1996 MINUTES
FACTOR XIII SUBCOMMITTEE

Saturday, 22 June, 1996, 13.00 - 17.00
Room Safir, Fira Palace Hotel
Barcelona, Spain

Chair: Laszlo Muszbek, Hungary

1. The chairman of the session announced that Dr. Jan McDonagh, the chairperson of the Subcommittee, could not attend the meeting, and on the request of ISTH/SSC, Dr. Muszbek, as one of the two co-chairmen of the subcommittee, organized this meeting and serves as acting chairman. Any proposal made by the subcommittee will be forwarded to Dr. McDonagh.

2. Past year activity: a recommendation for the nomenclature of blood coagulation factor XIII (FXIII) that has been put in its final form is ready for publication in Thrombosis & Haemostasis. The manuscript has been sent to Dr. McDonagh but, probably due to miscommunication, no further decision has been made in this matter. Dr. Muszbek will try to contact her again and, hopefully, the manuscript will reach SSC in the near future.

3. The chairman announced a few changes in the program. Two speakers, Drs. Vivien Yee and Róza Ádány, due to other engagements, could not attend the meeting. As a replacement, Dr. Bishop had an additional presentation on "FXIII replacement therapy in the treatment of inflammatory bowel disease: An animal model study."

4. Program: A. Progress report on the ETRO survey of factor XIII deficient patients. Rainer Seitz (Langen, Germany) and Alberto Tosetto (Vicenza, Italy) summarized the present stage of the survey initiated by the Factor XIII Working Party of the European Thrombosis Research Organization (ETRO) two and a half years ago. Sending a questionnaire to major European haemostasis centers and to all ETRO laboratories and publishing the questionnaire in major thrombosis haemostasis journals have resulted in the collection of data from 72 patients of 62 families. Now a second questionnaire has been formulated and mailed to all responders. The data collected so far have revealed some interesting aspects of FXIII deficiencies. The severity of this bleeding diathesis seems to vary more considerably than it was believed and no obvious relationship with the level of FXIII seems to exist. At this point, however, it was emphasized that the data obtained are based on different FXIII assays performed in different laboratories and caution in the interpretation of the results is needed. This problem underlines the need of standardization of FXIII assays and repeating the measurements on plasma samples distributed to three laboratories with special expertise on assaying FXIII. There is no common policy on the supplementation therapy of patients with severe FXIII deficiency. Some laboratories consider it absolutely essential to initiate life-long replacement therapy at the time of the diagnosis while at the other extreme some laboratories recommend replacement therapy only when bleeding complications occur or before surgical interventions. A consensus conference seems to be needed in this respect. It could also be firmly established that impaired wound healing, although frequently (30-40%) accompanying FXIII deficiency, is not an essential symptom of the disease.

Interestingly, and unexpectedly, there were reports on a few heterozygous patients with mild to moderate bleeding tendency.

One of the aims of the survey was to collect samples from a relatively higher number of FXIII deficient patients for molecular genetic analysis. This work has been started, some samples have been collected and analyzed in the Department of Clinical Chemistry, University of Helsinki and part of the results have already been published. Hanna Mikkola from Helsinki presented a comparative report on the molecular genetics of factor XIII deficiencies reviewing their own results and the data reported in the literature. Among others, an interesting case was presented in which the nature of the molecular genetic defect explains the relatively mild phenotype of a patient with severely decreased FXIII activity.

The following recommendations were made by the subcommittee:

a. The survey is to be kept open to be able to register further patients and the establishment of a permanent registry was proposed.

b. There was general agreement on the benefit of extending the survey beyond Europe. However, no one among the participants was willing to accept such a responsibility. Therefore, it was decided to go on with the survey in Europe and bring up this matter again at the next subcommittee meeting.

c. To be able to reach more solid conclusion on the relationship of FXIII activity and the severity of bleeding diathesis, it was recommended to collect plasma and possibly platelet samples from the patients included in the registry and analyze them for FXIII activity and antigen in three independent laboratories (Vicenza, Langen and Debrecen).

B. At the previous subcommittee meeting the issue of establishing an international reference FXIII preparation was raised and at the present meeting Dr. Paul Bishop (Seattle, USA) presented data on the properties of recombinant cellular FXIII (FXIII A2) to decide if such a preparation could satisfy the requirements of a reference preparation. The data presented on the stability, kinetic parameters and the comparison of rFXIII with native FXIII are promising and clearly suggest the feasibility of such a move.

It was recommended that an action plan on steps to achieve this goal be worked out for the next subcommittee meeting.

C. The policy of the laboratory diagnosis of FXIII deficiency was dealt with by Dr. Muszbek (Debrecen, Hungary). It was recommended to abandon the use of the clot solubility test as the sole screening test for FXIII deficiencies because it misses part of the FXIII deficiencies. An algorithm for achieving the diagnosis was presented and agreed upon by the attendees. The issue of the poor standardization of FXIII assays was elaborated and illustrated by several examples. A preliminary evaluation of the single commercially available FXIII activity assay (Behringwerke, Germany) was presented and weaknesses and advantages of the assay were pointed out.

The need for critical, independent, multicenter evaluation of new commercially marketed FXIII assays was emphasized by the discussants. A proposal for the policy of such evaluations will be worked out by a group of experts for the next subcommittee meeting. It was recommended that the appropriate committee of the International Federation of Clinical Chemistry be contacted and that we join forces concerning the standardization and evaluation of FXIII assays.

1997 MINUTES

FACTOR XIII SUBCOMMITTEE

Saturday, 7 June, 1997, 13:00-16:30
Tiziano, Fortezza da Basso
Florence, Italy

Chair: L. Muszbek, Hungary
Co-Chairs: P. Board, Australia; C. Greenberg, USA; A. Ichinose, Japan;
J. McDonagh, USA

Attendance: about 100 attendees.

1. **Nomenclature**

At an earlier meeting of the Subcommittee a proposal for the recommended terminology concerning blood coagulation factor XIII had been presented and discussed. At that time, the Subcommittee suggested slight modifications and asked L. Muszbek (Hungary) to resubmit the modified version to the Subcommittee for approval. The revised version was presented by L. Muszbek and discussed by the members of the Subcommittee. The proposal consists of three parts, the main points are outlined below:

a. Nomenclature of blood coagulation factor XIII (FXIII) from different sources.

Blood coagulation factor XIII present in the plasma (having the tetrameric structure A_2B_2):

plasma FXIII, (pFXIII)

Blood coagulation factor XIII present in cells (in platelets, megakaryocytes, monocytes and macrophages and having the dimeric structure A_2):

cellular FXIII (cFXIII)

Recombinant cellular factor XIII:

recombinant FXIII (rFXIII)

b. Designation of blood coagulation factor XIII subunits.

Recommended term for the potentially active subunit present both in plasma and cellular FXIII:

A (FXIII-A)

Recommended term for the inhibitory subunit present in plasma but not in cellular FXIII and also present as non-complexed free form in the plasma:

B (FXIII-B)

c. Designation of activation intermediates and end-products of blood coagulation factor XIII.

Active form of blood coagulation factor XIII in general:	FXIIIa
Thrombin-cleaved inactive form of plasma FXIII:	pFXIIIa'
Thrombin-cleaved inactive form of cellular FXIII:	cFXIIIa'
Thrombin-cleaved active form of FXIII:	FXIIIa*
Non-cleaved active form of FXIII:	FXIIIa¡
Thrombin-cleaved inactive form of subunit A:	A'
Thrombin-cleaved active form of subunit A:	A*
Non-cleaved active form of subunit A:	A¡
Activation peptide cleaved off from the A subunit:	AP-FXIII

Points (a) and (b) were unanimously approved by Subcommittee members, point (c) was supported by the majority. Regarding point (c), some questioned the need for such a detailed proposal on activation intermediates while the supporters argued that abbreviations for activation intermediates and end-products are already in use in the literature and the lack of accepted terminology causes considerable confusion). L. Muszbek was given the task of preparing the final form of the manuscript which will then be submitted to SSC for vote and publication.

2. Registry of FXIII deficient patients

A European registry has been set up by the European Thrombosis Research Organization Factor XIII Working Party. The question was whether to expand the European registry to make it a world-wide registry and thus make it a Subcommittee activity.

R. Seitz (Germany) gave an account of the present stage of the registry and outlined certain points of clinical appearance and supplementation therapy of FXIII deficiency based on the information provided by the survey. H. Mikkola (Finland) presented the results of a comprehensive molecular genetic investigation of FXIII deficient patients that was made possible by the European registry. A. Ichinose (Japan) and P. Board (Australia) provided additional information on FXIII-deficient patients in Japan and Australia, respectively.

Although a world-wide registry on FXIII-deficient patients did not seem a realistic goal for the near future, the creation of a database on the molecular genetic defects of FXIII-deficient patients was approved by the Subcommittee. A. Inbal (Israel) volunteered to set up the database.

