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Multiplate® Platelet Aggregation Findings Are Dependent on Platelet Count but Can Be Corrected by Use of a Ratio

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Abstract: Impedance aggregometry (Multiplate®) detects the effects of platelet aggregation inhibitors and can predict thrombotic complications after coronary and cerebrovascular stent interventions. The bedside method uses whole blood samples not corrected for platelet count. It is claimed but not proved that the findings are unrelated to platelet count in the physiological range. We therefore investigated in the experimental study: (1) whether impedance aggregometry findings and platelet count are correlated and (2) whether the aggregation/platelet count ratio expresses platelet function independent of platelet count. Following ethics committee approval, platelet-rich plasma from healthy probands was diluted with platelet-poor plasma to obtain different platelet counts. Thereafter, platelet count was measured and samples were subjected to impedance aggregometry using thrombin receptor activating peptide (TRAP) for platelet activation. In all probands, impedance aggregometry findings and platelet count were highly correlated (r = 0.88 to 0.94; $p < 0.05$). The combination of all experiments revealed the proportionality between impedance aggregometry findings and platelet count (n = 31, r = 0.78, $p = 0.0001$). In contrast, the ratio of impedance aggregometry findings and platelet count was not significantly correlated with platelet count (r = 0.017; $p = 0.3$) and thus constitutes a specific measure for platelet function. In conclusion, impedance aggregometry findings subsequent to the activation with TRAP are dependent on both platelet function and platelet count. Normalization of impedance aggregometry findings for platelet count can be achieved by a ratio resulting in more specific results.

Keywords: multiplate-device; impedance aggregometry; platelet aggregation; platelet count

1. Introduction

The use of conventional laboratory assays—the Born aggregometry—for the assessment of the platelet function has the disadvantages of being time and resource consuming with poor quality assurance [1]. Whole blood multiple impedance aggregometry with the Multiplate® device (Roche, Mannheim, Germany) has shown a number of advantages in the evaluation of the platelet function. Use of whole blood samples avoids the need for the centrifugation step and the eventual activation of platelets during preparation. Moreover, time requirement for measurement is markedly reduced in comparison to Born aggregometry. In addition, the computerized design and the availability of standardized activators simplify the measurements and therefore allow bedside monitoring of the platelet function [2,3].

Impedance aggregometry has been demonstrated to be useful in several settings [4–6]. In cardiac surgery, an impedance aggregometry-based algorithm was demonstrated to reduce the requirements of blood, plasma and platelet transfusion [7–9]. In patients with coronary artery disease and percutaneous

coronary intervention, impedance aggregometry was useful for the detection of aspirin responsiveness and resistance [10]. In 40 patients, who underwent transcatheter aortic valve implantation due to severe aortic stenosis, multiple aggregometry was useful to detect high reactivity despite the use of clopidogrel (in 42%) and aspirin (in 11%) and the risk of thromboembolic events [6,11]. In patients with intracranial hemorrhage impedance aggregometry was used to evaluate the need for platelet concentrates [12].

As whole blood samples subjected to impedance aggregometry are not corrected for platelet count, an effect of platelet count on the readings of the device can be assumed. However, it is the general opinion, that platelet count affects impedance aggregometry values only in thrombocytopenia with less than 100,000 mm^{-3} [13,14]. In contrast, a recent study demonstrates, that light transmission aggregometry, but not Multiplate® findings, are independent of platelet count in the physiological range [15].

In our study we examined the effect of platelet count on impedance aggregometry findings. We hypothesized that impedance aggregometry findings are dependent on platelet count, and that the correction of impedance aggregometry findings for platelet count using the ratio of the variables results in a variable which is independent of platelet count.

2. Materials and Methods

Blood Sampling: After approval of the ethics committee of the University Hospital Essen, blood samples were drawn for the experimental study from 8 probands without a history of antiplatelet therapy intake or thrombotic disorder. For collection, hirudin-containing tubes (2.7 mL, 0.045 mg r-hirudin; S-Monovette®, SARSTEDT, Nümbrecht, Germany) were used.

Blood fractionation: Platelet-rich plasma (PRP) and platelet-poor plasma (PPP), respectively, were obtained by centrifugation of blood samples at 110 g for 15 min or 1800 g for 10 min at 24 °C.

Dilution of platelets: After measuring the platelet concentrations in PRP using a Coulter counter (KX-21N™ Automated Hematology Analyzer, Sysmex, Kobe, Japan), the cell suspension was diluted with PPP to achieve different platelet concentrations. Resultant platelet concentrations were controlled and ranged from 55,000 to 580,000 mm^{-3} platelets.

