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OC01 - ID 277

THE C-TYPE LECTIN CD93 REGULATES PLATELET ACTIVATION INDUCED BY PAR4 STIMULATION

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Background/Aims: CD93 is a member of group XIV in the C-type lectin superfamily and plays a prominent role in inflammation, vascular diseases, and cancer. In endothelial cells, CD93 is an adhesion molecule regulating angiogenesis through the interaction with the extracellular matrix protein Multimerin-2. CD93 also regulates leukocyte recruitment and migration, as well as efferocytosis. The extracellular domain of CD93 can be released as a soluble factor by proteolytic shedding and its plasma levels are increased in different disorders. Despite its emerging importance in several vascular contexts, the contribution of CD93 to platelet biology is still unknown.
In this study we investigated the role of CD93 in platelet activation induced by physiological platelet agonists.
Materials and Methods: Platelet aggregation, secretion, protein phosphorylation and platelet-neutrophil aggregate formation induced by several agonists were investigated in whole blood and purified human and murine platelets by optical aggregometry, immunoblotting, and flow cytometry.
Results: CD93 is expressed in human and murine platelets, as demonstrated by immunoblotting and immunofluorescence microscopy analyses. Stimulation of human platelets with thrombin or collagen induced CD93 tyrosine phosphorylation and shedding, promoting its release as a soluble extracellular domain. Platelets from CD93-knockout (KO) mice aggregated normally when stimulated with convulxin, thrombin or U46619, but displayed a defective aggregation upon selective stimulation of PAR4. Moreover, integrin $\alpha_{IIb}\beta_3$ activation and α-granule release induced by PAR4-activating peptide were significantly reduced in CD93KO platelets, and formation of platelet-neutrophil aggregates was also impaired. These functional defects were mirrored by a reduction of protein tyrosine phosphorylation and protein kinase C activation. Interestingly, analysis of platelet response upon multiple stimulations over time with low doses of PAR4-activating peptide revealed a more pronounced desensitisation of CD93-deficient platelets compared to wild type cells.
Conclusions: This study reveals a novel role for CD93 in platelet function as a selective regulator of PAR4-mediated signaling.

OC02 - ID 239

GPVI DRIVES PULMONARY INFLAMMATION BY MEDIATING LOCAL PLATELET-NEUTROPHIL COMPLEX FORMATION AND NEUTROPHIL ACTIVATION IN A MURINE MODEL OF ARDS

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Background/Aims: The Acute Respiratory Distress Syndrome (ARDS) is a detrimental inflammatory disease state associated with high mortality. Especially, the infiltration of neutrophils into the pulmonary airspace is causative for the acute inflammation and lung injury. A role of platelet glycoprotein (GP)VI to host defense in a model of pneumonia-derived sepsis was suggested, but the underlying mechanisms remained elusive.
We aimed to mechanistically dissect the contribution of GPVI to thrombo-inflammation in the acute phase of experimental ARDS in mice.
Materials and Methods: GPVI was depleted in wild-type mice by injecting JAQ1 antibody. Acute alveolar inflammation was induced by intranasal instillation of lipopolysaccharide (LPS). A bronchoalveolar lavage (BAL) was performed after 4 hours and infiltrated cells, myeloperoxidase (MPO) activity, hemoglobin content and a set of cytokines were determined. MPO activity and Evans blue dye extravasation in the processed lung were analyzed. Furthermore, high resolution, multi-color confocal microscopy of cryosections and the ventilated lung (intravital confocal microscopy) was performed to assess cell-cell interactions during acute inflammation.
Results: Control mice developed a profound inflammatory response to LPS characterized by pulmonary and blood neutrophilia, hypothermia, and increased blood lactate levels. In contrast, GPVI depleted mice were clearly protected from pulmonary and systemic compromises as evidenced by reduced hypothermia and blood lactate levels, while inflammatory bleeding was not increased. This was accompanied by a markedly mitigated pulmonary neutrophilic infiltration and reduced platelet-neutrophil complex (PNC) formation in lung tissue as detectable in cryosections and by intravital microscopy. Remarkably, the extent of neutrophil extracellular trap (NET) formation was significantly reduced in GPVI-depleted animals compared to control. BAL analyses revealed diminished inflammatory cytokine levels and neutrophilic infiltrates in GPVI-depleted mice.
Conclusion: GPVI drives alveolar inflammation by promoting neutrophil recruitment, PNC formation, and NETosis. GPVI inhibition might be a promising strategy to reduce the acute pulmonary inflammation causing ARDS.
Keywords: lung inflammation, GPVI, neutrophilic granulocyte recruitment, platelets, intravital confocal microscopy

OC03 - ID 263

TEMPORAL LABELLING UNCOVERS FUNCTIONAL AND MOLECULAR CHANGES OF PLATELETS AS THEY AGE IN THE CIRCULATION

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Background/Aims: Levels of young newly formed platelets, have been clinically correlated with adverse cardiovascular outcomes, which may be underpinned in-part by their hyper-reactivity. However, our understanding of the molecular differences between platelets of different ages remains incomplete and is hindered by existing technical limitations.

Materials and Methods: Antibody-based in vivo 'temporal labelling' was applied in healthy C57Bl6 mice to sub-divide, track and study platelets as they age in the circulation during their 5-day lifespan. Ex-vivo calcium-flux, p-selectin expression and aggregation studies were performed along with in-vivo pulmonary embolism studies. Surface receptor expression was determined following sequential blood sampling, and qRT-PCR coupled to unbiased computational analysis performed to explore mRNA content.

Results: Relative to older platelets, young platelets (<24h) responded with greater calcium flux and degranulation, and more readily contributed to thrombi in vitro and in vivo; these responses were independent of platelet size. Age-associated decrease in thrombotic function was accompanied by significant decreases in the surface expression of GPVI and CD31 (PECAM-1). Interestingly old platelets (day 5) exhibited significantly higher expression of CD9. Platelet mRNA content also decreased with age, but the rate of loss varied by individual mRNA, and displayed apparent conservation for those mRNAs encoding granule proteins.

Conclusions: Temporal labelling in vivo defines circulating platelets by age and permits the molecular characterisations necessary to interrogate platelet aging. Here we dispel commonly held beliefs about platelet age and size, identify age-associated losses in key receptors that may underpin changes in hemostatic function, and gain novel insights into the regulation of platelet mRNAs during healthy turnover. This approach will act as a tool to provide future insights into how different platelet-age profiles contribute to health and disease.

OC04 - ID 301

ULTRA-HIGH THROUGHPUT SCREENING IDENTIFIES NOVEL CALCIUM-DEPENDENT ANTIPLATELET DRUGS WITH DISCRIMINATIVE MODE OF RECEPTOR-DEPENDENT ACTION

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Background/Aims: Cytosolic Ca^{2+} is an important platelet second messenger released in response to collagen and thrombin receptors, glycoprotein VI (GPVI) and protease activated receptor 1 and 4 (PAR1/4), respectively. Calcium release triggers downstream platelet responses involved in arterial thrombosis and hemostasis. Recently, we established a well-plate-based $[Ca^{2+}]_i$ assay to screen for potential inhibitory small molecules, interfering in the GPVI and PAR1/4 responses. By ultra-high throughput screening of a large compound library, we aimed to find new inhibitors that can differentiate between GPVI and PAR1/4 activation-induced Ca^{2+} release.

Materials and Methods: Washed human platelets were loaded with Calcium-6 dye and activated with collagen-related peptide (CRP) or thrombin in 96- or 1536-well plates by automated pipetting using the FlexStation or FLIPR-Tetra robots, respectively. Using a panel of 25 known antiplatelet agents, interfering in specific signalling steps (receptors, phospholipases, protein kinases, Ca^{2+}-ATPases), we selected 8 $[Ca^{2+}]_i$ trace parameters to develop an algorithm for predicting a given Ca^{2+} release and platelet activation profile. This algorithm was applied to select hit molecules from a 16,640 small molecule compound screening, performed in 1536-well plates.

Results: The panel of antagonists differed in their specific effects on CRP- or thrombin-induced $[Ca^{2+}]_i$ trace parameters. Using the algorithm and an ADME-Tox filtering tool, we selected 12 hit compounds for further study, which showed differential suppression effects on GPVI- or PAR1/4 responses. The selected hit compounds did not affect cell viability. After establishing IC_{50} values based on Ca^{2+} responses, two compounds were obtained that interfered with CRP-induced activation and one compound that inhibited thrombin-induced activation. The inhibitory mechanism was via so far poorly studied signalling targets. Several platelet function tests indicated broad applicability of the compounds.

Conclusions: Using an ultra-high-throughput screening assay of agonist-induced $[Ca^{2+}]_i$ release, we found potential calcium-dependent antiplatelet agents.

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OC05 - ID 233

INTEGRIN A5B1 IN HAEMOSTASIS AND ARTERIAL THROMBOSIS IN MICE

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Background: Integrins α5β1 and αIIbβ3 are the main platelet fibronectin receptors. It is known that α5β1 participates in the platelet adhesion, activation and aggregation on fibrillar fibronectin under flow conditions, but its importance in hemostasis and arterial thrombosis remains unknown.

Aim: The objective of this study was to characterize the role of α5β1 integrin in platelet responses to fibronectin and to determine its importance in haemostasis and experimental thrombosis in mice.

Methods: We generated a mouse strain which does not express α5β1 on platelets using the PF4Cre/lox recombination system. An in vitro flow based-assay was used to monitor platelet adhesion and thrombus formation. The role of α5β1 was assessed in experimental models based on $FeCl_3$-, mechanical- and laser-injuries. The tail-bleeding time assay was used to evaluate haemostasis.

Results: No obvious abnormalities were detected in PF4Cre- $\alpha5^{-/-}$ mice. Expression of the main platelet surface receptors (αIIbβ3, α2, α6, β1, GPIbα, GPV, GPIX, GPVI) was normal, except for α5 which was markedly reduced. Aggregation of PF4Cre-$\alpha5^{-/-}$ platelets, as well as P-selectin exposure, fibrinogen and annexin V binding were normal in response to a series of soluble agonists including ADP, thrombin and U46619. PF4Cre-$\alpha5^{-/-}$ platelet adhesion under flow was normal on fibrinogen, laminin (300 s^{-1}) or von Willebrand factor (1,500 s^{-1}), but displayed a marked decrease in adhesion, activation and aggregation on fibrillar fibronectin and collagen (300 s^{-1}). No defects were observed in three experimental models of arterial thrombosis. PF4Cre-$\alpha5^{-/-}$ mice presented no increase in tail-bleeding time.

Conclusion: This study shows that platelet α5β1 integrin is an important receptor for fibrillar fibronectin but is dispensable for haemostasis and arterial thrombosis.

OC06 - ID 235

CAROTID STENT THROMBOSIS IN ACUTE STROKE WITH TANDEM LESIONS: FOCUS ON THE MECHANISM AND ITS PREVENTION

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Conflicts of interest: Authors declare having no conflict of interest.

Background: In case of acute stroke with tandem lesions, emergent stenting of the carotid improves recanalization rates and clinical outcomes. During this procedure, dual-antiplatelet therapy is usually avoided to limit the risk of haemorrhagic transformation. As a consequence, early stent thrombosis (ST) occurs in around 20% of the patients and results in worsening of clinical outcomes. A better understanding of the mechanisms of ST opens new avenues to develop less thrombogenic stents or improved anti-platelet approaches. Recent results from our laboratory suggest that the blockade of platelet GPVI, impairs fresh thrombus formation and could therefore represent an attractive candidate to prevent ST.

Aims: The objective is to evaluate the role of rheology in carotid ST and the potential of glenzocimab, a GPVI blocker, in the prevention of ST.

Methods: We developed an on-scale macrofluidic flow chamber mimicking a human carotid artery, to investigate the thrombogenicity of clinically-used stents. Stent-induced flow disturbances were characterized using computational fluid dynamics (CFD). Platelet aggregation on stents was studied using fluorescence and scanning electron microscopy (SEM).

Results: Real-time video-microscopy showed that anticoagulated whole blood perfused at physiological shear rates, resulted in the accumulation of platelet thrombi on the stent struts. SEM showed that the platelets were highly activated. Regions of platelet aggregation were not randomly distributed; hot spots of thrombus formation were identified around the crossing of the stent mesh, suggesting a role of local rheology. CFD allowed for precise characterisation of the flow occurring at these hot spots and identification of thrombogenic flow profiles. Glenzocimab reduces platelet thrombus in a chamber implanted with a stent and coated with human atherosclerotic plaque homogenate, suggesting that GPVI plays an important role in ST.

Conclusions: Our results highlight the pro-thrombogenic potential of local stent-mediated blood flow disturbances. Moreover, glenzocimab opens new possibilities to safely prevent the occurrence of ST.

OC07 - ID 242

GPIBA SHEDDING IS RESTRICTED TO THE INNER PLATELET MEMBRANE

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Aim: GPIbα is an abundant transmembrane receptor on the platelet cytoplasmic membrane. Following strong and sustained platelet activation, ADAM17 catalyzes GPIbα proteolysis thereby releasing the soluble glycocalicin ectodomain. We aimed to understand the enzyme-substrate relationship in space and time.

Methods: ADAM17 and GPIbα were detected using labeled monoclonal antibodies in human resting platelets or after activation via GPVI and PARs, using immunofluorescence and western blot analysis.

Results: To chase glycocalicin, resting platelets were preincubated with anti-GPIbα monoclonal antibody before activation. After activation, the majority of antibody was detected in the platelet pellet with only 11.2±4.0% released in supernatant despite 82.1±4.9% of GPIbα effectively shed. Furthermore, preincubation of resting platelets with anti-GPIbα antibody 5G6, targeting the ADAM17 cleavage site, did not inhibit glycocalicin release (93±6% relative to control without 5G6). When 5G6 was present during activation, glycocalicin release was partially inhibited (59±11% relative to control, p=0.02; n=5). Finally, pretreatment of platelets with the membrane impermeable O-sialoglycoprotein endopeptidase removed a 45kDa N-terminal fragment of surface-exposed GPIbα but had no effect on the molecular weight or amount of glycocalicin released in platelet supernatant following activation-dependent shedding. Statistically, >80% of GPIbα was an ADAM17 substrate, while <2% of released GPIbα was surface-exposed. This agrees with our transmission electron microscopy findings using anti-ADAM17 immunogold labeling, showing ADAM17 resides almost exclusively (96±2% of label; n≥25 platelets) within the inner membranes of both resting and activated platelets. This intracellular detection was confirmed by immunofluorescence microscopy and flow cytometry where successful ADAM17-antigen detection required permeabilization with saponin. In addition, only membrane-permeable sheddase inhibitors marimastat, GM6001 and KP-457 significantly inhibited GPIbα shedding (88±5%, 88±4% and 95±1%, respectively). In contrast, membrane-impermeable ADAM17 inhibitors D1A12 (11±11%) and recombinant prodomain (7±10%; ≥6) could not inhibit metalloproteolysis of GPIbα.

Conclusion: The majority of GPIbα is a substrate for ADAM17, but surface-exposed GPIbα is not.

OC08 - ID 253

PLATELET-RELEASED MITOCHONDRIA INTERACT WITH NEUTROPHILS CAUSING ALTERATIONS IN THEIR PHENOTYPE

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Background: Activated platelets produce a heterogenous population of extracellular vesicles packaged with pro-inflammatory and pro-thrombotic molecules. A small subset of these vesicles contains functional mitochondria that actively respire and consume oxygen. Interactions between platelet vesicles and cells within the circulation have been described, however to date, mitochondria containing vesicles have not been defined as a separate population. Notably, mitochondria are damaged associated molecular patterns which raises the question whether mitochondria vesicles will interact with and affect immune cells.

Aims: To investigate if platelet vesicles containing mitochondria interact with neutrophils thereby changing their phenotype.

Methods: Washed platelets ($3x10^8$/ml) stained with MitoTracker Orange (20nm) were incubated with TRAP-6 (20μM; 2 hours, 37˚C) to stimulate vesicle production. Cell sorting was used to separate mitochondria negative (PMV) and positive (mitoPMV) vesicles. Isolated neutrophils were incubated with sorted vesicles (45 mins, 37˚C) and analysed for the expression of CD66b, CD11b and CXCR2 by flow cytometry. Data were analysed using Image J, FlowJo v.10 and GraphPad Prism.

Results: MitoPMVs accounted for 19±1% of the vesicle population, had high levels of P-selectin expression (96±1%, n=8) and interacted with and were internalised by neutrophils. Microscopy revealed mitoPMVs merged with the neutrophil mitochondria network. Furthermore, neutrophils incubated with mitoPMVs, but not those incubated with PMVs, showed significant increases in expression of CD66b (1.5±0.2-fold; n=6, p<0.05) and CD11b (1.3±0.1; n=6, p<0.05) with a concurrent reduction in CXCR2 expression (0.7±0.07-fold, n=6, p<0.05).

Conclusion: Mitochondrial transfer has been demonstrated in numerous cells highlighting a mechanism in which mitochondrial function may be improved. Here we show that mitochondria encapsulated within platelet vesicles interact with neutrophils causing an increase in activation and phagocytosis markers. Following initial interactions, the platelet-derived mitochondria can merge with existing neutrophil mitochondrial networks. Further work is required to understand if this interaction can enhance or alter neutrophil metabolic capacity.

OC09 - ID 255

TICAGRELOR PREVENTS INFECTIVE ENDOCARDITIS BY MITIGATING *STAPHYLOCOCCUS AUREUS* VIRULENCE

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Background: Infective endocarditis (IE) is a deadly disease mainly caused by the gram-positive and highly virulent bacteria Staphylococcus aureus (SA). Due to lack of efficacy of current antibiotic therapy, there is an urgent need to discover new strategies that could prevent this disease.

Aim: To assess the ability of the antiplatelet drug ticagrelor, which also displays antibacterial activity against gram-positive bacteria, to prevent SAIE.

Methods: We used a mouse model of infective endocarditis induced by a SAIE clinical isolate. Ticagrelor and clopidogrel were administered prior to local histamine infusion on the aortic valve and infection by SAIE isolate. Infected vegetation presence was determined by Gram staining on heart sections after three days. The antibacterial effect of ticagrelor on key mechanisms of infective endocarditis development was assessed in vitro.

Results: A single administration of ticagrelor at conventional antiplatelet dosage (3mg/kg) prior to infection significantly prevented the formation of infected vegetation, with IE in only 14.3% of ticagrelor-treated mice (n=21) compared to 55 % of vehicle-treated mice (n=20). Interestingly, clopidogrel treatment failed to prevent disease development with IE in 61.1% of the mice (n=18) which made it unlikely that solely the antiplatelet effect would explain IE prevention by ticagrelor (Figure 1). Ticagrelor dosed at plasma levels achieved in patients (0.75ug/ml - 1.25ug/ml) did not inhibit SAIE growth in liquid cultures but it caused drastic adherence defect on activated endothelial cells and extracellular components. Furthermore, we found that growing SA in the presence of ticagrelor altered the Agr regulator system, leading to reduced toxin production and toxin-induced platelet aggregation while preserving the ability of platelets to kill those bacteria.

Conclusion: Our study demonstrates unprecedented ability of ticagrelor to prevent IE by directly mitigating bacterial virulence. Hence, clinical trials using ticagrelor as adjunct therapy to antibiotics in patients at risk for IE are warranted.

OC10 - ID 275

MOLECULAR INSIGHTS INTO THE CAUSE AND TREATMENT OF CONGENITAL THROMBOCYTOPENIA IN MICE LACKING THE CO-INHIBITORY RECEPTOR G6B-B

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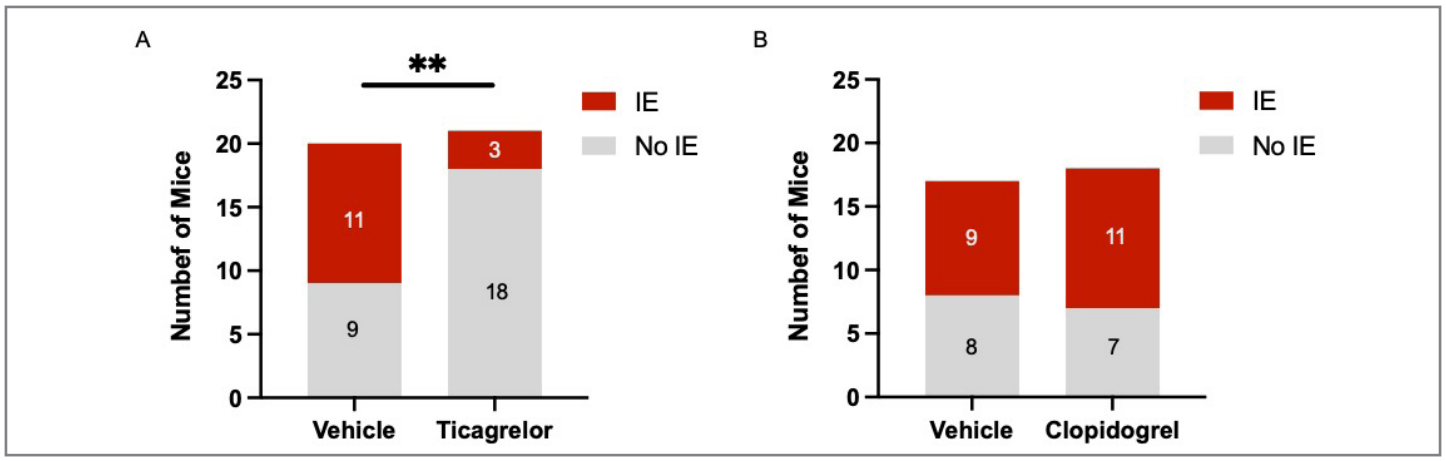
Background: Thrombocytopenia is a common platelet disorder with a variety of etiologies, including antibody-mediated platelet activation and clearance, aberrant megakaryocyte (MK) development, infection and trauma. Clinical outcomes vary from mild purpura and bruising to severe, life-threatening bleeding. Mice lacking the immunoreceptor tyrosine-based inhibition motif-containing co-inhibitory receptor G6b-B (Mpig6b, G6b knockout, KO) are born with a complex MK/platelet phenotype



OC09 - Figure 1.

characterized by severe macrothrombocytopenia, expansion of the MK population and myelofibrosis. Platelets are almost completely devoid of the GPVI-FcR g-chain collagen receptor complex and a subset have increased surface immunoglobulins. A strikingly similar phenotype has been recently reported in patients with null and loss-of-function mutations in MPIG6B.

Aims: To better understand the cause and treatment of macrothrombocytopenia and GPVI down-regulation in G6b KO mice.

