Multiplate: Applicability, Recommendations, Usefulness in Monitoring Platelet Inhibitors and Diagnosis of Platelet Disorders

Prof. Dr. Michael Spannagl

Munich University Hospital
platelet inhibitors are being applied in a large number of in- and out-patients in many clinics: no monitoring of platelet function available in a routine setting
Aspirin and clopidogrel resistance: an emerging clinical entity

Thomas H. Wang, Deepak L. Bhatt*, and Eric J. Topol

Viewpoint

Aspirin “resistance” and its impact on cardiovascular morbidity and mortality: it is real, clinically relevant and should be measured

Stephanie J Brister¹, Michael R Buchanan²
Multiplate®: detection principle

- analysis of platelet function in whole blood
- twin impedance sensor
- platelets aggregate on metal sensors and increase electrical resistance
- platelet function analysis on surfaces
platelet adhesion and aggregation on the Multiplate sensor wires shown by SEM
Why study platelet function in whole blood rather than in PRP?

- In platelet rich plasma (PRP) platelets are studied in isolation which is NOT their natural situation.

- In whole blood other blood cells are also present - erythrocytes and leucocytes which also influence platelet aggregation.

- In PRP not all platelets may be present, the most active platelets and larger platelets may have been lost during centrifugation.
- citrated blood is the typical sample anticoagulant used for platelet function analysis
- citrate complexes approx. 98% of the free calcium in the sample
- calcium is an important second messenger of platelet activation

→ when citrated blood is used for the analysis ensure a good filling of tubes and use the NaCl-CaCl$_2$-solution when performing the ADPtest, COLtest and TRAPtest
→ hirudin-anticoagulated tubes are used in the majority of publications on Multiplate
→ when heparin blood is analysed, we recommend the ADPtest HS for the monitoring of clopidogrel
Multiplate® instrument

- 5 channels for parallel tests
- electronic pipetting
- applicable for laboratory and near patient analysis
Performing the test

1. Put the test cell into the measuring position.
2. Attach the sensor cable.
3. Pipette 300 µl of saline + 300 µl of blood.*
4. Allow 3 minutes for warming and equilibration.
5. Add the activator after 6 minutes:
   - Print the results
   - Discard the test cell

* Usually hirudin or heparin blood
Multiplate is the most widely used aggregometer in Europe

→ more than 50 medline-listed publications since 2009

**Clopidogrel affects leukocyte dependent platelet aggregation by P2Y₁₂ expressing leukocytes**

Philipp Diehl · Christoph Olivier · Christoph Halscheid · Thomas Helbing · Christoph Bode · Martin Moser

**Heparin-induced multiple electrode aggregometry: a potential tool for improvement of heparin-induced thrombocytopenia diagnosis**

I. Elalamy, V. Galea, M. Hatmi† and G. T. Gerotziafas *

*Service d’Hematologie Biologique, Hopital Tenon, Paris; and †Departement de Medecine Moléculaire, Institut Pasteur, Paris, France

**Cross validation of the Multiple Electrode Aggregometry**

A prospective trial in healthy volunteers

Jolanta M. Siller-Matula; Ghazaleh Gouya; Michael Wolzt; Bernd Jilma

Department of Clinical Pharmacology, Medical University of Vienna, Austria

**The Antiplatelet Effect of Aspirin is Reduced by Proton Pump Inhibitors in Patients With Coronary Artery Disease**

Morten Würtz, Erik L Grove, Steen Dalby Kristensen and Anne-Mette Hvas

**Platelet Reactivity After Clopidogrel Treatment Assessed With Point-of-Care Analysis and Early Drug-Eluting Stent Thrombosis**

Dirk Sibbing, MD, Siegmund Braun, MD, Tanja Morath, MS, Julinda Mehilli, MD, Wolfgang Vogt, MD, Albert Schömig, MD, Adnan Kastrati, MD, Nicolas von Beckerath, MD
**Multiplate tests**

- Activation
- Inhibition

1. Release of arachidonic acid

- Aspirin ®
- NSAID

- TXA₂

- TRAP

- ADP

- Clopidogrel
- Prasugrel
- Cangrelor

**GpIIb/IIIa antagonists:**
- Reopro ® (abciximab)
- Aggrastat ® (Tirofiban)
- Integrillin ® (Eptifibatid)

**COX**
- Arachidonic Acid

**Activated platelet**
- Platelet activation
- GpIIb /IIIa receptor exposure
- Degranulation
Multiplate tracings – examples

