nucleotide polymorphism rs5918 (T1565C), which results in a leucine-to-proline substitution at codon 33 (Leu33Pro), also known as the human platelet antigen-1 (HPA-1) or PlA1/A2 polymorphism [181, 183]. This variant is relatively common in Caucasian populations, with an estimated prevalence of approximately 20% [184]. The protein encoded by *ITGB3* forms the β subunit of integrin α IIb β 3 (GPIIb/IIIa), the most abundant glycoprotein receptor on the platelet surface, with approximately 70,000 copies per resting platelet [185]. This integrin acts as a receptor for fibrinogen, von Willebrand factor, and vitronectin, mediating platelet adhesion and aggregation. Structurally, the extracellular portion of the receptor comprises an α IIb β -propeller and a $\beta 3$ A-domain, which together form the integrin head [186, 187]. In resting platelets, α IIb β 3 remains in a low-affinity conformation that prevents ligand binding; upon platelet activation, inside-out signalling induces a conformational change that exposes ligand-binding sites and enables aggregation [188]. Functionally, the rs5918 polymorphism has been associated with increased platelet reactivity. The rs5918 variant is thought to induce conformational changes in the GPIIb/IIIa receptor that enhance fibrinogen binding, lower the activation threshold to agonists such

as ADP and collagen, and promote α -granule release [184]. Consequently, platelets carrying the rs5918 variant may display a hyperreactive phenotype characterised by enhanced aggregation. As α IIb β 3 is the final common receptor mediating platelet cross-linking through fibrinogen, such changes may theoretically influence both thrombotic tendency and perioperative bleeding risk [36, 183]. Because of its apparent prothrombotic effect, rs5918 has been extensively studied in cardiovascular disease. Several studies have associated the rs5918 to increased susceptibility to myocardial infarction, coronary heart disease, stroke, and venous thromboembolism [183, 189]. Khatami et al. suggested that this polymorphism contributes to coronary heart disease susceptibility in certain populations [183], while Conkbayir et al. reported associations with lipid metabolism, including total cholesterol and HDL-C levels, which may further influence cardiovascular risk [190]. These findings support the view that *ITGB3* variation may affect thrombotic phenotype through both platelet and vascular mechanisms. However, the role of rs5918 in perioperative bleeding remains unclear. Mechanistically, carriers of the rs5918 might be expected to have a lower bleeding tendency due to enhanced platelet aggregability, whereas rs5918 homozygotes could theoretically be more susceptible to bleeding because of lower baseline platelet reactivity. Despite this plausible biological rationale, clinical findings have been inconsistent. Morawski et al. suggested that the effect of this polymorphism on bleeding may be modified by aspirin therapy [184]. However, larger clinical studies have not confirmed a robust and independent association between rs5918 and postoperative bleeding outcomes. In patients undergoing coronary artery bypass grafting (CABG), Welsby et al. found no significant relationship between this polymorphism and postoperative chest tube drainage or transfusion requirements [36]. Similarly, Greiff et al. did not validate its prognostic value for excessive postoperative bleeding [176]. More recently, Grinshtein et al. demonstrated that *ITGB3* variants were associated with thrombotic cardiovascular events, including recurrent myocardial infarction, but not with perioperative bleeding complications [191].

2.8.2. *FGB* (rs1800790)

Fibrinogen is a dimeric glycoprotein composed of three pairs of polypeptide chains (Aa, Bb, and γ), encoded by three distinct genes: the alpha fibrinogen gene (*FGA*), the beta fibrinogen gene (*FGB*), and the gamma fibrinogen gene (*FGG*) [82, 192]. Of these, synthesis of the beta chain is considered the rate-limiting step in fibrinogen production [193] and is regulated by a promoter region located on the long arm of chromosome four [194]. Therefore, genetic variation within the *FGB* promoter plays a key role in determining

plasma fibrinogen levels. Genetic polymorphisms in the promoter region of the *FGB* gene, including rs1800790 (-455 G/A) and rs1800787 (-148C>T), have been shown to influence fibrinogen levels [195]. The *FGB* rs1800790 (also known as -455G>A) polymorphism is one of the most extensively investigated variant and is located in the promoter region of the *FGB* gene [196]. The rs1800790 SNP involves a guanine-to-adenine (G>A) substitution. This G>A substitution alters transcriptional activity, with the A allele associated with increased gene expression and elevated plasma fibrinogen levels. This effect is mediated by altered nuclear protein binding and may increase basal transcription rates by about 50% [197]. Functional studies indicate that the A allele significantly increases the rate of gene transcription, particularly in response to interleukin-6 (IL-6) stimulation. This mechanism leads to a hyperfibrinogenaemic phenotype, where carriers of the A allele consistently demonstrate elevated plasma fibrinogen levels compared to homozygous wild-type (G/G) carriers [198]. Despite this, the rs1800790 SNP has not been consistently associated with bleeding in cardiac surgery [196]. In a study of 1,036 cardiac surgery patients, analysis of 22 polymorphisms in coagulation and fibrinolysis pathways identified several variants associated with postoperative bleeding (including *PROC*, *ITGA2*, *ABO*, and *F9* genes), whereas fibrinogen gene polymorphisms were not significant predictors [176]. Similarly, a study of 780 patients undergoing CABG identified seven genetic polymorphisms associated with bleeding, but fibrinogen-related variants were again not implicated [36]. Clinical studies have mainly focused on fibrinogen concentration rather than genetic determinants. Recent evidence indicates that the rs1800790 A allele may be associated with a prothrombotic rather than a bleeding phenotype. These findings suggest that increased fibrinogen levels do not necessarily lead to improved haemostatic balance and may instead reflect altered clot structure and resistance to fibrinolysis [199]. Behague et al. reported that the presence of the minor allele was associated with higher plasma fibrinogen levels, particularly among smokers, and correlated with increased severity of coronary artery disease [200]. Similarly, Al-Awad et al. demonstrated that rs1800790 significantly influenced thrombotic risk [195].

Overall, although the *FGB* rs1800790 polymorphism plays a significant role in regulating fibrinogen synthesis, its direct influence on bleeding risk remains unclear, and current evidence does not support its use as an independent predictor of perioperative bleeding in cardiac surgery.

2.8.3. *SERPINE1* (rs2227631) and plasma level of PAI-1

Plasminogen activator inhibitor-1 (PAI-1) is the primary physiological inhibitor of both tissue plasminogen activator (t-PA) and urokinase

plasminogen activator (u-PA) [201]. As a serine protease inhibitor (serpin), PAI-1 rapidly binds to and inactivates these activators, preventing the conversion of plasminogen to plasmin, a key step in regulating fibrinolysis and reducing blood clot breakdown [202]. PAI-1 levels exhibit significant inter-individual variability, a large portion of which is genetically determined by polymorphisms in the promoter region of the *SERPINE1* gene. The most extensively characterised variant is the -675 insertion/deletion polymorphism (4G/5G), which modulates transcription through a competitive binding mechanism. The 4G allele binds only a transcriptional activator, resulting in high PAI-1 expression and a “hypofibrinolytic” state associated with thrombotic risk [203–205]. In contrast, the 5G allele binds a repressor protein, reducing PAI-1 transcription and creating a profibrinolytic environment that is more susceptible to bleeding [206–208]. Parallel to the 4G/5G variant, the SNP at position -844 (A>G, rs2227631) has emerged as a potential modulator of the fibrinolytic phenotype. These loci are frequently in strong linkage disequilibrium and are often co-inherited. Research indicates that the -844 G allele has been associated with reduced PAI-1 expression, while the A allele is associated with higher PAI-1 levels [209, 210]. In the context of cardiac surgery, cardiopulmonary bypass (CPB) induces a systemic inflammatory response accompanied by a marked release of t-PA. In patients with genotypes associated with low PAI-1 expression (e.g., rs2227631 G/G or -675 5G/5G), this fibrinolytic activation may remain insufficiently suppressed, leading to hyperfibrinolysis and increased blood loss. Ozolina et al. suggested that the rs2227631 G/G genotype as a potential “bleeding phenotype”, demonstrating significantly higher postoperative chest tube drainage and biochemical evidence of enhanced fibrinolysis compared with A/A carriers [211, 212]. Conversely, the A/A genotype is associated with higher baseline PAI-1 levels, similar to the 4G/4G phenotype. While this may provide relative protection against surgical bleeding by effectively suppressing fibrinolysis, it may theoretically increase the risk of postoperative thrombotic complications [207]. Individuals with the A/G genotype typically display intermediate PAI-1 levels and fibrinolytic activity, representing a balanced phenotype between the haemorrhagic risk of G/G and the thrombotic risk of A/A [212]. Despite these findings, studies directly evaluating the impact of the rs2227631 polymorphism on bleeding, and vice versa, in patients undergoing cardiac surgery with CPB remain limited.

3. MATERIALS AND METHODS

3.1. Study design

This prospective observational study was conducted at the Heart Centre (Department of Cardiac Surgery and Department of Anaesthesiology) of Kauno Klinikos, Lithuanian University of Health Sciences, Kaunas, Lithuania. Written informed consent was obtained from all participants prior to inclusion, and the study was conducted in accordance with the Declaration of Helsinki. The study was conducted between October 2023 and November 2024. A total of 212 patients undergoing elective cardiac surgery with cardiopulmonary bypass (CPB) were selected. Seventeen patients were excluded: 13 due to re-sternotomy, 1 declined to participate during study, and 3 had incomplete data. Consequently, 195 patients were included in the final analysis. The study was conducted in collaboration with the Laboratory of Molecular Cardiology at the Lithuanian University of Health Sciences and the Department of Laboratory Medicine at Kauno Klinikos.

Inclusion criteria:

1. Age between 49 and 75 years (regardless of gender);
2. Written informed consent with signature;
3. Elective on-pump cardiac surgery patients:
 - coronary artery bypass grafting (CABG);
 - heart valve surgery (mitral, aortic or tricuspid valve replacement or repair);
 - combined cardiac procedures;
 - chronic aortic dissection (any type), or aneurysm of the thoracic aorta.

Exclusion criteria:

1. Emergency or urgent heart surgery;
2. Redo surgery;
3. Postoperative re-sternotomy for bleeding or cardiac tamponade after surgery;
4. Acute aortic dissection;
5. Heart transplantation or implantation of mechanical circulatory support devices (LVAD, RVAD, BiVAD);
6. Bone marrow disorders;
7. Co-morbid pathologies:
 - Chronic inflammatory diseases (Crohn's disease, ulcerative colitis);

- Severe hepatic or renal dysfunction, including viral hepatitis (A, B, C, D, E);
- Autoimmune diseases;
- Active oncological disease (including metastasis or recurrence).

3.2. Ethical approval

Ethical approval for this study (Approval No. BE-2-53; Protocol No. 1) was granted by the Kaunas Regional Biomedical Research Committee, Lithuanian University of Health Sciences, (Kaunas, Lithuania) on 31 July 2023.

3.3. Anaesthesia and cardiopulmonary bypass

In all cases, general anaesthesia and CPB were performed according to standard protocols. Midazolam or diazepam was prescribed for premedication. The same protocol was used for induction of anaesthesia in all patients: fentanyl 1–2 µg/kg (Fentanyl-Kalceks® 50 mcg/mL, AS Kalceks, Latvia), midazolam 0.05 mg/kg (Midozalamum®, Sun Pharmaceutical Industries Europe B.V., Hoofddorp, Netherlands), propofol 1.0–2.5 mg/kg (ANESIA® 10 mg/mL, UAB Norameda, Vilnius, Lithuania), rocuronium 0.08–0.1 mg/kg (Rocuronijev bromid Hameln® 10 mg/mL, Hameln Pharma GmbH, Germany), and sevoflurane (Sevofluran Piramal®, Piramal Critical Care B.V., Netherlands) at 1.0%–2.0% minimum alveolar concentration (MAC). Anaesthesia was maintained with fentanyl 3 mg/kg/h, midazolam 1–4 mg/h, and sevoflurane 2.0%–3.0% MAC during CPB. Antiplatelet therapy was discontinued 5–7 days before surgery in all patients. Before the start of CPB, heparin (Pan-Heparin Sodium®, Panpharma S.A./Rotexmedica GmbH, Germany) was administered at an initial dose of 300–400 IU/kg and 5,000–10,000 IU to achieve and maintain an activated clotting time (ACT) of more than 480 seconds during CPB.

A standard pulsatile CPB (STOCKERT S5) with an extracorporeal circuit consisting of a polypropylene membrane oxygenator (Sorin/INSPIRE HVR®, LivaNova, Italy) and a venous reservoir was used. The CPB perfusion pump was primed with 1.5 l of crystalloid solution (Ringer's acetate solution, Warsaw, Poland) containing 10,000 units of heparin. Weaning from CPB after the surgical procedure was performed once the patient had been warmed to at least 36°C. After weaning from CPB, protamine (Protaminsulfat®, LEO Pharma, Ballerup, Denmark) was initially administered at a dose of 1 mg per 100 units of heparin, with further doses given until ACT returned to baseline. All patients underwent median sternotomy. Venous return was achieved

using bicaval or single-stage venous cannulation, depending on the surgical procedure. The aorta was cross-clamped. Myocardial protection was achieved by antegrade cold crystalloid cardioplegia St. Thomas Cardioplegia (ST. THOMAS TIRPALAS®, Lithuania). Certain aspects of patient management, such as target mean arterial pressure and rate of rewarming, were determined by standard practice and not dictated by the study protocol.

3.4. Data collection and analysis

The following parameters were analyzed during the study:

- Demographic and anthropometric data: age, gender, BMI, BSA, and EBV;
- Clinical variables: LVEF, EuroSCORE II, Papworth Bleeding Risk Score, heart failure severity was classified using New York Heart Association (NYHA) functional classification). Comorbidities (including diabetes mellitus, arterial hypertension, obesity);
- Surgical and CPB parameters: type of surgery (coronary artery bypass grafting (CABG), heart valve repair or replacement and combined heart surgery (CABG + valve)), CPB time, aortic cross-clamp time and reperfusion time (all measured in minutes);
- Postoperative outcomes and complications: incidence of neurological complications, heart failure (HF), renal failure (RF), atrial fibrillation (AF), and atrioventricular (AV) block;
- Hospitalisation metrics: total length of hospital stay and intensive care unit (ICU) stay (both measured in days);
- Genetic analysis: determination of specific single nucleotide polymorphisms (SNPs), including *ITGB3* (rs5918), *FGB* (rs1800790) and *SERPINE1* (rs2227631);
- Laboratory data: standard coagulation tests (prothrombin time (PT), activated partial thromboplastin time (aPTT), International Normalised Ratio (INR), fibrinogen level), D-dimer, complete blood count (CBC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), PAI-1 plasma levels.
- Platelet function and coagulation monitoring: rotational thromboelastometry (ROTEM *Sigma*) and platelet aggregometry (PA) induced by adenosine diphosphate (ADP) and epinephrine;

Data were collected at four predefined time points (Table 1):

- T0 – baseline (preoperative);
- T1 – immediately after surgery (upon ICU admission);
- T6 – 6 hours postoperatively;
- T18 – 18 hours postoperatively.

Blood loss was quantified using the Hb/kg Index, defined as haemoglobin mass loss per kilogram of patient weight.

Table 1. *Timing of laboratory testing and clinical assessments*

Examinations	T0	T1	T6	T18
Gene variants (<i>ITGB3</i> , <i>FGB</i> , <i>SERPINE1</i>).	+			
Standard Coagulation Profile (PT, aPTT, INR), Fibrinogen level	+	+	+	+
Complete Blood Count (Hb, Hct, PLT, RBC)	+	+	+	+
ALT, AST	+			
D-dimer		+	+	+
PAI-1 plasma level	+			+
Thromboelastometry (EXTEM, INTEM, FIBTEM, PLTEM)	+ APTEM	+ HEPTEM		
Platelet Aggregometry (ADP, Epinephrine)	+	+		+

3.4.1. Measurement of PAI-1 concentration

Plasma concentrations of PAI-1 were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (abx050189 PAI-1 ELISA, Abnova Ltd., Cambridge, UK), following the manufacturer's instructions. The assay is based on a solid-phase sandwich ELISA principle and is designed for the quantitative determination of human PAI-1. Peripheral venous blood samples were collected into tubes containing sodium citrate as anticoagulant and centrifuged at $1,500 \times g$ for 10 minutes at 4 °C to obtain platelet-poor plasma. Plasma aliquots were stored at -80 °C until analysis and thawed only once prior to testing. Briefly, microplate wells pre-coated with a monoclonal antibody specific for human PAI-1 were incubated with standards, quality controls, and plasma samples. During the incubation period, PAI-1 present in the samples bound to the immobilized capture antibodies. After washing to remove unbound components, a horseradish peroxidase (HRP)-conjugated detection antibody specific for PAI-1 was added, forming an antibody-antigen-antibody sandwich complex. Following additional washing steps, a chromogenic substrate solution was added, and the enzymatic reaction was allowed to develop. The reaction was stopped by the addition of stop solution. The optical density of the samples was measured at 450 nm using an Infinite M Plex microplate reader (Tecan, Männedorf, Switzerland), and PAI-1 concentrations were calculated based on a standard calibration curve.

3.4.2. Genotyping

Genetic analyses were performed at the Laboratory of Molecular Cardiology, Institute of Cardiology, Lithuanian University of Health Sciences (LSMU). All participants were genotyped for single nucleotide polymorphisms (SNPs) in the *ITGB3* (rs5918), *FGB* (rs1800790), and *SERPINE1* (rs2227631). Genotyping was conducted using real-time polymerase chain reaction (RT-PCR) with TaqMan™ Genotyping Assays (Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's protocols. Each assay consists of two allele-specific, fluorescently labelled TaqMan® minor groove binder (MGB) hydrolysis probes and a pair of primers designed to amplify the target genomic region containing the SNP of interest. PCR amplification and allelic discrimination were performed using the QuantStudio™ 3 Real-Time PCR System (Thermo Fisher Scientific, USA), in accordance with the manufacturer's recommendations. The genotyping reaction mixture was prepared as described in Table 2 and dispensed into 96-well reaction plates. Each plate contained DNA samples from 95 study participants, while one well was used as a no-template control (NTC), in which 1 µL of nuclease-free water was added instead of genomic DNA. The thermal cycling conditions were as follows: initial enzyme activation at 95 °C for 10 minutes, followed by 40 cycles of denaturation at 95 °C for 15 seconds and annealing/extension at 60 °C for 90 seconds. Allelic discrimination was performed automatically by the QuantStudio software based on fluorescence signal detection (Figure 3).

Table 2. Preparation of the genotyping reaction mixture

Reagents	Volume per reaction (1×), µL
TaqMan® Universal Master Mix II, no UNG (Applied Biosystems by Thermo Fisher Scientific, Lithuania)	5.3
TaqMan® SNP Genotyping Assay (20×) (Applied Biosystems by Thermo Fisher Scientific, UK)	0.2
PCR-grade water	1.5
Total genotyping reaction mix volume	10.0
Genomic DNA (10 ng per reaction)	1.0
Total reaction volume per well	11.0

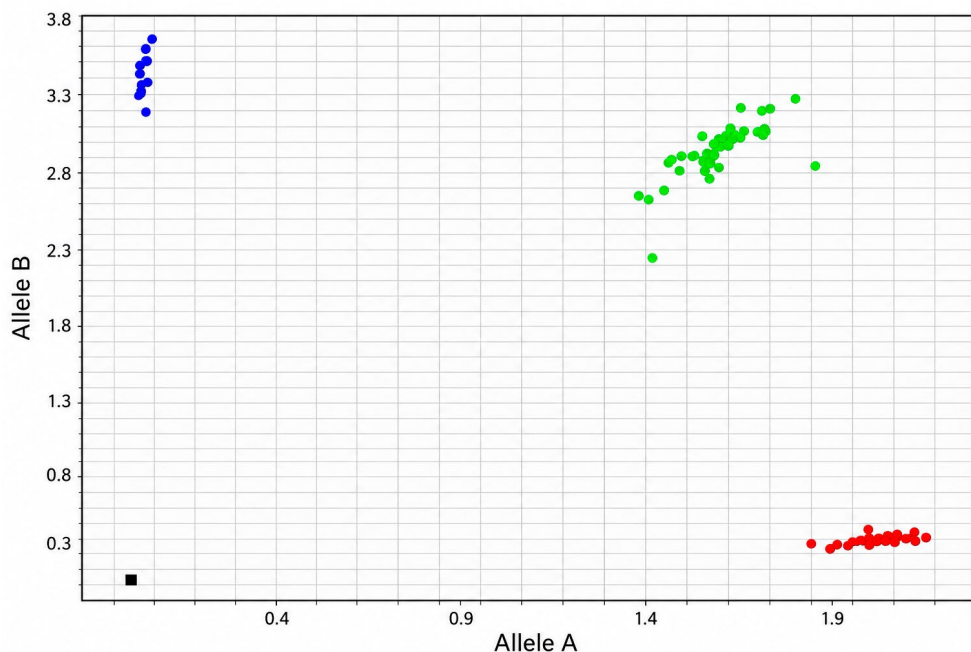


Fig. 3. Allelic discrimination plot showing the genotypes of the study participants

Homozygotes for allele 1 are represented by red dots, heterozygotes carrying both alleles by green dots, and homozygotes for allele 2 by blue dots. The black square in the lower left corner of the plot represents the no-template control (NTC).

3.4.3. Platelet aggregometry

Platelet aggregation was evaluated using light transmission aggregometry (LTA) according to the classical Born method. Venous blood samples (3.5 mL) were collected via atraumatic venepuncture into vacuum tubes containing 3.2% sodium citrate (Greiner Bio-One Vacuette, NC, USA, or BD Vacutainer, NJ, USA). Tubes were gently inverted and left upright at room temperature for 15 minutes to minimise pre-analytical activation. All samples were transported to the Laboratory of Molecular Cardiology (Institute of Cardiology, LSMU) and processed within one hour of collection. Platelet-rich plasma (PRP) was prepared by centrifuging whole blood at $100 \times g$ for 10 minutes at room temperature without the brake. Platelet-poor plasma (PPP), used as a 100% light transmission reference and for PRP dilution, if necessary, was obtained by further centrifuging the remaining blood at $2,500 \times g$ for 15 minutes. Aggregation was measured using an 8-channel TA-8V Thrombo-Aggregometer (SD Medical/STAGO, France) operated at 37°C.

For the assay, 450 µL of PRP was incubated and then stimulated with 10 µL of the following agonists: (1) adenosine diphosphate (ADP, final concentration 5 µM; Chrono-Log, PA, USA) and (2) epinephrine (ADR, final concentration 10 µM). The degree of aggregation was recorded in real time as the increase in light transmission through the sample over time, calibrated against the PPP blank control.

3.4.4. Viscoelastic Testing

Global haemostatic function was assessed using the ROTEM *Sigma* automated thromboelastometry system (Tem Innovations GmbH, Munich, Germany) (Figure 4). The analysis was performed according to the manufacturer's instructions at defined time points. Blood samples were collected via atraumatic venepuncture or central venous catheter into vacuum tubes containing 3.2% sodium citrate. To ensure clot stability and minimise pre-analytical errors, all samples were analysed within 30 to 60 minutes of collection. The ROTEM sigma system performed all pipetting and reagent mixing steps automatically using cartridge-based technology, significantly minimising operator-dependent variability. The following automated assays were performed for each patient using cartridges containing freeze-dried reagents:

- EXTEM: Activated by tissue factor to evaluate the extrinsic pathway, providing rapid screening of the coagulation system, including the contribution of fibrinogen and platelets.
- INTEM: Activated via the contact phase (ellagic acid) to evaluate the intrinsic pathway; it is sensitive to intrinsic factor deficiencies and the presence of heparin.
- FIBTEM: Utilises cytochalasin D to neutralise platelet function, thereby isolating the contribution of fibrinogen to total clot firmness.
- APTTEM: Provides an EXTEM-based analysis with the addition of aprotinin to inhibit fibrinolytic enzymes, allowing confirmation of hyperfibrinolysis.
- HEPTTEM: An INTEM-based assay containing heparinase to neutralise heparin (up to 7 IU/ml), enabling analysis of the underlying coagulation status during cardiopulmonary bypass (CPB) or before heparin neutralisation by protamine.
- Additionally, PLTEM (the platelet contribution to clot formation) was calculated as the difference between the EXTEM and FIBTEM assays (PLTEM = EXTEM – FIBTEM).



Fig. 4. *Rotational thromboelastometry (ROTEM Sigma)*

The following quantitative parameters were recorded and analysed for each assay (Figure 5):

- Clotting Time (CT): The time from the start of measurement until the initiation of clot formation is defined as an amplitude of 2 mm. CT reflects the initiation phase of coagulation, including coagulation factor activation, thrombin generation, and the onset of fibrin formation.
- Clot Formation Time (CFT, sec): The time from the onset of clotting, defined by CT, until a clot amplitude of 20 mm is reached. CFT reflects the kinetics of clot propagation and fibrin polymerisation and is influenced by fibrinogen function, platelet contribution, and Factor XIII-mediated clot stabilisation.
- Maximum Clot Firmness (MCF, mm): The maximum amplitude of the ROTEM curve represents the peak mechanical strength of the clot and reflects the combined stabilising effects of polymerised fibrin, platelets, and Factor XIII.
- A5 and A10 (mm): Clot amplitude was measured 5 and 10 minutes after CT, respectively. These parameters were recorded as early indicators of clot firmness and used to support faster assessment of clot formation.
- Maximum Lysis (ML, %): The percentage reduction in clot stability relative to MCF during the measurement; an ML > 15% within one hour was used to identify hyperfibrinolysis.

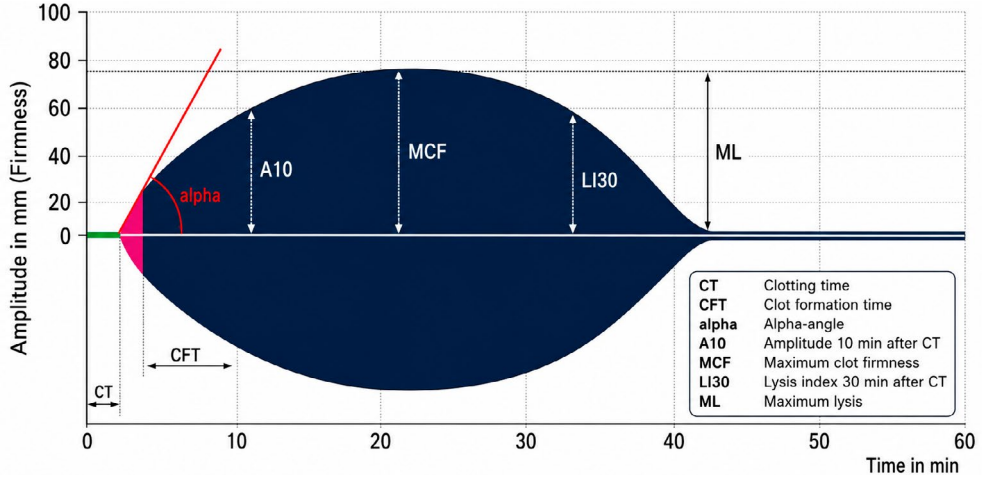


Fig. 5. ROTEM parameters and scaling

3.5. Assessment of blood loss: The Hb/kg Index

Perioperative blood loss was quantified using the Hb/kg Index, defined as haemoglobin mass loss normalized to patient body weight (g/kg). This physiologically based approach was first developed and described in our previous study [213], where it was proposed as a more accurate alternative to conventional volumetric methods of blood loss assessment. This approach integrates individual haemoglobin dynamics with patient-specific blood volume, providing a physiologically grounded measure of blood loss. Hb/kg Index was designed using following parameters:

- i. Estimated blood volume (EBV);
- ii. Initial haemoglobin mass;
- iii. Intraoperative haemoglobin mass loss;
- iv. Haemoglobin mass loss via chest tube drainage;
- v. Transfusion of packed red blood cells (PRBC) converted to haemoglobin mass;
- vi. Patient weight (kg).

(i) Estimated blood volume (EBV)

EBV was calculated using Nadler formula, which includes height, weight and sex-specific coefficients [214]:

$$EBV (L) = k_1 \times Height (m)^3 + k_2 \times Weight (kg) + k_3;$$

Where:

- For men: $k_1 = 0.3669$, $k_2 = 0.03219$, $k_3 = 0.6041$;
- For women: $k_1 = 0.3561$, $k_2 = 0.03308$, $k_3 = 0.1833$.

