

1996 MINUTES

FIBRINOGEN AND DIC SUBCOMMITTEE

Monday, 24 June, 1996, 8.00 - 12.00
Room Rossinni, Fira Palace Hotel
Barcelona, Spain

Chair: Willem Nieuwenhuizen, The Netherlands

This year's agenda comprised the following items:

W. Nieuwenhuizen gave an update on the reference material to harmonize the currently available quantitative "D-dimer" assays. The manuscript on this subject has been drafted and sent to the co-chairman for approval. The NIBSC has agreed to vial and distribute the reference material, which will consist of a pool of patient plasmas, and will have an assigned consensus value (see last year's report). The manufacturers will be notified and invited to report in the kit inserts how their calibrators relate to the reference material.

P. Gaffney reported on the calibration of an SSC plasma with respect to fibrinogen content. The Immuno Pharmaceutical Company (Vienna) has prepared a large batch of pooled human plasma which has been dispensed into 1 ml aliquots, lyophilized and stored at -20 degrees C. The Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH) has requested that this be calibrated in terms of a number of coagulation factors. Gaffney presented a summary of a collaborative study to calibrate this plasma standard in terms of fibrinogen (clottable form) using the International Standard for Plasma Fibrinogen (89/644) as a calibrator in an automated Clauss procedure. The Clauss procedure is based on the turbidity (light scattering) of plasma following the addition of thrombin. This study was conducted under the auspices of the Fibrinogen Subcommittee. Twelve laboratories were requested to assay the fibrinogen content of SSC plasma. The overall mean was 2.58 g/l with 95% confidence limits between 2.48-2.69. It was suggested that the SSC plasma standard be calibrated to contain 2.6 g/liter. It was suggested to name this material the Secondary Standard.

M. Matsuda presented a proposal for a nomenclature for abnormal fibrinogen. This nomenclature describes the mutation in amino acids and base pairs, and is more informative on the (point) mutation in the abnormal fibrinogen molecule than the currently used indication by the name of the place where the abnormality was first discovered (e.g. Tokyo II, Cedar Rapids which are identical). It was decided that this nomenclature be published as an official ISTH publication.

C. Francis presented a survey and comparison of the currently available assays for soluble fibrin. It is obvious that the problem of standardizing soluble fibrin is a major challenge. Several options are under investigation. Important information will come from the THROMBO study and the DIC Study (see below).

Drs. Taylor, (Chairman of the DIC Subcommittee) Francis and Nieuwenhuizen presented a draft protocol aimed at assessing the clinical utility of soluble fibrin assays in DIC. Several assays are

currently available, which are not (or may not be) fully comparable. DIC patients will be selected on very strict and specific criteria. Twelve clinicians have agreed to participate by supplying samples. The plan is to have results available in Florence in 1997 with a final report in 1998.

1997 MINUTES

Fibrinogen and Dic Subcommittees

Friday, 6 June, 1997, 8:00-12:00

Giotto I, Fortezza da Basso

Florence, Italy

Fibrinogen Chair: M.W. Mosesson, USA

Co-Chairs: F. Brosstad, Norway; M. Matsuda; W. Nieuwenhuizen, The Netherlands

DIC Chair: F. Taylor, USA

Co-Chairs: M. Blombäck, Sweden; M. Kazama, Japan; T. Matsuda, Japan; I. Bokarew, Russia; J.W. Ten Cate, The Netherlands; N. Sakuragawa, Japan

Dr. M. Mosesson chaired the first portion of the meeting dealing with fibrin sealants as chair of the fibrinogen subcommittee. Drs. M. Blombäck and F. Taylor chaired the second portion of the meeting dealing with soluble fibrin assays as chairs of the DIC subcommittee. The subjects reported and discussed by this subcommittee were as follows:

I. Fibrinogen Subcommittee

There was a wide ranging discussion concerning the characterization and rationale for standardization of fibrin sealants including present and past formulations, delivery devices, clinical applications. Discussants included Dr. D.L. Amrani (session moderator), Dr. U. Martinowitz, Dr. T. Seelich, Dr. M. Weinstein, Dr. M. MacPhee, Dr. M. Nowartarski. Approximately 120 persons were in attendance.

There is currently a broad range of formulations for fibrin sealants, which vary greatly with respect to the concentration of fibrinogen, factor XIII concentration, fibronectin concentration, and the presence and amounts of a number of other plasma constituents and/or additives. Specific issues that were raised included 1) the need for high fibrinogen concentrations; 2) the need for standard assay of mechanical strength and standardization of *in vitro* methods for measuring fibrin sealants; 3) the role of factor XIII; 4) the inclusion of antifibrinolytics and other agents. There is no accepted or preferred basis for such formulation, or objective criteria by which preparations are measured.

The discussants agreed by consensus that it would be valuable to develop a rationale and establish criteria for standardization and calibration of fibrin sealants. A working party was formed which will exchange written communications among interested participants and develop criteria for characterizing fibrin sealants as outlined above. Dr. Michael Mosesson will head up the working party. It is anticipated that information will be solicited and collected over the next six months, disseminated for consideration, and be ready for discussion at the next meeting of the SSC in 1998.

Dr. F. Dati (Behring Diagnostics) reported on a study of criteria for establishing a High Concentration Fibrinogen Standard. Dr. Dati presented a detailed analysis of the need for a high fibrinogen standard, problems in the measurement of fibrinogen, and standardization of testing methods. He made specific recommendations for a high fibrinogen plasma standard. It is expected that Dr. Dati will submit a written summary of his report for further consideration by the Fibrinogen Subcommittee.

II. DIC Subcommittee

A. Immediate, Practical Issues

1. Definition of DIC

In 1994-95, Drs. Müller Berghaus and Margareta Blombäck oversaw the development of a definition of DIC by the subcommittee: DIFF (DIC), Disseminated Intravascular Fibrin Formation, is an acquired process associated with disseminated soluble fibrin formation within the microvasculature.

2. Assay of Soluble Fibrin Formation

In 1996-97, Dr. Charles Francis enlisted the assistance of the DIC subcommittee and of Drs. F. Taylor and M. Blombäck in the development of a protocol for the assessment of the clinical relevance of assays of soluble fibrin using four different commercially available ELISA assay kits. A protocol was agreed upon which included collection of samples from patients with DIC (i.e., patients with culture positive sepsis or with specified trauma within 12 hours of injury) as well as from patients following myocardial infarction (Thrombo Study). During the first year (1996-97) of this two-year study, 12 members of the DIC subcommittee agreed to participate. Four members provided samples from 17 sepsis patients and Dr. Owenings provided samples from the 44 trauma patients. The results are summarized as follows:

PERCENT OF RESULTS WHICH WERE ELEVATED (SOLUBLE FIBRIN)

SOLUBLE FIBRIN ASSAY #	THROMBO (275-892)	TRAUMA (44)	SEPSIS (17)
1	30	48	60
2	21	95	75
3	55	70	94
4	90	73	81

The biochemical/immunologic rationale explaining the differences between these four tests, although of importance, is secondary to simply collecting data on the overall incidence of elevated concentrations of soluble fibrin in these three arbitrarily defined clinical conditions. It was agreed with Dr. Francis that at least 20 additional sepsis DIC samples be provided by Drs. Blombäck and Bredbecka (Sweden), Bokarew (Russia), and Sakuragawa and Wada (Japan) as well as possible additional samples from Drs. Levi (Netherlands), Brenner (Israel) and Falanga (Italy).

