41st Annual Scientific and Standardization Committee Meeting

June 10-12, 1995

Jerusalem, Israel
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Animal Models

Co-Chairs: Gerhard Johnson, USA; Thomas Griggs, USA

The subcommittee meeting was attended by 15 participants.

The group reviewed the project to establish an animal models registry and an electronic bulletin board using the Internet. The Registry of Animal Models of Thrombosis and Hemostasis will be available from "Sunsite" by file transfer protocol (FTP). The subcommittee has established and is operating a "listserv" bulletin board service on Sunsite. Access can be gained by sending a note or message to Dr. Griggs by: Phone: (919) 966-5207
Fax: (919) 966-1743
E-mail: trg@med.unc.edu
or letter: CB #7075, Division of Cardiology
UNC School of Medicine
Chapel Hill, NC 27599-7075

Recommendation:

The subcommittee voted to make a formal recommendation that the ISTH support this effort by establishing an ISTH "homepage" on the World Wide Web (WWW).

Activities for the coming year:
2. Categorize current and future entries in the registry regarding their utility in assessments of hemostatic components (platelets, fibrin formation, inhibitors, etc.) and thrombotic variables (flow, platelet, endothelial cell function, fibrin formation, fibrinolysis, etc.).
3. To undertake a detailed review of the utility of animal models for assessing therapeutic modalities in the prevention of restenosis following arterial interventions with the goal of establishing guidelines for standardization of animal experimentation in preclinical assessment.
Control of Anticoagulation

Chair: T. W. Barrowcliffe (UK)
Co-Chairs: G. Agnelli (Italy); A. M. H. P. van den Besselaar (Netherlands); B. Boneu (France); P. M. Mannucci (Italy); L. Poller (UK); S. M. Lewis (UK) Liaison Representative of ICSH

There were two main sessions, the first dealing with PT standardization and the second with control of heparin therapy.

PT Standardization

Reference Thromboplastins
1. Dr. Tripodi - The stocks of the WHO IRP for human thromboplastin (BCT/253) are almost exhausted. A multicenter study for replacement of BCT/253 is planned. The study will include two candidate replacement materials (human recombinant thromboplastin) and the three current WHO IRPs (BCT/253, RBT/90, and OBT/79). In addition, a human placenta thromboplastin will be included to assess the comparison with the human recombinant materials. The results of the study will be discussed by members of the working party and later by the SSC subcommittee on Control of Anticoagulation. One of the candidates will be chosen as the new IRP from human origin.

Reference Plasmas
2.(i) Dr. Van der Besselaar - There are several practical problems with the current INR/1S1 system. Few laboratories can perform the manual technique required for the IRPs. Instruments have an unpredictable influence on the ISI of thromboplastins. In some cases, there are minor statistical deviations from the WHO model for thromboplastin calibration. The ISI of several reagents is influenced by the citrate concentration used to anticoagulate the blood. This problem can be solved easily by adopting only one citrate concentration for all coagulation tests. It is strongly recommended to use 0.11 mol/l, because this concentration has been used in all multi-center study of lyophilized plasmas. ISI differences were observed between fresh (native) and lyophilized plasmas.
(ii) Dr. Hubbard - In a project on European Reference Plasmas, a comparison was made between lyophilized coumarin and artificially depleted plasmas for local calibration. INR equivalents of the lyophilized coumarin plasmas determined with different thromboplastins were relatively close to each other. Greater differences were observed with artificial plasmas.
(iii) Dr. Carroll - An international multi-center study was performed to assess the value of lyophilized plasma calibrants in correcting coagulometer effects on INRs (local ISI determination). Differences between artificially depleted and coumarin plasmas were not of clinical importance.
(iv) Dr. D’Angelo - A comparison was made between PTs of the VDGH lyophilized normal reference plasma and fresh normal plasmas with a number of different thromboplastins. Although there were statistical differences, the lyophilized plasma PT was close to MNPT (differences <3%) with almost all thromboplastins except for recombinant thromboplastins (differences about 10%). The lyophilized normal reference plasma can be used with a correction factor for determination of the PT-ratio.
3.(i) Dr. Van der Besselaar (on behalf of Dr. Poller) - The European Concerted Action on
Anticoagulation (ECAA) was described briefly. The aims of ECAA involving participants in EC and EFTA states will be to improve reliability of INRs using a new simplified system of ISI determination performed locally based on lyophilized calibrated plasmas, and to standardize clinical dosage by computerized programs of INR based oral anticoagulant dosage to improve clinical efficacy.

