Multiplate® analyzer
Selection of literature summaries
Blood platelets play a pivotal role in physiological hemostasis, but also in the development of arterial thrombosis (myocardial infarction and stroke). Platelet function testing is utilized in the analysis of inherited and acquired platelet function disorders, in the analysis of platelet function in anesthesia and intensive care, and for monitoring of platelet function antagonists.

The Multiplate® analyzer is an easy-to-use instrument to determine platelet function in small quantities of whole blood in a highly standardized manner. The 5 channel analyzer with a broad menu of CE marked tests has been featured by over 400 Medline-listed articles and has been reported by several groups to be useful in tailoring anti-platelet therapy as well as improving the management of bleeding complications in surgical procedures.

This folder contains a selection of literature summaries.
Prediction of coronary stent thrombosis based on Multiplate® platelet reactivity

High residual platelet reactivity in patients on clopidogrel therapy undergoing percutaneous coronary intervention (PCI) is associated with an increased risk of ischemic events.\(^1\)

Sibbing, Braun, Morath et al. (2009) investigated in a prospective trial if platelet reactivity to clopidogrel assessed with multiple electrode aggregometry (MEA, Multiplate\(^\text{®}\)) correlates with the risk of early drug-eluting stent thrombosis (ST).\(^2\) With 1,608 CAD patients enrolled who were scheduled for drug eluting stent PCI, this study is among the largest ones conducted on this topic. The primary end point was definite ST at 30 days.

Before PCI, all patients received 600 mg clopidogrel. Blood was obtained directly before PCI and tested with the Multiplate\(^\text{®}\) ADPtest more than 2 hours after clopidogrel loading. The upper 20% of patients according to Multiplate\(^\text{®}\) measurements (n = 323) were defined as clopidogrel low responders using a cut-off value of 42 U. The statistical analysis was based on the relations of different demographic, clinical, and procedural variables with the platelet reactivity to clopidogrel as obtained with MEA.

Low responders had an approximately 10 times higher risk of definite ST within 30 days compared to normal responders, (2.2% vs. 0.2%; odds ratio [OR]: 9.4; 95% confidence interval [CI]: 3.1 to 28.4; \(p < 0.0001\)) (Fig. 1). Armando Perez de Prado and colleagues however proposed to include further procedural and angiographic variables into the multivariable analysis, like vessel diameter, total stent length, and final pressure to implant the stent.\(^3\) After inclusion of these variables, MEA had even higher predictivity for the occurrence of ST (hazard ratio: 12.0, 95% confidence interval: 2.3 to 63.2; \(p = 0.003\)).\(^4\)

In Sibbing et al’s (2009) study hirudin anticoagulated blood was used for analysis. The optimal cut-off value to predict the occurrence of 30-day stent thrombosis according to ROC analysis was 47 U.

This study has been featured in an article in “www.theheart.org”\(^5\) where Sibbing stated: “We can’t say whether the MEA assay is better than other assays or not, but we are very happy with it, and we are convinced by the results we have seen in this study. It also works out at a reasonable cost for each test. When we started this study, we were not routinely measuring platelet responsiveness in the cath lab, but now we are measuring it with this device”.

### Definite stent thrombosis 30 days post DES-PCI\(^2\)

<table>
<thead>
<tr>
<th>Odds ratio</th>
<th>95% CI</th>
<th>(p)</th>
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<tbody>
<tr>
<td>9.4</td>
<td>3.1-28.4</td>
<td>(&lt;0.0001)</td>
</tr>
</tbody>
</table>

**Fig. 1**

MEA measurements are highly predictive for the occurrence of stent thrombosis
2010 Sibbing et al. also reported the six-month follow-up results of these study patients.\(^6\)

Low responders had an approximately 7 times higher risk of definite stent thrombosis within 6 months compared to normal responders, (2.5% vs. 0.4%; OR: 6.5; 95% [CI]: 2.4 – 17.0; \(p < 0.001\)) (Fig. 2).

In the same year Schulz and colleagues published 1-year follow-up data of the same patient cohort.\(^7\) Low responders had significantly more stent thrombosis (2.5% vs. 0.5%, HR 5.4, 95% CI 1.9-15.6, \(p = 0.002\)), Q-wave myocardial infarctions (2.5% vs 0.6%, HR 4.0, 95% CI 1.5-10.7, \(p = 0.005\)), and ischemic strokes (1.3% vs 0.2%, HR 5.4, 95% CI 1.2-24.0, \(p = 0.028\)) (Fig. 3).

Of note is the observation that the majority of low responders to clopidogrel identified by MEA suffer ischemic events early in the course following PCI, usually within 3 months.

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References
5. www.theheart.org/article/946661.do
Clinical relevance of the Multiplate® in neuroradiology

Müller-Schunk, Linn, Peters, Spannagl et al. conducted the first outcome related clinical trial with the Multiplate® analyzer, published in 2008 in AJNR. 50 neurologic patients scheduled for supra-aortic stent placement were enrolled. The aim was to determine platelet inhibition after clopidogrel treatment using the Multiplate® analyzer and correlate the results with the clinical outcome. Adverse events were registered in 10% of patients, all of them were clopidogrel non-responder as assessed with multiple electrode aggregometry (MEA). In contrast, none of the clopidogrel responders suffered an adverse event (Fig. 1). A statistically significant correlation of clopidogrel non-response and adverse events has been reported in this study.

Adverse events in supra-aortic stent placement

- Responders to clopidogrel
- Non-responders to clopidogrel

High platelet reactivity on clopidogrel is associated with increased risk of adverse events in neuroradiology patients
References

Various medical centers use the Multiplate® analyzer to assess and monitor platelet function in patients receiving anti-platelet drug therapy, like Aspirin® or clopidogrel, to minimize the risk of on-treatment complications.

High residual platelet reactivity (HPR) in patients on clopidogrel therapy undergoing percutaneous coronary intervention (PCI) is associated with increased risk of ischemic events (e.g. stent thrombosis (ST)). Prasugrel and ticagrelor are novel potent ADP-receptor inhibiting drugs that act more consistently and significantly reduce the risk of ischemic events compared to clopidogrel therapy. However, these drugs are also associated with a risk of increased major bleeding and other side effects that negatively impact compliance and patient outcomes.

