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Impact of fibrinogen-to-erythrocyte suspension ratio on mortality and functional outcomes in major perioperative bleeding (Approximate Dose-Equivalent of Fibrinogen-to-Erythrocyte Suspension (ADEFES) study): protocol for a prospective observational study

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ABSTRACT

Introduction Early and balanced replacement of blood products appears to be the key factor in improving outcomes of major bleeding patients including acute trauma, cardiac, obstetric and transplant surgery patients. Definitive clinical guidance regarding the optimal ratio of blood products, including those containing fibrinogen, is still lacking. Therefore, we tested the hypothesis that increasing the fibrinogen content to erythrocyte suspension ratio improves the mortality and functional outcomes of patients undergoing surgeries with expected major bleeding.

Methods and analysis The Approximate Dose-Equivalent of Fibrinogen-to-Erythrocyte Suspension (ADEFES) ratio is a multicentre, prospective, observational, cohort study of patients undergoing major surgical procedures with expected major perioperative bleeding (ie, requiring packed red blood cells (PRBC)>4U/24 hours). For 5U of cryoprecipitate and 1.5 U of fresh frozen plasma (FFP), the approximate dose-equivalent for fibrinogen is considered as 1 gram of fibrinogen. Association of the ADEFES ratio at 24 hours will be assessed on the primary objective, which will consist of the composite of 30-day all-cause mortality, 30-day bleeding-specific mortality and the 'highly-dependent scores' of Katz index of independence in activities of daily living.

Ethics and dissemination The study protocol was approved by the Ethics Committee of Ankara Bilkent City Hospital (approval no. E2-23-4265, dated 07 June 2023; Chair: Prof. Dr. F.E. Canpolat) and by the institutional review boards of all participating centres. The study will be conducted in accordance with the principles of the Declaration of Helsinki and the Strengthening the Reporting of Observational Studies in Epidemiology guidelines, as well as in compliance with national regulations on data protection and Good Clinical Practice standards. Written informed consent will be obtained from all participants prior to inclusion in the study.

The results of this study will be disseminated through peer-reviewed scientific journals, presentations at national and international conferences, and communication with relevant stakeholders including clinical practitioners and healthcare institutions. If applicable, study outcomes will also be shared via institutional newsletters and digital platforms to reach a broader audience in the medical community.

Trial registration number NCT06021184.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is a prospective, observational, multicentre cohort study conducted across multiple regions and institutions.
⇒ Fibrinogen dosage from various sources (cryoprecipitate, fresh frozen plasma and fibrinogen concentrate) is standardised using an approximate dose-equivalent formula.
⇒ A web-based electronic case report form ensures consistent and structured data collection.
⇒ Inclusion criteria are clearly defined, focusing on surgeries with expected major bleeding and transfusion need.
⇒ As an observational study, the lack of randomisation and intervention limits causal inference.

INTRODUCTION

Massive transfusion, defined as the transfusion of total or near-total body blood volume (10+ or 8+units of erythrocyte suspension (ES)) within 24 hours, remains one of the greatest challenges for the perioperative care team. In spite of balanced timely replacement of blood products, massive haemorrhage continues to be an important reason for morbidity and mortality in the perioperative period. While the replacement is easier when the teams are well prepared with type and cross match of appropriate products, a chaotic response may occur when the bleeding is unexpected and one is not prepared. Therefore, each discipline and division has protocols to handle this critical process, and studies continue to evolve and improve patient outcomes.[1–3]

Massive bleeding management mainly focuses on early resuscitation with blood products under minimal haemodilution with fluids. Regardless of the reasons for massive transfusion, the main goals behind the management of massive bleeding are to maintain oxygen delivery and organ perfusion and to prevent coagulopathy. While these goals can be achieved during bleeding by meticulous surgical management, haemodynamic control and the timely delivery of blood products, delays in management and the triad of hypothermia, acidosis and coagulopathy may contribute to increased morbidity and mortality in the perioperative period.[4 5]

There are a few well-established management approaches which contribute to improved clinical outcomes such as damage-control resuscitation, timely initiation of transfusion, early use of reversal agents and minimising early use of crystalloids.[6 7] The landmark PROPPR study reported that in the blood product replacement of massively bleeding trauma patients, a 1:1:1 transfusion ratio of fresh frozen plasma (FFP):platelets (PLTs):ES resulted in haemostasis in patients compared with a 1:1:2 ratio. The bleeding-related mortality was lower in the 1:1:1 group of patients within the first 24 hours; however, no significant difference was found after 24-hour or 30-day overall mortality.[8] In a cohort study performed in patients with severe blunt trauma, patients were divided into high FFP group (ratio >1) and low FFP group (ratio <1), and they found that a high FFP/red blood cell (RBC) ratio was associated with favourable survival. The authors emphasised the importance of revising current transfusion protocols to include a high FFP/RBC ratio and recommended further research on optimal patient management.[8]

In a systematic review of retrospective data on obstetric bleeding management, investigators showed that the amount of FFP administered was greater than the amount of ES transfused, and they concluded that a FFP/ES ratio of ≥1 could be recommended in the blood product transfusions during obstetric haemorrhage.[9] Similarly, higher rates of FFP/ES transfusion were associated with better outcomes in massive bleeding cardiac surgery patients.[10 11] It was found that in a study with complex cardiac surgery patients, there was less organ dysfunction in those who received high ratio transfusions of FFP:ES and PLT:ES and lower mortality in high ratio FFP:ES transfusions.[10] In the light of these publications, the following question inevitably comes to one's mind: *which component/s of FFP contribute to the improved outcomes?*

