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Abstract:

Introduction

Excessive bleeding after cardiopulmonary bypass (CPB) is risk factor for adverse outcomes after elective cardiac surgery (ECS). Differentiating between patients who bleed due to surgical issues and those whose excessive chest tube output (CTO) is due to coagulopathy, remains challenging. Bedside suitable tests to identify hemostatic disturbances and predict excessive bleeding are desirable. The study sought to evaluate prediction of excessive bleeding after ECS using two bedside suitable devices for platelet function and viscoelastic blood clot properties assessment.

Methods

We enrolled 148 patients (105 male and 43 female) undergoing ECS in a prospective observational study. Patients were characterized as bleeders if their 24 hour CTO exceeded the 75th percentile of distribution. Multiple electrode aggregometry (*MEA*, with ASPI, ADP and the TRAP test) and rotational thromboelastometry (*TEM,* with ExTEM, HepTEM and FibTEM test), were performed at three time points: preoperatively (T1), during CPB (T2), and after protamine administration (T3). The primary endpoint was CTO and the secondary endpoint was administration of blood products, 30-day and 1 year mortality.

Results

The best predictors of increased bleeding tendency were the tests performed after protamine administration (T3). At T3, patients characterized as bleeders had significantly lower MEA ASPI (median, 14 *vs.* 27 AUC, p=0.004) and ADP test values (median, 22 *vs.* 41 AUC, p=0.002) as well as TEM values expressed in maximum clot firmness after 30 min (MCF 30) for ExTEM (53 *vs.* 56 mm, p=0.005), HepTEM (48 *vs.* 52 mm, p=0.003) and FibTEM (8 *vs.* 11 mm, p<0.001) test. 24 hour CTO inversely correlated with both the MEA (ASPI test: r=-

0.236, p=0.004; ADP test: r=-0.299, p<0.001), and TEM MCF 30 (ExTEM: r=-0.295, p<0.001; HepTEM: -0.329, p<0.001; FibTEM: -0.377, p<0.001) test values.

Conclusion

Our study showed that MEA and TEM are useful methods for prediction of excessive bleeding after ECS. In order to prevent excessive postoperative CTO, hemostatic interventions with timely and targeted blood component therapy according to MEA and TEM results should be considered.

Keywords: multiple electrode aggregometry; rotational thromboelastometry; cardiac surgery; bleeding risk; transfusion

Introduction:

Cardiac surgery procedures with cardiopulmonary bypass (CPB) are often accompanied with an increased bleeding tendency. For these patients bleeding into the chest remains a common life-threating complication[1]. Excessive bleeding after CPB continues to be an important cause of morbidity and mortality[2]. Dixon B et al[3] reported chest tube output (CTO) as the strongest independent predictor of mortality[3]. Excessive hemorrhage results in the transfusion of allogenic blood products that expose the patient to multiple risks [4-6]. Disturbances of coagulation consequent to CPB have a multifactorial etiology, such as loss of platelet reactivity, coagulation factor consumption, hemodilution due to the CPB priming procedure etc.[7]. Extracorporeal circulation and heparin may induce many abnormalities in the coagulation system, including thrombocytopenia[2], platelet dysfunction[8, 9], coagulation factor deficiencies[10], residual heparin after protamine administration[11], and fibrinolysis[12]. Microvascular bleeding caused by dysfunctional platelets and an impaired coagulation cascade occur after CPB secondary to foreign surface contact[13]. Impaired platelets removed from the CPB circuit are presumed to be dysfunctional and are jointly responsible for postoperative bleeding [13].

However, the question „How to predict and prevent excessive postoperative bleeding?“ remains challenging. Differentiating between coagulopathy and inadequate surgical hemostasis as a cause for bleeding after CPB is sometimes difficult. Identifying abnormal alteration of platelet function and coagulation tests prior to and after CPB may make the important differentiation between coagulopathic (nonsurgical) bleeding requiring procoagulant blood components transfusion and surgical bleeding requiring surgical intervention. Reliable methods to predict patients with higher risk of coagulation impairments are lacking [14]. Conventional coagulation tests are not able to predict postoperative bleeding

[15, 16]. „Blind“ transfusion therapy with multiple blood products still occurs when bleeding is excessive, and transfusion of allogeneic blood products expose patients to multiple risks and increased expenses[4, 17-20]. The bedside suitable tests with capability for differentiating bleeders from nonbleeders would be useful in creating a transfusion algorithm to guide blood product targeted therapy. Rotation thromboelastometry (TEM) performed on whole blood samples provides information on the contribution of fibrinogen and platelets to clot formation. A significant reduction in the number of blood transfusions and mediastinal re-exploration for hemorrhage in cardiac surgery patients after institution of thromboelastography-guided blood product administration is described in retrospective study by Spiess et al[21]. Despite the fact that thromboelastometry use resulted in procoagulant blood products administration reduction, the precise relationship between thromboelastometry values and postoperative bleeding still remains unclear. Although obtaining evidence about the global coagulation cascade, this method is inaccurate for detecting isolated platelet dysfunction. Therefore, it seems that simultaneous use of TEM and platelet function assay could offer a comprehensive insight into the hemostatic properties. Multiple electrode aggregometry (MEA) uses the technique of whole blood aggregometry and quantifies platelet function by attachment of platelets activated with different stimulants onto metal electrodes leading to increase of electrical impedance.

This study sought to evaluate whether perioperative viscoelastic blood properties and platelet function measurements in three intraoperative measurement time points using concomitant TEM and MEA can identify patients with increased bleeding risk as measured by postoperative blood loss. We were also interested to determine whether above mentioned two *Point of Care (POC)* devices can predict patients at enhanced risk of blood products administration due to excessive blood loss. We hypothesized that MEA and TEM could detect patients with higher risk of excessive postoperative bleeding and transfusion requirements.

Patients and methods:

Patient selection:

This prospective observational study was approved by the Institutional Research Ethics Review Board and registered at the Clinical-trials.gov website (Identifier NCT01281397). Type and daily dose of antiplatelet therapy (APT), received preoperatively, and the interval from the last dose of clopidogrel (CLO) to surgical procedure was documented.

After approval by the local Medical University and University Hospital Center Ethics Committee and written informed consent, 148 consecutive patients (male, n=105 (70.9%); female, n=43 (21.9%)), scheduled for elective cardiac surgery (ECS) procedures requiring cardiopulmonary bypass (CPB) between July 2010 and January 2011 were prospectively studied. Criteria for excluding patients from the group of subjects were: age younger than 18 years, urgent procedure, patients with off-pump cardiac surgical procedure, on APT other than Aspirin (ASA) and CLO, patients with inaccurate APT administration documentation, urgent surgery, and patients requiring surgical exploration for excessive bleeding due to obvious surgical bleeding with a bleeding vessel identified.

In our study cohort APT was administered by the referral cardiologist. If patients were admitted to Department of Cardiac Surgery with a daily dose of 100 mg ASA, the drug was continued up to the day of surgery. If patients were admitted with a daily dose of 75 mg CLO, either alone or in addition to ASA, CLO was discontinued at Department of Cardiac Surgery prior to surgery with different time intervals from CLO cessation to surgery, from 2 to 8 days, respectively. Time from CLO cessation to surgery varied individually, based on the date of admission to Department of Cardiac Surgery and the date of the procedure. Patients received APT continuously for at least 10 days prior to admission to our department.

Perioperative management:

All patients had the same anesthetic and perfusion teams, and were admitted at least 1 day before surgery. Surgery was performed by using a standard technique. Surgical approach in all cases was through median sternotomy. All measurements were performed by research fellow, not directly included in treating patients. The nurses, anesthesiologists, and surgeons managing the patient's care were blinded to the both, TEM and MEA results. Surgery was performed in a single unit with standard surgical techniques. Surgical bleeding was controlled with diathermy and bone wax.