Furthermore, the treatment of patients with prasugrel or ticagrelor is up to 10 – 15 times more expensive compared to clopidogrel.

Several studies support that routine tailoring of anti-platelet therapy using Multiplate® testing has the potential to significantly reduce the incidence of major adverse ischemic complications.

A study of Hazarbasanov, Velchev, Finkov et al. (2012) aimed to analyze the clinical impact of a platelet function-guided anti-platelet therapy compared to standard dose clopidogrel treatment in patients after PCI. This prospective, randomized, open-label, interventional study enrolled 192 patients. The primary end point was the incidence of major adverse cardiac and cerebrovascular events such as cardiac death, myocardial infarction, stent thrombosis or ischemic stroke at six months (180 days).

Twenty-four hours before PCI, all patients received either 300 mg (patients with angina pectoris) or 600 mg (patients with acute coronary syndrome) loading dose of clopidogrel. In the tailored group, platelet function was tested with the Multiplate® ADPtest 24 hours after clopidogrel loading and at indicated time points. Patients with HPR (>46 U) received an additional 600 mg loading daily dose and 150 mg daily dose of clopidogrel for 30 days. Thereafter the 75 mg daily dose was resumed for the remainder of the study. Patients in the non-tailored group received a standard daily dose of 75 mg clopidogrel for 180 days after PCI.

During 6 months of follow-up, no ischemic events occurred in the Multiplate® tailored patient group. However, 5.3% of patients undergoing uniform standard dose clopidogrel therapy suffered severe cardiac and cerebrovascular complications such as cardiac death or myocardial infarction (Fig. 1).

Routine tailoring of anti-platelet therapy using Multiplate® testing has the potential to significantly reduce the incidence of major adverse ischemic complications.
Hazarbasanov et al.’s (2012) study demonstrated that a treatment strategy incorporating the Multiplate® analyzer to assess a tailored dose of clopidogrel may reduce the incidence of ischemic events in patients after PCI.

Another study by Sibbing, Mayer, Bernlocher et al. (2012) aimed to investigate whether Multiplate® tailored antiplatelet therapy with prasugrel in patients displaying HPR on clopidogrel treatment reduces the incidence of stent thrombosis after PCI. Before PCI, all patients received a loading dose of 600 mg clopidogrel. In the study design one group of patients received a standard clopidogrel treatment whereas another cohort of patients were switched from clopidogrel to prasugrel based on Multiplate testing. The primary outcome was the incidence of stent thrombosis within 30 days after PCI.

Stent thrombosis was 4-fold higher in the patient group without anti-platelet therapy adjustment compared to the patient group with Multiplate-tailored treatment (Fig. 2). Routine platelet function testing for guidance of anti-platelet treatment markedly reduced risk of stent thrombosis in patients with HPR while on clopidogrel therapy.

References
Cardiology consensus paper on high on-treatment platelet reactivity

The addition of ADP receptor antagonists to Aspirin® treatment reduces ischemic events in patients with cardiovascular disease. However, recurrent ischemic events during dual anti-platelet therapy, including stent thrombosis, remains a major concern. Platelet function measurements demonstrate a highly variable inhibition of ADP induced platelet function under clopidogrel treatment. High on-treatment platelet reactivity to adenosine diphosphate (ADP) is observed in a significant proportion of clopidogrel-treated individuals.

Multiple studies have demonstrated a clear association between high on-treatment platelet reactivity to ADP and the occurrence of adverse events. This review provides a consensus opinion on the definition of high on-treatment platelet reactivity to ADP based on various methods and proposes how identification of HPR may be used in patient care.

Multiplate® analysis was included in this consensus document. Bonello summarized various studies linking high on-treatment platelet reactivity to ischemic events. The best prediction of ischemic events was associated with the Multiplate® analyzer, with an odds ratio of 12.0, while other studies using for example the VerifyNow system showed an odds ratio of only 2-3 (Fig. 1).

The document also provides a consensus statement of the definition of high platelet reactivity to ADP on the Multiplate® analyzer, defined as an aggregation $>47$ U (468 AU*min).

### Multiplate – highest odds ratios in consensus paper

<table>
<thead>
<tr>
<th>Study</th>
<th>Endpoint</th>
<th>Odds ratio [Risk enhancement]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sibbing et al. (2009)</td>
<td>30-day ST</td>
<td>12.0</td>
</tr>
<tr>
<td>Cuisset et al. (2009)</td>
<td>1-month ST</td>
<td>5.8</td>
</tr>
<tr>
<td>Gurbel et al. (2008)</td>
<td>2-year post-PCI MACE</td>
<td>3.9</td>
</tr>
<tr>
<td>Breet et al. (2010)</td>
<td>1-yr death, MI, ST, and stroke</td>
<td>2.1</td>
</tr>
<tr>
<td>Marcucci et al. (2009)</td>
<td>1-yr CV death and nonfatal MI</td>
<td>2.8</td>
</tr>
<tr>
<td>Breet et al. (2010)</td>
<td>1-yr death, MI, ST, and stroke</td>
<td>2.5</td>
</tr>
<tr>
<td>Breet et al. (2010)</td>
<td>1-yr death, MI, ST, and stroke</td>
<td>2.2</td>
</tr>
<tr>
<td>Blindt et al. (2007)</td>
<td>6-month ST</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Fig. 1

Cardiology consensus paper is supporting best predictivity of Multiplate® analysis
References

Diagnostic accuracy of genotyping vs. phenotyping for the prediction of atherothrombotic events

Clopidogrel is a state of the art oral, thienopyridine class anti-platelet agent used in patients undergoing percutaneous coronary intervention (PCI). However, clopidogrel therapy has limitations due to inter-individual response variability and potential drug-drug interactions. Because clopidogrel is metabolized by a highly polymorphic cytochrome P450 (CYP) hepatic system, a possible strategy for identifying patients who will not properly respond to clopidogrel is to genotype the CYP2C19 enzyme. Considering that genotyping is a relatively costly and time-consuming method, several phenotyping methods have been proposed to monitor platelet function during clopidogrel treatment. The aim of the PEGASUS-PCI study by Siller-Matula et al. was to compare the diagnostic accuracy of genotyping vs. phenotyping for the prediction of ischemic and bleeding events in patients with coronary artery disease undergoing PCI with drug-eluting stents.