3. Further issues

R. Ádány (Hungary) reviewed data on the site of synthesis of FXIII subunits and made methodological recommendations for further studies on this area. P. Bishop (USA) presented an animal model of inflammatory bowel diseases which seems to be applicable for studying the beneficial effect of FXIII concentrate in such cases. C. Greenberg (USA) provided data on the use of site-directed mutagenesis in studying structural-functional aspects of FXIII subunits.

1998 MINUTES
FACTOR XIII
Monday, 22 June, 1998, 8:00-12:00
Cankarjev Dom
Ljubljana, Slovenia
Chair: L. Muszbek, Hungary
Co-Chairs: P. Board, Australia, C.S. Greenberg, USA,
A. Ichinose, Japan

The Subcommittee Meeting was chaired by A. Ichinose and L. Muszbek, C.S. Greenberg and P. Board could not attend. Attendance was approximately 50 throughout the meeting. Many valuable remarks, comments and questions came from the audience, and after each presentation there was a lively discussion. The Subcommittee concentrated on four different issues:

1. Role of FXIII-A subunit polymorphism in vascular diseases.
2. Methodology and the need for standardization of FXIII assays.
3. Animal models for inflammatory bowel disease (IBD) in which the effectiveness of FXIII supplementation could be tested.
4. FXIII B-subunit deficiencies.

1.

Dr. P. Grant gave an overview of the most recent findings on FXIII-A polymorphism in different vascular diseases. Val34Leu was the only mutation among the several they tested which as a protective mutation showed (inverse) correlation with arterial diseases. It was also demonstrated that the cardioprotective effect was lost if PAI-1 level was high or if the patients had insulin resistance. The wild type was more common in DVT as well. There was an interesting discussion about the possible mechanism of the protective effect with special reference to the fact that the mutation is three amino acid residues up-stream of the thrombin cleavage-site.

2.

Dr. Muszbek gave a critical review of FXIII methodology with a proposal for the requirements of reliable FXIII assays which are aimed to be used widely in clinical laboratories. Then, he presented two new assays, sample kits are about to be distributed among interested expert laboratories for evaluation. The functional assay was a modification of the UV spectrophotometric assay based on monitoring ammonia released during the transglutaminase reaction. The antigen assay was a one-step sandwich ELISA which detected only plasma FXIII, i.e., the tetrameric complex of the two subunits, and showed remarkable sensitivity.

Dr. Jennings from UK NEQAS presented the first external quality assessment survey on FXIII determination. A relatively high number of laboratories participated and the performance of classic clot solubility test was surveyed. There was a surprising variation in the set-up of the test and accordingly the results also varied considerably. Samples from patients with severe inherited and acquired deficiencies were not recognized as abnormal by 60% and 30% of the laboratories, respectively. There was a long discussion on the use of the clot solubility test, with the general conclusion that, although it still has a place in the diagnostics of FXIII deficiencies, the general practice of using it as a

"screening test" should be abandoned. UK NEQUAS was encouraged to continue the survey and to include FXIII determination in its coagulation survey profile.
Dr. Longstaff from NIBSC presented a talk on the possible developments of FXIII reference plasma. The audience highly supported the idea and the need for such a reference material was emphasised and supported. A list of participating expert laboratories is to be provided to NIBSC by the chairman of the Subcommittee.

3.

Dr. Bishop presented pieces of evidence demonstrating that dextrane sulphate-induced inflammatory bowel disease in mice could be a useful model for investigating the mechanism of beneficial effect of FXIII supplementation in IBD.

4.

Dr. Ichinose reviewed the cases of FXIII-B deficiencies and the underlying molecular genetic defects, and the classification of FXIII deficiencies was discussed.

In the general discussion it was recommended that promotion of the development of a reference plasma should be number one priority of the Subcommittee during the coming year.

1999 MINUTES

FACTOR XIII

Sunday, 15 August 1999

8:00 to 12:00 PM

Room 38

Washington Convention Center

Washington, DC

Chair: L. Muszbek, Hungary

Co-Chairs: P. Bishop, USA; P.G. Board, Australia;

C.S. Greenberg, USA; A. Ichinose, Japan

There was an acceptable attendance throughout the subcommittee meeting. The number of attendees varied between 50 to 70. A short additional presentation (Wartiowara and coworkers, University of Helsinki, "Different rate of thrombin activation of wild type and 34Leu mutant Factor XIII (FXIII) A") was added to the original program.

Dr. Yee (USA) provided the audience with a general state of the art overview on the three-dimensional structure of Factor XIII A subunit (FXIII-A) which was an introduction to some of the problems elaborated by other speakers.

Dr. Greenberg (USA) elaborated how site-directed mutagenesis could be utilized to study the biochemical mechanism of FXIII activation and inactivation. As an example he used mutations at the Ca^{2+} binding site of FXIII-A to demonstrate the usefulness of such an approach in studying structural-functional relationship in the mechanism of the activation process.

Dr. Adany (Hungary) gave a detailed methodological overview on measuring the gene expression by quantitative PCR, in general, and measuring the expression of FXIII-A, in particular. She demonstrated how to overcome technical difficulties and how to complete the findings with morphological techniques by monitoring the expression of FXIII-A during the process of differentiation of monocytes into macrophages.

Dr. Bishop (USA) demonstrated the biochemistry of gelatin cross-linking by activated FXIII (FXIIIa) and the techniques by which the altered physicochemical characteristics of non-fibrin gel can be studied.

Dr. Ichinose (Japan) gave an overview of FXIII deficiencies and proposed a new classification. He proposed to abandon the terms FXIII type I and type II deficiency and replace it with FXIII-

A, FXIII-B and, if such a case is found, combined subunit deficiency. Subcommittee members agreed with the proposal and by a unanimous vote suggested supplementing the formerly accepted, but not yet published, paper on FXIII nomenclature with the new classification.

Dr. Muszbek (Hungary) demonstrated a new one-step sandwich ELISA method that made it possible to measure the mass concentration of tetrameric plasma Factor XIII without any interference from free FXIII subunits, fibrinogen and other plasma components . He used this method to establish a reference interval for plasma FXIII mass concentration for the first time.

Dr. Grant (UK) summarized the findings of his group on Val34Leu FXIII-A polymorphism as a risk factor for arterial and venous thrombosis and showed the importance of its relationship to the expression of PAI-I. Dr. Balogh (Hungary) presented a study in which no increased risk for venous thrombosis could be related to this polymorphism.

Dr. Ariens (UK) presented data showing that the thrombin-induced release of activation peptide from plasma FXIII containing the mutant A subunit proceeded significantly faster than from the wild type plasma factor. The Finnish group (Wartiowara et al.) reported similar results for cellular FXIII. In three very recent publications it was reported that FXIII containing 34Leu allele had higher activity than wild type (Val/Val) FXIII. Evidence was presented that showed that this is not the case. Although in the mutant thrombin activation proceeds faster, fully activated mutant and wild type FXIII had identical specific activities.

At the previous subcommittee meeting it was decided to explore the possibility of preparing a reference plasma for FXIII measurement. Dr. Longstaff (UK) looked into this matter; however, FXIII did not present the required stability in the plasma preparations he investigated. It was agreed that a reference plasma would be essential for calibrating present and further assays and further effort is to be made in this direction.

FACTOR XIII

16 June
13:30 to 17:30
Room 0.5
Maastricht Meeting and Convention Center

Chairman: A. Ichinose--Japan
Co-chairmen: P. Bishop--USA; P.G. Board--Australia; C.S. Greenberg--USA;
L. Muszbek--Hungary

Unfortunately, factor XIII Subcommittee attracted only 20 participants this year, probably because a limited number of its members attended the associated meeting, the 1st North Sea Conference on Thrombosis and Hemostasis. Prof. Muszbek was the only co-chair who attended and presented. Six speakers discussed important issues in the factor XIII field as follows:

1) Control Mechanisms of the Gene Expression of the A Subunit for Human Factor XIII. A. Ichinose, M. Kida, and M. Souri (Japan)

To study the mechanism of gene regulation for coagulation factor XIII A subunit (XIIIA) Prof. Ichinose's group characterized its 5'-flanking region. Deletion analysis, DNase footprinting, electrophoretic mobility shift, and reporter gene assays demonstrated that promoter elements for a myeloid-enriched transcription factor (MZFR-1-like protein) and two ubiquitous transcription factors (NF-1 and SP-1) were important for the basal XIII expression. DNA sequences for binding of myeloid-enriched factors (GATA-1 and Ets-1) were recognized in an upstream region, and the GATA-1 element was found to be responsible for the enhancer activity. These transcription factors play a major role in the cell type-specific expression of XIIIA, which clearly differs from other transglutaminases.

