

**1995 MINUTES
PLATELET PHYSIOLOGY SUBCOMMITTEE**

Saturday, 10 June, 1995, 8.00 - 12.50

Chair: J.L. McGregor, France
Co-Chairs: C. Cerletti, Italy; K.J. Clemetson,
Switzerland; Gil White (USA);
Peter Newman (USA)

The platelet physiology subcommittee meeting in Jerusalem was divided into two parts which ran approximately for two hours (part I) and two hours and 20 minutes (part II). The meeting was attended by over 200 scientists.

Part I: Methods, standards and important practical issues resulting from platelet-leukocyte interactions. The first speaker reviewed the role of P-selectin, expressed by activated platelets, in inducing tissue factor (TF) expression by monocytes. This induction could be observed, albeit at a slightly reduced level, with purified P-selectin as well as the protein when expressed by CHO cells. A cofactor is thought, by some groups, to be present with P-selectin on platelets to induce higher levels of TF expression. Platelet chemokines, as well as some of their receptors, were then reviewed by the second speaker who showed the importance of chemokines (CXC, CC, SC) at sites of aggregation, injury, inflammation and neovascularization. A third speaker described an assay to evaluate the effect, on platelet rich plasma (PRP), of supernatants obtained from platelet-neutrophil interactions.

Results show that neutrophils inhibit platelets recruitment and 5 HT release. Such an assay may be used in characterizing cell-cell interactions and detect new aspects of pre- thrombotic states. However, observations from this assay may not always parallel those seen in whole blood. Platelet-neutrophil interactions were also investigated by another speaker through the use of flow cytometry techniques. Such cell-cell interactions were observed to be greatly reduced by monoclonal antibodies directed against P-selectin and CD18 but not other adhesion molecules present on platelets or leukocytes. The fifth author observed that large number of platelets (85%) adhere to extracellular matrix proteins (ECM) (at a shear rate of 1600 s⁻¹) compared to platelets (10-20%) adhering to HUVEC in a perfusion chamber. Neutrophils in such a set-up massively bound to adhering platelets. In such a system the authors quantitated rolling and adherence of leukocytes. The round table discussion showed that one of the components, possibly implicated with P-selectin in tissue factor expression by monocytes, was 12 HETE. The possibility of platelet GPIIb-IIIa, fibrinogen or fibrin being implicated, as well as CD11/CD18, in platelet-leukocyte interactions was discussed. The session showed the growing importance of platelet adhesion molecules, cytokines and metabolites in mediating platelet-leukocyte interactions. A large number of techniques are being set up to investigate such cell-cell interactions.

Part II: Cytoskeleton interaction in platelets. The first speaker reviewed platelet adhesion molecules and cytoskeleton interactions. Assay systems to study such molecules and cytoskeleton were described. Other speakers presented, using flow cytometry and/or electron microscopy, the effect of thrombin and other agonists on GPIb-IX movement. Techniques used

involved the use of a panel of monoclonal antibodies directed against different components of GPIb-IX-V, polyclonal antibodies to different domains of GPIb alpha as well as the use of permeabilized platelets. Differences in results were observed between two different groups with one observing a down regulation and the other seeing no differences in GPIb-IX expression. Conceivably, differences could come as a result of assay conditions (such as calcium concentrations), type of polyclonal or monoclonal antibodies used, concentration of fixative. Standardization of techniques used in the different laboratories and exchange of results could clearly help in resolving key issues.

The platelet physiology subcommittee is strongly interested in establishing an Internet network to allow members to select topics for future meetings, set up a forum to discuss issues of interest, allow the exchange of probes, antibodies and techniques. Subcommittee members strongly recommend that the ISTH support an effort to establish an ISTH "home page" on the World Wide Web (WWW).

1996 MINUTES

PLATELET PHYSIOLOGY SUBCOMMITTEE

Sunday, 23 June, 1996, 13.00 - 17.30

Room Rossini, Fira Palace Hotel

Barcelona, Spain

Chair: John L. McGregor, France

Co-Chairs: E. Angles-Cano, France; K. J. Clemetson, Switzerland

The platelet physiology subcommittee meeting in Barcelona was divided into two parts which ran respectively for one hour and forty-five minutes (part I) and two hours and 30 minutes (part II). The number of people attending this subcommittee meeting was estimated as between 70 to 80.

Part I: Platelet Physiology in Fibrinolysis (Co-Chair: E. Angles-Cano, France). The first speaker (Lindsey Miles, USA) reviewed new developments and frontiers on plasminogen binding and activation on platelets. The presence and the assembly of a number of molecules present on platelets and implicated in fibrinolysis was discussed. A new atherogenic and thrombogenic connection, between lipoprotein (a) and platelets was then reviewed by the second speaker (Eduardo Angles-Cano, France). The speaker showed the role of LP(a) in coronary heart disease. LP(a) was shown to bind to fibrin, extracellular matrix proteins and to a number of cells such as endothelial cells, mononuclear cells and activated platelets. Nuala Booth (UK) talked about platelets as fibrinolytic inhibitors and the role of PAI-1. Platelets were shown to contain small amounts of a-antiplasmin and PAI-1 is the major inhibitor of fibrinolysis in platelets.

Part II: New Platelet Inhibitors and Inhibitor Targets (K.J. Clemetson, Switzerland). Giovanni de Gaetano (Italy) reviewed the benefits and problems of new platelet inhibitors. The clinical efficacy of present drugs suggests either the search for new drugs and/or the use of available drugs in combination (for e.g. aspirin + ticlopedine). The next speaker (Graham Jamieson, USA) clearly showed the presence of a platelet high-affinity thrombin receptor, in addition to the cloned moderate affinity thrombin receptor, on the platelet surface. He presented new data on the platelet high-affinity thrombin receptor through the use of blocking monoclonal antibody and proteolysis studies. Graham Jamieson made the following suggestions to the physiology subcommittee: (1) Use highly purified a-thrombin (greater than 3000 U/mg) of stated specific activity; (2) Use of physiological relevant range of a-thrombin concentration (0.2U/ml); (3) Avoid the self-fulfilling prophecy. The third speaker (Lina Badimon, Spain) showed the platelet response to different types of ruptured plaque. She then presented the use of different platelet inhibitors, using blood that was not anticoagulated in contact with human vessel walls bearing atherogenic plaques, in a flow chamber at different shear rates. The last speaker (Mike Barnes, Cambridge) showed the presence of a new collagen receptor, not yet identified, that plays an important role in platelet activation by collagen. He used a series of synthetic peptides, derived from collagen, to indicate the presence of this new collagen receptor.