(ii) Dr. Wenzel - In Germany, a program has started to monitor the indications for oral anticoagulant treatment and the clinical efficacy of the treatment. Summary and recommendations for future work The following actions were agreed upon: i) WHO human thromboplastin reference - final report next year. ii) Reference plasmas - the subcommittee should work towards establishing recommendations on the preparation, calibration, and use of reference plasmas. iii) Revision of the WHO guidelines on thromboplastin calibration - this would be considered in detail at the next meeting. iv) A clinical study on the contribution of INR to the safety and efficiency of oral anticoagulant treatment was suggested by Dr. Wenzel - detailed proposals will be elaborated for the next meeting.

**Heparin**

1. The chairman gave an overview of the problems of standardization of heparin assays and suggested that establishment of heparin reference plasmas might help with standardization, not only for APTT but also anti-Xa and other methods. APTT

2.(i) Dr. Exner gave the final report of a collaborative study on the Proctor and Rapaport method. A combined reagent with purified phospholipids performed well and might be considered as a reference reagent for the INR system if adopted.

(ii) Dr. Denson considered the INR system unworkable in practice and recommended the measurement of heparin in units by anti-Xa assays.

(iii) Dr. Samama gave the results of a French multicenter study in which the therapeutic range of a new highly sensitive reagent was established by comparison with the existing reagent and also with heparin concentration measured by anti-Xa. Dr. Callahan described how one manufacturer approaches standardization of reagents on a lot to lot basis, and commented on the usefulness of plasmas with known heparin concentrations. Dr. van der Besselaar commented that if an INR scheme was adopted the effects of different instruments would be the same as for the PT, therefore, heparin reference plasmas would be required. In general, there was much support for the concept of heparin reference plasmas to calibrate the various reagents. Anti-Xa

3. Dr. Houbouyan presented the results of large French multicenter surveys (>1000 labs) of anti-Xa assays in the period 1989-1991. Although inter-laboratory variability was low, the cv’s between laboratories were quite high from 30-50% dispersion was greater for unfractionated heparin than for LMW heparin. A more recent survey with a limited number of labs indicated considerably lower inter-laboratory variability. Thrombin Generation

4. Dr. Hemker described alternative tests based on thrombin generation and emphasized the difference between measurement of the actual amounts of heparin in plasma, and its effect on the patient's clotting system. The amount of active heparin in plasma could now be assessed in molar quantities from the known specific activities of the molecules above and below the critical value of 5400. A different test, the endogenous thrombin potential (ETP), was proposed for measurement of the overall anticoagulant effect of heparin and was also sensitive to the effect of oral anticoagulants. Summary and Recommendations for Future Work

i) It was agreed that standardization not only of APTT but also other methods should continue to
be addressed.
ii) The possibilities of establishment of heparin reference plasmas could be explored further between now and the next meeting.
iii) Thrombin inhibitors, which were not discussed at the current meeting, were an important new area and would be included in the agenda for the next meeting.
At this meeting it had been decided that the subcommittee should only have a workshop and then join the Fibrinogen Subcommittee to discuss the study suggested by Dr. Francis on Soluble Fibrin in DIC patients. The subcommittee consists of more than 30 persons of whom seven persons attended the workshop, including all the co-chairs except for Dr. Muller-Berghaus.

The two issues that were taken into consideration were:
How should the subcommittee work, taking into consideration the suggestions by the ad hoc committee on subcommittees proposed by Dr. Meyer?
The study on soluble fibrin methods proposed by Dr. Francis.

I. It was found that many questions remain unsolved.
1. Definition ? no agreement was obtained in New York 1993 partly due to the fact that there was a misunderstanding on the voting procedure.
2. Diagnosis ? clinical criteria (scores, organ failures, etc.) are not yet set and tests to use including specificity and sensitivity of the tests are not yet set.
3. Furthermore, how to treat the patients is not solved since the above problems are not solved. This was underlined by a nice study reported on by Dr. Matsuda on patients diagnosed according to Japanese DIC definition in different groups (after surgery, medicine, newborns, etc.). He found that up to 70% of the patients with DIC in certain groups died.
II. It was agreed that the subcommittee was greatly in favor of a cooperative study with Dr. Francis. The patients suitable for such a study were discussed. We decided that patients with head trauma, multiple fracture trauma, and toxicosis should be included as we wanted to have as ?pure? patient material as possible. However it was also discussed and Dr. Francis later ? at the Fibrinogen Subcommittee meeting ? pointed out that it may be favorable to incorporate also patients with sepsis and other disorders involving DIC. The samples will probably be drawn only at admission. Protocols are being made by Dr. Francis and the finalized protocol will have to be agreed upon by the DIC subcommittee.