(ii) Initial haemoglobin mass

Initial Hb mass (g) was determined by multiplying the preoperative Hb concentration (g/L) by the EBV (L):

$$\text{Initial Hb mass} = \text{EBV (L)} \times \text{Hb}_{\text{concentration}} \text{ (g/L)}$$

(iii) Intraoperative haemoglobin mass loss

Intraoperative Hb mass loss was defined as the difference between initial haemoglobin mass before and immediately after surgery [215]:

$$\text{Hb mass loss (intra-op)} = \text{Initial Hb mass} - \text{Hb mass (post-op)}$$

(iv) Haemoglobin mass loss via CTD

CTD was recorded within 18 hours postoperatively. Fluid from chest tube was continuously collected and measured in the reservoir, which was connected to the vacuum system (GENTEC Model 882VR Continuous Suction Regulators, CA, USA). Aliquots of the drained fluid were sent to hospital laboratory where the haemoglobin concentration (g/L) was determined using automated haematology analyser. The total haemoglobin mass in the chest tube was then determined by multiplying the haemoglobin concentration to the CTD volume:

$$\text{Hb mass loss (CTD)} = \text{CTD}_{\text{Hb concentration}} \text{ (g/L)} \times \text{CTD}_{\text{volume}} \text{ (L)}$$

(v) Packed red blood cells (PRBC) transfusion

In general, one unit of PRBC increases the Hb concentration by about 10 g/L [216]. According to Lisander et al. one unit of PRBC contains about 52 ± 5.4 g Hb [217]. In our case, however, we found that one unit of PRBC contains at least 43 g of hemoglobin.

$$\text{Hb transfusion (g)} = \text{Number of PRBC units} \times 43 \text{ g}$$

The final formula

The formulation used in this study is consistent with our previously published methodology [213]. By integrating these parameters, the final Hb/kg Index was calculated to provide a physiologically relevant measure of blood loss normalised to patient body mass:

$$\text{Hb/kg Index} = \frac{\text{Hb loss (intra-op)} + \text{Hb loss (CTD)} - \text{Hb transfusion (PRBC)}}{\text{Body weight (kg)}}$$

3.6. Machine learning–based analysis of the Hb/kg Index

The Hb/kg Index used in this study is based on our previously established framework [213]. The distribution of the Hb/kg index across demographic and clinical subgroups (gender, BMI, and surgery type) was evaluated using independent t-tests and one-way ANOVA. Variables demonstrating statistical significance ($p < 0.05$) were prioritised for subsequent modeling, although the Lasso and Lasso-OLS frameworks independently conducted automated feature selection. Independent variables with absolute skewness greater than 2 were log-transformed to ensure stability and meet the assumptions of linear modeling. To identify robust independent predictors, a machine learning algorithm was applied to 86 variables, including:

- Linear and Regularized Models: Linear Regression, Lasso (L1 regularization, $\alpha = 0.1$), and a Lasso-OLS approach to balance predictive precision with clinical interpretability.
- Non-linear and Ensemble Methods: Support Vector Machine (SVM) with a Radial Basis Function (RBF) kernel ($C = 10$, $\epsilon = 0.1$), k-Nearest Neighbors (kNN) ($k = 8$), Decision Trees, and Extreme Gradient Boosting (XGBoost) using an ensemble of 100 trees.
- Deep Learning: A Deep Neural Network (DNN) was constructed with three hidden layers (40, 15, and 5 neurons) using ReLU activation. To prevent overfitting, dropout layers (20–30%) were integrated, and the output was constrained to non-negative values using a final ReLU neuron.

All models were rigorously validated using 10-fold cross-validation. Performance was quantified using the mean and standard deviation of the Mean Squared Error (MSE), Mean Absolute Percentage Error (MAPE), Spearman correlation coefficients, and the coefficient of determination (R^2).

3.7. Cluster stratification and validation

To classify clinical patterns of blood loss, an unsupervised K-Medoids clustering algorithm was implemented. This clustering framework was previously developed and validated in our recent study [Y], demonstrating its ability to identify physiologically distinct groups of blood loss severity [218]. This method stratified patients into distinct groups according to their physiological profiles and key predictors identified in the preceding regression analysis.

- Cluster Formation: All variables were standardised (z-score) to ensure equal weighting before partitioning the data based on pairwise dissi-

imilarities in multidimensional feature space. The optimal number of clusters was determined using the Elbow method and validated by the Silhouette score.

- **Threshold Determination:** Data-driven cut-off thresholds for the Hb/kg Index were calculated as the midpoints between the median values of adjacent clusters. These thresholds define three levels of blood loss severity:
 - Cluster 1 (Mild): $\text{Hb/kg} \leq 1.72$
 - Cluster 2 (Moderate): $1.72 < \text{Hb/kg} \leq 2.10$
 - Cluster 3 (Severe): $\text{Hb/kg} > 2.10$
- **Statistical Validation:** Differences between clusters were evaluated using ANCOVA (adjusted for age and gender), the Kruskal–Wallis test with Dunn’s post hoc analysis, and Chi-square (χ^2) tests for categorical variables.
- **Discriminative Performance:** The robustness of the Hb/kg index categories was assessed using ROC analysis. This validation analysis is consistent with previous published work [218]. A multinomial logistic regression model (one-vs-rest framework) was used to quantify the Area Under the Curve (AUC) and generate cluster specific ROC curves.
- **Effect Size and Ranking:** For each ANCOVA model, the proportion of explained variance was quantified using R^2 and partial eta squared (η_p^2). Dependent variables were ranked according to the magnitude of the main effect of blood loss category, using the (η_p^2) statistic from each model.

3.8. Sample size calculation and statistical analysis

Sample size calculation. The required sample size was determined before the study commenced to ensure adequate statistical power for identifying significant predictors of perioperative blood loss. Based on a power analysis using G*Power software (version 3.1.9.7), the calculation was performed for a multiple linear regression model (fixed model, R^2 deviation from zero). To achieve a statistical power ($1 - \beta$) of 0.80 with a significance level (α) of 0.05 and a medium effect size ($f^2 = 0.15$), assuming up to 15–20 potential independent predictors in the final model, the minimum required sample size was estimated at approximately 140–160 participants. The final cohort consisted of 195 patients. This sample size exceeds the minimum requirement, providing sufficient robustness for both conventional statistical analysis and machine learning algorithms, including 10-fold cross-validation.

Statistical analysis. Data were analysed using the Python programming language (version 3.12), including the scikit-learn package and the Keras

Python framework [5], as well as IBM SPSS Statistics version 31.0 (SPSS Inc., Chicago, IL, USA). Normality of data distribution was assessed using the Shapiro–Wilk test, and homogeneity of variances with Levene’s test. Descriptive statistics are presented as mean $\pm$ standard deviation (SD) for normally distributed continuous variables and as median [interquartile range, IQR] for non-normally distributed data. Categorical variables are reported as frequencies and percentages (n, %). Between-group comparisons were performed using the Student’s t-test for normally distributed continuous variables, the Mann–Whitney U test for non-normally distributed continuous variables, and the Pearson chi-square (χ^2) test for categorical variables. For longitudinal assessments of non-parametric parameters, overall differences across time points were evaluated using the Friedman test.

4. RESULTS

A total of 212 patients undergoing elective cardiac surgery with CPB met the inclusion criteria. Seventeen patients were excluded: one withdrew consent, three had incomplete data, and thirteen required re-sternotomy. The final study cohort included 195 patients: 134 men (68.7%) and 61 women (31.3%). Detailed demographic and clinical data are summarised in Table 3. The mean age was 65.8 ± 7.1 years. The mean weight was 84.3 ± 16.5 kg and the mean BMI was 28.5 ± 5.1 kg/m². The mean BSA was 1.96 ± 0.21 m², and EBV was 5.07 ± 0.83 L. The EuroSCORE II was 1.97 [1.04–3.74]. No in-hospital mortality was observed. Arterial hypertension was present in 79 patients (40.5%), hyperlipidaemia in 162 (83.1%), and diabetes mellitus in 32 (16.4%). The mean LVEF was 50.6 ± 7.5 %. NYHA class I–II was observed in 109 patients (55.9%) and class III–IV in 86 (44.1%). Surgical procedures included CABG in 101 patients (51.8%), valve surgery in 57 (29.2%) and combined procedures in 37 (19.0%). The mean duration of surgery was 202.4 ± 53.8 min, CPB duration was 97.4 ± 33.0 min and the cross-clamp time was 56.2 ± 25.6 min. PRBC transfusions were administered in 14 patients (7.2%). FFP was used in 3 patients (1.5%) and cryoprecipitate was used in 1 (0.5%). Active smoking was reported in 50 patients (25.6%). Preoperative antithrombotic therapy included aspirin (33.8%), P2Y12 inhibitors (1.5%), rivaroxaban (9.2%), and warfarin (1.5%).

Table 3. Patient characteristics

Demographics and anthropometrics	
Age (years)	65.8 ± 7.1
Gender, men, n (%)	134 (68.7%)
Height (cm)	171.8 ± 9.2
Weight (kg)	84.3 ± 16.5
BMI (kg/m ²)	28.5 ± 5.1
BSA (m ²)	1.96 ± 0.21
EBV, L	5.07 ± 0.83
Comorbidities	
Arterial hypertension, n (%)	79 (40.5%)
Hyperlipidaemia, n (%)	162 (83.1%)
Type 2 Diabetes Mellitus, n (%)	32 (16.4%)
Smokers (active), n (%)	50 (25.6%)
Cardiac status	
NYHA Class I–II, n (%)	109 (55.9%)
NYHA Class III–IV, n (%)	86 (44.1%)
LVEF, %	50.6 ± 7.5
EuroSCORE II	1.97 [1.04–3.74]
Papworth Bleeding Risk Score	1 [0–2]

Table 3 cont.

Surgical parameters and CPB	
CABG, n (%)	101 (51.8%)
Valve, n (%)	57 (29.2%)
Combined, n (%)	37 (19.0%)
Surgery duration, min	202.4 ± 53.8
CPB duration, min	97.4 ± 33.0
Aorta cross-clamp time, min	56.2 ± 25.6
Number of grafts	
No grafts, n (%)	56 (28.7%)
1 graft, n (%)	9 (4.6%)
2 grafts, n (%)	18 (9.2%)
3 grafts, n (%)	73 (37.4%)
4 and more grafts, n (%)	39 (20.0%)

Note: Data are presented as mean ± standard deviation (SD) or median [IQR] depending on distribution and frequency (percentage) for categorical variables. Abbreviations: BMI, body mass index; BSA, body surface area; EBV, estimated blood volume; EuroSCORE II, European System for Cardiac Operative Risk Evaluation II; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; CABG, coronary artery bypass grafting; CPB, cardiopulmonary bypass;

4.1. Validation of the Hb/kg Index

4.1.1. Subgroup analysis

The Hb/kg Index differed significantly between men and women (1.98 ± 0.69 vs. 1.76 ± 0.76 ; $p = 0.044$). No significant correlation was found between age and Hb/kg Index ($r = 0.035$, $p = 0.628$). The significant difference in the Hb/kg index was observed across BMI categories ($F(2,192) = 8.367$, $p < 0.001$, $\eta_p^2 = 0.08$). Patients in BMI Group I ($< 24.99 \text{ kg/m}^2$) and Group II ($25\text{--}29.9 \text{ kg/m}^2$) demonstrated similar values (2.08 ± 0.73 and 2.06 ± 0.71 , respectively), while Group III ($\geq 30 \text{ kg/m}^2$) showed significantly lower values ($1.64 \pm 0.64 \text{ kg/m}^2$). Post hoc analysis (Scheffe test) confirmed a significant difference between Group I and Group III ($p = 0.003$). No significant difference in the Hb/kg Index was observed based on the type of surgery ($F(2,192) = 1.436$, $p = 0.241$). However, patients with higher postoperative drainage ($\text{CTD} \geq 500 \text{ mL}$) had a significantly higher the Hb/kg Index compared with those with lower volumes ($\text{CTD} < 500 \text{ mL}$) (2.11 ± 0.68 vs. 1.85 ± 0.72 ; $t(193) = 2.88$, $p = 0.021$). Detailed results of the subgroup analysis are summarized in the Table 4.

Table 4. *The differences in Hb/kg Index according to gender, BMI categories, type of surgery, CTD groups and its association with age*

Variables	Groups, mean $\pm$ SD		Statistical test
Gender	Men (n = 134, 68.7%) Mean 1.98 $\pm$ 0.69	Women (n = 61, 31.3%) Mean 1.76 $\pm$ 0.76	t-test p = 0.044
Age	Pearson correlation coefficient, r = 0.035, p = 0.628		
BMI Groups	Group I** (<24.99 kg/m ² ; n = 56, 28.7%) Mean 2.08 $\pm$ 0.73	Group II (25–29.9 kg/m ² ; n = 67, 34.4%), Mean 2.06 $\pm$ 0.71	Group III** (≥ 30 kg/m ² ; n = 72, 36.9%) Mean 1.64 $\pm$ 0.64 ANOVA p < 0.001, η^2 = 0.08. difference between means of Group 1 and Group 3 Scheffe p = 0.003
Type of surgery	CABG (n = 101, 51.8%) Mean 1.99 $\pm$ 0.66	Valve procedures (n = 57, 29.2%) Mean 1.79 $\pm$ 0.78	Combined surgery (n = 37, 18.9%) mean 1.89 $\pm$ 0.75 ANOVA p = 0.241, η^2 = 0.015
CTD group	CTD Group < 500 mL (n = 145, 74.4%) Mean 1.85 $\pm$ 0.72	CTD Group $\geq$ 500 mL (n = 50, 25.6%) Mean 2.11 $\pm$ 0.68	t test p = 0.021

Note: Values are presented as mean $\pm$ standard deviation (SD). Group comparisons were conducted using independent samples t-test or one-way ANOVA with Scheffé post hoc test where appropriate. Correlation with age was assessed using Pearson's correlation coefficient. Statistical significance was defined as p < 0.05. Abbreviations: BMI, body mass index; CTD, chest tube drainage; CABG, coronary artery bypass grafting.

4.1.2. Correlation analysis

Spearman correlation analysis identified variables associated with the Hb/kg Index (Table 5 and Figures 6, 7). The strongest positive correlation was observed with intraoperative haemoglobin mass loss ($\rho = 0.82$, $p < 0.001$). A moderate positive correlation was found with baseline haemoglobin concentration ($\rho = 0.37$, $p < 0.001$). Significant negative correlations were observed with BMI ($\rho = -0.31$, $p < 0.001$) and PRBC transfusion related haemoglobin mass ($\rho = -0.29$, $p < 0.001$). Postoperative haemoglobin and RBC values at all measured time points (T1, T6 and T18) also demonstrated consistent negative correlation with the Hb/kg Index. Fibrinogen levels were inversely correlated with the Index at T1 ($\rho = -0.31$, $p < 0.001$), T6 ($\rho = -0.19$, $p = 0.009$) and T18 ($\rho = -0.28$, $p < 0.001$). CTD volume demonstrated weak, but statistically significant positive correlations with the Hb/kg Index, especially at 5 and 6 hours ($\rho = 0.23$ and 0.24 ; both $p = 0.001$).

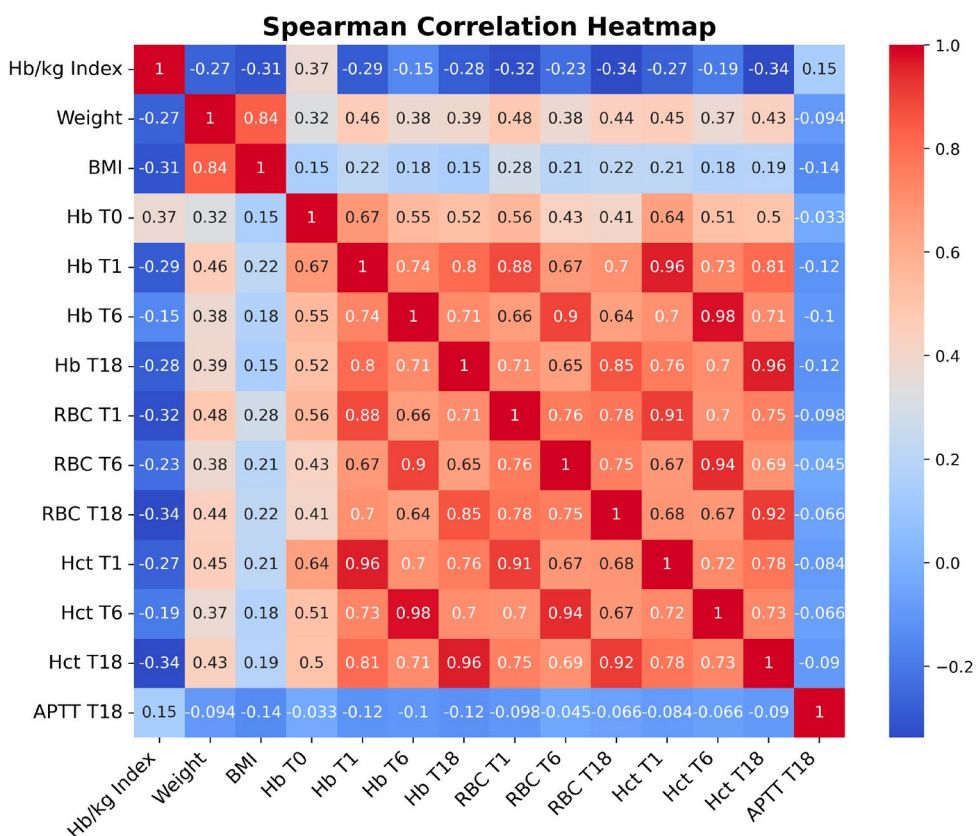


Fig. 6. Spearman correlation matrix: Hb/kg Index and selected variables

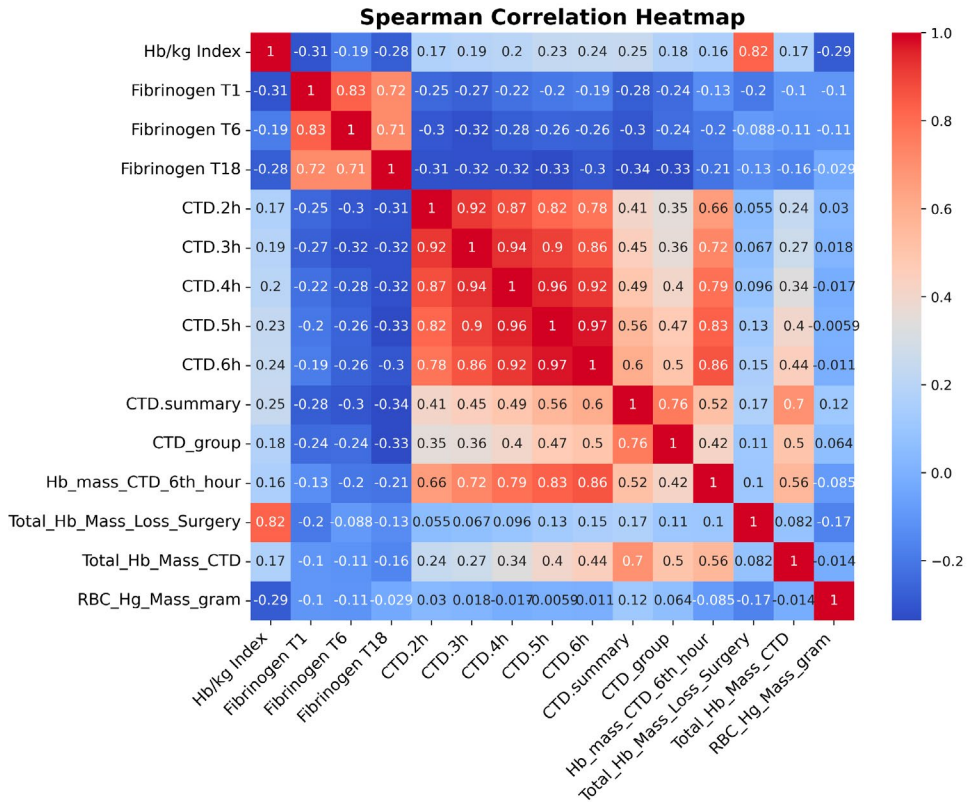


Fig. 7. Spearman correlation matrix: Hb/kg Index and selected variables

Table 5. Significant correlations between the Hb/kg Index and selected variables

Variables	Spearman, ρ	p-value
Intra-op Hb mass loss	0.82	< 0.001
BMI, kg/m ²	-0.31	< 0.001
Hb T0	0.37	< 0.001
Hb T1	-0.29	< 0.001
Hb T6	-0.15	0.032
Hb T18	-0.28	< 0.001
RBC T1	-0.32	< 0.001
RBC T6	-0.23	0.001
RBC T18	-0.34	< 0.001
Hct T1	-0.27	< 0.001
Hct T6	-0.19	0.007
Hct T18	-0.34	< 0.001
aPTT	0.15	0.040
Fibrinogen T1	-0.31	< 0.001

Table 5 cont.

Variables	Spearman, ρ	p-value
Fibrinogen T6	-0.19	0.009
Fibrinogen T18	-0.28	< 0.001
CTD 2h	0.17	0.021
CTD 3h	0.19	0.008
CTD 4h	0.20	0.005
CTD 5h	0.23	0.001
CTD 6h	0.24	0.001
CTD summary	0.25	< 0.001
CTD group	0.18	0.014

Note: Only statistically significant correlations ($p < 0.05$) are presented. Spearman's rank correlation coefficient (ρ) was used for analysis. Abbreviations: BMI – body mass index; Hb – hemoglobin; RBC – red blood cells; Hct – haematocrit; aPTT – activated partial thromboplastin time; CTD – chest tube drainage; CTD2h – CTD6h from 2 till 6 hours after surgery, CTD.summary – 18 hours after surgery. CTD Time points: T_0 – before surgery, T_1 – immediately after surgery, T_6 and T_{18} – 6 and 18 hours after surgery

4.1.3. Machine learning-based predictive modeling

Predictive modeling was performed to identify the key determinants of the Hb/kg Index and to evaluate the performance of different machine learning methods. Lasso regression was applied for feature selection and identified a limited set of clinically relevant predictors, including baseline haemoglobin, intraoperative haemoglobin mass loss, haemoglobin after surgery (T_1), BMI, and PRBC-related haemoglobin mass (Table 6).

Table 6. Predictors selected by Lasso-based feature selection for Hb/kg Index modeling

Variables	Lasso Coefficient	p-value
BMI	-0.18	<0.001
Hb T_0 (Pre-op Hb)	0.85	<0.001
Hb T_1 (immediately post-op Hb)	-0.69	<0.001
PRBC transfusion related Hb mass	-0.08	<0.001
Intra-op Hb mass loss	0.12	<0.001

Among all evaluated models, Lasso-OLS demonstrated the highest predictive performance, with a mean squared error of 0.08 ± 0.04 , a mean absolute percentage error of 0.18 ± 0.10 , a correlation coefficient of 0.92 ± 0.06 , and an R^2 of 0.82 ± 0.13 (Table 7). Linear regression showed comparable performance ($R^2 = 0.77 \pm 0.17$), while Lasso regression yielded slightly lower explanatory power ($R^2 = 0.68 \pm 0.07$). Tree-based and non-

linear models demonstrated lower predictive performance, including XGBoost ($R^2 = 0.65 \pm 0.21$), support vector machine ($R^2 = 0.64 \pm 0.15$), and deep neural networks ($R^2 = 0.42 \pm 0.21$). The lowest performance was observed for k-nearest neighbors ($R^2 = 0.26 \pm 0.12$) and decision tree models ($R^2 = 0.24 \pm 0.44$). Overall, regression-based models achieved the highest predictive accuracy, particularly the Lasso-OLS approach (Figure 8).

Table 7. Prediction accuracy for different statistical models

Model	MSE, mean $\pm$ SD	MAPE, mean $\pm$ SD	Corr, mean $\pm$ SD	$R^2 \pm$ SD
LR	0.12 ± 0.13	0.20 ± 0.11	0.90 ± 0.08	0.77 ± 0.17
Lasso	0.15 ± 0.07	0.31 ± 0.17	0.91 ± 0.06	0.68 ± 0.07
Lasso-OLS	0.08 ± 0.04	0.18 ± 0.10	0.92 ± 0.06	0.82 ± 0.13
SVM	0.17 ± 0.08	0.31 ± 0.19	0.83 ± 0.09	0.64 ± 0.15
kNN	0.35 ± 0.14	0.48 ± 0.29	0.58 ± 0.09	0.26 ± 0.12
DT	0.30 ± 0.09	0.33 ± 0.17	0.65 ± 0.19	0.24 ± 0.44
XGBoost	0.15 ± 0.05	0.27 ± 0.12	0.84 ± 0.11	0.65 ± 0.21
DNN	0.34 ± 0.36	0.42 ± 0.35	0.75 ± 0.20	0.42 ± 0.21

Abbreviations: LR, linear regression; Lasso, Lasso regression; Lasso-OLS, Least Absolute Shrinkage and Selection Operator and Ordinary Least Squares; SVM, support vector machine; kNN, k-nearest neighbour classifier; DT, decision tree; XGBoost, extreme gradient boosting; DNN, deep neural network.

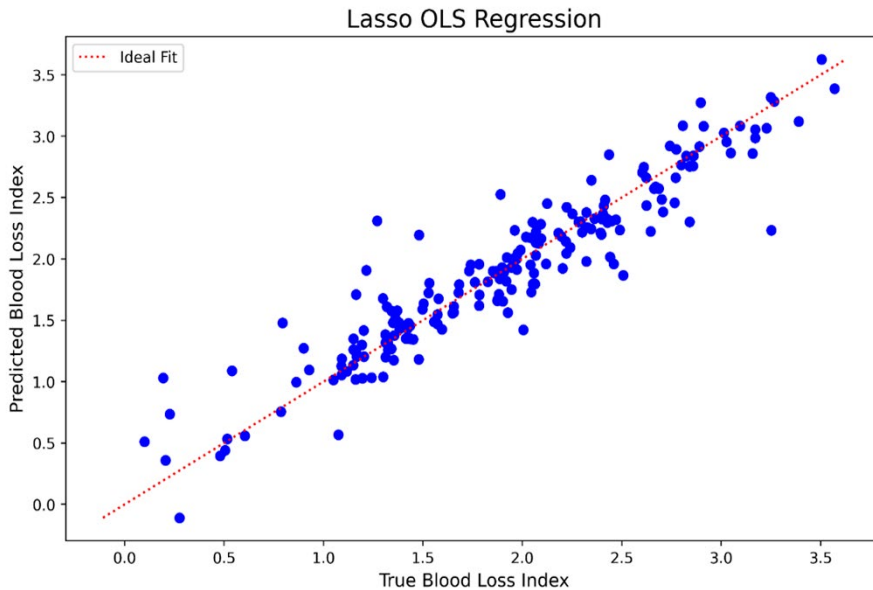


Fig. 8. Predicted versus observed values of the Hb/kg Index using the Lasso-OLS model

Each point represents an individual observation, and the dashed line indicates perfect agreement.

4.2. Identification of blood loss severity clusters

To categorize clinical patterns of perioperative blood loss, an unsupervised ML approach using K-Medoids clustering was applied. Variables included in the clustering procedure were selected based on their significant correlation with the Hb/kg index, thus focusing the model on clinically relevant predictors of cluster formation. The analysis identified three distinct blood loss severity clusters characterized by progressively increasing levels of Hb/kg Index. Data-driven Hb/kg thresholds of 1.72 and 2.10 were derived as optimal cut-off values between adjacent groups, providing stratification into Mild, Moderate, and Severe clusters (Table 8). These thresholds were calculated as midpoints between cluster medians, providing an objective classification framework.

Table 8. Descriptive statistics of patient clusters based on the Hb/kg Index

Clusters	n (%)	Blood loss category	Hb/kg range	Median	Mean
Cluster 1	76 (39%)	Mild	≤ 1.72	1.65	1.71
Cluster 2	46 (23.6%)	Moderate	$1.72 < \text{Hb/Kg} \leq 2.10$	1.80	1.76
Cluster 3	73 (37.4%)	Severe	> 2.10	2.41	2.44

Note: Hb/kg, the haemoglobin mass loss per kilogram of patient weight. The values represent the mean Hb/kg Index within each cluster identified by K-medoids clustering.

Discriminative performance of clusters. The discriminative ability of the derived clusters was assessed using ROC analysis (Figure 9). The Severe cluster showed the strongest discriminative performance (AUC = 0.790; 95% CI 0.721–0.859), indicating high accuracy in identifying patients with significant haemoglobin mass loss. In contrast, the Moderate cluster demonstrated weak separability (AUC = 0.576), while the Mild cluster showed moderate performance (AUC = 0.659). The overall micro-averaged AUC was 0.687.

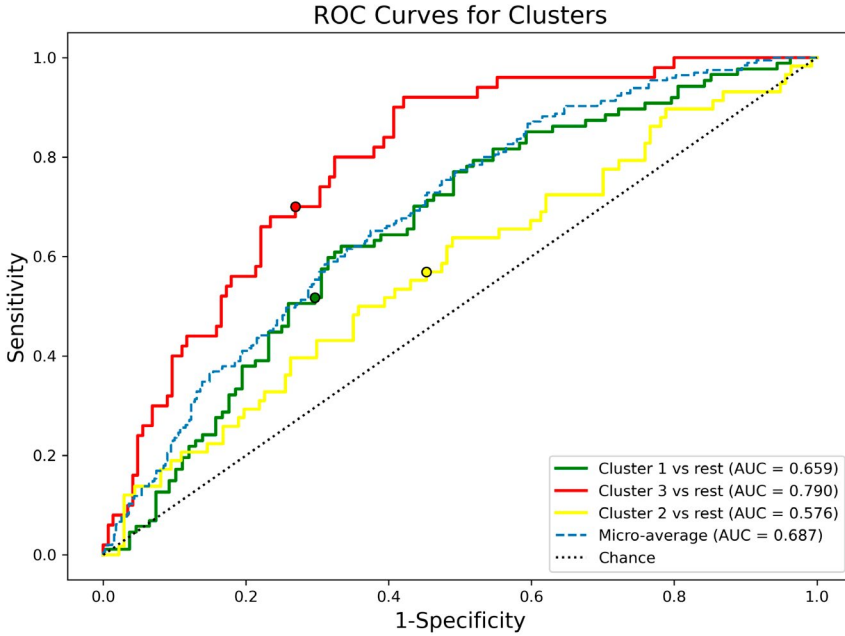


Fig. 9. Receiver Operating Characteristic (ROC) curves for predicting blood loss severity clusters based on the Hb/kg Index thresholds.

4.3. Clinical and anthropometric parameters across clusters

Adjusted two-way ANCOVA confirmed that the Hb/kg Index differed significantly across clusters ($F(2,186) = 250.44$, $p < 0.001$, $\eta_p^2 = 0.729$), explaining 80.3% of the variance ($R^2 = 0.803$) (Table 9). The Hb/kg Index increased from Mild to Severe cluster (1.22 ± 0.35 ; 1.94 ± 0.10 ; 2.65 ± 0.36 , respectively). No effect of gender ($p = 0.757$) or Cluster $\times$ Gender interaction ($p = 0.992$) was observed. Anthropometric parameters differed significantly between clusters. Weight decreased from Mild to Severe cluster (89.4 ± 18.2 kg, 84.7 ± 13.6 kg, and 78.9 ± 14.5 kg, respectively; $p < 0.001$), as well as BMI (30.5 ± 5.3 , 28.7 ± 4.9 , and 26.3 ± 4.1 kg/m², respectively; $p < 0.001$). BSA and EBV demonstrated a similar pattern, with lower values in the Severe cluster ($p < 0.001$ and $p = 0.002$, respectively). Baseline LVEF and creatinine levels did not differ significantly between clusters ($p = 0.199$ and $p = 0.791$, respectively). No significant differences were observed for EuroSCORE II ($p = 0.088$) and the Papworth Bleeding Risk Score ($p = 0.232$) (Table 9).