The audience indicated its approval for the introduction of a platelet bleeding disorder patient directory. Such a directory will be initiated with the help of scientists on both sides of the Atlantic. Dr. Debbie French from Prof. B. Coller's laboratory (Mt. Sinai, Hospital, NY) has already indicated her willingness to set up such a registry for Glanzmann's thrombasthnia patients.

Homepage address (<http://laennec2.univ-lyon1.fr/SCIENTIFIQUE/PLATELET/physiology.html>). This will provide a forum to discuss platelet physiology topics and to prepare future meetings. It will also contain a registry of genetic platelet bleeding disorders and schedule and reports of present and past meeting. All suggestions are welcome (mailbox: platelet@laennec.univ-lyon1.fr).

1997 MINUTES

Platelet Physiology SUBCOMMITTEE

Friday, 6 June, 1997, 13:00-17:00

Boticelli, Fortezza da Basso

Florence, Italy

Chair: J.L. McGregor, France

Co-Chairs: E. Angles-Cano, France; M. Berndt, Australia; C. Cerletti, Italy;
K. Clemetson, Switzerland; P. Newman, USA; G.C. White, USA

The platelet physiology subcommittee meeting in Florence was divided into three major parts. The two first parts ran for approximately two hours and the last part for one hour and 30 minutes. The number of people attending this subcommittee meeting was estimated to be 200.

Part I. Bioinformatics: Internet genomic registry of platelet congenital disorders (Co-Chair: J.L. McGregor, France).

The first speaker, Deborah French (USA) (dfrench@smtpink.mssm.edu), introduced the role of GPIIb/IIIa in platelet functions and the phenotyping of patients suffering from such a disorder. She did an excellent survey in putting together a registry (<http://scripps.edu/bcmd>) on point mutations, deletions and other defects, identified on Glanzmann thrombasthenic patients (21 individuals with GPIIb and 19 with GPIIIa defects) by their laboratory and workers in the field. She indicated the relevance of such work in prenatal diagnosis (countries where this was performed) and carrier detection. The second speaker, Alan Nurden (France), gave a very good review on the foundations that are necessary for setting up a strong registry on platelet genetic disorders. Points of importance raised by Dr. Nurden include: (1) What should go in the registry (clinical, biochemical and molecular information)? (2) Authenticity (reviewing the experimental data before insertion in the registry?) (3) Priority (what will be the attitude of journals toward the part of manuscripts made public via the Internet?) (4) New reports of previously encountered mutations, deletions, inserts, potential hotspots, etc. (5) How should the data be organized? Previous nomenclature, while useful, is outdated. (6) Who runs the database? Mount Sinai could be one of the centers with Dr. French being the database manager responsible for reviewing data and submissions. The third speaker, Kenneth Clemetson (Switzerland), gave a brief update on the possibility of setting up a registry for patients in France, Germany, Switzerland, Finland, the UK, etc., with the Bernard Soulier syndrome. He was followed by Dr. Satu Kaski (Prof. Kekomaki's laboratory, Finland) who presented data (three distinct types of mutation or deletion) on the Finnish Bernard Soulier (15 families, 22 patients in a population of five million). The fourth speaker, Dr. Dermot Kenny, presented a survey of Bernard Soulier syndrome in the Midwest region of the USA. The fifth speaker, Dr. Kenjiro Tanoue, (Japan), presented a survey of the Bernard Soulier population in Japan.

Part II. Characterization and standardization of the giant platelet syndromes (Co-Chair: Gilbert White, USA).

An attempt was made by the three speakers (Andreas Greinacher, Jim White and Paquita Nurden), after an introduction by the co-chair, to look at different giant platelet abnormalities (e.g., May-Hegglin, Fechtner syndrome, Mediterranean macrothrombocytopenia, Gray platelet syndrome, Medich inclusion disorder, Gainsville giant platelet disorder, Alport's syndrome, Montreal platelet syndrome, Chediak-Higashi syndrome) that can come under the classification of giant platelet syndrome. The co-chair stressed, in complete agreement with the speakers, that this is an area that requires further research in molecular medicine. Phenotypes of these patients should also be made available on the Internet to allow centers to have information on these anomalies.

Part III. Standardization in signal transduction measurements in platelets and quality control (Co-Chair: Kenneth Clemetson, Switzerland).

The first speaker, Koneti Rao (USA), gave an extensive review on platelet secretion disorders. He pointed out that many platelet secretion disorders were lumped together more out of convenience than on the basis of the mechanisms underlying the dysfunction. The second speaker, Gerard Mauco (France), presented the technical basis for inositol lipid metabolism. The third speaker, Jan Akkerman (Holland), gave an excellent review on quality control of platelet functional defects.

It will be suggested to the co-chairs of the three parts of this platelet physiology subcommittee to present a report, in view of the importance of the treated subjects, for publication (following acceptance by the publication review committee) in *Thrombosis and Haemostasis*.

1998 MINUTES
Platelet Physiology SUBCOMMITTEE
Sunday, 21 June, 1998, 8:00-12:00
Cankarev Dom
Ljubljana, Slovenia
Chair: M. Berndt, Australia;
Co-Chairs: C. Cerletti, Italy; H. Deckmyn, Belgium;
M. Hoffman, USA; J. L. McGregor, France; P. Newman, USA;
A. K. Rao, USA; G. C. White, USA

The Committee met under the chairmanship of Dr. M.C. Berndt (Australia) with co-chairmen, Dr. G.C. White (USA), Dr. K. Rao (USA), and Dr. H. Deckmyn (Belgium) also attending. Approximately 40 delegates attended the session. The subcommittee presented a final programme primarily covering recent basic and clinical insights into agonist-dependent signal transduction in platelets.

