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Comparison of heparin vs. lepirudin anticoagulated tubes for the assessment of ASS-induced platelet dysfunction using the Multiplate device

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Abstract

Background: The manufacturer of the Multiplate® device recommends usage of lepirudin anticoagulated blood samples. It was the aim of the present study to analyze a potential relationship between blood samples that were drawn into lepirudin versus heparin anticoagulated tubes.

Methods: In a prospective cohort, single-center study, patients scheduled for elective coronary artery bypass grafting were preoperatively screened for eligibility. Patients were enrolled into the study if they had ceased any antiplatelet therapy for at least five days prior to the planned surgical intervention. Lepirudin and heparin anticoagulated blood samples were taken at the evening before surgery (T1), 4 h after the first postoperative ingestion of 100 mg aspirin (T2) and five days after begin of daily aspirin therapy (T3).

Results: n = 75 patients were finally enrolled into the study. There was a significant correlation between lepirudin and heparin anticoagulated samples at each measuring point. Platelet aggregability was higher in lepirudin anticoagulated blood as compared to heparin anticoagulated blood.

Conclusions: Data of the present study show that heparin anticoagulated blood samples are suitable for the assessment of arachidonic acid induced platelet aggregation of unaffected platelets as well as platelets with aspirin associated reduced aggregability.

Key Words: Heparin, Multiplate, Multiple Electrode Aggregometry, Platelet function testing, Point-of-Care

Introduction

Conventional laboratory coagulation analyses (INR, aPTT, platelet count, fibrinogen concentration) are of limited use for the prediction and detection of perioperative coagulopathies and for the monitoring of their treatment [1]. Because the so-called "Point-of-Care" (POC) tests may partly compensate for the methodological limitations and diagnostic shortfalls of conventional coagulation testing, these methods are increasingly used

in perioperative care [2]. Generally, viscoelastic measures, which are used to analyze the plasmatic coagulation system, are differentiated from aggregometric measures. Aggregometric measures are used to screen for disorders of primary hemostasis, such as (acquired) platelet dysfunctions and allow the quantification of the effect of antiplatelet medications. In this context, aggregometric measures indicate low- or non-responsiveness to aspirin and/or clopidogrel [3].

The Multiplate® analyzer, which is based on Multiple Electrode Aggregometry (MEA, Roche AG, Grenzach, Germany) [4], is one of the standard devices for periprocedural monitoring of platelet function, both in internal medicine [5] and in perioperative care [6]. Considering the transient platelet activating effects of heparin [7, 8], the manufacturer recommends usage of lepirudin anticoagulated tubes for blood drawing. However, in comparison to lepirudin, usage of heparin anticoagulated tubes is more applicable because of their exceptional availability all over the hospital. Furthermore usage of heparin anticoagulated tubes is relative low-priced and favorable even economic reasons. Although recommended in commonly accepted hemotherapy algorithms [9, 10], accuracy of measures from heparin anticoagulated samples had not been studied yet. Therefore, it was the aim of the present study to evaluate the relationship of MEA analyses performed in lepirudin versus heparin anticoagulated blood samples.

Methods

Trial design

This prospective cohort, single-center study was conducted at the University Hospital Frankfurt am Main, Germany. The present study complies with the declaration of Helsinki and was approved by the local Scientific and Ethics Review Board (filed with the reference number 91-08).

Participants

Patients were suitable for this trial after two inclusion steps.

Step 1: Patients (age 18 years or older) scheduled for elective coronary artery bypass grafting (CABG) with cardiopulmonary bypass (CPB) were preoperatively screened for eligibility.

Step 2: Patients were enrolled into the study, if they had ceased any antiplatelet

therapy five days prior to the date of planned surgical intervention. All patients gave written informed consent. Pregnancy was defined as exclusion criterion.