It was agreed upon that we should propose that the subcommittee should continue but those who had to work during the time to come had to be a small group, especially the group who attended the meeting, as otherwise the work would be difficult to oversee. It was also suggested that with regard to next year's meeting we should have a small workshop and the official meeting together with the Fibrinogen Subcommittee meeting, just as this year, if the SSC agrees to this.
Factor VIII and Factor IX

Chair: Ian Peake, UK
Co-Chairs: Ernest Briet, Netherlands; Jorgen Ingerslev (secretary), Denmark; Carol Kasper, USA; Christopher Prowse, UK; Yvette Sultan, France

1. Chairman outlined the morning's program and stressed the shortage of time. At present there is one document with the SSC seeking a final approval for publication (In vivo recovery of factor VIII following transfusion, Dr. Christopher Prowse).

2. STANDARDIZATION
   a) Concentrate standards. Dr. Barrowcliffe announced that the 5th International Standard for Factor VIII concentrate was approved by the WHO, with a potency of 5.4 IU/ml. The 3rd International Standard for Factor II-IX-X concentrate had been assessed with consistency. He discussed the possibility of a common monocomponent factor IX concentrate. Dr. Weinstein reported on the progress of work on the FDA/EP common standard which should be complete by the end of 1995. Regarding a factor IXa standard, Dr. Gray discussed variations associated with different methodologies.
   b) Plasma standards. Dr. Barrowcliffe has produced a new Factor II-VII-IX-X plasma standard that will be calibrated this autumn. With regard to the 3rd International Standard for Plasma Factor VIII/ von Willebrand Factor, several large plasma pools have now assayed above 100% for factor VIII. He prompted investigators to send him results from their own laboratories. Dr. Kitchen described progress in assessing the SSC secondary plasma standard. These studies are soon to be completed, with stability testing and a few results to be included.
   c) Dr. Barrowcliffe described the Factor VIII and IX Concentrate Field Study involving mainly commercial laboratories. The variation seen was related to use of different assay methods, and analytical approaches. It was noted that the chromogenic assay showed a large variation which was discussed. Further studies will be overseen by a small steering group chaired by Dr. Christopher Prowse.
   d) Dr. Lee reported that she had found apparent different recoveries of factor VIII in the same patients with the same infusion by one-stage or chromogenic assay.

3. LABELING AND PURITY OF FACTOR VIII CONCENTRATES
   Dr. Tuddenham spoke to his paper on this issue which was followed by vigorous discussion. It was the feeling of the meeting that the Subcommittee should recommend that the use of non-scientific purity descriptive phrases should be discouraged in papers and the commercial literature. It should be recommended that products are described in terms of their final constituents and their production technology. The Chairman requested that interested parties write to him so that the final recommendations will be acceptable.

4. A STUDY TO MONITOR THE VIRAL SAFETY OF PCR SCREENED BLOOD PRODUCTS IN NON-INFECTED PATIENTS (NIPS)
   Dr. Berntorp proposed a new approach for safety assessment of viral transmission by blood products using NIPS in which PCR analysis would be used to monitor infection. It was decided to include this in proposed guidelines for the surveillance of viral transmission in PUPS (see below).
5. THE GENETIC BASIS OF FACTOR VIII INHIBITORS

In the absence of Dr. Hoyer, the Chairman outlined proposals for the study of the genetic basis of inhibitor development in severe haemophilia A with factor VIII gene inversions. This should either be in PUPS or in existing patients where the inhibitor status was known. Along with this, patients HLA class I and II characteristics would be assessed. Dr. Hoyer had proposed a prospective study and in the light of the comments made during the meeting, the Chairman would discuss the proposal further with him. It was clear that problems relating to the numbers of patients and their differing ethnic origins would make these studies difficult.

6. PROGRESS REPORTS AND FINAL REPORTS

a) Dr. Hill (on behalf of himself, Dr. Mannucci and Dr. Ludlam) outlined their proposals for surveillance of viral transmission in PUPS. There was considerable discussion and, following this, the Chairman requested that all interested parties should write to him, or the authors. A draft document would then be circulated to Subcommittee members and others for comment.

b) The following final reports were made (chaired by Dr. Kasper)
   - Registry of European-derived clotting factor concentrates (Dr. Costa e Silva)
   - Registry of North American-derived clotting factor concentrates (Dr. Menache)
   - Registry of North American Immune Tolerance Practices (Dr. diMichelle)
   - International Registry of Immune Tolerance Studies (Dr. Mariani)
   - Protocols for surveillance of inhibitor development in PUPS (introduced by Dr. Kasper, expanded by Dr. Klose)

The Chairman requested that these reports be sent to him as soon as available for assessment by the Subcommittee members prior to final submission to the SSC with a view to publication.