Dr. Koneti Rao spoke on the topic "Disorders of platelet activation" within a context of a proposed classification of congenital disorders of platelet function. He described several patients with abnormalities of platelet aggregation and emphasized the need for a detailed classification for those patients with disorders of platelet secretion and signal transduction. Analysis of these patients, one with low levels of Gαq and the other with defective pleckstrin phosphorylation, have provided important insights into signal transduction pathways induced by ADP and thrombin.

Dr. Michael Barnes described how studies from his laboratory with synthetic collagen sequence peptides have helped clarify the mechanisms by which platelets adhere to collagen and become activated. His studies have indicated that platelets primarily adhere to collagen via the integrin α2β1, but are activated via GPVI. Different sequences in collagen are involved in both these events.

Dr. Steve Watson further developed this theme in an elegant presentation describing research from his laboratory on collagen-dependent signaling pathways. These studies have made collagen-induced platelet activation one of the best understood signaling pathways and illustrated the value of employing multiple approaches in studies of signal transduction.

Dr. Michael Berndt presented a brief overview of genetic abnormalities in Bernard-Soulier syndrome and presented some recent data on structure-function of the GP Ib-IX-V complex using canine-human chimeras. A tabulation of the clinical reports and genotype of all reported Bernard-Soulier syndrome patients was presented as a framework for establishment of a Bernard-Soulier register and web site. There was discussion as to how new information/patients could be added. There was agreement by the subcommittee that the register and web site should proceed and that it would compliment the Glansmann's thrombosthemia register and web site.

The meeting closed with discussion of potential programme topics for the SSC meeting in Washington. There was general agreement that a session on evaluation of GP IIb-IIIa receptor function in the light of oral antagonists would represent an important topic. This and other

potential areas for subcommittee evaluation will be further considered in the coming months. There was a general view that earlier announcement of the programme would be valuable for attendees.

1999 MINUTES

Platelet Physiology

Saturday, 14 August 1999

1:00 to 5:00 PM

Room 38

Washington Convention Center

Washington, DC

Chair: M. Berndt, Australia;

Co-Chairs: C. Cerletti, Italy; M. Hoffman, USA; P.J. Newman, USA;

A. K. Rao, USA; G. C. White, USA

On Saturday afternoon, August 14th, the Platelet Physiology Subcommittee met to discuss the issue of "Assessment of GP IIb-IIIa Receptor Occupancy and Function during Therapy with GP IIb-IIIa Antagonists." The session was chaired by P.J. Newman, L.K. Jennings, and M. Hoffman. More than 300 attendees participated in the program.

Dr. Lisa Jennings opened the program with an overview of the biology of GP IIb-IIIa, the mechanism of action of the different antagonists, and the different pharmacokinetics of the currently available, FDA approved GP IIb-IIIa antagonists. She also reviewed the various oral GP IIb-IIIa antagonists remaining in phase trial. An issue emphasized by Dr. Jennings was the importance of choice of anticoagulant in sample preparation since citrate by chelating calcium alters the apparent receptor occupancy of the antagonist relative to native blood.

Dr. Jennings' presentation was followed by fine presentations describing approaches and instrumentation for assessment of GP IIb-IIIa occupancy and function. Dr. Alan Michelson outlined the use of whole blood flow cytometric approaches for assessment of occupancy and functional response. An advantage of flow cytometry is that only small volumes of blood need be analyzed. Potential disadvantages included the cost of instrumentation and the requirement for a highly trained and dedicated operator. Care also is required to minimize dilutional effects that would lead to dissociation of agonist from receptor leading to underestimation of receptor occupancy. Dr. David Varon described use of a prototype research cone and plate analyzer for rapid assessment of platelet function. The method involves image analysis to quantitate platelet adhesion to polystyrene from whole blood and is sensitive to plasma levels of von Willebrand factor, fibrinogen, and to GP IIb-IIIa occupancy by antagonists. The next three speakers described the use of commercially available instrumentation for assessment of GP IIb-IIIa occupancy. Dr. Robert Hillman from Accumetrics discussed the Ultergra™ RPF system for bedside assessment of oral and intravenous GP IIb-IIIa antagonists. Dr. Doug Christie from Dade

Behring discussed use of a high shear system, the PFA-100, for evaluation of platelet dysfunction in patients receiving GP IIb-IIIa antagonists. Finally, Dr. Bruce Lages of Xylum Corporation presented data using the Xylum Clot Signature Analyzer. All three instruments would appear to provide appropriate methodology for assessment of GP IIb-IIIa receptor occupancy and function that would be useful depending on the clinical and research context.

Dr. Koneti Rao discussed the impact of GP IIb-IIIa antagonists on thrombin generation. He provided data from *in vivo* assessment in primates for a potential anticoagulant effect of GP IIb-IIIa antagonists on top of that provided by heparin during coronary procedures. Dr. Dick Aster discussed the issue of thrombocytopenia associated with the use of GP IIb-IIIa inhibitors. The incidence of moderate to severe thrombocytopenia reported in the literature associated with the use of GP IIb-IIIa receptor antagonists varies between one and five percent. Dr. Aster presented preliminary data that the thrombocytopenia occurs by an immune mechanism involving circulating pre-existing antibody but that the epitopes recognized by the antibodies may be different depending upon the receptor antagonist that binds to GPIIb-IIIa. Finally, Dr. Barry Collier gave an elegant presentation describing when, where, and why GP IIb-IIIa receptor antagonists need to be monitored in the context of either acute or chronic therapy. It is hoped that Drs. Jennings and Collier will work with the subcommittee to prepare a discussion document on this topic for publication as a subcommittee report.