Anesthetic management

On the evening prior to surgery, patients were given 20 mg clorazepat dipotassium (Tranxilium®, Sanofi-Aventis GmbH, Hoechst, Germany). After routine monitoring was applied, general anesthesia was induced with 0.3–1 µg/kg sufentanil (Sufenta®, Janssen-Cilag GmbH, Neuss, Germany), 1–2.5 mg/kg propofol (Disoprivan®, AstraZeneca GmbH, Wedel, Germany), and 0.6 mg/kg rocuronium (Esmeron®, Essex GmbH, Munich, Germany). For the maintenance of general anesthesia, all patients received 1–2 Vol% sevoflurane (Sevoran®, Abbott, Wiesbaden, Germany) and intermittent boluses of sufentanil. Both isotonic crystalloid (Sterofundin®, B.Braun GmbH, Melsungen, Germany) and colloid fluids (6% HES 130/0.4, Voluven®, Fresenius Cabi, Bad Homburg, Germany) were perioperatively infused based on institutional standards.

Management of extracorporeal circulation

The extracorporeal circuit included a membrane oxygenator (Quadrox® oxygenator, Maquet Cardiopulmonary AG, Hirrlingen, Germany) and a roller pump system (HL20, Maquet Cardiopulmonary AG) equipped with a heat exchanger (Plegiox®, Maquet Cardiopulmonary AG). The circuit was primed with 500 ml crystalloid solution (Sterofundin®, B.Braun Melsungen AG), 500 ml colloid solution (6% HES 130/0.4, Voluven®, Fresenius Medical Care AG), and 250 ml 20% Mannitol (Mannitol Baxter®, Baxter, Unterschleissheim, Germany). Heparin (Heparin-Natrium Braun®, B.Braun Melsungen AG) was repeatedly administered after an initial bolus of 400 IU/kg to maintain an activated clotting time (ACT) of > 400 s. During CPB, a non-pulsatile flow was maintained at

2.6-3 l/min/m², and the mean arterial blood pressure was targeted to 50–70 mmHg with the addition of norepinephrine (Arterenol®, Sanofi-Aventis GmbH, Hoechst, Germany) if needed. Myocardial protection was achieved with cold blood cardioplegia (20°C). Antifibrinolytic therapy consisted of the application of 2 g tranexamic acid (Cyclocapron®, MEDA Pharma GmbH & Co KG, Bad Homburg, Germany) after the induction of anesthesia, and another 2 g was added into the priming volume of the heart-lung machine and again during CPB. Extracorporeal circulation was performed in mild hypothermia. When surgery was completed, patients were rewarmed to 36°C and weaned from CPB. To reverse the anticoagulant effects of heparin, protamine sulfate (Protaminsulfat, Novo Nordisk Pharma GmbH, Vienna, Austria) was administered, guided by the activated clotting time (ACT). If the target ACT was not obtained despite repeated heparin administrations 500 to 1,000 IU of antithrombin were infused. No procoagulatory therapy was performed before the administration of Protamine at the end of CPB.

Hematological Analyses

At each measuring point, we performed MEA analyses, blood gas analyses and conventional coagulation analyses. Preoperatively, blood samples were drawn by a single venous puncture with a 21G butterfly needle (Safety-Multifly® Set, Sarstedt AG&Co., Nümbrecht, Germany). Postoperatively, blood was drawn using the intraoperatively placed central line. For MEA analyses, the blood was collected into 2 ml heparin-anticoagulated and calcium-balanced tubes (Bloodgas-Monovette, Sarstedt AG&Co., Nümbrecht, Germany) and into 4.5 ml tubes containing the thrombin inhibitor lepirudin (25 µg/ml Refludan, Dynabyte, Munich, Germany). For blood gas analyses and the analyses of the platelet count, blood was collected into a 2 ml heparin anticoagulated and calcium balanced tube (Bloodgas-Monovette, Sarstedt

AG&Co) and a 4.7 ml EDTA tube (Sarstedt AG).