7. NEW PROPOSALS

a) Dr. E. Briet discussed the need to monitor inhibitor development in previously treated patients exposed to a new concentrate. Although it was felt that such practices are being conducted in many centers, it was thought that this could be included in the inhibitor surveillance guidelines proposed by the Subcommittee. Dr. Bri?t agreed to write to the Chairman outlining his proposal.

b) Dr. Verbruggen described a minor change in the Bethesda inhibitor assay to stabilize factor VIII in the control sample which resulted in a more sensitive and parallel assay. The possibility of a collaborative study was discussed.

c) Dr. J. Lusher proposed a worldwide pharmacokinetic study of factor VIII and IX in infants below the age of five with severe haemophilia. She asked interested doctors to contact either her or Dr. Laurian.

8. CONCLUSION

The Chairman thanked all participants and presenters and said that details of all projects would be sent to the Subcommittee members shortly. The arrangements for 1996 Meeting have yet to be finalized.
Haemostasis and Malignancy

Chair: Frederick R. Rickles (USA)

Seven members of the subcommittee participated in the business meeting:
Drs. Rickles, Donati, Falanga, Kwaan, Francis, Zacharski and Barbui.

The subcommittee met in business session only this year and discussed five major topics: (1) Frequency and format of the meetings of the subcommittee; (2) Content of the meetings; (3) Current activities for completion; (4) Publications; (5) Recommendations for the program for next year in Barcelona.

Frequency and Format: The members voted to meet every other year in the off-year with the caveat that many U.S. scientists expressed concern re: obtaining funding for travel to the off-year meetings.

Content: After an introduction by Dr. Donati regarding the concerns of the SSC, the subcommittee reaffirmed its desire to deal principally with the issues of standardization of methodology for measuring tumor-associated procoagulants and fibrinolytic proteins, development and maintenance of registries and recommendations for the design of clinical trials in cancer patients.

Ongoing Activities for Completion in 1995-96: Dr. Hau Kwaan will contact Dr. Manfred Schmitt to complete the recommendations for processing tissue and plasma samples for assay of fibrinolytic proteins. Dr. Anna Falanga will contact Dr. Paolo Prandoni regarding preparing a brief description of the study design of SOMIT (Screening for Occult Malignancy in Thromboembolism) for publication in Thrombosis and Haemostasis. Drs. Rickles and Kwaan will be contacting the major manufacturers of antithrombotic drugs (e.g. LMW heparin, etc.) and the principal investigators running trials of these agents to work with the subcommittee. It is our desire to see cancer survival data added to their current study forms and provide this data to the subcommittee on a regular basis. Dr. John Francis will coordinate the preparation of a registry of antibodies that cross-react with clotting and fibrinolytic proteins in animal models. Drs. Francis and Rickles will initiate a plan for standardization of tumor procoagulants to be presented to the subcommittee in Barcelona. Drs. Rickles and Dr. Mark Levine will be contacting the chairman of the major clinical cancer trials groups and the American Society of Clinical Oncology for the development of interactive programs with the subcommittee, including symposia, joint data collection (e.g. incidence of thrombosis in high risk cancer patients on clinical trials; predictive value of clotting tests for thromboembolism in cancer patients; etc.).

Publications anticipated for submission in 1995-96: (1) Recommendations for collection and processing of tumor and plasma samples for measure of fibrinolytic proteins; (2) SOMIT study design; (3) Recommendations for antithrombotic therapy (prophylaxis and therapeutic) for high-risk cancer patients; (4) Registry of cross-reacting antibodies for use in measuring coagulation proteins in tumor animal models.

Recommendations for program in Barcelona: (1) Standardization of assays for tumor procoagulants; (2) Standardization of collection and assay methods for tumor extracts for
fibrinolytic proteins in cancer patients; (3) Update on cancer clinical trials [(a) SOMIT; (b) EST 2491 - Molecular and plasma markers of coagulation and fibrinolysis in Acute Progranulocytic Leukemia; (c) Anticoagulant and fibrinolytic therapy of cancer; (d) Cancer mortality in patients treated with LMW heparin vs. unfractionated heparin]; (4) Report on integration of subcommittee activities with cancer clinical trials groups.
Lupus Anticoagulants and Phospholipid-dependent Antibodies

Chairman: Douglas A. Triplett - USA
Co-Chairmen: T. Barbui - Italy, J. Brandt - USA, R. Roubey - USA, I. Scharrer - Germany


B) Third International Workshop on Laboratory Diagnosis of Lupus Anticoagulants. University Institute of Biomedical Sciences, Favaloro Foundation, Buenos Aires, Argentina, May 6, 1995

C) Workshop on Anticardiolipin and Anti b2 Glycoprotein I Antibodies. University Institute of Biomedical Sciences, Favaloro Foundation, Buenos Aires, Argentina, May 6, 1995

D) International Survey on Lupus Anticoagulants - 4 Participants Preliminary summary of data

METHODOLOGY
A) Anticardiolipin Assays J. Brandt

Anticardiolipin Antibodies
Where Do We Go From Here?
1. Current Status:

Multiple methods of performing the assay, including specific protein-based assays.