PLATELET PHYSIOLOGY

15 June 2000

13:30 to 17:30

Room 0.8

Maastricht Meeting and Convention Center

Chairman: M.C. Berndt--Australia

**Co-chairmen: C. Cerletti--Italy; M. Hoffman--USA; P.J. Newman--USA;
P. Nurden--France; A.K. Rao--USA; S. WatsonUK**

Tremendous strides have been made in our understanding of the aberrant platelet mechanisms in some groups of patients with inherited platelet dysfunction. However, in a substantial number of such patients, the specific abnormal mechanisms are unknown. There is convincing information that at least some of these patients have defects in the early events of platelet activation/signal transduction. The theme of the present session was to review aspects of normal platelet signaling mechanisms and evidence for specific abnormalities in signal transduction events with the overall goals: a) to develop approaches applicable in defining the molecular mechanisms in patients with impaired platelet function; and b) to foster a collaborative approach involving a network of investigators with the required expertise.

Dr. C. Gachet, S. Levy-Toledano and M. Jandrot-Perrus reviewed the existing information on platelet ADP, thromboxane A₂ and collagen (GPVI) receptors respectively. Dr. J-W Akkerman reviewed the information on protein kinase C. Each of the speakers reviewed data from available specific knock-out mouse models as well. The second part of the session focussed on defects in patients with impaired platelet function. Dr. A.K. Rao provided an overview of the platelet signal transduction defects with specific emphasis on deficiencies of platelet phospholipase C- β 2 and G α _q. Drs. P. Nurden, M. Cattaneo and M. Hoylaerts described their studies on defects at the level of the ADP receptors. Drs. S. Watson and K. Clemetson presented information on abnormalities in platelet responses to collagen and in the involved receptors. Dr. C. van Geet presented studies on an inherited defect in G α _s hyperfunction.

Following presentations, there was an extensive discussion regarding the approaches needed to delineate the platelet mechanisms in the larger group of patients with abnormal aggregation responses and impaired hemostasis. It was concluded a working group be established on inherited platelet signal transduction defects that would evaluate the methods and develop guidelines regarding the laboratory studies needed to define the abnormal mechanisms in these patients. This group would address issues such as the specific platelet responses to be studied and the agonists involved. There was also a discussion regarding the need for a data base/registry and a Web site focussing on patients (and mouse knock-out models) with signal transduction defects and the investigators involved in these studies. It was concluded that a working group be established to address this. Other points of discussion included the need for assessing platelet adhesion and the importance of studying flow-related aspects of platelet function in such patients.

PLATELET PHYSIOLOGY

**6 July 2001
13:00 to 17:00
Room 252
Palais des Congrès**

Chairman: A.K. Rao--USA

Co-chairmen: M.C. Berndt--Australia; C. Cerletti--Italy; M. Hoffman--USA;
A.D. Michelson--USA; P.J. Newman--USA; P. Nurden--France; S. Watson--UK

The main theme of this year's session was platelet signaling and signal transduction defects. This was a continuation of the discussions initiated at the last SSC meeting in Maastricht. Drs. Deborah French and Dermott Kenny presented the reports on the existing databases on Glanzmann thrombasthenia and Bernard Soulier Syndrome (BSS), respectively. Both of the databases are running effectively. Dr. French indicated that all of the submissions were patients published in the literature. Dr. Kenny reported that the BSS web site had 1386 visitors so far. Dr. French agreed to update and incorporate a classification of thrombasthenia on the web site. Dr. Steve Watson discussed the ongoing efforts for establishing a database that covers platelet signaling and signal transduction defects. The main stumbling block is the need for financial support for setting up the database and this is still unresolved.

Several speakers reviewed the current information available with respect to different aspects of platelet signaling and information from specific knockout models and human platelet defects. Dr. John Hartwig reviewed aspects related to signaling and shape change. Dr. Guy Reed reviewed signaling mechanisms governing platelet secretion. At the receptor level, Dr. Athan Kuliopulos provided information on platelet thrombin receptors. Dr. Lawrence Brass described studies in mouse knock out models of $G_{\alpha i}$ family, including $G_{\alpha Z}$. Dr. Jean-Max Pasquet reviewed the signaling pathways involving tyrosine kinase/phosphatase pathways. Dr. Hidehiko Saito presented recently described mutations in non-muscle myosin heavy chain A in patients with May-Hegglin anomaly. Lastly, Dr. A. Koneti Rao summarized recent studies in patients with phospholipase C- $\beta 2$ and $G_{\alpha q}$ deficiency with respect to thrombin-induced responses.

At the end of the meeting there was a discussion regarding the potential topics for the next meeting of the SSC in Boston in 2002. In addition, there were discussions regarding the working group on platelet signal transduction defects and about the areas that it should address. These were extensive discussions on the need for developing guidelines on specific methods widely used to study platelets. The specific areas that were included: 1) preparation of human platelets for studies, 2) preparation of mouse platelets, 3) platelet aggregation, 4) shape change, 5) flow cytometry, and 6) adhesion. In addition it was felt that a working party on platelet signal transduction defects should address the issues regarding the laboratory evaluation of patients with platelet function defects. Dr. James Bussell announced to the Platelet Subcommittee regarding a registry being established on patients with non-immune thrombocytopenia to facilitate studies on the molecular basis of the platelet abnormality.

Platelet Physiology

July 19, 2002

09:00 to 13:00

Terrace Room

Boston Park Plaza Hotel

Chairman: A. Koneti Rao, USA

Co-chairs: M. Berndt, Australia; C. Cerletti, Italy; C. Hayward, Canada; M. Hoffman, USA; A. Michelson, USA;

P. Newman, USA; P. Nurden, France; S. Watson, UK

There were two main themes addressed in this meeting of the Platelet Physiology Subcommittee. The first one was platelet-leukocyte interactions, an area where tremendous new information has become available over the last few years. The second theme was platelet function disorders with a focus on a specific methodology (Platelet Function Analyzer, PFA-100).