Cardiopulmonary circuit consisted of the Medtronic Affinity Trillium membrane oxygenator, venous reservoir and PVC tubing (Medtronic, Minneapolis, MN, USA) and a Stoeckert III roller pump (Stoeckert, Munich, Germany). Flow at 2.2 l min-1 m-2 and mean blood pressure of 60 mmHg were targeted. Heart was arrested with cold blood cardioplegia. Systemic heparinisation aiming at an activated clotting time (ACT) >480 s was used, followed by full reversal with protamine after decannulation. A dose of 1 g tranexamic acid was given at the induction of anesthesia and after protamine administration. Inotropic support was initiated in order to maintain a cardiac index greater than 2.2 l min-1 m-2. Weaning from CPB was initiated once the patient’s rhythm had stabilized and normothermia had been achieved. Cardiotomy suction returned blood to the CPB circuit. Packed red blood cells (PRBC) were transfused if hematocrit was <20% during CPB and < 25% after terminating CPB, or when significant bleeding was obvious. Volume replacement in the intensive care unit was administered as deemed necessary by the attending physician using hydroxyethyl starch 6% 130/0.4 and lactated Ringer's solution, PRBC's were transfused if deemed necessary by the consultant anesthesiologist.

Fresh frozen plasma (FFP) was given in cases showing prolonged prothrombin time (less than 45%), or according to clinical decision by the consultant anesthesiologist. Fibrinogen cryoprecipitate and platelet transfusion were administered in cases of excessive bleeding at discretion of consultant anesthesiologist.

Blood Sampling:

Blood samples for MEA and TEM measurements were obtained at three time points on day of surgery via central venous port. For MEA tests blood was collected in 4 ml heparin (Lithium Heparin 68 I.U.) coated BD Vacutainer® plastic tubes. For TEM test blood was collected in 1.8 mL sodium citrate (0.109Molar/3.2% citrate concentration) Vacutainer® plastic tubes. Routine laboratory tests were performed each day from day before surgery to fourth postoperative day. The same person, not directly involved in patient care, performed all measurements. Blood samples for MEA were at rest for 30 minutes after blood withdrawal and MEA was performed subsequently. TEM tests were performed immediately after blood withdrawal.

To assess changes in viscoelastic blood properties and platelet function before, during and after CPB, samples were drawn before induction of anesthesia (T1), after release of aortic clamp (T2), and 15 min after heparin neutralization (T3).

We used two *point of care* (POC) devices for platelet function assessment and for monitoring of viscoelastic properties of blood clot.

Multiple electrode aggregometry (MEA):

Whole blood aggregation was determined using a new generation impedance aggregometer (Multiplate® analyzer, Dynabyte Medical, Munich). Detailed description of method has already been published [22]. Put briefly, MEA is based on the principle that blood platelets are non-thrombogenic in their resting state, but expose receptors on their surface when they get activated, which allow them to attach on vascular injuries and artificial surfaces. When

platelets stick on the Multiplate sensor wires, they enhance the electrical resistance between them, which is continuously recorded. Increase in impedance is expressed in arbitrary area under curve (AUC) units, highlighted as the parameter with the highest diagnostic power [22, 23]. Platelet aggregation was determined in response to stimulation with arachidonic acid with a final concentration of 0.5 mM (ASPI test designed to evaluate ASA effect) , adenosine diphosphate (ADP) with a final concentration of 6.4µM (ADP test designed to evaluate thienopyridines, such as CLO, effect) and thrombin receptor-activating peptide (TRAP-6), TRAP test, 32- µM final concentration. The TRAP test is sensitive to GPIIb-IIIa inhibitors only, and in absence of these drugs, represents the „natural“ aggregation potential of the platelets.

Modified rotational thromboelastometry (TEM):

TEM was performed according to the manufacturer' instruction using equipment and kits provided by TEM International GmbH, Munich, Germany. The following kits were used: intrinsically activated coagulation (InTEM) and extrinsically activated coagulation (ExTEM), which induce clot formation via the intrinsic pathway or the extrinsic, tissue-factor-dependent pathway, respectively; HepTEM assay (heparinase modified TEM), coagulation is triggered via the intrinsic pathway. FibTEM, a test measuring the contribution of fibrinogen, which is based on ExTEM but contains cytochalasin D to inhibit the contribution of platelets in order to give a measure for the contribution of fibrinogen to clot firmness. By digital data processing, the following typical variables are obtained: clotting time (CT), the time from the start of measurement until the onset of clotting; Clot formation time (CFT), the time between the onset of clotting and the moment when the clot firmness reaches an amplitude of 20 mm; A 10, A 20, A 30 corresponds to the maximum amplitude of the curve after 10, 20 and 30 minutes, respectively.

Primary and secondary outcome definition and evaluation:

Chest tube output (CTO) was determined as study's primary outcome. To estimate blood loss, we meticulously documented CTO, in first 24 postoperative hours and divided it by patient's weight. Drainage loss was assessed after completion of a 30-minute stabilization period. Blood loss during the stabilization period was not included in the definition of postoperative hemorrhage. Such loss may be caused by postural changes when transferring the patient from the operating room table to the bed or because of fluid in the pleural or mediastinal cavity, which may have arisen from the rinsing with water as an attempt to achieve surgical hemostasis. Intraoperative and postoperative transfusion requirements (PRBC in mL, FFP in mL, fibrinogen cryoprecipitate in grams and platelet concentrates in units) were determined as study's secondary outcome. Surgical reexploration of the mediastinum for excessive bleeding was noted, along with any surgical explanation for the bleeding.

Although some authors offer definitions of abnormal blood loss[16], we decided to make our own definition in order to adapt the volume of postoperative CTO to our study cohort. We believe that such a definition makes the most reliable correlation, and is not distorted to different perfusionistic, surgical and anesthetic techniques described by other authors. Postoperative CTO was recorded for the first 24 postoperative hours and divided by the patient's weight. Patients were characterized as bleeders if their 24 hour CTO (ml/kg) exceeded > 75^{th} percentile of distribution. A similar definition has already been described in the literature[24]. MEA and TEM variables were correlated to 24 hour CTO (ml/kg). Patients were divided with respect to presence of excessive bleeding, and MEA and TEM variables were compared between groups.

Statistical analysis:

The Kolmogorov-Smirnov test was used for evaluating the normality of distribution of all continuous variables. Correlation between CTO during the first postoperative 24h and MEA parameters was evaluated by Spearman's correlation coefficient. Mann-Whitney U test was

used to evaluate whether the medians on a test variables differ significantly between two groups. Chi-square statistic test was used to compare a frequency distribution of observed categoric variables between the two groups. Receiver operating characteristic curve (ROC) was constructed to assess the ability of MEA and TEM parameters to predict excessive postoperative blood loss [25]. A value of $p<0.05$ was considered statistically significant. For statistical analysis we used MedCalc® For Windows (MedCalc Software, Broekstraat 52, 9030 Mariakerke, Belgium).

Results:

All patients from sample were discharged from hospital. 24 hour CTO value of 12.46 ml/kg presented 75th percentile of distribution, thus cut off value for "bleeder category". The cohort group was divided into two subgroups according to occurrence of excessive postoperative bleeding. Patient's demographic, surgical, and baseline routine laboratory findings are presented in Table 1a and 1b. Differences in clinical and transfusion outcomes of patient groups according to bleeding tendency are presented in Table 2. Higher proportion of patients in "bleeder" category were preoperatively exposed to CLO (43.2% *vs*.22.5% , p=0.015, Table 1b). For patients exposed to CLO preoperatively, we observed shorter timeframe from clopidogel discontinuation to surgery in "bleeder" group (4 *vs.* 6 days, p= 0.024).

Two groups according to excessive bleeding occurrence were compared for clinical and transfusion outcomes (Table 2.). In „excessive bleeding" subgroup, higher proportion of patients were transfused with fresh frozen plasma (43.2% *vs.* 12.6%, p<0.001), fibrinogen concentrate (24.3% *vs.* 1.8% , p<0.001), and platelet concentrate (10.8% *vs.* 1.8% , p=0.016). Concerning clinical outcome, 1 year mortality was more frequently observed in „excessive bleeding" subgroup (12.1% *vs.* 1.9% , p=0.012).