The study enrolled 416 consecutive patients meeting the study criteria who received a 600 mg loading dose of clopidogrel followed by a daily maintenance dose of 75 mg. Genomic DNA for CYP2C19 genotyping was obtained from the patients’ blood samples. Patients with a loss of function CYP2C19*2 allele were classified as poor metabolizers, patients with a gain of function CYP2C19*17 allele as ultra-metabolizers, and patients with a CYP2C19*1 allele as regular metabolizers. The study had a follow-up of one year and the primary efficacy endpoint was the incidence of definite and probable stent thrombosis (ST). ROC curve analysis demonstrated that platelet aggregation assessed by MEA [ADPtest HS (ADP+PGE1) or ADPtest] was the best predictor of subsequent stent thrombosis (area under the curve = c-index = 0.9 and 0.78 respectively); whereas other assays (VASP, CPA and PFA-100) failed to distinguish between patients with and without ST (c-index < 0.67) (Table 1). Furthermore, MEA demonstrated much higher specificity and sensitivity than the VASP, CPA, PFA-100 assays and even genotyping of the CYP2C19*2 carrier status. Although all tests had a similarly high negative predictive value (93-100%), MEA ADPtest HS showed the highest positive predictive value (13% vs. 3-7% for the other assays) (Table 1).

The following devices were used to assess clopidogrel response post-PCI:

- Whole blood multiple electrode aggregometry (MEA, Multiplate® analyzer) with ADPtest and ADPtest HS (ADP + PGE1)
- PFA-100 (CADP cartridge)
- Cone and platelet analyzer (CPA, ImpactR)
- VASP phosphorylation assay

MEA outperforms genotyping and other platelet phenotyping methods in a comparative study.
Kaplan-Meier curves showed a very early separation of ST rates between clopidogrel low-responders (MEA aggregation ≥ 48 U, 12.5%) compared with the regular or ultra-responders (aggregation < 48 U, 0.3%; P < 0.001) (Fig. A), which was not shown for CYP2C19 genotyping (Fig. B). According to multiple logistic regression model Multiplate® ADP HS test was identified as an independent predictor of stent thrombosis (OR = 36.9, 95% CI 0.43-319).

In conclusion, the PEGASUS-PCI study showed that assessment of platelet response to clopidogrel by MEA was a better predictor of stent thrombosis than genotyping.

Table 1: Statistical estimates for the prediction of stent thrombosis by different assays for assessment of responsiveness to clopidogrel.

<table>
<thead>
<tr>
<th>Test</th>
<th>c-index (95% CI)</th>
<th>P</th>
<th>Cut-off</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
<th>Positive likelihood ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiplate ADPtest HS (U)</td>
<td>0.90 (0.86-0.95)</td>
<td>&lt; 0.001</td>
<td>48</td>
<td>90</td>
<td>83</td>
<td>13</td>
<td>100</td>
<td>3.5</td>
</tr>
<tr>
<td>Multiplate ADPtest (U)</td>
<td>0.78 (0.63-0.94)</td>
<td>0.002</td>
<td>46</td>
<td>70</td>
<td>67</td>
<td>7</td>
<td>100</td>
<td>2.1</td>
</tr>
<tr>
<td>VASP (% PRI)</td>
<td>0.62 (0.46-0.79)</td>
<td>0.204</td>
<td>42</td>
<td>70</td>
<td>38</td>
<td>3</td>
<td>98</td>
<td>1.1</td>
</tr>
<tr>
<td>PFA100: CADP-CT(s)</td>
<td>0.86 (0.48-0.84)</td>
<td>0.084</td>
<td>105</td>
<td>70</td>
<td>61</td>
<td>4</td>
<td>98</td>
<td>1.8</td>
</tr>
<tr>
<td>Impact-R: ADP (SC %)</td>
<td>0.62 (0.47-0.76)</td>
<td>0.205</td>
<td>4.6</td>
<td>90</td>
<td>36</td>
<td>3</td>
<td>98</td>
<td>1.4</td>
</tr>
<tr>
<td>Impact-R: ADP (AS µm²)</td>
<td>0.45 (0.25-0.65)</td>
<td>0.606</td>
<td>43</td>
<td>60</td>
<td>42</td>
<td>3</td>
<td>98</td>
<td>1.0</td>
</tr>
<tr>
<td>CYP2C19*2</td>
<td>0.56 (0.32-0.69)</td>
<td>0.950</td>
<td>*2/*2</td>
<td>30</td>
<td>71</td>
<td>3</td>
<td>93</td>
<td>1.0</td>
</tr>
</tbody>
</table>

A. Phenotyping

- MEA < 48 U (responder + ultra responder)
- MEA ≥ 48 U (poor responder)

B. Genotyping

- CYP2C19*1/*1 (regular metabolizer)
- CYP2C19*1/*2 or *2/*2 (poor metabolizer)

Adapted from Siller-Matula JM et al. (2012)

References
Drug tailoring algorithm with Multiplate® in PCI

A study by Christ et al. (2011) has shown that routine tailoring of anti-platelet therapy using Multiplate® testing helps prevent early definite stent thrombosis (ST) in compliant patients.

In this prospective, single center cohort study on-treatment platelet reactivity was measured via MEA in 507 patients undergoing PCI.

All patients undergoing stent placement received a personalized anti-platelet treatment of aspirin and either clopidogrel or prasugrel depending on the clinical status and platelet reactivity test result. STEMI patients received first line prasugrel, while all other patients received clopidogrel and were tested for their platelet response.

Patients with a low response to the anti-platelet treatment (ADPtest > 50 U) were either reloaded with clopidogrel (patients with prior cerebrovascular events) or with prasugrel (all other patients).

Definite events of ST within 30 days of study follow-up defined the primary endpoint, whereas probable ST and cardiovascular death events served as the secondary endpoints. After 30 days of study follow-up no primary endpoint events occurred in compliant patients undergoing Multiplate®-tailored dual anti-platelet therapy.