3. Assessment of Markers of Pre DIC

The remainder of the meeting was taken up by presentations by Dr. Hoots concerning his experience with anti-thrombin concentrates in neurotrauma patients and by Dr. Wada concerning his experience with assays of the pre DIC state in patients who went on to develop DIC (114 leukemia, 126 non-leukemic patients). Dr. Wada observed that hemostatic markers such as PAP (PIC), TAT, D-dimer and soluble fibrin that were already elevated began a further significant rise two to three days before the onset of DIC. On the other hand, protein C, protein S, and tissue factor levels did not change. It was agreed that continued examinations of markers of increased but compensated hemostatic activity (pre DIC) might be as important as evaluation of a definitive marker of DIC or decompensated hemostatic activity such as soluble fibrin. In line with this, Dr. Ten Cate and Dr. Taylor suggested that a controlled study using primates (baboons) also be considered as a means of evaluating assays of soluble fibrin and enzyme/inhibitor complexes as markers of decompensated and compensated responses of the hemostatic system, respectively. Accordingly, Drs. Taylor, Wada, and Sakuragawa agreed to run assays for soluble fibrin and for enzyme/inhibitor complexes including PAP (PIC), TAT, APC/PCI, TFPI/Xa, and for Factor VIIa on blood of baboons infused with very low (10² CFU/kg), low (10⁵ CFU/kg), medium (10⁷ CFU/kg), and high (10¹⁰ CFU/kg) concentrations of E. coli. The question is whether the above markers are of value in discriminating between a stressed but compensated hemostatic system (pre DIC) and a stressed and decompensated system. In theory, enzyme/inhibitor complexes consisting of regulators and mediator components might appear under conditions where the hemostatic system is stressed but compensated before markers of DIC such as soluble fibrin, DD dimers, or FDP appear. These studies will be done this year.

B. Less Immediate, Conceptual Issues

Assuming these studies described above are informative, Dr. Bokarew raised the serious question of whether we can provide a picture or concept which could be used 1) to interpret these studies, and 2) to connect the various clinical conditions in which we will find evidence of DIC (soluble fibrin) with the molecular and

cellular events unique to each condition which can be understood and used by the practicing physician. It was agreed among the chairs of the subcommittee to draft a preliminary report on this issue by the 1998 ISTH-SSC meeting in [Slovenia](#).
jwd/6/10/97

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jwd/6/10/97

1998 MINUTES
Fibrinogen Subcommittee
Sunday, 21 June, 1998, 8:00-12:00
Cankarjev Dom
Ljubljana, Slovenia
Chair: M. W. Mosesson, USA
Co-Chairs: F. Brosstad, Norway; M. Matsuda;
W. Nieuwenhuizen, The Netherlands

The Fibrinogen Subcommittee met to discuss a single subject, "Criteria for Characterization of Fibrin Sealants." Fibrin sealants are complex, multi-component, biological preparations that are used in a variety of clinical situations to promote hemostasis and wound healing.

There are few established criteria or standards for characterizing the content and functional behavior of these preparations. We had seven presentations on the subject, and a lengthy and lively discussion afterward concerned with criteria, methods for measurement, and possible standards. We agreed that certain characteristics such as fibrinogen content, thrombin concentration, and factor XIII concentration were of importance and furthermore, that standards for fibrinogen and thrombin be sought. We intend to pursue this goal by an interactive correspondence during the coming year. We will also use this means to define other characteristics of fibrin sealant content and extend the dialogue to include certain functional parameters.

1999 MINUTES

Fibrinogen

Saturday, 14 August 1999

8:00 to 12:00 PM

Room 38

Washington Convention Center

Washington, DC

Chair: M. W. Mosesson, USA

Co-Chairs: F. Brosstad, Norway; M. Matsuda, Japan;

W. Nieuwenhuizen, The Netherlands; R. McIntosh (ex officio), Scotland

Dysfibrinogens:

The subcommittee undertook a discussion of dysfibrinogenemia including a general overview of dysfibrinogens from the standpoint of structural and genetic abnormalities and correlative dysfunction. Drs. Michio Matsuda and Susan Lord made formal presentations. Drs. Jose Martinez and Steve Brennan were unable to be at the meeting. The subcommittee expressed the view that it would be useful to compile and publish an updated and annotated summary of dysfibrinogens, including afibrinogenemia and hypofibrinogenemia, with emphasis on those with known structural defects. The abnormalities should be primarily identified by the molecular defect but the city name, when available, should be retained. The subcommittee also recommended that the list should serve as a repository for reporting newly characterized dysfibrinogens that are not intended for full publication.

Dr. Michio Matsuda agreed to coordinate the compilation effort with a working party consisting of Drs. Susan Lord, Steve Brennan, Jose Martinez, Michael Mosesson, and Jan McDonagh. Dr. McDonagh has compiled an extensive database on dysfibrinogens and will make this information available to the working party.

Fibrinogen Plasma Standard:

Following the results of an international collaborative study presented by Patrick Gaffney, the Fibrinogen SSC agreed to recommend to the ECBS of the WHO that the UK NIBSC plasma preparation coded 98/612 should be adopted as the Second International Plasma Standard for Fibrinogen. This standard is defined to contain 2.2 mg of clottable protein per vial.

The subcommittee also discussed the requirement for a plasma standard (Dr. Nicodemo Weinstock presenting) containing high levels of fibrinogen. Dr. Ian Mackie also presented in relation to this topic. There was no consensus and the subcommittee agreed to discuss this question more fully at the next meeting.

Standardization and Fibrin Sealant:

Dr. Patrick Gaffney presented the results of a preliminary study that had been initiated at the Fibrinogen Subcommittee meeting in Ljubljana concerning a fibrinogen concentrate standard. The subcommittee agreed to proceed to an International Collaborative Study using the materials prepared by the UK NIBSC with a defined assay protocol for participants.

Dr. Ronald McIntosh summarized proposals for the standardization of the measurement of other components in fibrin sealants in addition to its principal constituent, fibrinogen. It was agreed that future discussion on the standardized of measurement of these components (e.g., thrombin and Factor XIII), should continue within the Fibrinogen Subcommittee, as this will provide continuity rather than have other constituents discussed as separate subjects in different subcommittees.