Comparison of MEA and TEM test results between „bleeder" and „non-bleeder" category and correlation of MEA and TEM values with CTO is presented for each time point in Table 3a (T1), 3b (T2) and 3c (T3), respectively.

At T1, significant differences between groups were found for MEA ASPI test (Median 20 *vs.* 40 AUC, p=0.043) and FibTEM A 20 (Median 16 *vs.* 18 mm, p= 0.044) (Table 3a). MEA ASPI test (p=0.019), InTEM A 20 (p=0.038), InTEM A 30 (p=0.035), FibTEM A 10 (p=0.021), FibTEM A 20 (p=0.01) and FibTEM A 30 (p=0.019) significantly correlated to 24 hour CTO.

At T2, significant differences between groups were found for MEA ASPI and ADP test values, as well as for HepTEM, ExTEM and FibTEM variables (Table 3b).

The best predictors of increased bleeding tendency were the tests performed after protamine administration (T3) (Table 3c). At T3, patients characterized as bleeders had significantly lower MEA ASPI (median, 14 *vs.* 27 AUC, p=0.004) and ADP test values (median, 22 *vs.* 41 AUC, p=0.002) as well as TEM values expressed in maximum clot firmness after 30 min (MCF 30) for ExTEM (53 *vs.* 56 mm, p=0.005), HepTEM (48 *vs.* 52 mm, p=0.003) and FibTEM (8 *vs.* 11 mm, p<0.001) test. 24 hour CTO inversely correlated with both the MEA (ASPI test: r=-0.236, p=0.004; ADP test: r=-0.299, p<0.001), and TEM MCF 30 (ExTEM: r=-0.295, p<0.001; HepTEM: -0.329, p<0.001; FibTEM: r=-0.377, p<0.001) test values.

At T3, both the MEA and TEM tests with the strongest correlations to 24 hour CTO were tested for accuracy in predicting excessive postoperative bleeding using ROC analysis. The accuracy of the ASPI test (AUC 0.636, p=0.008) is shown in Figure 1, the accuracy of the ADP test (AUC 0.667, p=0.001) is shown in Figure 2, the accuracy of the ExTEM alfa (AUC 0.691, p<0.001) is shown in Figure 3, the accuracy of the FibTEM A 30 (AUC 0.695, p<0.001) is shown in Figure 4, and the accuracy of the HepTEM A 30 (AUC 0.663, p=0.002) is shown in Figure 5. We used ACT test regularly after protamine administration. We compared ACT values between groups with respect to bleeding tendency and we found non-significant differences between groups (median 137 vs. 138 sec, p=0.755). In addition to, ACT value did not correlate to value of 24 hour chest tube output (Spearman's correlation coefficient -0.002, p=0.976).

Discussion:

Our study showed that MEA and TEM are useful methods for prediction of excessive bleeding after ECS. The best predictors of increased bleeding tendency were the tests performed after protamine administration (T3). One reason for such results is the fact that the effect of CPB on platelet function and viscoelastic blood properties and the consequent risk of bleeding were not explored in T1.

Hemostatic impairment after CPB is complex in nature due to many components of the hemostatic system involved in. In addition to insufficient surgical hemostasis, bleeding after CPB may be induced by many abnormalities in the coagulation system. Conventional laboratory tests are unable to decompose multifactorial coagulopathy pathogenesis as described by Paparella et al[26]. Impaired platelet function is thought to be the most common coagulation defect associated with CPB[2, 27]. In the absence of platelet mapping, both thromboelastometry and thromboelastography are poor indicators of impaired platelet function. Thus, concurrent use of both modified rotational thromboelastometry (TEM) and MEA should be the best show of hemostatic properties of blood.

Literature review

Conflicting data have been reported regarding ability of point-of-care clotting assays and platelet function assays to predict bleeding after cardiac surgery procedures.

Essel et coworkers [28] compared conventional thromboelastography to conventional coagulation tests. Whilst the bleeding time and platelet count had sensitivities similar to thromboelastography, thromboelastography specificity was greater. In addition, they suggested that patients with abnormal thromboelastography were at increased risk of bleeding but the excessive bleeding in the face of a normal thromboelastography implied surgical bleeding and FFP and platelets should not simply be used empirically[28].

Analyzing patients with coronary artery bypass surgery (CABG), Ti et al found moderate correlation between thromboelastography parameters, total blood loss and blood product requirements in „bleeders“ cohort subgroup[29]. The major drawback of this study was the fact only CABG surgery patients were evaluated. CABG surgery patients differ from patients undergoing „other than isolated CABG“ surgery in the use of anticoagulants, length of CPB, age of patients and preoperative status. Mengistu et al showed that patients requiring postoperative blood transfusion showed lower preoperative ADP-mediated aggregometry compared with nontransfused patients, whereas thromboelastography did not show differences preoperatively[14]. Contrary to results reported by Mengistu et al [14], comparison of patient groups with respect to transfusion requirements in our study revealed no differences in T1 MEA test values. However, transfuded patients had significantly lower T1 values of InTEM, ExTEM and FibTEM values. Forrestier et al showed decreased ADP-mediated platelet aggregation in patients with increased bleeding tendency after cardiac surgery[30]. Our results suggest similar phenomenon for ADP test values in T2 (Spearman $r = -0.230$, $p=0.005$) and T3 (Spearman $r = -0.299$, $p<0.001$), but not in T1 time point where ADP test value did not correlate to CTO volume (Spearman $r = -0.095$, $p=0.252$). Ray et al reported extensive bleeding in patients with reduced preoperative platelet aggregation[31]. This report is only partially in line with our results. Preoperatively (T1) significant differences between groups with respect to bleeding tendency were found for MEA ASPI test (Median 20 *vs.* 40 AUC, $p=0.043$), but not for ADP and TRAP test.

In contrast to our results, several studies found no correlation between clotting assays and bleeding outcomes. Nutall et al described post CPB bleeding prediction using thrombelastography. The thromboelastography values that were most sensitive and specific for differentiating bleeding from nonbleeding patients were within the normal range for thromboelastography[32]. The major drawback was the fact that patients were characterized

as bleeder by subjective designation of the surgical field by anesthesiologist and surgeon. Of note, the thromboelastography values did not correlate with blood loss.Dorman et al didn't find significant relationship between thromboelastography variables and blood losses[33]. Wang et al described similar results with lack of any correlation between thromboelastography variables and postoperative blood loss[34]. Ostrowski et al described correlation between preoperative thromboelastography data and blood product usage, but there was no correlation between thromboelastography data and chest tube drainage[35]. Cammerer et al reported risk stratification of patients with increased bleeding tendency by maximal clot firmness and alpha-angle, but low positive predictive values did not allow clinical application[24].

Conclusion

In order to prevent excessive postoperative CTO, hemostatic interventions with timely and targeted blood component therapy according to MEA and TEM results should be considered. TEM can be used to predict bleeding in cardiac surgery, but it can also be used to guide targeted blood products transfusion algorithms where its use had been associated with significant decreases in blood products administration[36].