Table 9. Patient characteristics across clusters

Characteristics	Mild group	Moderate group	Severe group	F	p-value	η_p^2
Hb/kg Index	1.22 ± 0.35	1.94 ± 0.10	2.65 ± 0.36	250.435	<0.001	0.729
Weight (kg)	89.4 ± 18.2	84.7 ± 13.6	78.9 ± 14.5	9.811	<0.001	0.095
BMI (kg/m ²)	30.5 ± 5.3	28.7 ± 4.9	26.3 ± 4.1	10.322	<0.001	0.099
BSA (m ²)	2.01 ± 0.23	1.97 ± 0.16	1.91 ± 0.20	7.989	<0.001	0.078
EBV (L)	5.17 ± 0.92	5.11 ± 0.64	4.94 ± 0.81	6.637	0.002	0.066
LVEF, %	51.93 ± 5.47	52.09 ± 6.04	49.37 ± 8.14	1.627	0.199	0.017
Creatinine	84.76 ± 22.48	87.27 ± 17.36	86.35 ± 17.49	0.235	0.791	0.002
EuroSCORE II	2.85 ± 2.33	2.56 ± 1.76	2.17 ± 1.63	2.460	0.088	0.026
Papworth Bleeding Risk Score	1.11 ± 0.90	0.96 ± 1.01	1.18 ± 1.05	1.473	0.232	0.015

Note: Values are presented as mean ± standard deviation (SD) after outlier removal within each Cluster × Gender cell using the 3×IQR rule. Group comparisons were performed using two-way analysis of covariance (ANCOVA), with Cluster and Gender as fixed factors and Age as a covariate, including the Cluster × Gender interaction included in the model. All reported F-tests refer to the main effect of Cluster (Type III sum of squares). Statistical significance was defined as $p < 0.05$. Partial eta squared (η_p^2) was interpreted according to Cohen's guidelines: 0.01, small; 0.06, medium; 0.14, large.

4.4. Perioperative changes in complete blood count parameters

Complete blood count parameters decreased across the perioperative period (Table 10). Haemoglobin levels decreased from 139.57 ± 13.31 g/L at baseline to 103.51 ± 14.72 g/L at T18 ($p < 0.001$). Haematocrit declined from $41.86 \pm 4.11\%$ to $30.44 \pm 4.34\%$. RBC count from $4.62 \pm 0.52 \times 10^{12}/L$ to $3.38 \pm 0.54 \times 10^{12}/L$ and platelet count from $221.25 \pm 57.76 \times 10^9/L$ to $160.05 \pm 46.33 \times 10^9/L$ (all $p < 0.001$). Figure 10.

Table 10. Dynamic changes in complete blood count parameters

Parameters	T0	T1	T6	T18	p-value
Haemoglobin (g/L)	139.57 ± 13.3	110.84 ± 13.3	109.96 ± 13.83	103.51 ± 14.72	<0.001
Haematocrit (%)	41.86 ± 4.11	32.87 ± 4.16	32.78 ± 3.98	30.44 ± 4.34	<0.001
RBC ($\times 10^{12}/L$)	4.62 ± 0.52	3.64 ± 0.50	3.62 ± 0.51	3.38 ± 0.54	<0.001
Platelets ($\times 10^9/L$)	221.25 ± 57.76	167.58 ± 48.65	171.35 ± 47.76	160.05 ± 46.33	<0.001

Note: Data are presented as mean ± standard deviation (SD). Overall differences across perioperative time points were assessed using the Friedman test.

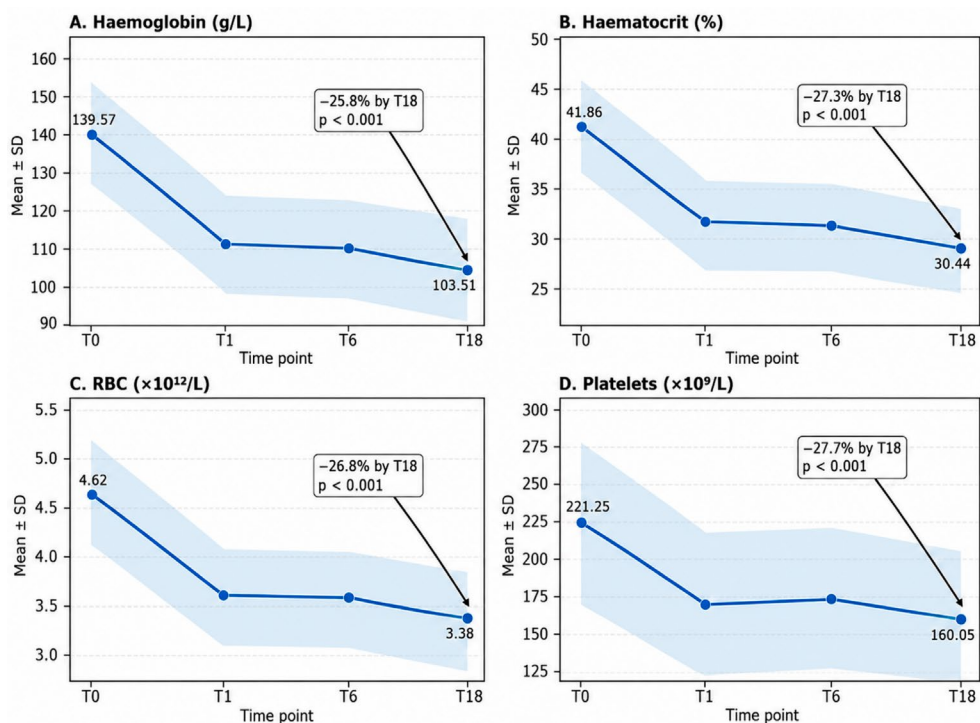


Fig. 10. Perioperative dynamics of complete blood count parameters (mean $\pm$ SD)

4.4.1. Correlations with the Hb/kg Index

Baseline haemoglobin, haematocrit, and RBC demonstrated positive correlation with the Hb/kg Index (all $p < 0.001$). In contrast, haemoglobin, haematocrit, and RBC values at T1, T6 and T18 negatively correlated with the Hb/kg Index. The strongest associations were observed for haematocrit and RBC at T18 (both $p < 0.001$). Platelet count did not show statistically significant correlations with the Hb/kg Index at any time point (Table 11).

Table 11. Correlation coefficients between the Hb/kg Index and complete blood count parameters at different time points

Parameters	T0	T1	T6	T18
Haemoglobin	0.375 ($p < 0.001$)	-0.287 ($p < 0.001$)	-0.153 ($p = 0.032$)	-0.283 ($p < 0.001$)
Haematocrit	0.308 ($p < 0.001$)	-0.273 ($p < 0.001$)	-0.193 ($p = 0.007$)	-0.336 ($p < 0.001$)
RBC	0.235 ($p < 0.001$)	-0.323 ($p < 0.001$)	-0.232 ($p = 0.001$)	-0.338 ($p < 0.001$)
Platelets	0.072 ($p = 0.319$)	-0.131 ($p = 0.067$)	-0.035 ($p = 0.631$)	-0.090 ($p = 0.211$)

Data are presented as Spearman's ρ (p-value).

4.4.2. Complete blood count dynamics across clusters

Haemoglobin levels varied significantly between clusters during the perioperative period. At baseline, the highest values were observed in the Severe cluster (144.5 ± 11.7 g/L) and the lowest in the Mild cluster (134.5 ± 14.6 g/L; $p < 0.001$). However, following surgery (T1), the Severe cluster demonstrated the most pronounced decline, reaching the lowest absolute values (106.1 ± 12.6 g/L) among all clusters ($p < 0.001$). While differences were not significant at T6 ($p = 0.112$), a significant divergence re-emerged by T18 ($p = 0.004$), when the Severe group failed to show recovery, maintaining a significantly lower haemoglobin concentration (98.4 ± 14.6 g/L) compared to the Mild cluster (107.0 ± 11.9 g/L). Haematocrit varied significantly between clusters at all time points: T0 ($p = 0.018$), T1 ($p < 0.001$), T6 ($p = 0.019$), and T18 ($p < 0.001$). The most significant difference between clusters observed at T1 and T18. RBC counts showed no significant differences at baseline ($p = 0.134$). However, an immediate post-surgical drop (T1) affected the Severe cluster ($p < 0.001$) and persisted at T6 ($p = 0.018$) and T18 ($p = 0.002$). The lowest RBC counts were found in the Severe cluster at the final observation point (T18: $3.20 \pm 0.59 \times 10^{12}/L$). In contrast to RBC, platelet counts did not differ significantly between clusters at any time point. These data are presented in Table 12 and Figure 11.

Table 12. Common blood count parameters across clusters at different time points

Time points	Mild group	Moderate group	Severe group	F	p-value	η_p^2
Haemoglobin concentration, g/L						
T0	134.5 ± 14.6	140.2 ± 10.3	144.5 ± 11.7	8.128	<0.001	0.080
T1	115.3 ± 14.0	111.0 ± 10.7	106.1 ± 12.6	7.863	<0.001	0.077
T6	112.9 ± 12.7	109.6 ± 12.9	107.4 ± 14.0	2.212	0.112	0.023
T18	107.0 ± 11.9	104.9 ± 16.0	98.4 ± 14.6	5.691	0.004	0.057
Haematocrit, %						
T0	40.6 ± 4.5	42.2 ± 3.3	42.9 ± 3.8	4.084	0.018	0.042
T1	34.0 ± 4.4	33.2 ± 3.6	31.4 ± 3.9	7.651	<0.001	0.075
T6	33.7 ± 3.9	32.8 ± 3.8	31.8 ± 4.0	4.039	0.019	0.042
T18	31.7 ± 3.5	30.7 ± 4.2	28.7 ± 4.0	9.065	<0.001	0.088
RBC, $10^{12}/L$						
T0	4.49 ± 0.49	4.68 ± 0.44	4.72 ± 0.49	2.031	0.134	0.021
T1	3.81 ± 0.53	3.68 ± 0.43	3.43 ± 0.44	11.853	<0.001	0.112
T6	3.74 ± 0.52	3.65 ± 0.48	3.47 ± 0.47	4.087	0.018	0.042
T18	3.54 ± 0.44	3.41 ± 0.50	3.20 ± 0.59	6.323	0.002	0.063

Table 12 cont.

Time points	Mild group	Moderate group	Severe group	F	p-value	η_p^2
Platelets, $10^9/L$						
T0	216.29 $\pm$ 53.40	218.13 $\pm$ 54.25	224.88 $\pm$ 57.39	0.369	0.692	0.015
T1	171.25 $\pm$ 41.48	168.71 $\pm$ 37.02	157.90 $\pm$ 40.74	1.318	0.270	0.026
T6	171.09 $\pm$ 47.07	173.02 $\pm$ 40.52	166.74 $\pm$ 41.43	0.080	0.923	0.010
T18	163.71 $\pm$ 46.17	160.20 $\pm$ 44.41	152.75 $\pm$ 37.97	0.517	0.597	0.017

Note: Values are presented as mean $\pm$ standard deviation (SD) after outlier removal within each Cluster $\times$ Gender cell using the $3 \times IQR$ rule. Group comparisons were performed using two-way analysis of covariance (ANCOVA), with Cluster and Gender as fixed factors and Age as a covariate, including the Cluster $\times$ Gender interaction included in the model. All reported F-tests refer to the main effect of Cluster (Type III sum of squares). Statistical significance was defined as $p < 0.05$. Partial eta squared (η_p^2) was interpreted according to Cohen's guidelines: 0.01, small; 0.06, medium; 0.14, large.

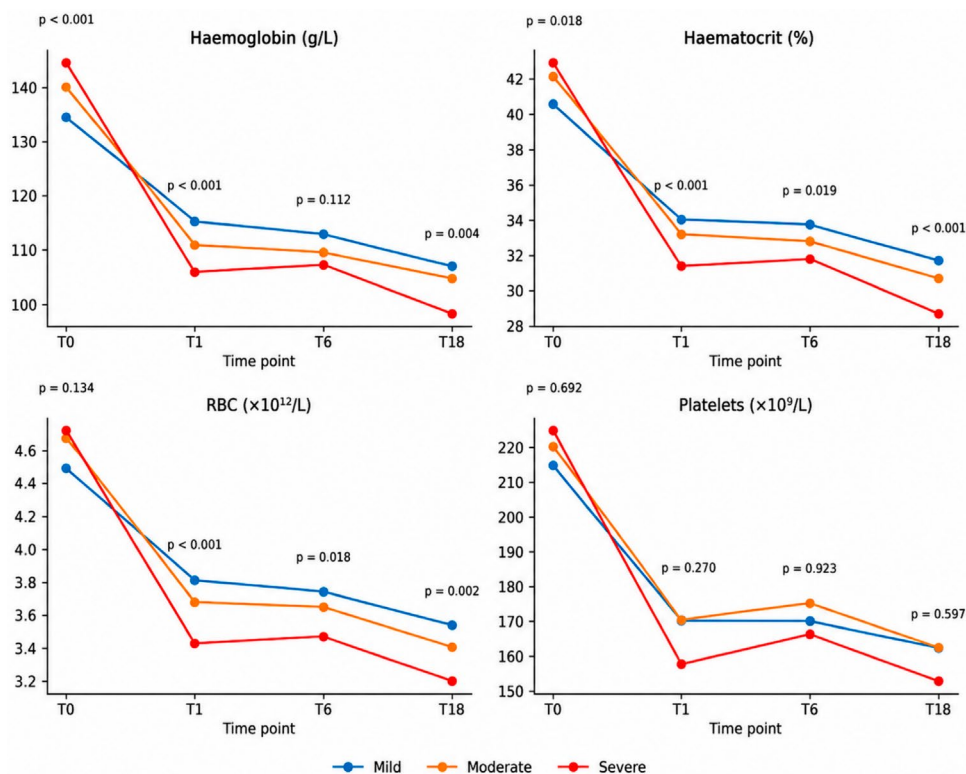


Fig. 11. Dynamics of CBC across clusters at perioperative time points

4.5. Standard coagulation tests (SCTs)

Coagulation parameters demonstrated significant changes (Table 13 and Figure 12). aPTT increased from baseline to its peak at T6 from 36.18 ± 10.36 s to 44.95 ± 12.03 s, then partially recovering to 39.35 ± 8.53 s at T18. INR increased from 1.04 ± 0.10 at baseline to 1.25 ± 0.12 at T18. In contrast, fibrinogen concentration decreased at T1 compared with baseline, from 3.86 ± 0.89 g/L to 2.87 ± 0.71 g/L, then recovered to 3.78 ± 0.74 g/L at T18. D-dimer was not measured at baseline (T0). D-dimer decreased from T1 to T18, from 0.66 ± 0.51 mg/L to 0.55 ± 0.37 mg/L (all $p < 0.001$).

Table 13. Dynamic changes in coagulation parameters at different time points

Parameters	T0	T1	T6	T18	p-value
aPTT, s	36.18 ± 10.36	43.93 ± 10.59	44.95 ± 12.03	39.35 ± 8.53	<0.001
INR	1.04 ± 0.10	1.20 ± 0.10	1.18 ± 0.11	1.25 ± 0.12	<0.001
Fibrinogen concentration, g/L	3.86 ± 0.89	2.87 ± 0.71	3.13 ± 0.74	3.78 ± 0.74	<0.001
D-dimer, mg/L		0.66 ± 0.51	0.64 ± 0.46	0.55 ± 0.37	<0.001

Note: Values are presented as mean $\pm$ SD. Overall differences across time points were assessed using the Friedman test.

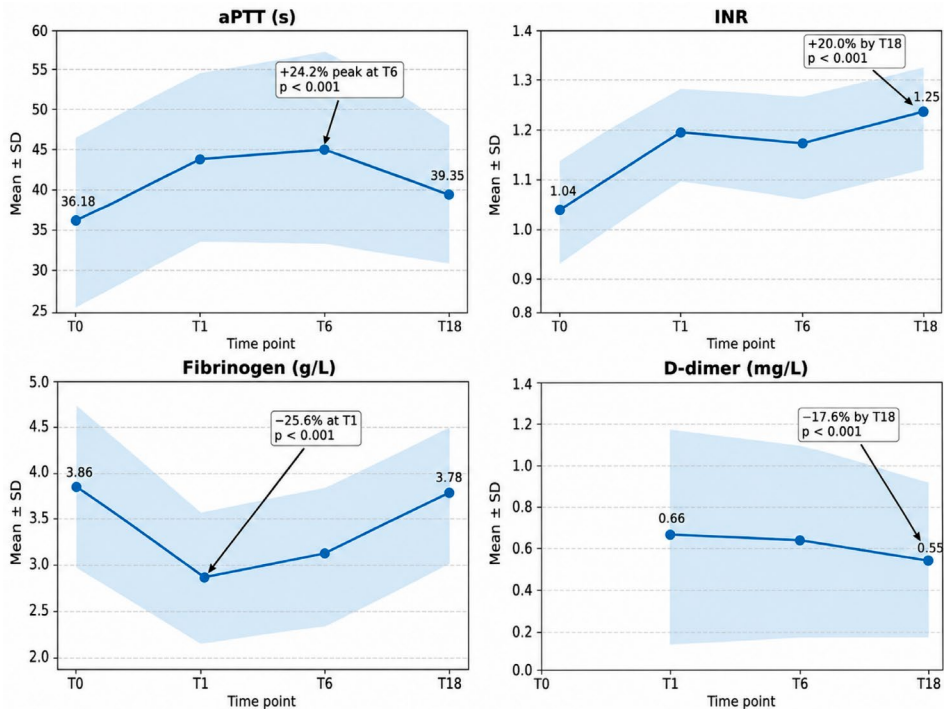


Fig. 12. Dynamics of coagulation tests across clusters at perioperative time points

4.5.1. Correlations with the Hb/kg Index

Correlation analysis demonstrated significant association between the Hb/kg Index and fibrinogen levels at multiple time points. The strongest negative correlation was observed at T1 and T18 ($\rho = -0.308$ and $\rho = -0.285$; both $p < 0.001$). In addition, significant association was observed T6 ($\rho = -0.180$, $p = 0.012$). No significant correlations were found between the Hb/kg Index and INR or D-dimer at any time point (all $p > 0.05$). Regarding aPTT, a significant positive correlation was observed at T18 ($\rho = 0.147$, $p = 0.040$).

Table 14. Correlation coefficients between the Hb/kg Index and complete blood count parameters at different time points

Parameters	T0	T1	T6	T18
aPTT, s	0.028 ($p = 0.700$)	0.045 ($p = 0.528$)	0.085 ($p = 0.239$)	0.147 ($p = 0.040$)
INR	-0.083 ($p = 0.249$)	0.126 ($p = 0.080$)	0.050 ($p = 0.490$)	0.089 ($p = 0.217$)
Fibrinogen concentration, g/L	-0.090 ($p = 0.210$)	-0.308 ($p < 0.001$)	-0.180 ($p = 0.012$)	-0.285 ($p < 0.001$)
D-dimer, mg/L		-0.041 ($p = 0.568$)	-0.099 ($p = 0.167$)	-0.109 ($p = 0.129$)

Data are presented as Spearman's ρ (p-value). Abbreviations: aPTT – activated partial thromboplastin time; INR – International Normalised Ratio. D-dimer was not available at baseline. T0, baseline; T1, early postoperative period; T6, 6 hours after surgery; T18, 18 hours after surgery.

4.5.2. Standard coagulation tests across clusters

The longitudinal analysis of standard coagulation parameters across clusters is summarized in Table 15 and Figure 13. Baseline fibrinogen concentration did not differ between clusters ($p = 0.057$). At T1, significant difference was observed ($p = 0.001$) with the lowest values in the Severe cluster (2.66 ± 0.57 g/L) compared with the Mild (3.15 ± 0.75 g/L) and Moderate (2.83 ± 0.57 g/L) clusters. No significant differences between clusters were detected at T6 ($p = 0.290$). At 18, fibrinogen concentration again differed significantly across clusters ($p = 0.003$), with the lowest levels in the Severe cluster (3.59 ± 0.57 g/L), compared with the Mild (4.04 ± 0.76 g/L) and Moderate clusters (3.68 ± 0.73 g/L). D-dimer did not differ significantly between clusters at any time point. aPTT also did not differ between clusters at T0, T1 and T6 ($p > 0.05$). only significant difference was observed at T18 ($p = 0.034$). INR show no significant differences ($p > 0.05$).

Table 15. Standard coagulation parameters across blood loss severity clusters at different time points

Time	Mild group	Moderate group	Severe group	F	p-value	η_p^2
Fibrinogen concentration, g/L						
T0	4.02 ± 0.99	3.83 ± 0.88	3.70 ± 0.75	2.911	0.057	0.030
T1	3.15 ± 0.75	2.83 ± 0.57	2.66 ± 0.57	6.780	0.001	0.068
T6	3.27 ± 0.81	3.12 ± 0.69	2.99 ± 0.71	1.248	0.290	0.013
T18	4.04 ± 0.76	3.68 ± 0.73	3.59 ± 0.57	5.904	0.003	0.060
D-dimer, mg/L						
T1	0.67 ± 0.41	0.60 ± 0.36	0.61 ± 0.40	0.771	0.464	0.008
T6	0.69 ± 0.41	0.58 ± 0.34	0.53 ± 0.30	2.744	0.067	0.029
T18	0.60 ± 0.35	0.54 ± 0.36	0.45 ± 0.26	1.785	0.171	0.019
aPTT, s						
T0	34.35 ± 6.98	34.74 ± 5.58	34.93 ± 6.33	0.192	0.825	0.002
T1	41.55 ± 6.93	43.26 ± 8.34	42.91 ± 6.92	1.703	0.185	0.018
T6	43.68 ± 11.04	45.00 ± 11.87	45.41 ± 10.95	0.057	0.945	0.001
T18	37.23 ± 4.32	40.94 ± 6.67	38.81 ± 4.55	3.435	0.034	0.036
INR						
T0	1.02 ± 0.08	1.05 ± 0.10	1.02 ± 0.09	0.474	0.623	0.005
T1	1.18 ± 0.08	1.21 ± 0.10	1.21 ± 0.11	1.029	0.359	0.011
T6	1.17 ± 0.10	1.17 ± 0.09	1.18 ± 0.10	0.138	0.872	0.002
T18	1.24 ± 0.10	1.25 ± 0.12	1.26 ± 0.12	0.194	0.824	0.002

Note: Values are presented as mean ± standard deviation (SD) after outlier removal within each Cluster × Gender cell using the 3×IQR rule. Group comparisons were performed using two-way analysis of covariance (ANCOVA), with Cluster and Gender as fixed factors and Age as a covariate, including the Cluster × Gender interaction included in the model. All reported F-tests refer to the main effect of Cluster (Type III sum of squares). Statistical significance was defined as $p < 0.05$. Partial eta squared (η_p^2) was interpreted according to Cohen's guidelines: 0.01, small; 0.06, medium; 0.14, large.

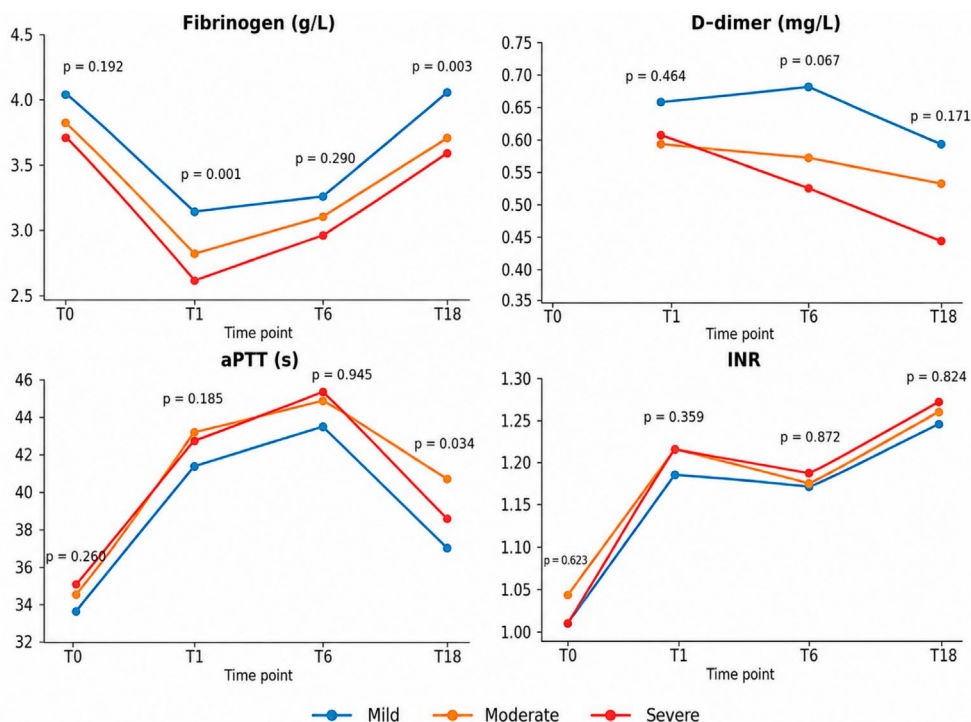


Fig. 13. Dynamics of SCTs across clusters at perioperative time points

4.6. Plasminogen Activator Inhibitor Type-1 (PAI-1)

Plasma levels of PAI-1 were measured in 127 patients at baseline (T0) and 18 hours postoperatively (T18). The mean baseline PAI-1 concentration was 35.14 ± 19.32 ng/ml, increasing to 46.32 ± 18.25 ng/ml. Due to non-normal distribution at both time points, changes over time were assessed using the Wilcoxon signed-rank test. Postoperative (T18) PAI-1 levels were significantly higher than baseline values ($Z = -4.974$, $p < 0.001$).

A weak but statistically significant positive correlation was observed between PAI-1 levels at T0 and T18 ($\rho = 0.213$, $p = 0.016$). No significant correlation was found between the Hb/kg Index and PAI-1 levels at T0 ($\rho = -0.066$, $p = 0.464$) and at T18 ($\rho = 0.127$, $p = 0.155$).

PAI-1 levels were further analysed across clusters using ANCOVA (Table 16). Mean baseline PAI-1 concentrations were 42.17 ± 25.87 in the Mild cluster, 28.40 ± 9.96 in the Moderate, and 31.64 ± 13.05 in the Severe clusters. No statistically significant differences in preoperative PAI-1 levels were observed ($F = 2.24$, $p = 0.111$, $p = 0.037$, $R^2 = 0.115$). Mean PAI-1 concentrations at T18 were 45.98 ± 17.42 in the Mild, 40.12 ± 17.54 in the

Moderate, and 48.94 ± 18.61 in the Severe cluster. Similarly, no statistically significant differences were found between clusters at T18, although a borderline trend was observed ($F = 2.74$, $p = 0.069$, $\eta_p^2 = 0.044$, $R^2 = 0.161$).

Table 16. Preoperative plasminogen activator inhibitor type-1 (PAI-1) across blood loss severity clusters at different time points ($N = 127$)

Time points	Mild group (N = 35)	Moderate group (N = 26)	Severe group (N = 66)	F	p-value	η_p^2
PAI-1 at T0	42.17 ± 25.87	28.40 ± 9.96	31.64 ± 13.05	2.24	0.111	0.115
PAI-1 at T18	45.98 ± 17.42	40.12 ± 17.54	48.94 ± 18.61	2.74	0.069	0.161

Note: Values are presented as mean $\pm$ standard deviation (SD) after outlier removal within each Cluster $\times$ Gender cell using the $3 \times \text{IQR}$ rule. Group comparisons were performed using two-way analysis of covariance (ANCOVA), with Cluster and Gender as fixed factors and Age as a covariate, including the Cluster $\times$ Gender interaction included in the model. All reported F-tests refer to the main effect of Cluster (Type III sum of squares). Statistical significance was defined as $p < 0.05$. Partial eta squared (η_p^2) was interpreted according to Cohen's guidelines: 0.01, small; 0.06, medium; 0.14, large.

4.7. Platelet aggregometry (PA)

PA demonstrated significant perioperative changes during the observation period (Table 17). ADP-induced PA decreased by 26.9% at T1 compared with baseline (from $73.06 \pm 14.48\%$ to $53.39 \pm 14.30\%$), followed by an increase to $66.11 \pm 10.67\%$ at T18. Epinephrine-induced PA showed a more pronounced decline, decreasing by 53.3% at T1 (from $77.72 \pm 14.47\%$ to $36.29 \pm 25.79\%$), with sustained suppression observed at T18 ($41.56 \pm 18.42\%$).

Table 17. Dynamic changes in platelet aggregation parameters

Time points	PA (ADP), %	PA (Epinephrine), %	p-value
T0	73.06 ± 14.48	77.72 ± 14.47	<0.001
T1	53.39 ± 14.30	36.29 ± 25.79	<0.001
T18	66.11 ± 10.67	41.56 ± 18.42	<0.001

Correlation analysis showed no significant associations between the Hb/kg Index and ADP-induced PA at T0 ($\rho = -0.103$, $p = 0.150$), T1 ($\rho = -0.078$, $p = 0.278$), or T18 ($\rho = -0.031$, $p = 0.663$). Similarly, no significant correlations were found between the Hb/kg Index and epinephrine-induced PA at T0 ($\rho = 0.016$, $p = 0.826$), T1 ($\rho = -0.021$, $p = 0.770$), or T18 ($\rho = 0.050$, $p = 0.485$). Table 18.