Characterization of Fibrin Sealants:

Following a presentation by Dr. Rainer Seitz, there was a strong view expressed in the meeting that the European Pharmacopoeia monograph "Fibrin Sealant" is not suitable in its present form for the regulatory characterization of new fibrin sealants. The subcommittee agreed to discuss the monograph at the next meeting with the intention of offering its recommendations to the European Pharmacopoeia and establishing its own view on constituents that should be measured in fibrin sealants.

Drs. Israel Nur and Gilbo Soe presented papers on characteristics of certain fibrin sealant preparations. The subcommittee will continue to hear papers on the characterization of fibrin sealant in the broader sense.

FIBRINOGEN

15 June 2000

8:00 to 12:00

Room 0.5

Maastricht Meeting and Convention Center

Chairman: S.T. Lord--USA

**Co-chairmen: M. Matsuda--Japan; R. McIntosh--UK; M.W. Mosesson--USA;
W. Nieuwenhuizen--The Netherlands**

Dr. R McIntosh was the presiding chairman at this meeting.

Dysfibrinogens

On behalf of Dr. Matsuda, Dr. McIntosh presented a summary table of newly identified structural alterations with city names as work-in-progress from the dysfibrinogens working party. Dr. Michel Hanss (France) presented a paper on the database of published fibrinogen variants that he has constructed and made available on the Internet.

With the agreement of Dr. Hanss the meeting recommended that together with the work-in-progress and the database of Dr. McDonagh (referred to in the 1999 meeting) the working party should include Dr. Hanss in their efforts to compile and publish an annotated summary of dysfibrinogens.

Fibrinogen Plasma Standards

Dr. Nico Weinstock (FRG) presented the case for a reference material prepared by adding fibrinogen concentrate to normal plasma to give a significantly higher than normal level of fibrinogen. Dr. Ian Mackie (UK) presented the case against a high fibrinogen plasma standard, maintaining that existing standards allow the determination of when levels are higher than normal.

The meeting agreed that there is a need to know when fibrinogen levels were above normal but it may also be desirable to quantify the higher than normal level. Therefore, calibration of a high fibrinogen plasma preparation of the type discussed at this meeting is critical to its use as a reference material. The meeting recommended that Dr. Weinstock should submit a report to the Subcommittee on the material coded S2 in his presentation. The Subcommittee would then consider whether or not the manufacture, assay performance and in particular, calibration of this preparation are suitable for its recommendation as an International Standard. Dr. Weinstock agreed to do this.

Standardization and Fibrin Sealant

Dr. Trevor Barrowcliffe (UK) presented results from the recently completed International Collaborative Study on the establishment of a fibrinogen concentrate standard. The meeting agreed that, subject to a satisfactory report on the study, the preparation coded "A" and "C" in the study should go forward to be recommended as the First International Standard for Fibrinogen Concentrate. It was also agreed that when reviewing the study report the Subcommittee should consider whether or not to recommend a reference method for use with this material.

It has been decided previously (1999 minutes) that studies on the standardization of the measurement of other components in fibrin sealant should continue with the Fibrinogen Subcommittee. Following on from this decision, the present meeting agreed that the priorities for these studies should be thrombin, Factor XIII and a functional measurement of fibrin sealant (i.e., the product resulting from the combination of the thrombin with fibrinogen) if it can be agreed that this is required.

Characterization of Fibrin Sealants

Dr. Peter Feldman (UK) presented "options for change" to the Current European Monograph on Fibrin Sealant. Dr. Rainer Seitz (FRG) outlined the aspects of the monograph being considered for a draft revision that he has been asked to prepare by the expert group 6B of the European pharmacopoeia. Dr. Gerhart Dickneite (FRG) described animal experiments on the role of Factor XIII in fibrin sealant and Dr. Ronald McIntosh gave an update on responses to an international survey asking for proposals to change the monograph.

Although it was agreed that the monograph should contain details of how Factor XIII should be measured in fibrin sealants there was no agreement on whether the Factor XIII content should be declared by the manufacturer or if this should be optional. This question turns on whether or not Factor XIII is always an essential component of fibrin sealant.

The Subcommittee agreed to set up a working party to formulate its views on revisions to the monograph to be submitted to group 6B through Dr. Seitz. Dr. Peter Feldman, Dr. Hubert Metzner (FRG), Dr. Anne Walton (UK) and Dr. Per Bengtsson (Sweden) volunteered to participate. The subcommittee permitted the working party to seek other expert views as required.

FIBRINOGEN
6 July 2001
08:00 to 12:00
Room 251
Palais des Congrès

Chairman: S.T. Lord--USA

Co-chairmen: P. Feldman--UK; R. McIntosh--UK; N. Weinstock--Germany

Dysfibrinogens

Dr. Hanss (France) summarized the current content of the on-line database of dysfibrinogens, which he compiled, and that is now also available on the ISTH web site. He described the specific information associated with each case in this database. Dr. Hanss proposed developing a form for on-line submission of further entries to the database. It was agreed that Dr. Hanss should moderate and update this data base. Investigators should submit new dysfibrinogens on-line to Dr. Hanss. As this database may provide the basis to associate specific structural changes with clinical symptoms, the subcommittee encouraged Dr. Hanss to include a description of the clinical data available for each patient and family member.

Fibrinogen in mice

Dr. Lord (USA) presented data on the potentially significant variations in "normal" plasma fibrinogen levels in laboratory mice, which appear to vary with diet, age and mouse strain. The mouse model is commonly used in experimental studies that relate fibrinogen levels to a variety of disease states. There appeared to be a consensus that standardization of the measurement of plasma fibrinogen in mice would be desirable. Dr. Lord undertook to return to the Subcommittee with proposals for a standardization exercise. The proposals will include a method to draw blood, specifying an anticoagulant, and the assay method itself (e.g, ELISA). A reference preparation for mouse plasma would be a desirable aspect of the standardization. The mouse standard would be related to previous functional measurements of either human or mouse fibrinogen.

Fibrinogen plasma standards

Dr. Weinstock (Germany) summarized the critical features of his subcommittee report that described the proposed high fibrinogen reference plasma. Dr. Weinstock also showed further results on the potential use of such a preparation in clinical laboratory measurements. Dr. Lord confirmed that Dr. Weinstock's report has been received and she indicated that this report had been reviewed by expert members of the Subcommittee. (Note added after the meeting: the statement made by the chair that the review process was complete was incorrect, as in fact, the review process is ongoing. This position will be clarified with Dr. Weinstock and Dr. Padilla, WHO, who was present at the meeting.)

Standardization of the measurement of components in fibrin sealants

Dr. McIntosh (UK) reported that it was his understanding that the revision of the fibrin sealant monograph is still under consideration by the European Pharmacopia.