- no platelet inhibition
  - TRAPtest: 113 U
  - ASPItest: 102 U
  - ADPtest: 89 U

- 100 mg aspirin qd
  - TRAPtest: 139 U
  - ASPItest: 17 U
  - ADPtest: 134 U

- 75 mg clopidogrel qd
  - TRAPtest: 98 U
  - ASPItest: 89 U
  - ADPtest: 31 U

- 100 mg aspirin + 75 mg clopidogrel qd
  - TRAPtest: 88 U
  - ASPItest: 8 U
  - ADPtest: 17 U

- tirofiban (Aggrastat® i.v.)
  - TRAPtest: 7 U
  - ASPItest: 3 U
  - ADPtest: 3 U
Analysis of ASPltest, ADPtest and TRAPtest in aspirin-treated individuals was performed at 30 and 60 minutes after blood sampling.

Regression analysis of the two determinations is shown here.

A good reproducibility is shown.
ASPItest: distributions and definition of low response in Munich university, hemostasis and transfusion medicine department.

- 57 blood donors
- 341 patients on Aspirin 100 mg / d

Blood examination using hirudin blood

ADPtest: distributions and definition of low response in Munich university, hemostasis department

206 blood donors

170 Patients on Clopidogrel 75 mg / d

≥ 45 U clopidogrel low response

< 45 U clopidogrel response

Cut off for clopidogrel response:

Examination using hirudin blood
Patient before neuroradiologic stenting: Aspirin 100 + Plavix 300 mg

Aspirin response

Plavix non-response
partial stent thrombosis in clopidogrel non-responder

resolved clot after 30 min tirofiban
After application of Aggrastat (GpIIb/IIIa receptor antagonist)

Müller-Schunk, Neuroradiology, University Hospital Munich
After cessation of Aggrastat

after 15 min

after 30 min

Müller-Schunk, Neuroradiology, University Hospital Munich
Early instent thrombosis (5-th day), as a result of high residual platelet activity

University National Heart Hospital - Sofia

I. Paskaleva, MD, PhD

Threevessel disease, implanted stent in left anterior descending (LAD) (15.01.2008), AMI with ST elevation (20.05.2008), PTCA.
Therapeutic intervention in case of non-response:

Stent thrombosis

F. Krötz, Cardiology, Munich University Clinic

Clopidogrel 150mg/d

Prasugrel 10mg/d
Resistance to aspirin

University National Heart Hospital - Sofia

I. Paskaleva, MD, PhD

Status after ACB x3, threevessel disease, TIA
Monitoring of Clopidogrel-Related Platelet Inhibition: Correlation of Nonresponse with Clinical Outcome in Supra-aortic Stenting

Dirk Sibbing, MD, Siegmund Braun, MD, Tanja Morath, MS, Julinda Mehilli, MD, Wolfgang Vogt, MD, Albert Schömig, MD, Adnan Kastrati, MD, Nicolas von Beckerath, MD

Multiple electrode aggregometry predicts stent thrombosis better than the vasodilator-stimulated phosphoprotein phosphorylation assay

J. M. Siller-Matula, * G. Christ, † I. M. Lang, ‡ G. Delle-Karth, † K. Huber § and B. Jilma *
Platelet Reactivity After Clopidogrel Treatment Assessed With Point-of-Care Analysis and Early Drug-Eluting Stent Thrombosis

Dirk Sibbing, MD, Siegmund Braun, MD, Tanja Morath, MS, Julinda Mehilli, MD, Wolfgang Vogt, MD, Albert Schömig, MD, Adnan Kastrati, MD, Nicolas von Beckerath, MD

- n=1,608 patients with coronary artery disease and planned DES implantation
- loading dose 600 mg clopidogrel
- Blood was obtained directly before PCI
- Multiplate ADP test in hirudin blood
- The primary end point was definite ST at 30 days

Aggregation results and cut-offs

- Non-response according to pre-defined cut-off: 42 U
- Non-response according to ROC analysis: 47 U

Wide variation of ADP-induced aggregation
Results: risk enhancement

Risk for definitive stent thrombosis within 30 days (%):
- Clopidogrel response
- Clopidogrel non-response

OR 9.8

Risk for definitive and probable stent thrombosis within 6 months (%):
- Clopidogrel response
- Clopidogrel non-response

OR 5.8
Results: Kaplan-Meier analysis

1 month follow-up

OR 9.8

6 month follow-up

"MEA measurements are highly predictive for the occurrence of ST"
Significantly more adverse events in clopidogrel non-responders (p<0.001)

"Near-patient testing of platelet inhibition before neurointerventional stent placement seems reasonable to adjust the antiplatelet protocol individually if required and has the potential to reduce thromboembolic complications..."