Methods: G6b KO mice were either treated with standard therapies for macrothrombocytopenia, or crossed with mice Rag1 KO or Syk R41A loss-of-function mice to rescue the phenotype. Platelet count and volume were measured using a blood analyzer. Platelet surface receptor expression and activation were measured by flow cytometry. Platelet aggregation and secretion were measured by lumi-aggregometry.

Results: Intravenous-immunoglobulin resulted in a transient partial recovery of platelet counts in G6b KO mice, whereas crossing these mice with Rag1 KO mice, lacking B and T cells, had no effect. Treatment with the thrombopoietin mimetic Romiplostim rescued platelet count, GPVI expression and the response of G6b KO platelets to the GPVI-specific agonist collagen-related peptide. Loss-of-function of Syk tyrosine kinase (R41A) and treatment with the Syk kinase inhibitor BI1002494 rescued platelet counts and GPVI expression in G6b KO mice, whereas the Src family kinase inhibitor dasatinib did not.

Conclusions: These findings provide mechanistic insights into the cause of the disease and mode of action of Syk inhibitors in the treatment of congenital macrothrombocytopenia caused by mutations in G6b.

OC11 - ID 298

REDUCED PLATELET GLYCOPROTEIN IBA SHEDDING ACCELERATES THROMBOPOIESIS AND COX-1 RECOVERY: IMPLICATIONS FOR ASPIRIN DOSING REGIMEN

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Background/Aims: Cardiovascular prevention with low-dose aspirin can be less effective in patients with a faster recovery of platelet cyclooxygenase (COX)-1 activity during the 24-hour dosing interval. We previously showed that incomplete suppression of TXA_2 over 24 hours can be rescued by a twice daily aspirin regimen.

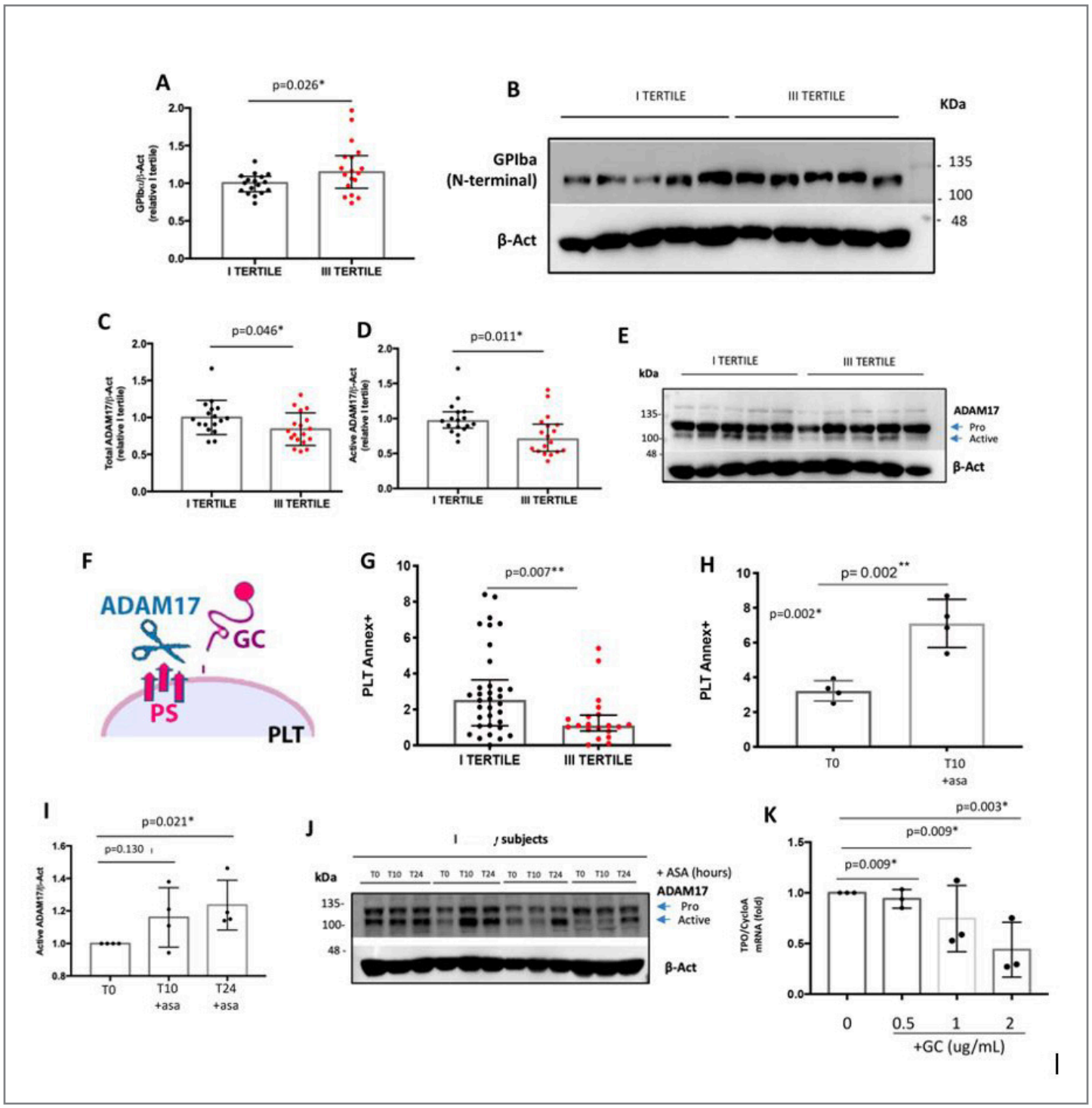
Here we show that reduced platelet glycoprotein (GP)Ibα shedding characterizes patients with accelerated COX-1 recovery and may contribute to higher thrombopoietin production and higher rates of newly-formed platelets, escaping aspirin inhibition over 24 hours.

Materials and Methods: Two-hundred aspirin-treated patients with high cardiovascular risk (100 with type 2 diabetes mellitus) were stratified according to the kinetics of platelet COX-1 activity recovery during the 10-24h dosing interval.

Results: Whole proteome analysis showed that platelets from patients with accelerated COX-1 recovery were enriched in proteins involved in cell survival, inhibition of apoptosis and cellular protrusion formation. In agreement, we documented increased plasma thrombopoietin, megakaryocyte maturation and proplatelet formation, and conversely increased platelet galactose and reduced phosphatidylserine exposure and

ADAM17 activation, translating into diminished GPIbα cleavage and glycocalicin release. Treatment of HepG2 cells with recombinant glycocalicin led to a dose-dependent reduction in liver thrombopoietin mRNA, suggesting that reduced GPIbα ectodomain shedding may unleash thrombopoiesis. A cluster of clinical markers, including younger age, non-alcoholic fatty liver disease, visceral obesity and higher thrombopoietin/glycocalicin ratio, predicted with significant accuracy the likelihood of faster COX-1 recovery and suboptimal aspirin response.

Conclusions: Circulating thrombopoietin/ glycocalicin ratio, reflecting a dysregulation of platelet lifespan and production, may provide a simple tool to identify patients amenable to more frequent aspirin daily dosing.



OC11 - Figure 1.

OC12 - ID 227

PLATELET RNA SEQUENCING FOR SLFN14 K219N DEFICIENCY SHOWS OVEREXPRESSION OF RIBOSOMAL GENES

Fabienne Ver Donck[1], Kato Ramaekers[1], Chantal Thys[1], Kathelijne Peerlinck[1,3], Chris Van Geet[1,2], Koji Eto[4,5], Veerle Labarque[1,2], Kathleen Freson[1]

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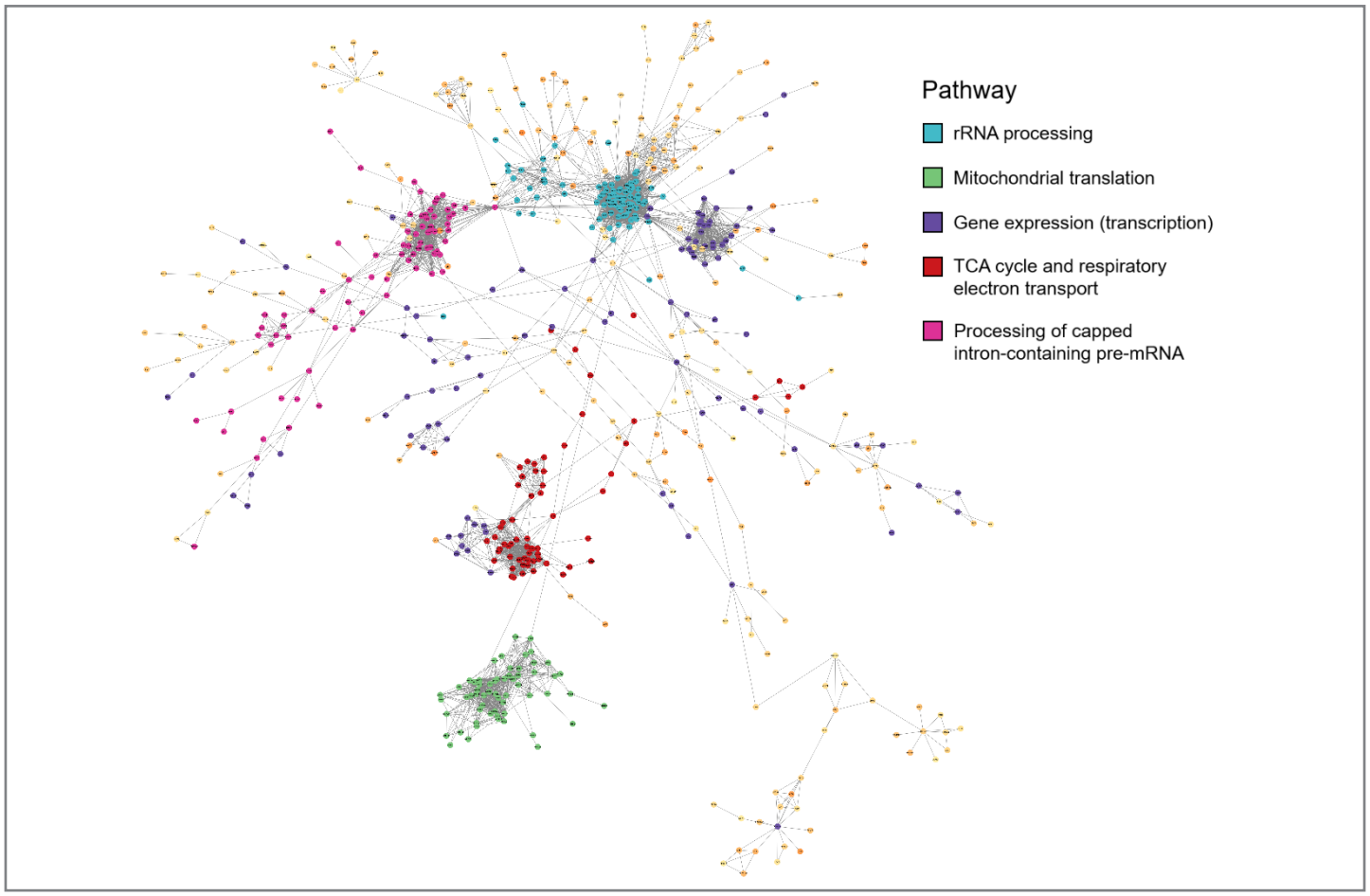
Background: Single nucleotide variants in SLFN14, which encodes an RNA endoribonuclease protein, are known to cause inherited thrombocytopenia and impaired platelet aggregation and ATP secretion. Despite mild laboratory defects, these patients display an obvious bleeding phenotype. The function of SLFN14 in megakaryocyte and platelet biology is currently not well studied.
Aim: This study aims to characterize the platelet transcriptome in patients with a SLFN14 K219N variant using RNA sequencing and to model the disease using the immortalized MegaKaryocyte Cell Line imMKCL (Nakamura et al. Cell Stem Cell 2014).
Methods: Patients' platelet RNA was extracted and used for total RNA sequencing. Differential expression analysis and pathway enrichment was performed using the DESeq2 R package and Cytopscape3, respectively. The K219N variant was introduced in imMKCL using CRISPR/Cas9.
Results: The SLFN14 K219N variant was detected by WES in thrombocytopenia patients from a large pedigree. Platelet RNA was sequenced for two patients and 19 healthy controls. Differential gene expression analysis yielded 2999 and 2888 significantly (|log2FC|>1, FDR<0.05) upregulated and downregulated genes, respectively. While upregulated genes were enriched for pathways relating to (mitochondrial) translation (represented by >60 different ribosomal genes shown in the blue and green pathway nodes), transcription and splicing (Figure 1), no significant pathways were found for downregulated genes. Heterozygous SLFN14 K219N imMKCL showed a pronounced defect in megakaryocyte differentiation and proplatelet-formation compared to the wild type condition. An almost complete block in megakaryopoiesis was detected for the homozygous SLFN14 K219N imMKCL. SLFN14 defective megakaryocytes shown rRNA degradation and contain numerous multilamellar bodies as well as dysmorphic mitochondria.
Conclusion: Our results indicate a dysregulation of gene translation and transcription as potential disease mechanism underlying SLFN14-related thrombocytopenia.



OC12 - Figure 1. Gene network illustrating pathway enrichment for significantly upregulated genes in SLNF14 K219N defective platelets.

OC13 - ID 228

STRUCTURAL STUDIES OF GLYCOPROTEIN VI: FROM EXTRACELLULAR DOMAINS TO FULL-LENGTH

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Background / Aims: Glycoprotein VI (GPVI) is the major signalling receptor for collagen on platelets and is a promising anti-thrombotic target. We have raised a series of high affinity blocking nanobodies to the extracellular immunoglobulin (Ig) domains in GPVI. Currently, our structural knowledge is limited to these domains. Our understanding of GPVI presentation and activation at the cell surface however is hindered by our lack of structural knowledge for the rest of the receptor.

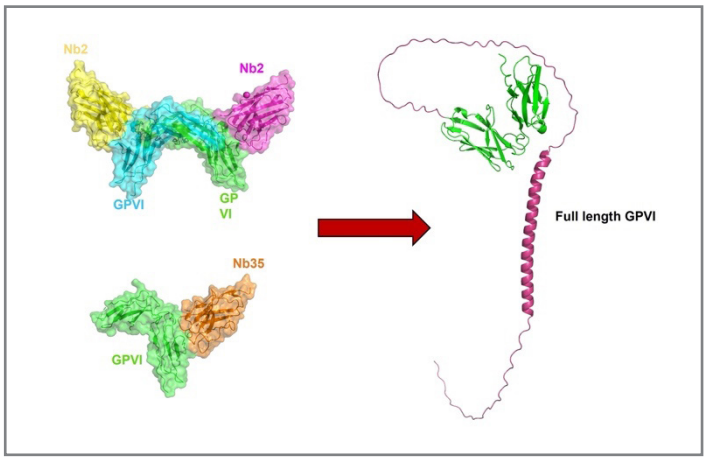
Our aims are to solve the structures of several of the inhibitory nanobodies in complex with GPVI to map their binding sites and mode of inhibition. Secondly, we aim to solve the full-length membrane bound structure of GPVI in complex with the associated FcRγ signalling chain.

Methods: We used x-ray crystallography to solve the complex of extracellular GPVI with two inhibitory nanobodies. Using styrene maleic acid (SMA) polymers, we have extracted GPVI-FcRγ from the membrane of transfected cells and use cryo-electron microscopy to solve the full-length structure. GPVI-mutants were expressed in DT40 B cells; a NFAT reporter assay was used to measure activation.

Results: Solving the structures of GPVI bound to two inhibitory nanobodies by x-ray crystallography revealed a common inhibitory binding site and we identified Arg46 as critical for inhibition. In addition, we observed a novel domain swapped GPVI dimer conformation which through mutation and functional studies we link to biological activity.

We have extracted the full-length GPVI-FcRγ complex from the membrane using SMA and have used negative stain and cryo-electron microscopy to gain single particle images in the presence and absence of collagen to move towards a full-length structure.

Conclusions: Using nanobodies we have identified both a potent inhibitory binding site within GPVI and a biologically active GPVI dimer conformation. The relationship of these recombinant structures to the full-length receptor is under investigation.



OC13 - Figure 1.

OC14 - ID 234

LIGHT-INDUCED ION INFLUX TRIGGERS MEGAKARYOCYTE POLARIZATION

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Background/Aims: Bone marrow megakaryocytes (MKs) extend proplatelets into sinusoidal blood vessels where these proplatelets undergo fission to release platelets. The molecular mechanisms that regulate megakaryocyte differentiation, polarization and proplatelet formation are only poorly understood. Here, we expressed an optogenetic construct in primary MKs, which allows us to study these complex processes by spatiotemporally controlling cellular activity using light. A widely used optogenetic construct is Channelrhodopsin2 (ChR2), which is a blue light-activatable, non-selective cation channel from the green alga Chlamydomonas reinhardtii. We modified ChR2 to obtain higher calcium conductance and named the protein ChR2 XXM2.0. We expressed ChR2 XXM2.0 in MKs to manipulate calcium signaling by light in a high spatiotemporal manner in order to better understand the influence of changes in intracellular Ca^{2+} levels on MK function.

Materials and Methods: ChR2 XXM2.0 was expressed in bone marrow-derived MKs after virus transduction. Whole cell patch-clamp was used to test the functionality of the channel. After spreading on fibrinogen, MKs were globally or locally illuminated and subsequent MK behavior was analyzed by confocal microscopy. Inhibitors and genetically modified mouse models were applied to identify key proteins involved in light-induced cellular response.

Results: ChR2 XXM2.0 localized in the plasma membrane and the demarcation membrane system of MKs. Blue light induced a significant photocurrent in ChR2 XXM2.0 expressing MKs, which indicates cation influx. Global illumination of ChR2 XXM2.0 expressing MKs spread on fibrinogen resulted in intracellular calcium increase, stress fiber formation and further MK spreading. Local illumination triggered MK polarization and motility towards the direction of light. Using inhibitors and genetically modified mouse models, we show that MK polarization was dependent on calcium influx, Cdc42 and myosin IIA activity as well as integrin αIIbβ3 activation.

Conclusions: We established optogenetics in bone marrow-derived MKs. Light-induced local cation influx triggered Cdc42-, myosin IIA- and integrin αIIbβ3 activation dependent MK polarization.

OC15 - ID 237

PHARMACOLOGICAL INHIBITION OF SHP2 INHIBITS MEGAKARYOPOIESIS, THROMBOPOIESIS AND MPL SIGNALLING

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Background: The non-transmembrane protein-tyrosine phosphatase (PTP) Src homology (SH2) domain-containing PTP 2 (Shp2), encoded by the proto-oncogene PTPN11, has been implicated in many cellular processes, including survival, proliferation and differentiation of multiple cell types, including megakaryocytes (MKs). Shp2 has been demonstrated to regulate signalling from a variety of tyrosine kinase-linked receptors, including cytokine and growth factor receptors and is a well-known positive regulator of the Ras-MAPK pathway. Targeting Shp2 pharmacologically, therefore represents a therapeutic strategy for many Ras-driven cancers.
Aims: Investigate the role of Shp2 in mouse and human megakaryocytopoiesis and thrombopoietin (Tpo) receptor Mpl signalling, through the use of a specific allosteric Shp2 inhibitor, SHP099.
Methods: MK maturation, Mpl signalling, proplatelet formation and platelet release were analysed in primary mouse and human haematopoietic progenitors treated with SHP099, by flow cytometer, capillary-based immunoassays and microscopy assays respectively. SHP099 was also tested on human MK cultured ex vivo in an artificial three-dimensional (3D) miniature marrow bioreactor system.
Results: Treatment of primary bone marrow (BM)-derived MKs with 10 µM SHP099 affect slightly cell viability, however it resulted in an inhibition of proliferation and maturation, with decreased number of mature MKs and reduced ploidy, as well as a decreased number of proplatelet-forming MKs. Similar results were observed in human $CD34^+$-derived MKs. SHP099 also inhibited the number of platelet released from $CD34^+$-derived MKs, showing that Shp2 activation is required for platelet formation. Moreover, Tpo-induced Erk1/2 and Akt phosphorylation was significantly decreased in $CD34^+$-derived MKs, demonstrating that Shp2 is a positive regulator of Mpl signalling. Using a 3D ex vivo BM niche model made of silk fibroin, confocal microscopy analysis also revealed a reduction in human proplatelet formation and branching, and a decreased number of platelets released in the presence of SHP099.
Conclusions: Shp2 activation is essential for mouse and human megakaryocytopoiesis and in mediating Mpl signal transduction.

OC16 - ID 254

PLATELET DYSFUNCTION IN HFPEF PATIENTS: REDUCED PLATELET ACTIVATION AND THROMBUS FORMATION UNDER FLOW

Giorgia D'Italia[1], Titus Lemmens[1], Simone Wielders[1], Daniëlle M Coenen[2], Sanne GJ Mourmans[3], Ahmad Al-Abadi[1], Joël Franssen[3], Samah Toutouh[1], Bibian ME Tullemans[1], Jerremy Weerts[3], Arantxa Barandiaran Aizpurua[3], Vanessa PM van Empel[3], Blanche Schroen[3], Judith MEM Cosemans[1]

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Background/Aims: Heart failure with preserved ejection fraction (HFpEF) results from a complex interplay of systemic syndromes, including diabetes and hypertension, characterized by chronic low-grade inflammation and microvascular dysfunction (MVD). In recent years, the importance of platelets in vascular inflammation and endothelial dysfunction emerged, suggesting an unexplored role for platelets in MVD. However, the role of platelets in HFpEF is still poorly examined. The aim of this study is to investigate whether HFpEF patients present alterations in platelet functions.
Materials and Methods: Endothelial and platelet activation markers were measured in plasma from HFpEF patients and age- and sex-matched controls. Moreover, platelet integrin $\alpha_{IIb}\beta_3$ activation and platelet α-granule secretion were measured by flow cytometry using freshly isolated platelets from HFpEF patients (N=118) and controls (N=38-50, depending on assay), stimulated with different agonists. Microfluidics assays were performed with whole blood from HFpEF patients and controls to measure platelet adhesion, platelet activation markers (CD62P, fibrin(ogen), annexin A5), and thrombus growth under arterial flow conditions.
Results: VCAM-1, a marker for endothelial cell activation, is increased in HFpEF patients compared to controls, likely reflecting the inflammatory state in HFpEF. In contrast, platelet activation markers - β-TG, PF4, TSP-1 - are decreased. Moreover, platelets from HFpEF patients show decreased integrin $\alpha_{IIb}\beta_3$ activation and α-granule secretion upon stimulation with collagen-related peptide and TRAP-6, and increased platelet integrin activation upon stimulation with ADP. Under coagulating conditions, HFpEF patients show an overall reduction in platelet activation, thrombus contraction and density, and fibrin formation under flow.
Conclusions: In sum, our preliminary results show endothelial cell activation and platelet dysfunction in HFpEF patients compared to controls, suggesting a possible unrecognized role of platelets in HFpEF.