Platelet-Leukocyte Interactions

Several speakers reviewed recent information on different aspects of platelet-leukocyte interactions. Dr. Bruce Furie reviewed interactions between P-selectin and PSGL-1. Dr. Jose Lopez reviewed the interactions between GPIb and MAC-1. Dr. Chiara Cerletti focused on the signaling interactions between platelets and leucocytes with a focus on Src kinases. Dr. Michael Berndt reviewed the regulation of P-selectin binding to PSGL-1 by elastase and cathepsin G. Lastly, Dr. Alan Michelson reviewed recent information on circulating monocyte-platelet aggregates as a sensitive marker of in vivo platelet activation in patients.

Working Group on Platelet Function Disorders

The second half of the session focused on presentations by the Working Group on Platelet Function Disorders. Despite major advances in our understanding of platelet physiology and available newer methods, our understanding of the mechanisms in patients with platelet function disorders remains low. In the vast majority of patients with inherited abnormalities in platelet responses, the underlying mechanisms leading to the platelet dysfunction are unknown. The focus of this session was on the role of PFA-100 in the diagnosis of platelet function disorders, excluding von Willebrand disease. PFA-100 has become widely available and is being used in the evaluation and management of patients with vWD and platelet function disorders.

Dr. Catherine Hayward presented information on developing evidence-based approaches to the diagnosis of platelet disorders. She presented information from recent ongoing studies in her laboratory, including the Clinical History Assessment Tool (CHAT). Several speakers addressed

various aspects of PFA-100 in the diagnosis of platelet function disorders (excluding von Willebrand disease): Drs. Diane Nugent, Marco Cattaneo, Thomas Ortel, Berndt Jilma and Paul Harrison. The information presented included studies with the PFA-100 in the general group of patients referred for the evaluation of bleeding disorders, patients with menorrhagia, and those on platelet inhibitory drugs. Following these presentations, there was a discussion regarding the advantages and limitations of PFA-100. It was also felt that the working group on platelet function disorders should continue to explore the need for setting up collaborations between various laboratories with relevant expertise to define the underlying mechanisms in patients with inherited disorders of platelet function.

Dr. James Bussell presented information on the ongoing studies and on the registry of patients with non-immune thrombocytopenias.

Platelet Physiology

July 12, 2003

14:00 to 18:00

Hall 9

The International Convention Center, Birmingham

Chairman: A. Koneti Rao, USA

Co-chairs: M. Berndt, Australia; M. Cattaneo, Italy; C. Cerletti, Italy; C. Hayward, Canada; J. López, USA; A. Michelson, USA; P. Nurden, France; S. Watson, UK

The platelet subcommittee discussed three main themes at this meeting. The first theme was in the area of application of expression profiling and proteomics to the study of platelets. The second theme was on the molecular mechanisms in defective platelet production and congenital thrombocytopenias, and the third set of discussions focused on GPIIb-IIIa antagonists and their impact on activation of the integrin complex.

I. Genomics, Proteomics: Methodology and Relevance.

As a part of this session, Dr. Wadi Bahou summarized recent information on the application of expression profiling to human platelets. Dr. Steve Watson summarized recent studies on the platelet proteome. These presentations and the subsequent discussions focused on the methodology, their limitations when applied to platelets, and relevance of these techniques to the study of platelets. Both speakers emphasized the need for additional studies to define the optimal ways to apply these techniques to study platelets.

II. Molecular Mechanisms in Defective Platelet Production and Congenital Thrombocytopenias. (Working Group on Platelet Function Disorders)

This part of the session had five speakers. The goal of these presentations was to summarize recent information on the molecular and genetic mechanisms in the areas of platelet production and congenital thrombocytopenias. Dr. Ramesh Shivdasani presented new information obtained from the various knock-out models, including for NF-E2 and tubulin 1. Dr. Andrew Leavitt presented studies on the regulation of GPIIb-IIIa function and the interactions with caspase-12 and transcription factor NF-E2. Dr. A. Koneti Rao presented new evidence in human platelets on a mutation in transcription factor CBFA2 and its association with impaired activation of GPIIb-IIIa and platelet PKC-theta deficiency. Dr. Carlo Balduini presented evidence supporting a unifying theme for MYH-9 gene mutations in congenital thrombocytopenias, encompassing the May Hegglin anomaly, the Fechtner syndrome, the Epstein syndrome and others. Lastly, Dr. Jim Bussel described the current activity with regards to the development of a Registry of Non-immune Thrombocytopenias. He invited members to participate in the Registry and described the information being collected along with the procedures. The sub-committee endorsed this

effort.

III. GPIIb-IIIa Antagonists and Activation of Integrin Complex: Evidence and Clinical Relevance.

Evidence from previous studies have suggested that GPIIb-IIIa antagonists may lead to conformational changes in the complex and to its activation. However, the biological and clinical significance is controversial. Four speakers presented evidence and different aspects related to this theme. Drs. Jose Lopez, A. Lawrence Frelinger, Paquita Nurden, and Stan Heptinstall presented their studies on this issue and there was a discussion regarding the data presented and their significance. It was recognized that additional studies were needed to define the clinical relevance of the observations presented.

Platelet Physiology

June 18, 2004

8:30 to 12:30

Cipressi Room

Fondazione Giorgio Cini

Chairman: A. Koneti Rao, USA

Co-chairs: M. Cattaneo, Italy; C. Cerletti, Italy; C. Hayward, Canada; J. Lopez, USA;
A. Michelson, USA; D. Nugent, USA; P. Nurden, France; S. Watson, UK

The Platelet Physiology Subcommittee discussed aspirin resistance as the major topic at this year's session. Although there are a large number of publications on various aspects of aspirin resistance, there does not appear to be a well-defined definition of this entity. Moreover, issues such as clinical relevance and biochemical mechanisms are far from clarified. In the first part of the subcommittee meeting, the focus was on aspirin resistance. There were five presentations. They were targeted to address the issues of definition, clinical relevance and mechanisms of aspirin resistance. Dr. Alan Michelson (USA) provided an overview of the current status, and he summarized some of the studies published to date. Dr. Kandice Kottke-Marchant (USA) discussed the studies performed at the Cleveland clinic focusing on the clinical relevance of aspirin resistance. Dr. Thomas Kunicki (USA) discussed recent studies on aspirin resistance in subjects with arterial diseases using the platelet function analyzer. Dr. Fabio Pulcinelli (Italy) discussed their studies with respect to aspirin resistance in patients with ischemic heart disease. Dr. Marco Cattaneo (Italy) discussed the issue of definition of aspirin resistance.