Table 18. Correlation coefficients between the Hb/kg Index and complete blood count parameters at different time points

Parameters	T0	T1	T18
PA (ADP)	-0.103 (p = 0.150)	-0.078 (p = 0.278)	-0.031 (p = 0.663)
PA (epinephrine)	0.016 (p = 0.826)	-0.021 (p = 0.770)	0.050 (p = 0.485)

PA parameters induced by ADP and epinephrine across clusters are summarized in Table 19. No statistically significant differences were observed between clusters for ADP-induced or epinephrine-induced PA at any measured time point (all p-values > 0.05).

Table 19. Platelet aggregation with ADP and epinephrine between clusters at different time points

Time	Mild group	Moderate group	Severe group	F	p-value	η_p^2
PA (ADP), %						
T0	74.9 ± 10.7	72.9 ± 18.3	73.4 ± 10.7	0.223	0.800	0.002
T1	55.6 ± 12.7	52.3 ± 15.6	51.8 ± 14.9	0.617	0.541	0.007
T18	66.5 ± 9.8	67.4 ± 10.5	65.5 ± 8.4	1.152	0.318	0.012
PA (Epinephrine), %						
T0	79.0 ± 11.1	80.4 ± 8.5	80.7 ± 6.5	0.328	0.721	0.004
T1	38.4 ± 27.7	36.3 ± 27.0	34.1 ± 23.1	0.292	0.747	0.003
T18	40.9 ± 17.0	41.8 ± 19.6	41.5 ± 18.8	0.486	0.616	0.005

Note: Values are presented as mean ± SD. F – F-statistics from ANCOVA models adjusted for gender and age as covariates. Degrees of freedom for analysis were $df1 = 2$, $df2 = 180-188$. PA – platelet aggregation induced by ADP (5 µM) or epinephrine (5 µM).

4.8. ROTEM parameters

4.8.1. Perioperative changes in ROTEM parameters

Viscoelastic haemostatic properties were assessed in a subgroup of 79 patients (40.5% of the study cohort) before (T0) and immediately after surgery (T1) (Table 20). Clotting time (CT) demonstrated minimal perioperative changes: EXTEM CT remained stable (p = 0.542), while INTEM CT showed a slight but statistically significant prolongation (p = 0.011). In contrast, clot firmness parameters (A10 and MCF) showed a consistent and significant decrease in both EXTEM and INTEM assays (all p < 0.001). The most pronounced reductions were observed in fibrinogen-related parameters (FIBTEM), where FIBTEM A5, A10 and MCF all decreased significantly (all p < 0.001). Conversely, platelet-related parameters (PLTEM) remained

stable. No statistically significant differences were observed in any amplitude of PLTEM parameters (A5, A10, MCF; all $p > 0.05$). Residual heparin effect was assessed by comparing INTEM and HEPTTEM CT after surgery. A statistically significant difference was observed with median HEPTTEM CT of 185 [175–191] s, compared with 195 [187–212] s in INTEM CT ($p < 0.001$). In 26 patients (33%), the INTEM/HEPTTEM CT ratio exceeded 1.1.

Table 20. *Dynamic changes in ROTEM parameters before and after CPB (N = 79)*

Parameters	Baseline (T0)	Postoperative (T1)	p-value
EXTEM CT, s	64 [60–69]	63 [60–68]	0.542
EXTEM A10, mm	59 [56–62]	57 [53–61]	< 0.001
EXTEM MCF, mm	66 [64–69]	64 [61–68]	< 0.001
INTEM CT, s	193 [184–202]	195 [186–212]	0.011
INTEM A10, mm	55 [53–58]	53 [50–57]	< 0.001
INTEM MCF, mm	63 [60–66]	61 [57–65]	< 0.001
FIBTEM A5, mm	13 [11–16]	11 [9–14]	< 0.001
FIBTEM A10, mm	15 [12–17]	12 [10–15]	< 0.001
FIBTEM MCF, mm	16 [14–19]	14 [12–17]	< 0.001
PLTEM A5, mm	35 [33–38]	35 [33–39]	0.772
PLTEM A10, mm	44 [42–46]	44 [42–47]	0.630
PLTEM MCF, mm	50 [49–53]	50 [48–52]	0.195

Data are presented as median and interquartile range (IQR). P-values calculated using Wilcoxon signed-rank test. Note: CT — clotting time; A5/A10 — clot amplitude at 5 and 10 minutes; MCF — maximum clot firmness; EXTEM — extrinsic test; INTEM — intrinsic test; FIBTEM — fibrinogen-specific test; PLTEM — calculated platelet contribution (EXTEM A-amplitude minus FIBTEM A-amplitude).

4.8.2. Correlation with Hb/kg Index

Correlation analysis of perioperative ROTEM parameters and the Hb/kg Index is presented in Table 21 and Figure 14. Initial FIBTEM parameters, including A5 ($\rho = -0.231$, $p = 0.039$), A10 ($\rho = -0.242$, $p = 0.032$), and MCF ($\rho = -0.236$, $p = 0.036$), demonstrated statistically significant inverse correlations with the Hb/kg Index. Postoperatively (T1), these associations persisted and became more pronounced (all $p < 0.01$).

EXTEM and INTEM parameters were not associated with the Hb/kg Index at baseline. At T1, inverse correlations were found for EXTEM A10

($\rho = -0.318$, $p = 0.004$) and MCF ($\rho = -0.311$, $p = 0.005$), as well as for INTEM A10 ($\rho = -0.322$, $p = 0.004$) and MCF ($\rho = -0.343$, $p = 0.002$). EXTEM CT and INTEM CT were not associated with the Hb/kg Index at either time point (all $p > 0.05$).

For PLTEM, a positive correlation with the Hb/kg Index was observed for A10 at baseline ($\rho = 0.263$, $p = 0.019$), while A5 and MCF were not significant. At T1, no significant correlations were found for any PLTEM parameters.

HEPTEM parameters, which performed only after surgery (T1), demonstrated significant inverse correlations with the Hb/kg Index, including A5 ($\rho = -0.285$, $p = 0.011$), A10 ($\rho = -0.270$, $p = 0.016$), and MCF ($\rho = -0.298$, $p = 0.008$). However, HEPTEM CT did not reach statistical significance ($\rho = 0.214$, $p = 0.058$). APTEM assessed only at baseline, did not demonstrate any statistically significant correlations with Hb/kg Index (all $p > 0.05$).

Table 21. Correlations of perioperative ROTEM parameters with the Hb/kg Index loss

ROTEM parameters	Baseline (T0)		Postoperative (T1)	
	ρ	p-value	ρ	p-value
EXTEM CT, s	0.046	0.685	0.105	0.355
EXTEM A10, mm	-0.107	0.349	-0.318	0.004
EXTEM MCF, mm	-0.220	0.052	-0.311	0.005
INTEM CT, s	-0.176	0.121	0.180	0.112
INTEM A10, mm	-0.091	0.424	-0.322	0.004
INTEM MCF, mm	-0.182	0.109	-0.343	0.002
FIBTEM A5, mm	-0.231	0.039	-0.337	0.002
FIBTEM A10, mm	-0.242	0.032	-0.338	0.002
FIBTEM MCF, mm	-0.236	0.036	-0.315	0.005
PLTEM A5, mm	0.205	0.071	-0.142	0.215
PLTEM A10, mm	0.263	0.019	-0.083	0.465
PLTEM MCF, mm	0.167	0.142	-0.077	0.499
HEPTEM CT, s	—	—	0.214	0.058
HEPTEM A5, mm	—	—	-0.285	0.011
HEPTEM A10, mm	—	—	-0.270	0.016
HEPTEM MCF, mm	—	—	-0.298	0.008

Table 21 cont.

ROTEM parameters	Baseline (T0)		Postoperative (T1)	
	ρ	p-value	ρ	p-value
APTEM CT, s	-0.109	0.340	—	—
APTEM A5, mm	-0.084	0.464	—	—
APTEM A10, mm	-0.097	0.395	—	—
APTEM MCF, mm	-0.163	0.151	—	—

Abbreviations: MCF: Maximum Clot Firmness; PA: Platelet Aggregation; PLTEM: calculated platelet contribution to clot firmness (EXTEM – FIBTEM); A5: clot amplitude at 5 min; A10: clot amplitude at 10 min;

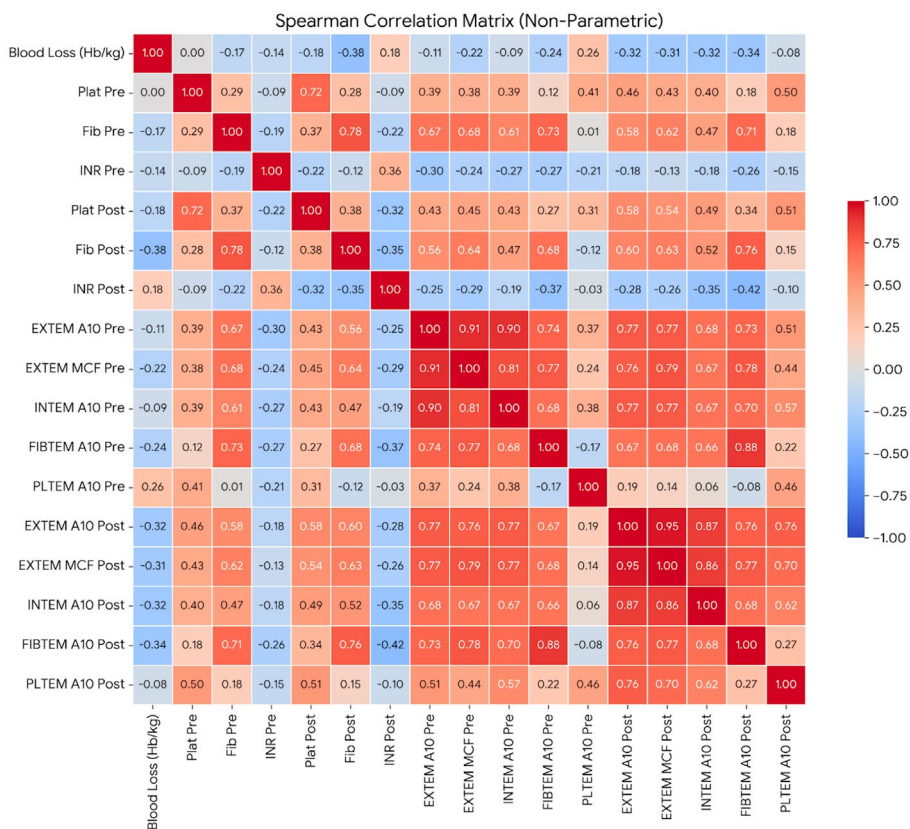


Fig. 14. Correlation analysis of perioperative ROTEM parameters and the Hb/kg Index

4.8.3. ROTEM parameters across clusters

At baseline, most ROTEM parameters did not differ significantly (Table 22), including EXTEM CT ($p = 0.443$), EXTEM A10 ($p = 0.632$), EXTEM MCF ($p = 0.165$), INTEM CT ($p = 0.737$), INTEM A10 ($p = 0.791$), or INTEM MCF ($p = 0.461$). However, fibrinogen-related parameters (FIBTEM) showed significant differences between groups. FIBTEM A10 decreased from 16.35 ± 4.22 mm in the Mild cluster to 13.72 ± 3.24 mm in the Severe cluster ($F = 3.462$, $p = 0.037$, $\eta_p^2 = 0.088$). FIBTEM MCF decreased from 18.06 ± 4.80 mm to 15.10 ± 3.60 mm ($F = 3.571$, $p = 0.033$, $\eta_p^2 = 0.090$). All PLTEM parameters did not differ between clusters.

After surgery EXTEM CT, A10 did not differ between clusters ($p > 0.05$), only EXTEM MCF was significantly lower in the Severe group ($F = 3.909$, $p = 0.024$, $\eta_p^2 = 0.098$). INTEM A10 ($F = 3.566$, $p = 0.033$, $\eta_p^2 = 0.090$) and INTEM MCF ($F = 4.991$, $p = 0.009$, $\eta_p^2 = 0.122$) demonstrated statistically significant difference, while INTEM CT was not significant ($p = 0.366$). The most pronounced differences were seen in fibrinogen-dependent parameters. FIBTEM A10 decreased from 14.53 ± 3.79 mm in the Mild cluster to 11.47 ± 2.95 mm in the Severe cluster ($F = 4.990$, $p = 0.006$, $\eta_p^2 = 0.132$). FIBTEM MCF also decreased from 16.26 ± 4.59 mm to 12.81 ± 3.46 mm ($F = 4.908$, $p = 0.010$, $\eta_p^2 = 0.120$). In contrast, neither PLTEM A10 ($p = 0.754$) nor PLTEM MCF ($p = 0.811$) did not differ between clusters.

Table 22. ROTEM parameters across groups ($N = 79$)

ROTEM parameters	Mild group ($N = 31$)	Moderate group ($N = 19$)	Severe group ($N = 29$)	F	p-value	η_p^2
Before surgery (T0)						
EXTEM CT, s	65.00 ± 9.54	64.58 ± 6.36	65.72 ± 9.52	0.823	0.443	0.022
EXTEM A10, mm	59.32 ± 3.97	59.21 ± 6.01	58.14 ± 3.87	0.461	0.632	0.013
EXTEM MCF, mm	67.65 ± 3.56	67.32 ± 5.21	65.41 ± 3.41	1.847	0.165	0.049
INTEM CT, s	191.68 ± 27.46	189.74 ± 20.31	193.31 ± 40.51	0.307	0.737	0.008
INTEM A10, mm	55.61 ± 4.70	56.47 ± 5.64	54.72 ± 4.31	0.235	0.791	0.006
INTEM MCF, mm	63.68 ± 4.71	64.89 ± 7.89	61.79 ± 3.67	0.783	0.461	0.021
FIBTEM A10, mm	16.35 ± 4.22	15.32 ± 3.32	13.72 ± 3.24	3.462	0.037	0.088
FIBTEM MCF, mm	18.06 ± 4.80	16.89 ± 3.67	15.10 ± 3.60	3.571	0.033	0.090
PLTEM A10, mm	43.03 ± 2.76	45.53 ± 3.81	45.00 ± 2.71	2.677	0.076	0.069
PLTEM MCF, mm	49.52 ± 2.79	52.32 ± 3.30	50.97 ± 2.31	1.455	0.240	0.039

Table 22 cont.

ROTEM parameters	Mild group (N = 31)	Moderate group (N = 19)	Severe group (N = 29)	F	p-value	η_p^2
Immediately After surgery (T1)						
EXTEM CT, s	62.94 ± 5.43	67.42 ± 10.77	63.46 ± 5.22	0.464	0.631	0.013
EXTEM A10, mm	58.68 ± 4.93	57.16 ± 7.54	54.69 ± 5.00	2.756	0.070	0.071
EXTEM MCF, mm	66.26 ± 4.31	65.16 ± 6.34	62.52 ± 4.04	3.909	0.024	0.098
INTEM CT, s	190.94 ± 30.96	197.84 ± 15.72	215.52 ± 74.63	1.018	0.366	0.028
INTEM A10, mm	54.84 ± 6.09	53.79 ± 6.05	51.28 ± 4.33	3.566	0.033	0.090
INTEM MCF, mm	62.45 ± 4.32	62.26 ± 5.26	58.79 ± 3.83	4.991	0.009	0.122
FIBTEM A10, mm	14.53 ± 3.79	13.00 ± 3.20	11.47 ± 2.95	5.490	0.006	0.132
FIBTEM MCF, mm	16.26 ± 4.59	14.58 ± 3.70	12.81 ± 3.46	4.908	0.010	0.120
PLTEM A10, mm	44.26 ± 2.89	44.58 ± 5.54	43.72 ± 3.16	0.28	0.754	0.008
PLTEM MCF, mm	50.10 ± 2.49	51.11 ± 4.24	49.83 ± 2.58	0.21	0.811	0.006

Note: Values are presented as mean ± standard deviation (SD) after outlier removal within each Cluster × Gender cell using the 3×IQR rule. Group comparisons were performed using two-way analysis of covariance (ANCOVA), with Cluster and Gender as fixed factors and Age as a covariate, including the Cluster × Gender interaction included in the model. All reported F-tests refer to the main effect of Cluster (Type III sum of squares). Statistical significance was defined as $p < 0.05$. Partial eta squared (η_p^2) was interpreted according to Cohen's guidelines: 0.01, small; 0.06, medium; 0.14, large.

4.8.4. The diagnostic accuracy of ROTEM

The diagnostic ability of ROTEM parameters for detection of hypofibrinogenaemia (<2.5 g/L) and thrombocytopenia ($<150 \times 10^9/L$) is presented in Table 23 and Figure 15 (A, B).

For hypofibrinogenaemia FIBTEM A10 demonstrated excellent diagnostic accuracy with an AUC of 0.888 (95% CI 0.797–0.979, $p < 0.001$) (Figure 14A). ROC analysis identified an optimal cut-off value of 12 mm (maximum Youden's index = 0.649), providing high sensitivity (96%) and specificity (69.1%). At this threshold, the negative predictive value (NPV) was 97.4%. FIBTEM MCF demonstrated a similar performance (AUC = 0.895, 95% CI 0.807–0.994, $p < 0.001$).

For thrombocytopenia, PLTEM A10 (AUC = 0.755) and EXTEM A10 (AUC = 0.752) showed a highest AUC value among evaluated parameters. Using an EXTEM A10 cut-off of 57 mm (positive if ≤ 57 mm) yielded a sensitivity of 82% and specificity of 63% (PPV = 55%, NPV = 87%). A PLTEM A10 cut-off of 43 mm provided a more balanced profile, with sensitivity of 71% and specificity of 69% (PPV = 56%, NPV = 81%). Figure 14B.

Table 23. *Diagnostic Accuracy and Cut-off Values of Postoperative ROTEM Parameters for Predicting Hypofibrinogenaemia and Thrombocytopenia*

	ROTEM parameters	Cut-off (mm)	Sensitivity	Specificity	PPV	NPV	AUC	95% CI	p-value
Fibrinogen (2.5 g/l)	FIBTEM A10, mm	≤ 12	95.8	69.1	57.5	97.4	0.888	0.797–0.979	< 0.001
	FIBTEM MCF, mm	≤ 14	100.0	65.5	55.8	100.0	0.895	0.807–0.984	< 0.001
Platelets ($< 150 \times 10^9/L$)	EXTEM A10, mm	≤ 57	82.1	62.7	54.8	86.5	0.752	0.634–0.870	< 0.001
	EXTEM MCF, mm	≤ 64	78.6	62.7	53.7	84.2	0.744	0.625–0.872	< 0.001
	PLTEM A10, mm	≤ 43	71.4	68.6	55.6	81.4	0.755	0.638–0.872	< 0.001
	PLTEM MCF, mm	≤ 48	50.0	84.3	63.6	75.4	0.735	0.614–0.856	< 0.001

Note: Sensitivity, specificity, PPV, and NPV are presented as percentages (%). AUC values and 95% CI were determined using ROC curve analysis. The p-value indicates the statistical significance of the AUC. Abbreviations: A10, clot amplitude at 10 minutes; AUC, area under the receiver operating characteristic curve; CI, confidence interval; EXTEM, extrinsic pathway test; FIBTEM, fibrinogen-specific thromboelastometry test; MCF, maximum clot firmness; NPV, negative predictive value; PLTEM, calculated platelet contribution to clot firmness (EXTEM MCF – FIBTEM MCF); PPV, positive predictive value; ROTEM, rotational thromboelastometry.

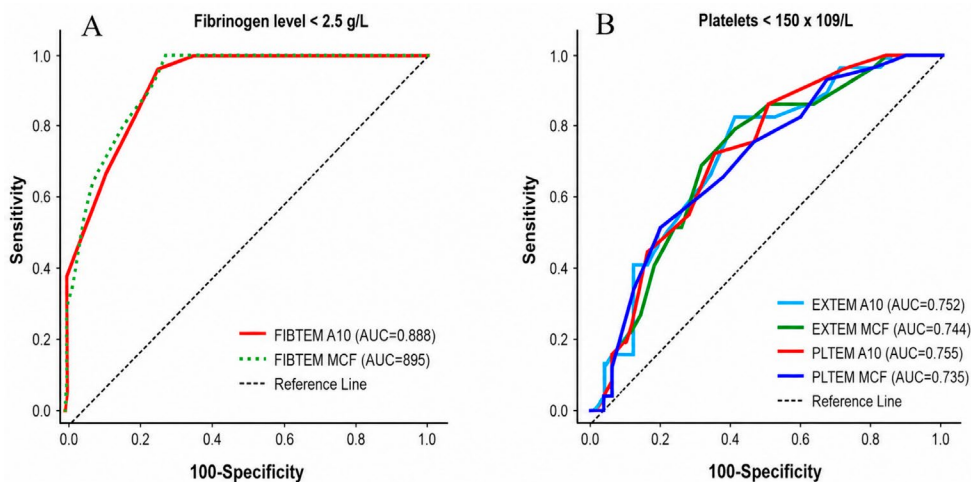


Fig. 15. ROC-curves for ROTEM parameters

4.9. Chest tube drainage: volume versus content

Chest tube drainage (CTD) volume. CTD volume varied significantly between clusters at T1, T2, T4, T5, and T6 (all $p < 0.01$), with the highest volumes consistently observed in the Severe cluster. However, these differences were not significant at T3 ($p = 0.075$). The cumulative drainage volume at 18 hours (Summary CTD) demonstrated no statistically significant difference between ($F = 2.361$, $p = 0.097$, $\eta_p^2 = 0.025$) (Table 24).

Haemoglobin mass loss via chest tube drainage. The actual haemoglobin mass loss demonstrated a more robust clinical pattern (Table 24). Haemoglobin mass loss via CTD did not differ between clusters at T1 ($F = 3.001$, $p = 0.052$, $\eta_p^2 = 0.032$). However, at T6 differed between clusters ($F = 5.426$, $p = 0.005$, $\eta_p^2 = 0.055$), with the highest value in the Severe cluster (19.2 ± 12.9 g) compared with the Mild cluster (14.0 ± 9.2 g). The cumulative haemoglobin mass loss via CTD (Summary T18) demonstrated a statistically significant difference between clusters ($p = 0.011$) ($F = 4.633$, $p = 0.011$, $\eta_p^2 = 0.048$). Figure 16.

Table 24. Hb mass loss via CTD and CTD volume

Time points	Mild group	Moderate group	Severe group	F	p-value	η_p^2
Hb mass loss via chest tubes at different time points, g						
T1	4.1 ± 2.3	4.9 ± 2.6	4.4 ± 2.3	3.001	0.052	0.032
T6	14.0 ± 9.2	13.1 ± 8.7	19.2 ± 12.9	5.426	0.005	0.055
Summary (T18)	15.0 ± 9.3	14.8 ± 10.0	21.5 ± 16.5	4.663	0.011	0.048
Chest tube drainage volume at different time points, mL						
T1	43 ± 22	55 ± 21	53 ± 21	5.873	0.003	0.067
T2	73 ± 43	87 ± 36	99 ± 56	5.430	0.005	0.056
T3	102 ± 65	115 ± 52	128 ± 64	2.268	0.075	0.027
T4	121 ± 71	134 ± 66	160 ± 81	4.908	0.008	0.050
T5	131 ± 71	153 ± 75	160 ± 96	8.928	< 0.001	0.088
T6	160 ± 80	175 ± 92	219 ± 111	5.802	0.004	0.059
Summary CTD (T18)	343 ± 175	341 ± 171	427 ± 186	2.361	0.097	0.025

Note: Values are presented as mean ± standard deviation (SD) after outlier removal within each Cluster × Gender cell using the 3×IQR rule. Group comparisons were performed using two-way analysis of covariance (ANCOVA), with Cluster and Gender as fixed factors and Age as a covariate, including the Cluster × Gender interaction included in the model. All reported F-tests refer to the main effect of Cluster (Type III sum of squares). Statistical significance was defined as $p < 0.05$. Partial eta squared (η_p^2) was interpreted according to Cohen's guidelines: 0.01, small; 0.06, medium; 0.14, large.

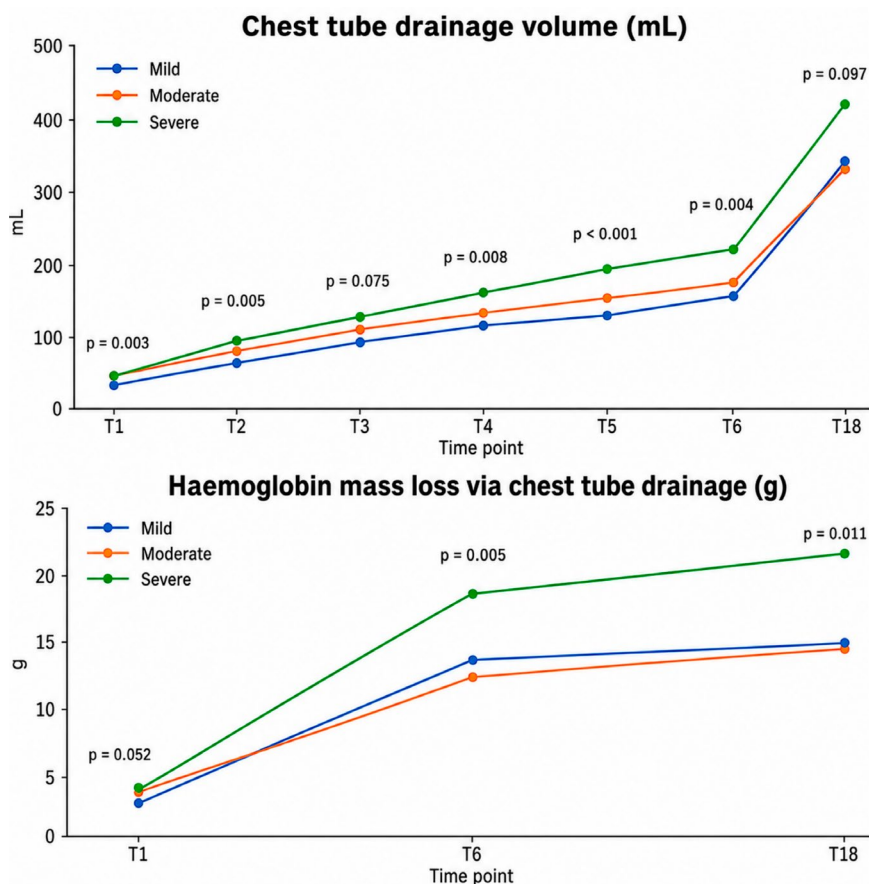


Fig.16. Hb mass loss via CTD and CTD volume between clusters at different time points

4.10. Type of surgery

The distribution of surgical procedures and the extent of coronary revascularisation across clusters are shown in Table 25. The type of surgery did not differ significantly between clusters; however, a borderline difference was observed (Pearson's χ^2 (4) = 9.425, $p = 0.051$). Patients in the Mild cluster more frequently underwent isolated valve procedures (40.8%), while the Moderate and Severe clusters predominantly comprised patients undergoing CABG (60.9% and 57.5%, respectively).

The number of bypass grafts differed significantly across clusters (Pearson's χ^2 (4) = 14.248, $p = 0.027$), with a weak but significant positive ordinal association (Kendall's $\tau_c = 0.178$, $p = 0.005$). In the Mild cluster, 39.5% of patients had no coronary grafting. In contrast, a higher proportion

of patients in the Moderate and Severe clusters received three or more grafts (73.9% and 63.1%, respectively). The proportion of patients receiving four or more grafts was approximately twofold higher in the Moderate (26.1%) and Severe (24.7%) clusters compared with the Mild cluster (11.8%).

Table 25. *Surgical characteristics across groups*

Parameters	Mild group n (%)	Moderate group n (%)	Severe group n (%)	p-value
Type of surgery				
CABG	31 (40.8)	28 (60.9)	42 (57.5)	0.051
Valve procedures	31 (40.8)	8 (17.4)	18 (24.7)	
Combined surgery (CABG + valve)	14 (18.4)	10 (21.7)	13 (17.8)	
Number of grafts				
No grafts	30 (39.5)	8 (17.4)	18 (24.7)	0.027
1-2 grafts	14 (18.4)	4 (8.7)	9 (12.3)	
3 grafts	23 (30.3)	22 (47.8)	28 (38.4)	
4 and more grafts	9 (11.8)	12 (26.1)	18 (24.7)	

Note: Values are presented as counts and percentages (n, %). Group comparisons for categorical variables were performed using the Pearson chi-square (χ^2) test. For ordinal variables, Kendall's tau-c was also used to assess the direction and strength of ordinal associations. CABG: coronary artery bypass grafting.

No significant differences were observed between clusters in the duration of surgery ($p = 0.082$), cardiopulmonary bypass (CPB) time ($p = 0.729$), or aortic cross-clamp time ($p = 0.280$).

Table 26. *Surgery and cardiopulmonary bypass parameters across clusters*

Characteristics	Mild group	Moderate group	Severe group	F	p-value	η_p^2
Duration of Surgery, min	205.9 $\pm$ 53.9	213.3 $\pm$ 53.8	191.9 $\pm$ 52.61	2.537	0.082	0.026
CPB duration, min	98.3 $\pm$ 35.2	96.8 $\pm$ 31.7	96.2 $\pm$ 32.5	0.318	0.729	0.003
Cross-clamp time, min	59.6 $\pm$ 27.2	55.2 $\pm$ 25.3	53.9 $\pm$ 24.4	1.283	0.280	0.013

Note: Values are presented as mean $\pm$ standard deviation (SD) after outlier removal within each Cluster $\times$ Gender cell using the 3 $\times$ IQR rule. Group comparisons were performed using two-way analysis of covariance (ANCOVA), with Cluster and Gender as fixed factors and Age as a covariate, including the Cluster $\times$ Gender interaction included in the model. All reported F-tests refer to the main effect of Cluster (Type III sum of squares). Statistical significance was defined as $p < 0.05$. Partial eta squared (η_p^2) was interpreted according to Cohen's guidelines: 0.01, small; 0.06, medium; 0.14, large.