According to the study results Christ et al. proposed the following treatment algorithm for the personalized anti-platelet therapy guided with Multiplate®:

- STEMI patients are treated first line with Prasugrel*.
- NSTEMI and stable CAD patients receive clopidogrel first line and are subsequently monitored using Multiplate® ADPtest > 12 hours after clopidogrel loading.
- An intensified anti-platelet regimen is initiated in patients with clopidogrel low response**.

A personalized anti-platelet treatment algorithm is helpful to prevent stent-thrombosis after PCI.
*In case of contraindications to prasugrel (history of stroke, intracranial hemorrhage, low body weight (< 60 kg), old age (> 75 y) first line therapy with clopidogrel (600mg loading, 75mg maintenance dose) is considered.

**Clopidogrel low-responders receive an intensified drug regimen: Either prasugrel loading (60mg) and 10 or 5 mg in the maintenance phase (depending on age and body weight) or, if contraindications for prasugrel are present, repeated loading with clopidogrel (600mg) is considered. Assessment of response to intensified drug therapy with repeated platelet function testing.

References
Inhibition of platelet activation in myocardial infarction or stroke patients via anti-platelet drugs is a well established therapy for the prevention of vascular events. The golden standard is the dual anti-platelet therapy with clopidogrel and Aspirin. As clopidogrel is a pro-drug, requiring two-step hepatic metabolism, there is a wide inter-patient variability in response to clopidogrel. Many factors, such as genetic polymorphism of hepatic enzymes, gender, age, obesity, co-morbidities or co-medication, lead to diminished clopidogrel responsiveness in approximately 20% - 25% of patients.1

For instance, high residual platelet reactivity (HPR) in patients on clopidogrel therapy undergoing percutaneous coronary intervention (PCI) is associated with increased risk of ischemic events (e.g. stent thrombosis). Prasugrel and ticagrelor are novel potent ADP-receptor inhibiting drugs which were shown to act more consistently and significantly reduce the risk of ischemic events compared to clopidogrel therapy. However, these drugs are also associated with a risk of increased major bleeding and other side effects which negatively impact on compliance and patient outcome. Further, the treatment of patients with prasugrel or ticagrelor is much more expensive and is not yet widely used in many countries. Thus, clopidogrel treatment at standard dose is still a state of the art therapy.

However, clopidogrel low/non-responder patients who are at increased risk of ischemic events, would benefit from switching from clopidogrel to more potent platelet inhibitors; whereas clopidogrel normal responders would still be benefiting from safe and cheap therapy without exposure to increased bleeding risk.

The MADONNA (Multiple electrode Aggregometry in patients receiving Dual antiplatelet therapy to guide treatment with Novel platelet Antagonists) study of Siller-Matula et al.2 could prove that tailored anti-platelet treatment using Multiplate® results in an improved therapeutic efficacy with an equal safety compared to the standard treatment.

This prospective non-randomized non-blinded study with 798 patients on clopidogrel and undergoing PCI compared two cohorts (tailored and non-tailored treatment) with a follow-up of one month. Patients in the non-tailored cohort (n=395) received only one clopidogrel loading dose of 600 mg at least 2 h before PCI. In the tailored group (n=403) clopidogrel responsiveness was repeatedly determined via ADPtest using multiple electrode aggregometry (MEA) and non-responders (≥ 50 U) received repeated loading doses of clopidogrel or prasugrel.

Tailoring anti-platelet therapy with Multiplate® results in an improved therapeutic efficacy with an equal safety compared to the standard treatment.

Tailoring platelet inhibitory drugs with Multiplate® reduces the incidence of major cardiac events.
The primary efficacy end point was the incidence of definite and probable stent thrombosis during a 30-day follow-up, whereas the secondary efficacy parameters were acute coronary syndrome (ACS) and cardiovascular death. During 1-month follow-up, significantly less events of definite and probable stent thromboses occurred in the Multiplate-tailored patient cohort (0.2%) than in the non-tailored group (1.9%) (Fig. 1). In agreement with this, the incidence of ACS was also significantly lower in the guided than in the non-guided group (0% vs. 2.5%; \( p = 0.001 \)), whereas no difference in the event rates of cardiac death (2% vs. 1.3%; \( p = 0.422 \)) or major bleedings (1% vs. 0.3%; \( p = 0.186 \)) were found between guided vs. non-guided group.

The present study demonstrated that platelet function testing using Multiplate® analyzer results in more efficient personalized and safe therapeutic strategy than standard treatment.

References
Definition of a therapeutic window for platelet inhibition in PCI

High residual platelet reactivity (HPR) in patients on clopidogrel therapy undergoing percutaneous coronary intervention (PCI) is associated with an increased risk of ischemic events. However, an enhanced response to clopidogrel is associated with a higher risk of bleeding. Adverse ischemic advents as well as increased bleeding are both determinants affecting mortality.

Because Sibbing et al. established the optimal cut-off values in the Multiplate® ADPtest of 468 AU•min for clopidogrel low responders and 188 AU•min for enhanced clopidogrel responders previously. In the present study they aimed to explore a potential therapeutic window with 2533 patients undergoing PCI. All patients were pre-loaded with 600 mg clopidogrel and ADP-induced platelet aggregation was measured directly prior procedure. The primary efficacy end point was the 30-day incidence of definite or probable stent thrombosis (ST), whereas the primary safety end point was the incidence of in-hospital major bleeding according to Thrombolysis In Myocardial Infarction (TIMI) criteria. Using the pre-defined cut-off values, patients were allocated to 3 different groups: 975 patients (38%) were classified as enhanced responders (≤188 AU•min), 428 patients (17%) as low responders (≥468 AU•min), and 1,130 patients (45%) as normal responders (189 - 467 AU•min).

The primary efficacy end point (definite or probable ST) occurred in 22 patients (0.9%), and the primary safety end point (TIMI-major bleeding) was observed in 34 patients (1.3%). Clopidogrel low responders had the highest incidence of ST (2.8%), whereas enhanced responders had the highest incidence of major bleeding (2.2%). No significant differences were observed between normal and enhanced responders for ST (p = 0.38) or between normal and low responders for bleeding (p = 0.72). The overall risk of combined adverse events was significantly lower in normal responders compared with the remaining patient cohort of high and low responders (N=1,403; OR: 0.40; 95% CI: 0.22 to 0.75; p= 0.003) (Fig. 1).