Dr. McIntosh (UK) summarized the steps taken to approve the report from Dr. Barrowcliffe (UK) on the collaborative study (initiated by the SSC Fibrinogen subcommittee in 1999) to establish an international standard for fibrinogen concentrate. The WHO has adopted the recommended preparation from this report as the First International Fibrinogen Concentrate Standard. Dr. Lord will report on this procedure to this year's SSC Annual Business Meeting.

Dr. Longstaff (UK) and Dr. Chang (USA) presented a proposal to characterize and calibrate a new reference preparation for thrombin. Thrombin is a key component of Fibrin Sealant kits and the subcommittee had previously decided to provide continuity by considering the standardization of other Fibrin Sealant components in addition to fibrinogen. The proposal from Drs. Longstaff and Chang was generally accepted with specific agreement that 100IU/vial was sufficient for a reference preparation; an intermediate purity preparation formulated in human albumin would be acceptable; results from a functional assay (thrombin/fibrinogen clotting time) would be preferred in assaying the potency and the calibration should be carried out against both existing NIH and WHO reference materials. The calibration of thrombin activity using clotting time should use the First International Fibrinogen Concentrate Standard, or material calibrated against that standard. The remaining stocks of the NIH and WHO thrombin standards are low, so there is an urgent need to establish a new reference material. Thus, Drs. Longstaff and Chang will report on their progress to next year's SSC meeting in Boston.

On behalf of Dr. Barrowcliffe (UK), who was detained at another subcommittee meeting, Dr. McIntosh presented summary slides on the progress of proposals to establish reference materials for the measurement of FXIII. These proposals will also be discussed at the FXIII subcommittee meeting. The purpose of presenting them here was to determine whether or not there would be value in including a candidate preparation that could be used for the measurement of FXIII in Fibrin Sealant kits. It was agreed that the Fibrinogen Subcommittee would welcome the opportunity to work with the FXIII Subcommittee on this study. Furthermore, it was suggested that the inclusion of a preparation to determine if FXIII can be accurately measured in a concentrated solution of fibrinogen would be particularly useful. In addition to the assay methods proposed by Dr. Barrowcliffe, data on measuring FXIII using the solubility of fibrin in a suitable solvent, e.g., urea or chloroacetic acid (the latter is reference method in the Fibrin Sealant Monograph), would also be useful.

Fibrinogen

July 18, 2002

13:00 to 17:00

**Whittier Hill Room
Boston Park Plaza Hotel**

Chairman: S. Lord, USA

Co-chairs: J. Koopman, The Netherlands; R. McIntosh, UK; N. Weinstock, Germany

Dr. Nicodemo Weinstock presented the summary of the evaluation procedure for a high fibrinogen standard. The project was started at the XVIIth ISTH Congress in Washington (1999), where there was considerable support for the type of standard presented. The main problem is that fibrinogen is a risk factor for CHD, but studies show large discrepancies in fibrinogen levels measured.

The results of a large European study were presented and discussed at the SSC Annual Meeting in Maastricht (2000), where those attending the Subcommittee meeting voted for establishment of a high fibrinogen standard.

The complete approved report of the international study (19 laboratories in 10 countries) was presented at the XVIIIth ISTH Congress in Paris (2001), where the participants voted for the introduction of the presented standard. In addition, the results were evaluated by scientists and clinicians not involved in the study, reaching general agreement. Stability of the plasma preparation was extensively evaluated. Comparison with 1st International fibrinogen standard (WHO) showed acceptable agreement. The audience agreed to present the data to the SSC Business Meeting and to recommend it as the first International High Fibrinogen Reference Plasma with a potency of 5 g/l.

Dr. Colin Longstaff reported on a collaborative study of thrombin standards in fibrin sealants. Two thrombin preparations were prepared, 10000 Ampoules each, approximately 100 units per ampoule (similar to current 1st international standard).

The goal was to replace the currently used standards that are running low and to re-unite IU and NIH-U to a single unit. Preliminary results from 25 participating laboratories were presented.

Each lab received four ampoules of A (current international standard), two ampoules of B (US standard), and four ampoules each of the two new preparations, C and D.

Overall variability was 8.8 % in clotting and 6.5 % in chromogenic assays. There were no differences between human and bovine fibrinogen as substrate concerning activity and the value for plasma as substrate lower than with fibrinogen was not significant. Chromogenic assays displayed a trend towards higher levels of thrombin activity. Candidate C seemed to be quite similar to B, whereas D was close to A concerning amount of thrombin activity in clotting and chromogenic assays.

Sample D was selected as best candidate for determination of final potency. Geometric mean is 110 units per ampoule using A and B as standards and human and bovine fibrinogen as well as plasma.

There are no stability data yet, but since there are only very small quantities of preparations A and B left, replacement is urgent. Dr. Barrowcliffe commented that differences between IU and NIH U are up to 15 % in earlier studies, therefore, a common standard is a great achievement.

Carl-Erik Dempfle proposed a collaborative study on measurement of fibrinogen in animal plasma. Results of different studies on fibrinogen in various animal models showed a high degree of fibrinogen levels reported. A specific problem is imposed by the fact that current fibrinogen assays are optimized for use with human plasma, not with animal fibrinogen, and human plasma/fibrinogen is used as calibrator. Reactivity with enzyme (bovine thrombin,...) may be different from human fibrinogen, and the fibrin polymerization rate may be different from human fibrin. Presence of fibrin(ogen) degradation products influences clotting rate (underestimation of fibrinogen concentration). In optical methods, turbidity of fibrin clots may be different from the human system. Clot turbidity is also influenced by presence of fibrin(ogen) degradation products (overestimation in PT-derived fibrinogen). For PT-derived fibrinogen: Differences in PT influence rate of thrombin formation, and fibrin structure (overestimation of fibrinogen concentration in prolonged PT / high INR). It is suggested to study mouse, rat, rabbit and pig, and dog fibrinogen.

Study proposal consists of Phase 1: collection of plasma samples using defined protocol for blood collection, plasma preparation and storage. Fibrinogen concentration is measured by clot recovery method. Assays are screened. In Phase 2, aliquots of 3 plasma samples per species are given to participating laboratories for analysis. Phase 3 includes normal range studies for various animal species and subspecies, using the pooled plasma material of same species for calibration. Audience and readers are asked for support with collection of animal plasma samples, and analysis of samples. For help and support: E-mail: carl-erik.dempfle@med.ma.uni-heidelberg.de

Kunihiko Nakahara from Iatron Inc. presented data on a new soluble fibrin assay. The assay is based on MAb IF-43. IF-43 does not react with fibrinogen, but reacts with FM and fragments E of fibrin in immunoblot. In native material, the epitope alpha 52-78 is exposed only when FM binds to fibrinogen (or fragment D), resulting in a trimolecular complex with an apparent MW of 1 000 kD, equaling trimer of 2 x fibrinogen and 1 x FM. Using IF-43, a latex enhanced photometric immunoassay (LPIA) was developed. The assay uses serial dilutions of desAABB-FM in normal plasma for calibration. Human plasma may be replaced by bovine plasma for preparation of the calibrators. There was no correlation with D-dimer assay (Iatron) in clinical plasma samples. The time course in clinical samples (before/after surgery) differs both from TAT and D-dimer, with highest levels observed on days 2 and 3 after surgery. Elevated levels of soluble fibrin were found in patients with disseminated intravascular coagulation.

