Abstracts with Multiplate at GTH 2013

P1 Thromboembolism

Thursday, 21 February 2013 / 13:00 – 14:00

P1-17 Release of S1P by platelet sphingosine kinase 2 intrinsically controls platelet homeostasis and arterial thrombosis in mice

Urtz N.1, von Bruhl M.-L.1, Gartner F.1, Schubert I.1, Rahimi F.1, Barocke V.1, Tirniceriu A.1, Beil J.1, Chandraratne S.1, Beerli C.2, Ledieu D.2, Persohn E.2, Billich A.2, Baumruker T.2, Massberg S.3 1Deutsches Herzzentrum, TU Munchen, Munchen, Germany, 2Novartis Institutes for BioMedical Research, Basel, Switzerland, 3Klinikum Groshadern, LMU Munchen, Munchen, Germany

P2 Haemorrhagic Diathesis

Thursday, 2 February 2013 / 13:00 – 14:00

P2-94 Anaemia does not impair platelet aggregation and clot formation: An in vitro Experiment

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P6 Laboratory Issues

Friday, 22 February 2013 / 13:00 – 14:00

P6-2 Cycling induces a hypercoagulable state

Posthuma J.J.1, Loeffen R.1, van Oerle R.1, Henskens Y.M.C.2, ten Cate H.1, Spronk H.M.H.1, van der Meijden P.E.J.1 1Maastricht University Medica Center, Laboratory for Clinical Thrombosis and Haemostasis, Maastricht, Netherlands, 2Maastricht University Medica Center, Department of Internal Medicine, Central Diagnostic Laboratory, Maastricht, Netherlands

P6-9 Rotem® and Multiplate®: A comparison of arterial vs. venous measurements. Results of a pilot study in patients undergoing major orthopedic surgery

Oswald E.1, Finsterwalder T.1, Innerhofer N.1, Haas T.1, Mittermayr M.1, Strohmaier S.2, Innerhofer P.1 1Univ. Klinik f. Anasthesie, Innsbruck, Austria, 2Medizinische Universitat Innsbruck, Department fur Medizinische Statistik, Informatik und Gesundheitsokonomie, Innsbruck, Austria

P6-10 Effect of thrombocytopenia on platelet function in point-of-care monitoring

Wetzel L.1, Pramhas S.1, Keresztesi L.1, Kozek-Langenekcer S.2, Scharbert G.1 1Medical University of Vienna, Abteilung fur Allgemeine Anasthesie, Intensivmedizin und Schmerztherapie, Wien, Austria, 2Evangelical Hospital Vienna, Abteilung fur Anasthesie, Intensivmedizin und Schmerztherapie, Wien, Austria

P6-31 Experiences from the analysis of platelet function in blood donors

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P9 Genetics In Blood Coagulation

Friday, 22 February 2013 / 13:00 – 14:00

P9-2 A novel ADP-receptor mutation in a large Turkish family with bleeding symptoms

Ekhlasi-Hundrieser M.1, Detering C.1, Halves C.1, von Depka Prondzinski M.1 1Werlhof-Institut, Hannover, Germany

P9-8 Determination mutation status of platelet polymorphisms in patients with acute myocardial infarction

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P11 Acquired Haemostatic Disorders

Friday, 22 February 2013 / 13:00 – 14:00

P11-10
Chronic garlic supplement intake does not potentiate the platelet inhibitory effect of diclofenac

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P12 Miscellaneous

Friday, 22 February 2013 / 13:00 – 14:00

P12-6
Impaired platelet aggregation – a hint for bacterial contamination?

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P1-17

Release of S1P by platelet sphingosine kinase 2 intrinsically controls platelet homeostasis and arterial thrombosis in mice

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Background: Platelets are known to play a crucial role in hemostasis. Their activation at sites of vascular injury prevents live-threatening blood loss and promotes wound healing. Sphingosine kinase 1 and 2 (Sphk) catalyze the conversion from sphingosine (Sph) to the bioactive metabolite sphingosine-1-phosphate (S1P). Although platelets express both Sphk1 and 2 and are able to secrete S1P and thereby contribute to balance the plasma S1P level, little is known about a potential intrinsic effect of these lipids on platelets. Here, we therefore investigated the role of Sphk1 and 2 in the regulation of platelet function.

Methods and results: We found a 100-fold reduction in intracellular S1P levels in Sphk2-/- compared to Sphk1-/- or wildtyp (wt) platelets analyzed by mass spectrometry. Moreover Sphk2-/- platelets failed to secrete S1P into the extracellular space. Interestingly, the release of arachidonic acid (AA) metabolites in response to stimulation by thrombin was significantly reduced in Sphk2-/- as compared to wt platelets. Beyond that we found in Sphk2 deficient mice a decrease in blood aggregation after ADP stimulation assessed by whole blood impedance aggregometry in vitro. Finally Sphk2 knock-out mice show significantly reduced and delayed arterial thrombus formation in response to ferric chloride injury compared to wt mice.