Platelet function testing by MEA can assess the quality of clopidogrel response balancing bleeding and thrombotic risk.
The present study provides an evidence for the existence of a so-called therapeutic window or a “sweet spot” of P2Y12-receptor inhibition, as patients with platelet aggregation values within the “normal range” (189 - 467 AU•min) have a low risk for both adverse events, ST and major bleeding (Fig. 2). Thus, platelet function testing with MEA provides important information on individual patient risk status under ADP-receptor inhibitor treatment.

**References**


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www.roche.com
High residual platelet reactivity in patients on clopidogrel therapy has been associated with increased risk of thrombotic events in PCI; however, it is also recognized that enhanced response to clopidogrel carries an associated risk of increased major bleeding. Both conditions significantly increase mortality.

The association of enhanced platelet response to clopidogrel treatment in PCI as evidenced by low levels of platelet reactivity measured with the Multiplate® analyzer and its association with bleeding was first elucidated by Sibbing, Schulz, Braun, Morath et al. (2010).1

Sibbing’s group evaluated 2,533 CAD patients scheduled for drug-eluting stent PCI. All patients received a clopidogrel loading dose of 600 mg. Blood was obtained directly before PCI, more than 2 hours after clopidogrel loading and tested with the Multiplate® ADPtest.

ROC analysis identified the optimal cut-off of 19 U to identify enhanced responders. The incidence of major bleeding was significantly higher in enhanced clopidogrel responders (n = 975) as compared with the remaining patients (n = 1,558) [21 (2.2%) vs. 13 (0.8%); OR 2.6, 95% CI 1.3 –5.2; P = 0.005] (Fig. 1).
References

Multiplate® analysis: Prediction of bleeding risk and blood transfusion in cardiac surgery

Reece et al. (2011) investigated the Multiplate® analyzer for determining platelet aggregation in 44 patients during coronary artery surgery. The results demonstrated that platelet aggregation was reduced during and after cardiopulmonary bypass surgery (CPB) as compared to baseline values with evidence of slight recovery in platelet function post chest closure. Patients receiving transfusion products displayed lower levels of ADP and TRAP induced platelet aggregation than patients not receiving transfusions (Fig. 1). It was concluded that the Multiplate® analyzer can detect platelet dysfunction in the setting of CPB.

Rahe-Meyer et al. (2008) showed in a study of 100 surgical patients that in the case of possible Aspirin® ingestion, the Multiplate® system is a better predictor of platelet related coagulopathy or transfusion than patient self-reporting.

Ranucci et al. (2011) aimed to determine if a preoperative test of platelet function could determine postoperative risk of excessive bleeding and transfusion requirements. They tested 87 patients who discontinued thienopyridine treatment no more than 7 days prior to surgery.

Multivariable linear regression analysis confirmed the Multiplate® ADPtest (p = 0.007) as independently associated with postoperative bleeding. The accuracy of prediction was good with an area under the curve of 0.71, p = 0.013. Using the Youden index the best cut-off value for the ADPtest was determined to be 31 U with an associated sensitivity of 72%, specificity of 66%, negative predictive value of 92%, and positive predictive value of 29% (Fig. 2). The incidence of excessive bleeding was significantly higher in patients with ADPtest results below the cutpoint (31 U) in comparison to patients with ADPtest results higher than the cutpoint (29% vs. 8%).

Patients with an ADPtest < 31 U are at 3.5 times higher risk of massive bleeding during cardiac surgery
Platelet dysfunction and bleeding in cardiac surgery

- Patients with excessive bleeding

Cut-off value 31 U

Sens = 72%
Spec = 66%
NPV = 92%
PPV = 29%

ADP - induced platelet aggregation [U]

Number of patients [n]

Fig. 2

References
Multiplate® – Point-of-care testing for guided hemostatic therapy in cardiac surgery

Perioperative coagulopathy and the use of allogenic blood products are independent risk factors for increased mortality and major perioperative adverse events in patients undergoing cardiac surgery. Therefore, early and specific diagnosis of the underlying hemostatic pathology and tailored treatment have a significant importance in clinical routine.

Standard laboratory coagulation tests are of limited diagnostic value due to the pathophysiological complexity of perioperative bleeding and a long turnaround time. In contrast, thromboelastometry (Rotem®) and multiple electrode aggregometry (MEA; Multiplate®) deliver test results in a short time and provide complementary information on hemostasis.

The trial of Weber et al. (2012)¹ aimed to study the efficacy of hemostatic therapy guided either by conventional coagulation analyses (platelet count, hemoglobin concentration, fibrinogen concentration, INR, aPTT) or point-of-care (POC) testing via Rotem® and Multiplate® in coagulopathic patients undergoing complex cardiac surgery.

In this prospective study 100 patients with diffuse bleeding after heparin reversal or increased blood loss during the first 24 postoperative hours were randomized either to the conventional laboratory testing group (N=50) or POC-guided group (N=50).

The primary outcome measure was the number of transfused units of packed erythrocytes during the first 24 h after inclusion, whereas the secondary outcome measures were post-operative blood loss, use and costs of hemostatic therapy, and clinical outcome parameters.

Patients from the POC-guided group had less blood loss at each of the post-operative measuring points (Fig. 1a) and received less cumulative dosages of packed erythrocytes (Fig. 1b). Further, significantly less patients from the POC-guided group received fresh frozen plasma (FFP) transfusion (Fig. 1c). Cumulative platelet concentrates (PC) transfusion requirements were also lower in the POC patient cohort (Fig. 1d).

Point-of-care coagulation testing with the Multiplate® and thromboelastometry (Rotem®) reduces allogenic blood transfusion and is associated with improved clinical outcomes.
Besides this, patients in the POC-guided group had shorter postoperative ventilation times and duration of intensive care unit (ICU) stay as well as lower incidence of composite adverse events than patients in the conventional group.

Further, Kaplan-Meier analysis demonstrated lower mortality of the POC-guided patients during a 6-month follow-up (4% vs. 20%; p=0.013) (Fig. 2).