Following these presentations, there was a vigorous discussion on various aspects of aspirin resistance. A Working Group on Aspirin Resistance was established with Dr. Alan Michelson as the Chairperson and Dr. A. Koneti Rao as the Co-Chair. The Working Group will generate a document that summarizes the current status as well as a recommendation to be submitted for publication in Journal of Thrombosis and Haemostasis.

Clopidogrel is widely used as an antiplatelet agent with or without aspirin. Dr. Paul Gurbel (USA) summarized their studies on clopidogrel resistance.

Working Party on Congenital Platelet Function Defects

Under aegis of this Working Party on Platelet Function Analyzer, Dr. Catherine Hayward presented a report on the advantages and shortcomings of the evaluation of platelet function disorders using the Platelet Function Analyzer (PFA). In addition, she presented to the subcommittee studies on the patterns of practice in clinical laboratory testing of platelet disorders, a compilation of the results of a North American survey. This survey included approximately 50 laboratories in North America involved in platelet function testing using the optical aggregometry.

As a follow-up from last year's meeting of the Working Group on Platelet Function Defects, Dr. Alan Nurden presented his efforts in establishing a network for studying patients with inherited disorders of platelet function in France, as a model for others to follow.

Congenital Thrombocytopenia Registry

This registry was intended to encompass patients with non-immune thrombocytopenias. Dr. Bussel presented his efforts in establishing an ongoing registry on patients with type II von Willebrand disease.

Working Party on Platelet Genomics and Proteomics

Dr. Steve Watson presented a report on behalf of this Working Party, summarizing aspects of the potential and the pitfalls in the application of proteomics to platelets. He summarized the contents of a document prepared for eventual submission for publication in the Journal of Thrombosis and Haemostasis.

Platelet Physiology

7 August 2005

8:30 to 12:00

**Tumbalong Meeting Room
Sydney Convention and Exhibition Centre**

Chairman: A. Michelson, USA

Co-chairs: J. Bennett, USA; M. Cattaneo, Italy; C. Cerletti, Italy; C. Hayward, Canada; D. Kenny, Ireland;

J. Lopez, USA; D. Nugent, USA; P. Nurden, France; A. Koneti Rao, USA; S. Watson, UK

This year's Platelet Physiology Subcommittee Program in Sydney Australia was divided into 3 parts:

1. Aspirin and Clopidogrel Resistance (co-chairs Alan Michelson and Marco Cattaneo)

Dr. Alan Michelson (U.S.A.) spoke on "*Platelet Response Variability to Aspirin And Clopidogrel*" and Dr. Diane Nugent (U.S.A.) spoke on "*Evaluation Of Platelet Response To Aspirin And Clopidogrel*". A general discussion followed. The Working Party on Aspirin Resistance (Chair: Alan Michelson) has just published (June 2005) an official communication of the SSC: Michelson AD, Cattaneo M, Eikelboom JW, Gurbel P, Kottke-Marchant K, Kunicki TJ, Pulcinelli M, Cerletti C, Rao AK. Aspirin Resistance: position paper of the Working Group on Aspirin Resistance. *Journal of Thrombosis and Haemostasis* 2005;3:1309-1311. A future official communication of the SSC on the subject of clopidogrel "resistance" is planned.

2. Inherited Thrombocytopenias (co-chairs Alan Michelson and Paquita Nurden)

Dr. Carlo Balduini (Italy) spoke on "Classification Of Inherited Thrombocytopenias", Dr. Catherine Hayward (Canada) spoke on "Clinical Challenges With Assessing Inherited Platelet Disorders Associated With Thrombocytopenia", and Dr. Amy Geddis (U.S.A.) spoke on "A Registry And Shared Resource For Congenital Thrombocytopenias". A general discussion followed. Drs. Amy Geddis and Jim Bussel have set up a Non-immune Thrombocytopenia Registry.

3. The Platelet Transcriptome (co-chairs Willem Ouwehand and Koneti Rao)

Dr. Willem Ouwehand (United Kingdom), on behalf of the Bloodomics consortium, spoke on "*Transcription Profiling In Human Megakaryocytes And Platelets With The Aim To Discover Novel Genetic Markers For The Prediction Of Thrombus Formation*", Dr. Andrew Weyrich (U.S.A.) spoke on "*Mixed Messages: Are Platelet RNAs Important for Cellular Function?*", Dr. Wadie Bahou (U.S.A.) spoke on "*Progress And Limitations Of Platelet Transcript Profiling*", Dr. Koneti Rao (U.S.A.) spoke on "*Expression Profiling In Platelet Function Disorders*", and Dr. Dermot Kenny (Ireland) spoke on "*Translating The Platelet Transcriptome*". A general discussion followed. The Working Party on the Platelet Proteome (Chair: Steve Watson) has just published an official communication of the SSC: Watson SP, Bahou WF, Fitzgerald D,

Ouwehand W, Rao AD, Leavitt AD. Mapping the platelet proteome: a report of the ISTH Platelet Physiology Subcommittee. *Journal of Thrombosis and Haemostasis* 2005; early edition online. A future official communication of the SSC on the subject of the platelet transcriptome is planned.

In addition to those listed above, the following manuscript is undergoing SSC review prior to submission to the *Journal of Thrombosis and Haemostasis* as an official communication of the SSC: Hayward CPM, Harrison P, Cattaneo M, Ortel TL, Rao AK. Closure time in the evaluation of platelet disorders and platelet function: a report of the Working Party on the PFA-100.