Conclusions: We demonstrate here that Sphk2 is the major isoform responsible for the generation of S1P in platelets and plays a pivotal intrinsic role in the control of platelet activation. Correspondingly, Sphk2 deficient mice are protected from arterial thrombosis after vascular injury.

P2-94

Anaemia does not impair platelet aggregation and clot formation: An in vitro Experiment

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Aim: Red blood cell transfusions are recommended for improvement of haemostasis as anaemia is believed to impair platelet function and clot formation.1 Studies supporting this strategy used the obsolete bleeding time or achieved reduced haemoglobin concentrations by dilution.2 The aim of the current study was to evaluate the effect of reduced haemoglobin concentrations on coagulation in vitro without haemodilution for sample preparation.

Methods: Blood was drawn from 13 volunteers. Samples were adjusted to haemoglobin concentrations of 10, 7 and 3g/dl. Platelet function was investigated using multiple electrode aggregometry (MEA) with various agonists and clot formation was assessed using rotational thrombelastometry (ROTEM) (EXTEM and INTEM-test).

Results: The MEA parameters area under the curve and maximum aggregation did not differ significantly between haemoglobin concentrations indicating undisturbed platelet function. Velocity of aggregation was significantly faster at haemoglobin concentration 7g/dl than at baseline using the agonists ADP ($p < 0.05$), arachidonic acid ($p < 0.05$) and TRAP ($p < 0.01$). In ROTEM clot formation time (CFT) was significantly shorter at Hb 7g/dl than at baseline in EXTEM ($p < 0.01$) and INTEM ($p < 0.05$). At Hb 3 g/dl the CFT was significantly shorter than at baseline in EXTEM and INTEM ($p < 0.05$). Maximum clot strength (MCF) was significantly higher at lower Hb values in all ROTEM tests ($p < 0.05$).

Conclusion: Platelet aggregation and clot formation are not impaired by moderate to severe anaemia. Our data indicate activated platelet reactivity as well as faster and stronger clot formation at lowered haemoglobin concentrations. Recommendations of red blood cell transfusion for improved haemostasis need to be revised.

References: 1. Valeri CR, Crowley JP, Loscalzo J. Transfusion 1998 2. Iselin BM, Willimann PF, Seifert B, et al. Br J Anaesth 2001

P6-2

Cycling induces a hypercoagulable state

Posthuma J.J.1, Loeffen R.1, van Oerle R.1, Henskens Y.M.C.2, ten Cate H.1, Spronk H.M.H.1, van der Meijden P.E.J.1 1Maastricht University Medica Center, Laboratory for Clinical Thrombosis and Haemostasis, Maastricht, Netherlands, 2Maastricht University Medica Center, Department of Internal Medicine, Central Diagnostic Laboratory, Maastricht, Netherlands

Aim: Although a clear association between exercise and activation of coagulation has been demonstrated, evidence is fragmented and the trigger of the haemostatic processes remains unknown. Therefore, we investigated the haemostatic profile in young cyclist after strenuous exercise.

Methods: Venous blood was collected from 17 male cyclists (age: 22±2.9 years) before and after 4 hours cycling (≈120 km). Whole blood cell count and von Willebrand factor (vWf) levels were determined, platelet reactivity was assessed by Multiplate (collagen, ADP, TRAP, and ASPI), rotational thromboelastography by means of ROTEM (ExTem, InTem and NaTem), thrombin generation through 0 and 1 pM tissue factor (TF) triggered CAT analysis.

Results: Compared to baseline, white blood cell count was elevated after exercise, which was predominantly caused by an increase in monocytes (1.3±0.45 fold) and neutrophils (2.5±1.1 fold). Remarkably, eosinophils decreased with 52% and hematocrit and hemoglobin levels remained constant. Platelet aggregation in response to ADP, ASPI and TRAP was enhanced after cycling exercise as indicated by increased maximal aggregation and area under the curve. VWf levels increased from 110±44% to 193±72% after exercise, which points to endothelial activation. ROTEM Ex- and InTem were not altered upon exercise, whereas the NaTem analysis was characterized by a shortened clotting time and enhanced clot formation (MCF and alpha), suggesting the presence of an endogenous trigger of coagulation. This was confirmed by thrombin generation analysis, which was increased in both the absence and presence of 1 pM TF. Despite inhibition of the TF-pathway through addition of active site inhibited factor VII (ASIS), thrombin generation was still enhanced upon cycling, suggesting the contribution of the intrinsic pathway of coagulation.