The inconsistent results that were obtained in the various studies could be due to differences in the TEM and thromboelastography tests that were applied (i.e. clotting activation via intrinsic or extrinsic pathway), concentration of the stimuli, as well, as due to differences in the recording time of blood loss [37, 38]. The definition of what constitutes abnormal postoperative bleeding is difficult. Although blood loss is a continuous variable, a separation of normal and abnormal bleeding based on single numerical value is necessary to allow meaningful comparisons with previous studies. Ti et al[16] described the drawbacks of chest tube output quantification. Blood loss measured from the chest and mediastinal tubes consists of a mixture of fluids, including actual blood loss, serous drainage, and fluid left in the pleural

cavity. Also, the actual blood loss through these tubes is the sum of coagulopathic bleeding and surgical bleeding from wound edges which may partially explain low specificity values as obtained by ROC analysis. In present study, drainage loss was assessed after completion of a 30-minute stabilization period. Blood loss during the stabilization period may be caused by postural changes when transferring the patient from the operating room table to the bed or because of fluid in the pleural or mediastinal cavity, which may have arisen from the rinsing with water as an attempt to achieve surgical hemostasis. We decided to monitor chest tube output in first 24 hours after surgery. Drainage loss after this period was not considered to be of "hemostatic disturbances" origin, because it most likely originates from a surgical source (eg, detached arterial clip). Concerning serous drainage, chest output output observed in the first 24 postoperative hours in present study was never clear and serous, but sanguinary and accompanied with the drop in hemoglobin levels, thus indicating hemorrhage. Standard goal-directed restrictive fluid therapy was employed in all cases. When executed meticulously, as it is in our institution, it is unlikely to reduce albumin to a level that could be connected with such a significant decline in colloid osmotic pressure in patients with preoperatively normal protein level (measuring protein level is a part of routine preprocedural panel of tests, albumin level is not).

In our study the attending clinicians, both the anesthesiologists and surgeons were blinded to MEA and TEM results, therefore administering procoagulant blood products mainly on the clinical grounds. The use of procoagulant blood products certainly affected the number of bleeders and amount of bleeding. This decrease in amount of bleeding would reduce the degree of correlation between blood loss and both MEA and TEM parameters by increasing the number of false-negative results, and reducing the sensitivity of MEA and TEM parameters. Finally, MEA and TEM are useful in distinguishing patients by excessive bleeding and provide correlations with CTO. Values above the cut-off values do not imply the

fact that the patients will not bleed, however they may advise to pay attention to surgical cause of bleeding. Hemostatic management algorithm with the timely and targeted procoagulant blood component therapy administration according to MEA and TEM should be evaluated in context of outcomes such as mortality, bleeding and transfusion requirements.

REFERENCES

1. Fergusson DA, Hebert PC, Mazer CD, Fremes S, MacAdams C, Murkin JM, Teoh K, Duke PC, Arellano R, Blajchman MA, Bussieres JS, Cote D, Karski J, Martineau R, Robblee JA, Rodger M, Wells G, Clinch J, Pretorius R (2008) A comparison of aprotinin and lysine analogues in high-risk cardiac surgery. N Engl J Med 358:2319-2331.

2. Woodman RC, Harker LA (1990) Bleeding complications associated with cardiopulmonary bypass. Blood 76:1680-1697.

3. Dixon B, Santamaria JD, Reid D, Collins M, Rechnitzer T, Newcomb AE, Nixon I, Yii M, Rosalion A, Campbell DJ (2012) The association of blood transfusion with mortality after cardiac surgery: cause or confounding? Transfusion doi: 10.1111/j.1537-2995.2012.03697.x

4. Practice Guidelines for blood component therapy: A report by the American Society of Anesthesiologists Task Force on Blood Component Therapy (1996) Anesthesiology 84:732-747.

5. Lackritz EM, Satten GA, Aberle-Grasse J, Dodd RY, Raimondi VP, Janssen RS, Lewis WF, Notari EP, Petersen LR (1995) Estimated risk of transmission of the human immunodeficiency virus by screened blood in the United States. N Engl J Med 333:1721-1725.

6. Tremolada F, Chiappetta F, Noventa F, Valfre C, Ongaro G, Realdi G (1983) Prospective study of posttransfusion hepatitis in cardiac surgery patients receiving only blood or also blood products. Vox Sang 44:25-30.

7. Fries D, Streif W, Haas T, Kuhbacher G (2004) [Dilutional coagulopathy, an underestimated problem?]. Anasthesiol Intensivmed Notfallmed Schmerzther 39:745-750.

8. Edmunds LH, Jr., Ellison N, Colman RW, Niewiarowski S, Rao AK, Addonizio VP, Jr., Stephenson LW, Edie RN (1982) Platelet function during cardiac operation: comparison of membrane and bubble oxygenators. J Thorac Cardiovasc Surg 83:805-812.

9. Harker LA, Malpass TW, Branson HE, Hessel EA, 2nd, Slichter SJ (1980) Mechanism of abnormal bleeding in patients undergoing cardiopulmonary bypass: acquired transient platelet dysfunction associated with selective alpha-granule release. Blood 56:824-834.

10. Mammen EF, Koets MH, Washington BC, Wolk LW, Brown JM, Burdick M, Selik NR, Wilson RF (1985) Hemostasis changes during cardiopulmonary bypass surgery. Semin Thromb Hemost 11:281-292.

11. Gundry SR, Drongowski RA, Klein MD, Coran AG (1989) Postoperative bleeding in cardiovascular surgery. Does heparin rebound really exist? Am Surg 55:162-165.

12. Tanaka K, Takao M, Yada I, Yuasa H, Kusagawa M, Deguchi K (1989) Alterations in coagulation and fibrinolysis associated with cardiopulmonary bypass during open heart surgery. J Cardiothorac Anesth 3:181-188.

13. Rinder CS, Bohnert J, Rinder HM, Mitchell J, Ault K, Hillman R (1991) Platelet activation and aggregation during cardiopulmonary bypass. Anesthesiology 75:388-393.

14. Mengistu AM, Wolf MW, Boldt J, Rohm KD, Lang J, Piper SN (2008) Evaluation of a new platelet function analyzer in cardiac surgery: a comparison of modified thromboelastography and whole-blood aggregometry. J Cardiothorac Vasc Anesth 22:40-46.

15. Spiess BD, Tuman KJ, McCarthy RJ, DeLaria GA, Schillo R, Ivankovich AD (1987) Thromboelastography as an indicator of post-cardiopulmonary bypass coagulopathies. J Clin Monit 3:25-30.

16. Ti LK, Cheong KF, Chen FG (2002) Prediction of excessive bleeding after coronary artery bypass graft surgery: the influence of timing and heparinase on thromboelastography. J Cardiothorac Vasc Anesth 16:545-550.

17. Goodnough LT, Soegiarso RW, Birkmeyer JD, Welch HG (1993) Economic impact of inappropriate blood transfusions in coronary artery bypass graft surgery. Am J Med 94:509-514.

18. Lackritz EM, Satten GA, Aberle-Grasse J, Dodd RY, Raimondi VP, Janssen RS, Lewis WF, Notari EPt, Petersen LR (1995) Estimated risk of transmission of the human immunodeficiency virus by screened blood in the United States. N Engl J Med 333:1721-1725.

19. Nightingale CH, Robotti J, Deckers PJ, Allmendinger PD, Lowe R, Low HB (1987) Quality care and cost-effectiveness. An organized approach to problem solving. Arch Surg 122:451-456.

20. Tremolada F, Chiappetta F, Noventa F, Valfre C, Ongaro G, Realdi G (1983) Prospective study of posttransfusion hepatitis in cardiac surgery patients receiving only blood or also blood products. Vox Sang 44:25-30.

21. Spiess BD, Gillies BS, Chandler W, Verrier E (1995) Changes in transfusion therapy and reexploration rate after institution of a blood management program in cardiac surgical patients. J Cardiothorac Vasc Anesth 9:168-173.

22. Toth O, Calatzis A, Penz S, Losonczy H, Siess W (2006) Multiple electrode aggregometry: a new device to measure platelet aggregation in whole blood. Thromb Haemost 96:781-788.

23. Calatzis A WB, Krueger (2004) A new approach to platelet function analysis in whole blood- the multiplate analyzer. Platelets 15:479-517.

24. Cammerer U, Dietrich W, Rampf T, Braun SL, Richter JA (2003) The predictive value of modified computerized thromboelastography and platelet function analysis for postoperative blood loss in routine cardiac surgery. Anesth Analg 96:51-57, table of contents.