The treatment algorithm based on the POC-testing with Rotem® and Multiplate® resulted in significant costs savings of hemostatic therapy. As depicted in table 1, the average patient cost for the POC-guided coagulation treatment was 1,528 € vs. 3,109 € for the conventional management.

Hemostatic therapy algorithms guided by the POC testing not only reduced patient exposure to allogenic blood products and decreased number of transfused units of packed erythrocytes but also lowered fresh frozen plasma and platelet concentrate usage. Further, POC-guided algorithm significantly improved clinical outcome and provided significant costs benefits compared to the standard treatment.

### Cumulative costs in Euro [€] for applied treatments

<table>
<thead>
<tr>
<th></th>
<th>Conventional group</th>
<th>POC-guided group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packed erythrocytes [72 €/U]</td>
<td>18,648</td>
<td>13,176</td>
</tr>
<tr>
<td>FFP [0.162 €/g]</td>
<td>13,530</td>
<td>4,665</td>
</tr>
<tr>
<td>PC [231 €/U]</td>
<td>28,755</td>
<td>15,123</td>
</tr>
<tr>
<td>Fibrinogen [233 €/g]</td>
<td>28,755</td>
<td>27,727</td>
</tr>
<tr>
<td>PCC [114 €/600 IU]</td>
<td>10,944</td>
<td>6,726</td>
</tr>
<tr>
<td>rVIIa [2,784 €/240 kIU]</td>
<td>44,544</td>
<td>5,568</td>
</tr>
<tr>
<td>Total blood products and hemostatic therapy</td>
<td>155,531</td>
<td>76,397</td>
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<tr>
<td>Rotem®</td>
<td>–</td>
<td>4,093</td>
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<tr>
<td>Multiplate®</td>
<td>–</td>
<td>2,427</td>
</tr>
<tr>
<td>Cumulative [€]</td>
<td>155,431</td>
<td>82,918</td>
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<tr>
<td>Mean costs per patient [€]</td>
<td>3,109</td>
<td>1,658</td>
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</table>

Tab. 1 according to Weber et al.¹

FFP = fresh frozen plasma; IU = international units; PC = pooled platelet concentrate; PCC = prothrombin complex concentrate; rVIIa = recombinant activated factor VIIa concentrate.

### References

Multiplate® – A rapid functional assay in heparin-induced thrombocytopenia (HIT)

Heparin-induced thrombocytopenia (HIT) is a potentially severe complication of anticoagulant therapy with unfractionated heparin and less likely with low molecular weight heparin. Some patients develop abnormal antibodies of the IgG class against heparin when it is bound to the platelet protein platelet factor 4 (PF4). This antigen–antibody complex subsequently binds to the FcγIIa receptor on the platelet surface and leads to platelet activation and resulting thrombocytopenia. This thrombocytopenia may be associated with venous or arterial thrombosis but is rarely associated with bleeding. Thus, an accurate and rapid diagnostic tool is crucial to determine HIT.

The accuracy of HIT diagnosis may be improved by the combination of an immunoassay with a functional assay. An enzyme-linked immunooassay (ELISA) detects the presence of anti-PF4-heparin antibodies and is suitable to exclude HIT with low clinical probability. The ^14C-serotonin release assay (SRA) is a functional test that is considered the gold standard. Another functional test for HIT diagnosis is light transmission aggregometry (LTA) using platelet-rich plasma (PRP). However, these functional assays are complex, time consuming, technically demanding, require qualified medical staff and consequently are not suitable for routine use.

Morel-Kopp et al.’s (2012) study^1 demonstrated, that the Multiplate® analyzer is not only an easy-to-use and rapid test system, but its high sensitivity and specificity are comparable to those of the accepted gold standard SRA. The prospective study of Galea et al. (2012) aimed to evaluate the usability of heparin-induced multiple electrode aggregometry (HIMEA) for HIT diagnosis^2 in combination with ELISA. HIMEA and LTA were compared to the gold standard SRA in 200 well-characterized patients with suspected HIT. LTA was performed on citrated platelet-rich plasma (PRP), whereas HIMEA was performed in citrated whole blood. HIMEA was performed by incubating 340 µL of donor whole blood for one minute with 200 µL of plasma from a HIT suspected patient and 40 µL of unfractionated sodium heparin (UFH) for a final concentration of 1 and 100 anti-Xa IU/mL. An additional aliquot was incubated with saline containing no heparin.

Table 1 shows sensitivity, specificity, positive and negative predictive values (PPV / NPV) for HIMEA and LTA in comparison to SRA results.

<table>
<thead>
<tr>
<th></th>
<th>PAT</th>
<th>HIMEA</th>
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<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>76</td>
<td>81</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>96</td>
<td>99</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>80</td>
<td>89</td>
</tr>
<tr>
<td>NPV (%)</td>
<td>97</td>
<td>98</td>
</tr>
</tbody>
</table>

The combination of ELISA with Multiplate® can eliminate false positive ELISA results and aid in the clinical management of patients with confirmed platelet-activating antibodies.
HIMEA was found to be more sensitive and more specific than LTA compared to the gold standard SRA.

Galea and colleagues proposed an algorithm for the interpretation of results of the combination of ELISA and HIMEA (Fig. 1).

The authors concluded that “HIMEA is a useful and simple tool […] for detection of platelet-activating antibodies […] and a more sensitive and more specific test than [LTA]. The combination of ELISA with very high-negative prognostic value and HIMEA with a very good specificity in a non-specialized laboratory would ideally eliminate false positive ELISA results and identify functional antibodies that are more correlated with clinical HIT”.2

References
Multiplate® analysis for the determination of Glanzmann thrombasthenia

Glanzmann thrombasthenia is a rare hereditary disease which can lead to severe mucocutaneous bleeds. Glanzmann thrombasthenia caused by a genetic defect of the GPIIb/IIIa receptor. The platelet GPIIb/IIIa receptor binds to fibrinogen and is the most important principle receptor for platelet aggregation. Quantitative and qualitative defects in GPIIb/IIIa receptors lead to deficient platelet aggregation followed by a tendency for increased bleeding.