During major bleeding, significant amounts of coagulation factors are lost along with plasma volume, in addition to the depletion of RBCs. FFP contains all coagulation factors including fibrinogen. Fibrinogen plays a vital role in the coagulation process by being converted into fibrin through the action of the enzyme thrombin. The resulting fibrin strands weave into a mesh that stabilises the PLT plug, forming a durable clot at the site of vascular injury. Fibrinogen also binds to glycoprotein IIb/IIIa receptors on activated PLTs, further strengthening the clot. Fibrinogen is one of the key factors, which its levels quickly drop to critical values during bleeding. It is a unique coagulation building block that plays a role in both primary and secondary haemostasis.[12] Based on the results of the RETIC trial, the effectiveness of fibrinogen supplementation in limiting blood loss appears to be strongly dependent on timing and plasma levels of fibrinogen >200 $mg \cdot dL^{-1}$.[13] The 5th Edition of the European Guidelines for the Management of Major Post-traumatic Hemorrhage and Coagulopathy recommends early and repeated monitoring of fibrinogen concentrations and/or polymerisation and rapid correction of deficiencies.[14] While RETIC stands out as a prospective randomised controlled study on the role of fibrinogen during massive transfusion in trauma patients, there are few studies in cardiac and transplantation surgery conducted under different designs that also support the early replacement of fibrinogen levels.[13–18] Regardless of the cause, early restoration of fibrinogen levels during massive bleeding reduces transfusion requirements.[1 19 20] In the FIBRES study, which evaluated the effectiveness of fibrinogen concentrate in cardiac surgery patients with bleeding, it was emphasised that fibrinogen concentrate was not inferior to cryoprecipitate, was easy to apply and provided a predictable effect.[21] Similarly, it was hypothesised that early replacement of fibrinogen in severely injured trauma patients may improve outcomes.[22] However, there is limited evidence to support or debunk this hypothesis both for cryoprecipitate and fibrinogen concentrate.[23–25]

Early and balanced replacement of blood products appears to be the key factor in improving outcomes of major bleeding patients including acute trauma, cardiac, obstetric and transplant surgery patients. Despite numerous well-designed clinical trials, we still lack precise clinical guidelines on the optimal ratio of blood products, including fibrinogen-containing ones, during major perioperative bleeding, which could provide valuable direction for a wide range of major surgeries. Therefore, in this large prospective observational study, we hypothesised that during major bleeding, increased ratio of fibrinogen content to erythrocyte suspension (Approximate Dose-Equivalent of Fibrinogen-to-Erythrocyte Suspension (ADEFES) ratio) improves mortality and functionality

outcomes of patients undergoing cardiac and non-cardiac surgeries.

METHODS AND ANALYSIS

Patients and public involvement

The patients, their parents and the public had no role in designing the ADEFES protocol or in writing the present manuscript. They had no role in the data collection period, before or after the study. They will not be involved in the statistical analysis, evaluation, interpretation or dissemination of the results. The study results will be published in a peer-reviewed journal and reported at one or more scientific conferences and social media platforms.

Participants

We will include the patients who will be going for surgeries with expected *major bleeding*, defined as expected bleeding, which may require transfusion of ≥4U of ES and at least one fibrinogen-containing product. Transfusion of 4–7 U ES will be considered as submassive, and ≥8 U ES will be considered as massive transfusion in the study.

At each study centre, a site PI will screen patients' admission records to identify potentially qualified participants, who are expected to have major bleeding requiring transfusion of blood products. Patients who meet the eligibility criteria (see below) and provide their written informed consent will be included in this study.

Inclusion and exclusion criteria

Patients are eligible for this study if they are 18 years and older, who will be undergoing surgery and expected to have major bleeding requiring submassive/massive blood transfusion. The study will include surgical patients who are expected to receive a transfusion of ≥4 U packed red cells (ES) and at least one fibrinogen-containing product (ie, FFP, fibrinogen concentrate or cryoprecipitate) within the 24 hours of surgery.

Minor surgeries, which expectedly will not require transfusion of blood products, will be excluded. Incidents of death before or during massive transfusion will cause the data to be withdrawn from the study, as well as the patients whose perioperative bleeding and transfusion data cannot be collected (eg, no study personnel available).

Study design

The reporting of this study protocol followed Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (figure 1). In order to standardise the fibrinogen content of different products, we can approximate their fibrinogen content equivalents. Approximate dose-equivalent (ADE) for fibrinogen (F); 1 g fibrinogen=5 U cryoprecipitate=1.5 U FFP will be evaluated as total grams of fibrinogen. This estimation is supported by data to harmonise dose comparisons across different sources of fibrinogen.[26–28] This estimation is based on typical fibrinogen content: one unit of FFP contains approximately 2–3 g of fibrinogen per litre. Assuming an average unit volume of ~300 mL, this corresponds to roughly 600–900 mg per unit. Therefore, 1.5 units of FFP approximate 1 g of fibrinogen, although content may vary significantly. One unit of cryoprecipitate contains approximately 200 mg of fibrinogen, so five units yield ~1 g. This conversion was applied to enable consistent quantification of fibrinogen administration across different product types, while acknowledging inherent variability in fibrinogen content. In accordance with this formula, we decided to investigate the effect of the ratio of 'Approximate Dose-Equivalent (ADE) of fibrinogen (F) to erythrocyte suspension (ES)' on composite objective (figure 2).