4.11. Clinical outcomes and risk factors

No statistically significant differences in postoperative complications were observed between clusters. The incidence of heart failure was comparable across groups ($p = 0.823$), as were the rates of atrial fibrillation ($p = 0.646$) and acute kidney injury (AKI; $p = 0.611$). No significant differences were observed for neurological complications ($p = 0.327$) or atrioventricular (AV) block ($p = 0.505$). In addition, ICU stay ($p = 0.752$) and total hospital stay ($p = 0.596$) did not differ significantly between clusters. Current smokers differed significantly across clusters (Pearson's $\chi^2 (2) = 11.305$, $p = 0.004$). The prevalence of smokers increased from the Mild to the Severe cluster, accounting for 13.2%, 28.3%, and 37.0% of patients, respectively. In contrast, alcohol consumption did not differ significantly between clusters (Pearson's $\chi^2 (2) = 1.046$, $p = 0.593$). The proportions of patients using alcohol were similar across the Mild, Moderate, and Severe clusters, with no significant linear trend observed.

4.12. The influence of gene variants on blood loss

Genetic analysis was conducted in 195 patients. Three single nucleotide polymorphisms (SNPs) were examined: *ITGB3* (rs5918), which encodes the platelet fibrinogen receptor subunit β_3 ; *FGB* (rs1800790), associated with the fibrinogen β -chain gene; and *SERPINE1* (rs2227631), associated with plasminogen activator inhibitor-1 (PAI-1). Genotype distributions for all polymorphisms were consistent with Hardy–Weinberg equilibrium.

4.12.1. *ITGB3* (rs5918) and its association with blood loss

For rs5918 the genotype frequencies were as follows: TT 74.9%, CT 22.1%, and CC 3.1%. The corresponding allele frequencies were 85.9% (T) and 14.1% (C). For rs5918 no statistically significant association with the Hb/kg Index was observed in any inheritance model (Table 27). The genotypic model was not significant ($p = 0.808$), nor were the additive (adjusted $p = 0.524$), dominant (adjusted $p = 0.485$), or recessive (adjusted $p = 0.946$) models. These results correspond to regression-based genetic models, whereas presents non-parametric comparisons using the Kruskal–Wallis test.

Table 27. *The Hb/kg Index across genotype groups*

SNP	Genotype	N	Hb/Kg index, Median [IQR]	p-value
ITGB3 (rs5918)	TT	146 (74.9%)	1.89 [1.62–2.18]	0.721
	CT	43 (22.1%)	2.05 [1.78–2.34]	
	CC	6 (3.1%)	1.99 [1.70–2.25]	
FGB (rs1800790)	GG	89 (45.6%)	1.93 [1.68–2.20]	0.667
	AG	82 (42.1%)	1.94 [1.70–2.21]	
	AA	24 (12.3%)	1.84 [1.60–2.10]	
SERPINE1 (s2227631)	AA	73 (37.4%)	1.89 [1.66–2.15]	0.769
	AG	96 (49.2%)	1.94 [1.70–2.22]	
	GG	26 (13.3%)	2.02 [1.75–2.30]	

Data are presented as median [interquartile range]. P-values correspond to the Kruskal–Wallis test.

Across all tested models, effect estimates were small, with narrow confidence intervals crossing zero and minimal explained variance ($R^2 \approx 0.002$). In the gene–phenotype scan, rs5918 was not associated with any of the analyzed clinical or laboratory variables after false discovery rate (FDR) correction. Similarly, no statistically significant differences in rs5918 genotype distribution were observed across the Hb/kg-defined blood loss severity clusters. Therefore, rs5918 did not show an association with the Hb/kg Index, perioperative blood loss clusters (Table 28). In an additional exploratory analysis, no statistically significant differences in total chest tube drainage volume (CTD summary) were observed across rs5918 genotype groups ($p = 0.241$) (Table 28). No consistent associations were found for *ITGB3* (rs5918) with any ROTEM parameters.

Table 28. *Total chest tube drainage volume (CTD summary) across genotype groups*

SNP	Genotype	N	CTD volume, Median [IQR], mL	p-value
ITGB3 (rs5918)	TT	146 (74.9%)	300 [250–400]	0.241
	CT	43 (22.1%)	375 [250–500]	
	CC	6 (3.1%)	550 [350–650]	
FGB (rs1800790)	GG	89 (45.6%)	350 [250–450]	0.484
	AG	82 (42.1%)	350 [200–450]	
	AA	24 (12.3%)	325 [238–400]	
SERPINE1 (rs2227631)	AA	73 (37.4%)	300 [250–400]	0.148
	AG	96 (49.2%)	350 [200–450]	
	GG	26 (13.3%)	400 [300–500]	

4.12.2. *FGB* (rs1800790) and its association with blood loss

Genotype frequencies were GG 45.6%, AG 42.1%, and AA 12.3%, with an allele frequency of 66.7% (G) and 33.3% (A). No statistically significant association with the Hb/kg Index was observed in the genotypic ($p = 0.566$), additive (adjusted $p = 0.618$), dominant (adjusted $p = 0.838$), or recessive (adjusted $p = 0.701$) models (Table 27). Effect sizes were small, with CI including zero and low explained variance ($R^2 \approx 0.001$).

No significant differences in genotype distribution were identified across the Mild, Moderate, and Severe clusters ($p = 0.667$). Similarly, no statistically significant differences in CTD summary were observed across rs1800790 genotype groups ($p = 0.484$; Table 28).

However, significant associations were observed between rs1800790 and fibrinogen levels. in the gene–phenotype scan, associations were identified at T0 (effect = 0.283, $p = 6.04 \times 10^{-5}$, FDR = 0.0086), T1 (effect = 0.261, $p = 0.000229$, FDR = 0.0163), and T6 (effect = 0.240, $p = 0.000738$, FDR = 0.035). These findings remained statistically significant after FDR correction.

In additive model, the A allele was associated with increased fibrinogen concentration across all time points:

- T0: $\beta = +0.365$ g/L (95% CI 0.190–0.539, $p = 0.000055$);
- T1: $\beta = +0.249$ g/L (0.118–0.379, $p = 0.000226$);
- T6: $\beta = +0.274$ g/L (0.126–0.422, $p = 0.000334$);
- T18: $\beta = +0.205$ g/L (0.067–0.343, $p = 0.00370$).

Median fibrinogen levels also increased stepwise across genotype groups (GG, AG, AA) at all time points (Table 29 and Figure 17). In addition, repeated-measures analysis demonstrated a significant overall genetic effect ($p = 0.000042$) and a significant gene $\times$ time interaction ($p = 0.0086$).

Nominal associations were observed for *FGB* (rs1800790) with preoperative FIBTEMA5 ($p = 0.025$), A10 ($p = 0.034$), and MCF ($p = 0.063$). No associations were observed after surgery.

Table 29. Fibrinogen levels across rs1800790 (*FGB*) genotype groups

Time period	GG (n = 89)	AG (n = 82)	AA (n = 24)	p-value
Fibrinogen T0 (g/L)	3.49	3.83	4.38	0.00035
Fibrinogen T1 (g/L)	2.63	2.90	3.24	0.00119
Fibrinogen T6 (g/L)	2.85	3.07	3.62	0.00211
Fibrinogen T18 (g/L)	3.52	3.77	4.12	0.00893

Values represent median fibrinogen concentrations across genotype groups. P-values correspond to the Kruskal–Wallis test.

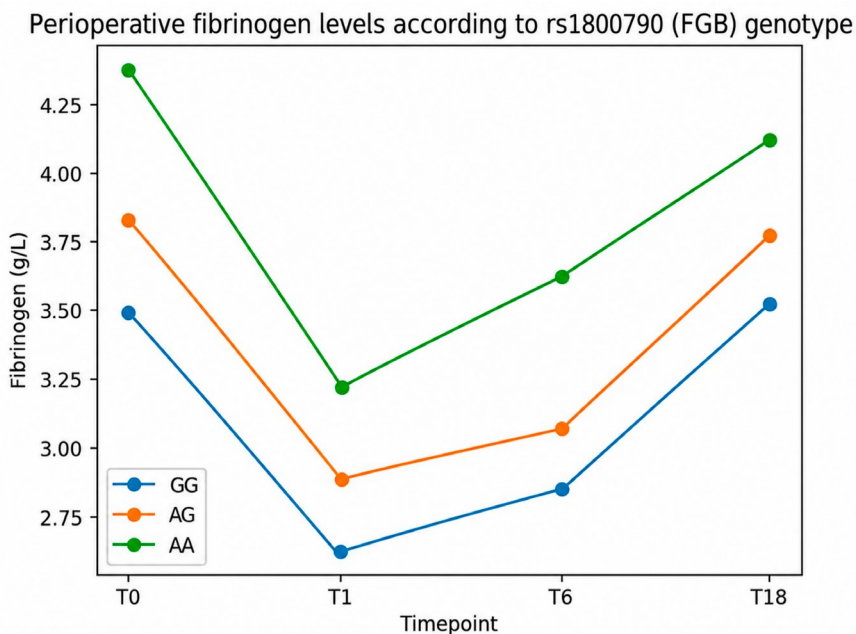


Fig. 17. Perioperative fibrinogen levels across rs1800790 (FGB) genotype groups

Data are presented as median values at each time point. A consistent genotype-dependent gradient (GG to AG to AA) was observed across all perioperative time points.

4.12.3. *SERPINE1* (rs2227631) and its association with blood loss

Genotype frequencies of rs2227631 were AA 37.4%, AG 49.2%, and GG 13.3%, with allele frequencies of 62.1% (A) and 37.9% (G). No association with the Hb/kg Index was found in the genotypic ($p = 0.724$), additive (adjusted $p = 0.789$), dominant (adjusted $p = 0.768$), or recessive (adjusted $p = 0.906$) models. Effect estimates were small, with confidence intervals crossing zero and negligible explained variance ($R^2 \approx 0.003$). No associations were detected in the gene–phenotype scan after FDR correction. Genotype distribution did not differ across Hb/kg Index based clusters ($p = 0.769$) (Table 27), and CTD summary did not differ across genotype groups ($p = 0.148$) (Table 28).

5. DISCUSSION

5.1. The Hb/kg Index and Clustering

Patients undergoing cardiac surgery are inherently at risk of perioperative bleeding [58]. Despite numerous methods described in the literature [12, 16, 58, 219, 220], a universally accepted gold standard for quantifying blood loss remains elusive [16]. In routine practice, bleeding is typically assessed via volumetric or visual estimation of CTD [219], both of which are subjective and prone to significant error [55–57]. Visual estimation consistently underestimates true blood loss, thereby limiting its clinical reliability [56]. The inaccuracy of volume-based assessment is further compounded by haemodilution from irrigation fluid and serous exudate [55]. Gerdessen et al. demonstrated that dilution can lead to a nearly twofold overestimation of perceived blood loss, reporting a mean perceived loss of 500 mL when actual loss was only 281.5 mL [221]. Clinically relevant thresholds for re-sternotomy, such as more than 200 mL/h [9] or more than 1.5 mL/kg/h over six hours [14] are practical but do not fully reflect the biological impact of bleeding. Furthermore, while early re-exploration for high output can minimise total loss [58], late-stage drainage often represents an accumulation of serum rather than active haemorrhage [55]. Consequently, CTD volume often fails to reflect the actual intravascular haemoglobin deficit [9, 221, 222]. Considering these limitations, several formula-based estimation methods have been developed to improve accuracy [12, 16, 57, 220, 223], and generally considered more reliable than visual assessments. However, these approaches remain limited, as they do not quantify actual haemoglobin (Hb) mass loss via CTD or do not adequately normalise the results to the patient's body weight [213].

To address these limitations, we developed the Hb/kg index, a new metric that quantifies actual haemoglobin mass loss normalized to patient weight [213]. Our method provides a physiologically grounded measure of blood loss, the robustness of which was confirmed through both statistical and machine learning analyses. Lasso-based regression modeling demonstrated high predictive performance R^2 score (0.82 ± 0.13) and correlation (0.92 ± 0.06). Moreover, the Index showed a strong correlation with intraoperative Hb mass loss ($\rho = 0.82$, $p < 0.001$) and was significantly influenced by anthropometric parameters. Importantly, the Index was inversely correlated with BMI ($\rho = -0.31$, $p < 0.001$), suggesting that patients with lower BMI experience a greater relative hemoglobin deficit for the same absolute blood loss.

Given that up to 92% of cardiac surgery patients receive blood products, transfusion is a critical factor in bleeding assessment [10, 224]. It is associated with increasing rate of morbidity and mortality [225]. While some authors

suggested that one unit of PRBC contains 52g of haemoglobin mass [217], we determined a value of approximately 43g based on national standards. In our study, PRBC-related haemoglobin mass demonstrated a significant inverse association with the Hb/kg index ($\rho = -0.29$, $p < 0.001$), confirming its essential role in objective quantification.

The clinical relevance of the Hb/kg Index becomes particularly evident when applied to data-driven stratification. Using an unsupervised machine learning approach (K-Medoids clustering), we identified three distinct blood loss severity clusters (Mild, Moderate, and Severe). These clusters were defined by objective Hb/kg cut-off values of 1.72 and 2.10. The robustness of this clustering was supported by a very large effect size for the Hb/kg Index ($\eta_p^2 = 0.729$) and strong discriminative performance in ROC analysis for the Severe cluster (AUC = 0.790; 95% CI: 0.721–0.859). These results demonstrate that the identified clusters represent true physiological gradients of haemoglobin loss rather than arbitrary statistical groupings [218].

Patients in the Severe cluster demonstrated significantly lower weight, BMI, and EBV ($p < 0.001$ and $p = 0.002$), indicating that reduced circulating volume contributes to increased vulnerability. This confirms that physiological impact of bleeding is strongly modulated by body size. The prominent role of weight ($F = 9.811$, $p < 0.001$, $\eta_p^2 = 0.095$) and BMI ($F = 10.322$, $p < 0.001$, $\eta_p^2 = 0.099$) in our clusters confirms a fundamental principle: identical absolute blood loss affects patients of different weight and BMI differently. Patients with lower BMI were markedly overrepresented in the Severe cluster, showing a higher relative deficit per kilogram. Significant negative associations between the Hb/kg Index and both weight ($\rho = -0.27$, $p < 0.0001$) and BMI ($\rho = -0.31$, $p < 0.0001$) reaffirm that these are not peripheral variables but critical determinants of bleeding severity. This is consistent with findings by Bhavsar, who demonstrated that morbidly obese patients ($\text{BMI} \geq 40 \text{ kg/m}^2$) had less postoperative bleeding and fewer transfusions, while underweight patients ($\text{BMI} < 18.5 \text{ kg/m}^2$) faced higher risk [226]. Individuals with a higher BMI typically have a greater EBV, providing a greater physiological reserve [227].

Our findings further highlight the inaccuracy of traditional metrics. We observed only a weak association between the identified clusters and CTD volume ($F = 2.361$, $p = 0.097$; $\eta_p^2 = 0.025$). In contrast, actual Hb mass loss via CTD showed a significant cluster effect ($F = 4.663$, $p = 0.011$; $\eta_p^2 = 0.048$), with an effect size nearly double that of volume alone. A key finding of our stratified analysis is the divergence in drainage reliability: in the Mild cluster, the Hb/kg Index correlated well with drainage Hb mass ($r = 0.39$), suggesting that in standard cases, CTD reasonably reflects loss. However, this correlation was absent in the Moderate and Severe clusters. In cases of

major haemorrhage, haemodilution obscures the true extent of blood loss, making the drainage fluid an inaccurate indicator. This paradox demonstrates the stability of the Hb/kg Index as a measure of blood loss.

From a clinical perspective, such precision is essential. While mild postoperative bleeding is often clinically insignificant, severe bleeding is linked to catastrophic outcomes, prolonged intensive care, and increased resource use [4, 43, 44, 46, 54]. Despite its impact, clinicians face a significant challenge: there is currently no universally accepted method for quantifying blood loss or a standardised classification for bleeding in cardiac surgery [219, 228]. As our findings confirm, existing definitions remain inconsistent and rely heavily on CTD volume [9, 14, 58, 229, 230], a metric that does not distinguish between active haemorrhage and late-stage serous accumulation [17].

The advantages of a weight-normalised approach (Hb/kg Index) therefore become clear when compared with other existing classifications. The Bleeding Academic Research Consortium (BARC) definition serves as a useful binary endpoint for trials but does not capture the full spectrum of bleeding [60]. The European Multicenter Study on Coronary Artery Bypass Grafting (E-CABG) and Universal Definition of Perioperative Bleeding (UDPB) classification systems are more comprehensive and holistic but remain limited by their reliance on clinical interventions, including transfusion or re-exploration thresholds, which vary between institutions [58, 230]. Similarly, the Advanced Trauma Life Support (ATLS) system categorizes haemorrhagic shock based on a theoretical 70 kg individual [189, 231, 232].

Despite these physiological insights, our cohort showed no statistically significant differences in the incidence of major complications, including AKI, AF, HF, and neurological complications across clusters. The absence of morbidity differences may reflect effective perioperative mitigation of blood loss consequences. Furthermore, standardised clinical protocols likely attenuated the clinical impact of bleeding across clusters, distinguishing our findings from previous research where massive haemorrhage was more strongly associated with poor outcomes [7, 8, 233].

5.2. Complete Blood Count and the Hb/kg Index

Analysis of complete blood count (CBC) parameters reveals a significant and systematic decline in haemoglobin, haematocrit, and RBC counts during the first 18 postoperative hours ($p < 0.001$). While these changes are expected in cardiac surgery due to haemodilution, surgical trauma and bleeding, their correlation with the Hb/kg Index provides critical evidence of the metric's clinical validity. A pivotal finding in this study is that patients in the Severe

cluster had significantly higher baseline haemoglobin levels than those in the Mild cluster (144.5 ± 11.7 g/L vs 134.5 ± 14.6 g/L; $F = 8.128$, $p < 0.001$, $\eta_p^2 = 0.080$). Paradoxically, this same group experienced the most drastic postoperative decline, reaching the lowest absolute haemoglobin levels by T18 (98.4 ± 14.6 g/L vs 107.0 ± 11.9 g/L in the Mild cluster; $F = 5.691$, $p < 0.004$, $\eta_p^2 = 0.057$). This suggests that a high initial haemoglobin concentration may mask the relative intensity of blood loss in the early stages, underscoring the necessity of the Hb/kg Index in identifying the true physiological deficit.

A similar pattern was observed for haematocrit. At baseline, patients in the Severe cluster had higher haematocrit values than those in the Mild cluster ($42.9 \pm 3.8\%$ vs $40.6 \pm 4.5\%$; $F = 4.084$, $p = 0.018$, $\eta_p^2 = 0.042$). This difference became more pronounced immediately after surgery, when the lowest haematocrit was observed in the Severe cluster ($31.4 \pm 3.9\%$ vs $34.0 \pm 4.4\%$ in the Mild cluster; $F = 7.651$, $p < 0.001$, $\eta_p^2 = 0.075$), remained significant at T6 ($31.8 \pm 4.0\%$ vs $33.7 \pm 3.9\%$; $F = 4.039$, $p = 0.019$, $\eta_p^2 = 0.042$), and persisted at T18 ($28.7 \pm 4.0\%$ vs $31.7 \pm 3.5\%$; $F = 9.065$, $p < 0.001$, $\eta_p^2 = 0.088$). This pattern closely mirrored haemoglobin dynamics and further supports the Hb/kg Index's ability to identify patients with greater cumulative perioperative haemoglobin loss.

The dynamic shift in correlation coefficients further reinforces the validity of the Index: a positive correlation exists at baseline ($\rho = 0.375$, $p < 0.001$), whereas postoperatively, this relationship inverts and strengthens by T18 for both haematocrit ($\rho = -0.336$) and RBC count ($\rho = -0.338$; both $p < 0.001$). The clinical relevance of this stratification is further supported by the RBC counts, which showed clear differences between clusters immediately after surgery ($F = 11.853$, $p < 0.001$, $\eta_p^2 = 0.112$) and at T18 ($F = 6.323$, $p < 0.002$, $\eta_p^2 = 0.063$). Notably, while the RBC line demonstrates clear stratification, platelet counts showed no significant differences across clusters ($p > 0.05$) or correlations with the Hb/kg Index at any time point. This implies that quantitative platelet deficiency was not the primary driver of bleeding severity in this cohort, directing the diagnostic focus toward plasma-based hemostatic factors.

5.3. Standard coagulation tests and the Hb/kg Index

As hemodynamics stabilise, the Hb/kg Index accurately reflects the actual haemoglobin deficit. This is mirrored in the dynamics of SCTs; however, INR and aPTT were largely non-effective in stratifying patients. Except for platelet count, these tests require plasma separation, resulting in a prolonged turnaround time [29]. In our study, INR showed no significant differences

between clusters at T1 ($F = 1.029$, $p < 0.359$, $\eta_p^2 = 0.359$) and at T18 $F = 0.194$, $p < 0.824$, $\eta_p^2 = 0.002$), and aPTT while achieved peak at T6 (44.95 ± 12.03 s), failed to differentiate blood loss severity during the critical early hours (T1 and T6; $p > 0.05$). In contrast, fibrinogen concentration emerged as the primary laboratory determinant of the Hb/kg Index and cluster classification. Fibrinogen levels dropped by approximately 25% immediately after surgery (T1: 2.87 ± 0.71 g/L vs T0: 3.86 ± 0.89 g/L). This aligns with the literature, which suggests that fibrinogen is the most vulnerable factor during CPB, often decreasing by an average of 36% [24]. In our cohort, fibrinogen was the only standard parameter that significantly differed between clusters immediately after CPB ($p = 0.001$). Patients in the Severe cluster exhibited the lowest levels (2.66 ± 0.57 g/L) compared to the Mild group (3.15 ± 0.75 g/L). The consistent negative correlation between the Hb/kg Index and fibrinogen at T1 ($\rho = -0.308$) and T18 ($\rho = -0.285$; both $p < 0.001$) confirms that the biological impact of bleeding is directly linked to the magnitude of fibrinogen depletion. Notably, while baseline fibrinogen did not differ significantly between clusters ($p = 0.057$), the emergence of significant differences postoperatively indicates that the rate of consumption during CPB, rather than the initial reserve, is a critical factor. This consumption is further amplified by surgical trauma and cardiotomy suction [20, 21], reinforcing the necessity of early and targeted fibrinogen replacement, as INR or aPTT fail to trigger this critical clinical intervention. Finally, the stability of D-dimer levels across clusters ($p > 0.05$) suggests that factor consumption, rather than hyperfibrinolysis, was the dominant mechanism underlying the increased blood loss in the Severe group. However, since SLTs do not fully reflect platelet function [129] and cannot detect qualitative dysfunction induced by CPB [31, 32], our reliance on fibrinogen dynamics becomes even more clinically justified when INR and aPTT fail to trigger necessary interventions.

5.4. Plasminogen Activator Inhibitor Type-1 (PAI-1) and the Hb/kg Index

The analysis of PAI-1 dynamics reveals a significant postoperative increase in plasma concentrations by T18 (46.32 ± 18.25 ng/ml vs 35.14 ± 19.32 ng/mL; $p < 0.001$). This elevation reflects a systemic antifibrinolytic response, which is a well-documented phenomenon following the extensive endothelial activation and surgical trauma associated with CPB [234]. The post-CPB surge in PAI-1 typically peaks within the first 24 hours, representing the organism's attempt to counteract the initial activation of the fibrinolytic system [98, 235]. However, despite this significant rise, PAI-1 levels showed no correlation with the Hb/kg Index and failed to differentiate between

clusters at either T0 ($p = 0.111$) or T18 ($p = 0.069$). This lack of association, combined with the observed stability of D-dimer levels across clusters, further supports our hypothesis that hyperfibrinolysis was not the primary driver of increased blood loss in the Severe group. While a borderline trend was noted at T18 ($p = 0.069$), the overall variance explained by the blood loss category remained low ($R^2 = 0.161$).

These findings suggest that, while the PAI-1-mediated inhibition of fibrinolysis is an integral part of the systemic inflammatory response to CPB, it operates independently of the mechanisms governing postoperative bleeding intensity in this cohort. In clinical terms, this reinforces the diagnostic focus on factor consumption, specifically fibrinogen depletion, rather than on the fibrinolytic shut-off mechanism's variability, as reflected in PAI-1 levels [236].

5.5. Platelet aggregometry and the Hb/kg Index

The analysis of platelet function showed a significant perioperative decline in both ADP- and epinephrine-induced aggregation immediately after CPB (T1; $p < 0.001$). ADP-induced PA decreased by 26.9%, while epinephrine-induced PA was more profoundly suppressed by 53.3%. This is consistent with the known effects of CPB, which impair platelet function by causing both quantitative and qualitative platelet abnormalities [102]. Despite this substantial impairment, PA parameters did not differ significantly between clinical clusters at any time point ($p > 0.05$). In addition, no significant correlations were found between the Hb/kg Index and PA induced by either agonist. This lack of association is particularly notable given the sustained suppression of epinephrine-induced PA observed at T18 (41.56 ± 18.42). These findings, together with the absence of correlation between the Hb/kg Index and quantitative platelet counts, suggest that neither quantitative nor qualitative platelet deficiencies were the primary contributors to bleeding severity in this cohort.

Although light transmission aggregometry (LTA) is considered the gold standard for assessing platelet function [34], its predictive value in our cohort was limited. Our results present a striking contrast to studies such as the PLATFORM trial, which identified post-CPB ADP-induced PA as the strongest predictor of severe bleeding ($AUC = 0.712$) [146].

The difference between our findings and the PLATFORM study may be due to several factors. Although light transmission aggregometry (LTA) is considered the gold standard [34], it measures only one aspect of haemostasis: the formation of the primary platelet plug. It does not account for interactions with coagulation factors or fibrin formation. Consequently, in cases of

significant fibrinogen depletion, the role of platelet dysfunction in the overall physiological deficit indicated by the Hb/kg Index may be less critical.

5.6. Viscoelastic assessment (ROTEM) and the Hb/kg Index

The etiology of bleeding following CPB is multifactorial, including haemodilution, consumption of coagulation factors, activation of the extrinsic and intrinsic system [20, 21, 52]. While conventional assays provide quantitative data, they fail to capture the functional adequacy of clot formation or qualitative dysfunction of platelets [30–32]. ROTEM addresses these limitations by providing a rapid (10–15 min) assessment of whole blood clot dynamics [135, 159, 163, 164, 237–239]. Given the strong association between early and maximal clot firmness, as well as the clinical advantage of obtaining results much earlier, the following analysis focuses primarily on A10 parameters [240–242].

Our analysis of viscoelastic properties provides clear evidence of the mechanisms contributing to blood loss, as indicated by the Hb/kg Index. A significant finding is the consistent and marked decrease in clot firmness parameters (A10 and MCF) in both EXTEM and INTEM assays after surgery ($p < 0.001$). The most profound decline was observed in FIBTEM parameters, which demonstrated the strongest associations with blood loss severity in terms of Hb/kg Index.

The primacy of fibrinogen in clot stability

Our results support the concept that fibrinogen is the “first factor to fall” in cardiac surgery with CPB [215]. While previous studies using absolute volume measurements reported correlations ($r = 0.52$) [243], these values often overlook individual patient reserves. Using of the Hb/kg Index provides a more physiologically calibrated correlation ($\rho = -0.338$), isolating the impact of fibrinogen on actual blood loss rather than fluid volume alone.

We observed a 26% reduction in plasma fibrinogen, with one-third of patients developing clinically significant hypofibrinogenaemia. This reduction is related to the combined effects of haemodilution, consumption and hyperfibrinolysis [50, 98, 244, 245]. The key findings regarding FIBTEM include: correlation of FIBTEM A10 with both Clauss fibrinogen level ($r = 0.783$) and the Hb/kg Index ($\rho = -0.338$, $p < 0.001$). Even at baseline, patients in the Severe cluster exhibited lower FIBTEM A10 values ($F = 3.462$, $\eta_p^2 = 0.088$, $p = 0.037$), suggesting a reduced initial capacity fibrinogen-dependent clot formation. Postoperatively, FIBTEM A10 and MCF demonstrated significant differences between clusters, with the lowest values consistently found in the Severe group (FIBTEM A10: $F = 5.490$,

$p = 0.006$, $\eta_p^2 = 0.132$ and FIBTEM MCF: $F = 4.908$, $p = 0.010$, $\eta_p^2 = 0.120$). While 33% of patients demonstrated a residual heparin effect (HEPTTEM/INTEM ratio >1.1 , $p < 0.001$), CT did not correlate with the Hb/kg Index. This confirms that the underlying issue is not associated with the initiation of clotting, but with the structural firmness of the clot, which is mainly influenced by fibrinogen levels.

Diagnostic thresholds: hypofibrinogenaemia and thrombocytopenia

In our study, the hypofibrinogenaemia was defined as < 2.5 g/L. This threshold was selected because preoperative levels below this limit are significantly associated with increased postoperative bleeding [86]. Furthermore, Ranucci et al. identified this level as a pragmatic trigger for early deficit detection [246], while high-profile randomized trial has used it as a therapeutic target [247]. FIBTEM demonstrated exceptional diagnostic accuracy for hypofibrinogenaemia (<2.5 g/L):

- FIBTEM A10: AUC = 0.888, cut-off ≤ 12 mm; (Sensitivity 95.8%, NPV 97.4%).
- FIBTEM MCF: AUC = 0.895, cut-off ≤ 14 mm; (Sensitivity 100%, NPV 100%).

Regarding thrombocytopenia ($150 \times 10^9/L$), this threshold identifies patients within continuum of risk, where decreasing platelet counts are independently associated with increased mortality, infection, and acute kidney injury [248, 249]. The diagnostic performance of ROTEM was as follows:

- PLTEM A10: AUC = 0.755, cut-off ≤ 43 mm; (Sensitivity 71.4%, NPV 68.6%).
- EXTEM A10: AUC = 0.752, cut-off ≤ 57 mm; (Sensitivity 82.1%, NPV 86.5%).