25. Metz CE (1978) Basic principles of ROC analysis. Semin Nucl Med 8:283-298.

26. Paparella D, Brister SJ, Buchanan MR (2004) Coagulation disorders of cardiopulmonary bypass: a review. Intensive Care Med 30:1873-1881.

27. Harker LA (1987) Acquired disorders of platelet function. Ann N Y Acad Sci 509:188-204.

28. Essell JH, Martin TJ, Salinas J, Thompson JM, Smith VC (1993) Comparison of thromboelastography to bleeding time and standard coagulation tests in patients after cardiopulmonary bypass. J Cardiothorac Vasc Anesth 7:410-415.

29. Ti LK, Cheong KF, Chen FG (2002) Prediction of excessive bleeding after coronary artery bypass graft surgery: the influence of timing and heparinase on thromboelastography. J Cardiothorac Vasc Anesth 16:545-550.

30. Forestier F, Coiffic A, Mouton C, Ekouevi D, Chene G, Janvier G (2002) Platelet function point-of-care tests in post-bypass cardiac surgery: are they relevant? Br J Anaesth 89:715-721.

31. Ray MJ, Marsh NA, Hawson GA (1994) Relationship of fibrinolysis and platelet function to bleeding after cardiopulmonary bypass. Blood Coagul Fibrinolysis 5:679-685.

32. Nuttall GA, Oliver WC, Ereth MH, Santrach PJ (1997) Coagulation tests predict bleeding after cardiopulmonary bypass. J Cardiothorac Vasc Anesth 11:815-823.

33. Dorman BH, Spinale FG, Bailey MK, Kratz JM, Roy RC (1993) Identification of patients at risk for excessive blood loss during coronary artery bypass surgery: thromboelastography versus coagulation screen. Anesth Analg 76:694-700.

34. Wang JS, Lin CY, Hung WT, O'Connor MF, Thisted RA, Lee BK, Karp RB, Yang MW (1992) Thromboelastogram fails to predict postoperative hemorrhage in cardiac patients. Ann Thorac Surg 53:435-439.

35. Ostrowsky J, Foes J, Warchol M, Tsarovsky G, Blay J (2004) Plateletworks platelet function test compared to the thromboelastograph for prediction of postoperative outcomes. J Extra Corpor Technol 36:149-152.

36. Royston D, von Kier S (2001) Reduced haemostatic factor transfusion using heparinase-modified thrombelastography during cardiopulmonary bypass. Br J Anaesth 86:575-578.

37. Reinhofer M, Brauer M, Franke U, Barz D, Marx G, Losche W (2008) The value of rotation thromboelastometry to monitor disturbed perioperative haemostasis and bleeding risk in patients with cardiopulmonary bypass. Blood Coagul Fibrinolysis 19:212-219.

38. Nielsen VG (2007) A comparison of the Thrombelastograph and the TEM. Blood Coagul Fibrinolysis 18:247-252.

Tables:

Table 1:

Basic demographic, laboratory and operative data statistics of patient groups according to bleeding tendency for continuous (Table 1a) and categorical (Table 1b) variables

Table 1a:

	24h chest tube output (ml/kg)	N	Percentiles			Mann-Whitney U
			25th	50th (Median)	75th	p
Age (years)	<75. Percentile	111	57.00	65.00	72.00	0.536
	>75 percentile	37	52.50	69.00	73.00	
Body mass index (kg/m2)	<75. Percentile	111	25.72	28.65	31.35	0.266
	>75 percentile	37	25.22	27.77	31.06	
Body surface area (m2)	<75. Percentile	111	1.83	1.99	2.13	0.136
	>75 percentile	37	1.77	1.89	2.11	
EURO score (%)	<75. Percentile	111	1.81	2.71	5.62	0.844
	>75 percentile	37	1.81	3.78	5.52	
Ejection fraction (%)	<75. Percentile	111	45.00	60.00	65.00	0.563
	>75 percentile	37	50.00	55.00	61.00	
Days after clopidogrel discontinuation	<75. Percentile	25	4.00	6.00	7.50	0.024
	>75 percentile	17	2.00	4.00	6.50	
Platelet count (x 10 9/l)	<75. Percentile	111	176.00	212.00	255.00	0.263
	>75 percentile	37	165.50	196.00	241.00	
Fibrinogen (g/l)	<75. Percentile	111	3.30	3.90	4.60	0.203
	>75 percentile	37	3.10	3.60	4.35	
Hemoglobin	<75. Percentile	111	121.00	133.00	145.00	0.183
	>75 percentile	37	126.00	140.00	146.00	
Hematocrit	<75. Percentile	111	0.37	0.40	0.43	0.183

	>75 percentile	37	0.38	0.42	0.44	
INR	<75. Percentile	111	0.94	0.98	1.06	0.245
	>75 percentile	37	0.93	0.96	1.01	
Cross-clamp time (min)	<75. Percentile	111	49.00	67.00	88.00	0.473
	>75 percentile	37	50.00	69.00	104.50	
Cardiopulmonary bypass time (min)	<75. Percentile	111	74.00	96.00	126.00	0.089
	>75 percentile	37	80.50	112.00	139.50	
Ventilation (h)	<75. Percentile	110	7.75	9.00	12.00	0.24
	>75 percentile	36	7.00	10.00	15.75	

Table 1b:

		24 hour chest tube output (ml/kg)				Chi Square
		<75. percentile N=111		>75. percentile N=37		
		N	%	N	%	p
Gender	Male	79	71.2%	26	70.3%	0.917
	Female	32	28.8%	11	29.7%	
Arterial hypertension	No	18	16.2%	7	18.9%	0.704
	Yes	93	83.8%	30	81.1%	
Diabetes mellitus	No	76	68.5%	27	73.0%	0.606
	Yes	35	31.5%	10	27.0%	
Smoking habit	No	88	79.3%	30	81.1%	0.813
	Yes	23	20.7%	7	18.9%	
Renal dysfunction	No	109	98.2%	35	94.6%	0.242
	Yes	2	1.8%	2	5.4%	
Beta blockers	No	28	25.2%	7	18.9%	0.434
	Yes	83	74.8%	30	81.1%	
Amiodarone	No	99	89.2%	33	89.2%	1
	Yes	12	10.8%	4	10.8%	
Calcium channel blockers	No	81	73.0%	25	67.6%	0.528
	Yes	30	27.0%	12	32.4%	
Clopidogrel	No	86	77.5%	21	56.8%	0.015
	Yes	25	22.5%	16	43.2%	
acetylsalicylic acid	No	41	36.9%	13	35.1%	0.844
	Yes	70	63.1%	24	64.9%	
Antiplatelet therapy	No	35	31.5%	9	24.3%	0.406
	Yes	76	68.5%	28	75.7%	
Lipid lowering drugs	No	28	25.2%	13	35.1%	0.243
	Yes	83	74.8%	24	64.9%	
Procedure type	Isolated CABG	62	55.9%	22	59.5%	0.257
	Valve procedure	27	24.3%	7	18.9%	
	Combined procedure	11	9.9%	7	18.9%	
	Other	11	9.9%	1	2.7%	
ISOLATED CABG	Other	49	44.1%	15	40.5%	0.702
	Isolated CABG	62	55.9%	22	59.5%	

Table 2:

Transfusion and clinical outcomes of patient groups according to bleeding tendency

