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Pneumatic tube transport affects platelet function measured by multiplate electrode aggregometry

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ABSTRACT

Multiple electrode aggregometry (MEA) is used to measure platelet function. Pneumatic tube transport systems (PTS) for delivery of patient samples to a central laboratory are often used to reduce turnaround time for vital analyses. We evaluated the effects of PTS transport on platelet function as measured by MEA. Duplicate samples were collected from 58 individuals. One sample was sent using PTS and the other was carried by personnel to the lab. Platelet function was measured by means of a Multiplate® analyzer using the ADP test, ASPI test, COL test, RISTO test and TRAP test.

Samples transported using PTS showed a reduction of AUC-values of up to a 100% of the average as compared to samples carried by personnel and a majority showed reductions of AUC-values greater than 20% of the average. Bias ± 95% limits of agreement for the ADP test were 26 ± 56% of the average. Bias ± 95% limits of agreement for the ASPI test were 16 ± 58% of the average. Bias ± 95% limits of agreement for the COL test were 20 ± 54% of the average. Bias ± 95% limits of agreement for the RISTO were 14 ± 79% of the average. Bias ± 95% limits of agreement for the TRAP test were 19 ± 45% of the average.

We conclude that PTS transport affect platelet activity as measured by MEA. We advise against clinical decisions regarding platelet function on the basis of samples sent by PTS in our hospital settings.

Introduction

Platelet activation and aggregation is an essential component of a wide range of thrombotic events from acute coronary syndromes and complications related to per-cutaneous coronary intervention (PCI) with stent implantation and cerebral infarction. Anti-platelet therapy in the form of the cyclooxygenase-1 inhibitor aspirin as well as the adenosine diphosphate (ADP) P2Y12 receptor inhibitors clopidogrel and prasugrel are taken by millions, 5-10% of the worldwide population, as primary or secondary prophylaxis against these thrombotic events [1].

Reduced platelet function, due to anti-platelet therapy, can also cause complications in the clinical setting. It increases the risk of bleeding as well as the risk of intracerebral hemorrhage and may cause hemorrhagic complications during PCI or surgery. A measurement of platelet function may explain why a certain patient is exhibiting persistent bleeding after surgery or trauma and also be used to evaluate pharmacological effects of anti-platelet therapy [1,2]. Measurements of platelet function have been shown to predict bleeding and thrombosis during surgery [3,4].

Light transmission aggregometry has traditionally been used to measure platelet function. Although currently the "golden standard" it has several limitations. It requires the preparation of platelet-rich plasma which is both labor intensive and operator dependent. Concerns have been raised that the spinning process may result in the selection of platelet sub-populations of different size, density and activity [5]. It has further been pointed out that the measurement is performed outside the complex biochemical environment of whole blood and thus under altered conditions than the physiologic conditions of platelets [6].

Multiple electrode aggregometry (MEA) has been developed to overcome these limitations by using whole blood instead of platelet-rich plasma. This eliminates the need for spinning and allows a measurement of platelet function under more physiologic conditions [1].

Many large hospitals use a pneumatic tube transport system (PTS) for delivery of patient samples to a central laboratory. This reduces the time delay for analyses and reduces the overall turnaround time. A short time delay for measurements of platelet function is important in view of patients who may bleed during surgery, patients bleeding spontaneously or patients admitted to the emergency unit after trauma. However one important concern is how PTS influences different biochemical analysis, especially cell count and their functions. Insignificant or no effects of PTS transport on routine hematology, coagulation as well as blood gas analyses have been previously shown [7–9]. It has been hypothesized that the accelerations and de-accelerations of the samples in the PTS affect platelet function. This effect of PTS transport on platelet activity remains controversial. Some authors showed the decrease of aggregation values after PTS transport [10,11] while others found that PTS transport does not affect the results of platelet function testing using a multiplate device [12]. An effect on platelet

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activity by PTS transport measured by the platelet function analyzer PFA-100® has also been shown [13].

The aim of this study is to evaluate the extent of pre-analytical effects caused by PTS transport in our center on platelet function measured by MEA.

Methods

Duplicate samples were collected from 32 healthy individuals, 8 patients from an intensive care unit, 4 patients from a coagulation clinic and 14 patients from a cardiovascular unit treated with aspirin, clopidogrel or both. According to the local regulations no ethical approval is required for a method evaluation of unidentified samples. However consent was obtained from all subjects for taking an additional tube. One sample was sent using the PTS and the other was carried by personnel to the lab. Mean transport time by the PTS was 5 minutes. Mean transport time for samples carried by personnel was 15 minutes.

Test-tubes containing hirudin were used for anti-coagulation purposes. The test-tubes were filled with 3 ml blood giving a final concentration of hirudin above 15 μg/ml in each test-tube. The samples were stored at room temperature for 30 minutes before analysis in accordance with the manufacturer's instructions and the maximum time between sample-collection and analysis did not exceed 2 hours. The samples sent by PTS and the samples carried by personnel were analyzed in random order.

The MEA instrument (Multiplate®, Roche, Mannheim, Germany) measures the increase in impedance between a pair of electrodes caused by platelet aggregation. Each test-cell has two pairs of electrodes. The continuous change in impedance between the electrodes is proportional to the amount of platelets adhering to them and the measurements are given in arbitrary units (U).