ADEF/ ES ratio = (approximate dose-equivalent of total grams of fibrinogen) / (erythrocyte suspension unit)

The ADEFES study is a national multicentre, prospective, observational, cohort study of patients undergoing major surgical procedures. The study will focus on major bleeding requiring submassive and massive transfusion practices perioperatively. The study is being conducted in Turkey across more than 25 tertiary healthcare centres, including university hospitals and high-volume state training and research hospitals. The coordinating centre is the Department of Anesthesiology and Reanimation at Ankara Bilkent City Hospital, affiliated with the University of Health Sciences, Gulhane Faculty of Medicine. The planned start date for patient enrolment is 1 July 2024, and data collection is expected to be completed by 31 December 2025.

Study protocol and data collection

Demographic, morphometric data, comorbidities, type of surgery and study site details will be collected. A web-based multicentre electronic case report form (e-CRF) application will be used for data collection, which also will store the data in a dedicated database. The e-CRF application was specifically developed and integrated into the IREA Network Platform. Each patient will be given a unique number code, which will be in a study site specific numeric order. Patient identifiable information will not be recorded, nor will it be available for data analysis. Investigators will be given secure login credentials (username and password) to enter the study data. Each study team member will be instructed on the study protocol and how to use the data entry system. A frequently asked questions tool and 'how to' video explaining the key parts of the study were prepared for participating study centres. Each study centre's investigator team will be coached by the site's primary investigator (PI) throughout the study in preplanned intervals. Main study PI will routinely organise video meetings to assess enrolment details and answer study centres' questions. All included patients will be treated according to the standard of care at each participating site. Patients' outcomes will be followed for the first 3 months after their first ES transfusion.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1-6	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1-6	
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	7, 8	
Objectives	3	State specific objectives, including any prespecified hypotheses	9	
Methods				
Study design	4	Present key elements of study design early in the paper	10	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	10, 11	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	9, 10	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case		
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9-12	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	11	
Bias	9	Describe any efforts to address potential sources of bias	None	
Study size	10	Explain how the study size was arrived at	13	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9-11	
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	12, 13	
		(*b*) Describe any methods used to examine subgroups and interactions	12, 13	
		(*c*) Explain how missing data were addressed		
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	12, 13 None None	
		(*e*) Describe any sensitivity analyses	12-13	
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	None	
		(b) Give reasons for non-participation at each stage	None	
		(c) Consider use of a flow diagram	None	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	None	
		(b) Indicate number of participants with missing data for each variable of interest	None	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	None	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	None	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	None	
		Cross-sectional study—Report numbers of outcome events or summary measures	None	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	None	
		(*b*) Report category boundaries when continuous variables were categorized	None	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	None	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	None	
Discussion				
Key results	18	Summarise key results with reference to study objectives	14-16	
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	16	
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-16	
Generalisability	21	Discuss the generalisability (external validity) of the study results	None	
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3	

Figure 1 STROBE statement—checklist of items that should be included in reports of observational studies. *Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies. Note: An explanation and elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org. STROBE, Strengthening the Reporting of Observational Studies in Epidemiology.