Gordon Lowe gave an update on fibrinogen and cardiovascular disease risk from the Fibrinogen Studies Collaboration (FSC), a collaborative meta-analysis of prospective studies of fibrinogen and risk of CHD and stroke. Since the collaboration started in 1999, individual data has been collected from 38 cohorts (total subjects included 137,000). After an additional 20 % of data,

10,000 coronary events and 2000 stroke events will be included.

At the next meeting of the ESC in 9/2002 in Berlin, preliminary analyses of CVD endpoints will be presented.

A sub-analysis on type of fibrinogen assay used and standardization issues will be discussed. Input from the Fibrinogen Subcommittee was invited. Discussions included recalibration with the high fibrinogen standard.

Next steps of Fibrinogen Subcommittee:

- Gordon Lowe: fibrinogen as a risk factor; standardization and re-evaluation of epidemiological studies.
- Nicodemo Weinstock: Establishment of generally accepted reference values and risk percentiles by re-measuring available samples of risk factor studies at a central laboratory using the newly established high fibrinogen standard. Correlation of high fibrinogen values from different fibrinogen assays.
- Carl-Erik Dempfle: Initiation of study on standardization of fibrinogen measurement in animal models, in collaboration with Susan Lord.
- Colin Longstaff: Stability studies on thrombin standards.

N. Weinstock
C.E. Dempfle
G.D.O. Lowe

Fibrinogen

July 12, 2003

14:00 to 18:00

Hall 10

The International Convention Center, Birmingham

Acting Chairman: S. Lord, USA

Co-chairs: J. Koopman, The Netherlands; R. McIntosh, UK; N. Weinstock, Germany

Approximately 50 persons attended this session, chaired by S. Lord. Co-chairs Koopman, McIntosh and Weinstock were not present.

S. Lord (USA) led a discussion of the proposal that the FXIII subcommittee merge with the fibrinogen subcommittee. Advantages and disadvantages were discussed. There is significant overlap, but concern was expressed that issues of interest to each group—for example, dysfibrinogen characterization in the fibrinogen SSC—would be diminished in a merged group. It was suggested that a joint session for the two subcommittees be organized in 2004, and the issue of merger reconsidered in light of the joint session.

M. de Maat (Netherlands) reported on the impact of fibrinogen heterogeneity on clinical assays and cell growth. For the clinical assays she focused on the forms of fibrinogen known as HMW, LMW and LMW' and the contribution of these forms to immunoassays for fibrinogen. The influence of the splice variants g' and aE were also reported. Examination of two fractions of fibrinogen showed endothelial cell growth and differentiation differed, indicating that specific forms of fibrinogen influence cellular properties.

H. Tanaka (Japan) reported on a new immunoassay to measure elastase-driven fibrinogen fragments in plasma. Characterization of a monoclonal antibody, IF123, showed both specificity and sensitivity. This assay enables the measurement of elastase degradation of fibrinogen as a marker for disease states such as sepsis.

S. Lord presented a proposal from **J. Koopman** (Netherlands) to standardize procedures to correlate structure and function of human fibrinogen variants. The proposal suggested a working plan to establish such procedures. Discussion of this plan led to the suggestion that a set of minimal standard parameters be listed on the WEB site of dysfibrinogens that is maintained by **F. Hanss** (France).

S. Lord discussed the project to set standards for fibrinogen-related measures in mice. She reported that the subcommittee on animal models planned to address similar issues for multiple coagulation factors. She reported that she will participate in this activity with the animal models subcommittee. She requested that interested individuals contact her to assist in which measurements and what methods would be recommended.

C. Longstaff (UK) reported on the newly established WHO/FDA standard for thrombin, which

is now available in vials of 110 units.

T. Barrowcliffe (UK) and **S. Raut** (UK) were prepared to report on standards for high fibrinogen in plasma and FXIII, respectively. Unfortunately, these individuals were detained at conflicting sessions and therefore unable to present at the fibrinogen session.

Factor XIII and Fibrinogen Joint Meeting

June 17, 2004

14:00 to 18:00

Cipressi

Fondazione Giorgio Cini

Chairmen:

Robert Ariëns (Factor XIII)

Nicodermo Weinstock (Fibrinogen)

Co-Chairmen:

Paul Bishop, Akitada Ichinose (Factor XIII)

Jaap Koopman, Susan Lord, Ron McIntosh (Fibrinogen)

Active Members:

Charles Greenberg, Hans Kohler, Laszlo Muszbek, Reiner Seitz (Factor XIII)

The FXIII and fibrinogen subcommittees held a joint meeting this year. There were approximately 30-70 interested researchers present and the presentations were followed by lively discussions. Thanks to recent efforts in the first international collaborative study for FXIII standardisation, a large part of the meeting was dedicated to the presentation of these data.

I. Measurements of FXIII

The meeting was opened with a presentation from **Trevor Barrowcliffe** (NIBSC, UK) regarding the general principles for standardisation of coagulation factors and inhibitors. Standardisation efforts are normally composed of several stages including preliminary investigation of materials suitable for standardisation, trial fills, stability studies, large-scale fills, international collaborative studies, and the final report for recommendation through the SSC/ISTH to the WHO. Once a standard has met approval by the WHO, continuity of the unit can be assured through either long-term usage of same standard, replacement by similar material, and/or crosschecks versus normal plasma or previous standards. Stability studies can be performed at a higher temperature to accelerate the procedure, but high-temperature degradation studies may overestimate stability. Stability studies are best performed in more than one lab. An important issue for standardisation is to compare 'like' with 'like', for example use a plasma standard for plasma measurements. Assay methodology can play a role in potential inconsistencies. However, reference methodology is normally not easily definable, though some pre-description for protocol may be useful.