OC17 - ID 262

ULTRASTRUCTURAL CHANGES IN PLATELETS ASSOCIATED WITH VIRAL XENOPHAGY IN SEVERE COVID-19 PATIENTS

Cédric Garcia[1,3], Jonathan Au Duong[2,3], Michael Poëtte[2,3], Agnès Ribes[1,3], Bruno Payre[4], Vincent Mémier[1], Pierre Sié[1,3], Vincent Minville[2], Sophie Voisin[1,3], Bernard Payrastre[1,3], Fanny Vardon-Bounces[2,3]

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[3] Inserm UMR1297 and Université Toulouse 3, Institut des Maladies Métaboliques et Cardiovasculaires, Toulouse, France
[4] Université Paul Sabatier, Faculté de Médecine de Rangueil, CMEAB, Toulouse, France

Background: Since the beginning of the COVID-19 pandemic, several groups have shown the implication of platelets in the thrombo-inflammatory reaction of sever COVID-19 patients. Mild thrombocytopenia correlating with the severity of the disease, changes in platelet gene expression, increased platelet functionality and presence of platelet-rich thrombi in the lungs have been reported. However, the potential role of platelets in the mechanism of viral clearance remains poorly characterized. While the ACE2 receptor of SARS-CoV-2 is not present on platelets, viral internalization in platelets has been shown to occur through different mechanisms.

Aims: To study the ultrastructure of platelets from patients with severe COVID-19 following their admission to the intensive care unit and to analyse the potential presence of SARS-CoV-2.

Methods: The ultrastructure of platelets and the presence of viral material was analysed by transmission electron microscopy, confocal imaging and immunoblotting in a prospective cohort of 29 patients with severe COVID-19 following their admission to the intensive care unit of the Toulouse Hospital.

Results: SARS-CoV-2 particles were found in a significant fraction of platelets, as assessed by immunogold labelling and immunofluorescence of Spike S and nucleocapsid proteins. Compared to platelets from healthy donors, we observed the presence of large intracellular vesicles and autophagolysosomes in severe COVID-19 platelets. These platelets showed large LC3-positive structures, increased levels of LC3B type II and a co-localization of LC3B with Spike S protein.

Conclusion: Our data indicate that in severe COVID-19 patients, platelets can uptake SARS-CoV-2 material and process it through a selective autophagy response reminiscent to xenophagy strongly suggesting that platelets contribute to viral clearance in the bloodstream.

OC18 - ID 264

SINGLE-CELL RNA SEQUENCING REVEALS ABERRANT MEGAKARYOCYTE-ERYTHROID PROGENITOR AND MEGAKARYOCYTE POPULATIONS IN THROMBOCYTOPENIC ETV6 VARIANT CARRIERS

Timothée Bigot[1], Elisa Gabinaud[1], Laurent Hannouche[1], Véronique Sbarra[1], Dominique Payet-Bornet[2], Marie Loosveld[2], Delphine Potier[2], Marie-Christine Alessi[1], Marjorie Poggi[2]

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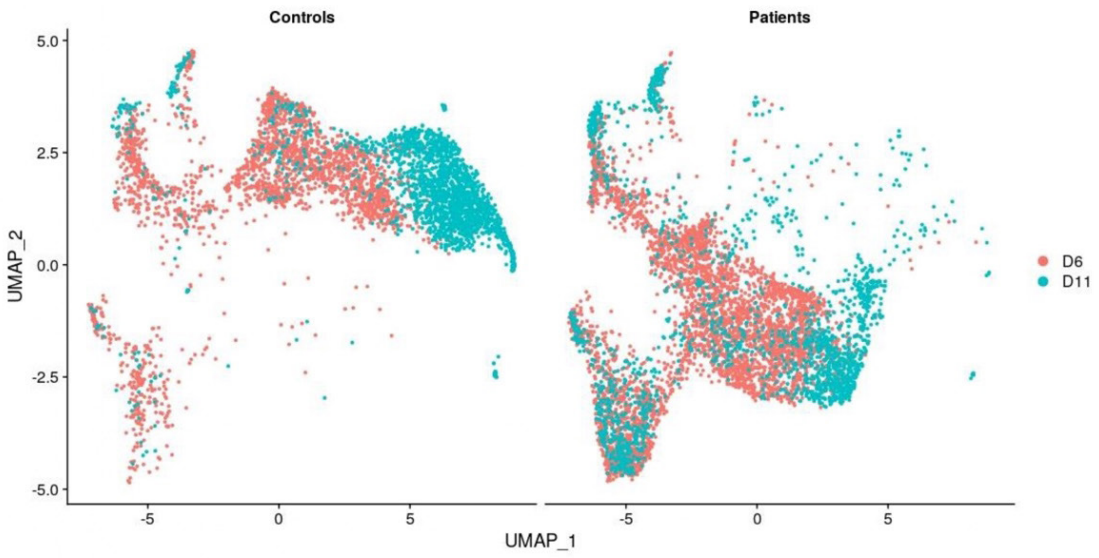
Background/Aim: Germline mutations in the ETV6 transcription factor gene are responsible for a familial thrombocytopenia and leukemia predisposition syndrome. Although previous studies showed that ETV6 plays an important role in megakaryocyte (MK) maturation and platelet formation, the mechanisms by which ETV6 dysfunction promotes thrombocytopenia remain unclear. Presuming that ETV6 mutations have selective effects at a particular cell stage, our objective is to study the effect of ETV6 variations on the differentiation of hematopoietic-stem/progenitor-cells (HSPC) at a single-cell level.

Materials & Methods: Peripheral $CD34^+$-cells were isolated from healthy controls and patients carrying two different ETV6 variants (P214L localized in the central and F417LfsTer4 in the ETS domains) and differentiate in vitro in MK. F417LfsTer4 has never been reported. Transcriptomic profiles were analyzed by scRNAseq at days 6 and 11 of culture.

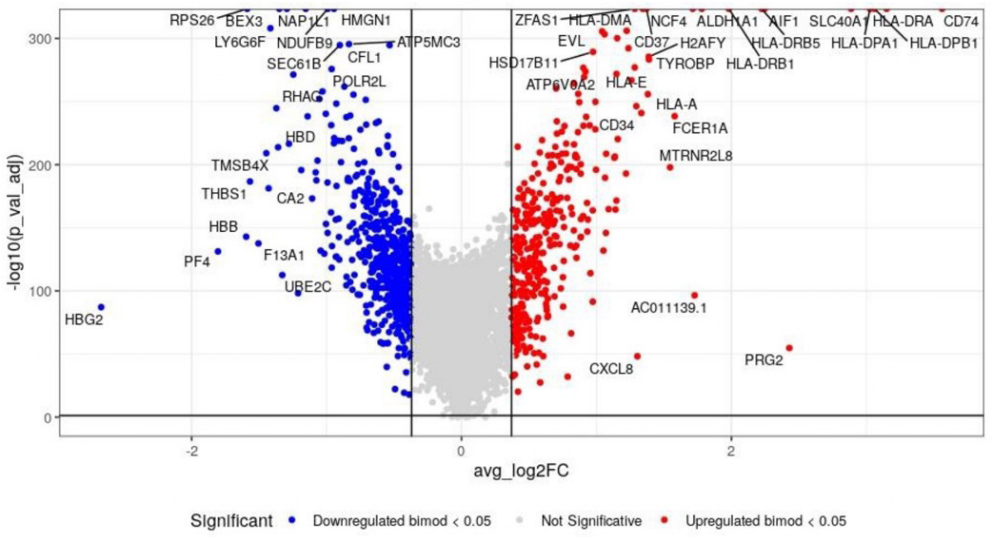
Results: The transcriptomic profiles of the F417LfsTer4 and P214L ETV6 are similar, indicating that the same dysregulation is observed with both variations. The patients' cells strongly differ from those of controls (Figure 1). ETV6 deficient condition is characterized by a higher proportion of HSPC (17±6.9 vs 3.0±0.7%,), a reduced proportion of megakaryocyte-progenitor/megakaryocyte (MKP/MK, 16.9±4.1 vs 38.5±1%) and a lack of platelets. Transcriptomic profiles of HSPC, common myeloid progenitor (CMP) and granulocyte/monocyte progenitor (GMP) were closed between wild-type and ETV6 variant cells. Divergence intensifies at megakaryocyte-erythroid progenitor (MEP) and MkP/Mk stages (Figure 2). Pathway analysis in these latter identified a down regulation of "mitochondrial pathways" (i.e. oxidative phosphorylation, respiratory electron transport chain, ATP synthesis) and an upregulation of "translation" pathways in patients.

Conclusion: ETV6 mutations affect early hematopoiesis, at the level of the CMP/MEP transition, resulting in aberrant MEP population and a defect in megakaryopoiesis. Analysis of the deregulated pathways in patients will contribute to better understand the ETV6 pathology and may represent potential targets for diagnosis and therapy purposes.



UMAP plot of cells at day 6 and 11 split by genotype.
"Ctrl" for controls and "Patients" for ETV6-variants carriers. Blue color labeled for cells analyzed at day 6 (D6) and orange color at day 11 (D11).

OC18 - Figure 1.



Volcano plot of up and down regulated genes in ETV6-variant carriers vs wild-type megakaryocytes
Downregulated genes in ETV6-variant carriers are in blue and upregulated genes are in red. Thresholds: p_adj_val < 0,05 & avg_log2FC > 0,37 or p_adj_val < 0,05 & avg_log2FC < -0,37 (corresponding to a Fold Change > 1,3 or < -1,3).

OC18 - Figure 2.

OC19 - ID 279

BLOOD PLATELETS AT THE CROSSROAD BETWEEN ALZHEIMER'S DISEASE AND AGEING-RELATED FRAILTY

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[4] Department of Medicine and Surgery, Division of Internal and Cardiovascular Medicine, University of Perugia, Italy

Background/Aim: In humans, age-related frailty is commonly associated to Alzheimer's Disease (AD). The physiopathological mechanism remains poorly understood, but an association with a low-grade chronic inflammation is recurrent. Beside their classical role, platelets are true inflammatory cells and are implicated in some age-related pathologies, including AD. Therefore, platelets may represent a link between frailty and AD. We investigated the correlation between platelet reactivity and the onset of frailty in a mouse model of AD.

Materials and Methods: APP23 mice, the murine model for AD, were analysed during their lifespan and experimental observations were collected at 3, 9 and 18 months of age in comparison with wild type (WT) controls. Development of frailty was assessed

according to the methodology proposed by Whitehead et al (J Gereontol. 2014;69:621-632). Platelet activation and reactivity as well as platelet-leukocytes aggregates were evaluated by flowcytometry.

Results: The calculated frailty index progressively increased with age in both WT and APP23 mice. In 18 months old mice the frailty score, for some specific evaluated parameters, was higher in APP23 mice compared to WT, indicating that the onset of AD is associated to a stronger propensity to develop frailty. Circulating platelets from aged APP23 mice displayed an overall hyperactivation state compared to age-matched controls, as demonstrated by a slightly higher degree of integrin aIIbb3 activation and P-selectin exposure. Aged APP23 platelets also revealed an evident hyperreactivity in terms of and secretion upon stimulation with specific soluble agonists. Importantly, the formation of platelet-leukocyte aggregates was significantly enhanced in blood from aged APP23 mice compared to WT controls.

Conclusion: These results indicate that the onset of frailty is more pronounced in APP23 mice and is associated with a hyperactivation of circulating platelets. These observations point toward a possible novel role of platelets in linking AD with age-related frailty.

OC20 - ID 288

HAEMORRHAGIC COMPLICATIONS IN CHOLERA – A POSSIBLE ROLE OF *VIBRIO CHOLERA* OUTER MEMBRANE VESICLES VIA IMPAIRMENT OF PLATELET FUNCTION

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Background: Vibrio cholerae, the causative pathogen of cholera, shed outer membrane vesicles (OMVs) during bacterial growth to modulate and evade host immunity. Haemorrhagic complications in cholera disease are associated with bacteraemia and increased mortality. Given the immune-sensing capacity of platelets, these OMVs may also affect platelet functions.

Aims: We aimed to investigate whether V. cholerae-derived OMVs may modulate haemostatic/pro-thrombotic platelet functions.

Methods: OMVs were isolated in the late logarithmic growth phase of V. cholerae. Platelets were incubated with OMVs in vitro to study platelet activation and aggregation using flow cytometry, light transmission aggregometry and Western Blot. Platelet spreading and cytoskeletal remodelling were assessed by (super-resolution) fluorescence microscopy. In vivo relevance was investigated in FeCl3-induced thrombosis mouse models after transfusion of OMV-treated platelets. To clarify whether OMV-mediated inhibition of platelet activation is species specific we analysed platelet degranulation and GPIIb/IIIa expression comparing OMVs from different bacteria via flow cytometry.

Results: V. cholerae-derived OMVs significantly reduced platelet degranulation (measured by surface P-selectin) and almost abolished GPIIb/IIIa activation. Investigation of intracellular signalling events revealed profound impairment of AKT phosphorylation, whereas phosphorylation of p38 and vasodilator-stimulated phosphoprotein (VASP) were unaffected. OMVs also diminished platelet aggregation and curtailed platelet spreading, which involved aberrant rearrangement of the actin cytoskeleton. In vivo, mice transfused with OMV-treated platelets were protected from FeCl3-induced thrombosis. Similar effects of OMVs on platelet CD62P exposure and GPIIb/IIIa activation were also observed for other Vibrio strains.

Conclusion: OMVs from V. cholerae strongly impair pro-thrombotic platelet functions in vitro and in vivo, mediated by inhibition of the central signalling hub AKT and dysregulation of actin polymerisation. Thereby, OMV-mediated platelet inhibition may represent an underlying mechanism that contributes to haemorrhagic complications in cholera patients.

OC21 - ID 295

TNF-ALPHA-INDUCED ENDOTHELIAL DYSREGULATION ENHANCES PLATELET ACTIVATION UNDER SHEAR AND IN STASIS: A MULTI-OMICS APPROACH

Claudia Schoenichen[1,2], Isabella Provenzale[1,3], Annekathrin Ludt[4], Fiorella Solari[5], Federico Marini[4], Audrey Cleuren[6], Kerstin Jurk[2], Monika Stoll[7], Albert Sickmann[5], Marijke J.E. Kuijpers[1,8], Johan W.M. Heemskerk[1,9]

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Background: Endothelial cells (EC) suppress platelet activation and coagulation to prevent blood clotting. Under inflammatory conditions, this negative regulation can be hampered and lead to thrombotic complications, but the mechanisms are largely unknown.
Aims: Unravel the pathways by which inflammatory EC influence platelet signaling using vessel-on-a-chip microfluidics and multi-omics approaches.
Methods: The transcriptomes of control-, TNFalpha- or LPS-treated human umbilical vein EC (HUVEC) were characterized by RNA-sequencing. Platelet activation and thrombus formation on the sub-confluent EC were monitored in whole-blood and high-shear conditions. Changes in phosphorylation levels of platelets exposed to (treated) EC were assessed by stable isotope phosphoproteomic analysis. Platelet activation in these conditions was measured by flow cytometry.
Results: Out of >18k stably expressed genes, the HUVEC transcriptome showed ~6.5k and ~0.7k expression changes (TNFalpha- or LPS-treatment vs. control, respectively, adjusted p-value <0.01), in particular of inflammatory pathways and secretory mediators. Pathway analysis via clusterProfiler and topGO revealed distinct inflammatory footprints of the treated EC. In high shear flow conditions on a collagen/tissue factor surface, the presence of sub-confluent EC potently suppressed platelet adhesion, thrombus and fibrin formation. When pre-treated with TNFalpha, the thrombo-protective and anticoagulant effect of the EC was significantly reverted. Platelet integrin activation was reduced by 80%, if platelets were exposed to healthy EC before agonist stimulation, as assessed by flow cytometry. When pre-treated with TNFalpha - but not LPS-, the protective effect of the EC was reverted by 50%. Key changes in the phosphoproteome (3.0k phospho-sites) in EC-exposed platelets are currently validated to uncover most relevant signaling alterations due to TNFalpha or LPS treatment of the cells.
Conclusion: The combined use of omics and microfluidic approaches reveals a multi-path regulation of inflammation-modulated interactions between EC and platelets.

OC22 - ID 297

CONFORMATIONAL CHANGE IN PLATELET $A_{IIB}B_3$ INDUCE PKC-DEPENDENT SERINE 284 PHOSPHORYLATION OF JUNCTIONAL ADHESION MOLECULE-A AND RAP1 ACTIVATION

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Background: In resting platelets tyrosine phosphorylated JAM-A suppresses $\alpha_{IIb}\beta_3$ signaling by recruiting CSK to the $\alpha_{IIb}\beta_3$-Src complex. Upon platelet activation JAM-A is rapidly phosphorylated on S^{284} residue.
Aim: To elucidate the role of ligand-binding to $\alpha_{IIb}\beta_3$ in JAM-A S^{284} phosphorylation.
Methods: We used Mn^{2+}/DTT/RGDS to induce conformation change in $\alpha_{IIb}\beta_3$ without inside-out signaling and ligand binding. Full $\alpha_{IIb}\beta_3$ activation was assessed by PAC-1 binding using flowcytometry. Immunoprecipitation and western blot analyses were performed to determine JAM-A Y^{280} or S^{284} phosphorylation. Jam-A null mouse platelets were used to detect Rap1-activation during outside-in signaling.
Results: We show that in resting platelets JAM-A is phosphorylated on Y^{280}. Upon agonist-induced activation of $\alpha_{IIb}\beta_3$, JAM-A is rapidly dephosphorylated on Y^{280} and concomitantly, phosphorylated on S^{284} in a PKC-dependent manner. We observed that conformational change in $\alpha_{IIb}\beta_3$ by Mn^{2+} (10μM) is sufficient for JAM-A S^{284} phosphorylation without requiring inside-out signaling. RGDS peptide (200μM) or DTT (5mM) also induced JAM-A S^{284} phosphorylation similar to that induced by Mn^{2+}. Moreover, this phosphorylation is independent of ligand binding to $\alpha_{IIb}\beta_3$ since the presence or absence of fibrinogen had no effect on Mn^{2+}-induced phosphorylation. We found that $\alpha_{IIb}\beta_3$-dependent S^{284} phosphorylation of JAM-A was abolished by pharmacological inhibitors of PLCγ, Src, and FAK suggesting that outside-in signaling is responsible for JAM-A S^{284} phosphorylation. Pan-PKC inhibitor bisindolylmaleimide I, but not calcium chelator BAPTA-AM inhibited S284 phosphorylation of JAM-A, highlighting the role for PKC in S^{284} phosphorylation of JAM-A. We found that aPKCζ specifically associated with JAM-A upon platelet activation and this association is inhibited by PKC inhibitor. Furthermore, outside-in signaling dependent Rap1 activation did not occur in Jam-A null platelets indicating that Jam-A regulates outside-in dependent Rap1 activation.
Conclusion: Our results suggest that conformational change in $\alpha_{IIb}\beta_3$ is sufficient to induce outside-in signaling dependent S^{284} phosphorylation of JAM-A and Rap1 activation.

OC23 - ID 231

DIVALENT LIGANDS ACTIVATE CLEC-2 IN MOUSE BUT NOT HUMAN PLATELETS

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Background/Aims: The platelet C-type lectin-like receptor 2 (CLEC-2) has been identified as a target for a new class of anti-thrombotic drugs due to its major role in driving occlusive thrombosis and minor role in haemostasis. Activation of CLEC-2 is mediated by clustering but its membrane organisation is controversial and its dependency on ligand valency is not known. The aims were to determine whether CLEC-2 is expressed as monomers or dimers in the membrane and investigate the dependency on ligand valency for activation of human and mouse platelets.

Materials and Methods: We used fluorescence correlation spectroscopy (FCS) and photobleaching to determine the stoichiometry of CLEC-2 in cell lines. We have investigated the ability of divalent and tetravalent ligands to CLEC-2 (including novel crosslinked nanobodies) to induce activation of human platelets and CLEC-2 humanised mouse platelets with aggregometry. We tested whether the novel nanobody ligands cause clustering of CLEC-2 and investigated the effect of Syk inhibition on CLEC-2 clustering by FCS.

Results: Contrary to previous reports, we show that CLEC-2 is expressed as a mixture of monomers and dimers in cell lines. Using human and mouse platelets, and cell lines transfected with low and high levels of CLEC-2, we report that divalent ligands serve as antagonists in human platelets and low-expressing cells lines and as agonists in mouse platelets and high-expressing cell lines. Further, we show that tetravalent nanobodies cause aggregation of human platelets and using FCS that multivalent ligands cause clustering of CLEC-2 in cell lines which is blocked by Syk inhibition.

Conclusions: These results provide evidence that CLEC-2 is present in both monomeric and dimeric forms. Divalent ligands can act as agonists and antagonists depending in part on receptor density. We therefore propose that divalent ligands should be considered as partial agonists.

OC24 - ID 241

PIM KINASE: A NOVEL REGULATOR OF MEGAKARYOCYTE AND PLATELET PRODUCTION AND FUNCTION

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Background: We have previously demonstrated anti-platelet and anti-thrombotic effects of Pim-1 kinase deletion and inhibition, mediated by modulation of platelet thromboxane A2 receptor signalling that is not associated with bleeding. In other cell types, Pim kinases have also been shown to also regulate CXCR4, a known mediator of megakaryocyte function. Despite this, the role of Pim kinase in megakaryocytes and platelet production and function remains to be fully elucidated.

Methods: For functional analysis, bone marrow derived WT megakaryocytes were treated with Pim kinase inhibitors and megakaryocyte maturation and proplatelet formation determined. For mechanistic studies, HEK293 cells transfected with a Flag or GFP- tagged TPαR and MEG01 cells (expressing CXCR4) were treated with Pim kinase inhibitors. Receptor levels were assessed using both flow cytometry and microscopy. TxB2 generation was measured by ELISA and calcium mobilisation was measured to determine effects on downstream signalling.

Results: Pim kinase expression in megakaryocytes is increased in response to TPO stimulation and treatment with Pim kinase inhibitors resulted in a delay in megakaryocyte maturation. Receptor based mechanistic studies, identified that Pim kinase regulates platelet TPαR signalling independently of COX1 and that Pim inhibition causes internalisation of both CXCR4 and TPαR when assessed using flow cytometry and fluorescence microscopy. Consistent with the receptors being internalised, a reduction in TxA2 and SDF1a mediated calcium mobilisation, and intracellular signalling was observed following treatment with Pim kinase inhibitors.