Multiple methods of establishing the reference (normal) range.

Multiple methods of performing the assay.

Problems with standards and reference materials.

2. Methods of performing the assay:

Is it beyond the scope of the LA/PDA Subcommittee to make specific recommendations in this fast-moving environment?

Are there recommendations that we can make in terms of validations of an assay?
Number of samples to be analyzed to demonstrate correlation between methods. Criteria for evaluating agreement between methods; linear regression analysis versus concordance between classification/titer (e.g. negative, low moderate, high)

Are there recommendations we should make regarding evaluation for background (non-specific) binding?

3. Methods for establishing reference (normal) ranges:

Minimum number of subjects to be included.

Handling of outliers (presumed positive); i.e. criteria for editing the data.

Type of subjects to include:
Gender
Age

Calculation of the reference interval
Mean \pm sd (arithmetic)
Mean \pm sd (log)
Percentile
Multiples of the median
a. Absorbance units
b. GPL/MPL units
Do we have adequate data to recommend a uniform approach at this time?

4. Reporting of results:

Should we recommend a uniform method? If so, which method?
Titer (e.g. negative, low/weak positive, moderate positive, high/strong positive). What criteria would be used to designate the titer?
MPL/GPL units
SD above mean or median (arithmetic or log transformed)
Multiples of the median

Do we have sufficient data to recommend one method? If not, what additional data is needed?

5. Standardization:

What form should a standard/reference material be in?
Lyophilized plasma
Lyophilized serum
Purified immunoglobulin
Pooled versus single donor

How should a value be assigned to such a material?
What would be the proposed use(s) of such a material?

Routine (day-to-day) calibration of clinical laboratory assays?
Calibration of working standards?
Calibration of manufacturers' kits?
Research programs
B) Nature of Antigen R. Roubey
Detection of Autoantibodies to Specific Protein
Antigens for the Diagnosis of the Antiphospholipid Antibody Syndrome

1. Scientific Background
Research suggests that autoantibodies associated with the antiphospholipid syndrome and detected in lupus anticoagulant and anticardiolipin assays are directed against one or more phospholipid-binding plasma proteins or protein-phospholipid complexes. The most common antibodies are directed against epitopes expressed on b2-glycoprotein I (b2GPI) or prothrombin.

Standard anticardiolipin: ELISAs detect antibodies with at least two distinct specificities:
Antibodies to b2GPI (predominantly bovine)
Authentic anticardiolipin antibodies
? Antibodies to other calcium-independent phospholipid-binding proteins in bovine or human sera

Standard lupus anticoagulant assays detect certain antibodies to:
b2GPI
Prothrombin
? Other coagulation factors

Issue: Are the data sufficient to warrant a re-evaluation of clinical laboratory testing?

2. Implications for Clinical Testing
Potential benefits of ELISAs using purified human protein antigens:
Decreased intra- and inter-laboratory variation due to standardization and quality control of antigens.
Detection of species-specific antibodies
No detection of authentic anticardiolipin antibodies which may not be associated with the antiphospholipid syndrome (improved specificity)
Possible improved prognostic capabilities if certain autoantibodies correlate with clinical subsets

Potential disadvantages of ELISAs using purified human protein antigens
Increased expense
Represents a significant change for clinicians, clinical laboratories
Issues: Should immunoassays (ELISAs) for autoantibodies to b2GPI be developed and evaluated for clinical use?

Should such assays be used clinically at this time?
3. Issues in Immunoassay Development

Methodology
Preparation of antigen, assessment of purity
Type of microtiter plate, coating conditions
Processing of samples
Many others

Quantitation of results
End-point titration vs. single dilution/reference curve
Effect of affinity
Establishment of a normal range
Need for positive standards (workshop)
Assignment of units
Quality controls, confidence limits, coefficient of variation

Clinical correlations
Sensitivity, specificity
Predictive values

4. Nomenclature
Issue: Should terminology change and how?

NOMENCLATURE

Growing problems with confusion in the literature (e.g., Anticardiolipin Antibodies [Type A and Type B]; b2 Glycoprotein I Dependent and Independent Antibodies; Anti-b2 GPI Antibodies)

STANDARDS

Is it possible for the Subcommittee to provide monospecific or affinity-purified IgG and IgM fraction with various relevant specificities?

Plans to approach this problem (workshop)? NIH support?