Conclusion: Cycling exercise induces a hypercoagulable state through both increased platelet reactivity and contact dependent activation of coagulation.

P6-9

Rotem® and Multiplate® : A comparison of arterial vs. venous measurements. Results of a pilot study in patients undergoing major orthopedic surgery

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Background: Rotational thromboelastometry (ROTEM®) and multiplate platelet functional analyzer (Multiplate®) are frequently performed by using arterial blood samples. However, both devices were licensed only for use in venous blood, and discussions have arisen on whether the two blood sources can be used interchangeably.

Methods: This is a prospective clinical pilot study conducted in 50 patients undergoing major orthopedic surgery. Arterial and venous blood samples were drawn simultaneously after line insertion (T0), intraoperatively (T1) and at the end of surgery (T2). At each time point the INTEM, EXTEM and FIBTEM assays as well as the ASPI, ADP and TRAP assays were performed on arterial and venous blood samples using the ROTEM® and the Multiplate® device, respectively.

Results: Venous and arterial measurement results correlated at each time point with a coefficient of 0.519 - 0.977. At the three measurement points only a few statistically significant deviations were detected for some of the ROTEM® parameters [INTEM coagulation time, EXTEM lysis index 30 min (T0), EXTEM and FIBTEM amplitude 30 min (A30) (T1), and EXTEM A30 (T2). For Multiplate® assays, we found significant differences in ASPI (T0), TRAP (T1), and ADP (T2) results. We observed that pathological conditions were detected with similar frequency regardless of the sampling site, except for Multiplate® TRAP at T0, where venous samples more frequently indicated low platelet aggregation ($p=0.0455$).

Conclusion: Arterial as well as venous blood samples detected coagulopathy and low platelet aggregation with similar frequency. Thus, it is very unlikely that the blood sampling site influences clinical decision-making.

P6-10

Effect of thrombocytopenia on platelet function in point-of-care monitoring

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Aim: Evaluate whether a decrease in platelet count is associated with a proportional decrease in platelet function measured in point-of-care (POC) tests targeting primary hemostasis.

Methods: Samples containing a decreased platelet count were generated from blood samples of healthy volunteers. Platelet count was adjusted to 80, 50 and 20 G/l. Two POC-tests were applied: multiple electrodes aggregometry (MEA, Multiplate ®) and a Cone and Plate(let) Analyzer (CPA) technology, measuring whole blood platelet adhesion under flow conditions (Diamed Impact-R®). We used the agonists ADP, ASPI, COL, RISTO and TRAP for both tests.

Results: Platelet counts of 80 G/l and below impaired platelet function significantly in all tests in the velocity and area under the curve (AUC) of MEA ($p < 0.05$). In the CPA the measured Surface Coverage (SC) was also impaired at platelet counts of 80 G/l and below using COL und RISTO. The Aggregate Size (AS) was only influenced significantly by platelet counts of 50 and 20 G/l when using RISTO.

Conclusion: Platelet function in MEA analysis showed a decrease proportional to platelet count at 80, 50 and 20 G/l. The SC of CPA was also significantly correlated to platelet count using the agonists RISTO and COL while AS was less influenced. All measurements worked correctly with low platelet counts. In vivo results have to be put in context with clinical symptoms of the patient.

Literature: Rossaint, R., Cerny V, et al. (2006). Key issues in advanced bleeding care in trauma. Shock 26(4): 322-31.

P6-31

Experiences from the analysis of platelet function in blood donors

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Issue: Analysis of platelet function results collected during routine testing of platelet donors over a 9 weeks period shows stability in platelet function using MEA.

Methods: Platelet function was tested in 623 samples (hirudin) using multiple electrode aggregometry (MEA, Multiplate® analyzer) after stimulation by arachidonic acid (ASPI, 0.5 mM) or ADP (6.4 µM). Aggregation was quantified by the area under the curve.

Results: 2.1 % (ADP) and 2.4 % (ASPI) of donors showed aggregation lower than normal. 9.1% (ADP) and 6.3% (ASPI) showed aggregation higher than normal. (53-122 U for ADP and 75-136 U for ASPI). Aggregation found were in good agreement with the reference ranges. When analyzed separately per week, quite stable aggregations were found, with mean value varying maximally by 5% (ADP) and 6% in comparison to the overall mean value found over 9 weeks.