Multiple Electrode Aggregometry (MEA)

MEA was performed using the Multiplate analyzer and a whole blood impedance aggregometer (Roche AG, Grenzach, Germany) based on the impedance aggregometry described by Cardinal and Flower [11]. The device has five test cells for parallel testing, and each test cell incorporates two independent sensor units. The analysis is based on the aggregation of the activated platelets onto metal sensor wires in the test cell, which increases the electrical impedance between the wires. For measurement purposes, 300 mL of preheated saline (37°C) and 300 mL of heparin-anticoagulated whole blood were placed into the test cell, and the sample was stirred using a Teflon-coated electromagnetic stirrer (800 rpm) over a 3-minute incubation period. Platelet aggregation was initiated using 0.5 mmol/L of arachidonic acid (ASPItest) with commercially available reagents. The increased impedance due to the attachment of platelets to the electrodes was continuously and separately measured by each sensor unit for 6 minutes. The data were transformed into arbitrary aggregation units and plotted as two separate aggregation curves against time. The platelet aggregation in each test was quantified by the area under the aggregation curve (AUC), which was given in arbitrary units called “aggregation units” (U). Standard quality control procedures for each device were routinely performed following the manufacturer’s recommendations.

Data collection

Demographic and clinical characteristics were recorded. Hematological analyses were performed at T1 (at the day before surgical intervention, thus before the first ingestion of Aspirin), T2 (4 h after the first ingestion of 100 mg Aspirin) and T3 (five days after start of daily aspirin therapy). At each measuring

point, blood gas analyses were performed to assess the pH and the corresponding concentrations of hemoglobin and ionized calcium as well as the platelet count.

Outcome analyses

Primary outcome variable

The area under the aggregation curve in the ASPItest of the MEA was defined as primary outcome.

Sample Size Analyses and Statistical Methods

The Sample size analysis was based on data obtained from the manufacturer of the MEA showing a difference in arachidonic acid induced platelet aggregability between heparin and lepirudin anticoagulated tubes of about 20-30 U in healthy individuals. The analysis (change to be detected 20 U, expected standard deviation of 40 U, desired power of 0.8 and an alpha of 0.025) revealed a required sample size of at least $n = 41$ patients to detect statistically significant differences. Considering results of meta-analyses showing a non-response to aspirin of about 20-28 % [12, 13], we decided to include $n = 75$ patients.

Spearman rank order correlations were performed to quantify the association between platelet aggregations in Heparin versus lepirudin anticoagulated blood. Bland-Altman Plots were created to analyze the agreement between lepirudin and heparin anticoagulated samples. The Mann-Whitney Rank Sum Test was used to analyze differences between heparin and lepirudin anticoagulated blood samples at each measuring point. The Wilcoxon Signed Rank test was used to analyze changes at T2 and T3 in comparison to the Baseline at T1. After the Bonferroni Holm adjustment for multiple testing ($p=0.05/2$), $p < 0.025$ was defined as the level of statistical significance. Depending on the distribution of the data (Kol-

mogorov-Smirnov-Test), results are given as mean \pm SD or median (25th and 75th percentiles). The statistical analyses were performed using SigmaStat 3.5 and SigmaPlot 12 (Systat Software GmbH, Erkrath, Germany) software.

Results

A total of $n = 122$ patients were assessed for eligibility. Of those, $n = 47$ did not fulfill the inclusion criteria and $n = 75$ patients were finally enrolled into the study. Table 1 shows demographic and clinical characteristics.

Figure 1 shows an example of arachidonic-acid induced platelet aggregation (ASPItest) analyzed from heparin-anticoagulated blood.



Figure 1: Arachidonic acid induced platelet aggregation (ASPItest). AUC 12 U indicates efficient therapeutic aspirin-induced inhibition of platelet aggregability.

Table 1: Baseline demographic and clinical characteristics

Sex [male]	52 (69)
Age [years]	75 ± 11
BMI [kg/m ²]	27 ± 5
ASA Score	3 (3/4)
euroSCORE	7.1 ± 3.1

The data are presented as numbers (%) or means ± standard deviation.

Figure 2 shows the results of *ex-vivo* induced platelet aggregation in the MEA. Platelet aggregation was higher in lepirudin anticoagulated blood as compared to heparin anticoagulated blood at T1 [69 (36/94) (median (25th / 75th percentiles) U vs. 85 (56/116) U, $p < 0.001$], T2 [27 (14/65) U vs. 48 (34/80) U, $p = 0.009$] and T3 [26 (11/60) U vs. 39 (25/64) U, $p = 0.016$], respectively.