Multiple impedance aggregometry: The undiluted and diluted PRP samples were then subjected to multiple electrode impedance aggregometry (Multiplate® analyzer, Roche, Mannheim, Germany) within 30 min to 2 h from blood sampling, and the platelet activator thrombin receptor activating peptide (TRAPtest) was used to induce platelet aggregation.

Statistics: The IBM SPSS statistics package version 22 was used for the statistical analysis. The Pearson correlation coefficient was used for linear correlation analysis. Differences between groups were evaluated after testing for normal distribution (Komolgorov-Smirnov test). A p-value of less than 0.05 was considered significant. For power-analysis, the software GPower 3.1 and the post hoc point biserial model were used [16].

3. Results

In the present study, platelet-rich plasma was diluted with platelet-poor plasma from the same patient to obtain samples with differing platelet counts but identical aggregation characteristics. Measurement of platelet function with impedance aggregometry and determination of platelet count with a Coulter counter demonstrated that impedance aggregometry readings increased with platelet count. The combined results of 8 experiments are shown in Figure 1 and demonstrate a significant correlation of 0.78 ($p = 0.0001$) in a broad platelet count range (55,000–580,000 mm^{-3}) As the regression line passes the origin of the graph, we normalized the impedance aggregometry findings for platelet count calculating the ratio of the variables. The results, shown in Figure 2, demonstrate that the ratio is independent of platelet count ($r = 0.78$, $p = 0.0001$).

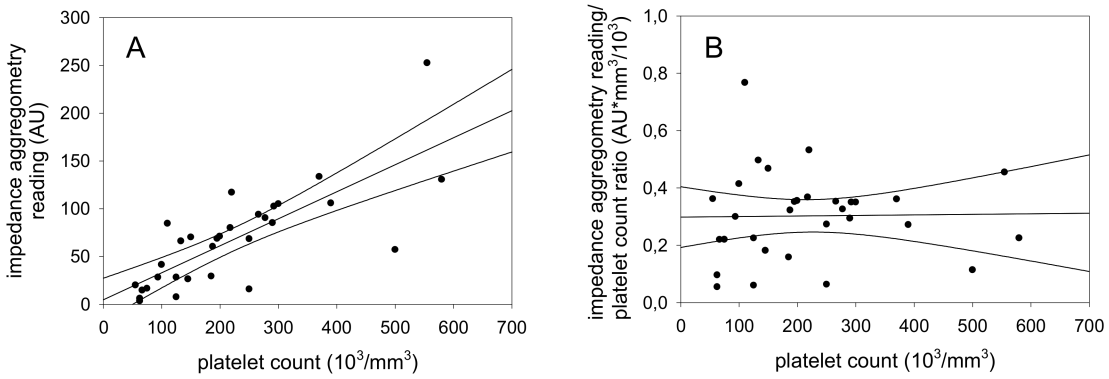

Figure 1. (**A**) The effect of platelet count on the impedance aggregometry reading and (**B**) the ratio of the impedance aggregometry reading and platelet count. Platelet-rich plasma samples were diluted with platelet-poor plasma to obtain different platelet counts. Impedance aggregometry readings were correlated with platelet count (n = 31, r = 0.78, p = 0.0001, power = 0.99). In contrast, the ratio of the impedance aggregometry reading and platelet count was independent of platelet count and thus specific for platelet function (n = 31, r = 0.16, p = 0.4). Shown are individual data points as well as linear regression lines and 95% confidence intervals.