Confirmation: study in neuroradiology
Multiple electrode aggregometry predicts stent thrombosis better than the vasodilator-stimulated phosphoprotein phosphorylation assay

J. M. SILLER-MATULA,* G. CHRIST,† I. M. LANG,‡ G. DELLE-KARTH,‡ K. HUBER§ and B. JILMA*

- n=416 pts with CHD undergoing PCI
- VASP assay and Multiplate ADPtest HS in hirudin blood
- 6 month follow-up

„The effectiveness of MEA in predicting stent thrombosis .. was higher than any of the values reported for tests assessing the antiplatelet effect of clopidogrel in order to predict thrombotic adverse events“

Clopidogrel effect and bleeding

ADP-induced platelet aggregation (AU*min)

Number of patients (n)

Incidence of in-hospital major bleeding (%)

Enhanced Responders
n = 975
P = 0.005

Remaining Patients
n = 1558

Cut-off 19 U

Major bleeding events

n = 2533
Impact of systemic inflammation on platelet aggregation in patients under chronic clopidogrel treatment

I. Bernlochner1, S. Steinhubl2, S.-L. Braun1, T. Morath1, J. Mehilli1, J. Stegherr1, A. Schömig1, N. von Beckerath1, A. Kastrati1, D. Sibbing1

Elevated levels of C-reactive protein, WBC count and fibrinogen were significantly associated with high on clopidogrel treatment platelet reactivity. Thus, a status of systemic inflammation significantly attenuates the level of platelet inhibition that is achieved by clopidogrel treatment.
• Assessment of platelet function in 188 patients under clopidogrel maintenance treatment (75 mg/d) with / without diabetes mellitus

D. Sibbing et al, Poster presentation: Assessment of Clinical Variables That Influence Platelet Aggregation in Patients Under Long-term Clopidogrel Treatment Using Multiple Electrode Platelet Aggregometry

GTH congress February 2008
Impact of common CYP2C19 genetic variants on the antiplatelet effect of chronic clopidogrel therapy
Aspirin monitoring

Control of Aspirin Effect in Chronic Cardiovascular Patients Using two Whole Blood Platelet Function Assays: PFA-100 and Multiple Electrode Aggregometry

K.-W. von Pape, M. Dzijan-Horn, J. Bohner, M. Spannagl, H. Weisser, and A. Calatzis

Whole Blood Multiple Electrode Aggregometry is a Reliable Point-of-Care Test of Aspirin-Induced Platelet Dysfunction

The antiplatelet effect of aspirin is reduced by proton pump inhibitors in patients with coronary artery disease

Morten Würtz,¹ Erik L Grove,¹ Steen D Kristensen,¹ Anne-Mette Hvas²
Assessment of residual platelet reactivity during aspirin treatment

Control of Aspirin Effect in Chronic Cardiovascular Patients Using two Whole Blood Platelet Function Assays: PFA-100 and Multiple Electrode Aggregometry

K.-W. von Pape, M. Dzijan-Horn, J. Bohner, M. Spannagl, H. Weisser, and A. Calatzis

n=76 patients under chronic aspirin therapy with 100 mg aspirin / d

n=57 blood donors

Multiplate ASPItest in hirudin blood
Fig. 3. Distribution of results of whole blood aggregometry (Multiplate).
Whole blood multiple electrode aggregometry is a reliable point-of-care test of aspirin-induced platelet dysfunction.
Aspirin: drug interactions

The antiplatelet effect of aspirin is reduced by proton pump inhibitors in patients with coronary artery disease

Morten Würtz,1 Erik L Grove,1 Steen D Kristensen,1 Anne-Mette Hvas2

418 stable patients with CAD
54 of whom treated with PPI

ASPIItest in citrated blood (1 mM)

„Patients with CAD treated with PPIs had a reduced platelet response to aspirin .. compared with patients with CAD not taking PPIs. Concomitant use of aspirin and PPIs might reduce the cardiovascular protection by aspirin.“

Heart 2010;96:368–371.
Patients with Previous Definite Stent Thrombosis Have a Larger Fraction of Immature Platelets and a Reduced Antiplatelet Effect of Aspirin Presentation

Morten Würtz, Erik L. Grove, Lise N. Wulff, Anne K. Kaltoft, Hans H. Tilsted, Lisette O. Jensen, Anne-Mette Hvas, Steen D. Kristensen
Aarhus University Hospital, Skejby, Aarhus, Denmark

→ 117 patients previously undergoing percutaneous coronary intervention (PCI)
→ 39 patients suffered stent thrombosis within two years of stenting and 78 patients served as controls matched in a 1:2 ratio with respect to age, gender, stent type, diabetes and PCI indication.