Discussion: Determination of platelet function in 623 samples over a 9 weeks period revealed a good agreement of results compared to the reference. Few individuals showed abnormal platelet aggregations. May be due to donor selection (donors with lower platelet counts being excluded) slightly higher aggregations (in average) were found (compared to the reference). The stable results over the observation period show good stability of the platelet function over time.

Table 1: Statistics of 623 platelet function analyses

	ADP	ASPI
mean ± SD	89 ± 21	111 ± 20
(mean - 2 SD) - (mean + 2 SD)	46 - 131	72 - 150
quantil 5 - quantil 95	56,1 - 125	85 -142
lower - upper end of reference range	53 - 122	75 -136
mean of reference range	87,5	105,5

[Tab.1]

Table 2: Analysis of results over the observation period

week	ADP (mean ± SD in U)	Ø of week in relation to Ø overall	ASPI (mean ± SD in U)	Ø of week in relation to Ø overall	n
1	91±19	103%	118±19	106%	62
2	91±20	103%	115±21	103%	82
3	87±20	98%	107±18	96%	67
4	86±22	96%	112±20	101%	70
5	91±20	102%	111±23	100%	74
6	88±21	99%	108±19	97%	75
7	85±22	95%	111±16	100%	60
8	87±22	98%	110±18	99%	80
9	91±24	103%	112±18	101%	53
all	89±21	100%	111±20	100%	623

[Tab.2]

P9-2

A novel ADP-receptor mutation in a large Turkish family with bleeding symptoms

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Aim: P2Y12 is a Gi-coupled platelet receptor (P2RY12) for adenosine diphosphate (ADP). Molecular defects in P2RY12 cause mild to moderate bleeding disorders. Herein, we report the detection of a novel homozygous missense mutation of the P2RY12 gene as well as its association with an impaired platelet aggregation in a large Turkish family involving consanguinity.

Method: Complete exons and exon-intron boundaries of P2RY12 were amplified by polymerase chain reaction and subsequently sequenced on an automated sequencing system. Coagulation parameters such as platelet numbers, light transmission aggregometry (LTA with collagen, epinephrine, ristocetin), whole blood impedance aggregometry (IA), PFA-100 using Col/EPI, Col/ADP, and P2Y12 cartridge were measured in all individuals.

Result: A homozygous missense mutation within P2RY12 was identified in several family members at nucleotide position 561 (CAT→CAA) resulting in an amino acid substitution at position 187 (H→Q) of the P2RY12 protein. Patients showed distinct abnormal platelet function. Mean Platelet number was 270/nL (normal 150-400/nL). PFA Col/ADP (normal 68-121) as well as PFA P2Y12 (normal < 106) were >300sec with normal PFA Col/Epi. LTA using Col was 40% (normal> 70), using ADP 15% (normal>60) and using ristocetin 52% (normal>65). Area under curve in IA using Col was 41 (normal 75-115) and using ADP 27% (normal 30-88). GP-Ib, -IIb, -IIIa, -IV and -IX expression were 97, 97, 26, 34 and 14% (normal>=70), resp.

Conclusion: We assume that the bleeding symptoms in this family are caused by a homozygous mutation in P2RY12 affecting platelet function by decreasing platelet aggregation. We provide evidence that His187 is likely to be involved in the ADP binding site or receptor activation. Sequencing analyses of remaining family memberssharing the propositus phenotype are ongoing. Knowledge of the underlying mutation(s) may help to understand the function of the protein and to improve the management of the patients.

P9-8

Determination mutation status of platelet polymorphisms in patients with acute myocardial infarction

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Aim: Antiaggregation therapy is still the most frequently used approach to present thrombotic events in cardiovascular diseases. Dual antiaggregation therapy (aspirin and clopidogrel) is the treatment of choise for preventing thrombotic complications in patients undergoing AMI.

Material and methods: Testing of 90 patients with AIM by PCR followed by melting curve analysis using a LightCycler 480 analyzer (ROCHE) were used to determine the frequencies P2Y12 (H1/H2 haplotyp; rs2046934), P2Y12 (34C>T; rs6785930), COX-1 (-842A>G; rs10306114), GP1bA (-5T>C; rs2243093), GP6 (T13254C; rs1613662) polymorfisms. Platelet aggregation was measured after dual antiaggregation therapy by light transmittance aggregometry (LTA; APACT 4004) and by multiple electrode aggregometry (MEA; Multipate) after stimulation with arachidonic acid and ADP with prostaglandin E1.

Results: Cut off for patients with efficient therapy by ASA is 25 % of aggregated platelets on LTA (resp. 220 AUC/min - by MEA). Cut off with efficient therapy thienopyridins is 45% of aggregated platelets LTA (resp. 298 AUC/min - by MEA). The incidence of platelets gene polymorphisms are presented.