Sanj Raut (NIBSC, UK) presented a report on the first international collaborative study for a FXIII standard. Preliminary studies had shown improved consistency between activity assays when FXIII deficient plasma was used as diluent rather than buffer. Large-scale fills and the questionnaire/recruitment stages were successfully completed to proceed with the international collaborative study. Samples used were X - a 40 fold concentrate of FXIII, Y - the proposed first international standard for FXIII, A - plasma lot 2 and B - plasma lot 3. FXIII deficient plasma

was provided as reagent to all participating labs. In total, 23 labs returned data, there were 23 activity and 10 antigen measurements. Potency and variability versus pooled plasma and candidate Y were assessed. There was a good consistency of the measurement of FXIII activity for candidate Y with a potency of 0.91 u/ml and an inter-laboratory CV of 11.5%. Antigen measurements were mostly consistent with this although showed somewhat greater variability. Data for the FXIII concentrate showed some discrepancy between methods and this will need further consideration. A detailed report has been submitted to all co-chairs, active members of the FXIII SSC and participants of the Collaborative Study for feedback and approval prior to submission to the SSC.

Laszlo Muszbek (Hungary) discussed the measurement of FXIII activity in tissue sealants and concentrates. The activation of FXIII is greatly enhanced by the conversion of fibrinogen into fibrin and its subsequent polymerisation. There are therefore important considerations to be made whether FXIII activity is measured in the presence or absence of polymerising fibrin. Dilution of samples in buffer or FXIII deficient plasma can change the concentration of fibrin(ogen) present and hence activation. As a result of the presence of fibrinogen it was found that FXIII was best measured in plasma as opposed to citrate buffer. In addition FXIII appears to be more stable when diluted in plasma when compared with buffer. When fibrin sealants are diluted 20-40 fold a physiological concentration of FXIII is found.

In the last presentation of this section, **Janos Kappelmayer** (Hungary) discussed data regarding the assessment of FXIII expression on various cells by flow cytometry. Lymphocytes in M5 leukaemia stained strongly positive to both CD14 and FXIII A-subunit. FXIII A-subunit expression correlated positively with CD14. The expression level of FXIII A-subunit increased significantly from M0 to M0-2 and the highest staining was observed in M4. FXIII A-subunit expression on cells was in increasing order M0, M4, M5, CMML and PLT. It was concluded that FXIII A-subunit is an early marker of haematopoietic development in monocyte lineage.

II. Developments in treatment of FXIII deficiency

Aki Ichinose (Japan) presented an update on the studies on FXIII gene-knockout mice. Transglutamination can be considered as a major post-translational modification in proteins. There are up to 9 different human transglutaminases known to date, and an array of diseases ranging from neurological disorders, bleeding/thrombosis, cancer and hepatic disorders amongst others have been associated with malfunctions of transglutaminases. In order to determine respective functions of FXIII, a mouse model was established in which the FXIII A-subunit and B-subunit genes were targeted respectively. FXIII B-subunit gene knockout was performed by targeting exons 1 and 2. Blotting confirmed that B-subunit was absent in plasma. Although B is a carrier for A, some A-subunit appeared to be still circulating in the plasma of B-knockout mice. Interestingly, no major pathological defect was found for the FXIII B-deficient mice. It may be possible that B knockout mice are normal unless challenged for bleeding. To a certain extent in agreement with this, B-deficiency in humans is normally associated with a milder bleeding disorder than A-deficiency. The A knockout mice showed complete absence of A in plasma as determined by blotting. These mice showed excessive bleeding. Male mice died earlier from bleeding than female mice if the latter were not used for breeding. However, the female mice showed excessive bleeding, with necrosis and bleeding in the uterus and placenta, upon

pregnancy. Miscarriage in the A-subunit knockout mice was due to bleeding and not implantation defects for example. It was concluded that FXIII gene knockout mice provide a good model for human FXIII deficiency.

In the next presentation, **Ken Lewis** (USA) discussed recent data on the biochemical – physiological analysis of recombinant FXIII A-subunit infusion in humans. Single doses up to 50 U/kg of rFXIII A appeared safe, with a dose-response of 1.77% per U/kg. Five daily doses were assessed and the relationship of FXIII half-life to doses and B-subunit levels were investigated. Pharmacodynamics showed a much reduced half-life in a FXIII B-deficient subject. In normal subjects, there were differences in the levels of total A-subunit, A2B2 complex, and B-subunit levels in response to rFXIII A infusion. There was normal affinity of rFXIII A for B, leading to spontaneous and rapid A2B2 formation. Activity levels increased more than A2B2 levels suggesting saturation of B. The approximate half-lives were determined as 30 hrs for A2, 8.5 days for A2B2 and a surprisingly short 16.7 hrs for B. Overall, rFXIII A appeared to behave according to expectations based on these pre-clinical and biochemical studies.

III. Fibrinogen interactions

Leonid Medved (USA) presented structural data on molecular interaction between thrombin and fibrinogen E-region. X-ray crystallography was performed on the complex of thrombin and the fibrinogen E-region produced by cleavage with the leech enzyme hementin. Hementin uniquely produces an E-fragment with intact fibrinopeptides A and B. The crystal complex showed two thrombin molecules on either side of the fibrinogen E-region and was resolved at a resolution of 3.6 Å. It was shown that thrombin interacts with the E-region of fibrinogen through exosite I. Superimposing the structures of hementin E-fragment with that of the thrombin exosite I showed that the exosite is only partly involved. The orientation of thrombin on the E-region through exosite I means that the catalytic triad is located to the side of the molecule at a certain distance from the fibrinopeptides. A model was proposed to explain the preferred cleavage of FpA. FpA extends to the catalytic triad of thrombin, whereas FpB demonstrates a more random orientation. After cleavage of FpA, FpB assumes a conformation that orientates it towards the thrombin catalytic triad. Molecular modelling was used to support this theory and the structural changes in molecular confirmation were shown by computed animation.

IV. Clinical implications

Gordon Lowe (UK) discussed the role of fibrinogen in atherothrombotic disease. There is strong support for a consistent association between fibrinogen levels and atherothrombotic disease. The questions that remain include whether fibrinogen is a causal factor for disease and, related to this, whether lowering fibrinogen concentration reduces risk. Several potential mechanisms were discussed. Fibrinogen is involved in fibrin clot formation, fibrin structure/function, platelet aggregation, cell adhesion, erythrocyte aggregation and determines plasma viscosity. It is perhaps the latter that in addition to fibrinogen itself shows the most significant association with disease. Associations have been described between fibrinogen and/or plasma viscosity with carotid intima-media thickness or claudication. In the latter, bezafibrate reduced fibrinogen by around 14%, along with decreased cholesterol and red cell aggregation, and improved walking distance. Exercise has been shown to lower fibrinogen and to alter plasma viscosity. There is

evidence to suggest that not only increased fibrinogen associates with vascular disease but also alterations in structure/function of fibrinogen, such as effects of oxidation and other post-translational modifications. Fibrinogen levels significantly alter the risk for myocardial infarction or stroke even after adjustment for all known other risk factors. However, associations between genetic polymorphisms that alter gene expression and risk for disease are inconsistent. It was concluded that fibrinogen levels are significantly associated with vascular disease and that this association may be causal (although this requires further investigation), but that at the moment there does not seem to be a clinical utility in measuring fibrinogen, as fibrinogen-lowering drugs are currently not an option for the treatment of vascular disease.