The platelets are activated by the addition of different aggregation agonists to the respective test-cells. The ADP test uses ADP as an agonist making the test sensitive to ADP-receptor inhibitors such as clopidogrel. The ASPI-subtest uses arachidonic acid as an agonist making the test sensitive to cyclooxygenase-1 inhibitors such as aspirin. The TRAP test uses thrombin receptor peptide as an agonist. This test has a low sensitivity towards aspirin and clopidogrel but is also inhibited by GpIIbIIIa-antagonists. The COL test uses collagen as an agonist which binds to platelet membrane receptor glycoprotein VI. COL test is affected by COX-1 inhibitor such as aspirin The RISTO test uses ristocetin as an agonist which complexes with von Willebrand-factor (VWF), making the test sensitive to von Willebrand disease.

300 μL 0.9% NaCl and 300 μL hirudin blood was added to each test-cell and left to incubate and warm to 37 ° C over three minutes. 20 μL of each reagent was added to the corresponding test-cell, with the exception of ristocetin of which 50 μL was added, in order to activate the platelets. This gives the final amounts of 0,0065 μM ADP, 0,4839 μM ASPI, 0,0032 μg COL, 0,3076 μg RISTO and finally 0,0322 μM TRAP in each corresponding well. All pipetting steps were performed using the electronic pipette connected to the instrument in accordance to the instructions presented on-screen by the instrument itself.

The difference in measurements between each pair of samples was calculated and expressed in percent of the average using a Bland-Altman diagram. In calculating the difference between samples, the measurements of samples transported by PTS were subtracted from measurements of samples carried by personnel and this sum was divided by the average of both samples and multiplied by 100 to yield the difference expressed as percent of the average. The bias, defined as mean difference, and 95% limits of agreement, defined as mean difference ± 2 SD of the difference, as well as the corresponding confidence intervals were calculated as described by Bland and Altman [14].

We also split the samples further into two groups defined by the presence or absence of antiplatelet therapy. We compared the difference between these two groups using an independent t-test with respect to the differences between aggregation values of PTS transported samples and samples transported by personnel.

Statistical analyses were performed using SPSS for Windows 17.0 (SPSS Inc., Chicago, Il, USA) and diagrams were prepared using GraphPad Prism 4.0 (GraphPad Software Inc., Ca, USA).

Results

Samples transported using PTS showed a reduction of up to a 100% of the average as compared to samples carried by personnel and a majority showed reductions greater than 20% of the average. The agreement between samples sent by PTS and samples carried by personnel are presented in Fig. 1. Mean, median, SD and P-values of aggregation values are presented in Table 1. No statistically significant difference between the group currently receiving antiplatelet therapy and the group currently not receiving antiplatelet therapy with regard to the difference in aggregation values of samples transported by PTS and samples transported by personnel were found.

Bias ± 95% limits of agreement for the ADP-subtest were 26 ± 56% of the average. Bias ± 95% limits of agreement for the ASPI test were 16 ± 58% of the average. Bias ± 95% limits of agreement for the COL-subtest were 20 ± 54% of the average. Bias ± 95% limits of agreement for the RISTO were 14 ± 79% of the average. Bias ± 95% limits of agreement for the TRAP test were 19 ± 45% of the average.

Discussion

We have compared platelet function measured by MEA of samples transported by PTS with samples transported by personnel. The main finding is that PTS transport in our center affects platelet function leading to a reduction in platelet aggregation measured by MEA. This finding is in accordance to the report of Bolliger et al. and Boeer et al., but in contrast to the report by Braun et al. [10–12]. This suggests that PTS systems differ in their effect on platelet function.

The effect of PTS transport on platelet aggregation demonstrated in this study was substantial and stable across all five subtests of the MEA-assay. This effect was large enough to influence clinical decision-making. For example a clinician might be interested in a measurement of platelet function in the setting of evaluating the pharmacological effects of anti-platelet therapy [15]. A sample exhibiting a reduction in platelet aggregation because of PTS transport may then cause the clinician to overestimate the effect of anti-platelet therapy.

When split into two groups defined by the presence or absence of antiplatelet therapy, there was no statistically significant difference between the two groups with regards to the difference in aggregation time between samples transported by PTS and samples transported by personnel which suggests that PTS transport does not specifically impact samples taken from patients undergoing antiplatelet therapy.

There are ongoing discussions relating to the concepts of aspirin resistance and clopidogrel resistance in the context of cardiovascular secondary prevention [10,11,15,16]. Due to the lack of standardization in the field of platelet aggregometry both concepts are not well defined. Prevalence rates for aspirin resistance range from 2% to 57% and clopidogrel resistance range from 5% to 30% [15,16]. These are characterized by an inadequate response to aspirin and clopidogrel medication respectively and samples show normal platelet aggregation in spite of medication. A clinician may falsely conclude that a patient is exhibiting an adequate aspirin or clopidogrel responsiveness while the reduction in platelet aggregation supporting this is actually due to the effect of PTS on the sample and not the administered medication.

A number of hypotheses as to the cause of this reduction of platelet aggregation after PTS transport have been proposed. All implicate the shear stress imposed on the sample by the accelerations and de-acceleration in the PTS.