Conclusion: Pim kinase is a positive mediator of megakaryocyte and platelet function with multiple mechanisms of action. Pim kinase inhibitor mediated reduction of platelet and megakaryocyte TPαR and CXCR4 receptor levels and signalling could be an advantageous novel targeting strategy, especially in inflammatory conditions such as rheumatoid arthritis and SLE, that are associated with increased circulating plasma levels of thromboxane A2 and SDF-1a, reactive thrombocytosis, platelet hyperreactivity and thrombosis.

OC25 - ID 246

HOW TO ASSESS THE ABILITY OF PLASMA EXCHANGE TO REDUCE ON COVID-ASSOCIATED THROMBO-INFLAMMATION WITH AN *IN VITRO* PLATELET-ENDOTHELIUM CO-CULTURE MODEL

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Background/Aims: Coronavirus disease (COVID)-19 is particularly characterized by vascular inflammation with platelet activation and endothelial dysfunction. During the pandemic, therapeutic plasma exchanges (TPEs) have been used to reduce the cytokine storm in the circulation and delay or prevent patient admission in ICU (Clinical trial NCT04751643). This procedure consists in

replacing the inflammatory plasma by transfused fresh frozen plasma and is often used to remove pathogenic molecules from plasma (autoantibodies, immune complexes, toxins…). The aim of this study is to implement an in vitro model of platelet-endothelial cell interactions to assess the alteration of these interactions by plasma from COVID-19 patients and to which extent TPEs reduce this alteration.

Materials and Methods: Platelets (Plts) were isolated from healthy donors and were then co-cultured with an endothelial EA.hy926 cells (ECs) monolayer during 24h in presence of plasma from hospitalized COVID-19 patients (CPs), before and after TPE. Activation of Plts and ECs was assessed by flow cytometry with the specific markers CD62P, CD63, Toll-like receptor (TLR)-2 and TLR-4, or CD31, CD54 and CD141, respectively. Immunomodulatory molecules such as interleukin (IL)-6, IL-8, IL-10 or MCP-1 were assessed by ELISA immunoassays in supernatants of cell culture. Alteration of endothelial permeability by COVID-19 plasma was measured by assessing fluorescent dextran permeability and modification of the Trans-Endothelial Electric Resistance (TEER).

Results: CP plasma upregulated the activation markers of ECs. CP plasma induced platelet activation which was even higher upon co-culture with ECs. Moreover, CP plasma had an effect on TLR-expression by platelets cocultured with ECs. Finally, plasma recovered from patients after TPE showed a reduced activating effect, to some extent, on platelet-endothelial interactions.

Conclusion: Our results, obtained thanks to a robust co-culture in vitro model, provide new insights about platelets interactions with endothelium in COVID-19-associated inflammation and in particular the benefits brought by TPE.

OC26 - ID 273

IDENTIFICATION OF NOVEL INHIBITORS OF THE PLATELET COLLAGEN RECEPTOR GPVI BY A PHENOTYPIC SCREENING ASSAY

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Background: Current antiplatelet drugs are often associated with significant risks of bleeding and haemorrhage by seriously compromising platelet function. Finding antiplatelet drugs modulating thrombus formation without significantly altering haemostasis is a need. The collagen receptor glycoprotein VI (GPVI) is a promising candidate target, since not only is uniquely expressed in platelets and megakaryocytes, but also its blockade inhibits thrombosis.

Aim: To identify GPVI inhibitors by phenotypic screening that can progress into preclinical trials as novel antithrombotics.

Materials and Methods: Platelet-based intracellular calcium mobilization (FLIPR Calcium 4) phenotypic assay was used to identify inhibitors of platelet activation via GPVI. Label-free dynamic mass redistribution assay was carried out to confirm ligand binding. Series of derivatives of the hits obtained were synthesized by using a medicinal chemistry strategy. Functional studies were based on platelet aggregation and spreading assays. Viability Calcein-AM assay by flow cytometry was used to test the potential toxicity of ligands. In vitro ADME profiling assays were carried out to check metabolic stability and plasma protein binding for the most potent inhibitor.

Results: Two molecules showing efficacy at inhibiting intracellular calcium release and platelet aggregation through GPVI modulation, SIL-ENA and SEDN2, were identified as ligands. SEDN2 was the most potent aggregation inhibitor and was used as a scaffold to obtain 19 chemical derivatives. Two of those derivatives showed a potent antiplatelet activity and were prioritized, together with SEDN2, for preclinical thrombosis studies. The candidates have negligible effects in platelet viability at micromolar doses. They showed significant inhibition of platelet adhesion in spreading assays. SEDN2 showed good ADME properties in human plasma but lower stability and lower binding to plasmatic proteins in mouse plasma, indicating interspecies differences.

Conclusion: Novel antiplatelet molecules targeting GPVI were identified and prioritized by using phenotypic screens, functional studies and ADME characterization.

OC27 - ID 286

SARS-COV-2 INFECTION IS ASSOCIATED WITH ACTIVATED BUT EXHAUSTED PLATELETS

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Background and Aims: COVID-19 is commonly associated with thrombotic and bleeding complications. Aim of this study was to characterize some parameters of platelet function in patients with COVID-19 without (mild) or with pneumonia (severe).

Methods: We studied 33 patients with mild COVID-19 (54% male; median age 67 (23-86) years), 43 with severe COVID-19 (60% male, 61 (25-88) years), and 13 healthy controls (HC) (63% male, 48 (29-70) years). We measured: 1) the following markers of platelet activation by flow cytometry: platelets-monocytes (PMA) and platelets-granulocytes (PGA) aggregates and platelets expressing p-selectin; 2) platelet aggregation (PA) induced by ADP 1 µM and collagen 1 µg/mL, by light transmission aggregometry; 3) platelet adenine nucleotides (ATP and ADP) content, by luminometer;

4) capacity of platelets to form thrombi on collagen (10 µg/mL)-coated microchannels perfused by whole blood at constant shear rate (300/s); D-dimer plasma levels were measured only in severe COVID-19.

Results: Activated platelets were increased in mild and severe COVID-19 compared to HC (table 1) and positively correlated with D-dimer levels (PMA: r=0.4433, p=0.0026; PGA: r=0.5310, p=0.0002); PA was not significantly different in mild and severe COVID-19 compared to HC (table 1). In 24% mild and 43% severe COVID-19 patients the platelet ADP and ATP content were lower than the normal range. Platelets from both mild and severe COVID-19 patients showed a reduced capacity to form thrombi on collagen-coated microchannels compared to HC (table 1). There was a statistically significant inverse correlation between D-dimer levels and platelet ADP content (r=-0.4550, P=0.0047).

Conclusions: The platelet ADP content was reduced in COVID-19 patients, likely as a consequence of in vivo secretion by activated platelets, and was associated with impaired formation of platelet thrombi on collagen under controlled blood flow conditions.

SUBJECTS		FLOW CYTOMETRY			LIGHT TRANSMISSION AGGREGOMETRY			LUMINO METER	MICROFLUIDIC SYSTEM	
						COLLAGEN 1 µg/mL			Platelet thrombi	
		PMAs (%)	PGAs (%)	Platelet P-selectin (%)	ADP 1 µM LT (%)	TIME LAG (min)	LT (%)	ADP (nmol/10^8plt)	SC (%)	FI
CONTROLS	N=13	8.6 (6.7-13.0)	5.2 (4.6-6.0)	1.5 (1.3-1.9)	35.3 (13.9-70.1)	0.45 (0.4-0.6)	73.7 (66.9-76.4)	2.87 (2.5-3.7)	19.6 (17-22)	712 (566-1110)
MILD COVID-19 PATIENTS	Normal ADP content (>2.3 *nmol/10^8plt*) (n=25)	17.0# (13.5-27.5)	7.3# (5.4-9.0)	2.0# (1.5-4.0)	49.6# (21.9-73.1)	0.50* (0.4-0.5)	77.1* (71.6-81.8)	3.16* (2.9-4.0)	13.4# (11-19)	884# (697-977)
	Low ADP-content (<2.3 *nmol/10^8plt*) (n=8)	15.2# (11.9-42.5)	6.0# (4.3-11.4)	4.4# (1.5-7.0)	59.9# (12.1-62.8)	1.00* (0.6-1.2)	72.8* (66.5-76.5)	1.92* (1.4-2.2)	16.2# (13-17)	630# (514-866)
	All (n=33)	16.7 (13.2-31.6)	7.1 (5.1-9.8)	2.1 (1.5-4.4)	54.8 (20.5-70.9)	0.50 (0.4-0.6)	75.2 (69.6-80.7)	3.07 (2.4-3.8)	14.5 (11-18)	791 (570-957)
SEVERE COVID-19 PATIENTS	Normal ADP-content (>2.3 *nmol/10^8plt*) (n=18)	14.3# (9.5-18.9)	5.1# (3.1-7.7)	3.4# (2.6-4.4)	48.2# (10.2-74.8)	1.0# (0.4-1.2)	79.8* (54.5-83.5)	2.97* (2.5-4.4)	16.3# (5-24)	615# (342-1045)
	Low ADP-content (<2.3 *nmol/10^8plt*) (n=24)	15.2# (10.9-29.0)	6.4# (4.5-11.0)	3.1# (1.9-5.2)	38.5# (27.2-72.1)	1.17# (1.0-1.3)	68.8* (48.4-74.6)	1.81* (1.5-2.0)	18.4# (9-25)	477# (343-621)
	All (n=42)	15.6 (10.2-26.0)	5.8 (4.0-10.3)	3.3 (2.2-4.7)	38.5 (22.5-74.3)	1.09 (0.6-1.3)	71.6 (58.7-79.5)	2.1 (1.8-2.8)	17.6 (8-24)	498 (344-657)
Controls vs all mild vs all severe COVID-19 patients		*p=0.0003*	*p=0.0085*	*p=0.0742*	*p=0.5970*	*p=0.0005*	*p=0.2945*	*p<0.0001*	*p=0.2015*	*p=0.0086*
Controls vs all mild COVID-19 patients		*p<0.0001*	*p=0.0039*	*p=0.0138*	*P=0.1952*	*P=0.0007*	*p=0.1319*	*p=0.488*	*P=0.0082*	*P=0.2710*
Controls vs all severe COVID-19 patients		*p=0.0006*	*P=0.1946*	*p<0.0001*	*p=0.1730*	*P=0.2542*	*p=0.1689*	*P=0.0022*	*P=0.2163*	*P=0.0196*

PMAs= platelets/monocytes aggregates; PGAs= platelets/granulocytes aggregates; LT= Light transmission, SC= Surface Coverage, FI= fluorescence intensity.
Data are expressed as medians with interquartile ranges (25%-75%). One-way Analysis of Variance (ANOVA) test or t-tests were used when normal distribution was satisfied. Statistical significance was assumed for p-values <0.05. *$p<0.05$, #p= *ns* for differences between patients with normal vs low platelet ADP content.

OC27 - Table 1. Platelet parameters in patients with mnild or severe COVID-19 as a function of platelet ADP (nmol/10^8 platelets) content.

OC28 - ID 302

KINETICS OF CIRCULATING EXTRACELLULAR VESICLES AFTER LOW-DOSE ASPIRIN ADMINISTRATION IN PATIENTS AT CARDIOVASCULAR RISK

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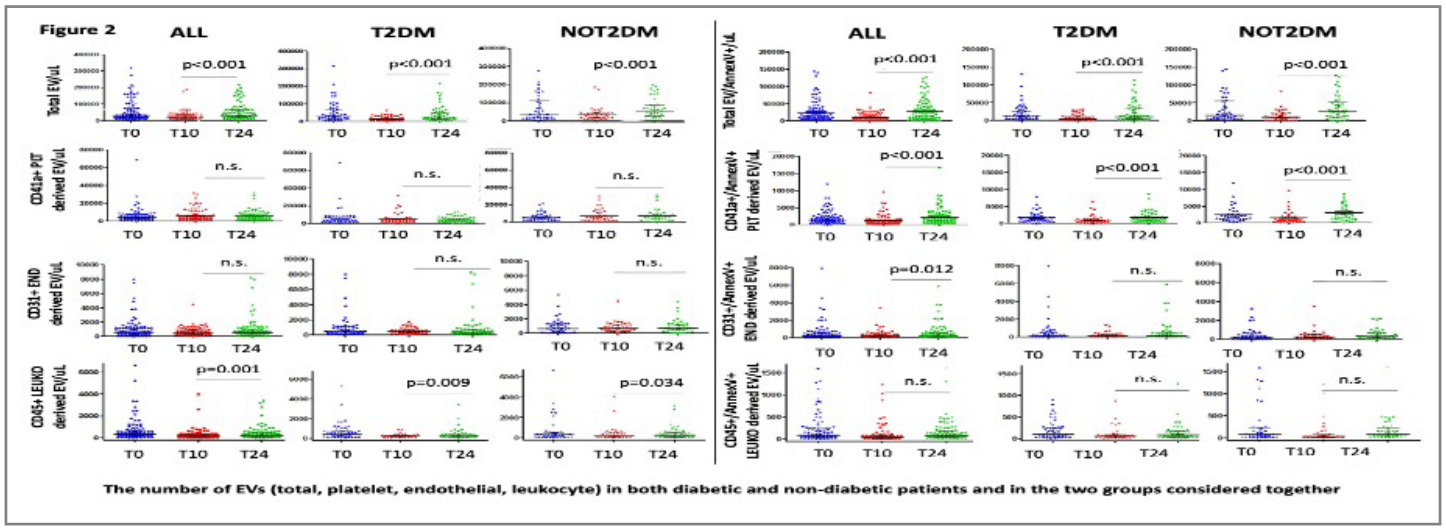
Backgrounds/Aims: Extracellular vesicles (EVs) are small vesicles deriving from all cell types during cell activation. They have been regarded as a link between the immune and the cardiovascular systems, and as predictors of cardiovascular events. We speculated that low-dose aspirin (ASA) may affect the release of EV within the 24-hour dosing interval, and that vice versa EV may affect the rate of cyclooxygenase (COX)-1 recovery and the subsequent duration of ASA effect.

Materials and Methods: We enrolled 45 patients with and 39 without type 2 diabetes mellitus (T2DM), at high cardiovascular risk, on chronic low-dose ASA treatment. The levels of total EVs and specific subtypes, such as platelet, endothelial, leucocyte derived EVs in patients with and without T2DM, were assessed immediately before, and after 10 and 24 hours after a witnessed ASA administration. The kinetics of platelet COX-1 recovery was also characterized by measuring serum thromboxane (TX) B_2 at the same time points. Nine healthy subjects were enrolled to verify the variability of EV number on circadian measurements.

Results: The numbers of circulating EVs (total: all p<0.001, T2DM p<0.001, NOT2DM p<0.001; and leukocyte: all p=0.001, T2DM p=0.009, NOT2DM p=0.034) and AnnexinV+ EVs (total all p<0.001, T2DM p<0.001, NOT2DM p<0.001; platelet: all p<0.001, T2DM p<0.001, NOT2DM p<0.001; endothelial all p=0.012) were reduced at T10 vs. T24. In healthy subjects, no difference was observed in the levels of EV except for leukocyte-derived EV.

Conclusions: In ASA-treated subjects, daily drug administration acutely inhibits, after 10 hours, the release of total EV, total AnnexinV+ EV, platelet AnnexinV+ EV, leukocyte EV, and endothelial AnnexinV+ EV. After excluding a circadian variation in EV levels, the inhibition after 10 hours since a witnessed ASA administration, suggests that COX-1 dependent mechanisms may be involved in this inhibition. Our findings suggest an unappreciated effect of ASA, possibly contributing to the cardioprotective effects of this drug.

Keywords: Extracellular vesicles, platelets, aspirin, thromboxane B_2, type 2 diabetes mellitus, cardiovascular risk



OC28 - Figure 1.

PO01 - ID 256

PIKFYVE-DEPENDENT PHOSPHOINOSITIDE DYNAMICS IN MEGAKARYOCYTE/PLATELET GRANULE INTEGRITY AND PLATELET FUNCTIONS

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Background and aim: Secretory granules are key elements for platelet functions. Their biogenesis and integrity are regulated by fine-tuned mechanisms that need to be fully characterized. Here, we investigated the role of the phosphoinositide 5-kinase PIKfyve and its lipid products, phosphatidylinositol 5 monophosphate (PtdIns5P) and phosphatidylinositol (3,5) bisphosphate (PtdIns(3,5)P_2) in granule homeostasis in megakaryocytes and platelets.

Materials and Methods: We used several megakaryocytic cell models (MEG-01 cell line, imMKCLs and primary megakaryocytes) and human platelets. PIKfyve invalidation was performed by pharmacological inhibition using STA 5362 or genetic silencing.

Results: We unveiled that PIKfyve expression and its lipid product levels increased with megakaryocytic maturation. In megakaryocytes, PtdIns5P and PtdIns(3,5)P_2 were found in alpha and dense granule membranes with higher levels in dense granules. Pharmacological inhibition or knock-down of PIKfyve in megakaryocytes decreased PtdIns5P and PtdIns(3,5)P_2 synthesis and induced a vacuolar phenotype with a loss of alpha and dense granule identity. Permeant PtdIns5P and PtdIns(3,5)P_2 and the cation channel TRPML1 and TPC2 activation were able to accelerate alpha and dense granule integrity recovery following release of PIKfyve pharmacological inhibition. In platelets, PIKfyve inhibition specifically impaired the integrity of dense granules culminating in defects in their secretion, platelet aggregation and thrombus formation.

Conclusions: These data demonstrated that PIKfyve and its lipid products PtdIns5P and PtdIns(3,5)P_2 control granule integrity both in megakaryocytes and platelets.

PO02 - ID 230

INCREASED PLATELET DISTRIBUTION WIDTH IS ASSOCIATED WITH MORTALITY RISK: PROSPECTIVE FINDINGS IN AN ITALIAN ADULT GENERAL POPULATION

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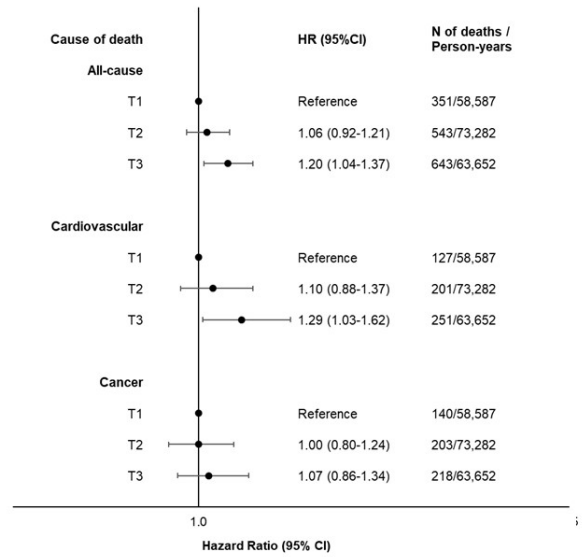
Background/Aims: Platelet Distribution Width (PDW) is a marker of platelet size heterogeneity used as redout of the combination of processes leading to platelet production and destruction to diagnose impaired megakaryocyte differentiation and thrombopoiesis. Recently, PDW was also reported to tag platelet activation variability, both ex vivo and in vitro. As platelets participate in the pathogenesis of many acute and chronic diseases, we evaluated the possible relationship between PDW values and all-cause and cause-specific mortality.

Materials and Methods: Longitudinal analysis was performed on 17,334 participants with available baseline PDW measurements (52% women, mean age 55.6 ±12y) in the Moli-sani study cohort, without a history of haematological diseases. PDW measurements were categorized in tertiles, the lowest acting as the reference. Multivariable Cox-proportional hazard model was used to estimate the association of PDW and mortality.

Results: In the whole population sample, the mean value of PDW was 16.4 ±0.58 fL. Over a median follow-up of 11.6y (interquartile range 10.7-12.5), 1,535 deaths (37.7% CVD and 36.5% cancer) were verified. As compared to those in the first tertile of PDW (14.6-16.0 fL), individuals within the highest tertile (16.6-20.4 fL) had an increased risk of all-cause (HR:1.20; 95%CI: 1.04-1.37) and CVD mortality (HR:1.29; 1.03-1.62). (Figure). Additionally, all-cause and CVD mortality increased by 5% and 7%, respectively, for each increase in 1 standard deviation (0.58 fL) of PDW (HR:1.05; 1.00-1.10 and 1.07; 0.99-1.16, respectively). Subgroup analyses by age classes (35-65y, ≥65y) showed that the association of PDW with all-cause and cancer mortality was more apparent in the elderly (HR:1.34; 1.14-1.58, p for interaction = 0.028 and HR:1.37; 1.01-1.85, p for interaction = 0.020, respectively, figure).

Conclusions: A PDW-associated increase of all-cause and CVD mortality risk, could be related to accelerated/altered platelet activation, production or destruction, all age-dependent processes leading to several clinical conditions and death.

Figure. Hazard Ratios for All-cause, CVD and Cancer Mortality according to tertiles of PDW in the Moli-sani cohort.



Model adjusted for age, sex, systolic blood pressure, atrial fibrillation, liver disease, hypercholesterolemia, cystatine C, Mediterranean Diet score, physical activity/leisure time, smoking, diabetes, waist-to-hip ratio; haematocrit, platelet count, mean platelet volume, white blood cell count.

PO02 - Figure 1.

PO03 - ID 240

AMBIGUOUS ROLE OF F11RECEPTOR/ JUNCTIONAL ADHESION MOLECULE-A IN HUMAN BLOOD PLATELET ADHESION AND THROMBUS FORMATION

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Background/Aims: F11Receptor/Junctional Adhesion Molecule-A (F11R/ JAM-A) is a transmembrane protein, which belongs to the immunoglobulin superfamily of cell adhesion molecules. F11R /JAM-A is expressed in endothelial cells, epithelial cells and in blood platelets. In the endothelium and epithelium, it is involved in the formation of intercellular tight junctions. In blood platelets F11R /JAM-A participates in adhesion under static conditions and negatively regulates the activation of the platelet αIIbβ3 integrin. The purpose of presented study was to understand whether F11R/ JAM-A is involved in platelet adhesion and thrombus formation under flow conditions.

Materials and Methods: Platelet adhesion to fibrinogen and/ or F11R/JAM-A and thrombus formation on collagen under flow conditions were performed using the VenaFlux (Cellix) system. Citrated whole blood from healthy volunteers was used. Changes in F11R/JAM-A/ expression on blood platelets due to platelet activation were assessed using flow cytometry. F11R/JAM-A expression in adhered blood platelets was visualized by confocal microscopy.

Results: Immobilized F11R/JAM-A alone did not support platelet immobilization under flow conditions in the shear force range of 1.5 to 40 dynes/cm2. However, the presence of immobilized F11R/ JAM-A modified platelet adhesion to fibrinogen depending on the proteins concentration. Thrombus formation was unaffected by soluble monomeric form of F11R/JAM-A, but was decreased by soluble dimeric form of the protein. Activation of blood platelets with ADP increased the amount of F11R/JAM-A on the platelets. Adhered, spread platelets showed higher F11R/JAM-A staining than non-spread platelets.