Prof. Ichinose also reported that G/A polymorphism at -246 bp in the 5'-flanking region of the XIIIA gene is not responsible for variable plasma XIIIA levels among individuals. Since it is inconsistent with the result obtained by a British group of Dr. Anwar, this discrepancy must be discussed in the future session. Finally, he showed the Val34-Leu polymorphism was absent in both Japanese healthy individuals and cases with ischemic heart disease.

2) Sensitivity of Factor XIII Screening Tests: Data from UK NEQAS Surveys. I. Jennings, and F.E. Preston (UK)

Dr. Jennings reported different sensitivities of various screening methods for XIII activity from a survey involving 160 centers. XIII deficient plasma, XIII inhibitor plasma, deficient plasma with replacement therapy were examined for clot solubility by Ca + Urea, Ca + Acetic acid, Ca + MCA, Ca/Thr + Urea, Ca/Thr + Acetic acid, or Ca/Thr + MCA. In general, thrombin methods are more sensitive than Ca alone methods, and tend not to miss abnormal XIII levels. Factor XIII levels obtained by these clot solubility tests vary widely between laboratories at both very low levels and normal levels. These results clearly indicate the necessity of standard methods for

routine assay for plasma XIII. Accordingly, this Subcommittee decided to seek for recommended tests for screening of abnormal factor XIII levels.

3) The Specific Activity of Variant A Subunits. L. Muszbek (Hungary)

As the site of Val34-Leu polymorphism is close to the Arg 37-Gly 38 activation cleavage site, Professor Muszbek investigated if this polymorphism influences the rate of thrombin-induced XIII activation. The initial rate of release of activation peptide (AP) by thrombin from Leu 34 homozygous XIII was more than double the rate measured with the Val 34 wild type. Heterozygous XIII showed intermediate rate of AP release. Similarly, the transformation of zymogen XIII into an active transglutaminase in the presence of thrombin and Ca ion occurred more with the Leu 34 variant than with Val34. Earlier activation of Leu34 XIII resulted in higher initial rate of fibrin cross-linking. Although the activation of leu34 XIIII proceeded at a faster rate, there was no difference in the specific activities of fully activated XIII of different Val34-Leu genotype.

Accordingly, the completed activation of XIIIA must be achieved in the functional assay for XIII activity employing enough amounts of thrombin and proper incubation time.

4) Molecular Modeling of the Interaction of Thrombin with XIII-A Variants. I. Komaromi and L. Muszbek (Hungary)

Dr. Komaromi performed molecular modeling to understand how XIII reaches the active conformation and to get semiquantitative estimation on the reaction path of its catalytic reaction. The geometry corresponding to the active conformation has been determined. The active conformation has an open active site arrangement which is now accessible for substrates. Simulations predict four possible substrate orientations on the XIIIA surface. Comparing the results of pure quantum chemical and mixed quantum chemical/molecular mechanical calculations suggests the crucial role of electrostatic field at the reaction center in catalytic activity. Dr. Komaromi claimed that on the basis of their results, a model for the activation mechanism, active conformation and enzymatic reaction of XIIIA can be constructed at atomic and subatomic level.

5) Effect of Activation Peptide on the Expression and Activity of rXIIIA in Eukaryotic Cells. S.K. Woo, I. Kim, and S.I. Chung (Korea)

In Dr. Kimís effort to understand the influence of AP on the expression, the cellular stability of rXIIIA, and insight into generation of enzyme activity, deletion mutants: -11A, -37A, -37A/Ala314 were constructed and expressed in S.cerevisiae and CHO cells. The expression of various XIIIA gene was analyzed by RT-PCR, western blot analysis, and immunoprecipitation. The full-length gene expressed a thrombin-dependent XIII activity in the cytosol. The ó11A gene expressed a comparable level of proenzyme with a partial activity independent of thrombin but still maintained the fibrin substrate specificity of a native enzyme. The ó37A gene transfected cells failed to show either enzyme activities or protein in spite of presence of comparable mRNA level whereas ó37A/Ala314 gene transfected cells expressed comparable levels of inactive protein. These results suggest that the full-length amino-terminal sequences of XIIIA AP are

required for a stable expression of XIIIA AP are required for a stable expression of XIIIA. First 11 residue is required for complete masking of active site and the 12-37 residue of AP was able to prevent cellular clearance of an active form of XIIIA.

6) Stability of Factor XIII in Plasma and Possibility of XIII Concentrate Standard. T. Barrowcliffe (UK)

Dr. Barrowcliffe reported that he developed two XIII concentrates which are considerably stable at both 4 and 20 degrees when measured by a chromogenic assay. Although these concentrates look more promising than the lyophilized plasma presented last year, there is still uncertainty because of the difference in XIII values between two labs currently involved. Accordingly, this Subcommittee decided to call volunteers to help to solve this issue.

7) General Discussion

Three topics were chosen to be discussed in the next session in Paris; proper screening methods for factor XIII with high sensitivity not to miss its deficiency; reassessment of the assay method for XIII activity regarding thrombin concentration; and collaboration to seek for a standard material for XIII.

FACTOR XIII
7 July 2001
08:00 to 12:10
Room 241
Palais des Congres

Chairman: A. Ichinose--Japan
Co-Chairmen: B. Bishop--USA; P.G. Board--Australia; C.S. Greenberg--USA;
L. Muszbek--Hungary

Fortunately, the factor XIII Subcommittee had more than 100 participants this year, probably because almost all of its members attended the ISTH congress, as usual. Thus, the chairman of this committee would like to suggest that the SSC meeting be associated certain closely related meetings, in order to discuss important issues in the factor XIII field with a large number of members.

1) Recent Progress in Factor XIII Science: Gene Targeting of Factor XIII.

CHARACTERISATION OF COAGULATION FACTOR XIIIA DEFICIENT MICE by Gerhard Dickneite (Germany).
A German group have established a transgenic factor XIIIA deficiency mouse model (FXIIIA knock-out (KO) mice) with an exon 7 deletion of the XIIIA gene by homologous recombination in embryonic stem cells. Transglutaminase activity in plasma was <5 % in homozygous XIIIA KO mice, no gamma-dimerization of fibrin in the plasma of the XIII KO mice could be detected. Mortality rate was higher in the XIII KO mice compared to normal mice because of bleeding episodes (one-year survival: 70 % in FXIII KO mice vs. 100 % in normal mice). When examined for the bleeding disorder in more depth, XIII deficient mice were found to have an increased bleeding time. Thrombelastography experiments demonstrated impaired clot formation in the XIII KO mice, the maximal amplitude was decreased and premature clot destruction was observed. It was concluded that these KO mice represent a good model to study the impact of factor XIII deficiency.

GENE TARGETING OF FACTOR XIII IN MICE by Akitada Ichinose, Shiori Koseki, Masayoshi Souri, Naoki Takeda, Gerhard Dickneite (Japan, Germany)
Prof. Ichinose's group have identified a number of mutations in the XIIIA and the XIIIB genes in patients' genomic DNA and also analyzed the molecular mechanisms using in vitro procedures. However, one cannot understand completely the clinical pathological mechanisms of this disease in vivo. To generate its disease model and ascertain the role of XIIIB in vivo, XIIIB knock-out (XIIIB KO) mice have been established. Both homozygous and heterozygous KO mice showed no marked difference from the wild-type mice in general appearance. Although XIIIB KO mice had somewhat prolonged bleeding time of their tail tips than the wild-type mice, this result should be reproduced by more standardized bleeding test.

As to XIIIA KO mice, three pairs of the homozygous XIIIA KO male and female mice were mated. All female mice became pregnant, and one of these mice died after 2 weeks because of massive bleeding from its vagina. Another female mouse also bled from its vagina and had

abortion. These observations remind us the spontaneous absorption in human female patients with factor XIII deficiency. Further analysis of these XIII KO mice would lead to understanding the physiological and pathological functions of XIII in vivo.

2) Clinical Research on Factor XIII Deficiency

GEFFXIII: A FRENCH WORKING GROUP ON FXIII, LOOKING FOR EXTENSION AND CONNECTIONS by Dreyfus M. (GEFFXIII; Arnutti B, Barrois D, Beurrier P, Borg JY, Claeyssens S, Doki-Thonon M, Gaillard S, Garnier JM, Girardel JM, LeBerre D, Pautard B, Pernod G, pollet F, Pouzol P, Seaume , Torchet MF, Wibaut B)
A working group including all the French physicians in charge of FXIII severely deficient patients has been organized in 2000 and named GEFF XIII (Groupe d'Etudes Francophone du FXIII). This group of physicians from 16 centers conducted the first study to assess the tolerance and safety of the FXIII plasma concentrate Fibrogammin P (Centeon Aventis). Eight out of the 19 patients had previous histories of intra-cerebral hemorrhages (ICH). Seven out of 8 episodes of IHC occurred in non-treated children before the age of 11, indicating the need for a systematic prophylactic replacement therapy in these patients.