Hans Kohler (Switzerland) discussed the clinical relevance of FXIII assays. It was noted that there are several different types of FXIII assays and that there are differences in the definition of activity in some of these. The NEQAS study has shown that with some FXIII assays in some labs there is a high percentage of misclassification of FXIII deficiency with measurements up to 50% of normal, which clearly identifies the need for standardisation. A better characterisation of FXIII deficiency is obtained through the usage of ELISA methods for the separate FXIII subunits. An additional problem is different responses of activity assays to the Val34Leu polymorphism. A poor correlation exists between activity assays that measure either incorporation of a small amine or the generation of ammonia by NADH respectively, but this correlation improves on separate analysis of the Val34Leu genotypes. Assays that are sensitive to the activation step by thrombin (which is affected by Val34Leu) may need to be altered to achieve full activation before assay. It was also noted in the discussion that it is important to include a blank to control for basal NADH oxidation of plasma in assays that are based on the measurement of the amount of ammonia released during the cross-linking reaction.

The joint meeting of the FXIII and fibrinogen SSC's was concluded at 6.15 pm, after a lively discussion regarding assay methodological and other issues.

Fibrinogen

August 7, 2005

8:30 to 13:30

Ballroom 1

Sydney Convention and Exhibition Centre

Chairman: Nicodemo Weinstock

Co-Chairmen: Jaap Koopman, Susan Lord, Moniek de Maat, Leonid Medved, John Weisel

Active Members: Michel Hanss, Wolfgang Miesbach

The FXIII and fibrinogen subcommittees held a joint meeting this year. There were more than 150 interested researchers present and the presentations were followed by lively discussions. Due to a busy agenda and discussions the meeting was about one hour over time but it was appreciated that most of the delegates were present till the end.

Michel Hanss described the exhaustive and continuous numbering of published variants has been started on the Internet beginning in the year 2000 with 219 cases thanks to the GEHT (groupe d'étude d'hemostase et thrombose) website , <http://www.geht.org/databaseang/fibrinogen> (SSC 2000). It now references 363 entries with a noticeable increase in a/hypofibrinogens and amyloidosis related dysfibrinogens. The referenced cases are identified with affected protein/gene position main symptoms, first author and year of publication. Minimum biological and clinical characteristics have been proposed in order to provide possible on-line registration of new cases. This meeting gave a good opportunity to enlarge the database due to new contacts with researchers in this field such as Wolfgang Miesbach, Shirley Utte de Willige and others.

Susan Lord spoke about recombinant fibrinogen polymerization and Factor XIII binding. In particular, this research project concerned the specific binding of Factor XIII to the γ' chain of fibrinogen. In adding Factor XIII to normal plasma fibrinogen, the final turbidity increased considerably. However, the recombinant $\gamma\gamma$, $\gamma\gamma'$ or mixtures of them did not show any effect on turbidity. These results may contribute to the standardization of determination fibrinogen levels.

Leonid Medved presented a review of fibrinogen polymerization and fibrinolysis. As part of his review, he included new x-ray crystallographic data on the binding of thrombin to fibrinogen, which provides evidence on the mechanisms involved. For the process of fibrinolysis, there are epitopes that are cryptic in fibrinogen and exposed through conformational changes and are important for binding of tPA and plasminogen. The new view facilitates the understanding of fibrin clot formation and dissolution now becoming more important for the understanding of bleeding and thromboembolic diseases in patients.

John Weisel spoke about the unique and remarkable viscoelastic properties of fibrin and their clinical significance: clot stiffness is necessary for hemostasis, the strength and integrity of the clot may be more important than parameters more commonly measured in the coagulation lab,

clot plasticity may be necessary to prevent obstruction, they determine whether embolization may occur and the response to treatments such as angioplasty, thrombolysis and surgery, and epidemiological studies suggest a correlation between clot stiffness and MI. Fibrin is a polymer with both elastic and viscous aspects, which were outlined, but we know little about the origin of either of these components. The research required to determine these mechanisms will require interactions between clinicians and basic scientists.

Wolfgang Miesbach presented studies of 350 cases involving 170 distinct mutations, of which 56% were asymptomatic, 25% bleeding or easy bruising, and 20% were thrombotic. There were two mechanisms involved in the thrombotic cases, either defective thrombin binding or defective lysis. Arterial thrombosis was overrepresented in these cases. The cases were characterized in terms of clinical symptoms and the molecular defects. In the same families with identical defect there could be a heterogeneous or even opposite manifestation of symptoms (e.g., bleeding and thrombosis). These cases will be further studied to try to determine the mechanism or to find further markers that could explain these unexpected results.

Jaap Koopman proposed that we standardize our toolbox used to characterize fibrinogen. There are two general sources of variation, natural variation in structure/function and methodological variation. There is little consistency in the parameters that are studied or in the analytical methods or the reference material. There is much natural variation in fibrinogen that is important for its characteristics. The parameters to be used to characterize fibrinogen need to be standardized. The following methods were suggested: SDS-PAGE, HIC (HMW/LMW ratio), ion exchange chromatography, turbidity curves, FPA/B release, and FXIII, plasminogen and fibronectin impurities.

Nicodemo Weinstock stressed that fibrinogen - though it is well known to be the central molecule in the clot formation and shows risk odd ratios comparable or higher than cholesterol - has been almost forgotten by the clinical community. The consequence: the number of fibrinogen determinations is dramatically falling. The reasons are: 1.) poor standardization for fibrinogen measurements 2.) mostly incorrect normal values determined in the 70ties (e.g. 150-450 mg/dl) 3.) strong variation of "normal mean values" (e.g from 230 up to 390 mg/dl) and cut offs(280; 300, 350, 380, 400) reported in large risk studies made in the 90ties. Therefore the opinions of what is normal, what is high or low, who or when persons are at risk, vary from lab to lab. The consequence is confusion and the feeling that fibrinogen is an invaluable parameter. The opinion was that these items should be overcome as soon as possible and that this should be part of the fibrinogen subcommittee work to be done in the next years.

The fibrinogen subcommittee decided to form working parties for further studies e.g.:

1. standardization of nomenclature (Koopman, Medved, Weisel)
2. reference values (Miesbach)
3. introduction of the new high fibrinogen standard (Weinstock)
4. studies on different methods of fibrinogen determination and correlation to CRP in cardiovascular disease study on 15,000 patients starting in October 2005 (Pretz)
5. standardization of methods (Koopman)

Fibrinogen

Chairman: Nicodemo Weinstock

Co-Chairmen: Moniek de Maat, Jaap Koopmann, Leonid Medved, John Weisel, Dieter Peetz

Active Members: Michel Hanss, Bernhard Laemmle, Michael Meyer, Winfried Plesch, Benny Sørensen, Michael Spannagl, Erwin Strasser

The FXIII and fibrinogen subcommittees held again a joint meeting this year. There were more than 80 interested researchers present and the presentations were followed by lively discussions.