Conclusions:

- F11R/JAM-A alone does not support platelet adhesion under flow conditions, but plays a regulatory role in platelet adhesion to other proteins.
- F11R/JAM-A plays a limited role in thrombus formation under flow conditions in vitro.
- Platelet membrane F11R/JAM-A abundance increases upon platelet activation.

Acknowledgements: This work was supported by the National Science Centre grant OPUS (UMO-2020/37/B/NZ3/00301).

PO04 - ID 248

APOLIPOPROTEIN CIII IMPAIRS PLATELET FUNCTION

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Background/Aims: Apolipoprotein CIII (apoCIII) is associated with triglyceride-rich lipoprotein metabolism and cardiovascular risk. It is present on various lipoproteins and plasma apoCIII correlates with triglyceride levels. In vitro apoCIII induces enhanced monocyte adhesion to endothelial cells, thereby promoting inflammatory diseases like atherosclerosis. Development and progression of atherosclerotic lesions is also fostered by activated platelets, which govern both inflammatory and thrombotic processes. Therefore, we aimed to determine how apoCIII impacts on platelet function.

Materials and Methods: Effects of apoCIII on platelet function were analysed in vitro by pre-incubating platelets with apoCIII before assessing platelet responsiveness to agonists using flow cytometry, light transmission and multiple electrode aggregometry, ELISA and Western Blot. Fluorescence microscopy was used to evaluate platelet adhesion to coated surfaces or endothelial cells under static conditions or capillary shear, respectively, as well for investigation of platelet spreading and cytoskeletal remodelling. In vivo relevance was tested in a model of $FeCl_3$-induced thrombosis using mice transfused with apoCIII-treated platelets.

Results: Surprisingly, despite its proinflammatory effect on monocytes, apoCIII strongly reduced platelet aggregation in response to various agonists using isolated platelets, platelet-rich plasma or whole blood. While platelet adhesion to collagen, fibronectin or endothelial cells was not impaired, apoCIII-treated platelets displayed aberrant reorganisation of the actin cytoskeleton as well as altered morphology with decreased size and lamellipodia formation. Further, apoCIII impaired degranulation and diminished GPIIb/IIIa activation. Investigation of intracellular signalling revealed no changes in Calcium mobilization, but slightly reduced phosphorylation of AKT and increased VASP-phosphorylation upon apoCIII pre-treatment. In mice transfusion of apoCIII-treated platelets delayed $FeCl_3$-induced thrombosis.

Conclusion: Although elevated apoCIII plasma levels are associated with higher risk for cardiovascular disease, apoCIII impairs pro-thrombotic platelet function. Thus, apoCIII might influence the thrombotic risk in these patients.

PO05 - ID 278

MICRORNA-223 IS A REGULATOR OF PLATELET PROCOAGULANT ACTIVITY

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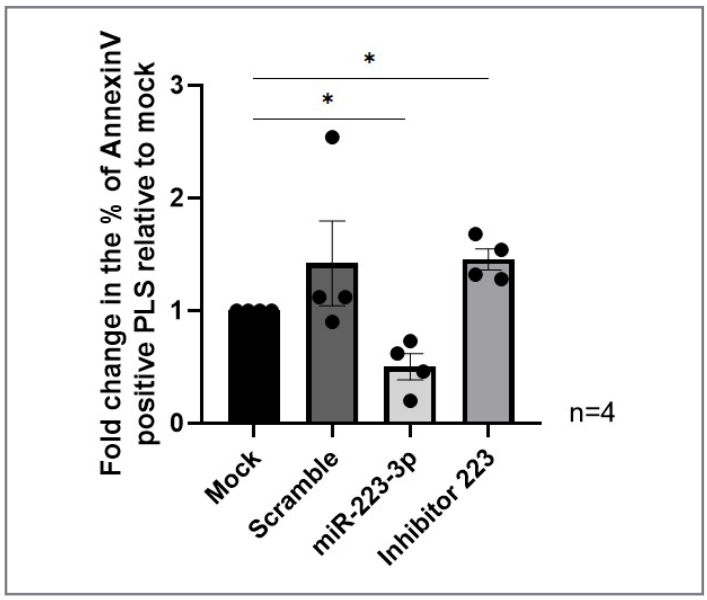
Background: Platelets are anucleate cells mostly involved in haemostasis through their aggregating properties and their ability to promote thrombin generation at their activated surface. Platelet reactivity is variable among individuals and microRNAs (miRNAs) may regulate platelet function. Among platelet-derived miRNAs, miR-223-3p is the most highly expressed miRNAs in platelets and several studies have reported an association between miR-223-3p level and platelet reactivity or recurrence of cardiovascular events, but the impact of miR-223-3p on platelet function is poorly understood.

Aim: To investigate the role of miR-223-3p on platelet reactivity in platelet-like structures (PLS) derived from human hematopoietic stem cells.

Materials and Methods: CD34+-derived megakaryocytes were transfected with a scramble miRNA, a miR-223-3p mimic or inhibitor and PLS were collected for functional tests. PLS production was quantified using a Tali Image-Based cytometer. GPIIbIIIa activation and procoagulant activity were assessed using flow cytometry.

Results: miR-223-3p mimic induced a 50±22% decrease of PLS procoagulant activity (n=4, p=0.022, Figure), while miR-223-3p inhibitor induced a 45±6% increase of the procoagulant profile compared to mock condition. miR-223-3p level did not affect PLS production and GPIIbIIIa activation.

Conclusion: miR-223-3p regulates procoagulant activity of PLS derived from human hematopoietic stem cells.



PO05 - Figure 1.

PO06 - ID 282

SMALL-MOLECULE CYCLOPHILIN INHIBITORS AS A POTENTIAL THERAPEUTIC APPLICATION TO LIMIT THROMBOSIS

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Background: Cyclophilins (CyP) are involved in various pathways regulating platelet function. CyPA is a Ca^{2+} regulator and is involved in integrin $\alpha_{IIb}\beta_3$ bidirectional signaling, while CyPD mediates opening of the mitochondrial permeability transition pore resulting in procoagulant platelet formation. Platelet CyP has therefore been suggested as a therapeutic target to limit thrombosis. Many studies utilize the immunosuppressant Cyclosporin A (CsA) or derivatives. However, these have several disadvantages including side-effects unrelated to CyP inhibition. Among others, CsA was shown to enhance platelet aggregation, which was suggested to be responsible for the thrombotic complications observed in patients treated with CsA. Therefore, potent CyP inhibitors unrelated to CsA are needed.

Aim: Test the effect of three novel non-peptidic small-molecule CyP inhibitors (SMCypIs), on platelet activation, aggregation and procoagulant platelet formation, in comparison with CsA.

Methods: Washed human platelets were incubated with 4 µM CsA or 125 µM SMCypI prior to activation. Procoagulant platelet formation was measured using flow cytometry, by analyzing AnnexinV binding and P-selectin expression, as well as tetramethylrhodamine methyl ester staining demonstrating an intact mitochondrial membrane potential. P-selectin expression and activated integrin $\alpha_{IIb}\beta_3$ were measured using flow cytometry. Platelet aggregation was measured using light transmission aggregometry.

Results: Addition of each SMCypI resulted in a decrease in $AnnexinV^+$/$P\text{-selectin}^+$ platelets upon dual-agonist activation, comparable to the inhibition observed with CsA. Similarly, SMCypIs prevented the dual-agonist induced loss of mitochondrial membrane potential analogous to CsA. No effect was observed on P-selectin expression and integrin $\alpha_{IIb}\beta_3$ activation after thrombin, collagen related peptide or ADP activation. SMCypIs induced a moderate inhibition of sub-optimal concentrations of thrombin-, collagen- and ADP-induced platelet aggregation, while a significant increased aggregation was observed with CsA.

Conclusions: Three novel non-peptidic SMCypI reduced procoagulant platelet formation and moderately reduced platelet aggregation. Further experiments are needed to demonstrate its potential therapeutic application to limit thrombosis.

PO07 - ID 283

ELTROMBOPAG IRON-CHELATING EFFECTS ON THROMBOPOIESIS

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Background/Aims: Thrombocytopenic disorders can be treated with Thrombopoietin-receptor agonists. Among these, Eltrombopag has been shown to increase the platelet count in patients with Inherited Thrombocytopenia by promoting proplatelet formation by megakaryocytes.[1] Though, patients with the same genetic defect may respond differently to the treatment, and sometimes the drug is ineffective.[1] Besides its ability to stimulate the thrombopoietin-receptor, Eltrombopag exhibits dose-dependent opposing effects on in vitro megakaryopoiesis due to its iron-chelating effects.[2] In the bone marrow, iron levels direct the lineage commitment of megakaryocyte/erythroid progenitors.[3] Whilst iron's role in the maturation and function of red blood cells has been long established, its implication in thrombopoiesis is poorly understood. It is known that iron deficiency or misdistribution in patients is associated with thrombocytosis, but thrombocytopenia may also occur. Despite this knowledge, the relationship between the iron-chelating effects of Eltrombopag and thrombopoiesis has never been investigated.

Materials and Methods: Primary megakaryocytes were differentiated and analyzed as previously described.[1] UT7 megakaryocytic cell line was purchased from ATCC. Samples were stimulated with Hemin (25μM), Eltrombopag (10μg/mL), or Deferoxamine (DFO, 10μM) to modulate iron availability. Calcein-AM was used to assay the intracellular labile iron pool (LIP). Relevant regulators of iron uptake and storage were monitored by western blot.

Results: We demonstrated that increased LIP and iron storage, as well as decreased cellular iron uptake, feature the maturation of megakaryocytes before proplatelet formation occurs. Pharmacological modulation of LIP through chelation with Eltrombopag or DFO, or overload by hemin, impaired iron homeostasis, and consequent thrombopoiesis in a dose-dependent manner. Also, either iron deficiency or overload affected cytoskeleton reorganization in a megakaryocytic cell line in adhesion onto extracellular matrix components.

Conclusions: Taken together, our results show that iron homeostasis control is crucial to platelet biogenesis and a key aspect to consider when defining the posology of Eltrombopag.

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PO08 - ID 294

RIVAROXABAN ATTENUATES SECOND WAVE OF PLATELET AGGREGATION BY INHIBITION OF PLATELET-DERIVED FACTOR XA

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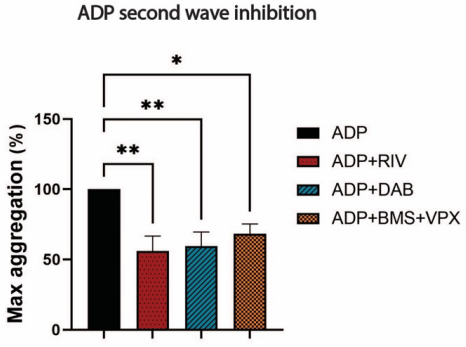
Background: It is well-known a low concentration of ADP mediated aggregation gives rise to a two phased aggregation, the second wave being dependent on platelet degranulation. Thrombin and its platelet receptor PAR1 have been shown to have a critical role in ADP-induced platelet secretion and the second wave of aggregation. If thrombin is present as an active enzyme is unknown.

Aim: To elucidate the role of coagulation factors in the second wave of platelet aggregation.

Methods: We have used flow cytometry and western blotting to study binding of drugs and coagulation factors to platelets. We have used light transmission aggregometry and flow cytometry to study activation of platelets.

Results: We investigated the contents of washed platelets and discovered the presence of FX, but not Xa. Furthermore, FX was released from the platelets and bound to the surface upon ADP mediated activation of platelet-rich plasma (PRP). The released FX was converted to the active enzyme FXa and contributed to the second phase aggregation since this second wave was blocked by rivaroxaban and by apixaban too, which both are specific inhibitors of FXa. How FX is activated upon release from the platelets is an on-going study. Rivaroxaban inhibited the platelet activation measured as platelet-bound fibrinogen and P-selectin by flow cytometry, as much as the specific thrombin inhibitor dabigatran or by blocking the trombin receptors PAR1 and PAR4 with vorapaxar and BMS986120.

Conclusions: We have discovered direct oral anticoagulants directed towards Xa or thrombin inhibited the second wave of aggregation in PRP and attenuated platelet activation. This new mechanism of action may contribute to the antithrombotic effects of these drugs. These new finding may be useful in further understanding the efficacy of these drugs.



Riveroxaban inhibits ADP mediated second wave of aggregation in platelets
Low level ADP stimulation of platelets in citrated plasma induces two phased platelet activation which is inhibited by dabigatran, rivaroxaban and thrombin receptor inhibitors vorapaxar (PAR1) and BMS986120 (PAR4) as measured by light transmission aggregometry. Data represents relative max aggregation where ADP mediated maximum aggregation is considered as 100%. Values presented are mean ± SEM; * P ≤ 0.05, ** P ≤ 0.01, and *** P ≤ 0.001; n=10.

PO08 - Figure 1.

PO09 - ID 296

CIRCULATING MIRNAS RELEASE PREDICTS SUBOPTIMAL RESPONSE TO ASPIRIN IN PATIENTS AT HIGH CARDIOVASCULAR RISK WITH AND WITHOUT TYPE 2 DIABETES MELLITUS

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Background: The recovery rate of platelet COX-1 activity during the 12 to 24h dosing interval of aspirin administration, in aspirin-treated subjects, is characterized by substantial interindividual variability. Circulating myeloid-related protein (MRP)-8/14 is an inflammatory protein associated with residual thromboxane (TX)-dependent platelet activation in aspirin-treated patients with acute coronary syndrome.

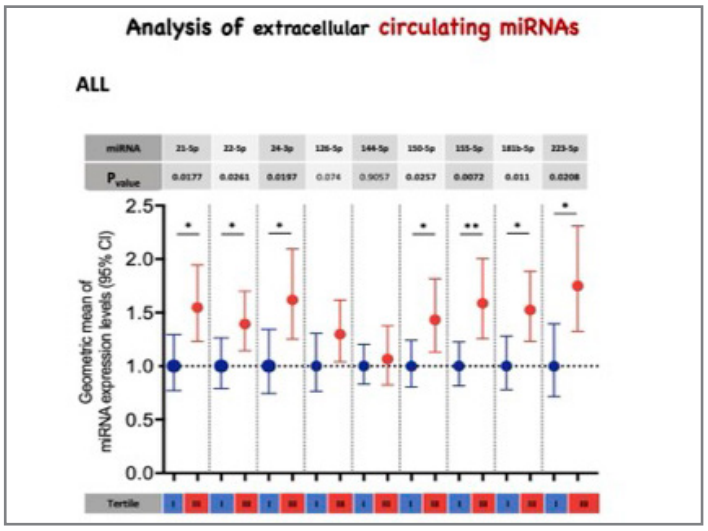
Aims: To identify any circulating miRNAs associated with a suboptimal ASA response in patients at high cardiovascular (CV) risk.

Methods: Two-hundred high CV risk patients (100 with type 2 diabetes mellitus (T2DM)) in chronic treatment with ASA (100 mg/day), for cardiovascular prevention, were enrolled. Blood sampling was performed at 10 (T10) and 24 hours (T24) after a witnessed ASA administration. Patients were stratified in tertiles according to serum TXB_2 slope. First vs. third tertile were compared. Circulating miRNAs custom array cards were applied to assay the expression levels of 14 miRNAs in plasma. We also measured plasma myeloid-related protein (MRP)-8/14 as an inflammatory index and predictor of cardiovascular events.

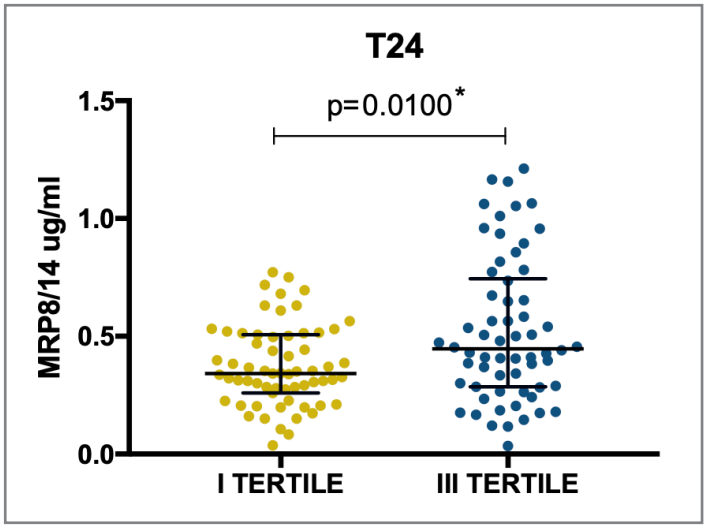
Results: miRNA-21-5p (p=.017), 22-5p (p=.026), 24-3p (p=.020), 150-5p (p=.026), 155-5p (p=.007), 181b-5p (p=.011), 223-5p (p=.021) were significantly lower in first vs. third tertiles at 24 hours after ASA administration in all patients (Fig.1). MRP-8/14 were higher in third vs. first sTXB2 slope tertile in all patients (Fig.2). MRP-8/14 was directly correlated with miRNA-21-5p (rho=.279, p=.008), 22-5p (rho=.264, p=.012), 24-3p (rho=.239, p=.023), 150-5p (rho=.236, p=.025), 155-5p (rho=.270, p=.011), 181b-5p (rho=.240, p=.023) and 223-5p (rho=.244, p=.030) in all patients (data not shown).

Conclusions: MRP 8/14 may contribute to circulating miRNA release and response variability to ASA. Vice versa, shorter duration of aspirin effect at 24 hours in third $sTXB_2$ slope tertile patients translates into higher degree of TX-dependent platelet activation, possibly promoting the release of both circulating MRP8/14 and miRNAs.

Reduced levels of circulating miRNAs may be a potential biomarker for predicting response to ASA treatment in high-risk cardiovascular patients.



PO09 - Figure 1.



PO09 - Figure 2.

PO10 - ID 226

IMPACT OF THROMBOCYTOPENIA, TEMPERATURE CHANGES AND ACIDIC/ALKALINE CONDITIONS ON HAEMOSTATIC AND IMMUNOMODULATORY FUNCTIONS OF PLATELETS

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Introduction: Thrombocytopenia and acidosis frequently occur during pathophysiological settings such as infections or inflammation. Although, platelet function regarding temperature changes, platelet count and pH levels have been evaluated in platelet transfusion and storage settings the precise impact of these environmental changes on haemostatic and immunomodulatory functions of platelets are still unclear.

Methods & Aim: Thus, we investigated the effects of thrombocytopenia, temperature changes, as well as acid and alkaline conditions on platelet functions in vitro by flow cytometry. Different platelet densities, 20.000, 100.000 and 200.000 platelets/µl; incubation at 4°C, 22°C and 37°C; and blood adjusted to pH

6.9, 7.2 and 8.1 were evaluated regarding platelet-mediated immunomodulatory functions such as formation of platelet-neutrophil/monocyte aggregates, platelet-induced leukocyte activation (activated CD11b, CD62L) as well as platelet reactivity (activated GPIIb/IIIa, CD62P, CD63, CD40L) in response to TRAP-6 and ADP stimulation.

Results: Platelet activation in particular degranulation processes were only mildly affected by decreasing platelet densities. More pronounced changes were observed by decreasing/increasing temperatures as well as alkalization, which led to hyporeactivity of platelets. Acidic conditions affected granule exocytosis, leading to increased expression of CD62P, CD63 and CD40L in response to submaximal concentrations of TRAP-6. Platelet-neutrophil/monocyte aggregates were slightly increased under acidic conditions whereas alkalization and incubation at 4°C decreased hetero-aggregate formation.

Conclusion: Taken together, our results indicate that platelet function is differently affected by environmental changes such as temperature and pH levels. While platelet densities only mildly impacted platelet function, we show that working temperature condition and pH levels, which can be affected by the choice of anticoagulant as well as by inflammatory conditions, substantially affect platelet function and platelet-leukocyte interplay.

PO11 - ID 249
EFFECTS OF NO-GC STIMULATORS AND ACTIVATORS ON PLATELET BIOMECHANICS

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Introduction: Cyclic guanosine monophosphate (cGMP) is a second messenger produced by the enzyme guanylyl cyclase (GC) in response to nitric oxide (NO). NO-sensitive GC (NO-GC) is expressed in a variety of cell types including smooth muscle cells and platelets. In platelets, the NO-GC/cGMP pathway is thought to inhibit platelet aggregation via changes of the cytoskeleton. However, the molecular mechanism underlying platelet inhibition and its correlation with cytoskeleton stiffness is poorly understood.

Methods: To study the role of NO-GC/cGMP in platelet stiffness and inhibtion, we applied scanning ion conductance microscopy (SICM), a nanopipette-based noncontact imaging method, to resolve the morphology and stiffness of single platelets at high spatial resolution. We quantified morphological changes in platelets using a deep learning convolutional neural network. Actin polymerization and platelet activation were measured by F-actin and P-selectin co-staining, respectively.

Results: We found that stimulation of human and murine platelets with the NOGC stimulator Riociguat (10 µM, ten minutes) or with the NO-GC activator Cinaciguat (10 µM, ten minutes) downregulated P-selectin expression and decreased stiffness by fifty percent compared to vehicle (DMSO) treated control. Also, the platelet shape became more round, while the platelet area was unaffected. F-actin polymerization was inhibited by fifty percent by Riociguat and Cinaciguat.

Conclusions: These observations can be linked to the decreased stiffness of the drug-treated platelets. Remarkably, none of these changes were observed in platelets isolated from platelet-specific NO-GC knockout mice, demonstrating a functional role of the NO-GC/cGMP pathway and the feasibility of its pharmacological targeting to modulate platelet cytoskeleton biomechanics.

Acknowledgments: This project is funded by the Deutsche Forschunggemeinschaft (DFG, German Research Foundation-Projektnummer 335549539/GRK2381).