Conclusion: The frequencies of tested platelets gene polymorphisms implicated in influencing susceptibility to patients with AIM or to failure of antiplatelet therapy, were 10-40% in young patients with AIM. ASA and clopidogrel resistance may be cause by several factors. If aspirin or clopidogrel resistance is detected by laboratory tests, the patient´s compliance with therapy should be discussed first of all. Than we need to follow up poor absorption, inadequate dosing, genetic factors, and interactions with concomitant medications.
Both aggregation methods are suitable for monitoring antiplatelet therapy with aspirin and clopidogrel. The advantage of both LTA and MEA is that they are capable of rapid assessment.
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P11-10

Chronic garlic supplement intake does not potentiate the platelet inhibitory effect of diclofenac

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Garlic consumption has been associated with an increased perioperative bleeding risk in several case reports. We could previously demonstrate that ingestion of raw garlic in healthy volunteers does not impair platelet function. The effect of longterm intake of garlic supplements and the potential additive effect of non-steroidal drugs on platelet function is not known. The aim of this study was to evaluate the effect of a single oral dose of 100mg diclofenac on platelet function in healthy volunteers after 21 days of daily garlic supplement intake. After IRB approval, 32 volunteers were randomized to receive dried garlic powder (KWAI® Forte 300 mg, Cassella-med, Germany) or placebo once daily for 21 days. After 21 days, all volunteers received a single oral dose of diclofenac 100mg. Platelet function was evaluated prior to the first dose of KWAI® or placebo (A), after 21 days (B) and 5 hours after the ingestion of diclofenac (C), after a wash out period of 7 days (D), and 5 hours after a second dose of diclofenac (E). Platelet function was evaluated using multiple electrode aggregometry (MEA; Multiplate ®).
Eleven volunteers in the KWAI group and 14 in the Placebo group completed the study, 7 were excluded. Diclofenac therapy alone did significantly impair platelet function. Platelet function was not impaired by diclofenac in the KWAI group. Garlic supplement therapy does not impair platelet function and does not potentiate the platelet inhibiting effect of diclofenac.

Table 1: Values are given as mean and SD standard deviation; no adjustment for multiple testing was done.

	A: Baseline	B: 21 days of study drug intake.	C: + Diclo-fenac	D: Wash out period	E: Diclofenac alone
KWAI MEA AUC (ASPI)	840 (SD 190)	962 (SD 161) ns	821 (SD 318) ns	854 (SD 260)	506 (SD 357)^{xx}
Placebo MEA AUC (ASPI)	1006 (SD 130)	1073 (SD 213) ns	642 (SD 318) ^{x}	921 (SD 214)	548 (SD 368)^{xx}

[Table 1: Results]
ns: not significant vs. A; ^{x}: significant vs. A; ^{xx} significant vs. D

P12-6

Impaired platelet aggregation – a hint for bacterial contamination?

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Background: Bacterial contamination of platelet concentrates (PCs) represents an ongoing risk in transfusion medicine (bacterial contamination rate up to 0.15%) and is more pronounced in units stored longer than 4 days. As a consequence the shelf-life of PCs has been reduced in Germany to 4 days (using bacterial detection or pathogen reduction it may be prolonged up to 5 days). We investigated different technologies for bacterial detection and measuring platelet aggregation of artificially contaminated apheresis PCs (APCs).

Methods: Bacterial strains (*B. thuringiensis*, *S. pyogenes, K. pneumoniae*, *E. coli*) were inoculated separately into APCs (5-30 CFU/APC) immediately after production and incubated under routine storage conditions for 4 days. Platelet function was analysed daily with 1) Multiplate Analyzer (Roche) upon addition of collagen; 2) aggregometry by Born upon addition of ADP, collagen, arachidonic acid, TRAP6 and ristocetin. The bacterial load was determined by the BactiFlow ALS (Biomerieux AES Chemunex) and classical plate assay. Sterile APCs from the same study period served as negative controls.

Results: Platelet aggregation was reduced in bacterially contaminated products in comparison to sterile APCs during storage. The level of reduction as well as the applicability of the different agonists was depended on donors and bacterial strain growth properties in the product. Hence slower growth of bacterial cells corresponded with a delayed detection of reduction in platelet aggregation.

Conclusion: Contamination of APCs with rapid growing bacterial strains results in a reduction of platelet aggregation. Therefore testing of platelet function during storage could not only improve quality and efficiency of platelet transfusion, it could also prevent transfusion of bacterially contaminated PCs. Further studies are needed to evaluate the details with respect to the different bacterial strains and test systems.