Leonid Medved : spoke on fibrinogen nomenclature, making recommendations based on the commonly used terminology for the regions, modules and domains of fibrinogen. With increasing information on fibrinogen structure now available, a more detailed description is possible and some older terminology is no longer appropriate and should be avoided. A proposal for fibrinogen and fibrin nomenclature will be prepared in the form of a paper for publication.

John Weisel: continued with nomenclature related to fibrin polymerization. Since the polymerization sites in fibrin have been partially characterized and named, these names should be incorporated into the nomenclature of the field. In addition, terminology for the intermediates in fibrin polymerization were proposed. Finally, there was discussion of ‘crosslinking’ versus ‘ligation’ to describe the action of Factor XIIIa on fibrin.

Jaap Koopman: described the difficulties of standardization of names of fibrinogen variants, with over a million possibilities. Therefore, it is only possible to name the most common variants, based on primary amino acid sequence, modification of amino acids, source of production/synthesis, and oligomeric structure. A logical system for abbreviations describing these aspects of fibrinogen variants was proposed.

Moniek de Maat: then provided evidence on the effects of fibrinogen heterogeneity on fibrinogen assays. In comparison of clottable protein assays, clotting rate assays, PT-derived assays, and other newly developed assays, normal fibrinogen behaves consistently but variant fibrinogens demonstrate different functional properties and give different results for these assays.

For all of these presentations above, the proposals will be formalized in two papers, approved by the Fibrinogen SSC, and published in the ISTH journal, so that these aspects of fibrinogen nomenclature will become widely known and used, Dr. Weinstock urged timely preparation and publication of these papers.

Dieter Peetz: presented the study design of the Prevent-it (Proteomics, Genomics and Vascular Endothelial Dysfunction) trial, an epidemiological prospective cohort study for cardiovascular risk stratification. Primary objective of the Prevent-it trial is the development of a score for cardiovascular risk stratification taking into account biochemical variability (including fibrinogen), exogenous influencing factors and the extent of subclinical disease. Overall 16.400 German inhabitants between age 35 and 75 will be included and examined at baseline (complete clinical examination) as well as 2.5 years (follow-up 1, interview) and 4.5 years (follow-up 2, complete examination as at baseline) afterwards. Baseline and Follow-up 2 examinations include

blood sampling with acquisition of plasma/serum, DNA, RNA and platelet rich plasma aliquots stored at -80 °C to establish a large prospective biobank. Primary objective of the Prevent-it trial regarding fibrinogen measurement is the evaluation of a “standardized” fibrinogen cutoff (same cutoff for fibrinogen methods in routine use) included in a cardiovascular risk score. Prerequisite for this aim is the standardization of methods including the new first high fibrinogen reference material. This project will be performed in cooperation with Dade Behring. Fibrinogen methods to be performed are: clotting rate methods [Clauss diluted (optical/mechanical) and Clauss undiluted (optical/mechanical)], PT-derived methods (turbidimetric), immunological (nephelometric) and indirect methods (viscosimetry). Additionally, in a whole genome approach association studies regarding fibrinogen will be possible as samples will be measured with the Affymetrix 500k array set.

Dr. de Maat announced that she would like to cooperate in this study by measurement of selected samples (nested case-control sets) with specialized assays for fibrinogen variants.

Winfried Plesh: stressed that OAC-control with a point of care testing device shows some relation between fibrinogen concentration and INR, but this influence is comparable to differences seen by the Prothrombin time method. Thus INR determination by POCT gives reliable results. Some studies will be done with blood of dysfibrinogenic patients and patients having large amounts of fibrinogen subspecies.

Benny Sørensen: spoke on the importance of the different patho-ethiology of acquired fibrinogen deficiencies. He considered acquired reduced levels due to e.g. fibrinolysis and increased consumption or to conditions of dysfunctional fibrinogen, defect synthesis and the bleeding tendency related to different diseases like Multiple Myeloma and Autoimmune diseases. He stressed on the importance of combined conditions like reduced and dysfunctional fibrinogen and exogenic interferences with polymerization, they are often the reason for massive bleeding and specially ongoing bleedings. There are critical levels for substitution when fibrinogen is < 100 mg/dl but sometimes substitution is even helpful when the levels were > than 200 mg/dl. Further items were the dependence of fibrinogen levels as measured by different methods. It was decided to prepare a publication on suggestions how and when to substitute. Dead line for all SSC members to get in contact with Benny to give comments and suggestions will be September this year and a peer reviewed paper should be prepared as an SSC Official communication the end of November 2006. The work will be done in strong collaboration with Wolfgang Korte, Uri Seligsohn and Michael Spannagl.

Erwin Strasser: presented data on the effect of haemodilution. He compared different solutions like Ringer, HAES or saline on different tests specially focusing on fibrinogen. Clotting tests were compared with immunological tests (RID) and clot firmness as measured by ROTEM-technique. Clotting tests were significantly influenced by dilution but results differed with different test and different solutions. The study will go on trying to find out if and how these differences are related to the clinical situation.

Michael Spannagl: spoke on proficiency testing and the central role of influence the of fibrinogen. There is a large difference between proficiency testing in coagulation compared to clinical chemical parameters. In coagulation we still need consensus values and it will take time

and a lot of standardization work to get to target values, if ever. To get suitable control material - that fairly compares to a fresh pooled plasma - is difficult and partly a problem of costs. At the end of his talk he presented a new study focused on fibrinogen and patient substitution in haemostaseology intensive critical care called HICC. This study approved by the SSC Fibrinogen will begin in September this year.

Nico Weinstock: showed that using D-Dimer, Fibrinogen and CRP in Pulmonary Embolism(PE) the PPV (positive predictive value) could be raised from about 25% to 80%. Thus the intruding differential diagnosis of PE will be greatly facilitated. Using these algorithm 90% of all patient can correctly classified in PE-pos. or PE-neg. avoiding the very unpractical, costly and/or dangerous Pulmonary Angiography or Helical Computer tomography.

Nico Weinstock: presented and discussed the value of Clauss fibrinogen. The clear message was: **CLAUSS does not measure** functional fibrinogen not even **fibrinogen!!!** Clauss measures a time of clot formation. This time depends on many - functional fibrinogen independent – factors. His suggestions were to routinely use derived fibrinogen in combination with the Clauss method. Whenever there is a difference between the two methods there will be a surprising result hid behind. “We will get a lot more of information on coagulation and coagulation defects in doing so”. Many results presented by the different speakers at this meeting support these facts.