This group plans future studies on the retrospective collection of data on patients undergoing surgery, systematic characterization patients' genotype, prenatal diagnosis, standardization of the FXIII assays, etc.

3) Activation and Its Implication of "Variant A subunits" of Factor XIII.

CHARACTERIZATION OF ENHANCED CATALYTIC EFFICIENCY OF RABBIT FACTOR XIII IN COMPARISON WITH HUMAN ENZYME by Lee S.Y., Lee I.H., Oh J.T., Kim I.G. and Chung S.I. (Catholic Univ., Seoul National Univ., and Korea)
Dr. Chung purified rabbit and human enzyme and characterized their enzymatic, physicochemical and structural properties. Rabbit factor XIIIA (RXIIIA), like the human enzyme (HXIIIA), showed the same mechanism where the deamidation step (k3) during the acyl enzyme formation step was found to be rate-limiting. However, the kinetic efficiency measured by methylamine incorporation into acetylated oxidized B chain of insulin showed rabbit enzyme was significantly greater than human enzyme (V/K: 427.45 for RXIIIA; 62.12 for HXIIIA). Structure modeling of RXIIIA by a fit to the known HXIIIA 3D structure coordinates again showed a similar resemblance of active site pocket and Ca ion binding domains. In light of the report that Val34Leu variant retained greater catalytic activity, the N-terminal activation peptide domain of rabbit enzyme, which is quite heterogeneous from human, may contribute in the enhancement of catalytic efficiency in an unknown manner and correlates well with the previously reported rat enzyme catalytic efficiency and its amino acid sequence.

EFFECT OF VAL34LEU PENOTYPE ON THE ACTIVATION OF FACTOR XIII: HOW IMPORTANT IS IT? by Laszlo Muszbek (Hungary)
To address the problem regarding Val34Leu, Prof. Muszbek reported the following: As observed with purified proteins in the absence of plasma, the rate of FXIII-A cleavage and fibrin polymerization was higher with the homozygous Leu/Leu variant than with wild type (Val/Val) FXIII. However, when plasma or whole blood from patients of various genotypes were

compared, Val34Leu polymorphism did not seem to be a major contributor to the speed of FXIII activation. The onset of fibrinogen clotting, i.e., the release of fibrinopeptide A and fibrin polymerization were of predominant importance in determining the time course of FXIII activation. The release of FXIII-A activation peptide always lagged behind the release of fibrinopeptide A and activated FXIII-A never appeared in the fluid phase. Prof. Muszbek concluded that the appearance of polymerizing fibrin and its accelerating effect on FXIII activation are the dominant factors that regulate the process of FXIII activation in whole blood.

FACTOR XIII VAL34LEU: RELATION TO THROMBOTIC DISORDERS by Peter J. Grant (UK)
Prof. Grant reviewed existing reports including his own on the implication of Val34Leu polymorphism of the XIIIA gene. The factor XIII genes are highly polymorphic. There are 5 common coding polymorphisms in the factor XIII A gene, of which a valine to leucine transition at residue 34 is of interest due to its vicinity to the thrombin cleavage site and its relation to thrombotic disorders. The relationship between Val34Leu and thrombosis has now been analyzed in approximately 18 epidemiological studies, of which 7 regard patients with cardiovascular disease, 6 venous thrombosis and 5 cerebrovascular disease. Several of these reports have shown that Val34Leu is protective against thrombosis in different vascular beds.

FACTOR XIII VAL34LEU: EFFECTS ON FIBRIN STRUCTURE AND FUNCTION by Robert A.S. Ariens (UK)
Factor XIIIA Val34Leu occurs three amino acids upstream of the thrombin cleavage site between arginine 37 and glycine 38. Dr. Ariens previously reported that the substitution of Val 34 with Leu accelerates the thrombin cleavage of the factor XIII activation peptide. He showed that early covalent cross-linking of the fibrin clot by factor XIII Leu34 reduced lateral aggregation of the fibrin fibers, leading to a reduction in fiber thickness from 121.0 +/- 23.9 nm to 75.7 +/- 11.3 nm and alteration in rates of fibrinolysis of the fibrin clot. The effect of Val34Leu on fibrin structure and function appeared to alter the interaction with platelets and was dependent on fibrinogen levels. Dr. Ariens concluded that Val34Leu is the first example of a mutation in the factor XIII activation peptide that alters Ttransglutaminase and fibrin structure and function.

4) Improvement of Screening Tests for Fzactor XIII.

SCREENING FOR FXIII ACTIVITY IN PLASMA by Lewis K.B., Heffernan J., Khuu Kien, Bishop P.D. (USA)
Although a variety of assays exist for screening FXIII levels in plasma samples, in many cases the reagents are not readily available and the protocols are not easily transferred to different laboratories. Rather than develop a new assay, an American has chosen to evaluate the Berichrom assay (not commercially available in the US). Where necessary, they have made simple modifications using readily available reagents. The principal modification has been to increase the thrombin concentration in the assay. A second modification has been the use of an absolute rFXIII [A2] standard rather than a pooled human plasma standard. With these modifications, FXIII activity levels have been measured in plasma from humans and other animal species.

5) Search for Standard Materials for Factor XIII.

SEEKING STANDARDIZATION MATERIALS FOR FACTOR XIII by Paul Bishop (USA)
Dr. Bishop proposed the following objectives: 1) Comparing the accuracy of factor XIII assays in common use for clinical evaluation. 2) Establishing an international standard for factor XIII activity. 3) Defining a specific activity for factor XIII. He also proposed to establish study participants and a work plan:

1) Solicit laboratories interested and willing to participate in such study.
2) Identify and designate reference plasma of normal FXIII activity (a lot of pooled normal plasma) and a FXIII deficient reference plasma.
3) Determine which assays will be employed by the various participating laboratories
4) Establish a protocol for testing the accuracy and availability and uniformity of FXIII assays in common use.
5) Discuss the possibility of providing FXIII (A2B2) plasma concentrate and & preparation of pure recombinant FXIII (A2) as an external reference.

FACTOR XIII AS A COMPONENT OF THE FIBRIN SEALANT, "BOLHEAL(R)," AND AN ASSAY FOR FACTOR XIII ACTIVITY by Hiroshi Kaetsu (Japan)
Factor XIII, one of the active ingredients of the fibrin sealant, "Bolheal(R)," is included in the fibrinogen component. Fibrinogen and factor XIII are purified individually in the production process of the fibrinogen component of Bolheal(R). Dr. Kaetsu uses the amine incorporation method as a routine assay for factor XIII activity. This method gave the dilution linearity in samples of "standard human plasma," "purified factor XIII," and "the fibrinogen component of Bolheal(R)." The linear range of the assay system was 0.05-2 units/ml for both factor XIII concentrate and fibrinogen concentrate including factor XIII. Attention needs to be paid as to whether or not the values of factor XIII activity obtained with different assay systems are comparable.

Dr. Kaetsu proposed a process to establish the reference preparations of factor XIII in which "standard plasma" is established as the first step, and thereafter "purified factor XIII standards" are evaluated, based on the value of activity in "standard plasma."

PROGRESS REPORT ON STANDARD FACTOR XIII MATERIAL by Trevor W Barrowcliffe (UK)
Last year, Dr. Barrowcliffe reported that he developed two XIII concentrates which are considerably stable at both 4 and 20 degrees when measured by a chromogenic assay. He also extended this study at higher temperatures, such as 37 and 45 degrees, where the two concentrates showed much less stability of 40-60%. Since these concentrates showed more than 90% stability, these should be used as one of candidates of XIII concentrates in the standardization study.

Dr. MacIntosh, co-chairman of the Fibrinogen Subcommittee, raised several issues regarding factor XIII materials and assay method; some fibrinogen preparations and clot solubility tests need to be?included in the future study.

6) General Discussion and Topics for 2002

Since the meeting was behind the schedule, and passed 10 min over 12:00, it was strongly suggested by a congress personnel to conclude the discussion. Accordingly, the chairman of this subcommittee just announced that factor XIII assay and material standardization activities would be continued during the year with reports at next year's meeting in Boston. Laboratories, institutes, and companies which are interested in this collaborative task force were requested to send an E-mail to Akitada Ichinose, <aichinos@med.id.yamagata-u.ac.jp>.

Factor XIII

July 19, 2002
14:00 to 18:00
Stanbro Room
Boston Park Plaza Hotel

Chairman: A. Ichinose, Japan
Co-chairs: RAS Ariens, UK; P. Bishop, USA; C. S. Greenberg, USA; L. Muszbek, Hungary

The session was opened with apologies of absence from Drs. Ichinose, Bishop, Greenberg and Muszbek. Drs. Ariens (UK) and Seitz (Germany) resided as Acting Chairs. There were approximately 75 people present and the presentations were followed by lively discussions.

1. Dr. Kohler (University of Bern, Switzerland) *"Determination of FXIII activity using different methods. Influence of FXIIIA Val34Leu"* .