The detection of Glanzmann thrombasthenia using light transmission aggregometry is time-consuming and weakly standardized. The study of Awidi, Maqablah, Dweik et al. (2009) investigated the suitability of Multiplate® analysis using whole blood for the diagnosis of patients with Glanzmann thrombasthenia.

Blood samples were taken from patients with Glanzmann thrombasthenia who were not taking platelet function inhibiting medications for at least 7 days. Platelet aggregation in platelet-rich plasma was monitored by LTA (Fig. 1), whereas platelet aggregation in whole blood was studied by Multiplate® (Fig. 2) after stimulation of platelets with ADP (ADPtest), collagen (COLtest) or ristocetin (RISTOtest). In both LTA and Multiplate® a very low level of aggregation was witnessed in response to all examined agonists. Thus, Multiplate® analysis provides comparable results to LTA.

Awidi et al.’s (2009) study demonstrated that MEA performed via the Multiplate® analyzer is a fast and standardized method to assess severe platelet function disorders like Glanzmann thrombasthenia in whole blood.

In comparison to optical aggregometry (LTA) Multiplate® provides a sensitive, rapid and standardized determination of patients with Glanzmann thrombasthenia.
References
Multiplate® analysis in von Willebrand disease (vWD)

The diagnosis and subtyping of von Willebrand Disease (vWD) requires a combination of testing methods and is a recognized challenge for the laboratory. In particular, the identification of subtype 2B requires platelet aggregation testing with low doses of ristocetin. Ristocetin induced platelet aggregation (RIPA) has traditionally been performed using light transmittance aggregometry (LTA). A study by Valarche et al. (2011) compared whole blood impedance aggregometry (WBI) using the Multiplate® analyzer with the classic diagnostic method LTA in order to evaluate the sensitivity of multiple electrode aggregometry (MEA) for identifying vWD in heparinized blood. It was shown that MEA was as sensitive as LTA in detecting vWD with sensitivities of 66% and 65% respectively for any vWD type. There was a high correlation (76%) between the two methods as illustrated in Table 1. Additionally, MEA correctly identified the difficult subtype 2B in three of three patients, two of whom were considered thrombocytopenic. Thus, MEA appears to be a reliable tool for the evaluation of vWD in association with other standard test systems.

Multiplate® analysis is a reliable tool for the evaluation of vWD in combination with other diagnostic markers
Tab. 1

LTA and MEA results of 30 patients with inherited, acquired and platelet-type (PT) von Willebrand disease (vWD)

<table>
<thead>
<tr>
<th>Patients</th>
<th>vWD type</th>
<th>Platelet count (10^9 L^-1)</th>
<th>LTA RH (min^-1) (&gt; 50)</th>
<th>LTA RL (min^-1) (&lt; 20)</th>
<th>MEA RH (AU*min) (&gt; 900)</th>
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<tr>
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<td>PT</td>
<td>231</td>
<td>ND</td>
<td>ND</td>
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<tr>
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<td>PT</td>
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<td>ND</td>
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<td>30</td>
<td>Acq</td>
<td>386</td>
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<td>380</td>
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</tbody>
</table>

LTA = light transmission aggregometry; ND = not done; MEA = multiple electrode aggregometry; MEA RH = RISTOtest high; MEA RL = RISTOtest low.

Measurement of RIPA was performed with LTA at 1.4 mg/mL (LTA RH) and 0.8 mg/mL (LTA RL) final ristocetin concentrations, or with MEA at 0.77 mg/mL (MEA RH) and 0.2 mg/mL (MEA RL) final ristocetin concentrations.

Normal ranges indicated in brackets in the LTA and WBI columns were based on the mean ±2 SD of 30 healthy volunteers.

Results highlighted in bold and italic are out of normal range.

Results highlighted in blue correlate between LTA and MEA, whereas the results shaded in grey show discrepancy between the two methods.

References
Evaluation of pediatric reference values for Multiplate® impedance aggregometry

Hereditary platelet disorders are rare but can lead to bleeding syndromes appearing during infancy or early childhood.

Patients with platelet dysfunction disorders possess a higher risk of bleeding complications during complex surgeries or percutaneous injuries. Evaluation of reference diagnostic parameters is necessary for the comparison between normal and disordered platelet function.

The study of Halimeh, de Angelis, Sander et al. (2010) evaluated pediatric reference values for the Multiplate® system in infants and children from different age groups (0.1-12 months, 1.1-4 years, 5-9 years and 10-18 years).

Platelet aggregation was evaluated using the ADPtest (ADP, 6.5 µM), COLtest (collagen, 3.2 µg/mL), TRAPtest (thrombin receptor activating peptide-6 (TRAP-6), TRAP-6, 32 µM), ASPItest (arachidonic acid, 0.5 mM) and RISTOtest (Ristocetin, 0.77 mg/mL).

Infants aged 0.1-12 months displayed significantly lower aggregation values in TRAPtest and ASPItest compared with the older age groups (Fig. 1).

Therefore adult reference values can provide a valid guidance for the evaluation of platelet function of children at age > 1 year.

**Aggegometry values in healthy infants, children and adolescents**

<table>
<thead>
<tr>
<th>Age Group</th>
<th>ADPtest</th>
<th>COLtest</th>
<th>TRAPtest</th>
<th>ASPItest</th>
<th>RISTOtest</th>
</tr>
</thead>
<tbody>
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<td>0.1 - 12 months</td>
<td><strong>60</strong></td>
<td><strong>60</strong></td>
<td><strong>80</strong></td>
<td><strong>80</strong></td>
<td><strong>160</strong></td>
</tr>
<tr>
<td>1.1 - 4 years</td>
<td>80</td>
<td>80</td>
<td>100</td>
<td>100</td>
<td>140</td>
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<tr>
<td>5 - 9 years</td>
<td>100</td>
<td>100</td>
<td>120</td>
<td>120</td>
<td>160</td>
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<tr>
<td>10 - 18 years</td>
<td>120</td>
<td>120</td>
<td>140</td>
<td>140</td>
<td>180</td>
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</table>

**Fig. 1**  **P < 0.001**

Adult reference values can provide a valid guidance for the evaluation of platelet function of children at age > 1 year.
References

Platelet function testing in clinical guidelines

Multiplate® analysis is included in the latest Clinical and Laboratory Standards Institute guideline on platelet function testing (H58-A).1

With the increasing evidence of a poor response to clopidogrel being associated with an increased risk for stent thrombosis and other adverse events, platelet function testing (PFT) has been incorporated into PCI treatment guidelines.