Although EXTEM A10 and PLTEM A10 were significant predictors of thrombocytopenia, their diagnostic power (AUC 0.752–0.755) was notably lower than that of fibrinogen-specific assays. The high Negative Predictive Value (NPV) of FIBTEM indicates that a normal trace reliably excludes fibrinogen deficiency, preventing unnecessary and costly replacement therapy.

Our results support the 2024 EACTS/EACTAIC Guidelines, which recommend higher fibrinogen targets (≥ 2.0 g/L) and Class IIa viscoelastic-guided therapy [250], moving away from the conservative 2017 thresholds (< 1.5 g/L) [215]. Using the Hb/kg Index, we show that bleeding risk increases significantly when fibrinogen drops below 2.5 g/L (FIBTEM A10 < 12 mm). Effective post-CPB management requires a shift from concentration-based metrics to a dual assessment of mass-based deficits (Hb/kg Index) and structural clot integrity (ROTEM).

5.7. Surgical procedures and clinical outcomes

A key question in cardiac surgery is whether the extent of surgical trauma and the duration of CPB determine the severity of blood loss. In our cohort, the type of surgery and duration of CPB ($p = 0.729$) or cross-clamp time ($p = 0.280$) did not differ significantly between clusters. This finding is important, as it suggests that weight-adjusted blood loss in terms of the Hb/kg Index, is not simply a result of longer or more complex procedures, but instead reflects individual haemostatic failure.

Although the broad category of surgery (CABG vs. Valve) showed only a borderline difference ($p = 0.051$), we identified a significant ordinal association between the number of bypass grafts and blood loss severity clusters ($p = 0.027$ and Kendall's $\tau_c = 0.178$, $p = 0.005$). Patients in the Moderate and Severe clusters were more likely to receive three or more grafts (73.9% and 63.1%, respectively) compared to the Mild cluster. The nearly twofold higher proportion of patients receiving four or more grafts in the Severe cluster (24.7% vs. 11.8%) suggests that extensive coronary revascularisation may contribute to increased blood loss.

The comparable rates of postoperative complications, including AKI ($p = 0.611$), heart failure, and ICU stay across clusters, indicate that our cohort was managed within a robust clinical framework, where blood loss, although varying in severity, did not result in immediate major organ dysfunction.

The smoking paradox. An interesting finding in our study is the increasing prevalence of current smokers from the Mild cluster (13.2%) to the Severe cluster (37.0%, $p = 0.004$). Although tobacco use is traditionally associated with a prothrombotic state, driven by increased platelet activation, elevated fibrinogen, and greater tissue factor activity [251, 252]. Smoking is significantly linked to an increased risk of major bleeding, including intracranial, gastrointestinal, and airway haemorrhage, with a clear dose-response relationship based on smoking intensity. The heightened bleeding risk is attributed to smoking-induced vascular damage rather than just impaired hemostasis. Chronic tobacco smoke exposure leads to endothelial dysfunction, structural instability in arterial walls, and damage related to hypertension, all contributing to an increased risk of bleeding [253]. Statistical evidence highlights the increased risk of major bleeding among individuals with a significant smoking history. Those with more than 40 pack-years of smoking are at a considerably higher risk compared to never-smokers, with a hazard ratio of 1.59 (95% CI: 1.45–1.73; $p < 0.001$) [253]. Furthermore, a nationwide analysis of over 5.4 million surgical cases from the ACS-NSQIP database confirmed that smoking is associated with a modestly increased risk of RBC transfusion [254]. These results support the ERAS

Cardiac Society guidelines, which recognize tobacco use as a contributor to bleeding, metabolic, and infectious complications. They recommend quit smoking at least four weeks prior to elective surgery (Class I, Level C-LD) [255]. In our research, the significant number of smokers in the Severe cluster suggests that the vascular fragility caused by smoking might make patients more likely to exhibit a “consumptive” haemostatic profile during CPB.

5.8. The influence of gene variants on blood loss

Identifying genetic predictors of perioperative blood loss involves analysing SNPs associated with platelet and fibrinogen function, and fibrinolysis [171, 172]. Among these, the ITGB3 (rs5918) SNP is a strong candidate because of its crucial role in platelet-fibrinogen interactions [181, 182]. The rs5918 SNP is associated with a hyperreactive phenotype, characterized by an increased affinity for fibrinogen and a reduced activation threshold for agonists such as ADP and collagen [184, 188]. Theoretically, carriers of this prothrombotic variant are expected to have a lower bleeding tendency [36, 183]. However, our analysis demonstrated a clear dissociation between this genotype and clinical outcomes. No statistically significant association with the Hb/kg Index was observed across genotypic ($p = 0.808$), additive ($p = 0.524$), dominant ($p = 0.485$), or recessive ($p = 0.946$) inheritance models. Furthermore, rs5918 distribution did not differ across the Hb/kg-defined blood loss clusters. No significant differences were observed for the Hb/kg Index across any inheritance model ($R^2 = 0.002$). Our findings are consistent with several large-scale studies that have not validated rs5918 as a prognostic marker for postoperative bleeding. Specifically, Welsby et al. and Greiff et al. found no significant association between this polymorphism and chest tube drainage or transfusion requirements in cardiac surgery patients [36, 176]. Similarly, Grinshtein et al. reported that ITGB3 variants were associated with adverse thrombotic cardiovascular events but not to perioperative bleeding complications in CABG cohort [191]. Our study found no significant association, despite the confirmed presence of the variant (CT and CC genotypes comprised 25.2% of the cohort). This suggests that clinical factors and the substantial haemostatic effects of CPB are likely more influential than the relatively minor impact of this platelet receptor SNP.

***FGB* (rs1800790)**

Genetic polymorphisms in the promoter region of the *FGB* gene, including rs1800790 (−455 G/A) and rs1800787 (−148C>T), have been shown to influence fibrinogen levels [195]. The *FGB* rs1800790 SNP is one of the most extensively investigated variant and is located in the promoter region

of the FGB gene [196]. The most significant finding of our study was the strong association between the *FGB* (rs1800790) and fibrinogen levels during the perioperative period ($p < 0.001$). We observed a consistent, genotype-dependent gradient ($GG < AG < AA$) at all time points. The A allele was associated with higher fibrinogen concentrations, with a baseline effect of $\beta = +0.365$ g/L ($p = 0.000055$). This SNP effect persisted post-surgery (T1: $\beta = +0.249$ g/L) and remained significant throughout the 18-hour recovery period ($p = 0.00370$). However, despite this clear biochemical impact, the clinical influence of this polymorphism on bleeding remains controversial. The rs1800790 SNP has not been consistently associated with bleeding in cardiac surgery [196]. In a study of 1,036 cardiac surgery patients, analysis of 22 polymorphisms in coagulation and fibrinolysis pathways identified several variants associated with postoperative bleeding, while the fibrinogen gene SNP was not a significant predictor [176]. Similarly, a study of 780 patients undergoing CABG identified seven genetic polymorphisms associated with bleeding, but fibrinogen-related variants were not implicated [36]. Clinical studies have primarily focused on fibrinogen concentration rather than genetic determinants. Recent evidence indicates that the rs1800790 A allele may be associated with a prothrombotic rather than a bleeding phenotype. These findings suggest that increased fibrinogen levels do not necessarily result in improved haemostatic balance and may instead reflect altered clot structure and resistance to fibrinolysis [199]. Behague et al. found that the minor allele was associated with higher plasma fibrinogen levels, particularly among smokers, and with greater severity of coronary artery disease [200]. Similarly, Al-Awad et al. demonstrated that rs1800790 significantly influenced thrombotic risk [195]. Our findings support these observations. In carriers of the AA genotype for this SNP, we observed a nominal improvement in baseline ROTEM parameters, particularly FIBTEM A5 ($p = 0.025$) and A10 ($p = 0.034$). However, this biochemical advantage did not result in a statistically significant reduction in the Hb/kg index ($p = 0.667$) or total chest tube drainage ($p = 0.484$). Although the *FGB* rs1800790 polymorphism plays a significant role in regulating fibrinogen synthesis, its direct influence on bleeding risk remains unclear. Current evidence does not suggest that it can serve as an independent predictor of perioperative bleeding in cardiac surgery.

***SERPINE1* (rs2227631)**

PAI-1 levels vary significantly between individuals, primarily due to genetic factors, particularly polymorphisms in the promoter region of the *SERPINE1* gene. The most extensively studied variant is the -675 insertion/deletion polymorphism (4G/5G), which affects transcription. The 4G allele

leads to higher PAI-1 expression, increasing the risk of thrombosis due to a hypofibrinolytic state [203–205]. In contrast, the 5G allele binds a repressor protein, reduces PAI-1 transcription, and creates a profibrinolytic environment that is more susceptible to bleeding [97–99]. The SNP at position -844 (A>G, rs2227631) may influence the fibrinolytic phenotype. The -844 G allele is linked to reduced PAI-1 expression, whereas the A allele is associated with higher PAI-1 levels [100, 101].

CPB triggers a systemic inflammatory response, accompanied by a marked release of t-PA. In patients with genotypes associated with low PAI-1 expression (e.g., rs2227631 G/G or -675 5G/5G), this fibrinolytic activation may remain insufficiently suppressed, leading to hyperfibrinolysis and increased blood loss. Ozolina et al. suggested that the rs2227631 G/G genotype may represent a “bleeding phenotype,” with significantly higher postoperative chest tube drainage and biochemical evidence of enhanced fibrinolysis compared with A/A carriers [211, 212]. Conversely, the A/A genotype is associated with higher baseline PAI-1 levels. While this may provide relative protection against surgical bleeding by effectively suppressing fibrinolysis, it may theoretically increase the risk of postoperative thrombotic complications [207]. Individuals with the A/G genotype typically display intermediate PAI-1 levels, representing a balanced phenotype [212].

Despite these findings, our study found that *SERPINE1* (rs2227631) SNP showed no correlation with Hb/kg-defined blood loss clusters ($p = 0.769$) or total drainage volume ($p = 0.148$). These results highlight that, for clinicians and researchers, systemic factors in terms of contact activation and surgical trauma are more influential during the immediate post-CPB period than the evaluated SNPs, underscoring their importance in understanding blood loss.

CONCLUSIONS

1. The Hb/kg Index provides an objective physiological measure for quantifying haemoglobin mass loss per kilogram of patient weight in cardiac surgery, demonstrating strong predictive performance in LASSO-OLS modelling ($R^2 = 0.82$).
2. Unsupervised K-medoids clustering identified three blood loss severity clusters (Mild, Moderate, and Severe) based on data-driven Hb/kg thresholds of 1.72 and 2.10. This framework reflects clinically meaningful gradients of blood loss, with the highest discriminative accuracy for the Severe cluster (AUC = 0.790).
3. Viscoelastic testing demonstrated superior discriminatory value for blood loss severity than standard coagulation tests and platelet aggregometry. FIBTEM A10 (cut-off ≤ 12 mm) identified postoperative hypofibrinogenaemia (< 2.5 g/L) with excellent diagnostic accuracy (AUC = 0.888; NPV = 97.4%).
4. Postoperative plasma PAI-1 concentrations increased significantly, reflecting a systemic fibrinolytic response to cardiopulmonary bypass and surgical trauma, but showed no significant association with the Hb/kg Index or clusters.
5. *ITGB3* (rs5918) or *SERPINE1* (rs2227631) SNP showed no significant associations with Hb/kg Index or clusters. In contrast, the *FGB* (rs1800790) SNP was strongly associated with fibrinogen levels at all perioperative time points ($p < 0.001$), supporting its role as an important determinant of the perioperative haemostatic phenotype.

PRACTICAL RECOMMENDATIONS

1. The Hb/kg Index may be considered for integration into routine cardiac surgery practice as an adjunctive tool to quantify perioperative blood loss. It provides a more physiologically relevant estimate of haemoglobin deficit, which may improve clinical decision-making, particularly in patients with lower body mass. Further multicentre validation is required before widespread standardisation.
2. The identified Hb/kg thresholds (1.72–2.10) may be used as a framework for risk stratification into Mild, Moderate, and Severe blood loss clusters. This approach supports early identification of patients at higher risk of significant haemoglobin loss and may help guide postoperative management strategies. However, external validation is required before routine clinical implementation.
3. The monitoring of haemostasis should primarily focus on fibrinogen status, as reduced fibrinogen levels and impaired fibrin-based clot firmness are associated with increased perioperative blood loss. Platelet count should remain part of routine monitoring, but alone may be insufficient to reflect clinically relevant haemostatic impairment. In this context, FIBTEM and PLTEM provide more clinically relevant functional information and may better support targeted haemostatic management. A FIBTEM A10 (≤ 12 mm) can be used in the perioperative period to identify clinically significant hypofibrinogenaemia (< 2.5 g/L), while PLTEM A10 (≤ 43 mm) can be used to identify thrombocytopenia ($< 150 \times 10^9/L$) and guide timely replacement therapy.
4. The FGB (rs1800790) polymorphism showed a significant and consistent association with perioperative fibrinogen levels, highlighting its potential relevance as a genetic marker of the perioperative haemostatic phenotype. However, the investigated SNPs did not provide sufficient predictive value for blood loss severity or Hb/kg Index values to justify their routine use for perioperative bleeding risk stratification in cardiac surgery. Therefore, bleeding risk assessment should continue to rely primarily on clinical, procedural, and laboratory parameters.
5. Patients with lower body weight, BMI, BSA, active smoking status, or those undergoing CABG procedures (≥ 3 grafts), may be at increased risk of higher Hb/kg Index loss.

- 5.1. In patients with lower body weight, BMI, or BSA, even moderate Hb/kg loss may have a clinically significant implication. Clinicians should anticipate anaemia, reduced haemodynamic reserve, and the need for earlier haemostatic or transfusion interventions.
- 5.2. In patients undergoing CABG procedures involving three or more grafts, enhanced perioperative surveillance, blood conservation measures, and early haemostatic monitoring should be considered. Clinicians should anticipate a higher likelihood of prolonged operative exposure, increased postoperative drainage, coagulopathy, and the potential need for earlier targeted haemostatic intervention.
- 5.3. Active smokers scheduled for elective cardiac surgery should be encouraged to stop smoking before surgery, preferably at least four weeks in advance, as part of perioperative risk optimisation and to reduce postoperative complications.
6. The identified preoperative risk factors, including lower body weight, active smoking status, planned surgical complexity, baseline fibrinogen level and platelet counts, haemostatic markers may provide a basis for developing a preoperative bleeding risk score using the Hb/kg Index framework.

In summary, this work establishes the Hb/kg Index as a clinically valid, objective, and scalable tool for quantifying and stratifying perioperative blood loss. By integrating machine learning, viscoelastic haemostatic assessment, and multidimensional clinical data, it provides a novel framework for understanding bleeding mechanisms and improving perioperative risk assessment. The findings support a paradigm shift towards function-based, patient-specific evaluation of haemostasis, with potential implications for optimising targeted haemostatic therapy and improving outcomes in cardiac surgery.

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LIST OF PUBLICATIONS

The list of publications related to the dissertation

1. **Orazymbetov, Yerlan**, Serik Aitaliyev, Povilas Jakuška, Audronė Veikutienė, Tadas Lenkutis, Rassul Zhumagaliyev, Aušra Saudargienė, and Rimantas Benetis. “Calculation of blood loss in cardiac surgery: How should we monitor?” *Perfusion (United Kingdom)*, 2025, DOI: 10.1177/02676591251370110.
2. **Orazymbetov, Yerlan**, Serik Aitaliyev, Povilas Jakuška, Audronė Veikutienė, Tadas Lenkutis, Rassul Zhumagaliyev, Aušra Saudargienė, and Rimantas Benetis. “Perioperative blood loss in cardiac surgery: Validation of machine learning-derived clusters,” *Perfusion (United Kingdom)*, 2026, DOI: 10.1177/02676591261440347.
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Conference presentations and abstracts related to the dissertation

1. **Orazymbetov, Y.**, Aitaliyev, S., Jakuška, P., Veikutienė, A., Lenkutis, T., Zhumagaliyev, R., Saudargienė, A., & Benetis, R.. (2025). Calculation of Blood Loss in Cardiac Surgery: How Should We Monitor?. *Health for All 2025 “Healthy Beginnings, Hopeful futures”*. Abstract Book: April 4th, 2025, 41-43. <https://hdl.handle.net/20.500.12512/251430>
2. **Orazymbetov, Y.**, Aitaliyev, S., Jakuška, P., Veikutienė, A., Lenkutis, T., Zhumagaliyev, R., Arslan, S., & Benetis, R.. (2025). Quantification of blood loss using the Hb/kg index in cardiac surgery. PROCEEDINGS materials of the V International conference of students and young scientists «FROM EXPERIENCE TO PROJECT», 26 April, 2025. Almaty, Kazakhstan. 34-34. <https://hdl.handle.net/20.500.12512/254008>

SUMMARY IN LITHUANIAN

IVADAS

Širdies ir kraujagyslių ligos išlieka pagrindine mirštamumo priežastimi Europos Sąjungoje – kasmet jos lemia daugiau nei 1,6 milijono mirčių. Tai sudaro 33 proc. visų mirčių [1]. Nors laikui bėgant krešėjimo sutrikimų diagnostika ir gydymas pagerėjo, perioperacinis kraujavimas išlieka reikšminga problema širdies chirurgijoje [2], [3]. Gausaus kraujavimo dažnis siekia nuo 8 proc. iki 15 proc. ir tebėra svarbia klinicine problema [4], [5]. Pakartotinių operacijų dėl kraujavimo dažnis svyruoja nuo 2,3 proc. iki 8 proc. [6]. Pakartotinė chirurginė intervencija yra nepriklausomas nepalankių baigčių (insulto, sternotominio pjūvio pooperacinės žaizdos infekcijos, hemodializės ir kt.) prognostinis veiksnys [7] ir yra susijusi su didesniu sergamumu bei mirštamumu [8], [9]. JAV atliktas 798 chirurginių skyrių tyrimas parodė, kad nuo 8 proc. iki 93 proc. pacientų turėjo poreikį donorinio kraujo transfuzijoms [10], kas, finansiniu požiūriu, ženkliai didina išlaidas ir kaštus [11].

Nepaisant klinikinio aktualumo, nėra visuotinai priimto pooperacinio kraujo netekimo kiekybinio įvertinimo ir klasifikavimo metodo [12]. Buvo sukurta keletas klasifikavimo sistemų, pagrįstų krūtinės pooperacinės žaizdos drenažo tūriu, kraujo transfuzijomis ar pakartotinėmis operacijomis. Pavyzdžiui, Kirklin ir Barratt-Boyes kriterijai rekomenduoja nedelsiant atlikti resternotomiją bei chirurginę reviziją, jei krūtinės pooperacinės žaizdos drenažo (KŽD) kiekis viršija 5 ml/kg per bet kurią vieną valandą arba 3 ml/kg per valandą tris valandas iš eilės [13]. Kai kurie autoriai aktyvų kraujavimą apibrėžia kaip kraujo netekimą, viršijantį 1,5 ml/kg per valandą šešias valandas iš eilės [14], o kiti nurodo daugiau nei 200 ml kraujo netekimą per 1 valandą [9]. Tuo tarpu Masačusetso Pagrindinės ligoninės klinikiniam protokole pakartotinei chirurginei intervencijai inicijuoti naudojamos pagal kūno svorį koreguotos netekto tūrio gairės (ml/kg) [15]. Nors šie metodai turi prognostinę vertę, jiems būdingi reikšmingi trūkumai. Vizualus krūtinės pooperacinės žaizdos drenažo įvertinimas yra netikslus, nes drenažo turinyje yra kraujo ir audinių skysčių mišinys, todėl jis gali būti pervertintas arba nepakankamai įvertintas [9], [14], [16], [17]. Be to, šie metodai dažnai neatsižvelgia į paciento svorį. Pavyzdžiui, tas pats 1000 ml kraujo netekimas turi labai skirtingą hemodinaminį poveikį 60 kg sveriančiam pacientui palyginus su 100 kg sveriančiu pacientu.

Kraujavimo priežastimi širdies chirurgijoje gali būti įvairūs veiksniai: apie trečdalis atvejų susiję su chirurginiais šaltiniais, o likusieji – su krešėjimo sutrikimais [18]. Dirbtinė kraujo apytaka (DKA) sukelia reikšmingus hemostazės sutrikimus, lemiančius koagulopatiją [19]. Tai paprastai atsiranda dėl

hemodiliucijos, kurią sukelia DKA aparato užpildymas skysčiais, krešėjimo faktorių suvartojimas, audinių faktoriaus sukeltas išorinio krešėjimo kelio aktyvinimas bei vidinio krešėjimo kelio aktyvinimas dėl kraujo kontakto su nebiologiniais DKA aparato paviršiais [19], [20], [21]. Be to, likutinis nefrakcionuotas heparinas taip pat prisideda prie hemostazės sutrikimų [22], [23]. Visi šie veiksniai kartu sukelia sudėtingus ir dinamiškus hemostazės pokyčius [24].

Patofiziologiškai pusiausvyrą tarp kraujavimo ir normalios hemostazės lemia trombino susidarymo, trombocitų aktyvacijos bei agregacijos, fibrino krešulio formavimosi bei fibrinolizinės sistemos aktyvumo sąveika [25]. Atsiranda vis daugiau įrodymų, kad fibrinolizė turi lemiamą vaidmenį, ypač pacientams, kuriems atliekama širdies operacija su dirbtine kraujo apytaka [26]. Nustatyta, kad pooperacinis kraujavimas koreliuoja su fibrinogeno koncentracija ir trombocitų funkcija – tai nepriklausomi hemostazės ir kraujavimo prognostiniai veiksniai [27], [28].

Nors standartiniai laboratoriniai tyrimai tradiciškai naudojami perioperaciniam kraujavimui valdyti, jų klinikinį naudingumą riboja ilgas rezultatų gavimo laikas, reikalingas plazmai atskirti [29]. Clauss metodas leidžia nustatyti fibrinogeno kiekį, tačiau neatspindi krešulio formavimosi funkcijos adekvatumo [30]. Panašiai, trombocitų skaičius suteikia tik kiekybinę informaciją ir negali nustatyti kokybinių sutrikimų, kuriuos sukelia antitrombocitinė terapija ar dirbtinė kraujo apytaka [31], [32]. Dėl šių ribotumų didėja susidomėjimas greitaisiais diagnostiniais tyrimais prie paciento lovos „point-of-care“ (POC). Krešulio formavimosi viskoelastinių savybių vertinimas naudojant viskoelastinius tyrimus (VET) gali sumažinti kraujo transfuzijų poreikį iki 50 proc. [33]. VET leidžia nustatyti krešulio susidarymo laiką, jo kokybę, kiekį ir stabilumą. Taip pat jis įvertina bendrą hemostazės lygį be heparino poveikio, kas yra itin svarbu po operacijų su dirbtine kraujo apytaka [33]. Vertinant trombocitų reaktyvumą, auksiniu standartu išlieka šviesos pralaidumo agregometrija, pagrįsta trombocitinės plazmos optinio tankio pokyčio, išreikšto santykiniais procentais, matavimu vykstant trombocitų agregacijai [34]. Kai kurie autoriai nustatė, kad perioperacinis trombocitų agregacijos vertinimas gali prognozuoti reikšmingo kraujavimo riziką širdies chirurgijos pacientams [35].

Perioperacinio kraujavimo komplikacijos išlieka rimtu iššūkiu. Genetiniai veiksniai daro įtaką kraujo netekimo variabilumui po širdies operacijų. Vis daugiau duomenų rodo, kad genetinių tyrimų ir klinikinių duomenų vertinimas kartu reikšmingai pagerina kraujavimo rizikos prognozavimo tikslumą, palyginti su vien klinikiniais duomenimis [36]. Dauguma krešėjimo proceso reakcijų vyksta trombocitų ir fibrinogeno receptorių paviršiuje [37], [38]. Be to, plazminogeno aktyvatoriaus inhibitorius-1 (PAI-1) atlieka svarbų vaidme-

nį slopindamas fibrinolizę, blokuodamas plazminogeno vartimą plazminu [39], [40]. Todėl genetiniai polimorfizmai, veikiantys trombocitų receptorių ir fibrinogeno receptorių bei PAI-1, yra reikšmingi biožymenys, galimai nurodantys padidėjusį polinkį kraujuoti.

Nepaisant reikšmingos pažangos hemostazės tyrimo srityje, vis dar sudėtinga prognozuoti, kurie pacientai patirs reikšmingą kraujo netekimą. Siekiant užpildyti šią spragą, šiame darbe pristatoma multimodalinė diagnostinė sistema, integruojanti naują Hb/kg indeksą su mašininio mokymosi pagrindu veikiančia stratifikacija ir išsamia klinikinių, hemostazinių bei genetinių veiksnių, lemiančių kraujo netekimą širdies chirurgijoje, analize.

TYRIMO TIKSLAS IR UŽDAVINIAI

Tyrimo tikslas

Sukurti ir patvirtinti Hb/kg indeksą kaip naują metodą širdies chirurgijoje perioperacinio kraujo netekimo kiekybiniam įvertinimui bei nustatyti jo sąsajas su hemostatiniais ir genetiniais veiksniais, apsprendžiančiais kraujo netekimo sunkumą.

Tyrimo uždaviniai

1. Sukurti ir patvirtinti naują faktinio kraujo netekimo skaičiavimo metodą, išreikštą hemoglobino masės netekimu paciento kūno svorio vienetui (Hb/kg indeksas).
2. Patvirtinti mašininio mokymosi būdu nustatytas grupes (lengvo, vidutinio ir sunkaus kraujo netekimo), pagrįstas Hb/kg indeksu.
3. Įvertinti standartinių krešėjimo tyrimų, trombocitų agregometrijos (TA) ir viskoelastinių tyrimų (VET) perioperacinę dinamiką skirtingose Hb/kg indeksu pagrįstose grupėse.
4. Išanalizuoti perioperacines plazmos plazminogeno aktyvatoriaus inhibitoriaus-1 (PAI-1) koncentracijas ir įvertinti jų sąsają su perioperaciniu kraujo netekimu.
5. Ištirti *ITGB3* (rs5918), *FGB* (rs1800790) ir *SERPINE1* (rs2227631) vieno nukleotido polimorfizmų (VNP-ų) sąsajas su kraujo netekimo sunkumu ir nustatyti jų klinikinę reikšmę vertinant kraujavimo riziką širdies chirurgijoje.

Darbinė hipotezė

Šio tyrimo darbinė hipotezė teigia, kad perioperacinis kraujo netekimas širdies chirurgijoje gali būti tiksliau įvertintas naudojant Hb/kg indeksą (he-

moglobino masės netekimas kilogramui kūno svorio), palyginti su tradiciniais vizualiniais ar netekto tūrio pagrindu veikiančiais metodais. Daroma prielaida, kad Hb/kg indeksas suteikia objektyvų būdą suskirstyti pacientus į kraujo netekimo grupes, kurių kiekviena pasižymi skirtingais hemostaziniais profiliais, perioperacinės eigos dinamika ir klinikiniais požymiais. Tikimasi, kad šios grupės labiausiai siesis su VET parametrais, o ne su standartiniais krešėjimo tyrimais ar trombocitų agregometrija. Kraujo netekimo sunkumą lemia genetiniai veiksniai, susiję su fibrinogeno sinteze, trombocitų funkcija (ITGB3) ir fibrinolizės reguliacija (SERPINE1), kurie nulemia individualią paciento perioperacinio kraujavimo riziką.

Tyrimo mokslinis naujumas

Šio tyrimo mokslinis naujumas grindžiamas nauju požiūriu į faktiškai netekto kraujo apskaičiavimą remiantis Hb/kg indeksu (hemoglobino masės netekimas vienam kilogramui paciento kūno svorio), turinčiu prognostinę vertę.

Pirma, Hb/kg indeksas buvo sukurtas ir patvirtintas kaip objektyvus ir fiziologiškai pagrįstas rodiklis, leidžiantis kiekybiškai įvertinti faktinį perioperacinį kraujo netekimą. Pagrindiniai indekso komponentai yra paciento kūno svoris, intraoperacinis hemoglobino masės netekimas, hemoglobino netekimas per krūtinės pooperacinės žaizdos drenus (KŽD) ir donorinių eritrocitų transfuzijos. Nustatyta, kad Hb/kg indeksas pasižymi aukštu prognozuojamumu taikant daugiafaktorinius modeliavimo metodus. Iš visų vertintų modelių geriausius rezultatus parodė Lasso OLS modelis: su mažiausia vidutine kvadratine paklaida ($0,08 \pm 0,04$) ir mažiausia vidutine absoliučia procentine paklaida ($0,18 \pm 0,10$), taip pat su didžiausia koreliacija ($0,92 \pm 0,06$) ir R^2 reikšme ($0,82 \pm 0,13$). Šie rezultatai rodo, kad perioperacinis kraujo netekimas, įvertintas Hb/kg indeksu, paaiškinamas kliniškai reikšmingais kintamaisiais, patvirtinant siūlomo modelio patikimumą ir fiziologinį pagrįstumą.

Antra, šiame darbe pristatomas naujas kraujo netekimo stratifikavimo metodas, naudojant neprižiūrimo mokymosi (angl. unsupervised machine learning) algoritmus (K-medoids). Analizė išskyrė tris skirtingas pooperacinio kraujo netekimo grupes (lengva, vidutinė ir sunki), kurios apibrėžiamos objektyviais, duomenimis pagrįstais Hb/kg indekso slenkstiniais dydžiais – 1,72 ir 2,10. Patvirtinimas naudojant ROC analizę, parodė, kad modelis efektyviai atskiria kraštutinius fenotipus. Sunkios grupės diskriminacinė geba buvo didžiausia (AUC = 0,790; 95 proc. PI 0,721–0,859). Svarbu pažymėti, kad KŽD tūris tarp grupių reikšmingai nesiskyrė ($p = 0,097$), tuo tarpu hemoglobino masės netekimas per KŽD buvo reikšmingas ($p = 0,011$), kas pabrėžia vizualinių ar netektu tūrių pagrįstų metodų ribotumus.