This presentation was opened with a reminder that there is no clear perspective on which type of FXIII activity assay is the best to use for any given situation. Two particular assays were discussed (i) biotin incorporation assay (PefaKit by PentaPharm) and (ii) photometric assay (Berichrom). Genetic polymorphisms of FXIII such as the Val34Leu polymorphism affect the rate of FXIII activation. The Leu allele increases the rate of FXIII activation and this may be detected when using the PefaKit assay, but will only be detected with the Berichrom assay if low levels of thrombin are used to activate FXIII. When FXIII is fully activated there is no longer any discrimination in specific activity measured with genotype variation. The question therefore is how much thrombin is generated in vivo? Previous work by Kohler et al, Thrombosis and Haemostasis, 1995, has shown that low levels of thrombin are likely to be generated (approximately 0.1u/ml) in vivo, and that there is only partial activation of FXIII. Thus, the best assay to achieve high sensitivity that can discriminate for FXIII polymorphisms is a biotin incorporation assay using low levels of thrombin to activate FXIII. To determine antigen levels of FXIII, an ELISA approach may be taken; alternatively it is possible to achieve full activation of FXIII using high levels of thrombin concentration and then assessing FXIII activity using either a photometric or biotin incorporation assay.

2. Dr. Jennings (University of Sheffield, UK) *"Performance and variability of FXIII screening".*

Dr. Jennings was representing the UK NEQAS for blood coagulation screening which is currently using approximately 150 laboratories to screen for FXIII assays. Currently only approximately 20-30 laboratories use more direct FXIII assays but the majority of labs (approximately 120-130) use the clot solubility test to assess FXIII levels. In theory the clot solubility test is meant to be sensitive to approximately 1% of FXIII in plasma. The laboratories involved were given 3 plasma samples (i) normal plasma (ii) FXIII deficient plasma and (iii) FXIII deficient plasma at trough level from a patient that was about to receive prophylactic treatment. Each of the laboratories reported the level of FXIII measured and how they interpreted their result. There was a great variation of levels reported, particularly in the sample from the FXIII deficient patient at trough level (sample iii) where 70% of the laboratories classified this patient in the "normal" category. It was found that labs using a calcium-based clot solubility assay misclassified the result as normal whereas those using thrombin in their assay detected low levels. Other parameters used in the clot solubility test that affect measurement of FXIII were the

lysing reagents urea or acetic acid. In May 2002 a further screening exercise was performed. The samples were (i) FXIII deficient patient (prior to treatment at trough level) plasma taken into citrate and (ii) a pool of normal donor plasma taken into both EDTA and citrate. Again there was great variation in levels reported from the laboratories. It has been found that many variables influence the reported FXIII level; these include calcium, thrombin preparation, urea, volume of plasma/reagents, incubation time, the source of the method and normal range. Different FXIII assays also report variations for a given sample especially the Berichrom assay which reported a range of between 0-55U/dl of FXIII for a given FXIII deficient sample. There is clearly a need for a good standardization method for FXIII measurement as there is too much variation with the current methods employed. The conclusions drawn from this presentation were (i) thrombin-based clotting assays are more sensitive than calcium-based clotting assays but care must be taken to ensure that thrombin preparations do not contain calcium, (ii) there is a lack of accuracy and precision evident in FXIII assays, and (iii) the clinical relevance of ‘mild’ FXIII deficiency is unresolved.

3. Dr. Ariens (University of Leeds, UK) *"Genetics of fibrin structure/function"*.
Dr. Ariens opened with an introduction to FXIII and fibrinogen and showed the vast number of polymorphisms present in both FXIII and fibrinogen. Two polymorphisms of FXIII, Val34Leu in the A-subunit and His95Arg in the B-subunit, alongside two polymorphisms of fibrinogen, AaThr312Ala on the alphaC domain of the alpha-chain and BbArg448Lys on the beta-chain of fibrinogen, were expanded upon in greater detail. These polymorphisms to a certain degree affected fibrin structure (with the exception of His95Arg which was not examined). Fibrinogen level also plays a role in the outcome of fibrinogen structure, so there is an environmental effect also acting on the genotype effect. Fibrin structure was also shown to be affected (compared to controls) in plasma from first-degree relatives of patients with 2- or 3-vessel coronary artery disease. The heritability of fibrin structure was examined and showed 39% heritability of permeation(ks) in a twin study. This is lower than that observed from the heritability of fibrinogen levels (approximately 50%) and other coagulation zymogens (61-75%). This has been attributed to environmental factors which have been shown to affect fibrin structure (e.g dimethylbiguanide used for treatment of diabetes, calcium ions and ionic strength and thrombin). The conclusions of the presentation were that genetic polymorphisms affect cross-linking and fibrin structure and that environmental effects also play a role.

4. Dr. Barrowcliffe (NIBSC, UK) *" Pilot study for standardization; presentation and discussion"*.
Dr. Barrowcliffe introduced the process for preparing standards
(1) Preliminary investigation of materials
(2) Trial fills
(3) Stability studies and assays on trial fills
(4) Large-scale fills
(5) International collaborative study
(6) Report to WHO
Phases 1-3 have now been completed and the organization of phase 4 is beginning. The types of materials assayed for FXIII standards are plasma, concentrates, fibrin sealants (fibrinogen components) and recombinant FXIII is also being considered. In the SSC meeting last year Dr. Barrowcliffe reminded us of the stability of two FXIII concentrates which were ampouled.

Preparation B was shown to have a stability of 0.05% per year at -20ºC, which was better than preparation A; therefore, further work will continue with preparation B. Proposals for the materials to be used include: plasma (NIBSC and normal pool), concentrate (one or two preparations), fibrinogen concentrate and recombinant FXIII (although this may need further consideration). Participants will be manufacturers of preparations, national control labs and clinical/academic labs. The methods to be employed will be chromogenic and antigen assessment. A preliminary collaborative study has begun with four laboratories with the aims of comparing dilution of samples in FXIII deficient plasma with buffer, comparing the Dade and Pentapharm assays, and to look at studies using fibrinogen concentrates. The samples include two FXIII concentrates, a fibrinogen concentrate and plasma. This preliminary study will be scaled up to include 10-20 labs. Persons wishing to collaborate with this study should please contact Dr. Barrowcliffe .

5. Dr Seitz (Paul-Ehrlich-Institut, Germany) *"Preliminary Results of PEI Pilot Collaborative Study"*

Dr Seitz presented preliminary results as a participating laboratory, following on from Dr. Barrowcliffe's introduction to the preliminary collaborative study that has started with four laboratories. The aims were to compare FXIII concentrates versus plasma that are diluted in either FXIII-deficient plasma or buffer. The samples were plasma (1U/ml of FXIII content), FXIII concentrate (30U/ml FXIII content) and FXIII concentrate (50U/ml FXIII content). Preliminary results show that pre-dilution of FXIII sample in FXIII deficient plasma gives a better result than dilution in buffer containing 1% BSA. Low levels of FXIII have been found in the fibrinogen concentrates and there is a need for a comparison of methods.

6. Dr. Seitz (Paul-Ehrlich-Institut, Germany) *"Preliminary Results: Novel FXIII Assay Based on Cross-linking of Peptides"*

Dr. Seitz gave an introduction to the development of a novel assay to measure FXIIIa cross-linking ability. The concept relies on the use of two peptides that are cross-linked to each other by FXIIIa. One of the peptides contains a His-tag that may be bound to Ni-NTA, and the second peptide is conjugated with FITC to allow detection. Preliminary data show that this assay is feasible but requires further development. Dr. Seitz was interested in looking for a collaborative study with a manufacturer to aid in the development of this novel assay system.

Factor XIII

Saturday July 12th, 2003
9.00 to 13.00
Hall 9
Birmingham International Conference Centre

Chair: RAS Ariëns-UK
Co-Chairs: A Ichinose-Japan, P Bishop-USA
Active Members: CS Greenberg-USA, L Muszbek-Hungary

Apologies were received from Dr Greenberg. All other chairs and members were present. The FXIII SSC meeting had a full programme, all speakers and approximately 130 people in the audience attended the meeting. The presentations were of a high standard and ranged from novel methodology including gene knockout, atomic force microscopy, site-directed mutagenesis to study protein interactions and protein chemistry to development of the first international standard for FXIII. The presentations of Dr Lim and Dr Raut were exchanged, as Dr Raut had to be at another SSC meeting in the late morning.