Figure 3 shows the correlation of platelet aggregation between heparin and lepirudin anticoagulated tubes at the measuring points. We found a highly significant correlations (T1: $r = 0.904$, $p < 0.001$; T2: $r =$

0.813 , $p < 0.001$; T3: $r = 0.773$, $p < 0.001$) at each of the measuring points.

Figure 4 shows Bland-Altman Plots analyzing the agreement between lepirudin and heparin anticoagulated samples. In comparison to lepirudin anticoagulated tubes, *in-vitro* aggregability was lower in heparin anticoagulated samples at T1 (-22 ± 16 U), T2 (-13 ± 19 U) and T3 (-10 ± 19 U), respectively.

Table 2 shows physiologic basic conditions for hemostasis and the platelet count at T1, T2 and T3, respectively.

Discussion

It was the aim of the study to compare the extent of platelet aggregability assessed in the MEA using lepirudin or heparin anticoagulated tubes, whereas anticoagulation with lepirudin was considered to be the gold standard for MEA analyses [4, 14]. We found highly significant correlations between the MEA results obtained from lepirudin and heparin anticoagulated tubes at each of the measuring points. In principle, anticoagulation with heparin resulted in lower aggrega-

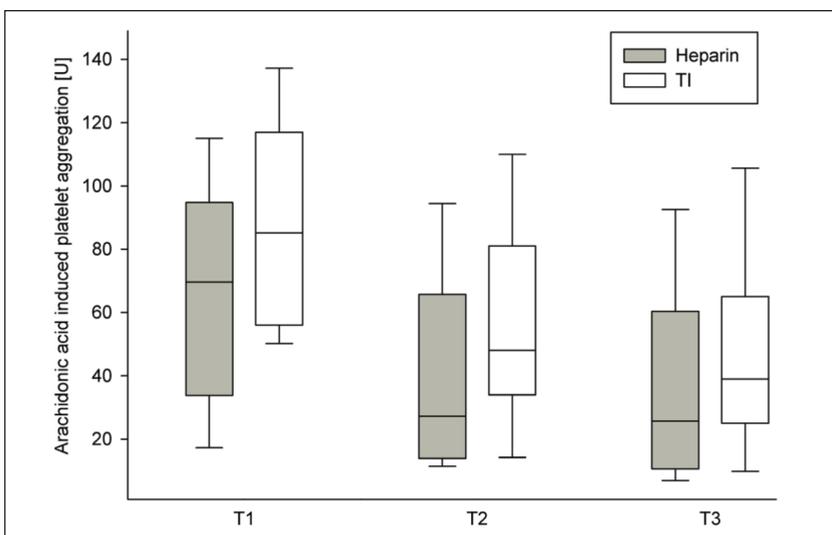


Figure 2: Ex-vivo induced platelet aggregation following stimulation with arachidonic acid at T1 (before the first ingestion of Aspirin), T2 (4 h after the first ingestion of 100 mg Aspirin) and T3 (five days after start of daily aspirin therapy)