Working Parties of the SSC Platelet Physiology Subcommittee:

- **Platelet Proteome**
 - Chair: Steve Watson
 - Members: Wadie Bahou, Desmond Fitzgerald, Andrew Leavitt, Willem Ouwehand, Koneti Rao
- **Platelet Transcriptome**
 - Chair: Willem Ouwehand
 - Members: Wadie Bahou, Dermot Kenny, Koneti Rao, Steve Watson, Andrew Weyrich
- **Evaluation of Platelet Function Disorders**
 - Chairs: Marco Cattaneo, Cathy Hayward, Koneti Rao
 - Members: Carlo Balduino, Amy Geddis, Alan Michelson, Diane Nugent, Paquita Nurden, Koneti Rao, Steve Watson
- **Aspirin Resistance**
 - Chair: Alan Michelson
 - Members: Marco Cattaneo, Chiara Cerletti, John Eikelboom, Paul Gu rbel, Kandice Kottke-Marchant, Thomas Kunicki, Fabio M. Pulcinelli, Koneti Rao
- **Clopidogrel Resistance**
 - Chair: Alan Michelson
 - Members: Marco Cattaneo, Christian Gachet, Chiara Cerletti, Paul Gu rbel, Diane Nugent, Paquita Nurden, Koneti Rao

Registries of the SSC Platelet Physiology Subcommittee:

- **Bernard-Soulier Syndrome** (Dermot Kenny)
- **Glanzmann Thrombasthenia** (Debbie French)
- **Non-immune Thrombocytopenia** (Amy Geddis/Jim Bussel)

Alan D. Michelson, M.D.
Chair, Platelet Physiology Subcommittee, SSC/ISTH

Platelet Physiology

Chairman: A. Michelson, USA

Co-chairs: J. Bennett, USA; M. Cattaneo, Italy; C. Cerletti, Italy; C. Hayward, Canada; D. Kenny, Ireland;
J. Lopez, USA; D. Nugent, USA; P. Nurden, France; S. Watson, UK

The meeting focused on the standardization of the measurement of platelet function in patients. The topics and speakers were:

- turbidometric platelet aggregometry (Lisa Jennings, Memphis , U.S.A.)
- whole blood platelet aggregometry (Stan Heptinstall, Nottingham , U.K.)
- VerifyNow (Rob Hillman, San Diego , U.S.A.)
- platelet function analyzer (PFA)-100 (Catherine Hayward, Hamilton , Canada)
- Impact cone and plate(let) analyzer (David Varon, Jerusalem, Israel)
- flow cytometry (Alan Michelson, Worcester , U.S.A.)
- thromboxane metabolites (Paola Patrignani, Chieti , Italy)
- other methods (Paul Harrison, Oxford , U.K.)

Official publications of the SSC Platelet Physiology Subcommittee in the past year were:

- Michelson AD, Cattaneo M, Eikelboom JW, Gurbel P, Kottke-Marchant K, Kunicki TJ, Pulcinelli FM, Cerletti C, Rao AK, on behalf of the Platelet Physiology Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Aspirin resistance: position paper of the Working Group on Aspirin Resistance . *Journal of Thrombosis and Haemostasis* 2005;3:1309-1311
- Watson SP, Bahou WF, Fitzgerald D, Ouwehand W, Rao AK, Leavitt AD. Mapping the platelet proteome: a report of the ISTH Platelet Physiology Subcommittee. *Journal of Thrombosis and Haemostasis* 2005;3:2098-2101
- Hayward CPM, Harrison P, Cattaneo, M, Ortel TL, Rao AK. Platelet function analyzer (PFA)-100 closure time in the evaluation of platelet disorders and platelet function. *Journal of Thrombosis and Haemostasis* 2006;4:312-319

Registries of the SSC Platelet Physiology Subcommittee are:

- Bernard-Soulier Syndrome (Dermot Kenny)
- Glanzmann Thrombasthenia (Debbie French)
- Non-immune Thrombocytopenia (Amy Geddis/Jim Bussel)

It was resolved to form a Working Group on Platelet Aggregation for the purpose of attempting to gain consensus on how to measure platelet aggregation in the clinical laboratory.

Alan D. Michelson, M.D.
Chair, Platelet Physiology Subcommittee, SSC/ISTH

Platelet Physiology

Chair: A. Michelson (USA)

Co-Chairs: J. Bennett (USA), M. Cattaneo (Italy), P. Harrison (UK), C. Hayward (Canada), D. Kenny (Ireland), D. Nugent (USA), P. Nurden (France), S. Watson (UK)

This year's SSC Platelet Physiology Subcommittee Program was held on July 7, 2007 from 8:00 am – 12:00 pm in Geneva, Switzerland. The meeting focused on the standardization of turbidometric platelet aggregation. The topics and speakers were:

1. *The History of Turbidometric Platelet Aggregation*, Gustav Born, London, U.K.
2. *The Molecular Basis of Platelet Aggregation*, Alan Nurden, Pessac, France
3. *The Clinical Utility of the Measurement of Platelet Hypoaggregability*, Paul Harrison, Oxford, U.K.
4. *The Clinical Utility of the Measurement of Platelet Hyperaggregability*, Paul Bray, Philadelphia, U.S.A.
5. *Clinical and Laboratory Standards Institute (CLSI) Standardization of Platelet Aggregation*, Alvin Schmaier, Cleveland, U.S.A.
6. *UK National External Quality Assessment Scheme (NEQAS) Survey of Platelet Aggregation*, Ian Jennings, Sheffield, U.K.
7. *Statistical Analyses of Reference Ranges for Platelet Aggregation*, Catherine Hayward, Hamilton, Canada
8. *SSC Working Party on Platelet Aggregation Survey*, Marco Cattaneo, Milan, Italy

These speakers were followed by a general discussion on the road forward with regard to the standardization of turbidometric platelet aggregation.

Registries of the SSC Platelet Physiology Subcommittee are:

- Bernard-Soulier Syndrome (Dermot Kenny)
- Glanzmann Thrombasthenia (Debbie French)
- Non-immune Thrombocytopenia (Amy Geddis/Jim Bussel)

Alan D. Michelson, M.D.