The meeting was opened by **Dr Ariëns**, University of Leeds, UK, who put forward for discussion the issue of whether the FXIII SSC should continue as a separate committee or whether it could merge with that of fibrinogen to a combined Fibrinogen and FXIII subcommittee. Dr Ariëns pointed out that most subcommittees relate to work in areas of research that involve many proteins and genes such as that of fibrinolysis, coagulation inhibitors and contact activation, and that there are only three subcommittees out of 20 that are dealing with one coagulation factor in isolation: the SSC for FXIII, fibrinogen and von Willebrand factor. Dr Ariëns suggested that it might be a good idea to combine the FXIII and fibrinogen SSC in one subcommittee as the two proteins are functionally closely involved in the final stages of the coagulation cascade. It was also suggested that thrombin could be included in this new SSC as well. Advantages of a possible merger would be that there would be a larger platform to discuss novel ideas regarding fibrinogen, FXIII and thrombin, with people present from various expertise. Work relating to standardization would be easier to perform and to discuss. Issues regarding standardization for FXIII, fibrinogen and thrombin are more often related than not, such as in the case of fibrin glue for example, that contains both FXIII and fibrinogen.

The idea for a merger with the fibrinogen SSC was met by a short but lively discussion. Dr Lord, University of North Carolina, USA, Acting Chair of the fibrinogen SSC, was supportive of a possible merger and mentioned that the idea would also be discussed in the fibrinogen subcommittee to be held the same afternoon. Dr Bishop, Zymogenetics, expressed concern that there would not be enough space on the agenda of a merged subcommittee to discuss all relevant issues. Dr Muszbek, Debrecen University, Hungary, said that the SSC for FXIII and fibrinogen used to be combined several years ago and that it was then decided to separate them. He

expressed concern that issues relating to fibrinogen would dominate meetings of a merged SSC. Dr Kohler, University of Bern, Switzerland, argued the opposite, and suggested FXIII issues may dominate those of fibrinogen. Overall, there seemed to be concern regarding dominance of one of the factors over the other and a possible lack of agenda space in meetings of a merged SSC. Dr Ariëns suggested that space issues could possibly be solved with longer agendas if necessary. Dr Muszbek suggested organizing a trial of a combined SSC, by holding a combined meeting for FXIII and fibrinogen at the next SSC. This suggestion was welcomed by Dr Ariëns. The agenda item was closed with a call to everyone in the audience to express their opinion on the matter by filling in a questionnaire form, so that a recommendation on this issue in some form or the other could be presented by the chair at the SSC Business meeting.

The main programme of the FXIII SSC was opened with a presentation from **Dr Ichinose**, Yamagata University, Japan, on abortion in FXIII gene-knockout mice. Dr Ichinose started his presentation with two minutes silence in honour of the work of Dr Peter Steinert who recently passed away. Dr Steinert has characterised many of the transglutaminase genes. Dr Ichinose presented data on the molecular biology of FXIII deficiency. There are approximately 500 cases of FXIII A-subunit deficiency known. Mutations occur over the whole molecule. There do not appear to be any particular hotspots for mutations in this gene. B-subunit deficiency is rare, currently only 4 cases are known with a characterized mutation in the B-subunit gene. FXIII deficiency is associated with severe bleeding diathesis, in particular umbilical cord, intracraneal and peritoneal bleeding, with poor wound healing and with miscarriage in pregnancy. Dr Ichinose has developed a FXIII A-subunit gene-knockout model in mice for the study of miscarriage in FXIII deficiency. The main symptoms in the A knockout mice were vaginal and intrauterine bleeding. Dr Ichinose concluded from this mouse model that spontaneous abortion in FXIII deficiency is caused mainly by bleeding.

Dr Kohler, University of Bern, Switzerland, presented data on the role of FXIII in vascular disease. Dr Kohler mentioned the importance of distinguishing between chronic and acute vascular disease. The first interest in the role of FXIII in vascular disease was developed by findings of an association between FXIII Val34Leu and vascular disease. Recent studies suggest that this polymorphism affects fibrin structure function through an interaction of FXIII Val34Leu and fibrinogen levels. Other studies have shown an interaction between insulin resistance and FXIII subunit levels, B-subunit levels in particular. Factor XIII levels have been shown to relate to the extent of coronary artery disease. In the Northwich Park Heart Study II, FXIII A-subunit levels were reduced in the acute phase of the disease, with no difference in B-subunit levels. The reduction in FXIII A is possibly due to activation and increased consumption. This is in agreement with a study in acute stroke, where FXIII A-subunit was reduced in patients with poor survival and the level inversely correlated with F1+2. In another study of acute pulmonary embolism, FXIII A-subunit levels were also reduced, with normal B-subunit levels. The relationship was linear with occlusion rate, and Dr Kohler suggested that FXIII consumption is

related to the thrombus size. The relationship between FXIII and vascular disease indicate the importance of standardization of the assays to measure FXIII levels (activity, A-subunit and B-subunit).

Results from a pilot study for the standardization of FXIII activity assays were reported by **Dr Raut**, National Institute for Biological Standards and Control, UK. Phase 4 (out of 7) of the study was performed. Data were obtained on two FXIII concentrates and 1 fibrinogen concentrate. The aims of were to compare the Berichrom FXIII activity assay and the new Pentapharm FXIII activity assay. The two assays use different principles to measure FXIII activity, in the Pentapharm assay incorporation of pentylamine into fibrinogen by FXIII is measured, whereas in the Berichrom assay the amount of ammonia released during the cross-linking reaction is measured. Another aim was to analyse assay differences in the presence or absence of fibrinogen. Collaborating laboratories were that of Dr Muszbek, Dr Seitz and Dr Barrowcliffe. It was found that differences between the two assays were large when buffer was used as diluent, whereas differences were small with FXIII deficient plasma as diluent. There was a high variability of measurements in fibrinogen concentrates. Dr Raut ended with an update on the development of the first international standard for FXIII. Recombinant FXIII was suggested as possible additional standard material, and a resource laboratory for this material would need to come forward. To establish an International standard for FXIII, 12-20 laboratories will have to participate and laboratories willing to participate are asked to come forward. Please contact the chair of this SSC or Dr Raut if you are interested. In the discussion, Dr Muszbek suggested that the difference between variability between buffer and deficient plasma as diluent is due to the presence of fibrinogen in plasma, which greatly enhances activation rates of FXIII. This may lead to differences between assays that employ different thrombin concentrations and have different sensitivity to the activation step.

Dr Weisel, University of Pennsylvania, USA, presented data on the effect of fibrin a- and g-chain cross-linking by activated FXIII on fibrin structure and function. Recombinant Aa251 fibrinogen lacks the aC domain and its connector polypeptide, and is therefore deficient of the a-chain FXIII cross-linking sites. Dr Weisel showed data using this fibrinogen to quantify the relative effects of a- and g-chain cross-linking on fibrin structure and elastic properties. Experiments were performed with purified FXIII from pooled plasma, which was fully activated to avoid any effects of the Val34Leu polymorphism on fibrin structure and stiffness. Recombinant Aa251 fibrinogen produced clots with smaller pores, thinner fibers and increased number of branchpoints when compared with normal fibrinogen. Cross-linking by pooled plasma FXIII had relatively little effect on overall fibrin structure, but there was a dramatic effect on stiffness of the clot. Cross-linked Aa251 fibrin was much less stiff and showed more non-elastic slippage of the protofibrils than cross-linked normal fibrin. This effect was due to the absence of a-chain cross-linking in Aa251 fibrin. Gamma chain cross-linking also contributed to clot stiffness as Aa251 fibrin showed reduced stiffness in the absence of FXIIIa when compared to

Aa251 fibrin in the presence of FXIIIa, the latter of which is expected to show normal -gamma chain cross-linking. The effects of alpha and gamma-chain cross-linking were also noted on the lysis speeds of the fibrin clots as analysed by laser scanning confocal microscopy.

After a short coffee break of 15-20 minutes, the meeting was resumed with a presentation on the use of FXIII peptides to investigate thrombin-FXIII interaction by **Dr Maurer**, University of Louisville, USA. Using a variety of novel methods, including 2D NOESY spectra, MALDI-TOF and pulsed alkylation mass spectrometry, Dr Maurer investigated the relative roles of P1-4 of the FXIII activation peptide in the interaction with thrombin. It was found that the P4 residue, which is the site of a common Val34Leu polymorphism, plays an important role in the interaction with thrombin. A change of Val to Leu change at this position dramatically improved the kcat as well as Km. Substitution of P4 with Ile or Ala improved the Km but the kcat worsened. The future plans are to apply the same methodology to study interaction with the whole FXIII protein, possibly as a recombinant. The aim is to obtain a fuller understanding of the conformational changes that occur in FXIII upon binding to thrombin.

In the next presentation by **Dr Philippou**, Imperial College, UK, FXIII and thrombin interactions were investigated from the thrombin point of view. Dr Philippou used a library of 58 thrombin mutants encompassing a total of 78 mutated surface exposed residues, to study their interaction with FXIII. The mutants were screened with a pentylamine incorporation assay using casein, fibrinogen or fibrin as substrate, and the results were confirmed by kinetic analysis of the activation peptide release by HPLC. The results showed that residues R78/R180/D183, W50, E229, R233 of thrombin were involved in direct interaction with FXIII during its activation. Additional thrombin residues H66, Y71, N74 were involved in binding to fibrin to cause the fibrin-enhancement effect of FXIII activation. Thrombin has many substrates, and general implications were drawn from these findings for the mechanism by which thrombin chooses its substrate. It was concluded that thrombin's fate is directed primarily by competition between its cofactors. In the specific case for FXIII for example, activation is directed by competition between fibrin and thrombomodulin for their overlapping binding site on thrombin. A model was proposed to explain the enhancement effect of fibrin on FXIII activation.