Trečia, VET tyrimai parodė, kad nuo fibrinogeno priklausomas krešulio tvirtumas (FIBTEM A10 ir MCF) yra pagrindinis veiksnys, lemiantis kraujo netekimo sunkumą. FIBTEM A10 (12 mm) pasižymėjo puikiu diagnostiniu tikslumu nustatant pooperacinę hipofibrinogenemiją ($< 2,5$ g/l) ir yra svarbus nustatant kraujo netekimo sunkumą širdies chirurgijoje (AUC = 0,888). Tuo tarpu standartiniai krešėjimo tyrimai ir trombocitų agregometrija parodė ribotą diskriminacinę vertę tarp tiriamųjų grupių.

Ketvirta, sukūrus ir patvirtinus Hb/kg indeksą, šiame tyrime atsižvelgiant į Hb/kg indeksą išskirtos kraujo netekimo pacientų grupės perioperacinei plazminogeno aktyvatoriaus inhibitoriaus-1 (PAI-1) koncentracijai plazmoje kaip fibrinolitinio atsako žymeniui nustatyti. Tai suteikė naują galimybę ištirti ryšį tarp fibrinolizės ir objektyviai kiekybiškai įvertinto kraujo netekimo sunkumo.

Galiausiai, šis tyrimas pateikia integruotą vertinimą tikslinio VNP panelio, atspindinčio pagrindinius hemostazės kelius: trombocitų agregaciją (ITGB3 rs5918), fibrinogeno sintezę (FGB rs1800790) ir fibrinolizės reguliaciją (SERPINE1 rs2227631), siejant juos su perioperacinio kraujo netekimo sunkumu ir prognostine verte širdies chirurgijoje. Nors FGB (rs1800790) reikšmingai veikė fibrinogeno koncentraciją, tirtų VNP bendras indėlis į faktinio kraujo netekimo sunkumą buvo ribotas.

REZULTATAI

Bendrieji duomenys. Viso 212 pacientų, kuriems buvo atlikta planinė širdies operacija naudojant dirbtinę kraujo apytaką (DKA), atitiko įtraukimo kriterijus. Septyniolika pacientų buvo ekskliuduoti: vienas atšaukė sutikimą, trijų duomenys buvo nepilni, o trylikai reikėjo pakartotinės sternotomijos. Galutinę tiriamąją imtį sudarė 195 pacientai: 134 vyrai (68,7 proc.) ir 61 moteris (31,3 proc.). Išsamūs demografiniai ir klinikiniai duomenys pateikti 3 lentelėje.

Vidutinis pacientų amžius buvo $65,8 \pm 7,1$ metų. Vidutinis kūno svoris – $84,3 \pm 16,5$ kg, o kūno masės indeksas (KMI) – $28,5 \pm 5,1$ kg/m². Vidutinis kūno paviršiaus plotas (KPP) siekė $1,96 \pm 0,21$ m², o cirkuliuojančio kraujo tūris (CKT) – $5,07 \pm 0,83$ l. EuroSCORE II mediana buvo 1,97 [1,04–3,74]. Stacionarinio mirtingumo nenustatyta. Arterinė hipertenzija nustatyta 79 pacientams (40,5 proc.), hiperlipidemija – 162 (83,1 proc.), o cukrinis diabetas – 32 (16,4 proc.). Vidutinė kairiojo skilvelio išstūmio frakcija (LVEF) buvo $50,6 \pm 7,5$ proc.. NYHA I–II funkcinė klasė nustatyta 109 pacientams (55,9 proc.), o III–IV funkcinė klasė – 86 (44,1 proc.). Chirurginės intervencijos apėmė aortos vainikinių arterijų jungčių operacijas (AVJO) 101 pacientui (51,8 proc.), vožtuvų operacijas – 57 (29,2 proc.) ir kombinuotas operaci-

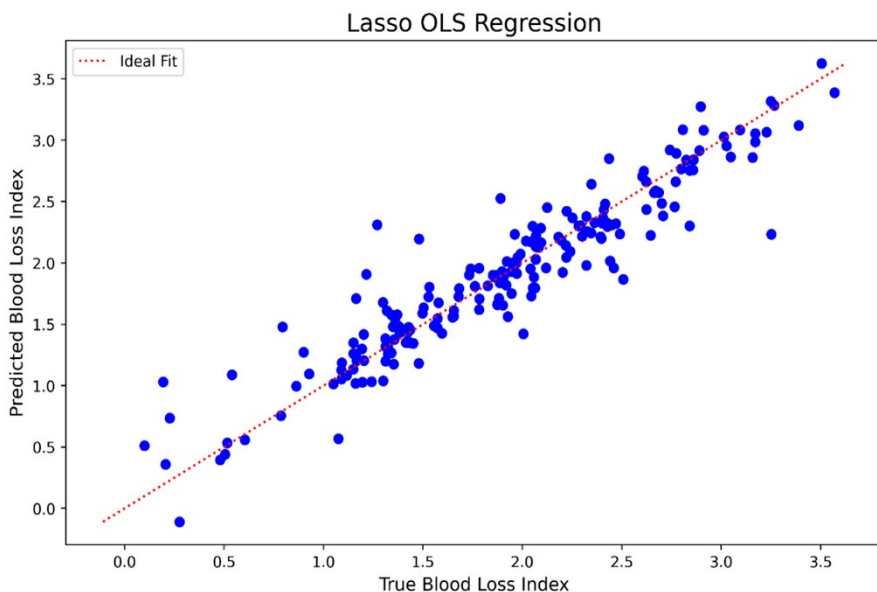
jas – 37 (19,0 proc.). Vidutinė operacijos trukmė buvo $202,4 \pm 53,8$ min, dirbtinės kraujo apytakos trukmė – $97,4 \pm 33,0$ min., o aortos užspaudimo laikas – $56,2 \pm 25,6$ min. Donorinių eritrocitų (DE) transfuzijos buvo taikytos 14 pacientų (7,2 proc.), šviežiai šaldyta plazma (ŠŠP) – 3 pacientams (1,5 proc.), o krioprecipitatas – 1 pacientui (0,5 proc.). Aktyvus rūkymas nustatytas 50 pacientų (25,6 proc.). Priešoperaciniu laikotarpiu vartota antitrombozinė terapija; aspirinas (33,8 proc.), P2Y12 inhibitorius (1,5 proc.), rivaroksabanas (9,2 proc.) ir varfarinas (1,5 proc.).

Mašininio mokymosi pagrindu sukurtas prognostinis modeliavimas

Prognostinis modeliavimas buvo atliktas siekiant nustatyti pagrindinius Hb/kg indekso raktinius veiksnius ir įvertinti skirtingų mašininio mokymosi metodų veiksmingumą. Požymių atrankai buvo taikyta Lasso regresija, nustatytas ribotas kliniškai reikšmingų prediktorių rinkinys: pradinė hemoglobino koncentracija, intraoperacinis hemoglobino masės netekimas, hemoglobino koncentracija po operacijos (T1), KMI ir su donoriniais eritrocitais susijusi hemoglobino masė (6 lentelė).

Iš visų vertintų modelių geriausią prognostinį tikslumą parodė Lasso-OLS metodas: vidutinė kvadratinė paklaida siekė $0,08 \pm 0,04$, vidutinė absoliuti procentinė paklaida – $0,18 \pm 0,10$, koreliacijos koeficientas – $0,92 \pm 0,06$, o $R^2 = 0,82 \pm 0,13$ (7 lentelė).

Linijinė regresija parodė panašų veiksmingumą ($R^2 = 0,77 \pm 0,17$), o Lasso regresijos aiškinamoji galia buvo šiek tiek mažesnė ($R^2 = 0,68 \pm 0,07$). „Tree-based“ ir nelinijiniai modeliai pasižymėjo mažesniu prognostiniu tikslumu: XGBoost ($R^2 = 0,65 \pm 0,21$), atraminių vektorių mašina ($R^2 = 0,64 \pm 0,15$) ir gilieji neuroniniai tinklai ($R^2 = 0,42 \pm 0,21$). Žemiausias veiksmingumas nustatytas k-artimiausių kaimynų metodui ($R^2 = 0,26 \pm 0,12$) ir sprendimų medžio tipo modeliams ($R^2 = 0,24 \pm 0,44$). Apskritai, regresija pagrįsti modeliai pasiekė didžiausią prognostinį tikslumą, ypač Lasso-OLS metodas (8 pav.)



8 pav. *Prognuozuotos ir stebėtos Hb/kg indekso reikšmės, naudojant Lasso-OLS modelį*

Kiekvienas taškas atitinka atskirą stebėjimą, o punktyrinė linija žymi tikslų atitikimą.

Kraujo netekimo sunkumo grupių nustatymas

Siekiant suskirstyti perioperacinio kraujo netekimo klinikinius modelius, buvo taikytas neprižiūrimo mašininio mokymosi metodas, naudojant K-medoidų klasterizavimą. Į tiriamųjų grupių nustatymo analizę įtraukti kintamieji buvo parinkti pagal jų reikšmingą koreliaciją su Hb/kg indeksu, taip modelį orientuojant į kliniškai svarbius grupių formavimo veiksnius.

Analizė nustatė tris skirtingas kraujo netekimo sunkumo grupes, apibūdinamas nuosekliai didėjančiu Hb/kg indekso lygiu. Duomenimis pagrįstos Hb/kg slenkstinės vertės – 1,72 ir 2,10 – buvo nustatytos kaip optimalūs atskyrimo taškai tarp gretimų grupių, leidžiantys suskirstyti pacientus į lengvo, vidutinio ir sunkaus kraujo netekimo grupes (8 lentelė). Šios slenkstinės vertės buvo apskaičiuotos kaip vidurio taškai tarp grupių medianų, taip sukuriant objektyvią ir duomenimis pagrįstą klasifikavimo sistemą.

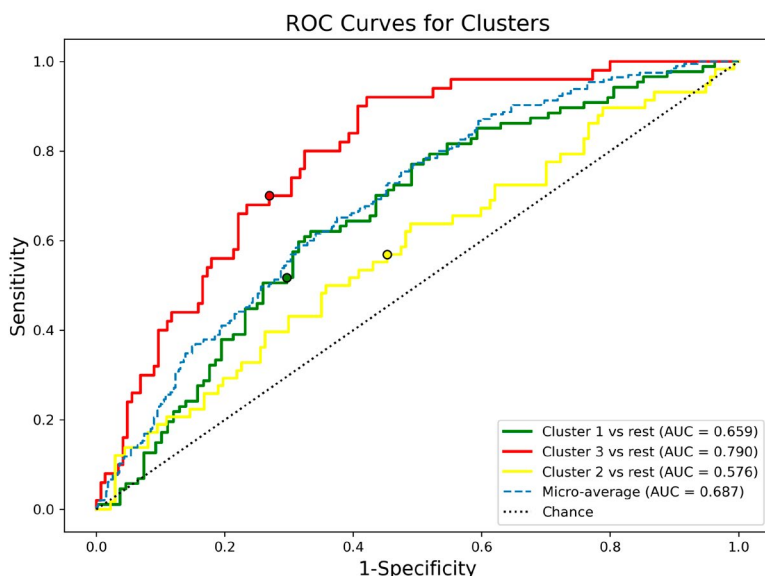
8 lentelė. Pacientų grupių aprašomoji statistika pagal Hb/kg indeksą

Grupės	n (proc.)	Kraujo netekimo laipsnis	Hb/kg indeksas	Mediana	Vidurkis
1 Grupė	76 (39)	Lengvas	$\leq 1,72$	1,65	1,71
2 Grupė	46 (23,6)	Vidutinis	$1,72 < \text{Hb/Kg} \leq 2,10$	1,80	1,76
3 Grupė	73 (37,4)	Sunkus	$> 2,10$	2,41	2,44

Pastaba: Hb/kg – hemoglobino masės netekimas vienam paciento kūno svorio kilogramui. Pateiktos reikšmės atspindi vidutinį Hb/kg indekso dydį kiekvienoje grupėje, nustatytoje taikant K-medoidų klasterizacijos metodą.

Grupių diskriminacinė geba

Sukurtų grupių diskriminacinė geba buvo įvertinta naudojant ROC analizę (8 pav.). Sunkaus kraujo netekimo grupė pasižymėjo geriausiu diskriminaciniu tikslumu ($\text{AUC} = 0,790$; 95 proc. PI 0,721–0,859), kas rodo aukštą gebėjimą identifikuoti pacientus, patyrusius reikšmingą hemoglobino masės netekimą. Tuo tarpu vidutinio kraujo netekimo grupė pasižymėjo silpnu atskiriamumu ($\text{AUC} = 0,576$), o lengvo kraujo netekimo grupė – vidutiniu diskriminaciniu tikslumu ($\text{AUC} = 0,659$). Bendras mikro-vidurkio AUC rodiklis buvo 0,687.



9 pav. ROC kreivės, skirtos prognozuoti kraujo netekimo sunkumo grupes pagal Hb/kg indekso slenkstines reikšmes

Klinikiniai ir antropometriniai rodikliai grupėse

Pakoreguota dvikryptė ANCOVA analizė patvirtino, kad Hb/kg indeksas reikšmingai skyrėsi tarp grupių ($F(2,186) = 250,44$, $p < 0,001$, $\eta_p^2 = 0,729$), paaiškindama 80,3 proc. variacijos ($R^2 = 0,803$) (9 lentelė). Hb/kg indeksas nuosekliai didėjo nuo lengvo iki sunkaus kraujo netekimo grupės (atitinkamai $1,22 \pm 0,35$; $1,94 \pm 0,10$; $2,65 \pm 0,36$). Lyties poveikis ($p = 0,757$) ar grupės ir lyties sąveika ($p = 0,992$) nenustatyti. Antropometriniai rodikliai tarp grupių reikšmingai skyrėsi. Kūno svoris mažėjo nuo vidutinio iki sunkaus kraujo netekimo grupės (atitinkamai $89,4 \pm 18,2$ kg; $84,7 \pm 13,6$ kg; $78,9 \pm 14,5$ kg; $p < 0,001$), taip pat ir KMI ($30,5 \pm 5,3$; $28,7 \pm 4,9$; $26,3 \pm 4,1$ kg/m²; $p < 0,001$). Kūno paviršiaus plotas (KPP) ir cirkuliuojančio kraujo tūris (CKT) rodė panašią tendenciją – mažiausios reikšmės nustatytos sunkaus kraujo netekimo grupėje (atitinkamai $p < 0,001$ ir $p = 0,002$).

Pradinė kairiojo skilvelio išstūmio frakcija (KSIF) ir kreatinino koncentracija tarp grupių reikšmingai nesiskyrė (atitinkamai $p = 0,199$ ir $p = 0,791$). Taip pat nenustatyta reikšmingų skirtumų vertinant EuroSCORE II ($p = 0,088$) ir Papworth kraujavimo rizikos balą ($p = 0,232$) (9 lentelė).

9 lentelė. Pacientų charakteristikos tiriamosiose grupėse

Pacientų charakteristikos	Lengvo laipsnio kraujotekimo grupė	Vidutinio laipsnio kraujotekimo grupė	Sunkaus laipsnio kraujotekimo grupė	F	p	η_p^2
Hb/kg indeksas	1,22 ± 0,35	1,94 ± 0,10	2,65 ± 0,36	250,435	<0,001	0,729
Kūno masė (kg)	89,4 ± 18,2	84,7 ± 13,6	78,9 ± 14,5	9,811	<0,001	0,095
KMI (kg/m ²)	30,5 ± 5,3	28,7 ± 4,9	26,3 ± 4,1	10,322	<0,001	0,099
KPP (m ²)	2,01 ± 0,23	1,97 ± 0,16	1,91 ± 0,20	7,989	<0,001	0,078
Kraujo tūris (l)	5,17 ± 0,92	5,11 ± 0,64	4,94 ± 0,81	6,637	0,002	0,066
KSIF, proc.	51,93 ± 5,47	52,09 ± 6,04	49,37 ± 8,14	1,627	0,199	0,017
Kreatininas	84,76 ± 22,48	87,27 ± 17,36	86,35 ± 17,49	0,235	0,791	0,002
EuroSCORE II	2,85 ± 2,33	2,56 ± 1,76	2,17 ± 1,63	2,460	0,088	0,026
Kraujavimo rizika (Papworth Score)	1,11 ± 0,90	0,96 ± 1,01	1,18 ± 1,05	1,473	0,232	0,015

Pastaba: reikšmės pateikiamos kaip vidurkis ± standartinis nuokrypis (SN), pašalinus išskirtis kiekviename *Klasterio* × *Lyties* derinyje pagal $3 \times IQR$ taisyklę. Grupių palyginimui atlikta dvifaktoriinė kovariacinė analizė (*ANCOVA*), kurioje *Klastervis* ir *Lytis* buvo įtraukti kaip fiksuoti veiksniai, *Amžius* – kaip kovariatas, o į modelį taip pat įtraukta *Klasterio* × *Lyties* sąveika. Visi pateikiami F kriterijai nurodo pagrindinį *Klasterio* poveikį (*III tipo kvadratų suma*). Statistinis reikšmingumas nustatytas, kai $p < 0,05$. Dalinis eta kvadratas (η_p^2) interpretuotas pagal Coheno rekomendacijas: 0,01 – mažas; 0,06 – vidutinis; 0,14 – didelis poveikis.

Bendras kraujo tyrimas grupėse

Hemoglobino koncentracijos perioperaciniu laikotarpiu reikšmingai skyrėsi tarp grupių. Pradiniame etape (T0) didžiausios reikšmės buvo nustatytos sunkaus kraujo netekimo grupėje ($144,5 \pm 11,7$ g/l), o mažiausios – lengvo kraujo netekimo grupėje ($134,5 \pm 14,6$ g/l; $p < 0,001$). Tačiau po operacijos (T1) sunkaus kraujo netekimo grupėje buvo stebimas ryškiausias Hb sumažėjimas, siekiantis mažiausias absoliučias reikšmes tarp visų grupių ($106,1 \pm 12,6$ g/l; $p < 0,001$). Nors skirtumai T6 etape nebuvo statistiškai reikšmingi ($p = 0,112$), T18 etape vėl išryškėjo reikšmingas skirtumas ($p = 0,004$), kai sunkaus kraujo netekimo grupėje Hb atsistatymo nestebėta ir išsilaikė reikšmingai mažesnė hemoglobino koncentracija ($98,4 \pm 14,6$ g/l) palyginti su lengvo kraujo netekimo grupe ($107,0 \pm 11,9$ g/l).

Hematokrito reikšmės tarp grupių reikšmingai skyrėsi visais laiko momentais: T0 ($p = 0,018$), T1 ($p < 0,001$), T6 ($p = 0,019$) ir T18 ($p < 0,001$). Didžiausi skirtumai tarp grupių buvo stebimi T1 ir T18 etapuose. Eritrocitų kiekis pradiniame etape reikšmingai nesiskyrė ($p = 0,134$). Tačiau iškart po operacijos (T1) ryškus sumažėjimas buvo nustatytas sunkaus kraujo netekimo grupėje ($p < 0,001$), o sumažėję rodikliai išliko T6 ($p = 0,018$) ir T18 ($p = 0,002$) etapuose. Mažiausias eritrocitų kiekis buvo nustatytas sunkaus kraujo netekimo grupėje paskutiniame etape (T18: $3,20 \pm 0,59 \times 10^{12}/l$). Priešingai nei eritrocitų, trombocitų kiekio reikšmingų skirtumų tarp grupių nestebėta nei viename tyrimo etape. Šie duomenys pateikti 12 lentelėje ir 10 paveiksle.

12 lentelė. Bendro kraujo tyrimo rodikliai grupėse skirtingais tyrimo etapais

Etapas	Lengvo laipsnio kraujo netekimo grupė	Vidutinio laipsnio kraujo netekimo grupė	Sunkaus laipsnio kraujo netekimo grupė	F	p	η_p^2
Hemoglobino koncentracija, g/l						
T0	134,5 ± 14,6	140,2 ± 10,3	144,5 ± 11,7	8,128	<0,001	0,080
T1	115,3 ± 14,0	111,0 ± 10,7	106,1 ± 12,6	7,863	<0,001	0,077
T6	112,9 ± 12,7	109,6 ± 12,9	107,4 ± 14,0	2,212	0,112	0,023
T18	107,0 ± 11,9	104,9 ± 16,0	98,4 ± 14,6	5,691	0,004	0,057
Hematokritas, proc.						
T0	40,6 ± 4,5	42,2 ± 3,3	42,9 ± 3,8	4,084	0,018	0,042
T1	34,0 ± 4,4	33,2 ± 3,6	31,4 ± 3,9	7,651	<0,001	0,075
T6	33,7 ± 3,9	32,8 ± 3,8	31,8 ± 4,0	4,039	0,019	0,042
T18	31,7 ± 3,5	30,7 ± 4,2	28,7 ± 4,0	9,065	<0,001	0,088
Eritrocitai, 10 ¹² /l						
T0	4,49 ± 0,49	4,68 ± 0,44	4,72 ± 0,49	2,031	0,134	0,021
T1	3,81 ± 0,53	3,68 ± 0,43	3,43 ± 0,44	11,853	<0,001	0,112
T6	3,74 ± 0,52	3,65 ± 0,48	3,47 ± 0,47	4,087	0,018	0,042
T18	3,54 ± 0,44	3,41 ± 0,50	3,20 ± 0,59	6,323	0,002	0,063
Trombocitai, 10 ⁹ /l						
T0	216,29 ± 53,40	218,13 ± 54,25	224,88 ± 57,39	0,369	0,692	0,015
T1	171,25 ± 41,48	168,71 ± 37,02	157,90 ± 40,74	1,318	0,270	0,026
T6	171,09 ± 47,07	173,02 ± 40,52	166,74 ± 41,43	0,080	0,923	0,010
T18	163,71 ± 46,17	160,20 ± 44,41	152,75 ± 37,97	0,517	0,597	0,017

Pastaba: reikšmės pateikiamos kaip vidurkis ± standartinis nuokrypis (SN), pašalinus išskirtis kiekviename *Klasterio* × *Lyties* derinyje pagal $3 \times IQR$ taisyklę. Grupių palyginimui atlikta dvifaktorinė kovariacinė analizė (*ANCOVA*), kurioje *Klastervis* ir *Lytis* buvo įtraukti kaip fiksuoti veiksniai, *Amžius* – kaip kovariatas, o į modelį taip pat įtraukta *Klasterio* × *Lyties* sąveika. Visi pateikiami *F* kriterijai nurodo pagrindinį *Klasterio* poveikį (*III tipo kvadratų suma*). Statistinis reikšmingumas nustatytas, kai $p < 0,05$. Dalinis eta kvadratas (η_p^2) interpretuotas pagal Coheno rekomendacijas: 0,01 – mažas; 0,06 – vidutinis; 0,14 – didelis poveikis.

Standartiniai krešėjimo tyrimai

Krešėjimo rodikliai parodė reikšmingus pokyčius (13 lentelė). ADTL pagilėjo nuo pradinės reikšmės iki didžiausios T6 etape – nuo $36,18 \pm 10,36$ s iki $44,95 \pm 12,03$ s, o vėliau iš dalies sumažėjo iki $39,35 \pm 8,53$ s T18 etape. TNS padidėjo nuo $1,04 \pm 0,10$ pradiniam etape iki $1,25 \pm 0,12$ T18 etape

Tuo tarpu fibrinogeno koncentracija po operacijos (T1) sumažėjo, palyginti su pradine – nuo $3,86 \pm 0,89$ g/l iki $2,87 \pm 0,71$ g/l, o vėliau atsistatė iki $3,78 \pm 0,74$ g/l T18 etape. D-dimerai pradiniam etape (T0) nebuvo tirti. Nuo T1 iki T18 jo koncentracija sumažėjo nuo $0,66 \pm 0,51$ mg/l iki $0,55 \pm 0,37$ mg/l (visais atvejais $p < 0,001$). Koreliacinė analizė parodė reikšmingą Hb/kg indekso ryšį su fibrinogeno koncentracija skirtingais laiko etapais. Stipriausia neigiama koreliacija nustatyta T1 ir T18 ($\rho = -0,308$ ir $\rho = -0,285$; abiem atvejais $p < 0,001$). Taip pat reikšminga sąsaja nustatyta T6 ($\rho = -0,180$, $p = 0,012$). Tuo tarpu reikšmingų korelacijų tarp Hb/kg indekso ir TNS ar D-dimerų nenustatyta (visais atvejais $p > 0,05$). Su ADTL nustatyta silpna, bet reikšminga teigiama koreliacija T18 etape ($\rho = 0,147$, $p = 0,040$). Išilginė standartinių krešėjimo rodiklių analizė grupėse pateikta 15 lentelėje ir 12 paveiksle. Pradinė fibrinogeno koncentracija tarp grupių reikšmingai nesiskyrė ($p = 0,057$). Tačiau T1 etape nustatytas reikšmingas skirtumas ($p = 0,001$): mažiausios reikšmės buvo sunkaus kraujo netekimo grupėje ($2,66 \pm 0,57$ g/l), palyginti su lengvo kraujo netekimo grupe ($3,15 \pm 0,75$ g/l) ir vidutinio kraujo netekimo grupe ($2,83 \pm 0,57$ g/l). T6 etape skirtumų tarp grupių nenustatyta ($p = 0,290$). T18 etape fibrinogeno koncentracija vėl reikšmingai skyrėsi tarp grupių ($p = 0,003$), mažiausios reikšmės išliko sunkaus kraujo netekimo grupėje ($3,59 \pm 0,57$ g/l), palyginti su lengvo kraujo netekimo grupe ($4,04 \pm 0,76$ g/l) ir vidutinio kraujo netekimo grupe ($3,68 \pm 0,73$ g/l).

D-dimerų koncentracija tarp grupių reikšmingai nesiskyrė nei viename etape. ADTL taip pat nesiskyrė tarp grupių T0, T1 ir T6 etapuose ($p > 0,05$), o vienintelis reikšmingas skirtumas nustatytas T18 etape ($p = 0,034$). TNS tarp grupių reikšmingai nesiskyrė nei viename vertinimo etape ($p > 0,05$).

15 lentelė. Standartiniai kresėjimo rodikliai tiriamosiose grupėse skirtingais tyrimo etapais

Etapas	Lengvo laipsnio kraujo netekimo grupė	Vidutinio laipsnio kraujo netekimo grupė	Sunkaus laipsnio kraujo netekimo grupė	F	p	η_p^2
Fibrinogeno koncentracija, g/l						
T0	4,02 ± 0,99	3,83 ± 0,88	3,70 ± 0,75	2,911	0,057	0,030
T1	3,15 ± 0,75	2,83 ± 0,57	2,66 ± 0,57	6,780	0,001	0,068
T6	3,27 ± 0,81	3,12 ± 0,69	2,99 ± 0,71	1,248	0,290	0,013
T18	4,04 ± 0,76	3,68 ± 0,73	3,59 ± 0,57	5,904	0,003	0,060
D-dimerai, mg/l						
T1	0,67 ± 0,41	0,60 ± 0,36	0,61 ± 0,40	0,771	0,464	0,008
T6	0,69 ± 0,41	0,58 ± 0,34	0,53 ± 0,30	2,744	0,067	0,029
T18	0,60 ± 0,35	0,54 ± 0,36	0,45 ± 0,26	1,785	0,171	0,019
ADTL, s						
T0	34,35 ± 6,98	34,74 ± 5,58	34,93 ± 6,33	0,192	0,825	0,002
T1	41,55 ± 6,93	43,26 ± 8,34	42,91 ± 6,92	1,703	0,185	0,018
T6	43,68 ± 11,04	45,00 ± 11,87	45,41 ± 10,95	0,057	0,945	0,001
T18	37,23 ± 4,32	40,94 ± 6,67	38,81 ± 4,55	3,435	0,034	0,036
TNS						
T0	1,02 ± 0,08	1,05 ± 0,10	1,02 ± 0,09	0,474	0,623	0,005
T1	1,18 ± 0,08	1,21 ± 0,10	1,21 ± 0,11	1,029	0,359	0,011
T6	1,17 ± 0,10	1,17 ± 0,09	1,18 ± 0,10	0,138	0,872	0,002
T18	1,24 ± 0,10	1,25 ± 0,12	1,26 ± 0,12	0,194	0,824	0,002

Pastaba: reikšmės pateikiamos kaip vidurkis ± standartinis nuokrypis (SN), pašalinus išskirtis kiekviename *Klasterio* × *Lyties* derinyje pagal $3 \times IQR$ taisyklę. Grupių palyginimui atlikta dvifaktorinė kovariacinė analizė (*ANCOVA*), kurioje *Klastervis* ir *Lytis* buvo įtraukti kaip fiksuoti veiksniai, *Amžius* – kaip kovariatas, o į modelį taip pat įtraukta *Klasterio* × *Lyties* sąveika. Visi pateikiami F kriterijai nurodo pagrindinį *Klasterio* poveikį (*III tipo kvadratų suma*). Statistinis reikšmingumas nustatytas, kai $p < 0,05$. Dalinis eta kvadratas (η_p^2) interpretuotas pagal Coheno rekomendacijas: 0,01 – mažas; 0,06 – vidutinis; 0,14 – didelis poveikis.