Results.
Platelet aggregation assessed by Multiplate® aggregometry was significantly higher in cases when induced by arachidonic acid (p = 0.02) and by collagen (p <0.0001).
Platelet aggregation was increased when assessed by VerifyNow® (p = 0.09, ns).
Soluble serum P-selectin levels did not differ between groups (p = 0.92)

Conclusion.
Patients with previous ST had an increased residual platelet aggregation compared to matched controls.
enhanced risk of bleeding events

“Sweet spot”

enhanced risk of ischaemic events

cut-off ADPtest
19 U
JTH Sibbing et al 2010

cut-off ADPtest
42 U JACC Sibbing et al, 2009 /
47 U JACC Sibbing D et al, 2009
according to ROC analysis

Residual platelet activity

Risk of any event

Risk of any event

Ferreiro, Sibbing, Angiolillo, modified

Thrombosis and Haemostasis 103.6/2010
Adequate response to ADP P2Y12 receptor blocking medication was defined as ADP-test < 45 units (AUC).

Results are expressed in U (AUC).
7 Whole Blood Impedance Aggregometry

7.1 Introduction/Principle

Recently, a new method for measuring whole blood impedance aggregometry was introduced based on a single-use test cell, with a total of four silver-coated electrodes that form two independent sensor units (MEA).36

During a brief period of equilibration, as an alternating current is applied across the electrodes, a monolayer of platelets forms on the exposed portions of the electrodes, resulting in a stable impedance value. An aggregating agent is added to the cuvette and stimulated platelets aggregate to the platelet monolayer on the immersed electrodes. The accumulation of platelets results in an increase in electrical resistance within the circuit (i.e., as platelets aggregate, the impedance increases). In impedance aggregometry, the extent and rate of aggregation are measured and quantified in ohms and ohms per minute (the measurement of electrical resistance). In MEA analysis, the results are expressed in arbitrary “aggregation units” (AU). The change in impedance is displayed as a function of time on a strip chart recorder or monitor using computer software. Formation of aggregates in the sample has no effect on the measured impedance unless they adhere to the electrodes. There are several factors that may affect the impedance: physical integrity of the electrode, sample temperature, stirring speed, and the HCT.25,87,88

In conclusion, aspirin and clopidogrel produce stronger signals in the MEA compared to several other methods."


**Multiplate**

- **Effect size:** >10

**PFA-100**

- **Effect size:** 2

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Cross validation of the Multiple Electrode Aggregometry - A prospective trial in healthy volunteers
Jolanta M. Siller-Matula; Ghazaleh Gouya; Michael Wolzt; Bernd Jilma
*Department of Clinical Pharmacology, Medical University of Vienna, Austria*
Correlation to optical aggregometry

"MEA is capable of detecting the effect of clopidogrel treatment and the results of MEA, prior to, and after clopidogrel treatment, correlate well with LTA."


"The present findings show that this new POC whole blood impedance aggregometer has the capability to detect residual platelet reactivity with good agreement with LTA."

Comparison of platelet aggregation using light transmission and multiple electrode aggregometry in Glanzmann thrombasthenia

ABDALLA AWIDI¹, AHMAD MAQABLAH¹, MANAR DWEIK¹, NAZZAL BSOUl¹, & AHMAD ABU-KHADER²

¹Department of Medicine, Hemostasis and Thrombosis Laboratory, Faculty of medicine, Amman, Jordan and ²Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, The Hashemite University, Zarqa, Jordan
## LTA

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP (&gt; 70)</td>
<td>&lt; 5</td>
<td>0</td>
</tr>
<tr>
<td>COL (&gt; 70)</td>
<td>&lt; 5</td>
<td>1</td>
</tr>
<tr>
<td>RISTO (&gt; 70)</td>
<td>72</td>
<td>82</td>
</tr>
</tbody>
</table>