Dr Reynolds from Zymogenetics, USA, presented the first data regarding pharmacokinetics and safety of a recombinant FXIII preparation in healthy human volunteers. Recombinant FXIII A-subunit was produced in yeast, and administered at doses of 2, 5, 10, 25 and 50 u/kg to 8 healthy subjects. Placebo was administered in 2 subjects. The highest dose of Rec FXIII was chosen such that it would increase the baseline level of FXIII by 100%. Levels were monitored using the Berichrom FXIII activity assay. Halflife of FXIII after injection was 9-11 days, which is in good agreement with previous findings using plasma substitution. There was a specific increase of FXIII of 1.77% per 1U/kg. The product was found to be safe as adverse effects such as headache, cramp, pain in limb, prolongation of thrombin time, abdominal pain or cough were all absent.

There was an increase in D-dimer and a slight drop in FXIII B-subunit levels at high concentrations of recombinant FXIII.

Dr Lim, University of Leeds, UK, presented data using novel technology of atomic force microscopy (AFM) to investigate the structure of fibrin(ogen) and FXIII. AFM was developed in 1986 for applications in physics, but it only since recently that it is being used more often in lifesciences. AFM allows real-time visualization under physiological aqueous conditions at almost unlimited resolution potential. Topographic images are generated by a piezo-electric scanner that moves or taps a microtip over the sample. The resolution is determined by the size of the tip with which the microscope scans or 'taps' the surface. Current tips used are around 10-30 nm in diameter, but future usage of tips of around 1 nm using carbon nanotubes should improve resolution. For comparison, length of a fibrinogen molecule is around 45 nm. Further advantages of AFM are that it can be used to measure attractive and repulsive forces on a single molecule level. Potential limitations of AFM are that the sample can be altered or damaged by the tip movement. AFM images were shown of fibrinogen molecules, showing their characteristic three-nodular appearance. Images were also shown of fibrin polymerization at various time-frames after the addition of thrombin, showing growing protofibrils that aggregate laterally and branch out. The first AFM images of FXIII were produced, showing a tetrameric structure that appears to be in agreement with the models of tetramer association based on transmission electron microscopic images of FXIII published by Carrell et al in 1989.

An update of the ETRO FXIII deficiency registry was presented by **Dr Ivaskevicius**, Frankfurt Red Cross Blood Center, Germany. A background was given about the structure of the FXIII A- and B-subunit genes and FXIII deficiency, which is an autosomal recessive disorder that affects around 1 subject in every 1-3 million. The registry had 72 entries in 1996, 96 in 2000 and 109 in 2003, with entries from a total of 20 different countries. Around 45% of the entries have also been genetically characterized. A particular mutation in intron E (IVS5-1 G>A) was most common, possibly due to an ancient founder effect, as it was found in several countries. Dr Ivaskevicius continued the presentation with data showing the usage of Denaturing HPLC (Wave technology) to screen DNA for mutations. DHPLC proves to be a useful and rapid methodology for screening, although some mutations are not detected. The average detection rate is reported to be around 95%. Further characterization in healthy subjects is required to exclude the possibility that a certain change found in the DNA of the FXIII deficient patients is a common genetic polymorphism.

The meeting was closed at 12.55.

The **questionnaire slip** was returned by 48 people, of whom
17 or **35% voted in favor** of a merger of the FXIII and fibrinogen SSC,

29 or **61% against** and
2 or **4% said they were indifferent.**

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 rFXIIIA 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 rFXIIIA infusion. There was normal affinity of rFXIIIA 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, rFXIIIA 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 intimamedia 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.

Factor XIII

7 August 2005
8:30 to 12:00
The Ballroom 1
Sydney Convention and Exhibition Centre

Chair: Robert Ariëns, UK

Co-chairs: Paul Bishop , USA , Akitada Ichinose , Japan , Hans Kohler, Switzerland ,Rainer Seitz , Germany

Active Members: Laszlo Muszbek , Hungary , Muriel Maurer, USA , Ivaskevicius , Germany

SSC approval sought for:

- Endorsement of International Registry for FXIII deficiency (Ivaskevicius)
- Nomenclature of FXIII (Muszbek)

Ongoing activities:

- Antigen potency estimation for 1 st International FXIII Standard
- Development of a standard for concentrates and recombinant FXIII

The FXIII subcommittee held a joint meeting with the fibrinogen subcommittee this year. We had a very busy agenda for both subcommittees, due to which the total joint meeting exceeded its allocated timeslot of 3.5 hours by up to almost 1 hour. Apologies were received from Bishop and Maurer, all other chairs and active members were present. The meeting was attended by around 80-100 delegates.

The FXIII session was opened by **Akitada Ichinose** (Japan), who provided an overview of the diseases with which transglutaminases are associated. In addition to thrombosis, cardiovascular disease and bleeding, these include neurological disorders, cancer, celiac disease and Huntington disease. Ichinose gave an overview also of the activities of the FXIII standard working party, which has been active since 2002 and has developed the 1 st International Standard for FXIII, approved by the SSC and WHO in late 2004.

Ivaskevicius (Germany) presented data from a registry for FXIII deficiencies previously endorsed by ETRO. Currently the registry contains around 100 entries, but the estimated number of cases of FXIII deficiency worldwide is somewhere between the figures of 6,500-19,500. The registry provides important information for the management of FXIII deficient patients. It will also aid in our understanding of genotype-phenotype relationships for FXIII, and structure – function relationships. It is proposed that this registry should be expanded as a true international registry. The SSC is asked to endorse the development of this new, expanded registry for FXIII deficiency.

A new method measuring FXIII activity was presented by the group of **Rainer Seitz** (Germany), using a biotinylated selection peptide bound to a streptavidin coated microtiter plate. FXIII in a sample is activated outside the plate by thrombin; the reaction is stopped by hirudin. A fluorescence labelled detection peptide is incubated in the plate together with the mixture containing the activated FXIII. The reaction is stopped by EDTA and the plate washed with urea, before bound fluorescence is measured. Multiple variations of the detection peptide can be used as a powerful tool to study the enzymatic characteristics of FXIII.

The measurement of FXIII activity in concentrates was discussed by **Laszlo Muszbek** (Hungary). Different FXIII activity assays behave differently with regards to the diluent of the concentrate. Choice of diluent includes buffer, FXIII deficient plasma or FXIII free fibrinogen. The latter two appear to be the materials of choice for the accurate and consistent measurement of diluted FXIII concentrates. It is proposed to use FXIII free fibrinogen at a concentration of 2 mg/ml for future standardisation studies of concentrates.

FXIII nomenclature was discussed by **Laszlo Muszbek**. Nomenclature of FXIII had been previously considered at the SSC meeting in Florence , 1997. At that time a draft proposal was made and it was decided to test its use by researchers in the haemostasis and thrombosis field. The nomenclature proposal was revisited at this meeting (attached) and it was unanimously accepted in unmodified form by everyone present. It is proposed to seek endorsement of this FXIII nomenclature by the SSC, after which it is planned to submit an SSC brief communication outlining the details to the Journal of Thrombosis and Haemostasis on behalf of the FXIII subcommittee.

Sanj Raut (UK) presented data from a collaborative pilot study by the FXIII standard working party (SWG) for the measurement of FXIII antigen in the 1 st international standard for FXIII. During the international collaborative study in 2003-2004 to develop the 1 st IS for FXIII, antigen levels had already been determined at 0.93 (GCV 14%). At that time, however, it was decided not to assign this value as yet, as different methods for FXIII antigen determination (anti-A/anti-A, anti-A2B2/anti-A, anti-B/anti-A sandwich ELISA, and A2B2 Laurell) had been employed. As the tetrameric form of FXIII (A2B2) is the prevalent and (potentially) active form of FXIII in plasma, it was decided that this should provide the 'gold' standard for FXIII antigen determination. The current SWG pilot study is based on the use of one A2B2 ELISA kit, performed in 5 laboratories, using a protocol developed by the NIBSC. Preliminary data showed an antigen potency estimate of 0.91 (GCV 1.8%), which is in close agreement with the previous study using various antigen assays. The FXIII SWG proposes to await full analysis of the data and, if confirmatory, to pool the estimates from both studies for the assignment of a FXIII antigen potency estimate for the 1 st IS for FXIII. Approval for this antigen estimate by the SSC and WHO will be requested in due course.