Plazminogeno aktyvatoriaus inhibitorius-1 (PAI-1)

PAI-1 koncentracija plazmoje buvo ištirta 127 pacientams prieš operaciją (T0) ir praėjus 18 valandų po operacijos (T18). Vidutinė pradinė PAI-1 koncentracija buvo $35,14 \pm 19,32$ ng/ml, o T18 etape ji padidėjo iki $46,32 \pm 18,25$ ng/ml. Kadangi abiem laiko momentais duomenys neatitiko normaliojo skirstinio, pokyčiai buvo vertinti naudojant Wilcoxon ženklų rangų testą. Nustatyta, kad pooperacinės (T18) PAI-1 reikšmės buvo statistiškai reikšmingai didesnės nei pradinės ($Z = -4,974$, $p < 0,001$). Nustatyta silpna, tačiau statistiškai reikšminga teigiama koreliacija tarp PAI-1 koncentracijų T0 ir T18 etapuose ($\rho = 0,213$, $p = 0,016$). Tuo tarpu reikšmingos koreliacijos tarp Hb/kg indekso ir PAI-1 koncentracijos nei prieš operaciją ($\rho = -0,066$, $p = 0,464$), nei po jos ($\rho = 0,127$, $p = 0,155$) nenustatyta. PAI-1 koncentracijos papildomai analizuotos grupėse taikant ANCOVA (16 lentelė). Vidutinės pradinės PAI-1 reikšmės buvo $42,17 \pm 25,87$ lengvo kraujo netekimo grupėje, $28,40 \pm 9,96$ vidutinio kraujo netekimo grupėje ir $31,64 \pm 13,05$ sunkaus kraujo netekimo grupėje. Statistiškai reikšmingų skirtumų tarp grupių prieš operaciją nenustatyta ($F = 2,24$, $p = 0,111$, $\eta_p^2 = 0,037$, $R^2 = 0,115$). T18 etape vidutinės PAI-1 koncentracijos buvo $45,98 \pm 17,42$ lengvo, $40,12 \pm 17,54$ vidutinio ir $48,94 \pm 18,61$ sunkaus kraujo netekimo grupėse. Taip pat nenustatyta statistiškai reikšmingų skirtumų tarp grupių, nors stebėta ribinė tendencija ($F = 2,74$, $p = 0,069$, $\eta_p^2 = 0,044$, $R^2 = 0,161$).

Trombocitų agregometrija (TA)

Trombocitų agregacijos tyrimas parodė reikšmingus perioperacinius pokyčius stebėjimo laikotarpiu (17 lentelė). ADF sukelta trombocitų agregacija T1 etape sumažėjo 26,9 proc., palyginti su pradine reikšme (nuo $73,06 \pm 14,48$ proc. iki $53,39 \pm 14,30$ proc.), o T18 iš dalies atsistatė iki $66,11 \pm 10,67$ proc.. Epinefrino sukelta trombocitų agregacija sumažėjo dar ryškiau – T1 etape ji sumažėjo 53,3 proc. (nuo $77,72 \pm 14,47$ proc. iki $36,29 \pm 25,79$ proc.), ir trombocitų funkcijos slopinimas išliko T18 etape ($41,56 \pm 18,42$ proc.). Koreliacinė analizė neparodė reikšmingų sąsajų tarp Hb/kg indekso ir ADF sukeltos trombocitų agregacijos nei T0 ($\rho = -0,103$, $p = 0,150$), nei T1 ($\rho = -0,078$, $p = 0,278$), nei T18 ($\rho = -0,031$, $p = 0,663$) etapuose. Taip pat nenustatyta reikšmingų korelacijų tarp Hb/kg indekso ir epinefrino sukeltos trombocitų agregacijos T0 ($\rho = 0,016$, $p = 0,826$), T1 ($\rho = -0,021$, $p = 0,770$) ar T18 ($\rho = 0,050$, $p = 0,485$) (18 lentelė). ADF ir epinefrino sukeltos trombocitų agregacijos rodikliai skirtingose grupėse pateikti 19 lentelėje. Nenustatyta statistiškai reikšmingų skirtumų tarp grupių nei ADF, nei epinefrino sukeltos trombocitų agregacijos požiūriu nei viename vertinimo etape (visos p reikšmės $> 0,05$).

ROTEM parametrai

Viskoelastinės hemostazės savybės buvo įvertintos 79 pacientų pogrupyje (40,5 proc. visos tiriamosios imties) prieš operaciją (T0) ir iškart po jos (T1) (20 lentelė). Krešėjimo laikas (CT) parodė minimalius perioperacinius pokyčius: EXTEM CT išliko stabilus ($p = 0,542$), tuo tarpu INTEM CT šiek tiek, bet statistiškai reikšmingai pailgėjo ($p = 0,011$).

Priešingai, krešulio tvirtumo parametrai (A10 ir MCF) reikšmingai sumažėjo tiek EXTEM, tiek INTEM tyrimuose (visais atvejais $p < 0,001$). Ryškiausias sumažėjimas nustatytas su fibrinogenu susijusiuose parametruose (FIBTEM), kur FIBTEM A5, A10 ir MCF reikšmingai sumažėjo (visais atvejais $p < 0,001$). Tuo tarpu trombocitų funkciją atspindintys PLTEM parametrai išliko stabilūs – A5, A10 ir MCF reikšmingų pokyčių nerodė (visi $p > 0,05$).

Liekamasis heparino poveikis buvo vertintas lyginant INTEM ir HEPTTEM CT po operacijos. Nustatytas statistiškai reikšmingas skirtumas: HEPTTEM CT mediana buvo 185 [175–191] s, palyginti su 195 [187–212] s INTEM CT ($p < 0,001$). 26 pacientams (33 proc.) INTEM/HEPTTEM CT santykis viršijo 1,1.

ROTEM parametrų ir Hb/kg indekso koreliacinė analizė pateikta 21 lentelėje ir 13 paveiksle. Pradiniai FIBTEM parametrai – A5 ($\rho = -0,231$, $p = 0,039$), A10 ($\rho = -0,242$, $p = 0,032$) ir MCF ($\rho = -0,236$, $p = 0,036$) – parodė statistiškai reikšmingas atvirkštines koreliacijas su Hb/kg indeksu. Po operacijos (T1) šios sąsajos išliko ir tapo dar ryškesnės (visi $p < 0,01$).

EXTEM ir INTEM parametrai pradiniam etape su Hb/kg indeksu nesusiję. Tačiau T1 laiko taške nustatytos reikšmingos atvirkštinės koreliacijos: EXTEM A10 ($\rho = -0,318$, $p = 0,004$), EXTEM MCF ($\rho = -0,311$, $p = 0,005$), INTEM A10 ($\rho = -0,322$, $p = 0,004$) ir INTEM MCF ($\rho = -0,343$, $p = 0,002$). EXTEM CT ir INTEM CT su Hb/kg indeksu nesusiję nei viename vertinimo etape (visi $p > 0,05$).

PLTEM atveju nustatyta teigiama koreliacija su Hb/kg indeksu tik pradiniam etape A10 parametrai ($\rho = 0,263$, $p = 0,019$), tuo tarpu A5 ir MCF reikšmingų sąsajų neturėjo. T1 etape reikšmingų koreliacijų su PLTEM parametrais nenustatyta.

HEPTTEM parametrai, vertinti tik po operacijos (T1), parodė reikšmingas atvirkštines koreliacijas su Hb/kg indeksu: A5 ($\rho = -0,285$, $p = 0,011$), A10 ($\rho = -0,270$, $p = 0,016$) ir MCF ($\rho = -0,298$, $p = 0,008$). Tačiau HEPTTEM CT nepasiekė statistinio reikšmingumo ($\rho = 0,214$, $p = 0,058$). APTEM vertintas tik pradiniam etape, reikšmingų koreliacijų su Hb/kg indeksu neparodė (visi $p > 0,05$).

ROTEM parametrai tarp grupių

Pradiniame etape dauguma ROTEM parametrų reikšmingai nesiskyrė (22 lentelė), įskaitant EXTEM CT ($p = 0,443$), EXTEM A10 ($p = 0,632$), EXTEM MCF ($p = 0,165$), INTEM CT ($p = 0,737$), INTEM A10 ($p = 0,791$) ar INTEM MCF ($p = 0,461$). Tačiau su fibrinogenu susiję parametrai (FIBTEM) parodė reikšmingus skirtumus tarp grupių. FIBTEM A10 sumažėjo nuo $16,35 \pm 4,22$ mm lengvo kraujo netekimo grupėje iki $13,72 \pm 3,24$ mm sunkaus kraujo netekimo grupėje ($F = 3,462$, $p = 0,037$, $\eta_p^2 = 0,088$). FIBTEM MCF sumažėjo nuo $18,06 \pm 4,80$ mm iki $15,10 \pm 3,60$ mm ($F = 3,571$, $p = 0,033$, $\eta_p^2 = 0,090$). Visi PLTEM parametrai tarp grupių nesiskyrė.

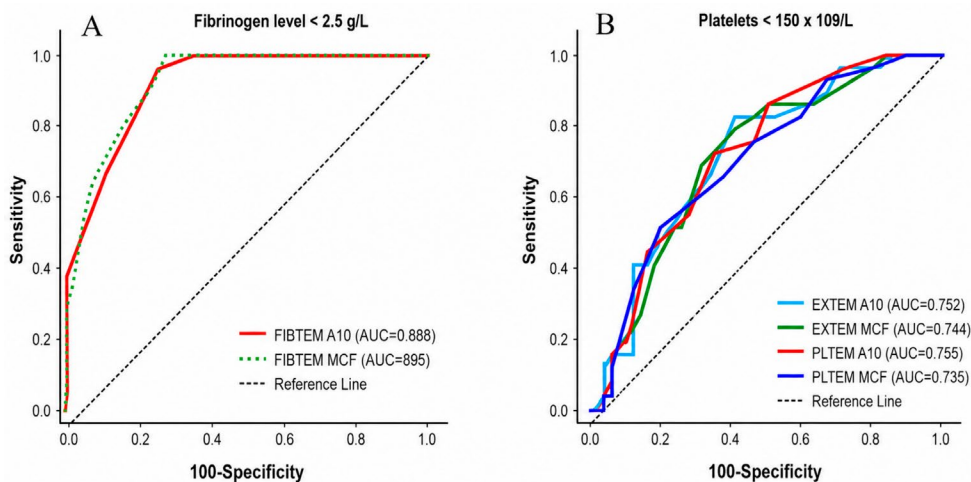
Po operacijos EXTEM CT ir A10 tarp grupių nesiskyrė ($p > 0,05$), tik EXTEM MCF buvo reikšmingai mažesnis sunkaus kraujo netekimo grupėje ($F = 3,909$, $p = 0,024$, $\eta_p^2 = 0,098$). INTEM A10 ($F = 3,566$, $p = 0,033$, $\eta_p^2 = 0,090$) ir INTEM MCF ($F = 4,991$, $p = 0,009$, $\eta_p^2 = 0,122$) parodė statistiškai reikšmingus skirtumus, tuo tarpu INTEM CT nebuvo reikšmingas ($p = 0,366$). Ryškiausi skirtumai nustatyti su fibrinogenu susijusiuose parametruose. FIBTEM A10 sumažėjo nuo $14,53 \pm 3,79$ mm lengvo kraujo netekimo grupėje iki $11,47 \pm 2,95$ mm sunkaus kraujo netekimo grupėje ($F = 4,990$, $p = 0,006$, $\eta_p^2 = 0,132$). FIBTEM MCF taip pat sumažėjo nuo $16,26 \pm 4,59$ mm iki $12,81 \pm 3,46$ mm ($F = 4,908$, $p = 0,010$, $\eta_p^2 = 0,120$). Priešingai, nei PLTEM A10 ($p = 0,754$), nei PLTEM MCF ($p = 0,811$) tarp grupių nesiskyrė.

ROTEM diagnostinis tikslumas

ROTEM parametrų diagnostinis gebėjimas nustatant hipofibrinogenemiją ($< 2,5$ g/l) ir trombocitopeniją ($< 150 \times 10^9/l$) pateiktas 23 lentelėje ir 14 paveiksle. Vertinant hipofibrinogenemiją, FIBTEM A10 parodė puikų diagnostinį tikslumą – AUC = 0,888 (95 proc. PI 0,797–0,979; $p < 0,001$) (15A pav.). ROC analizė nustatė optimalę ribinę vertę – 12 mm (maksimalus Youden indeksas = 0,649), kuris užtikrino didelį jautrumą (96 proc.) ir specifiškumą (69,1 proc.). Esant šiai ribai, neigiama prognostinė vertė (NPV) siekė 97,4 proc.. FIBTEM MCF parodė panašų diagnostinį tikslumą (AUC = 0,895; 95 proc. PI 0,807–0,994; $p < 0,001$). Vertinant trombocitopeniją, didžiausi AUC rodikliai nustatyti PLTEM A10 (AUC = 0,755) ir EXTEM A10 (AUC = 0,752) parametrams. Naudojant EXTEM A10 ribinę reikšmę 57 mm (teigiamas rezultatas, jei ≤ 57 mm), gautas 82 proc. jautrumas ir 63 proc. specifiškumas (PPV = 55 proc., NPV = 87 proc.). PLTEM A10 ribinė reikšmė 43 mm suteikė labiau subalansuotą diagnostinį profilį – 71 proc. jautrumą ir 69 proc. specifiškumą (PPV = 56 proc., NPV = 81 proc.) (15B pav.).

23 lentelė. Pooperacinių ROTEM parametrų diagnostinis tikslumas ir ribinės vertės, prognozuojant hipofibrinogenemiją ir trombocitopeniją

	ROTEM parametrai	Ribinės vertės (mm)	Jautrumas	Specifiškumas	PPV	NPV	AUC	95 proc. CI	p
Fibrinogenas (2,5 g/l)	FIBTEM A10, mm	≤ 12	95,8	69,1	57,5	97,4	0,888	0,797–0,979	< 0,001
	FIBTEM MCF, mm	≤ 14	100,0	65,5	55,8	100,0	0,895	0,807–0,984	< 0,001
	EXTEM A10, mm	≤ 57	82,1	62,7	54,8	86,5	0,752	0,634–0,870	< 0,001
Trombocitai ($< 150 \times 10^9/l$)	EXTEM MCF, mm	≤ 64	78,6	62,7	53,7	84,2	0,744	0,625–0,872	< 0,001
	PLTEMA10, mm	≤ 43	71,4	68,6	55,6	81,4	0,755	0,638–0,872	< 0,001
	PLTEM MCF, mm	≤ 48	50,0	84,3	63,6	75,4	0,735	0,614–0,856	< 0,001



15 pav. ROTEM parametrų ROC kreivės

Krūtinės poop. žaizdos drenažo tūris ir sudėtis

Krūtinės pooperacinės žaizdos drenažo (KŽD) tūris reikšmingai skyrėsi tarp grupių T1, T2, T4, T5 ir T6 tyrimo etapais (visi $p < 0,01$), o didžiausias tūris nustatytas sunkaus kraujo netekimo grupėje. Tačiau T3 etape šie skirtumai nebuvo statistiškai reikšmingi ($p = 0,075$). Bendras sukauptas drenažo tūris per 18 valandų (KŽD suma) tiriamose grupėse reikšmingai nesiskyrė ($F = 2,361$, $p = 0,097$, $\eta_p^2 = 0,025$) (24 lentelė).

Tuo tarpu stebėti ženklėsni faktinio hemoglobino masės netekimo skirtumai tarp grupių (24 lentelė). Hemoglobino masės netekimas per drenus (KŽD) T1 etape tarp grupių nesiskyrė ($F = 3,001$, $p = 0,052$, $\eta_p^2 = 0,032$). Tačiau T6 etape nustatytas reikšmingas skirtumas tarp grupių ($F = 5,426$, $p = 0,005$, $\eta_p^2 = 0,055$), didžiausios reikšmės stebėtos sunkaus kraujo netekimo grupėje ($19,2 \pm 12,9$ g), palyginti su lengvo kraujo netekimo grupe ($14,0 \pm 9,2$ g).

Suminis hemoglobino masės netekimas per KŽD 18 valandų laikotarpiu (T18 suma) taip pat parodė statistiškai reikšmingus skirtumus tarp grupių ($F = 4,633$, $p = 0,011$, $\eta_p^2 = 0,048$) (15 pav.).

Operacijos tipas

Chirurginių procedūrų pasiskirstymas ir aortos vainikinių arterijų jungčių operacijų (AVJO) apimtis tiriamosiose grupėse pateikta 25 lentelėje. Operacijos tipas tarp grupių statistiškai reikšmingai nesiskyrė, tačiau nustatytas ribinis skirtumas (Pearson χ^2 (4) = 9,425, $p = 0,051$). Lengvo kraujo netekimo grupės pacientams dažniau buvo atliekamos izoliuotos vožtuvų ope-

racijos (40,8 proc.), tuo tarpu vidutinio ir sunkaus kraujo netekimo grupėse vyraavo AVJO operacijos (atitinkamai 60,9 proc. ir 57,5 proc.). Nuosrūvių skaičius tarp grupių skyrėsi statistiškai reikšmingai (Pearson $\chi^2(4) = 14,248$, $p = 0,027$), taip pat nustatyta silpna, bet reikšminga teigiama ranginė sąsaja (Kendall $\tau_c = 0,178$, $p = 0,005$). Lengvo kraujo netekimo grupėje 39,5 proc. pacientų nebuvo atliekama aortos vainikinių arterijų jungčių operacija. Tuo tarpu vidutinio ir sunkaus kraujo netekimo grupėse didesnei pacientų daliai suformuoti trys ar daugiau nuosrūvių (atitinkamai 73,9 proc. ir 63,1 proc.). Keturių ir daugiau nuosrūvių dažnis buvo apytiksliai du kartus didesnis vidutinio kraujo netekimo (26,1 proc.) ir sunkaus kraujo netekimo (24,7 proc.) grupėse palyginti su lengvo kraujo netekimo grupe (11,8 proc.).

Operacijos trukmė ($p = 0,082$), dirbtinės kraujo apytakos (DKA) trukmė ($p = 0,729$) ir aortos užspaudimo trukmė ($p = 0,280$) tarp tiriamųjų grupių reikšmingai nesiskyrė.

Klinikinės išeitys ir rizikos veiksniai

Pooperacinių komplikacijų dažnis tarp grupių statistiškai reikšmingai nesiskyrė. Širdies nepakankamumo dažnis buvo panašus visose grupėse ($p = 0,823$), kaip ir prieširdžių virpėjimo paroksizmų ($p = 0,646$) bei ūminio inkstų nepakankamumo (ŪIN; $p = 0,611$) atvejų. Neurologinių komplikacijų ($p = 0,327$) bei atrioventrikulinės (AV) blokados ($p = 0,505$) dažnis tarp grupių taip pat nesiskyrė. Be to, gydymo trukmės intensyvios terapijos skyriuje (ITS) ($p = 0,752$) ir bendro hospitalizacijos laiko ($p = 0,596$) reikšmingų skirtumų nenustatyta.

Rūkančiųjų dalis tarp grupių skyrėsi statistiškai reikšmingai (Pearson $\chi^2(2) = 11,305$, $p = 0,004$). Rūkančiųjų skaičius tiriamose grupėse didėjo didėjant kraujo netekimo kiekiui: nuo lengvo kraujo netekimo grupės iki sunkaus kraujo netekimo grupės – atitinkamai 13,2 proc., 28,3 proc. ir 37,0 proc.. Tuo tarpu alkoholio vartojimas tarp grupių reikšmingai nesiskyrė (Pearson $\chi^2(2) = 1,046$, $p = 0,593$). Pacientų, vartojančių alkoholį, proporcijos buvo panašios tarp lengvo, vidutinio ir sunkaus kraujo netekimo grupių.

Genų variantų įtaka kraujo netekimui

Genetinė analizė buvo atlikta 195 pacientams. Buvo ištirti trys vieno nukleotido polimorfizmai (VNP): ITGB3 (rs5918), koduojantis trombocitų fibrinogeno receptoriaus subvienetą β_3 ; FGB (rs1800790), susijusį su fibrinogeno β -grandinės genu; ir SERPINE1 (rs2227631), susijusį su plazminogeno aktyvatoriaus inhibitoriumi-1 (PAI-1). Visų tirtų polimorfizmų genotipų pasiskirstymas atitiko Hardy–Weinberg pusiausvyrą.

ITGB3 (rs5918) ir jo sąsaja su kraujo netekimu

Rs5918 genotipo dažniai buvo: TT 74,9 proc., CT 22,1 proc. ir CC 3,1 proc.. Atitinkami alelių dažniai buvo 85,9 proc. (T) ir 14,1 proc. (C). Šiam polimorfizmui statistiškai reikšminga sąsaja su Hb/kg indeksu nenustatyta nė viename paveldėjimo modelyje (27 lentelė). Genotipinis modelis nebuvo reikšmingas ($p = 0,808$), taip pat nereikšmingi buvo adityvinis (koreguotas $p = 0,524$), dominantinis (koreguotas $p = 0,485$) ir recesyvinis (koreguotas $p = 0,946$) modeliai. Šie rezultatai atitinka regresinius genetinius modelius, o pateikiami neparametriniai palyginimai taikant Kruskal–Wallis testą.

27 lentelė. *Hb/kg indeksas genotipų grupėse*

VNP	Genotipas	N	Hb/kg indeksas Mediana [IQR]	p
<i>ITGB3</i> (rs5918)	TT	146 (74,9 proc.)	1,89 [1,62–2,18]	0.721
	CT	43 (22,1 proc.)	2,05 [1,78–2,34]	
	CC	6 (3,1 proc.)	1,99 [1,70–2,25]	
<i>FGB</i> (rs1800790)	GG	89 (45,6 proc.)	1,93 [1,68–2,20]	0.667
	AG	82 (42,1 proc.)	1,94 [1,70–2,21]	
	AA	24 (12,3 proc.)	1,84 [1,60–2,10]	
<i>SERPINE1</i> (s2227631)	AA	73 (37,4 proc.)	1,89 [1,66–2,15]	
	AG	96 (49,2 proc.)	1,94 [1,70–2,22]	
	GG	26 (13,3 proc.)	2,02 [1,75–2,30]	

Visuose taikytuose modeliuose poveikio įverčiai buvo maži, su siaurais pasikliautiniais intervalais, apimančiais nulį, ir minimalia paaiškinta variacija ($R^2 \approx 0,002$). Geno- fenotipo analizėje rs5918 nebuvo susijęs su jokiais analizuotais klinikiniais ar laboratoriniais rodikliais po klaidingų atradimų dalies (FDR) korekcijos.

Taip pat nenustatyta statistiškai reikšmingų rs5918 genotipo pasiskirstymo skirtumų tarp Hb/kg indeksu apibrėžto kraujo netekimo sunkumo grupėse. Todėl rs5918 sąsajos su Hb/kg indeksu ir perioperacinio kraujo netekimu grupėse nenustatyta (28 lentelė). Papildomoje tiriamojoje analizėje pagal suminių krūtinės pooperacinės žaizdos drenažo tūrį (KŽD suma) taip pat nenustatyta reikšmingų skirtumų tarp rs5918 genotipo grupių ($p = 0,241$) (2 lentelė). Be to, *ITGB3* (rs5918) nebuvo nuosekliai susijęs su jokiais ROTEM parametrais.

28 lentelė. Bendras netekto kraujo tūris per krūtinės pooperacinės žaizdos drenus genotipų grupėse

VNP	Genotipas	N	KŽD tūris Mediana [IQR], ml	P
ITGB3 (rs5918)	TT	146 (74,9 proc.)	300 [250–400]	0,241
	CT	43 (22,1 proc.)	375 [250–500]	
	CC	6 (3,1 proc.)	550 [350–650]	
FGB (rs1800790)	GG	89 (45,6 proc.)	350 [250–450]	0,484
	AG	82 (42,1 proc.)	350 [200–450]	
	AA	24 (12,3 proc.)	325 [238–400]	
SERPINE1 (rs2227631)	AA	73 (37,4 proc.)	300 [250–400]	0,148
	AG	96 (49,2 proc.)	350 [200–450]	
	GG	26 (13,3 proc.)	400 [300–500]	

***FGB* (rs1800790) ir jo sąsaja su kraujo netekimu**

Genotipo dažniai buvo: GG 45,6 proc., AG 42,1 proc. ir AA 12,3 proc., o alelių dažniai – 66,7 proc. (G) ir 33,3 proc. (A). Statistiškai reikšminga sąsaja tarp rs1800790 ir Hb/kg indekso nenustatyta nė viename paveldėjimo modelyje: genotipiniame ($p = 0,566$), adityviame (koreguotas $p = 0,618$), dominantiniame (koreguotas $p = 0,838$) ar recesyviniame (koreguotas $p = 0,701$) modeliuose (27 lentelė). Poveikio dydžiai buvo maži, pasikliautiniai intervalai apėmė nulį, o paaiškinta variacija buvo žema ($R^2 \approx 0,001$). Taip pat nenustatyta reikšmingų genotipo pasiskirstymo skirtumų tarp lengvo, vidutinio ir sunkaus kraujo netekimo grupių ($p = 0,667$). Analogiškai, reikšmingų skirtumų pagal suminį krūtinės pooperacinės žaizdos drenažo tūrį (KŽD suma) tarp rs1800790 genotipo grupių nenustatyta ($p = 0,484$; 28 lentelė). Vis dėlto geno–fenotipo analizėje nustatytos reikšmingos sąsajos tarp rs1800790 ir fibrinogeno koncentracijos T0 etape (efektas = 0,283, $p = 6,04 \times 10^{-5}$, FDR = 0,0086), T1 etape (efektas = 0,261, $p = 0,000229$, FDR = 0,0163) ir T6 etape (efektas = 0,240, $p = 0,000738$, FDR = 0,035). Šie rezultatai išliko statistiškai reikšmingi ir po FDR korekcijos.

Adityviniame modelyje A alelis buvo susijęs su didesne fibrinogeno koncentracija visais vertinimo etapais.

- T0: $\beta = +0,365$ g/l (95 proc. CI 0,190–0,539, $p = 0,000055$);
- T1: $\beta = +0,249$ g/l (0,118–0,379, $p = 0,000226$);
- T6: $\beta = +0,274$ g/l (0,126–0,422, $p = 0,000334$);
- T18: $\beta = +0,205$ g/l (0,067–0,343, $p = 0,00370$),

Fibrinogeno koncentracijos mediana taip pat nuosekliai didėjo tarp genotipo grupių (GG, AG, AA) visuose vertinimo etapuose (29 lentelė ir 16 pav.). Be to, pakartotinių matavimų analizė parodė reikšmingą bendrą genetinę poveikį ($p = 0,000042$) bei reikšmingą geno ir laiko sąveiką ($p = 0,0086$).

Buvo nustatytos nominalios sąsajos tarp FGB (rs1800790) ir priešoperacinio FIBTEM A5 ($p = 0,025$), A10 ($p = 0,034$) ir MCF ($p = 0,063$). Tačiau po operacijos reikšmingų sąsajų nenustatyta.

***SERPINE1* (rs2227631) ir jo sąsaja su kraujo netekimu**

rs2227631 genotipo dažniai buvo: AA 37,4 proc., AG 49,2 proc. ir GG 13,3 proc., o alelių dažniai – 62,1 proc. (A) ir 37,9 proc. (G). Statistiškai reikšminga sąsaja tarp rs2227631 ir Hb/kg indekso nenustatyta nė viename modelyje: genotipiniame ($p = 0,724$), adityviame (koreguotas $p = 0,789$), dominantiniame (koreguotas $p = 0,768$) ar recesyviniame (koreguotas $p = 0,906$) modeliuose. Poveikio įverčiai buvo maži, pasikliautiniai intervalai apėmė nulį, o paaiškinta variacija buvo nereikšminga ($R^2 \approx 0,003$).

Geno–fenotipo analizėje reikšmingų sąsajų po FDR korekcijos nenustatyta. Taip pat genotipo pasiskirstymas tarp Hb/kg indeksu pagrįstų tiriamųjų grupių reikšmingai nesiskyrė ($p = 0,769$) (27 lentelė), o suminis krūtinės pooperacinės žaizdos drenažo tūris (KŽD suma) tarp genotipo grupių taip pat nesiskyrė ($p = 0,148$) (28 lentelė).

IŠVADOS

1. Hb/kg indeksas - tai objektyvus fiziologinis rodiklis, įgalinantis širdies chirurgijoje kiekybiškai įvertinti hemoglobino masės netekimą paciento kūno svorio kilogramui, ir pasižymintis dideliu prognostiniu tikslumu taikant LASSO-OLS modeliavimą ($R^2 = 0,82$).
2. Neprižiūrimas K-medoidų klasterizavimas nustatė tris kraujo netekimo sunkumo grupes (lengvo, vidutinio ir sunkaus laipsnio), remiantis duomenimis pagrįstomis Hb/kg ribinėmis vertėmis – 1,72 ir 2,10. Šis metodas atspindi kliniškai reikšmingus kraujo netekimo laipsnių skirtumus, o didžiausias diskriminacinis tikslumas nustatytas sunkaus kraujo netekimo klasteriui ($AUC = 0,790$).
3. Nustatant kraujo netekimo sunkumo laipsnį viskoelastiniai tyrimai parodė aukštesnę diskriminacinę vertę nei standartiniai kraujo krešėjimo tyrimai bei trombocitų agregometrija. FIBTEM A10 (ribinė vertė ≤ 12 mm) nustatė pooperacinę hipofibrinogenemiją ($< 2,5$ g/l), pasižymėdamas puikiu diagnostiniu tikslumu ($AUC = 0,888$; $NPV = 97,4$ proc.).

4. Plazmos PAI-1 pooperacinės koncentracijos reikšmingai padidėjo, atspindėdamos sisteminį fibrinolizinį atsaką į dirbtinę kraujo apytaką ir chirurginę traumą, tačiau reikšmingos sąsajos su Hb/kg indeksu ar kraujo netekimo grupėmis nebuvo nustatyta.
5. *ITGB3* (rs5918) ir *SERPINE1* (rs2227631) VNP neparodė reikšmingų sąsajų su Hb/kg indeksu ar nustatytomis kraujo netekimo grupėmis. Tuo tarpu *FGB* (rs1800790) VNP buvo reikšmingai susijęs su fibrinogeno koncentracija visais perioperaciniais tyrimo etapais ($p < 0,001$), kas rodo, kad jis vaidina svarbaus veiksnio vaidmenį perioperacinės hemostazės fenotipe.

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The list of publications (last 5 years)

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