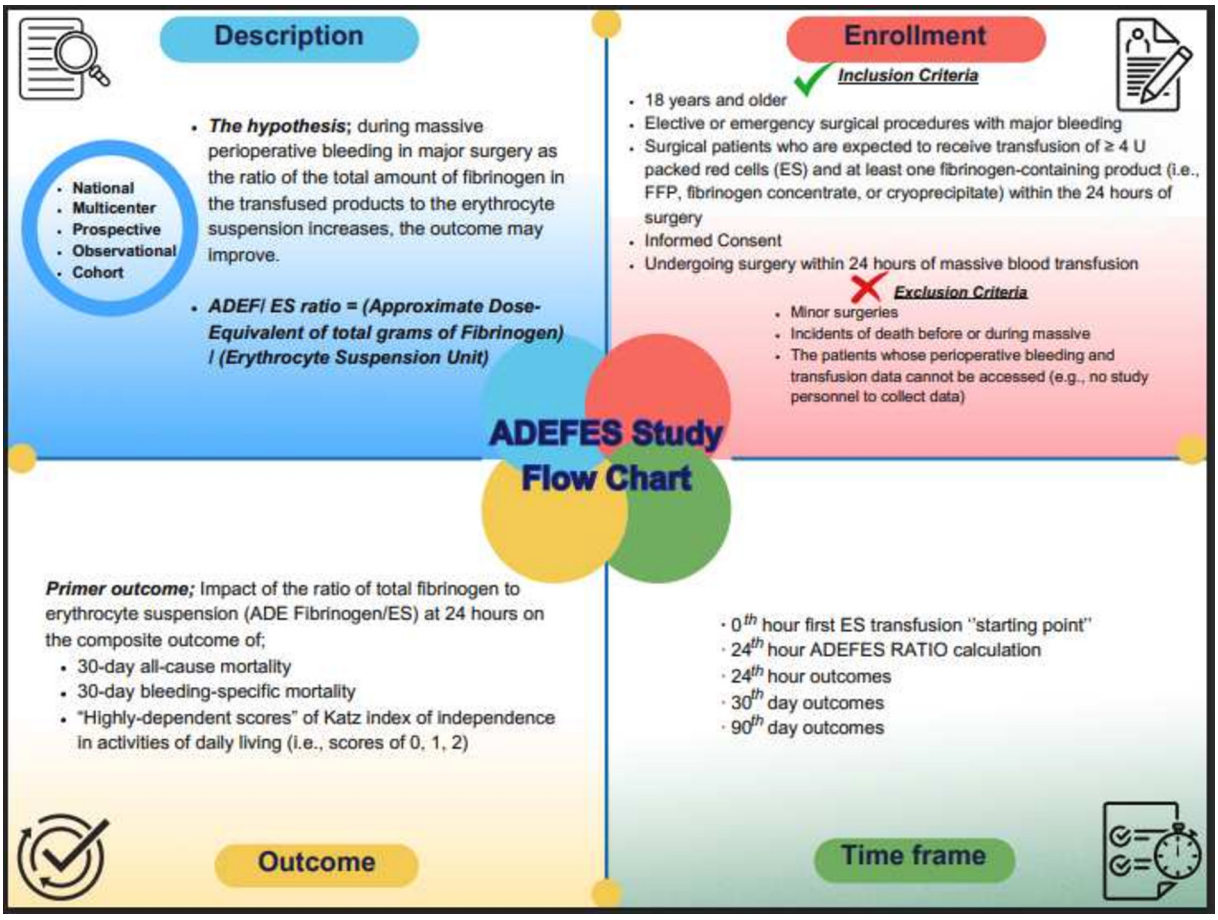

Figure 2 Quick look flowchart of ADEFES Study. FFP, fresh frozen plasma.

Bleeding management and blood product transfusions will be done according to the national/international guidelines. All site investigators will be instructed to follow.

Study objectives

Primary objective

Impact of the ratio of total fibrinogen to ES (ADE fibrinogen/ES) at 24 hours on the composite objective of 30-day all-cause mortality, 30-day bleeding-specific mortality, and the 'highly-dependent scores' of Katz index of independence in activities of daily living is the primary objective of this study. The 6-item Katz Basic Activity of Daily Living score measured an individual's ability to bathe, dress, eat, go to the toilet, transfer from bed to a chair and walk across a room. Each item is scored as 0 (completely dependent) and 1 (completely independent), with a score of 0 being a lower score and 6 with a higher score, suggesting greater independence. The primary composite objective is dichotomous and will be coded as 'poor outcome' (including 30-day all-cause mortality, bleeding-specific mortality or Katz score of 0–2) vs 'favourable outcome' (Katz score ≥3 and survival). Moreover, we will report each component separately in secondary analyses to provide full transparency and allow readers to assess the individual contributions to the composite endpoint.

Secondary objectives

The secondary objectives will explore the associations between the ADEF/ES ratio (independent variable) and the following dependent clinical variables:

1. Individual components of the composite outcome (eg, 30-day mortality alone).
2. *Postoperative all-cause and bleeding-specific mortality* at 24 hours, 30 days and 3 months.
3. *Composite morbidity beyond 90 days*, defined as persistent complications using International Classification of Diseases-9 codes (binary: presence or absence).
4. *Incidence of complications* at 24 hours, 30 days and 3 months.
5. *Length of stay* in the intensive care unit (ICU) (> 3 days) and total hospital stay (> 10 days).
6. *Functional status at discharge*, categorised as: able to perform daily activities, able to perform daily activities with assistance, completely dependent.
7. *Katz index of independence in activities of daily living* at 24 hours, 30 days and 3 months, analysed both in general and stratified by surgical type. Scores of 0–2 will be classified as poor and ≥3 as favourable outcomes (binary: 0–2 vs. ≥3).
8. *Surgery-specific mortality* (all-cause and bleeding-specific) at 24 hours, 30 days and 3 months.

9. *Relationship between tranexamic acid use* (dose and timing) and mortality.
10. *Baseline (admission) plasma fibrinogen levels* and their association with bleeding volume.
11. *Association between baseline plasma fibrinogen levels and ADEF/ES ratio*, exploring whether lower levels lead to increased fibrinogen administration.

In specific analyses where prediction of the ADEF/ES ratio itself is of interest, initial fibrinogen level, total bleeding volume and transfusion practices will be treated as *independent variables* and ADEF/ES as the *dependent variable*.

As part of the secondary analyses, the association between early ADEF/ES ratios (calculated at 6 and 12 hours postoperatively) and clinical objectives will also be explored.

Functional outcomes such as the Katz index are included in the primary and secondary analyses to capture the broader clinical impact of transfusion strategy. Although not exclusively determined by the ADEF/ES ratio, functional status may be influenced by the adequacy and timing of haemostatic support, which can affect recovery trajectories and independence at discharge. Confounders such as baseline comorbidity and American Society of Anesthesiologists (ASA) classification will be accounted for in adjusted models.

Statistical analysis

Categorical variables will be defined as frequencies and percentages and analysed with the $\chi 2$ test or Fisher's exact test. Continuous variables will be presented as mean±SD or median and IQR, and to compare the differences between the two groups, the independent samples t-test will be used when the variance homogeneity assumption is met, while the Welch t-test will be used when the variance homogeneity assumption is not met. T-tests are planned for exploratory group comparisons involving continuous variables (eg, comparing mean ADEF/ES ratios or mean initial fibrinogen levels between outcome groups). If the assumptions of homogeneity of variance are not met, Welch's t-test will be applied instead. All categorical comparisons (eg, presence or absence of complications) will use the $\chi 2$ test or Fisher's exact test, as appropriate. The primary objective is a composite of mortality within the first 30 days and low Katz scale scores. This primary objective is dichotomous, and patients will be classified as 'poor outcome' (death or Katz 0–2) versus 'favourable outcome' (survival and Katz ≥3). Logistic regression will be used to assess the relationship between the ADEF/ES ratio and the composite outcome. Prior to multiple logistic regression, the abovementioned univariate analyses will be conducted, and variables with a p value <0.1 in univariate testing will be included in the multiple logistic regression model.