Chair, Platelet Physiology Subcommittee, SSC/ISTH

Platelet Physiology

4 July 4
Vienna, Austria

Chair: *Marco Cattaneo, Italy*

Co-Chairs: *Joel S. Bennett, USA; Paul Harrison, UK; Catherine P. M. Hayward, Canada; Dermot Kenny, Republic of Ireland; Alan D. Michelson, USA; Diane Nugent, USA; Steve Watson, UK*

The session was mostly focused on the reports from the Working Party on Platelet Aggregation, which formed in July 2006, during the chairmanship of Alan D. Michelson.

Marco Cattaneo, Chairman of the Working Party, briefly outlined the aim and the activities of the Working Party. Aim of the Working Party is to achieve a consensus on recommendations on how to perform light transmission aggregometry in clinical and research laboratories. The first step was to organize a worldwide survey on how light transmission aggregometry is performed in real life. To this end, the Working Party prepared a questionnaire of 129 questions, with the help of experts in the field. The questionnaire was submitted online in February 2007 to ISTH members who expressed their interest to participate, and also to members of External Quality Assurance Organizations (e.g., EQAT, NASCOLA, CISMEL, NEQAS-BC and others). Analysis of the questionnaire was completed in June 2008. Catherine P.M. Hayward reported on the main results of such analysis. In total, 244 clinical laboratories and 115 research laboratories from all over the World, but more frequently from Europe (45%) and North America (34%), responded to the questionnaire. It was quite evident from the results of the survey that there is an extreme heterogeneity in the methodology that the responding laboratories follow to perform light transmission aggregometry. This finding confirms that there is an urgent need for the standardization of the technique.

The second step in the activity of the Working Party aimed at achieving a consensus on how light transmission aggregometry should be performed. In consideration of the lack of published scientific evidence, it was decided to try and reach the consensus using the RAND methodology. The RAND methodology is intended to obtain a formal consensus among expert groups about the appropriateness of health care interventions, particularly when scientific evidence is absent, scarce and/or heterogeneous. Technically, for each clinical scenario, a form is prepared, where each member of a group of experts scores appropriateness from 1 (completely inappropriate) to 9 (fully appropriate). Ballots are blinded to the other members. The two extreme scores (highest and lowest of the experts) are discarded, and the area containing the majority of the ballots defines the classification of the intervention (inappropriate, uncertain, appropriate). Eleven experts were chosen among the current Co-chairs of the subcommittee, the past Co-chairs who served during the chairmanship of Alan D. Michelson (2005-2007) and those who had experience on LTA standardization in other organizations. Forty-eight RAND forms, each containing a statement on how LTA should be performed, were prepared and submitted to each expert. A second run of RAND forms was done a few months later, after modification of some statements, based on the comments and suggestions of the experts during the first run. Presentation of the consensus reached among experts during the first two RAND runs at the meeting of the SSC Subcommittee on Platelet Physiology in Vienna is considered part of the long process to reach a final consensus, because all the comments and criticisms that emerged during the discussion of the consensus will be useful to improve the quality of the consensus.

The last part of the session included two presentations on other platelet function tests.

Paul Harrison analysed most commonly used tests, such as the PFA-100 device, the Verfy Now device and the Multiplate whole blood aggregometer. Each of these tests uses whole blood and therefore avoids most of the issues involved in blood processing for light transmission aggregometry. With the PFA-100 he presented a summary of the literature post our previous SSC document on this device published in JTH in 2006. There are now more than 500 papers published on this device and 200 more since 2006. The consensus statements on the PFA-100 still hold as a screening device. Paul Harrison also presented that there is a new PFA-100 P2Y cartridge in development which may have the potential for measuring responsiveness to clopidogrel and related drugs. He also presented an overview of the meta-analyses of the PFA-100 CEPI cartridge that have been published recently suggesting that the test is predictive of MACE in high risk individuals on aspirin treatment.

He pointed out that this is clearly not true aspirin resistance and that is likely to be a measure of platelet reactivity and is impacted by VWF levels. He then proceeded to discuss platelet reactivity and shortened CT's measured by the aspirin independent CADP cartridge. There is a growing literature on this latter topic and he also presented some unpublished data from large studies within Oxford (>600) and UMASS (>500). These studies demonstrated contrasting data on the predictive value of shortened CADP CT's with >100 MACE in each. A meta-analysis of shortened CADP CT's should be feasible soon. With the VerifyNow device he presented an overview of the 3 cartridges each designed to test the major classes of anti-platelet drugs and highlighted some of the significant recent literature on each. There is increasing evidence that each test can predict poor outcomes in small studies and he discussed some of the large trials that are either planned or in progress, which will determine whether there is clinical benefit in identifying poor responders and adjusting their anti-platelet therapy. It should be feasible to perform a meta-analysis of each test in the future. Lastly he presented an overview of the new Multiplate whole blood aggregometry system which is increasingly popular in Europe and the UK. There is a growing literature beginning to show a number of applications of this technology particularly in measuring drug responsiveness and identifying platelet defects. However, the test is relatively new and more trials are required.

Finally, Larry Frelinger updated the group on less commonly used and newly developed tests of platelet function: Vasodilator-Stimulated Phosphoprotein (VASP), TEG® PlateletMapping™ System, IMPACT® Cone and Plate(let) Analyzer, PlaCor PRT7000™, ThromboVision T-Guide®, Serum TXB₂, Urinary 11-dehydro TXB₂. Dr. Frelinger's presentation could be summarized as follows: 1) Platelet function as determined by urinary 11dhTXB₂ predicts adverse events in selected populations of aspirin-treated patients, 2) Platelet function as determined by VASP and TEG PlateletMapping predict adverse events in selected populations of clopidogrel-treated patients, 3) VASP assay may be useful to guide therapy, but larger studies are needed, 4) More studies are needed to determine the clinical utility of new point-of-care assays: Impact, ThromboVision T-Guide, PlaCor PRT7000.

Submitted by M. Cattaneo