College of American Pathologists Standards Committee

NIBSC - Preliminary efforts, T. Barrowcliffe

International Anti b2 GPI Workshop Letter:
D. Ware Branch
David L. McGlasson

UPDATE ON CLINICAL TRIALS

Randomized clinical trial on the management of antiphospholipid-antibody syndrome: a proposal
FUTURE PLANS


42nd Annual Meeting, Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis (June 22-24, 1996)
Perinatal Haemostasis

Chair: M. Andrew (Canada)
Co-Chairpersons: *J. Bonnar (UK); M. Hellgren (Sweden) *Absent

I. Vitamin K Deficiency
1. Introduction and report on the vitamin K international symposium by Dr. T. Sutor (Germany). Unfortunately, several presenters did not arrive. However, the extra time was effectively used for a full subcommittee discussion on recommendations to be included in an official publication from ISTH. Issues discussed were: terminology, definition, diagnosis, classification, prophylaxis.

II. Coagulation Protein Concentrates

Homozygous Protein C Deficiency

1. Ms. L. Mitchell (Canada) presented data on the subcutaneous administration of protein C in rabbits. Survival of protein C in an active form was observed for 77 hours.
2. Dr. M. David (Canada) reported on the successful subcutaneous administration of protein C concentrate in a young child for 48 hours.
3. Dr. P. Shwartz (Austria) updated the subcommittee on the intravenous use of protein C concentrate worldwide.
4. A presentation of the questions regarding dosage, response, pharmacokinetics, and risks of the use of coagulation protein concentrates in newborns was given by Dr. M. Dreyfus (France). There was general support from the subcommittee for the establishment of a registry.

III. International Trials in Perinatal Haemostasis

1. A brief summary of childhood thrombophilia was given by M. Andrew (Canada). There was agreement that the name of the subcommittee should be changed to Perinatal/Pediatric Subcommittee. Enormous interest was expressed by the audience. There was agreement that international studies in the prevention and treatment of thrombotic disease were needed and could be facilitated by the subcommittee.

2. A summary on the use of estrogen in women was given by Dr. J. Deuketis (Canada). The discussion focused on the need for clinical trials but that they may not be feasible (legally) in some countries.

IV. Mini Reports

1. Dr. Suzuki (Japan) reported on the use of the thrombostat 4000 in evaluating prethrombostat effects of pregnancy and oestrogen use.

2. Dr. C. Kaplan (France) reported on the treatment of the fetus with allo-immune thrombocytopenia. The need for individual clinicians to collaborate on studies addressing optimal treatment was accepted by the subcommittee.
Plasma Coagulation Inhibitors

Chair: T. Koide (Japan)
Co-chairs: R.M. Bertina (The Netherlands); B. Dahlbäck (Sweden); S. Iwanaga (Japan);
D. Lane (UK)

The program consisted of the following two parts:
I. Protein C and Related Subjects; and
II. Anticoagulant Serpins.

I. Protein C and Related Subjects
The meeting began with a presentation by Dr. A.R. Hubbard on "The Calibration of the SSC Plasma Standard for Protein C and Protein S." He presented results from 12 laboratories and was able to assign potency for each standard, PC 0.98 iu/ml (cv 6.0%); PS (total) 94.7 iu/ml (cv 4.8%); PS (free) has not yet been finalized but will be shortly.

Next, Dr. R.M. Bertina summarized the status of "Standardization of the APC-Resistance Test." The many problems were reviewed. There are tests based upon different principles, APTT, PT, Factor Xa clotting and diluted RVVT. How should the normal range be made (excluding factor V R5O6Q individuals) and what concentrates of APC be used? Should normal ranges for males and females be different?

Dr. Tripodi informed the meeting that plasma from normal and factor V R5O6Q individuals has been collected for a proposed multicenter study of APC-resistance methods, which is about to be initiated. The protocol of the study was reviewed.

Dr. S. Rosén also presented a report on "APC-Resistance Test: Assay Performance and Influence of Sample Handling." Local plasmas and instruments are very important in determining response of APC-resistance assays. Care in sample handling is very important. Using factor V deficient plasma highly specific assay for the factor V R5O6Q can be devised. A new chromogenic substrate assay using factor V deficient plasma has been devised and, again, it gives very high specificity for the mutation.

The data on nine patients with severe PC deficiency was presented by Dr. H. P. Schwarz. The most complication-free treatment is PC (70 u/kg) twice a week (in acute phase) and oral anticoagulants. However, in spite of this, blindness is a major outcome of severe PC deficiency. The reason for onset of blindness is uncertain.

Then, Dr. B. Dahlbäck presented "Protein S Deficiency of Type I and Type III Are Phenotypic Variants of One Genetic Disease: Proposal of a Revised Nomenclature." A study of 18 families with PS deficiency showed that the same families exhibit type I and III phenotypes. Two subtypes are now suggested. Type I, low free PS, normal or low total PS, and low functional activity; type II, low functional but normal free PS.

Following the talk, Dr. Martine Aiach made a short communication, "The Protein S Ser 460 to Pro Mutation (Heerlen Polymorphism) Is Associated with Type III Deficiency," against Dr. B.
Dahlbäck's proposal. Dr. Bertina suggested a working party might be convened to discuss further classification.