Since the surgical procedure is a strong predictor of complications and mortality, it is critical to stratify and adjust for its type and acuity when examining the relationship between major bleeding and the outcome. We will also adjust the analysis of the primary objective for the following factors: age, ASA score, baseline haemoglobin levels, bleeding-related cofactors and comorbidities (eg, baseline factors and diseases impacting bleeding and coagulation such as aspirin intake or von Willebrand Disease) and tranexamic acid dose given within the 3 hours of initial or surgical trauma.

The univariate logistic regression model will be used to examine the relationship between each response variable and baseline and intraoperative risk factors. The primary dependent variable is the binary composite outcome (death or Katz score 0–2 = 'poor outcome'; survival and Katz≥3 = 'favourable outcome'). Secondary dependent variables include 30-day mortality, 90-day morbidity, ICU length of stay >3 days and functional status at 3 months. Potential risk factors identified in univariate analysis will be used in a multiple logistic regression model to assess the relationships between each response variable and risk factors. A backward variable selection procedure will be used, with an inclusion criterion of p=0.10. While the ADEF/ES ratio is treated as an independent predictor variable in analyses of clinical outcomes, in separate exploratory models where the determinants of the ADEF/ES ratio itself are of interest, it will serve as the dependent variable, with variables such as initial fibrinogen level, total bleeding volume and transfusion practices entered as predictors. Additionally, potential confounding due to institutional and surgical team variability will be addressed by including centre-level and procedure-related variables in multiple logistic regression models. If clustering by centre is evident, mixed-effects logistic regression models with hospital as a random effect will be considered. Stratified analyses by surgical specialty and institution type will also be performed.

As a secondary analysis, a receiver operating characteristic (ROC) curve will be constructed to evaluate the predictive performance of the ADEF/ES ratio in identifying patients with poor outcomes (ie, the composite binary outcome of mortality or low Katz score). First, ROC analysis will be conducted with 80% of the dataset (training set), and the optimal cut-off point will be determined using the Youden index [Youden's index (J)=max (sensitivity+specificity - 1)]. Then, the remaining 20% of the dataset (test set) will be used to evaluate the performance of the cut-off point determined in the ROC analysis. AUC, accuracy, sensitivity, specificity, positive predictive value, negative predictive value and F1 measure will be used as performance measures.

Given the multicentre and multidisciplinary nature of the study, we anticipate potential variability due to differences in patient characteristics, surgical techniques and institutional practices. To account for these sources of heterogeneity, regression models will be adjusted for relevant covariates including age, comorbidities, ASA score, surgery type and intraoperative parameters. If centre-level variability proves substantial, mixed-effects models including hospital as a random effect will be considered. Stratified analyses by surgical specialty will

also be performed. In exploratory analyses, propensity score adjustment or matching may be employed to further assess the robustness and consistency of observed associations.

In addition to the cumulative 24-hour ADEF/ES ratio, subgroup analyses will also evaluate ratios at 6 and 12 hours postoperatively to assess the impact of early haemostatic support on clinical outcomes.

Two-sided values of $p<0.05$ will be considered statistically significant.

Sample size estimation

The primary objective is a binary composite variable, where patients who die within 30 days or have a Katz score of 0, 1 or 2 are classified as having 'poor outcomes', and those who survive and score 3 or higher on the Katz index are considered to have 'favourable outcomes'. This composite outcome was treated as the dependent variable (Y) and the ADEF/ES ratio as the independent variable (X) for the power analysis.

Although the ADEF/ES ratio is a continuous variable in the actual statistical model, it was dichotomised solely for the purpose of sample size estimation using a hypothetical threshold to represent 'high' versus 'low' ADEF/ES values. The primary objective is a binary composite variable, defined as 'poor outcome' (30-day all-cause mortality, bleeding-specific mortality or Katz score 0–2) versus 'favourable outcome' (survival with Katz score ≥3). Under this binary logistic regression model, a sample size of 1679 patients provides 90% power at a 0.05 significance level to detect a reduction in the probability of poor outcomes from 0.250 to 0.125, corresponding to an OR of 0.50. This binary categorisation of the ADEF/ES ratio was applied solely for the purpose of sample size calculation, assuming a clinically meaningful threshold. In the actual analysis, the ADEF/ES ratio will be treated as a continuous independent variable in logistic regression models.

To account for covariates in the full multiple logistic regression model, we assumed an R^2 value of 0.20 between the independent variable of interest and other predictors. With higher assumed R^2 values of 0.30 and 0.40, the estimated power was 86% and 81%, respectively, still with a minimum required sample of 1679 patients. In the actual data analysis, the ADEF/ES ratio will be treated as a continuous variable within a multiple logistic regression model. While the assumed effect size (OR=0.5) was not drawn from a specific published study, it was informed by preliminary observations in our pilot cohort, where patients with higher ADEF/ES ratios had lower rates of poor outcomes. We acknowledge that any deviation of the actual model from the assumptions used in the sample size calculation may influence the final statistical power. This will be taken into consideration during analysis and interpretation of results.

Study oversight

The data monitoring board (DMB) will evaluate the progress of the study, monitor data entry quality and recruitment goals throughout the study, and at the end of the completion of study enrolment, they will ensure accurate and complete reporting. DMB will assess the enrolment details of study sites, assess for any potential patient safety concerns and, accordingly, will provide recommendations regarding adjustments to the protocol if necessary. They will ensure the adherence to ethical standards and regulatory requirements.