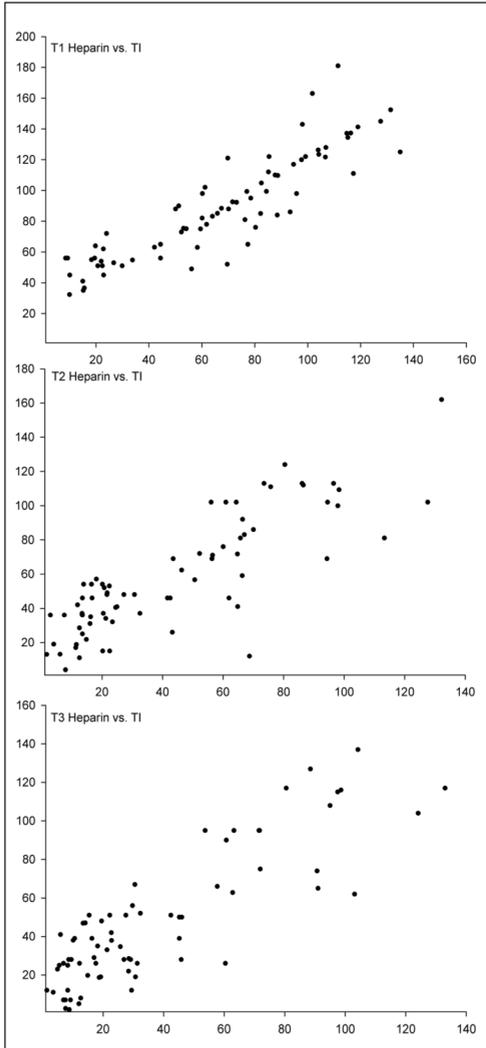


Figure 3: Correlation of arachidonic acid induced platelet aggregability between Heparin- (x-Axes) and Thrombin-Inhibitor (y-Axes) anticoagulated tubes at T1 (before the first ingestion of Aspirin), T2 (4 h after the first ingestion of 100 mg Aspirin) and T3 (five days after start of daily aspirin therapy)

bility as compared to lepirudin-induced anticoagulation of the sample. The main result of our study was that heparin anticoagulated tubes are feasible for monitoring of both, unaffected and (most likely aspirin associated) limited platelet aggregability.

Based on the results of former investigations, usage of lepirudin anticoagulated

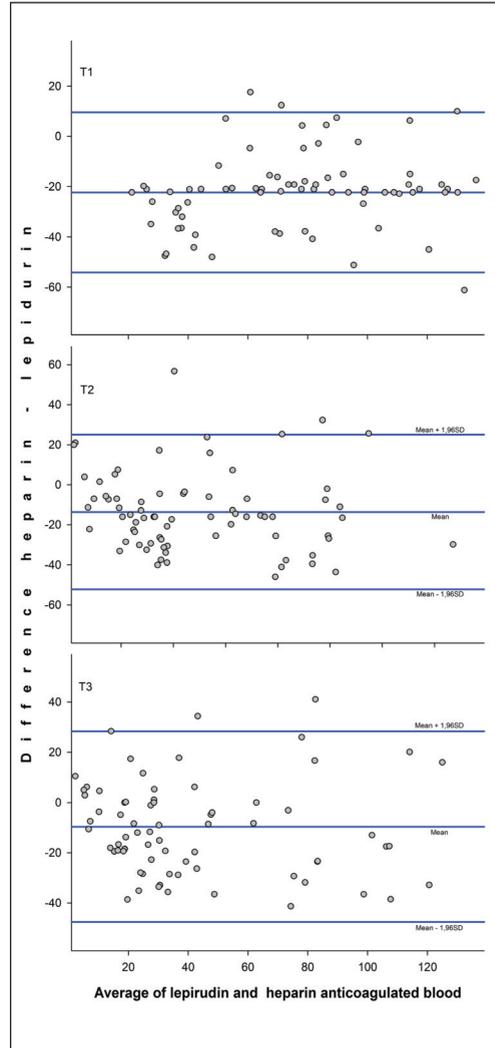


Figure 4: Bland-Altman Plots showing the agreement between lepirudin and heparin anticoagulated tubes at T1, T2 and T3.

blood was considered superior to citrate anticoagulated blood because spontaneous platelet aggregation, which was observed in citrate anticoagulated blood [4], was significantly inhibited by the enzyme apyrase. Furthermore, in contrast to citrate, lepirudin keeps up physiologic calcium concentrations which are necessary for platelet aggregation.

Table 2: Physiologic basic conditions for hemostasis at each measuring point

	T1	T2	T3
T [C°]	36,7 ± 0,7	36,8 ± 0,6	36,9 ± 0,7
pH	7,39 ± 0,05	7,36 ± 0,06	7,38 ± 0,04
Cai [mmol/l]	1,20 ± 0,05	1,20 ± 0,05	1,19 ± 0,04
Hb [g/dl]	11,2 ± 1,6	9,2 ± 1,4	9,2 ± 1,3
Platelet count [nl]	230 ± 64	166 ± 41	191 ± 46

The data are presented as means ± standard deviation. T = Temperature, C = Celsius, Cai = ionized calcium, Hb = Hemoglobin.