		CTO 24h (ml/kg)				
		<75. percentile N=111		>75. percentile N=37		Chi square
		N	%	N	%	p
Transfuded fresh frozen plasma	No	97	87.4%	21	56.8%	<0.001
	Yes	14	12.6%	16	43.2%	
Transfuded packed red blood cells	No	24	21.6%	5	13.5%	0.282
	Yes	87	78.4%	32	86.5%	
Transfuded Fibrinogen concentrate	No	109	98.2%	28	75.7%	<0.001
	Yes	2	1.8%	9	24.3%	
Transfuded platelet concentrate	No	109	98.2%	33	89.2%	0.016
	Yes	2	1.8%	4	10.8%	
30 day mortality	Alive	110	99.1%	35	94.6%	0.092
	Dead	1	0.9%	2	5.4%	
1 year mortality	Alive	103	98.1%	29	87.9%	0.012
	Dead	2	1.9%	4	12.1%	
1 year re-hospitalization	No	88	83.8%	27	81.8%	0.789
	Yes	17	16.2%	6	18.2%	
	24 hours chest tube output (ml/kg)	n	25th percentile	50th (Median)	75th percentile	Mann-Whitney U
Ventilation (h)	<75. percentile	110	7.75	9.00	12.00	0.24
	>75 percentile	36	7.00	10.00	15.75	
ICU stay (days)	<75. percentile	111	2.00	2.00	3.00	0.489
	>75 percentile	37	1.00	2.00	3.50	
Postoperative hospital stay (days)	<75. percentile	111	7.00	7.00	9.00	0.84
	>75 percentile	37	6.50	7.00	10.00	

Table 3

Table 3a:

Comparison of the multiple electrode aggregometry and rotational thromboelastometry results between groups with respect to bleeding tendency and correlations of parameters with 24 hours chest tube output (ml/kg). Results of tests are observed prior to induction of anesthesia (T1).

	According to 24 hour chest tube output (mL/kg)							CTO 24 H mL/kg	
T1	BLEEDER			NON-BLEEDER			Mann-Whitney U p	Spearman r	P
	25th percentile	Median	75th percentile	25th percentile	Median	75th percentile	P		
ASPI (AUC)	12	20	53	21	40	67	0.043	-0.193	0.019
ADP (AUC)	57	72	89	63	77	93	0.265	-0.095	0.252
TRAP (AUC)	95	105	120	95	109	122	0.849	-0.118	0.153
EXTEM CT (sec)	60	65	71	54	60	70	0.120	0.099	0.231
EXTEM CFT (sec)	65	77	93	58	73	89	0.233	0.115	0.166
EXTEM alfa (degree)	72	76	80	73	76	80	0.701	-0.102	0.218
EXTEM A 10 (mm)	56	60	62	57	61	66	0.191	-0.150	0.069
EXTEM A 20 (mm)	62	66	68	63	67	71	0.190	-0.157	0.056
EXTEM A 30 (mm)	64	67	69	63	68	72	0.296	-0.143	0.082
INTEM CT (sec)	150	164	191	147	169	200	0.820	-0.076	0.356
INTEM CFT (sec)	63	70	79	54	65	84	0.333	0.143	0.083
INTEM ALFA (degree)	74	76	78	75	77	80	0.173	-0.151	0.066
INTEM A 10 (mm)	54	57	60	54	59	64	0.177	-0.157	0.057
INTEM A 20 (mm)	60	63	66	60	65	69	0.112	-0.171	0.038
INTEM A 30 (mm)	60	64	67	61	65	69	0.134	-0.174	0.035
FIBTEM CT (sec)	50	55	59	48	53	58	0.346	0.093	0.260
FIBTEM A 10 (mm)	13	15	19	14	17	22	0.093	-0.190	0.021
FIBTEM A 20 (mm)	14	16	20	15	18	24	0.044	-0.210	0.010
FIBTEM A 30 (mm)	14	17	20	15	19	24	0.062	-0.193	0.019

Table 3b:

Comparison of the multiple electrode aggregometry and rotational thromboelastometry results between groups with respect to bleeding tendency and correlations of parameters with 24 hours chest tube output (ml/kg). Results of tests are during cardiopulmonary bypass after aortic cross clamp removal.

		According to 24 hour chest tube output (mL/kg)							CTO 24 H mL/kg	
T2		BLEEDER			NON-BLEEDER			Mann-Whitney U	Spearman r	p
		25th percentile	Median	75th percentile	25th percentile	Median	75th percentile	p		
ASPI (AUC)		4	9	13	6	15	33	0.004	-0.203	0.013
ADP (AUC)		23	30	46	29	48	67	0.002	-0.230	0.005
TRAP (AUC)		73	109	128	92	116	142	0.098	-0.130	0.115
EXTEM CT (sec)		64	73	87	66	76	90	0.609	-0.048	0.563
EXTEM CFT (sec)		114	139	165	95	118	144	0.004	0.272	0.001
EXTEM alfa (degree)		62	67	69	65	69	74	0.010	-0.245	0.003
EXTEM A 10 (mm)		41	44	48	45	49	54	0.001	-0.308	<0.001
EXTEM A 20 (mm)		48	52	56	53	57	61	0.001	-0.301	<0.001
EXTEM A 30 (mm)		50	55	59	56	59	63	<0.001	-0.311	<0.001
HEPTEM CT (sec)		187	200	221	175	200	223	0.825	0.043	0.602
HEPTEM CFT (sec)		99	130	161	84	102	124	0.001	0.261	0.001
HEPTEM ALFA (degree)		64	68	72	68	72	76	0.013	-0.199	0.015
HEPTEM A 10 (mm)		39	44	47	44	48	52	<0.001	-0.329	<0.001
HEPTEM A 20 (mm)		46	51	54	51	55	59	<0.001	-0.322	<0.001
HEPTEM A 30 (mm)		50	53	56	53	57	62	<0.001	-0.331	<0.001
FIBTEM CT (sec)		59	64	72	62	67	81	0.111	-0.154	0.061
FIBTEM A 10 (mm)		7	9	12	8	11	14	0.044	-0.236	0.004
FIBTEM A 20 (mm)		8	10	12	9	12	15	0.029	-0.223	0.007
FIBTEM A 30 (mm)		8	10	12	9	12	16	0.002	-0.282	0.001

Table 3c:

Comparison of the multiple electrode aggregometry and rotational thromboelastometry results between groups with respect to bleeding tendency and correlations of parameters with 24 hours chest tube output (ml/kg). Blood sampling was performed 15 min after protamine administration.

		According to 24 hour chest tube output (mL/kg)							CTO 24 H mL/kg	
T3		BLEEDER			NON-BLEEDER			Mann-Whitney U	Spearman r	p
		25th percentile	Median	75th percentile	25th percentile	Median	75th percentile	p		
ASPI (AUC)		8	14	22	10	27	51	0.013	-0.236	0.004
ADP (AUC)		15	22	36	22	41	66	0.002	-0.299	<0.001
TRAP (AUC)		78	93	113	77	103	133	0.133	-0.144	0.081
EXTEM CT (sec)		72	83	96	68	77	94	0.197	0.191	0.020
EXTEM CFT (sec)		131	166	212	105	141	172	0.005	0.271	0.001
EXTEM alfa (degree)		53	59	64	59	66	73	0.001	-0.317	<0.001
EXTEM A 10 (mm)		36	42	47	41	46	52	0.005	-0.280	0.001
EXTEM A 20 (mm)		45	51	55	49	54	60	0.005	-0.284	<0.001
EXTEM A 30 (mm)		48	53	57	52	56	61	0.005	-0.295	<0.001
HEPTEM CT (sec)		190	209	230	160	183	205	<0.001	0.348	<0.001
HEPTEM CFT (sec)		126	164	227	105	132	176	0.006	0.254	0.002
HEPTEM ALFA (degree)		55	62	66	60	67	72	0.004	-0.278	0.001
HEPTEM A 10 (mm)		33	39	43	38	43	47	0.002	-0.289	<0.001
HEPTEM A 20 (mm)		40	46	50	45	50	54	0.002	-0.322	<0.001
HEPTEM A 30 (mm)		44	48	52	47	52	55	0.003	-0.329	<0.001
FIBTEM CT (sec)		68	75	85	57	65	86	0.012	0.240	0.003
FIBTEM A 10 (mm)		5	8	9	7	9	13	0.002	-0.313	<0.001
FIBTEM A 20 (mm)		6	8	10	8	11	14	0.001	-0.354	<0.001
FIBTEM A 30 (mm)		6	8	11	8	11	14	<0.001	-0.377	<0.001

Figures

Figure 1.