Recognising the inherent limitations of drawing causal inferences from observational data, we considered incorporating selected elements of *target trial emulation*, as outlined by Hernán *et al* and Hubbard *et al*, to enhance the validity of our findings.[29 30] While this approach does not replace a randomised controlled trial, it provides a structured framework for improving comparability and reducing confounding.

DISCUSSION

Despite advances in surgery and transfusion practices, bleeding continues leading to poor outcomes and death. Throughout history, major natural disasters and wars have been the cornerstone of the development of knowledge about massive bleeding and transfusion practices. The lessons learnt from the painful events in history can be divided into undoubted main headings: measures to prevent and/or rapidly control massive bleeding and provision of early haemostatic resuscitation. The WHO underlines that blood transfusion saves lives and improves health, but many patients requiring transfusion do not have timely access to safe and sufficient blood supply.[31] It is now unquestionable that replacing blood loss early and in the most physiological ratio saves lives. However, the research on this timely replacement ratio has at the least been challenging. The relation between the number of erythrocyte units transfused and clinical outcomes was assessed in detail by Johnson *et al*'s study.[32] The incidence of composite morbidity increased in a curvilinear manner over the entire range of erythrocyte units administered. Infections and thrombotic events occurred four to five times more commonly than renal, respiratory or ischaemic events at the higher transfusion rates. Mortality also increased in a dose-related linear manner.[32 33] Restricting blood transfusion could be beneficial to some extent but may not be the right approach for every patient. Therefore, the most logical way for massive bleeding is to control it early and replace products in a balanced ratio, but accomplishing these goals has been challenging. Replacing blood loss with a balanced approach (eg, 1:1:1 or 1:1:2) essentially helps to preserve fibrinogen levels by allowing early application of fibrinogen containing blood products.

In this respect, it is thought that sufficient replacement of fibrinogen, which its levels are known to decrease rapidly during bleeding, may provide significant improvement in massive bleeding and transfusion process.[13–20] Generally, fibrinogen concentrate and/or cryoprecipitate are used for fibrinogen replacement, and FFP is used

for plasma replacement, because its fibrinogen content is relatively smaller. The use of fibrinogen replacement products varies, while cryoprecipitate is used prominently in the USA, fibrinogen concentrate is mostly preferred in the European countries. There are also situations where both are used. Sometimes in cases of emergency bleeding, fibrinogen concentrate is used first while waiting for the cryoprecipitate preparation.

In notable massive transfusion studies, two main problems were reported as limitations: (1) relatively small number of patients with massive bleeding and (2) the duration of time required for cryoprecipitate preparation. A total of 43 patients from 202 trauma centres in the UK participated in the CRYOSTAT-1 RCT, in which the mean time to first cryoprecipitate was 60 min versus 108 min, roughly a 40-min difference between the groups.[24] Similarly, in the CRYOSTAT-2 study, only about 25% of the 1531 study patients appeared to have received massive transfusions. The timing of the first cryoprecipitate dose delivery was about 68 min versus 120 min in the study groups. The authors reported the cryoprecipitate preparation time as the most common reason for the delay in its delivery.[34] As experienced in many studies, specific product delivery within the prescribed time frame is a difficult endeavour.

The positive effects of fibrinogen replacement on outcome have been shown in various surgeries, and such practices have helped to develop massive transfusion algorithms.[14] However, there has been an ongoing debate about which fibrinogen-containing product would be more effective. Cryoprecipitate and fibrinogen concentrate are constantly compared in terms of availability, safety/effectiveness and cost.[33 35 36] Some studies showed that miraculous results cannot be achieved with early administration of products containing fibrinogen, and blood loss and the need for transfusion do not decrease.[23–26 29–34] In the CRYOSTAT 2 study, massive bleeding was 23% in the cryoprecipitate group and 21% in the standard care group, injury severity score <40 patients were very few and the final cryoprecipitate administration time was about 120 min.[34] Researchers stated that the potential main limitation of their study was that cryoprecipitate administration was significantly delayed in both groups due to the time needed for the product's preparation. Possibly, FFP transfusion within the scope of the massive transfusion protocol (MTP) may have partially decreased the time delay and positively affected the study. FFP is often underestimated as a source of fibrinogen. However, in MTPs, large amounts of FFP are replaced in the early period. It is known that FFP is not an ideal source of fibrinogen, but early FFP may still delay the decrease in plasma fibrinogen levels.

In the ADEFES study, all fibrinogen-containing products will be evaluated on an equivalence basis in patients with major bleeding. In order to put forward an initiative that is applicable in all countries, the relationship between the ratio of ADEF equality (products containing different levels of fibrinogen will be formulated according to their average content and reported as a single item) to the amount of ES at the 24 hours after the first ES transfusion and the outcome will be examined. In addition, it will be obvious what percentage of the fibrinogen content is obtained from which of the replacement products. In the ADEFES study, emergency trauma, orthopaedics, obstetrics, cardiac and all major bleeding surgeries will be examined. Besides, other factors contributing to bleeding such as hypothermia treatment, surgery details, tranexamic acid application and amount of fluid replacement will be placed under the magnifying lens. Besides all this, we expect that monitoring the details of the patients' bleeding and transfusion processes, as well as their laboratory values, will more clearly reveal the seriousness of the patients' clinical conditions, which will help to assess the nuances of care after the fact.

The ADEFES study will focus on both mortality and quality of life as a composite primary objective. The most important rate-limiting step in effectiveness research is undoubtedly the safety concern. ADEFES will monitor arterial and venous thromboembolic complications as well as complications related to organ injuries for up to 3 months.