PFT is now supported by a Class IIb recommendation in the 2012 American College of Cardiology Foundation PCI guidelines on UA/NSTE-ACS patients.2

The Society of Thoracic Surgeons recommends perioperative assessment of platelet function in their 2012 update guideline on use of anti-platelet drugs in patients having cardiac and noncardiac operations.3 Platelet function analysis with Multiplate® is recommended for surgical patients on dual anti-platelet therapy, to limit blood transfusion (Class IIb) and for making decisions about surgical timing “rather than arbitrary use of a specified period of surgical delay” (Class IIa).

2012 ACCF/AHA update guideline for UA/NSTEMI PCI patients
Supporting the use of platelet function testing

<table>
<thead>
<tr>
<th>Oral anti-platelet agents</th>
<th>Recommendation</th>
<th>Evidence Level</th>
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<tbody>
<tr>
<td>Aspirin</td>
<td>I</td>
<td>A</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>I</td>
<td>A</td>
</tr>
<tr>
<td>Prasugrel</td>
<td>I</td>
<td>B</td>
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<tr>
<td>Ticagrelor</td>
<td>I</td>
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</table>

Several anti-platelet agents with level I recommendations

Relatively careful recommendation for drug selection aided by platelet function testing

Tab. 1: adapted from Jneid et al. (2012).2

Cardiology and surgical guidelines recommend adoption of platelet function testing into clinical algorithms
The European Society of Anaesthesiology recommends in their 2013 guidelines on the management of severe perioperative bleeding the preoperative platelet function testing in patients on anti-platelet therapy or with positive bleeding anamnisis (Class IIc).  

Such guidelines divide their advice into Class I, Class Ila, Class Iib, or Class III.

Class I represents conditions or recommendations for which there is general agreement or evidence, or both, that a procedure is useful or effective, while Class Ila and Iib recommendations represent conditions in which opinions diverge, with Class Ila carrying a weight of evidence or opinion in favor of the usefulness or effectiveness of a procedure, and Class Iib carrying a weight of evidence in which the usefulness or efficacy of the procedure is less well established.  

Typically as new markers gain more evidence over time the recommendations in guidelines become stronger, which is also expected for platelet function testing.

References
Cross validation of multiple electrode aggregometry – A comparative trial in healthy volunteers

At present dual drug therapy with Aspirin® and clopidogrel is the standard treatment of patients undergoing percutaneous coronary intervention (PCI). However, there is strong variability in the individual response to these anti-platelet drugs, that carries a high risk of bleeding by high-responders and an increased risk of stent thrombosis by low/non-responders.

There are several standard test systems which allow the determination of platelet function in patients undergoing clopidogrel and Aspirin therapy. Multiplate® analysis is a fast and easy-to-use platelet function test method that analyzes whole blood.

Siller-Matula, Gouya, Wolzt and Jilma (2009) investigated the sensitivity of Multiplate® analysis to clopidogrel and Aspirin effects and compared it with other platelet function tests such as the cone and platelet analyzer (CPA), PFA-100 and VASP phosphorylation assay. In this study design each of 9 healthy volunteers received a loading dose of 300 mg Aspirin and 300 mg clopidogrel on the first day, and a standard dose of 100 mg Aspirin and 75 mg clopidogrel on each of the three consecutive days. Blood samples were analyzed at baseline (pre Aspirin and clopidogrel intake), and 2, 4, 6 and 72 hours after drug ingestion. Clopidogrel effect was investigated by means of ADP-induced platelet aggregation, whereas Aspirin effect was measured by means of arachidonic acid-induced platelet aggregation. The effect size for the clopidogrel and Aspirin treatment was calculated as the average of the amplitude between baseline and maximal platelet inhibition at nadir for each patient. The best effect resolution for both Aspirin and clopidogrel according to the greatest signal magnitude was found for Multiplate® analysis (Tab. 1).

### TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Multiplate</th>
<th>PFA-100</th>
<th>VASP assay</th>
<th>CPA SC</th>
<th>CPA AS</th>
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<td>2.0</td>
<td>n.d.</td>
<td>2.3</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Tab. 1 according to Siller-Matula et al. (2009) the effect size for clopidogrel and Aspirin measured with different tests as median values. SC surface coverage; AS average size of platelet aggregation.

The Multiplate® analyzer proves best sensitivity to Aspirin and clopidogrel effects through demonstration of broadest effect size.
References
1 Siller-Matula, J., Gouya, G., Wolzt, M., Jilma B. (2009). Cross validation of the
Multiple Electrode Aggregometry. A prospective trial in healthy volunteers.

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Evaluation of Aspirin-induced platelet dysfunction based on Multiplate®

Aspirin® is a widely used inhibitor of platelet aggregation. Due to its anti-platelet effects, Aspirin intake is associated with increased bleeding risk during and after complex surgery.

Jambor, Weber, Gerhardt et al. (2009)¹ showed that multiple electrode aggregometry (MEA) is a reliable test to assess Aspirin-induced platelet dysfunction.

In Jambor et al.’s (2009) study each of 24 healthy adult volunteers received a single dose of 500 mg of acetylsalicylic acid (Aspirin). Blood samples were collected at several time points after Aspirin intake and analyzed with the Multiplate® system.

Platelet aggregation was determined following stimulation by arachidonic acid (ASPItest).

Time point 0 marks the baseline of platelet aggregation in healthy volunteers without Aspirin treatment.

Aspirin caused significant platelet inhibition four hours after intake and the inhibitory effect lasted 24 hours by all study participants. Platelet aggregation returned to the baseline range (60-120 U) in 33% of volunteers by 80 hours (day 3) and in 88% of probands by 124 hours (day 5) (Fig. 1).

This study showed that Multiplate® analysis is a reliable method for the assessment of Aspirin effect.

Multiplate® analysis is a reliable method for the assessment of Aspirin effects
References