"Database for Protein C and Protein S Gene Mutations." The recently published PC mutation database was summarized by Dr. P.H. Reitsma.

Dr. Sophie Gandrille also presented some additional data as "Mutations of Protein S Gene in 119 French Patients." Seventy-one percent of type I deficiency was successfully identified, while only 45% of type II deficiency was successfully identified.

Anticoagulant Serpins

Dr. D. A. Lane made a brief presentation on "The SSC Plasma Standard for Antithrombin." This standard was worked on by ten laboratories and assigned potency for standard as 0.96 iu/ml (antigen assay) and 0.92 iu/ml (activity measurement).

Dr. H. van Boven presented the data of "Natural History of Dutch Families with Inherited Antithrombin Deficiency." First, she showed several newly identified mutations of Type 1 ATIII deficiency including those caused by a missense mutation in a region other than a so-called pleiotropic effects mutation. She also showed a few Dutch families with combined deficiency of ATIII and factor V (factor V Leiden) whose risk factor for thrombosis are 92% in contrast to 54% for ATIII deficiency alone.

Finally, Dr. T. Koide presented the results from a study on "Expression and Characterization of Three Recombinant ATIII Mutants which Caused Pleiotropic Effects-Type ATIII Deficiency," in which he showed that three recombinant ATIII mutants (Oslo-type, Kyoto-type & Utah-type), which were grouped as "pleiotropic effects-type" mutants, have different properties from each other in secretion, intracellular degradation, abilities in heparin-binding and in the complex formation with thrombin. As a conclusion of his study, he suggested that a current subtype of "pleiotropic effects-type" mutants may be reconsidered if they should be classified into the same group.

Presentation by Dr. D.M. Tollefsen, "Insight into the Mechanism of Action of Heparin Cofactor II," was canceled.
Platelet Immunology

Chair: Cecile Kaplan, France
Co-Chairs: D. Beardsley, USA; J. Bussel, USA; T. Kunicki; USA; S. Shattil, USA

Minutes prepared by D. Beardsley and C. Kaplan.

This committee had a working session. Mainly three topics were discussed:
- Autoimmunity
- Alloimmunity
- Heparin-induced thrombocytopenia

The tasks for the coming years are as follow:
1) Autoimmunity:
Detection and characterization of platelet autoantibodies and antigenic targets: 
Recent studies of platelet autoantibodies in AITP have included a multicenter international workshop organized by Drs. P. Berchtold and P. Imbach. There was excellent correlation between results obtained by participating laboratories, particularly with regard to platelet associated antigen capture results. It could now be interesting to include in such workshop a standardization of monoclonal antibodies to be used. This is in order to have a standard that could be recommended to the majority of the laboratories active in the field. Platelet glycoprotein-specific antibody methods are superior to the measurement of platelet-associated IgG in the diagnosis of AITP. Direct assays gave more consistent results than indirect assays and were more valuable in diagnosing AITP. The reasons why it is important to determine the platelet bound antibodies include: 1) many false negatives can occur with indirect assays, especially if there is not a large amount of antibody present, 2) some antibodies may represent antibodies formed as a result of the AITP, rather than causing the disease, and 3) alloantibodies formed as a result of transfusion or pregnancy could co-exist with autoantibodies making it sometimes difficult to ascertain their origin.

A goal of this field should be to minimize the amount of blood needed to accomplish the direct assay of platelet-associated glycoprotein-specific antibodies. This can currently be done by the immunobead technique on 1x10^7 platelets, but this amount can probably be reduced further. The minimum number of platelets needed for direct MAIPA testing has to be determined. Concerning circulating autoantibodies, C. Kaplan reported that in special conditions, i.e. neonatal thrombocytopenias associated with "hidden maternal autoimmunity," the mothers had no detectable platelet-associated antibodies and that the only possibility for such a diagnosis was the characterization of circulating autoantibodies. It will be important to evaluate the feasibility of utilizing recombinant peptides for diagnostic assays for platelet antibodies. There is a great deal of work going on at present to identify the precise epitopes which are commonly the targets of platelet autoantibodies (N.R. Shulman's GPIb epitope is one example).

At the moment there is no current consensus regarding optimum management of pregnancy associated with anti-platelet autoimmunity. The question of whether fetal blood samplings must be performed at the end of pregnancy has been addressed by several groups. For the coming
year, it could be of interest to compare the results obtained by different teams in terms of number of thrombocytopenic fetuses and infants at birth or during the first days of life in order to evaluate more precisely the fetal/neonatal risks with the same criteria.

It is important to have a follow-up examination of the mother in the six month post-partum period, and when possible a platelet life span study, to accurately diagnose immune platelet destruction.