The study will be conducted as a national prospective, observational, multicentre, cohort study using a formula that plans to standardise fibrinogen content from different products under one single definition. The study will have several potential strengths: a very large cohort of patients, a national multicentre setting with different types of hospitals from multiple cities and regions of the country.

However, being an observational project—without any interventions—is an inevitable limitation coming by design. Given the large number of study sites, the success of the study will be highly dependent on the inclusion of surgical patients with potential major bleeding (to represent a true high enrolment of major bleeding) and compliance with the guidelines[37] on perioperative transfusion practices. Although the identity of the operating surgeon was not recorded—a potential source of unmeasured variability—detailed data on surgical specialties and operating institutions were collected and may sufficiently account for practice-related differences.

In conclusion, we anticipate that this study will make a significant global contribution to perioperative management strategies for massive transfusion. By planning to thoroughly analysing data from adult patients undergoing surgery with expected major bleeding, this research aims to enhance our clinical understanding of bleeding management and transfusion practices.

ETHICS AND DISSEMINATION

The study protocol was approved by the Ethics Committee of Ankara Bilkent City Hospital (approval no. E2-23-4265, 07 June 2023; Chair: Prof. Dr F.E. Canpolat). The committee granted permission for the study to be

conducted across all participating centres under a single centralised application.

This multicentre study is planned to involve the following institutions as participating centres under the study protocol: Ankara Bilkent City Hospital (07.06.2023, E2-23-4265), Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital (14.11.2023, E-21), Koşuyolu High Specialization Training and Research Hospital (10.11.2023), Istanbul Faculty of Medicine (Istanbul University) (04.03.2024, 46143867–19), Acibadem Mehmet Ali Aydinlar University School of Medicine (17.05.2023, 873), Sakarya University Faculty of Medicine and Sakarya Education and Research Hospital (31.07.2025, E-83529411-000-283709150), Kartal Dr. Lütfi Kırdar City Hospital (approval in progress), Fatih Sultan Mehmet Training and Research Hospital (29.09.2023, E-17073117–050.06.99-225543194), Kanuni Sultan Suleyman Training and Research Hospital (10.10.2023, 2023.11.162), Gazi University Faculty of Medicine (20.10.2023), Başakşehir Çam and Sakura City Hospital (approval in progress), Ataturk University Faculty of Medicine (approval in progress), Ahi Evren Thoracic and Cardiovascular Surgery Training and Research Hospital (approval in progress), Van Yuzuncu Yil University Faculty of Medicine (26.10.2023, 056), University of Health Sciences Bursa Yüksek İhtisas Training and Research Hospital (18.10.2023), Cukurova University Faculty of Medicine (12.12.2023), Suleyman Demirel University Faculty of Medicine (approval in progress) and Ondokuz Mayis University Faculty of Medicine (23.10.2023, E-15374210–811.99-2300113433). The final list may be updated as data collection progresses.

The study is registered at ClinicalTrials.gov (NCT06021184) as of 28 August 2023, last update submitted on 9 December 2024. The study is conducted in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines and the Strengthening the Reporting of Observational Studies in Epidemiology statement. Informed written consent was obtained from all participants or their legal representatives prior to inclusion.

Study results will be disseminated through peer-reviewed journals, scientific congress presentations and digital platforms, including the IREA Network website and its affiliated social media channels.