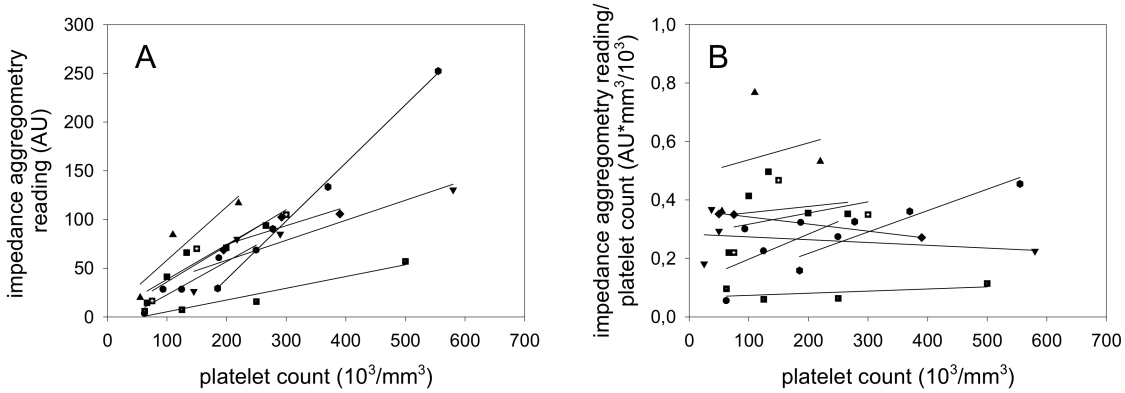

Figure 2. Platelet function as determined with impedance aggregometry in 8 healthy probands. Shown are individual dilution experiments demonstrating the effect of the platelet count on impedance aggregometry readings (**A**) before and (**B**) after correction for the platelet count. Individual correlation in (**A**) ranged from 0.88 to 0.94 ($p < 0.05$). Note, that more than 5-fold differences in aggregation between probands can be observed after correction for the platelet count (**B**).

Although Figure 1A shows a proportionality between the platelet count and impedance aggregometry findings, the variability of some samples was high. To decide whether this finding is due to "biological" differences in platelet function, individual regression curves of the 8 probands were calculated. The results demonstrate that there are marked differences between the healthy probands (Figure 2A). Normalization of impedance aggregometry findings by the platelet count in each of the 8 probands demonstrated that the ratio is capable to differentiate between the platelet aggregation characteristics independent of the platelet count (Figure 2B). The proband with the highest responsive platelets demonstrated a more than 5-fold increased aggregation in comparison to the lowest responsive proband.

4. Discussion

The results of the present study demonstrate that the platelet aggregation findings as obtained by impedance aggregometry are critically dependent on the platelet count: a strict proportional relation between both variables was demonstrated in samples with both reduced, normal and elevated platelet

count, respectively. Correction can easily be achieved by formation of a ratio of impedance reading and the correspondent platelet count.

The conventional procedure to determine platelet function is the Born aggregometry [1,17]. The method determines the transmission of light in a platelet-rich plasma sample upon induction of platelet aggregation by an activator. For this procedure, the platelet count is adjusted to a certain value for reasons of comparability of results. The method is technically demanding, requires a specialized laboratory and is not easy to standardize.

Impedance aggregometry, in contrast, relies on the aggregation of activated platelets to the surface of electrodes. Measurements are performed in anticoagulated whole blood samples and the samples are not adjusted for platelet count before the assay. Several standardized activators are commercially available; thus, a detailed analysis of platelet aggregation characteristics is feasible. Computerized design and the use of unprocessed whole blood enables the determination at the bedside.

The fact that impedance aggregometry uses unprocessed whole blood implicates that there is no correction for platelet count. As shown in the present study, impedance aggregometry readings are dependent on both the platelet function and platelet count. This fact might not be critical when the pattern of aggregation obtained by several activators is compared. For example, clopidogrel leads to a specific reduction of the ADP-induced platelet aggregation, while the response to the remaining activators (arachidonic acid, collagen, thrombin receptor activating peptide) is unaltered. Thus, the differences in impedance aggregometry readings with the different activators might give an impression for eventual action of platelet antagonists. In other clinical situations, the thrombotic risk might likely be defined by both the platelet count and platelet function. However, in other constellations, the interpretation of results might be impossible. For example, either tirofiban or thrombocytopenia can decrease impedance aggregometry findings and the diagnosis of etiology is impossible from the readout. In this case, correction of impedance aggregometry finding will lead to the right diagnosis.

There are, however, some disadvantages of the correction. At first, the complexity of the procedure increases and the use at the bedside might be impossible in some settings. Moreover, errors of both impedance aggregometry and the platelet count measurement increase the combined error, when using the calculation for correction. The combined error will probably increase especially in severe thrombocytopenia and might limit the advantages of correction. One limitation of the present study is the fact that only thrombin receptor activating peptide was used for platelet activation in the present study. However, the suggestion that different activators might lead to divergent platelet count dependencies of impedance aggregometry findings seems rather unlikely.

5. Conclusions

Impedance aggregometry readings obtained with thrombin receptor activating peptide activation of platelets are dependent on the platelet count even in the physiological platelet count range. The readings can be corrected by the ratio of the impedance aggregometry finding and platelet count. Further studies are required to investigate whether the correction results in a better prediction of clinical endpoints.

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