2) Alloimmunity:
- Nomenclature. There remains considerable controversy regarding a nomenclature system for platelet alloantigens. The possibility of a World Health Organization forum working out this issue was brought up in 1994 at the SSC meeting. The cooperation of the SSC as well as the ISBT should be incorporated to reach the best solution to this problem.
- Fetal/Neonatal Alloimmune Thrombocytopenia. Prospective studies have recently shown that alloimmunization is more frequent that previously thought. Results reported by W. Ouwehand (Great Britain), L. Marshall (Australia), and C. Kaplan (France) are concordant: immunization could occur in 1/500 pregnancies and 50% of the fetuses could be affected. A central registry for antenatal therapy comparison is a new concept presented by C. Kaplan of the European registry. Extending this to an international base should be explored. Statistical analysis must be done in order to have the same criteria for the definition of therapeutic effectiveness or failure. Proposals for the design and operation of such a registry should be brought forward for the 1996 SSC Platelet Immunology meeting.

3) Heparin-associated/induced Thrombocytopenia:
- Testing standardization
At the moment many laboratories hesitate between functional and antigen testing for the diagnosis. A standardization could be evaluated this coming year before the next meeting and could be discussed then.
- Therapy
The therapy of Heparin-induced thrombocytopenia is still a matter of concern. It could be worse than HIT by itself. For that purpose, T. Warkentin and A. Greinacher will prepare propositions after the clinical trials conducted this coming year.
Platelet Physiology

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).
I. Biomaterials

1. M. Andrews: Thrombotic complications in indwelling catheters in pediatric patients were considered. Thromboembolic complications by central catheters of 40-80%, and by femoral catheters of 10-40% (DVT). Umbilical catheters may produce peripheral ischemia. Benefit of low-dose heparin is unclear, whereas coumadin reduces the thrombotic events.

2. M. Barstad: Presented a novel technique to assay potential effects of X-ray contrast media (CM) on thrombus formation at arterial shear in native human blood. It was concluded that the effect of CM on the clotting time in classical coagulation assays and on platelet aggregation in PRP did not parallel the influence of CM on thrombus formation in native blood.

II. Aneurysms/Arterial Stenosis/Blood Flow

1. V. T. Turitto: Reported on computational analysis of pressure and flow in various types of intracranial aneurysms. A subcommittee report on these issues will be submitted to the ISTH/SSC publication committee in the near future.

2. P. A. Holme: Presented a novel technique to assay platelet activation in flowing native human blood. It was concluded that constant shear up to 2600s-1 did not activate platelets. However, significant platelet activation was observed at eccentric stenoses with shear rates exceeding 10500s-1 at the apex. This activation was potentiated when collagen-induced thrombus formulation was triggered at the apex. Activation markers used were PAC1, Annexin V, and microvesicles.

3. L. Harker: Reported on platelet activation epitopes in stroke and other thrombotic disorders. A number of activation markers were used, including PAC1, Annexin V, LIBS, F9F, microvesicles, and bTG. Platelet activation was observed during 1 to 3 days following the onset of the thrombotic events.

4. V. T. Turitto: Discussed the need for advanced computational routines of complex blood flow patterns, particularly at arterial stenoses and aneurysms. Application of these results should be used as rough estimates of flow geometry. Programs available are FIDAP, FLUENT, and Flow-3D. These programs require data on luminal geometry dimensions and boundary definitions.

III. Working Parties

1. G. Lowe: Informed the committee by message that the working party on red cells WBC count and other rheological variables from 25 studies are analysed. It is planned to present the analysis at Barcelona in 1996.
2. Ph. de Groot: Presented a study on mesothelial cell seeding on vascula graft. Fibronectin is necessary for cell attachment. The cells possesses anti-thrombotic properties and they remained attached to the graft for up to 2 hours at a shear rate of 1000s-1. This presentation was within the framework of the working party on the use of flow chambers.

IV. Future Activities

Following activities are planned to be reported on or finished within the next subcommittee meeting in Barcelona:

1. Final standardization of technique to study potential effects of CM on thrombus formation in flowing native human blood. Responsible: K. S. Sakariassen, S. R. Hanson and D. A. Gabriel

2. Initiate new working party on standardization of potential effects of CM on cultured human endothelium. Responsible: E. Dejana

3. Submit a Subcommittee paper on flow and pressure at intracranial aneuryours. Responsible: V. T. Turitto

4. Present a summary of the current situation of commercial available programs for computational routines of complex flow patterns. Responsible: E. Grabowski

5. Final analysis of data from working party on hematocrit and other rheological variables from 25 studies. Responsible: G. Lowe

6. Report on working party activities on standardization of the use of flow chambers. Responsible: J. Hubbell

Subcommittee publications