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PO12 - ID 250
A MULTI-TRAIT ASSOCIATION ANALYSIS OF PLATELET TRAITS AND BRAIN DISORDERS IDENTIFIES NOVEL SUSCEPTIBILITY LOCI FOR MAJOR DEPRESSION, ALZHEIMER AND PARKINSON DISEASE

Alfonsina Tirozzi[1], Miriam Shasa Quiccione[1], Chiara Cerletti[1], Maria Benedetta Donati[1], Giovanni de Gaetano[1], Licia Iacoviello[1,2], Alessandro Gialluisi[1,2]

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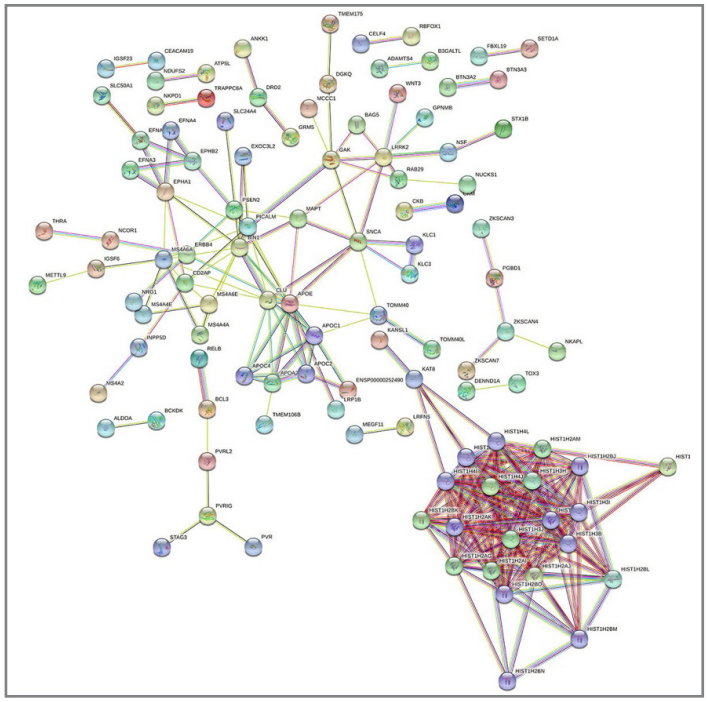
Background: Among candidate neurodegenerative/neuropsychiatric risk-predictive biomarkers, platelet count (Plt), mean volume (MPV) and distribution width (PDW) have been associated with the risk of Major Depressive Disorder (MDD), Alzheimer's (AD) and Parkinson's disease (PD), both through epidemiological and through genomic studies, suggesting partial co-heritability.

Method: We exploited these relationships for a multi-trait association analysis (MTAG), using publicly available summary statistics of genome-wide association studies (GWAS) of

three different platelet traits – Plt, MPV and PDW – and three neurodegenerative/neuropsychiatric disorders, AD, PD and MDD. Gene-based enrichment tests were carried out through MAGMA v1.08, while a network analysis of significantly enriched genes was implemented in dbSTRING v11.5.

Results: We analyzed 4,540,326 Single Nucleotide Polymorphisms (SNPs) shared among the analyzed GWAS, observing 149 genome-wide significant multi-trait LD-independent associations ($p<5\times10^{-8}$) for AD, 70 for PD and 139 for MDD. Among these, 27 novel associations were detected for AD (top hit at rs585021 within ITGB5 gene, $p=4.88\times10^{-14}$), 34 for PD (top hit at rs1372518 within SNCA gene, $p=3.33\times10^{-28}$) and 40 for MDD (top hit at rs200965 in a transcription factor binding site on 6p22.1, $p=4.07\times10^{-15}$). Out of 18,781 genes with annotated variants within ±10 kb, 62 genes were enriched for associations with AD, 70 with PD, and 125 with MDD ($p<2.7\times10^{-6}$). Of these, 7 genes were novel susceptibility loci for AD (EPPK1, TTLL1, PACSIN2, TPM4, PIF1, ZNF689, AZGP1P1), 2 for PD (SLC26A1, EFNA3) and 1 for MDD (HSPH1). The resulting network showed a significant excess of interactions (enrichment $p=1.0\times10^{-16}$) (Fig. 1).

Discussion: We identified novel genes involved in the organization of cytoskeletal architecture (EPPK1, TTLL1, PACSIN2, TPM4), in telomere shortening (PIF1) and regulation of cellular aging (ZNF689, AZGP1P1) and in neurodevelopment (EFNA3), gaining novel insights into the underlying biology of AD, PD and MDD.



PO12 - Figure 1. Interaction network of genes enriched for associations with AD, PD and MDD performed in STRING v11.5.

PO13 - ID 257

CLOT STRUCTURE CHANGES AND DECREASED PROCOAGULANT PLATELET DEVELOPMENT MEDIATED BY FIBRIN-GPVI INTERACTION

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Background/Aims: The GPVI-pathway, in the context of GPVI-collagen binding, has been previously implicated in platelet procoagulant activity. Involvement of the newly discovered fibrin-GPVI interaction on thrombosis has been reported. Nonetheless, whether GPVI-fibrin interaction contributes to the development of platelets, and the impact of interfering with this interaction on clot structure, remains to be established. The aims of our study were to investigate fibrin-GPVI mediated procoagulant platelet development and impact on clot structure.

Methods: Procoagulant platelet number were determined in platelet-rich plasma (PRP) clots by SEM and confocal microscopies. Procoagulant platelet number in fibrin(ogen) clots with washed platelets were compared with clots of fragment X, a fibrinogen degradation product with truncated αC-chains which are important for GPVI binding. PRP or whole-blood was used to investigate the effect of Affimer binding proteins or GPVI-signalling inhibitors (PRT-060318, ibrutinib and dasatinib) and eptifibatide control on clot density, thrombin generation, porosity and clot retraction.

Results: In the absence of collagen and presence of fibrin, clots 1) lacking GPVI, 2) in the presence of Affimers, 3) presence of GPVI-signalling inhibitors and 4) fibrin(ogen) clots compared to fragment X clots had decreased number of procoagulant platelets. Fiber density and peak thrombin generation were decreased in the presence of GPVI-signalling inhibitors, whilst clot pore size was increased. Only ibrutinib increased final clot weight following whole-blood clot retraction. The addition of eptifibatide exacerbated the effects of tyrosine kinase inhibitors on procoagulant platelet number, but not clot density or pore size. Whole-blood and platelet retraction were significantly impaired in the presence of eptifibatide.

Conclusions: Our results show a role of fibrin-GPVI interaction on procoagulant platelet development with downstream effects on clot structure. Our findings suggest that structural clot characteristics typically associated with a pro-thrombotic phenotype may be alleviated by targeting fibrin-GPVI interaction with important implications for novel anti-thrombotic intervention development.

PO14 - ID 259

PLASMA EXOSOMES REFLECT MYOCARDIAL INJURY DETECTED BY CARDIAC MAGNETIC RESONANCE IN STEMI PATIENTS

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Background: Exosomes are a subgroup of extracellular vesicles released by cells and detectable in body fluids. Their release and cargo are influenced by cellular microenvironment, thus mirroring cell/organ physio-pathological condition. The concentration and cargo of plasma exosomes released during ST-elevation myocardial infarction (STEMI) well reflect the clinical progression of the disease, suggesting their potential as biomarkers. Cardiac Magnetic Resonance (CMR) precisely detects STEMI-induced myocardial injury, by several parameters including microvascular obstruction (MVO) and myocardial salvage index (MSI), which predict functional recovery and risk of further cardiovascular events. However, it is not always applicable due to cost and availability reasons. The aim of our study was to assess whether plasma exosomes, specifically platelet-derived exosomes, reflect myocardial injury as detected by CMR after STEMI.

Methods: 42 STEMI patients were enrolled, underwent CMR within 1 week and concomitantly, blood was collected. Plasma exosomes were isolated by commercial kits, their concentration and size determined by Nanoparticle Tracking Analysis, and GPIIbIIIa expression assessed by ELISA kit.

Results: Patients with anterior STEMI and those with late revascularization (>3 h from symptoms onset) displayed a higher number of circulating plasma exosomes ($p<0.001$ and $p<0.05$, respectively). Exosome dimension was smaller in patients with MVO ($p<0.01$) and MSI< 0.5 ($p<0.05$). Similarly, the expression of platelet marker GPIIbIIIa was lower in patients with anterior STEMI ($p<0.01$) and MVO ($p<0.05$). Specifically, exosome GPIIbIIIa expression and dimension significantly discriminated between patients with and without MVO in ROC analysis, with areas under the curve ranging from 0.70 to 0.77.

Conclusions: The main finding of our study is that plasma exosome profile well reflects CMR-assessed myocardial injury after STEMI. In particular, the exosome dimension and the expression of platelet marker GPIIbIIIa are independently associated with MVO. Future studies with larger populations are required to confirm the role of platelet-exosomes in risk stratification after STEMI.

PO15 - ID 266

EFFECTS OF INOSITOL -1,3,4,5-TETRAKISPHOSPHATE (IP4) ON RAP1-GTP AND AKT PHOSPHORYLATION IN PERMEABILISED HUMAN PLATELETS

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Background: Inositol-1,3,4,5-tetrakisphosphate (IP4) is formed from inositol-1,4,5-trisphosphate (IP3) by IP3 -3 kinase. Its function is not known but has been suggested to be involved in Ca^{2+} entry, breakdown of IP3 and regulation of PI-3K. Rap1(A+B) is a small G protein that is highly expressed in human platelets and important in adhesion/aggregation reactions, thromboxane synthesis and cytoskeletal re-organisation. Rap1 is activated by increases of cytosolic Ca^{2+} and by a PI-3K dependent mechanism that may involve RASA3. How PI-3K leads to activation of Rap1 and how IP4 or PIP3 that both bind RASA3 affect Rap1 function is not known. Here we describe effects of IP4 on Rap1-GTP formation, Akt phosphorylation and aggregation in permeabilised platelets.

Methods: Fresh human platelets were isolated from donors (using local ethical guidelines) and resuspended in a normal Hepes Tyrode buffer or one that has higher K+ and low Na+ for permeabilization studies. Rap1-GTP formation was examined after addition of GTPγS (a non-hydrolysable analogue of GTP). Rap1-GTP was estimated using RalGDS-RBD beads followed by Western blotting. Akt phosphorylation was estimated with Western blotting and aggregation was determined using light transmission aggregometry.

Results: GTPγS induced the formation of Rap1-GTP in platelets permeabilised with saponin and not in intact cells. The activation was dose and time dependent. GTPγS also induced Akt phosphorylation and aggregation of permeabilised platelets. IP4 addition did not induce sufficient Rap1-GTP formation, Akt phosphorylation or aggregation of permeabilised platelets. However IP4 reduced GTPγS stimulated Akt phosphorylation and aggregation. IP4 also reduced high GTPγS stimulated Rap1-GTP levels.

Conclusions: The function of IP4 is not established. Here GTPγS is shown to activate Rap1, Akt phosphorylation and aggregation. As IP4 affected GTPγS induced responses this suggests IP4 can regulate platelet activation at the level of Akt and Rap1.

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PO16 - ID 268

CHARACTERISATION OF PLATELET ACTIVATION SURFACE MARKERS USING SPECTRAL FLOW CYTOMETRY

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Background/Aim: Platelet function is mediated through expression of specialised surface markers and dedicated signalling pathways. Changes in expression levels of these surface markers following platelet activation underlie haemostatic responses and thrombus formation. High-parameter immunophenotypic approaches (e.g. mass cytometry) are being applied to investigate these changes with greater analytical power than ever before. Here we have developed such an approach using spectral flow cytometry.

Materials and Methods: Blood was taken from healthy volunteers (n=22), centrifuged to produce platelet-rich plasma, and incubated (20min., 37°C) with ADP, U46619 (thromboxane A2 mimetic), TRAP-6 amide (SFLLRN), PAR-4 amide (AYGKPF), collagen-related peptide (CRP-XL), or vehicle. Spectral flow cytometry (Cytek Aurora 5-laser) was used to simultaneously determine the expression levels of 14 markers in platelets comprising cell surface receptors and activation-dependent proteins. Multi-dimensional data was analysed on a global and single platelet level using a combination of NovoExpress and GraphPad Prism software, and the FlowSOM algorithm.

Results: Relative to vehicle, changes in surface marker expression varied according to agonist. In global terms, expression of degranulation markers (CD62P, CD107a, CD63) increased on platelet activation, and expression of surface receptors (CD42a, CD42b, GPVI) decreased on activation. Interestingly, at the individual platelet level, those with the highest expression of degranulation were largely different from those with the greatest decrease in surface receptors.

Conclusions: Our spectral flow cytometry-based assay concurrently characterises the expression of 14 platelet surface markers and detects changes in response to activation consistent with established patterns. This approach offers an accessible and economic tool, which is as powerful as mass cytometry, for investigation of platelet heterogeneity, abnormalities, and activation-specific responses in both health and disease.

PO17 - ID 270

THE IMPACT OF PLATELET ISOLATION PROTOCOL ON THE RELEASE OF EXTRACELLULAR VESICLES

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Background: Platelet-derived extracellular vesicles (PEVs) are small vesicles released by activated platelets that are gaining growing interest in the field of vascular biology. The mode of platelet activation is a critical determinant of PEVs release, phenotype and function. However, only very limited information is available concerning the impact of the platelet purification procedure on PEVs release.

Methods: Washed or isolated platelets were separated by differential centrifugations. For washed platelets, the platelet pellet was washed by resuspension in PIPES buffer and finally resuspended in HEPES buffer. By contrast, isolated platelets were obtained by directly resuspending the platelet pellet in HEPES buffer, skipping the washing steps in PIPES buffer. PEVs release was induced in washed or isolated platelets by stimulation with different agonist and their concentration and size distribution analysed by Nanoparticle Tracking Analysis.

Results: Isolated platelets showed a higher release of PEVs upon adenosine diphosphate (ADP) stimulation compared to washed platelets, whereas PEVs released upon stimulation with strong agonists (thrombin, collagen, A23187, U46619) were similar in the two groups. This different responsiveness to ADP was also observed as a higher α-granules release and protein kinase C activation in isolated platelets compared to washed ones. Residual plasma contamination appeared to be essential for the ability of platelets to release PEVs in response to ADP.

Conclusions: In conclusion, our study strongly suggests that procedure adopted for platelets preparation is a critical determinant of PEVs release upon ADP stimulation.

PO18 - ID 271

ADENOVIRUS-BASED AND MRNA-BASED COVID-19 VACCINES DIFFERENTIALLY MODIFY THE PLATELET-IMMUNE CROSSTALK AND PLATELET COUNT HOMEOSTASIS IN HEALTHY SUBJECTS

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Background: Nuclei acid-based COVID-19 vaccines have proved highly effective in reducing the risk of hospitalization and death. As they were distributed for the first time on a large-scale population, the adenoviral-based vaccines were linked to a very rare thrombosis with thrombocytopenia syndrome and the interplay between vaccination and platelet activation increasingly gained attention.

Aim: To compare the effect of mRNA-based and adenovirus-based vaccines on platelets of young healthy adults.

Methods: We prospectively enrolled 15 healthy volunteers (53% females) who received two doses of the mRNA-based vaccine BNT162b2, 21 days apart, and 25 healthy volunteers (64% females) that received one dose of the adenovirus-based vaccine AZD1222, followed by one dose of BNT162b2 and we studied

their platelet response before and after each dose of the vaccine (3 and 10±2 days post-injection).

Results: The BNT162b2 vaccine induced a gradual increase of plasmatic IL-1beta concentration and platelet-leukocyte aggregate formation, in particular with B cells and CD25^{high}T cells, which correlated with the higher ability of this vaccine to evoke neutralizing antibodies against the Sars-COV2 spike.

Subjects receiving the AZD1222 vaccine experienced a transient but significant 20% decrease of the platelet count 3 days after the first injection, not detected after BNT162b2 vaccination.

This transient platelet count decrease after the AZD1222 injection temporally correlated with a 10-fold increase of the plasmatic concentration of IFN-gamma, higher basal integrin activation of circulating platelets, a 20% decrease of the neutrophil count, increase of neutrophil activation and phagocytic capacity and a 3-fold increase of circulating platelet-neutrophil aggregates.

Conclusion: Thus, we speculate that the platelet count decrease after AZD1222 vaccination is part of the innate antiviral response to the adenoviral vector and that the neutrophil-platelet crosstalk aimed at neutralizing the vector hampers the chances of the immune system to mount a strong adaptive response. Ongoing studies are testing this hypothesis.

PO19 - ID 274

OBESE PATIENTS SHOW ENHANCED *EX VIVO* COLLAGEN-INDUCED THROMBUS FORMATION IN FLOWING HUMAN BLOOD

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Background: We recently demonstrated that platelets from severely obese patients show higher surface expression levels of GPVI and CLEC-2, and up-regulated signalling pathways compared to lean-matched controls, revealing that obesity induces an hyperactivation on platelets (Barrachina et al. ATVB; 2021;41:478–490).

Aim: To evaluate the impact of obesity in thrombus formation and platelet reactivity by using a novel ex vivo microfluidics assay on whole blood.

Materials and Methods: Severely obese patients (with no major comorbidities) and lean-matched controls were recruited. Whole-blood microfluidic assays evaluated the shear-dependent thrombus formation process over collagen-H in non-coagulating conditions. Platelet adhesion and activation parameters (integrin activation, P-selectin, and phosphatidyl exposure) were recorded. Moreover, citrated blood was also perfused over a collagen surface to induce shear-dependent thrombus and fibrin formation, where tissue factor (TF) was partly included to induce activation of the extrinsic coagulation pathway promoting thrombin generation.

Results: In obese patients, increasing thrombus size and volume was observed on collagen. Adhesion parameters were slightly higher in obese patients whereas no major differences between groups were observed in activation parameters. Obese patients showed a slightly more rapid fibrin formation following adhesion to collagen in the presence of TF. In the latter conditions, there were also increased levels in coagulation parameters, such as fibrin formation and phosphatidylserine exposure, in blood from obese patients in comparison to the lean-matched subjects.

Conclusion: This is the first study evaluating the effect of obesity on ex vivo collagen-induced thrombus formation in flowing blood under both coagulating and non-coagulating conditions. Overall, we show that obesity enhances the platelet thrombotic phenotype to some extent. These results are in line with our previous studies on washed platelets and confirm an increased atherothrombotic risk in obese patients.

PO20 - ID 281

TETRAMERIC NANOBODIES AS NOVEL LIGANDS FOR GPVI AND CLEC-2

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Background: CLEC-2 and GPVI stimulate potent activation of platelets and are considered promising targets in preventing arterial/venous thrombosis and thromboinflammatory diseases. Nevertheless, there is a lack of agonists of known composition to study activation on both receptors. The available ligands include natural and synthetic collagens, purified snake venom toxins and cross-linked antibodies.

Aims: In this study, we have designed ligands of known valency for GPVI and CLEC-2 based on crosslinking of nanobodies and studied their ability to activate platelets.

Methods: We have dimerised nanobodies raised against the extracellular domains of GPVI and CLEC-2 them using a short crosslinking sequence and fused these with an Fc domain to generate tetrameric ligands. The nanobodies were expressed in HEK293T cells and purified by affinity chromatography using a protein A column. Tetrameric nanobodies have been characterised using light transmission aggregometry and western blotting. Affinity values were determined using surface plasmon resonance.

Results: Tetrameric nanobodies against CLEC-2 and GPVI were induce rapid and full aggregation of platelets at subnanomolar concentrations (n = 10). All donors showed similar sensitivity to the nanobody ligands. The affinity of the tetrameric ligands

were estimated to be 0.4nM and 0.02nM for CLEC-2 and GPVI, respectively, by surface plasmon resonance. Platelet aggregation induced by the new CLEC-2 and GPVI agonists was blocked by the Src and Syk kinase inhibitors, PP2 (10 uM) and PRT-060318 (10 uM), respectively, and by the divalent CLEC-2 antibody fragment AYP1 f(ab)2 or dimeric GPVI nanobody, Nb2, demonstrating that activation is mediated directly through these receptors.

Conclusions: tetrameric nanobodies induce strong platelet aggregation through CLEC-2 and GPVI with high affinity and specificity. These novel tools will enable further studies to probe GPVI and CLEC-2 function and their mode of activation.

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PO21 - ID 287

CHARACTERIZATION OF PLATELET-LEUKOCYTE INTERACTIONS IN COVID-19 PATIENTS

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Background: Severe Coronavirus disease 2019 (COVID-19) has been associated with a dysregulated cytokine production, lymphocyte and monocyte exhaustion, and immunothrombotic complications that reduce gas exchange in the lungs and contribute to multiorgan failure.

Aim: The objective of this study was to characterize the interplay between platelets and the dysregulated immune phenotype that drives disease severity.

Methods: To achieve this goal, we performed a high-throughput flow cytometric profiling of the phenotype and interactions of platelets circulating in the blood of Sars-COV2-positive subjects upon hospitalization. Patients were stratified into non-ICU (n=31), vaccinated non-ICU (n=19) and critically ill ICU (n=21) patients and compared to sex- and age-matched Sars-COV2-negative patients (n=29) and healthy volunteers (n=25). All participants gave written informed consent. The study was approved by the Ethics Committee of our institution.

Results: Platelets from ICU patients had dysfunctional mitochondria and a non-adhesive phenotype. Displayed significantly less glycoprotein (GP)Iba and GPVI on the surface and failed to present active integrin aIIbb3 and P-selectin on the plasma membrane in response to exogenous stimuli. Platelet hypo-responsiveness positively correlated with the Horowitz index (PaO2/FiO2 ratio), a measure of lung function, and with the D-dimer concentration, a surrogate marker of ongoing thrombosis. Despite the low adhesiveness, platelets of ICU patients bound avidly to innate immune cells. Interactions with monocytes and NK cells increased with severity, even though these leukocytes subpopulations were reduced in the circulation of ICU patients. Platelet-T cell aggregates were doubled in non-ICU patients compared to controls but were not detectable among the ICU patients. Conversely, platelets of vaccinated patients interacted with adaptive, but not innate immune cells.

Conclusions: In summary, platelets from COVID-19 patients present features of metabolic and functional exhaustion and bind primarily innate but not adaptive immune cells, thus promoting the dysregulated immune response that drives COVID19 severity.

PO22 - ID 272

PLATELET LIPIDOME FINGERPRINT OF OBESE PATIENTS: NEW ASSISTANCE TO CHARACTERIZE PLATELET DYSFUNCTION IN OBESITY

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Background: Obesity is associated with a pro-inflammatory and pro-thrombotic state that supports atherosclerosis progression and platelet hyperreactivity. During the last decade, the platelet lipidome has been considered a treasure trove as a source of biomarkers for preventing and treating different pathologies.

Aim: The goal of the present study was to determine the lipid profile of platelets from severely obese patients.

Materials and Methods: Twelve severely obese patients (BMI>40 kg/m^2) and their age- and sex-matched controls participated in the study. Platelets were isolated by an established method that limits contamination from other blood cells and plasma proteins. After that, lipids were extracted and major phospholipids, sphingolipids and neutral lipids were analyzed either by gas chromatography or by liquid chromatography coupled to mass spectrometry.