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REFERENCES

1 Cornero SG, Maegele M, Lefering R, *et al*. Predictive factors for massive transfusion in Trauma: a novel clinical score from an Italian Trauma Center and German Trauma Registry. *J Clin Med* 2020;9.
2 National Blood Authority (Australia). National health and medical research council. In: *Patient Blood Management Guidelines. Module 5*. Obstetrics and maternity,
3 Patil V, Shetmahajan M. Massive transfusion and massive transfusion protocol. *Indian J Anaesth* 2014;58:590–5.
4 Lier H, Krep H, Schroeder S, *et al*. Preconditions of hemostasis in trauma: a review. The influence of acidosis, hypocalcemia, anemia, and hypothermia on functional hemostasis in trauma. *J Trauma* 2008;65:951–60.
5 Pham HP, Shaz BH. Update on massive transfusion. *Br J Anaesth* 2013;111 Suppl 1:i71–82.
6 Holcomb JB, Jenkins D, Rhee P, *et al*. Damage control resuscitation: directly addressing the early coagulopathy of trauma. *J Trauma* 2007;62:307–10.
7 Holcomb JB, Tilley BC, Baraniuk S, *et al*. Transfusion of plasma, platelets, and red blood cells in a 1:1:1 vs a 1:1:2 ratio and mortality in patients with severe trauma. *JAMA* 2015;313:471.
8 Fujiwara G, Okada Y, Ishii W, *et al*. High fresh frozen plasma to red blood cell ratio and survival outcomes in blunt trauma. *JAMA Surg* 2024;159:1272–80.
9 Tanaka H, Matsunaga S, Yamashita T, *et al*. A systematic review of massive transfusion protocol in obstetrics. *Taiwan J Obstet Gynecol* 2017;56:715–8.
10 Delaney M, Stark PC, Suh M, *et al*. Massive transfusion in cardiac surgery: the impact of blood component ratios on clinical outcomes and survival. *Anesth Analg* 2017;124:1777–82.
11 Mazzeffi MA, Chriss E, Davis K, *et al*. Optimal plasma transfusion in patients undergoing cardiac operations with massive transfusion. *Ann Thorac Surg* 2017;104:153–60.
12 Grottke O, Mallaiah S, Karkouti K, *et al*. Fibrinogen supplementation and its indications. *Semin Thromb Hemost* 2020;46:38–49.
13 Innerhofer N, Treichl B, Rugg C, *et al*. First-line administration of fibrinogen concentrate in the bleeding trauma patient: searching for effective dosages and optimal post-treatment levels limiting massive transfusion-further results of the RETIC study. *J Clin Med* 2021;10.
14 Spahn DR, Bouillon B, Cerny V, *et al*. The European guideline on management of major bleeding and coagulopathy following trauma: fifth edition. *Crit Care* 2019;23.
15 Fenger-Eriksen C, Lindberg-Larsen M, Christensen AQ, *et al*. Fibrinogen concentrate substitution therapy in patients with massive haemorrhage and low plasma fibrinogen concentrations. *Br J Anaesth* 2008;101:769–73.
16 Görlinger K, Fries D, Dirkmann D, *et al*. Reduction of fresh frozen plasma requirements by perioperative point-of-care coagulation management with early calculated goal-directed therapy. *Transfus Med Hemother* 2012;39:104–13.
17 Rahe-Meyer N, Pichlmaier M, Haverich A, *et al*. Bleeding management with fibrinogen concentrate targeting a high-normal plasma fibrinogen level: a pilot study. *Br J Anaesth* 2009;102:785–92.
18 Solomon C, Pichlmaier U, Schoechl H, *et al*. Recovery of fibrinogen after administration of fibrinogen concentrate to patients with severe bleeding after cardiopulmonary bypass surgery. *Br J Anaesth* 2010;104:555–62.
19 Inaba K, Karamanos E, Lustenberger T, *et al*. Impact of fibrinogen levels on outcomes after acute injury in patients requiring a massive transfusion. *J Am Coll Surg* 2013;216:290–7.
20 Jarraya A, Khaled T, Kammoun M, *et al*. Golden hour for fibrinogen concentrate infusion to improve post partum hemorrhage. *Egypt J Anaesth* 2018;34:73–4.
21 Karkouti K, Callum J, Rao V, *et al*. Protocol for a phase III, non-inferiority, randomised comparison of a new fibrinogen concentrate versus cryoprecipitate for treating acquired hypofibrinogenaemia in bleeding cardiac surgical patients: the FIBRES trial. *BMJ Open* 2018;8.
22 Douin DJ, Fernandez-Bustamante A. Early fibrinogen replacement to treat the endotheliopathy of trauma: novel resuscitation strategies in severe trauma. *Anesthesiology* 2023;139:675–83.
23 Winearls J, Reade MC, McQuilten Z, *et al*. Fibrinogen in traumatic haemorrhage. *Curr Opin Anaesthesiol* 2021;34:514–20.
24 Curry N, Rourke C, Davenport R, *et al*. Early cryoprecipitate for major haemorrhage in trauma: a randomised controlled feasibility trial. *Br J Anaesth* 2015;115:76–83.
25 Curry N, Foley C, Wong H, *et al*. Early fibrinogen concentrate therapy for major haemorrhage in trauma (E-FIT 1): results from a UK multi-centre, randomised, double blind, placebo-controlled pilot trial. *Crit Care* 2018;22.
26 Graetz TJ, Nuttall G. *Intraoperative transfusion and administration of clotting factors*. Waltham, MA: UpToDate, 2025. Available: https://www.uptodate.com/contents/image?imageKey=HEME%2F102695
27 Levy JH, Goodnough LT. How I use fibrinogen replacement therapy in acquired bleeding. *Blood* 2015;125:1387–93.
28 Sørensen B, Bevan D. A critical evaluation of cryoprecipitate for replacement of fibrinogen. *Br J Haematol* 2010;149:834–43.
29 Hernán MA, Robins JM. Using big data to emulate a target trial when a randomized trial is not available. *Am J Epidemiol* 2016;183:758–64.
30 Hubbard RA, Gatsonis CA, Hogan JW, *et al*. “Target Trial Emulation” for observational studies - potential and pitfalls. *N Engl J Med* 2024;391:1975–7.
31 World Health Organization. Blood safety and availability. WHO fact sheets. Available: https://www.who.int/news-room/fact-sheets/detail/blood-safety-and-availability [Accessed 26 Aug 2025].
32 Johnson DJ, Scott AV, Barodka VM, *et al*. Morbidity and mortality after high-dose transfusion. *Anesthesiology* 2016;124:387–95.
33 Carson JL, Stanworth SJ, Roubinian N, *et al*. Transfusion thresholds and other strategies for guiding allogeneic red blood cell transfusion. *Cochrane Database Syst Rev* 2016;10.
34 Davenport R, Curry N, Fox EE, *et al*. Early and empirical high-dose cryoprecipitate for hemorrhage after traumatic injury. *JAMA* 2023;330:1882.
35 Stanford S, Roy A, Cecil T, *et al*. Differences in coagulation-relevant parameters: comparing cryoprecipitate and a human fibrinogen concentrate. *PLoS One* 2023;18.
36 Abrahamyan L, Tomlinson G, Callum J, *et al*. Cost-effectiveness of fibrinogen concentrate vs cryoprecipitate for treating acquired hypofibrinogenemia in bleeding adult cardiac surgical patients. *JAMA Surg* 2023;158:245–53.
37 Republic of Türkiye, Ministry of Health. National patient blood management action plan 2021–2025. Ankara Ministry of Health; 2021. Available: https://hastakanyonetimi.saglik.gov.tr/dokumanlar/1_2/National_Patient_Blood_Management_Action_Plan_EN.pdf