Receiver operating characteristic curve analysis for excessive bleeding prediction by the Multiplate ASPI test after protamine administration. The best predictive value corresponds to an ASPI test value of equal or less than 22 AUC (Area under the curve 0.636. 95% Confidence Interval 0.553-0.714. p=0.008).

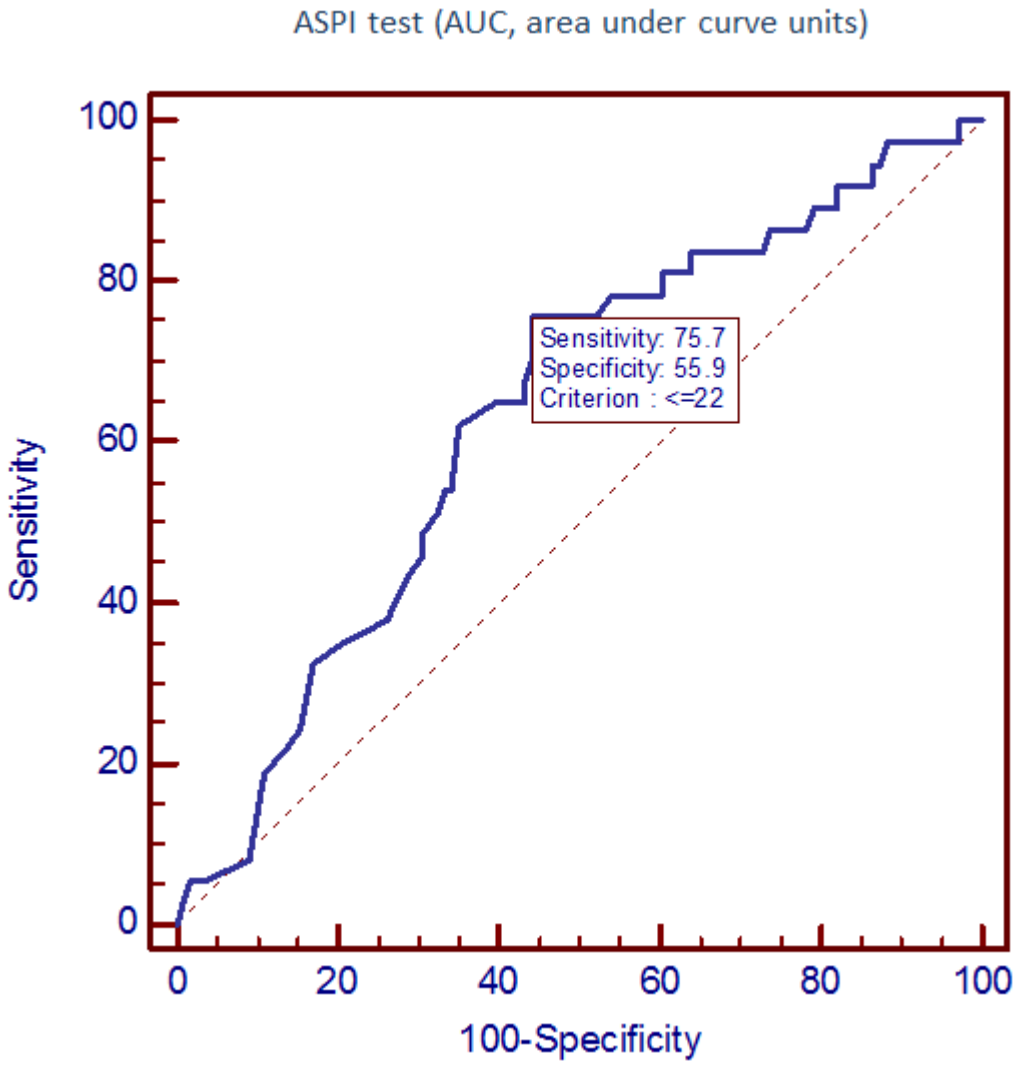

Figure 2.

Receiver operating characteristic curve analysis for excessive bleeding prediction by the Multiplate ADP test after protamine administration. The best predictive value corresponds to an ADP test value of equal or less than 36 AUC (Area under the curve 0.667. 95% Confidence Interval 0.585-0.742. p=0.001).

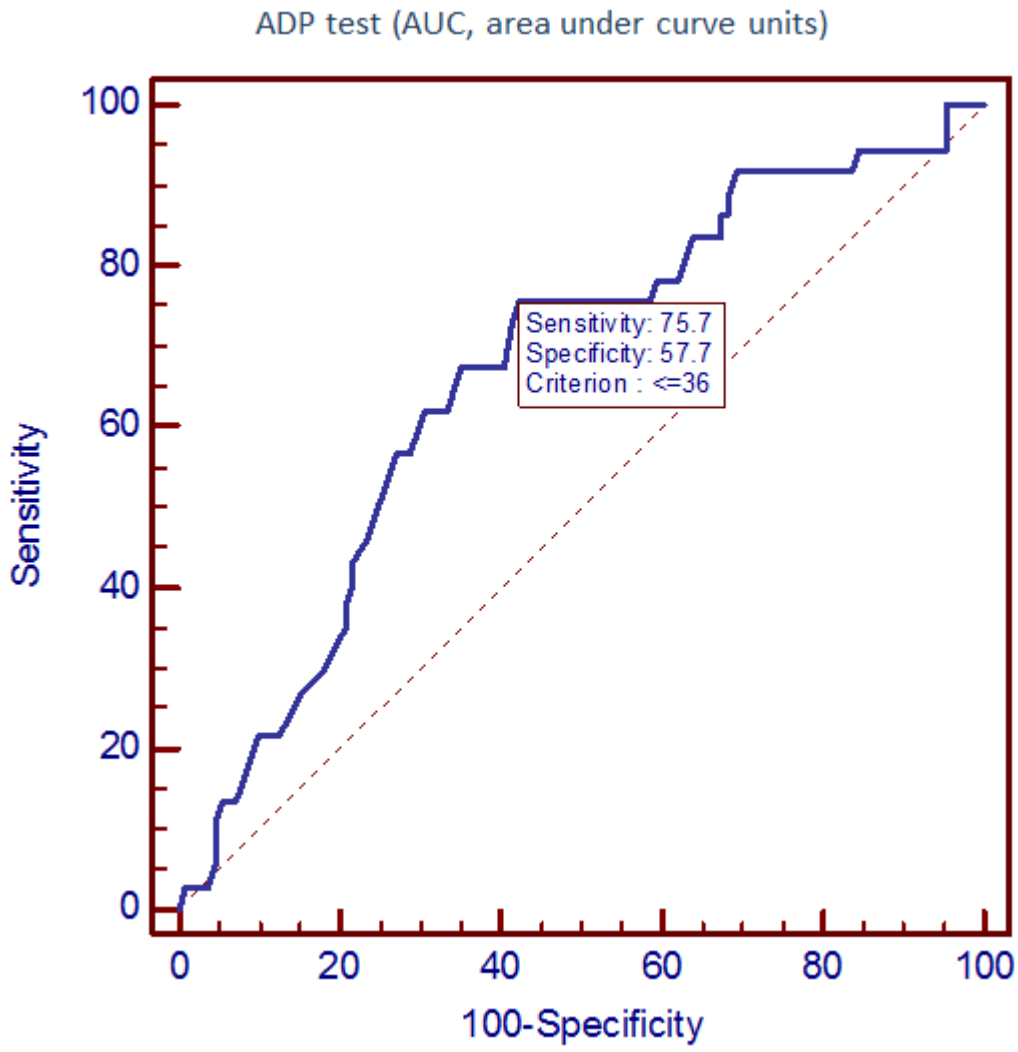

Figure 3.