Results: Despite a significant increase in obese patient's plasma triglycerides, there was no significant differences on the membrane levels of triglycerides in platelets among the two groups. In contrast, total platelet membrane free cholesterol was significant

decreased in the obese group. The profiling of phospholipids showed that phosphatidylcholine and phosphatidylethanolamine contents were significantly reduced in the obese group. However, no differences were found on the sphingomyelin and ceramides levels. The outline of glycerophospholipids and sphingolipids molecular species (fatty-acyl profiles) was similar in the two groups.

Conclusion: At summary, this lipidomics data indicate a unique lipid fingerprint in platelets from obese patients. These results may guide further studies and provide mechanistic-driven perspectives related to the hyperactivated state of platelets in obesity.

PO23 - ID 285

PROTEOMICS-BASED COMPREHENSIVE *IN SILICO* MODEL OF HUMAN PLATELET SIGNALLING FOR THE PERSONILIZED STUDIES OF HEMOSTASIS DISORDERS

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Background/Aims: Hereditary and acquired disorders of platelet number and function are often associated with life-threatening bleedings. Deciphering the underlying mechanisms of platelet malfunctioning and finding their relationship with the bleeding phenotype is complicated. Comprehensive computational approaches allow one to integrate fragmentary clinical and research data on platelet functioning and deduce the nature of platelet disorders. Here we aim at development of the computational model of platelet intracellular signaling and key functional responses, which would be applicable for the studies of platelet malfunctioning.

Materials and Methods: Computational model was constructed in the modular fashion and based on the ODEs set, which described the key platelet signaling pathways: platelet activation by ADP (P2Y1/P2Y12), thrombin (PAR1/PAR4), collagen (GPVI), podoplanin (CLEC-2) and thromboxane A2 (TP). Model covered functional responses: shape change, dense and alpha granule release, thromboxane A2 synthesis, inside-out and outside-integrin alphaIIbbeta3 activation. Model was constructed using COPASI software and Python. Basic model parameters were taken either from the literature or upon fitting of experimental data. Protein numbers for the generic "average healthy donor" model were taken from literature data. Personalized models were constructed using proteomics data from 5 healthy donors.

Results: The developed model could accurately describe available experimental data on platelet activation by ADP, thrombin, collagen/CRP, rhodocytin (CLEC-2 activator) and U46619 (TP-receptor activator). The model was also capable of accurately describing synergistic activation of platelets by the combination of different activators. It appeared that the key signaling proteins governing synergy were different isoforms of PI3K. Model personalization allowed to describe observed inter-donor variability in platelet responses.

Conclusions: Altogether, here we propose a novel comprehensive model covering key signaling and functional pathways of the platelets. The developed approach can be used for the studies of the acquired or hereditary platelet abnormalities.

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PO24 - ID 289

EXPLORING THE CARDIOVASCULAR RISK IN PEOPLE LIVING WITH HIV BY DETERMINING THE PLATELET AND ENDOTHELIAL EFFECTS OF INTEGRASE INHIBITORS USED IN CURRENT ANTI-RETROVIRAL THERAPIES

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Background & Aims: In an era of effective antiretroviral therapy (ART), people living with HIV (PLWH) have near-normal life-expectancies, but increased occurrence of cardiovascular disease (CVD) that is associated with ART. We have previously reported that certain classes of antiretroviral drugs such as nucleotide reverse transcriptase inhibitors (NRTIs) enhance platelet and endothelial activation promoting a pro-thrombotic phenotype. Little is known however about the impact of integrase inhibitors (INSTIs), commonly used in clinical settings. Here, we aimed to compare the effects of various IIs on platelet and endothelial activation.

Materials & Methods: Human Umbilical Vein Endothelial Cells (HUVEC) and platelets from HIV-negative donors were exposed in vitro to clinically relevant concentrations of the INSTIs, bictegravir, dolutegravir or a vehicle control for 48h (HUVEC) and 30min (platelets). Expression of ICAM-1 and E-Selectin on HUVEC were measured following a 4h, 6h and 24h stimulation with TNFα (10ng/ml) by flow cytometry. Platelet activation was assessed following treatment with ADP, TRAP6 or Collagen (1-30μM) by light transmission aggregometry and flow cytometry.

Results: None of the INSTIs affected endothelial ICAM-1 or E-Selectin expression (n=5). Platelet aggregation was also unaffected by INSTIs relative to control (n=4) and preliminary data suggests that integrin activation as well as α- and dense-granule release, had no significant changes between the control and drug-exposed groups.

Conclusions: In contrast to NRTIs, bictegravir and dolutegravir do not affect endothelial adhesion molecule expression nor platelet aggregation. Ongoing work includes further evaluation of platelet activation and assessing effects of INSTIs on platelet inhibitory responses as well as flow assays to examine platelet interaction with the vascular endothelium during ART exposure. Ultimately, this work may lead to the identification of treatment regimens with a lower CVD risk, providing a safer option when treating PLWH.

PO25 - ID 293

DETECTION OF PLATELET-ACTIVATING ANTIBODIES ASSOCIATED WITH VACCINE INDUCED THROMBOTIC THROMBOCYTOPENIA BY FLOW CYTOMETRY: AN ITALIAN EXPERIENCE

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Background and aim: Rare cases of thrombocytopenia and thrombotic complications after adenoviral vaccination (VITT) caused by platelet activating anti-platelet factor 4 (PF4)/polyanions antibodies, have been reported. The laboratory diagnosis of VITT, similarly to the diagnosis of heparin-induced thrombocytopenia (HIT), requires immunoassays for antibodies identification and platelet activating functional tests to confirm pathogenicity of anti-PF4/polyanions antibodies. We compared flow cytometry (FC) measurement of p-selectin exposure and heparin-induced platelet activation test (HIPA) as functional tests to confirm VITT diagnosis in two Italian centers.

Methods: Anti-PF4/polyanions antibodies were assessed using immunoloassays (ELISA Lifecode PF4 IgG and Asserachrom HPIA-IgG assays; HemosIL AcuStar HIT-IgG (PF4-H) assay) and functional assays (FC measurement of p-selectin exposure in platelet rich plasma and HIPA in the presence of VITT samples + buffer/low/high heparin concentration.

Results: Thirteen VITT patients [6 M/7 F; median age 56 (33-78)] (Table 1) were all positive to ELISA and negative to AcuStar assays. Functional assay by FC identified three different patterns: 1) a typical VITT p-selectin exposure pattern in 7 patients showing platelet activation with buffer only, which was moderately reduced with low heparin and completely abolished with high heparin doses (Fig.1A); 2) 4 VITT patients investigated under IvIg therapy showed low or no platelet activation (Fig.1B); 3) a typical HIT pattern – platelet activation with low heparin dose abolished by high heparin dose- was found in 2 VITT patients (Fig.1C). The HIPA testing was positive in 11 VITT patients. Three patients were no more positive to both ELISA and FC analysis after 7, 17 and 24 days of therapy (Fig.1A).

Conclusions: FC measurement of P-selectin exposure was a simple and reliable method 1) to detect platelet-activating anti-PF4/polyanions antibodies in VITT patients, 2) to discriminate VITT from HIT, thus helping for the choice of the anticoagulant treatment, 3) to follow the response to therapies.

PO26 - ID 299

APOLIPOPROTEIN A1 BECOMES TYROSINE PHOSPHORYLATED DURING PLATELET ACTIVATION BY *STAPHYLOCOCCUS AUREUS* ALPHA-TOXIN

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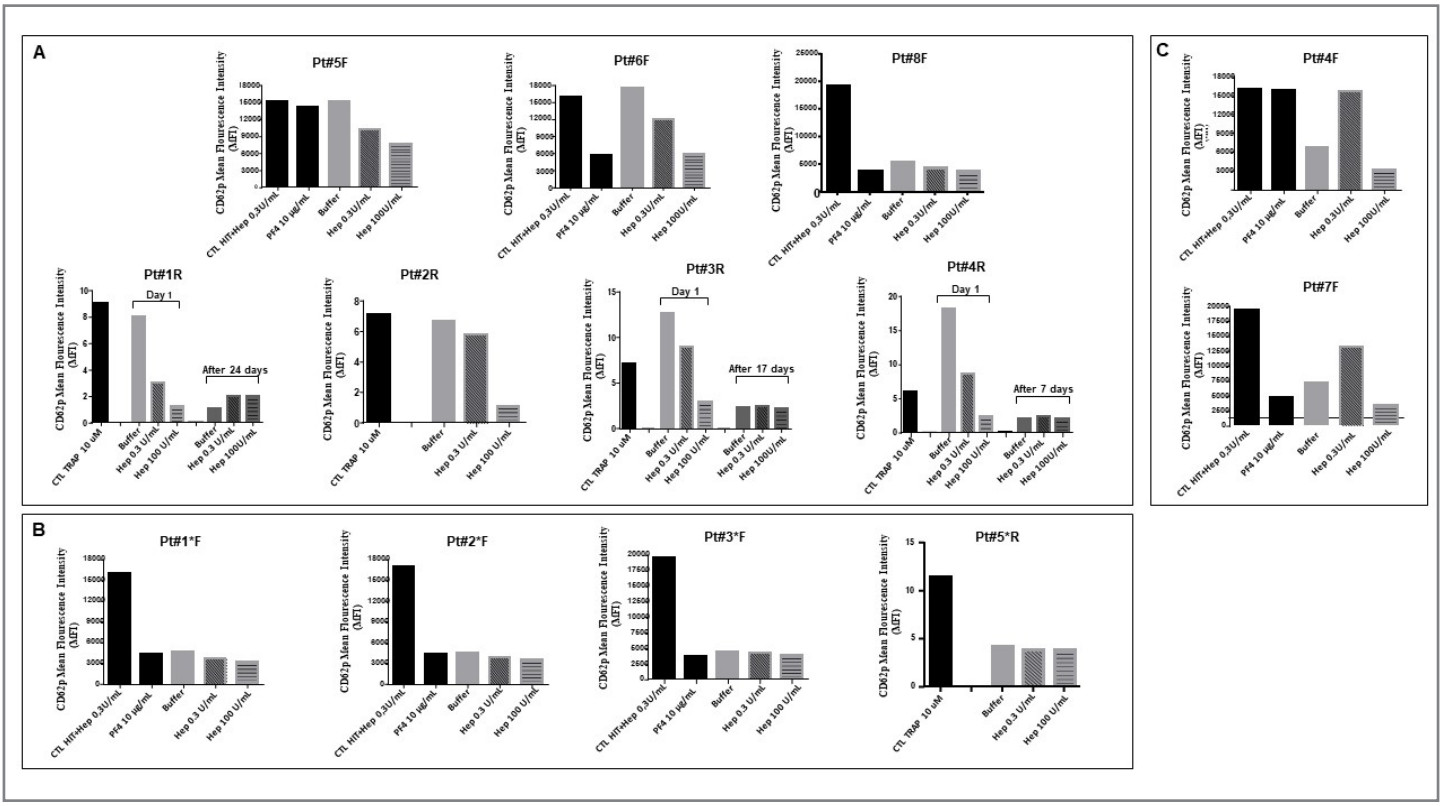
Background / Aims: Alpha-toxin, one of the key virulence factors of Staph. aureus, activates human platelets inducing characteristic changes in protein tyrosine phosphorylation. In comparison to thrombin and collagen, toxin-induced tyrosine phosphorylation is followed by a distinctive, nearly total dephosphorylation of proteins in the lower molecular range including a protein with the apparent molecular mass of 27 kDa.

Here, we reveal the identity of p27 and investigate the role of several protein kinases and phosphatases in toxin-induced p27 tyrosine phosphorylation.

Materials and Methods: Human platelets were isolated from whole blood of healthy volunteers, adjusted to 5x 10^8 platelets/ml apyrase-containing buffer and stimulated with alpha-toxin in presence or absence of inhibitors. Protein phosphorylation was detected by Western Blot and densitometrically quantified. For the identification of p27 platelets were stimulated and phosphorylated p27 was purified by three-step column chromatography (diethylaminoethyl cellulose, sulfopropyl sepharose, protein G sepharose) and further analysed by MALDI-TOF mass spectrometry.

Results: p27 was purified as described above and finally identified as apolipoprotein A1 (apo A1). In resting platelets apo A1 is not tyrosine phosphorylated. Upon toxin stimulation (0.3 µg/ml) apo A1 tyrosine phosphorylation reaches a maximum after 2-5 min, followed by a nearly complete dephosphorylation within 10 min. Preincubation with the structurally different SFK-inhibitors PP1, PP2, SU6654 and dasatinib significantly reduces toxin-induced apo A1 phosphorylation. Calpeptin (1 µM), an inhibitor of calpain, slightly increases and accelerates apo A1 phosphorylation, while dephosphorylation remains unaffected. Unspecific inhibition of tyrosine phosphatases by the use of sodium orthovanadate (30 µM), shifts the balance in favour of tyrosine phosphorylation. Toxin-induced apo A1 phosphorylation is accelerated, increased fourfold and dephosphorylation is inhibited.

Conclusion: Upon platelet stimulation apolipoprotein A1 undergoes changes in tyrosine phosphorylation. Src family kinases and calpain might participate in alpha-toxin-induced apo A1 tyrosine phosphorylation. Functional consequences have to be elucidated.



PO26 - Figure 1. A) Patients with a typical VITT pattern; B) Patients investigated under/after IVIG therapy; C) Patients with a HIT pattern. F= Florence, R= Rome. In Florence cohort positive controls were HIT samples+ 0.3 U/ml heparin. In Rome cohort positive controls were platelet stimulated by 10 uM TRAP.

VITT Cases	Age	Sex	Vaccine Type	Onset after administration (days)	Platelet count at the onset	Thrombosis	Hemosil AcuStar HIT-IgG (vn ≤1 U/mL)	Lifecode PF4 IgG test (O.D.)	Asserachrom HPIA IgG ELISA (O.D.)	Flow Cytometry (MFI)	Hipa test
Pt #1F*	57	F	AZ	6	10.000/uL	Portal vein; pulmonary embolism; splenic vein	0,04	Positive (2472)	/	Weakly Positive	Positive
Pt #2F*	73	F	AZ	6	10.000/uL	Pulmonary embolism; cerebral vein	0,15	Positive (1912)	/	Weakly Positive	Negative
Pt #3F*	75	F	AZ	7	23.000/uL	Cerebral vein; portal vein	0,06	Positive (3128)	/	Weakly Positive	Weakly Positive
Pt #4F	71	M	AZ	6	< 10.000/uL	Pulmonary embolism	0,04	Positive (2942)	/	HIT pattern	HIT pattern
Pt #5F	78	F	JJ	10	24.000/uL	Multiple lower limb vein	0,01	Positive (3339)	/	Positive	Positive
Pt #6F	41	F	AZ	9	49.000/uL	Cerebral vein	0,02	Positive (1473)	/	Positive	Positive
Pt #7F	61	M	AZ	7	20.000/uL	Multiple lower limb vein	0,51	Positive (3257)	/	HIT pattern	HIT pattern
Pt #8F	59	F	AZ	9	69.000/uL	Multiple lower limb vein; pulmonary embolism	0,19	Positive (3026)	/	Positive	Positive
Pt #1R	35	F	AZ	6	20.000/uL	Right superior sinus; right transverse sinus; intrahepatic portal vessels	0,06	/	Positive (1,23)	Positive	Positive
Pt #2R	67	M	AZ	11	25.000/uL	Portal vein; mesenteric veins; splenic veins	/	/	Positive (1,33)	Positive	Weakly Positive
Pt #3R	42	M	AZ	7	33.000/uL	Superior sagittal sinus (partial); right sigmoid and transverse sinuses	0,04	/	Positive (2,723)	Positive	Positive
Pt #4R	33	M	AZ	7	25.000/uL	Superior sagittal sinus; straight sinus; right transverse sinus; right jugular vein; left carotid bifurcation; pulmonary embolism; abdominal aorta	0,38	/	Positive (2,973)	Positive	Weakly Positive
Pt #5R*	34	M	JJ	11	66.000/ul	Right sigmoid and transverse sinuses; right jugular vein	0,16	/	Weekly Positive (0,790)	Weakly Positive	/

PO26 - Table 1. General characteristics of the study population, platelet count, site of thrombosis, tests. F= Florence, R= Rome.

PO27 - ID 260

CHARACTERISING THE NATIVE EXTRACELLULAR MATRIX IN AN ENDOTHELIALISED MODEL OF ATHEROTHROMBOSIS

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Background/Aims: Acute coronary syndrome occurs following rupture or erosion of an atherosclerotic plaque and exposure of extracellular matrix (ECM) proteins, initiating thrombus formation. The ECM is dynamic and complex, comprised of glycoproteins, collagens, and growth factors. Vascular dysfunction, typical in cardiovascular disease (CVD), is thought to alter ECM composition, however, the changes that occur and impact on thrombogenicity are not fully understood. The aim of this study was to characterise the changes in endothelial and smooth muscle derived ECM, induced by known cardiovascular risk factors and assess the impact on thrombus formation using an endothelialised in vitro model of thrombosis.

Materials and Methods: Gene expression of endothelial regulators of thrombosis and haemostasis were compared in human umbilical vein endothelial cells (HUVECs) and human coronary artery endothelial cells (HCAECs) using RT-qPCR. The composition of the ECM produced by HCAECs and human coronary artery smooth muscle cells (HCASMCs) under healthy conditions and following treatment with disease-related stimuli was identified using mass spectrometry. Thrombus formation on cell-derived matrices with and without endothelial cells was measured using fluorescent microscopy.

Results: Gene expression was significantly different in HCAECs compared with HUVECs ($P<0.05$), demonstrating differences in endothelial regulation of thrombosis. Methods were successfully developed to remove HCAECs and HCASMCs, leaving an intact ECM in sufficient quantities suitable for mass spectrometry. The ECM generated by HCAECs and HCASMCs varied considerably between the different cell treatments, and this was reflected in the thrombogenicity of the different cell-derived matrices, with significant differences in thrombus volume, size and number ($P<0.05$).

Conclusions: Understanding HCAEC mediated regulation of thrombosis on a native, human ECM representative of CVD will help to identify novel antithrombotic targets important for thrombosis but not haemostasis.

PO28 - ID 229

OBSERVATION OF DECREASED PLATELET FUNCTIONAL RESPONSES TO CONVENTIONAL ACTIVATION OVER SIX MONTHS PERIOD IN PATIENTS AFTER ACUTE CORONARY SYNDROME ON DUAL ANTI-PLATELET THERAPY

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Background: Dual anti-platelet therapy (DAPT) is a treatment of choice for prevention of thrombosis in patients after acute coronary syndrome (ACS). However, 10% of patients experience recurrent adverse cardiovascular events over the subsequent 30 days of DAPT. Moreover, data on long-term hemostatic effects of DAPT are scarce.

Aim: To assess the state of the hemostatic system in patients after percutaneous coronary intervention (PCI) for acute coronary syndrome over the 6 months period.

Methods: Ten post-PCI patients treated with a combination of aspirin and prasugrel (n = 5) or ticagrelor (n = 5) were enrolled in the study. Platelet functionality was assessed in 5 time points: day 0 (aspirin loading dose), day 1(prasugrel/ticagrelor loading dose), and on the 7th, 30th, and 180th days of full-dose therapy. Platelets were assessed with conventional light-transmission aggregometry (LTA) and low-angle light scatter aggregometry (LA). Platelet functional and signaling responses to conventional activation (ADP, PAR1-AP and collagen-related peptide, CRP) were assessed by continues and end-point flow cytometry and fluorescent microscopy. The study was approved by the local ethical committee (Vinogradov Clinical Hospital).

Results: Flow cytometry revealed significant decrease in surface expression of PS and PAC-1 after platelet activation with all agonists and CD62p with ADP in time points 1-180. The percentage of platelets with weak intracellular calcium response increased and did not recover after loading dose of DAPT. Meanwhile, platelet aggregation assay and fibrinogen binding showed a recover to the normal level at 180th day by the maximal signal intensity. Platelet disaggregation was tend to restore despite the impact of P2Y12 inhibitor. Meanwhile, the aggregation by LA was maintained on the impaired level until 180th day of treatment.

Conclusions: Our study revealed that standard aggregometry values and fibrinogen binding to platelets return to the pre-therapy levels over the 180-day period, although flow cytometry and low-angle light scatter aggregometry values did not change significantly.

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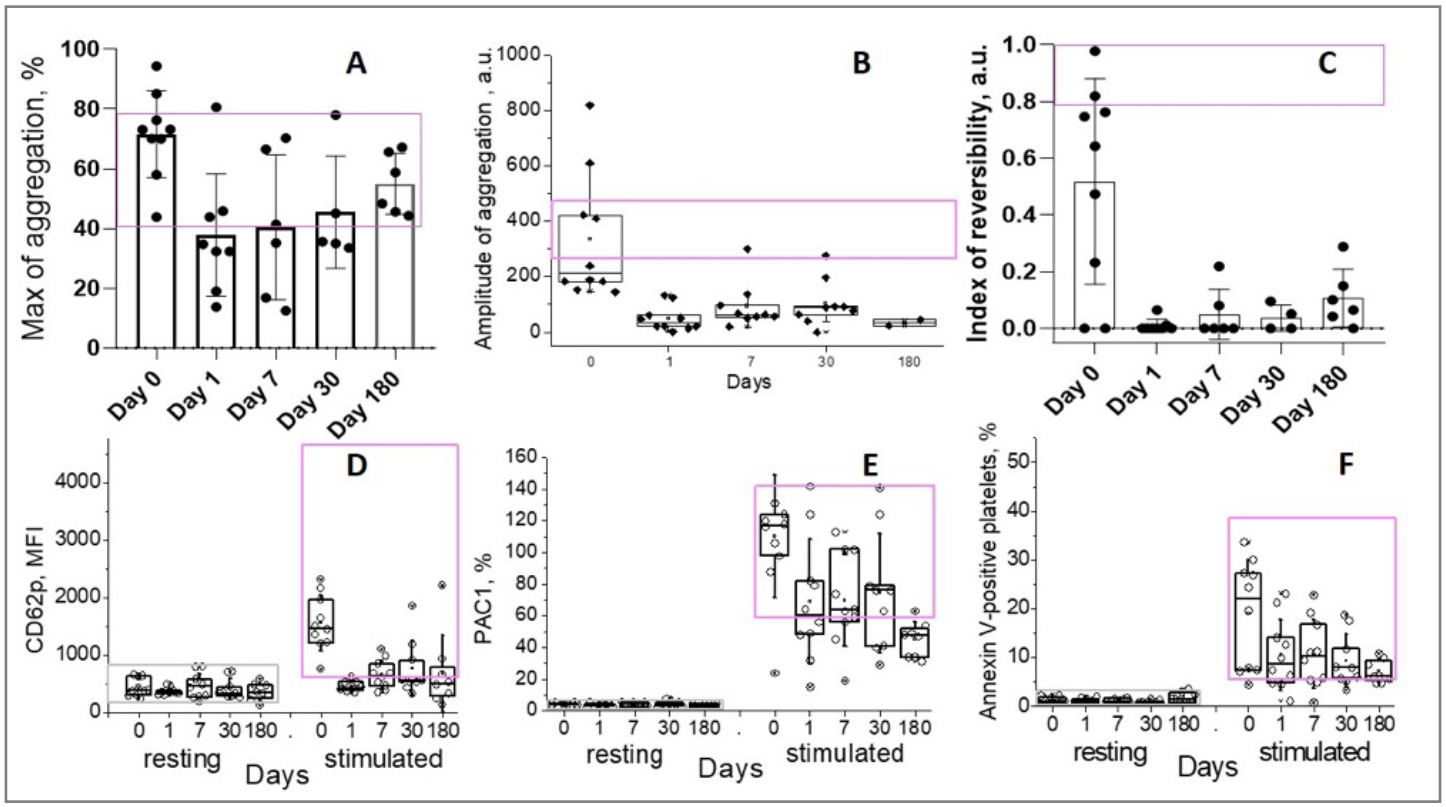

PO28 - Figure 1. Platelet functional responses. (A): The maximum of platelet aggregation in response to 2uM ADP. (B): The amplitude of platelet aggregation by LA. (C): The index of reversibility (the ratio of the aggregation at the 600th second to the maximum) in response to 5uM ADP. (D): P-selectin exposure in response to ADP; (E),(F): Glycoprotein IIbIIIa and phosphatidylserine (PS) exposure in response to CRP and PAR-1 AP.