→ in Multiplate also RISTOtest is abnormal in Glanzman thrombasthenia patients

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Glanzman thrombasthenia
38 years-old patient
urgent operation necessary (eye injury)
bleeding history: **disposition for hematoma, bleeding after tooth extraction**
family history: daughter with known vWS
factor VIII, vWF antigen + activity significantly reduced

<table>
<thead>
<tr>
<th>TRAPtest</th>
<th>ASPItest</th>
<th>ADPtest</th>
<th>COLtest</th>
<th>RISTOtest</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Area under the Curve:</strong></td>
<td><strong>Area under the Curve:</strong></td>
<td><strong>Area under the Curve:</strong></td>
<td><strong>Area under the Curve:</strong></td>
<td><strong>Area under the Curve:</strong></td>
</tr>
<tr>
<td>870 AU* min. (941 - 1583)</td>
<td>791 AU* min. (745 - 1361)</td>
<td>452 AU* min. (534 - 1220)</td>
<td>886 AU* min. (453 - 1166)</td>
<td>205 AU* min. (941 - 1563)</td>
</tr>
<tr>
<td>Aggregation:</td>
<td>Aggregation:</td>
<td>Aggregation:</td>
<td>Aggregation:</td>
<td>Aggregation:</td>
</tr>
<tr>
<td>RUD: 139.5 AU</td>
<td>RUD: 135.0 AU</td>
<td>RUD: 79.1 AU</td>
<td>RUD: 153.3 AU</td>
<td>RUD: 51.4 AU</td>
</tr>
<tr>
<td>Velocity:</td>
<td>Velocity:</td>
<td>Velocity:</td>
<td>Velocity:</td>
<td>Velocity:</td>
</tr>
<tr>
<td>RUD: 22.9 AU/min.</td>
<td>RUD: 16.8 AU/min.</td>
<td>RUD: 10.2 AU/min.</td>
<td>RUD: 21.1 AU/min.</td>
<td>RUD: 5.5 AU/min.</td>
</tr>
<tr>
<td>CC=1.000, DIF=0.747%</td>
<td>CC=0.999, DIF=10.367%</td>
<td>CC=0.999, DIF=13.274%</td>
<td>CC=1.000, DIF=4.907%</td>
<td>CC=0.998, DIF=9.002%</td>
</tr>
</tbody>
</table>
Detection of vWD using Multiplate

→ moderate sensitivity for type I vWD, good sensitivity for type II
→ moderate specificity (RISTOtest is aspirin-sensitive)
Heparin-induced multiple electrode aggregometry: a potential tool for improvement of heparin-induced thrombocytopenia diagnosis

I. ELALAMY,* V. GALEA,* M. HATMI† and G. T. GEROTZIAFAS*
*Service d'Hematologie Biologique, Hopital Tenon, Paris; and †Departement de Médecine Moléculaire, Institut Pasteur, Paris, France

Regular Article
Whole blood impedance aggregometry detects heparin-induced thrombocytopenia antibodies

Marie-Christine Morel-Kopp a,b,*, Margaret Aboud a,c, Chee Wee Tan a,b, Chandima Kulathilake b, Christopher Ward a,b

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b Department of Haematology and Transfusion Medicine, Royal North Shore Hospital, St Leonards NSW Australia
c Pacific Laboratory Medical Services (PaLMS), Royal North Shore Hospital, St Leonards NSW Australia
Conclusions

• Multiplate is being increasingly used for the monitoring of platelet function in cardiology and neuroradiology

• Low response to clopidogrel according to Multiplate has been shown to be a significant risk factor for ischemic events

• Clinical experiences concerning the use of Multiplate for optimizing anti-platelet therapy are promising

• Additional applications include the use of Multiplate for assessment of bleeding disorders

• Multiplate is today one of the most widely use platelet function analyzers in Europe
ROC-Kurve für die Prädiktion von MACE (kombinierter Endpunkt für Ischämien) mittels VASP

ROC-Kurve für die Prädiktion von Stentthrombosen mittels VASP und Multiplate (MEA)


ROC curve for MEA and definite or probable stent thrombosis during 6-month follow-up period. An area under the curve (AUC) of 0.74 was observed (P<0.001).
Sibbing D et al: Thrombosis and Haemostasis 2010

ROC-Kurve für die Prädiktion von Stentthrombosen mittels VASP und Multiplate (MEA)