Receiver operating characteristic curve analysis for excessive bleeding prediction by the TEM ExTEM test. The best predictive value corresponds to an ExTEM alfa angle value of equal or less than 63 degrees (Area under the curve 0.691. 95% Confidence Interval 0.610-0.764. p<0.001).

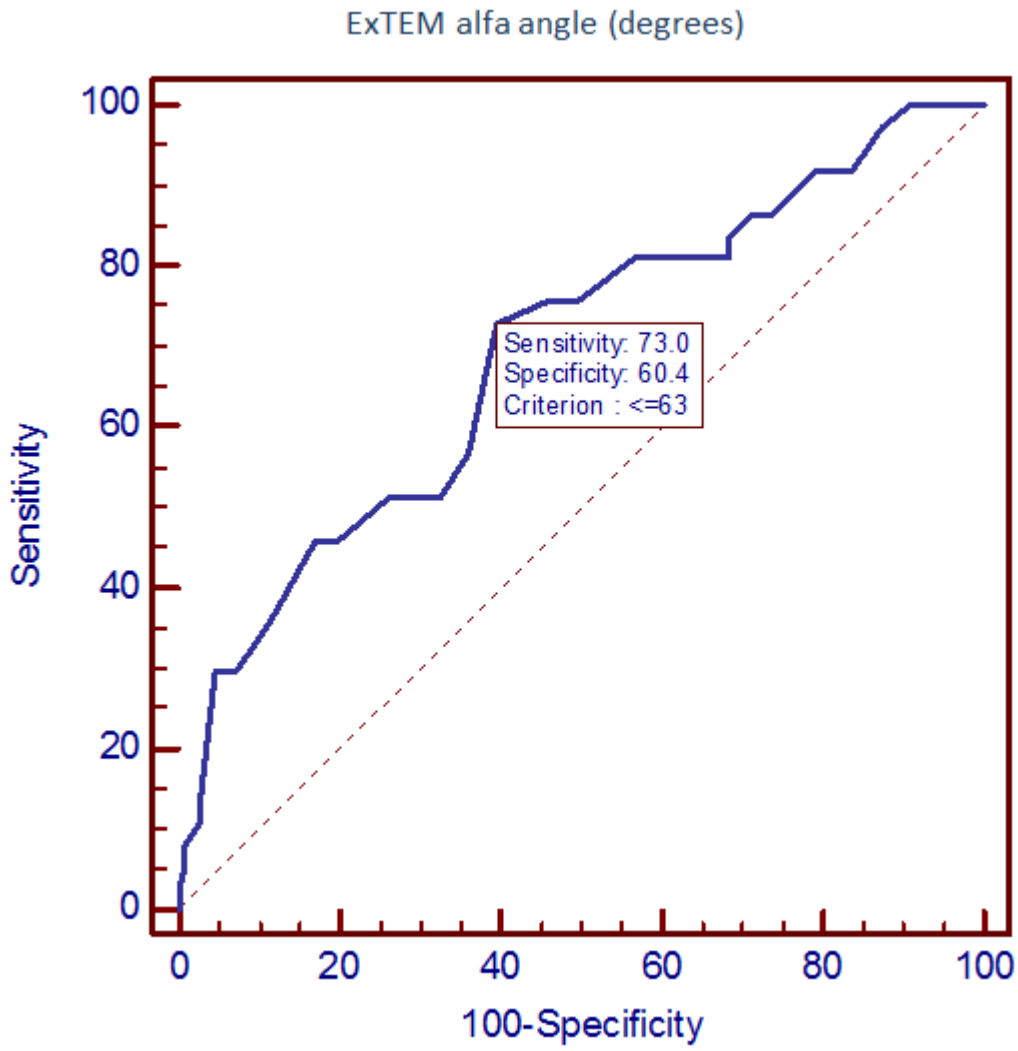

Figure 4.

Receiver operating characteristic curve analysis for excessive bleeding prediction by the TEM FibTEM test. The best predictive value corresponds to a FibTEM A 30 value of equal or less than 11 mm (Area under the curve 0.695. 95% Confidence Interval 0.614-0.768. p<0.001).

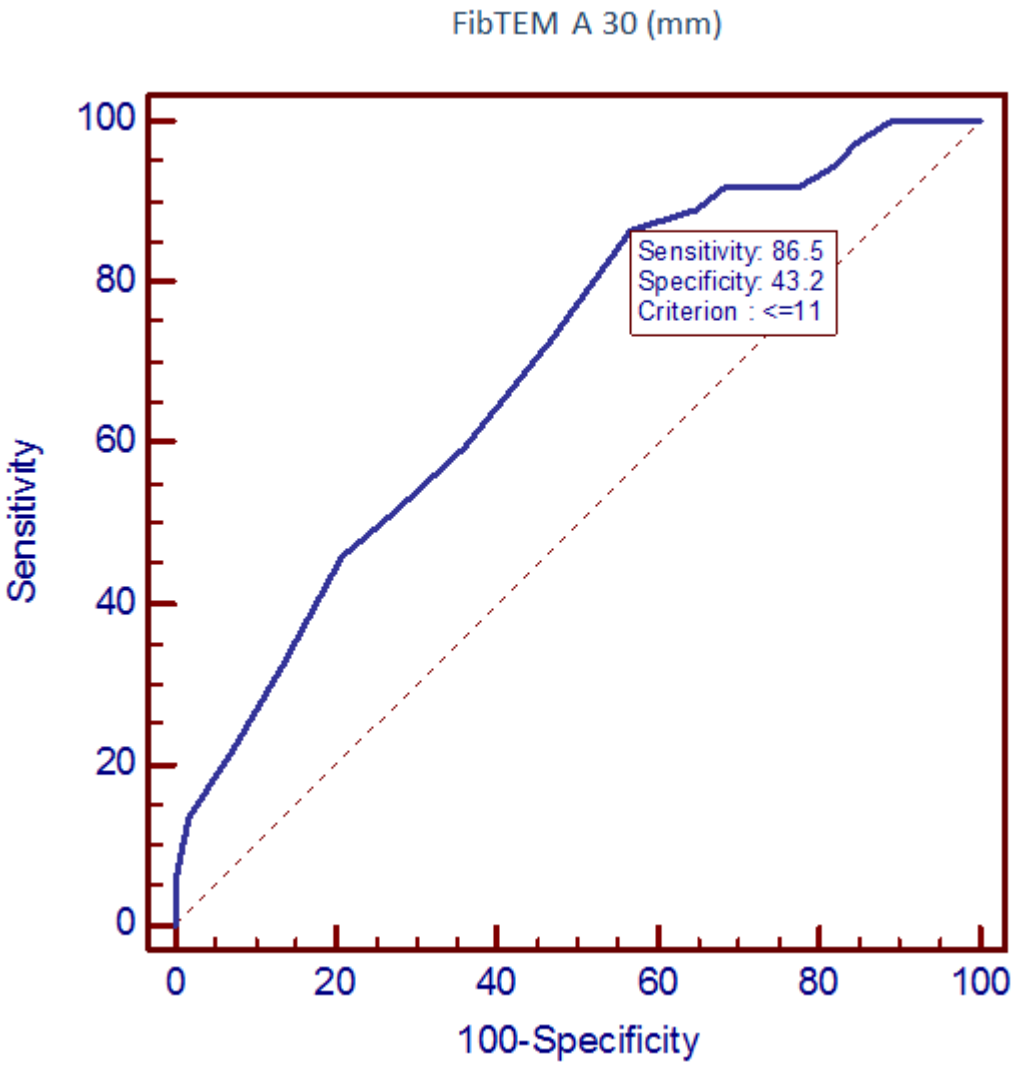

Figure 5.

Receiver operating characteristic curve analysis for excessive bleeding prediction by the TEM HepTEM test. The best predictive value corresponds to a HepTEM A 30 value of equal or less than 61 mm (Area under the curve 0.663. 95% Confidence Interval 0.580-0.738. p=0.002).