PO29 - ID 284

INTER-INDIVIDUAL VARIATION IN PLATELET AGGREGATION AND SECRETION RESPONSES IN HEALTHY VOLUNTEER DONORS

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Introduction & Aims: Platelet activation plays an important role in arterial thrombosis in coronary heart disease, stroke, and peripheral arterial disease. Therefore this study aims to determine if platelet aggregation is a true measure of global platelet responsiveness; or whether ATP secretion may reveal a different dynamic.

Methods: 108 normal healthy volunteers were assessed for indices of platelet function, in response to 3 different agonists over a physiological dose range as follows: thrombin receptor activating peptide (TRAP; 2.9-33uM), collagen-related peptide (CRP-XL; 0.1-2 ug/ml) and thromboxane A2-mimetic (U46619; 0.1-15 uM). Specifically, we examined the dose-response nature of each individual's responsiveness to platelet agonists in parallel assays of platelet aggregation (assessed by light transmission in PRP) and platelet ATP secretion (assessed by luminometry).

Results: The results show significant inter-individual differences in aggregation and secretion responses to all 3 agonists in the donor population. For example, the maximal extent of platelet aggregation varies in response to TRAP from a minimum 31.50% to maximum 120%). Similarly, the maximal extent of ATP secreted in response to TRAP from minimum 0.11 to maximum 4.67 pmoles / 10^6 platelets, demonstrating a 40 to 50 fold range in secretion capacity for this dense-granule component. A greater than 10 fold difference in secretion of ATP was also observed in response to CRP and U46619 in this donor cohort. There is a strong positive correlation between responses to all 3 agonists in the aggregation assays and, separately, in the secretion assays. However, there is no correlation between platelet secretion and aggregation responses.

Conclusion: Substantial inter-individual variations in platelet responses are observed in normal healthy donors using 3 different agonists (TRAP; CRP and U46619). Aggregation and secretion are independently regulated, suggesting that more studies are merited to investigate if ATP secretion might provide better insights into clinical risks in patient populations.

PO30 - ID 280

DEVELOPMENT OF A CELL BASED IN VITRO MODEL OF ISCHAEMIC REPERFUSION INJURY, TO IDENTIFY SMALL MOLECULE MULTICELLULAR DRUG TARGETS FOR THE PREVENTION AND TREATMENT OF ISCHAEMIC STROKE

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Ischemic stroke is one of the leading causes of morbidity and mortality worldwide. Despite successful reperfusion, stroke patients often suffer lifelong disabilities. The resupply of oxygen and nutrients is vital, however subsequent ischemia/reperfusion (I/R) injury can continue propagating cell injury via generation of

reactive oxygen species and inflammatory processes.
Platelets are established mediators of thrombosis and inflammation. However, little is known regarding the role for platelets in I/R injury. Whilst platelet activation leads to thrombus formation that causes the ischaemia, following reperfusion, it is unknown whether platelet activation is beneficial or problematic for recovery.
Multiple cells make up the neurovascular unit. Microglia, astrocytes, and neural cells as well as endothelial cells and smooth muscle cells cross talk to regulate cerebral blood flow and the blood brain barrier, which is disrupted during ischaemic reperfusion. There is therefore a real need to establish a multi-cellular model to enable the contribution and interplay of the different neurovascular unit cell types to be elucidated.
Monocultures of microglia (HMC-3), astrocytes (U-87) and microvascular endothelial cells (hCMECs/D3) were treated with activated platelet releasate, and cell activation, proliferation, migration and survival was monitored. Human washed platelet releasate was prepared by centrifugation following 30-minute treatment with 5mM CaCl2. Imaging and analysis performed using a HoloMonitor live cell imager over a 24-hour period demonstrated significant alterations in microglial and endothelial cell behaviour following treatment with activated platelet releasate vs non-activated and tyrodes-treated controls.
There are currently no approved therapies to treat I/R injury and thus it is imperative to identify therapeutic targets to decrease the burden of ischemic stroke. Establishment of a multicellular model of I/R injury, incorporating, microglia, astrocytes, microvascular endothelial cells and platelets will enable the identification of novel multicellular anti-platelet-based therapeutics that target both the thrombotic and inflammatory processes that occur in ischaemic injury.

ABS01 - ID 265

CHARACTERIZATION OF THE URIC ACID TRANSPORTER URAT1 (SLC22A12) IN PLATELETS AND MEGAKARYOCYTES

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Background: URAT1, encoded by the SLC22A12 gene in humans, acts as an organic anion transporter that plays a key role in uric acid and oxidative homeostasis. High levels of uric acid lead to urate crystals formation in the joints or in urine resulting in gouty arthritis or kidney stones, respectively. Less well-known, the epidemiological association of hyperuricemia with adverse cardiovascular events poses the question of the contribution of high uric acid circulating levels to platelet reactivity and thrombosis.

Aims: To characterize URAT1 expression and function in human platelets and megakaryocytes.

Methods: URAT1 expression was verified by immunoblotting and by flow cytometry using independent antibodies in platelets and the megakaryocytic cell line MEG-01, with HEK293 as positive controls. Platelet aggregation in response to classical platelet agonists (collagen, arachidonic acid, ADP and thrombin receptor activating peptide (TRAP)), in the presence or absence of pharmacological URAT1 inhibitors (lesinurad and verinurad) was verified by light transmission aggregometry in washed platelets prepared from whole blood of healthy male and female volunteers.

Results: URAT1 immunoreactivity was detected on the surface of and within platelets and MEG-01 cells, at the expected molecular weight of 65 kD. Cell fractionation experiments were consistent with flow cytometry results. Incubation of washed platelets with uric acid (50-100 µg/ml) did not induce spontaneous platelet aggregation, nor did it induce synergistic effects in the presence of low concentrations of classical platelet agonists. However, collagen-, arachidonic acid-, ADP-, and to a lesser extent TRAP-induced platelet aggregation was inhibited by pre-incubation of washed platelets with either lesinurad (IC_{50} 125 nM - 1.6 µM) or verinurad (IC_{50} 145 nM - 14 µM).

Conclusion: Human platelets and megakaryocytes express the URAT1 transporter. Whether platelets and megakaryocytes can sense and respond to uric acid fluctuations in their environment remains uncertain and merits further investigation.

ABS02 - ID 300

ZINC MAINTAINS THROMBUS STABILITY VIA MODULATION OF THE PLATELET CAMP/PKA PATHWAY

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Background/Aims: Zinc (Zn^{2+}) deficiency is a global nutritional issue, with the WHO estimating 31% of the global population have an element of zinc deficiency. One symptom of Zn^{2+} deficiency is increased bleeding through impaired haemostasis. Platelets are crucial to haemostasis, and are normally inhibited by endothelial derived prostacyclin (PGI_2) and Nitric oxide (NO) which induce Adenylyl Cyclase (AC) and cAMP signalling or sGC, and cGMP signalling respectively. In other cell types Zn^{2+} can modulate cAMP concentrations by changing AC and/or phosphodiesterase (PDE) activity. Therefore, we aim to investigate if Zn^{2+} can modulate platelet PGI_2 signalling.

Methods: Platelet aggregation, spreading and western blotting assays with Zn^{2+} chelating agents and cyclic nucleotide elevating agents, were completed in washed platelet and PRP conditions. In vitro thrombus formation with various Zn^{2+} chelating and cyclic nucleotide elevating agents was assessed in whole blood.

Results: To understand the inhibitory effect of Zn^{2+} chelation in platelet activation, we investigated if Zn^{2+} modulated platelet inhibitory pathways. Analysis of resting platelets incubated in the absence and presence of Zn^{2+} chelators showed an elevation in $pVASP^{ser157}$ whilst addition of the AC inhibitor SQ22536 blocked the Zn^{2+} chelation effects. Furthermore , spread platelets treated with Zn^{2+} prior to PGI_2 addition blocked PGI_2 mediated platelet reversal. Analysis of spread platelets treated with forskolin and milrinone indicated that Zn^{2+} blocked AC activity rather than PDE3 function. This data indicated that Zn^{2+} modulated PGI_2 signalling in an AC dependent manner. Importantly, further analysis of platelet aggregation and in vitro thrombus formation identified that PGI_2 function was potentiated in the presence of low doses of Zn^{2+} chelators.

Conclusion: Zn^{2+} chelation potentiates the effect of PGI_2 signalling, via elevation of AC activity, reducing a platelet's ability to overcome PGI_2 signalling. This may underpin why patients with reduced Zn^{2+} levels have an elevated bleeding diathesis.

ABS03 - ID 247

INHIBITION OF THE P75 PAN NEUROTROPHIC RECEPTOR PARTLY MITIGATES BDNF-INDUCED PLATELET AGGREGATION

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Background/Aims: The Brain-Derived Neurotrophic Factor (BDNF) promotes neuronal growth and survival, acting through the tropomyosin receptor kinase B (TrkB) and the 75 kDa pan-neurotrophic receptor ($p75^{NTR}$). This effect is further amplified by dimerization of these receptors. In addition to its cerebral expression, the quantity of BDNF in platelets can be 1000-fold greater than neurons. We have shown that washed platelets aggregate in response to BDNF via the activation of a truncated form of the TrkB receptor. Whether $p75^{NTR}$ is involved in BDNF-induced platelet aggregation remains unknown. This project aims to characterize $p75^{NTR}$ expression and function in platelets.

Materials and Methods: Washed platelets were prepared by sequential centrifugation from whole blood obtained from healthy volunteers. $p75^{NTR}$ expression was assessed by immunoblotting, flow cytometry and confocal microscopy, and quantified by ELISA (n=20). Platelets were activated using classical agonists (ADP, collagen, thrombin receptor activating peptide [TRAP]) and BDNF, in the presence of $p75^{NTR}$ inhibitor THX-B or its inactive analog, THX. Aggregation was measured by light transmission aggregometry. Signaling pathways downstream of BDNF activation were assessed by anti-phosphoprotein immunoblot.

Results: $p75^{NTR}$ immumoreactivity was detected on the surface of (28.3±19.4%) and within (56.4±18.4%) platelets. $p75^{NTR}$ levels were highly variable, with a 10-fold difference between highest (382 pg/ml) and lowest values (38 pg/ml), and did not change following platelet activation. Collagen-, ADP-, and TRAP-induced aggregation were not affected by incubation with the $p75^{NTR}$ inhibitor, THX-B. Inhibition of BDNF-induced platelet aggregation showed high interindividual variation: THX-B-induced responses ranged from no effect to a complete abrogation of platelet activation and aggregation in response to BDNF.

Conclusions: Pharmacological inhibition of $p75^{NTR}$ abrogated BDNF-induced platelet aggregation in some but not all healthy volunteers. Whether this variability in aggregation is related to the high interindividual variation in platelet $p75^{NTR}$ levels remains to be established.

ABS04 - ID 303

ACUTE E-CIGARETTE EXTRACT EXPOSURE *IN VITRO* LEADS TO A PROTHROMBOTIC PLATELET PHENOTYPE

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Introduction: In recent years electronic cigarette use has become more prevalent, in both smoking and non-smoking populations with people perceiving it as a risk-free smoking alternative. However, little is known about their effects on human health. Recent data suggest a possible association with cardiovascular pathology, which may include altered hemostasis, platelet function and thrombosis. In this study, we, therefore, aimed to determine the effects of e-cigarette extract on platelet function and generation.

Aims: To understand the effects on platelet function and generation after in vitro acute exposure to e-cigarette extract.

Methods: Platelet-rich plasma (PRP) was exposed to e-cigarette and cigarette extracts as well as vehicle for 60 minutes. Platelet function was assessed by aggregometry, and FACS-based assays for P-selectin and integrin $a_{IIb}b_3$. Megakaryocyte phenotype and platelet generation were examined in cultures exposed to e-cigarette extract using FACS-based analyses of MK receptor levels and assessment of platelet generation in a novel ex vivo heart lung system (https://www.biorxiv.org/content/10.1101/2021.11.01.466743v1).

Results: Acute exposure to e-cigarette extract caused a prothrombotic phenotype via increased maximal aggregation (49.4%± 0.7 vs 74.7%±1 p=<0.0001) as well as FACS-based assays showing significantly increased maximal response (2633.3± 251.7 vs 4147.6±45.1 p=<0.0001 and 1232.3± 121.7 vs 2675.6±23.5 p=<0.0001) and decreased EC_{50} (0.21 µg/ml± 0.05 vs 0.11 µg/ml±0.02 p=0.0044 and 0.22 µg/ml± 0.03 vs 0.105 µg/ml±0.04 p=0.001) for P selectin mobilisation and integrin $a_{IIb}b_3$ activation in PRP upon stimulation with CRP-XL when compared to vehicle. However murine megakaryocytes cultured from bone marrow showed no change in surface receptors or ability to generate platelets in the heart-lung system.

Conclusion: We conclude that acute exposure to e-cigarette extract has a prothrombotic effect on platelet function however no effect on platelet generation in vitro.

ABS05 - ID 238

THE ROLE OF PLATELETS AND RED BLOOD CELLS IN ABDOMINAL AORTIC ANEURYSM

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Background/Aims: Abdominal aortic aneurysm (AAA) represents a vascular disease with high morbidity and mortality. Recent clinical trials revealed no benefit administering potential growth restrictors; thus, no therapeutic approach is available to date. Certain factors have been shown to increase the risk for an AAA, e.g. smoking and high blood. 80% of all AAAs are accompanied by a growing platelet-rich intraluminal thrombus (ILT) which is relevant for AAA progression. However, the impact of platelets-RBC interaction in AAA progression are poorly understood.

Material and Methods: PPE-induced aneurysm formation was investigated via sonography over a 28-day time period in C57BL/6 wild type mice (sham vs. PPE) and GPVI knockout (KO)-mice. Flow cytometric analysis was performed with whole blood from mice and men as well as histological staining of the aortic wall.

Results: The aortic diameter of PPE-operated mice was significantly increased compared to sham-operated control mice. After formation of an established aneurysm (>150%) at day 28, platelet activation was determined by the measurement of active integrin αIIbβ3 and the degranulation marker P-selectin. The results indicate increased integrin αIIbβ3 activation and P-selectin externalization upon stimulation of the collagen receptor GPVI in PPE mice compared to sham-operated mice. Additionally, glycoprotein expression analysis revealed an upregulation of GPVI at the platelet membrane in PPE mice compared to sham-operated controls. The phosphatidylserine (PS) exposure analysis revealed enhanced procoagulant activity of platelets at different time points. First data showed reduced aneurysm progression in GPVI KO mice. Interestingly, GPVI deficient platelets showed reduced expression of P-selectin after stimulation with different agonist as well as reduced PS exposure 28 days after AAA. Histological sections of the aortic wall showed an accumulation of platelet-RBC- aggregates. These results of PPE-operated mice were supported by findings from human AAA samples from our biobank.

Conclusion: In summary, our results underline the critical involvement of platelets in the pathology of AAA and ILT formation.

ABS06 - ID 245

PLATELET APPR AS A BIOMARKER OF COGNITIVE STATUS IN PATIENTS DIAGNOSED WITH ALZHEIMER'S DISEASE

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Background/Aims: Neurons and platelets show common metabolic pathways and numerous studies have demonstrated the usefulness of monitoring brain disorders through platelet analysis. In this framework, the effects of a cognitive training program on patients affected by mild or moderate Alzheimer's disease (AD) were evaluated by neuropsychological/cognitive testing and ratio between two platelet isoforms of the amyloid precursor protein (APPr).

Materials and Methods: Participants were 59 patients with early AD enrolled in "My Mind Project" (Grant No. 154/GR-2009-1584108) and randomly assigned to control or trained group. Control group received 2 months of general psychoeducational training, whereas trained group underwent 2 months of comprehensive cognitive intervention. Cognitive and neuropsychological performance assessments as well as blood collection were performed at baseline, at the end of the psychoeducational training/cognitive intervention (FU1), and then at 6 (FU2) and 24 months (FU3). Platelets were separated from blood samples and membrane proteins were detected by Western blotting. The ratio between APP upper band (120–130 kDa) and APP lower band (110 kDa) was determined by image analysis.

Results: Some memory and attention tests, including forward verbal span, backward verbal span, prose memory, and the attentive matrices were significantly improved in trained vs. control patients at FU1 and FU2 compared to baseline (Δ values). At FU3, APPr and Mini Mental State Examination (MMSE) scores decreased in trained patients. Δ APPr correlated with the Δ scores of (i) MMSE at FU1, (ii) prose memory test at FU2, and (iii) Instrumental Activities of Daily Living (IADL), semantic word fluency test, Clinical Dementia Rating (CDR), and attentive matrices test at FU3, and all of these associations were found in the control group.

Conclusions: Our data demonstrate that platelet APPr correlates with key clinical variables suggesting it could be a peripheral biomarker of brain functioning in AD.

ABS07 - ID 261

THE EFFECT OF REELIN ON PLATELET-MEDIATED AB40-FIBRIL FORMATION

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Background/Aims: Alzheimer's disease (AD) is an age-dependent, progressive neurodegenerative disease leading to a cognitive decline without available therapy to this date. AD brain parenchyma is characterized by neurofibrillar tangles of hyperphosphorylated tau and accumulation of amyloid-β (Aβ), which is also found in cerebral vasculature of about 90 % of the Alzheimer patients, known as cerebral amyloid angiopathy (CAA). Platelets modulate synthetical Aβ into fibrillary Aβ in vitro and could be a main source of circulating Aβ in plasma. Moreover, platelets interact with reelin via glycoprotein Ib (GPIb), GPVI and amyloid precursor protein (APP). Reelin is an extracellular matrix glycoprotein not only in neuronal tissue, but also in blood plasma and platelet alpha granules. Reelin is important for neuronal development and plasticity by controlling tau hyperphosphorylation and neuronal cytoskeleton stability. Studies showed reelin as protective in Aβ42-fibril formation. The aim of this study is to investigate the impact of platelet reelin on Aβ40-fibril formation in vitro.

Material and Methods: Platelet culture experiments with murine platelets.

Results: Incubation of Aß with recombinant reelin showed an enhanced Aß-fibril formation compared to mock controls. To the contrary, in the presence of reelin a decreased Aβ-fibril formation was found in the platelet culture. In immunofluorescence microscopy reelin could be shown to co-localize with Aβ and Fibrinogen in platelet culture experiments.

Aβ-fibril formation in wildtype (WT) and reelin-deficient platelet culture was comparable. However, the addition of recombinant reelin to Aβ-stimulated WT platelets resulted in a reduced platelet aggregation. Using low-dose GPVI stimulated platelets, an enhanced fibril formation could be detected in reelin-deficient platelet culture with Aβ. Furthermore, reelin-deficient platelets were shown to release a reduced amount of fibrinogen in co-stimulation experiments with low dose CRP and Aβ.

Conclusions: Taken together these data indicate that extracellular reelin might have a protective effect on platelet-mediated Aß fibril formation.

ABS08 - ID 251

ROLE OF THE PROLINE-RICH TYROSINE KINASE PYK2 IN PLATELET ACTIVATION IN A MURINE MODEL OF ENDOTOXEMIA

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Background/Aim: Endotoxemia induced by lipopolysaccharide (LPS) in mice leads to inflammation, thrombosis, and sepsis. It is accompanied by hypercoagulability, increased platelet and leukocyte activation, platelet-leukocyte/neutrophil aggregate (PLA/PNA) formation, and disseminated intravascular coagulation[1]. Many different kinases regulate platelet activation and leukocyte interaction during thromboinflammation. The Proline-Rich Tyrosine Kinase Pyk2 is a non-receptor tyrosine kinase highly expressed in platelets where it controls adhesion, activation, aggregation, and thrombus formation[2,3].

In this work, the role of Pyk2 in platelet and neutrophil activation and platelet interaction with leukocytes was investigated in a murine model of endotoxemia.

Materials and Methods: LPS (Escherichia coli, O111:B4) was injected to induce endotoxemia in WT and Pyk2-KO mice. After four hours, vital parameters were checked, and blood was collected. PLA and PNA formation, platelet integrin $\alpha_{IIb}\beta_3$ activation, P-selectin expression and neutrophil $\alpha_M\beta_2$ activation were analysed by flow cytometry. Phosphorylation of signaling proteins was analysed ex vivo on isolated platelets in immunoblotting and aggregation was monitored in a Born lumiaggregometer.

Results: LPS-injected Pyk2-KO mice show a reduced disease score, but more severe thrombocytopenia compared to WT mice. LPS injected Pyk2-KO mice showed a lower number of circulating PLA and PNA as a consequence of a reduced P-selectin exposure on platelets in stimulated condition. LPS injection increases phosphorylation of MAP Kinases and Akt induced by thrombin receptor activating peptide in WT isolated platelets, which is completely abolished in Pyk2-KO platelets. No differences in term of aggregation were observed in the absence of Pyk2. Platelet $\alpha_{IIb}\beta_3$ and neutrophil $\alpha_M\beta_2$ activation either in resting and activated conditions were comparable in WT and Pyk2-KO mice.

Conclusions: Our results demonstrate that Pyk2 regulates MAPK Kinases and Akt phosphorylation, P-selectin expression in platelets and the interaction of platelets with leukocytes in a mouse model of LPS-induced endotoxemia, thus modulating the first trigger of thromboinflammation.

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AUTHORS INDEX