Aiming to avoid potential inaccuracy associated with the phenomena “transient heparin induced platelet activation” [7, 8, 15, 16], lepirudin was furthermore favored over heparin.

Major advantages of heparin anticoagulated tubes are that these tubes are low-priced and – because they are used as tubes for blood analyses – almost everywhere available all over a hospital. Furthermore, in contrast to liquid lepirudin anticoagulated tubes, heparin anticoagulated tubes do not need to be refrigerated at 2-8° Celsius. Therefore, for economical and infrastructural reasons, usage of heparin anticoagulated tubes is favorable over lepirudin.

Remarkably, the extent of aggregability differs in dependency of the form of lepirudin. Loreth et al. performed a study comparing blood samples anticoagulated with liquid versus dried lepirudin [14]. The authors observed different extents of aggregability with higher and more accurate and precise measures obtained from blood samples that were anticoagulated with the liquid form. Hence it has to be stated that results from MEA measures have to be interpreted with special regard to the used anticoagulants.

Besides the used anticoagulants, some other parameters and specificities of the blood sample have to be taken into account when interpreting MEA results. MEA measures have been shown to be affected by the platelet count. Hanke et al. showed that platelet aggregability was significantly reduced in samples with platelet counts < 100/nl

[17]. Thus, in thrombocytopenic patients, MEA analyses may misleadingly indicate platelet dysfunctions. For that reason, hemotherapy algorithm that use MEA analyses presume that platelet count was analyzed to be at least 70/nl. In the present study, the platelet count was analyzed at each measuring point. Table 2 shows that platelet count was > 100/nl at each of the measuring points.

Arachidonic acid induced platelet aggregability was reduced at measuring point T1 and T2 indicating sufficient and effective aspirin associated inhibition of platelet aggregability. However, decreased arachidonic acid induced aggregability may be of multifactorial origin. Besides negatively affected physiologic basic conditions for hemostasis like acidosis, hypocalcemia, hypothermia [18, 19] and anemia [20], interaction with colloids [21] and particularly the interaction with foreign surfaces during extracorporeal circulation induce platelet dysfunctions [1, 22, 23]. Table 2 shows physiologic basic conditions for hemostasis that were obtained at each measuring point. The results indicate that basic conditions for hemostasis were within physiologic reference values at each measuring point. However, our study was not designed for quantification of the antiaggregatory effects of aspirin but to study the relation of MEA results obtained from lepirudin versus heparin anticoagulated samples. Therefore, additional analyses like LTA measures or analyzing the plasma concentration of thromboxane had not been performed.

Limitations to the study

There were some limitations to the study. It would have been of scientific and clinical interest if we also had assessed thrombin and ADP-induced platelet aggregation in the MEA. Data of the present study indicate but do not prove that these tests can be performed reliably in heparin anticoagulated samples, too. Furthermore, we did not perform light transmission aggregometry (LTA, Born Aggregometry [24]), which represents the gold standard of platelet function testing. It would have been interesting to analyze potential relations between LTA and MEA analyses of heparin anticoagulated blood samples. Because of this important limitation, data of the present study could not be used to define reference values that may indicate therapeutic inhibition of platelet aggregation. Another limitation to the study was that we did not assess clinical outcome parameters and were consecutively not able to draw any conclusions with respect to potential bleeding disorders or thrombotic adverse events.

Conclusion

This prospective cohort study showed that heparin anticoagulated blood samples are suitable for the reliable assessment of arachidonic acid induced platelet aggregation at the bedside using the MEA.

Speculations

Data of the present study show that heparin anticoagulated tubes may be used for the assessment of platelet function at the bedside using the Multiplate® device. Because of the large availability of the heparin anticoagulated tubes all over the hospital, this may advance and facilitate periprocedural usage of aggregometric measures.

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