The effect of polymorphic variants on kinetic activity assays for FXIII was discussed by **Robert Ariens** (UK). Assays that are based on the kinetic measurement of early pentylamine substrate incorporation into fibrin, can be sensitive to differences in activation rate and hence to FXIII Val34Leu, a common polymorphism in certain populations (25% allele frequency in Caucasians). Ariens presented data of the modification of one such kinetic pentylamine assays into an end-stage, total activity assay for FXIII. The reaction mixture was incubated for 60

minutes rather than 5-10 minutes, and the measurement principle was based on dose-response curves of absorbency against plasma dilution rather than kinetic curves of absorbency against time. The modified assay proved sensitive to FXIII in the ng range with high specificity as demonstrated by parallelism of dose response curves for plasma, purified FXIII, recombinant FXIII-A and a FXIII concentrate for clinical use. Identical dose-reponse curves were found for FXIII VV, VL and LL samples with similar A2B2 antigen concentrations. It is proposed that this end-stage assay can be used as alternative to kinetic assays when it is desirable to measure total activatable FXIII.

TERMINOLOGY TO DESIGNATE DIFFERENT FORMS OF BLOOD COAGULATION FACTOR XIII
(Laszlo Muszbek)

Factor XIII normally present in the plasma (tetramer; A2B2):

Recommended term:
Plasma coagulation factor XIII (Plasma factor XIII)
Not recommended terms:
Fibrin stabilizing factor, Plasma protransglutaminase,
Fibrinoligase, Fibrinase, Laki-Lorand factor, Plasma transamidase.

Factor XIII of intracellular localization present in platelets, megakaryocytes, monocytes, macrophages(dimer; A2):

Recommended term:
Cellular coagulation factor XIII, (Cellular factor XIII).
Not recommended terms:
Platelet, monocyte, placenta, etc. factor XIII,
Cellular protransglutaminase.

PROPOSED FXIII TERMINOLOGY AND ABBREVIATIONS I.

Blood coagulation factor XIII: FXIII
Plasma factor XIII (A2B2): pFXIII
Cellular factor XIII (A2): cFXIII
Recombinant factor XIII (A2): rFXIII

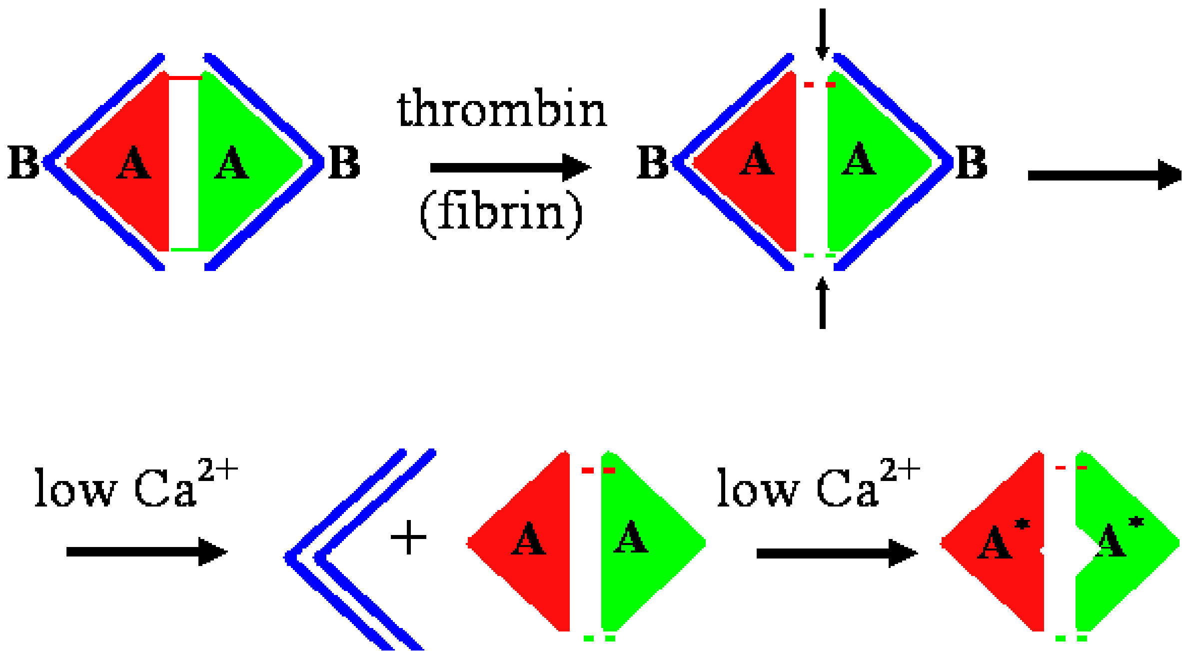
Potentially active FXIII subunit:

recommended term: A subunit of factor XIII (FXIII-A) not recommended terms: factor XIII a or *a* subunit, (FXIIIa, FXIII*a*, FXIII a, FXIII *a*, FXIII-a, FXIII-*a*)

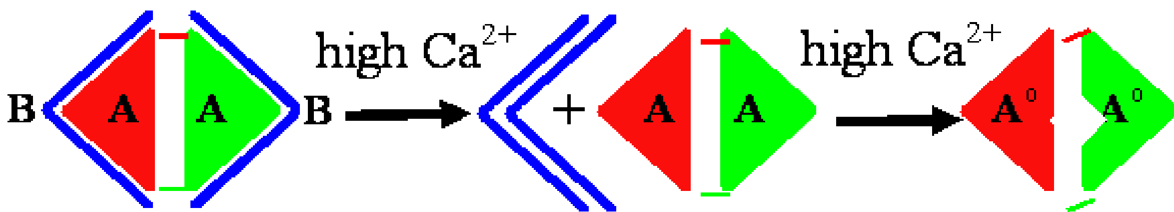
Inhibitory/carrier FXIII subunit:

recommended term: B subunit of factor XIII (FXIII-B) not recommended terms: factor XIII b, *b* or S subunit, (FXIIIb, FXIII*b*, FXIIIS, FXIII b, FXIII *b*, FXIII S, FXIII-b, FXIII-*b*, FXIII-S)

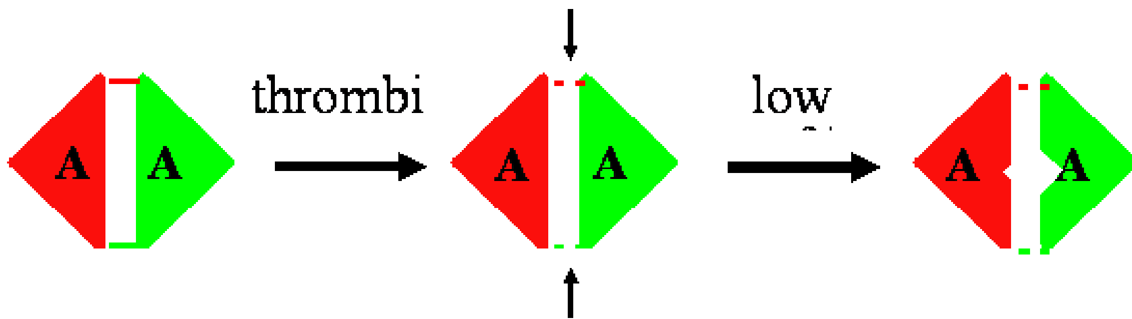
Proteolytic activation of plasma



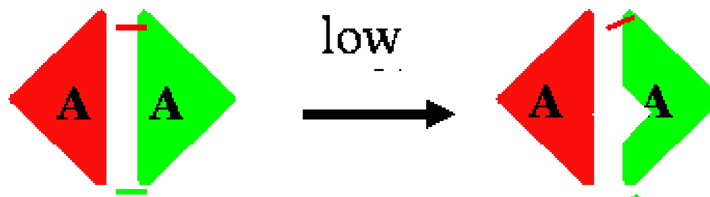
Non-proteolytic activation of plasma FXIII



Proteolytic activation of cellular



Non-proteolytic activation of cellular



PROPOSED FXIII TERMINOLOGY AND

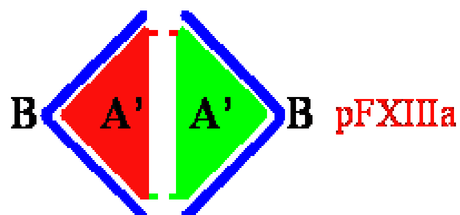
ABBREVIATIONS II.

Activated form of blood coagulation factor XIII in general:

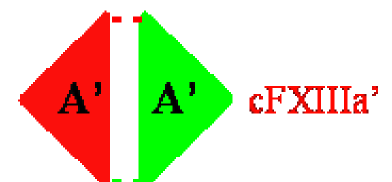
activated factor XIII (FXIIIa)

Activation peptide cleaved off from the A subunit by thrombin: factor XIII activation peptide (AP-FXIII)

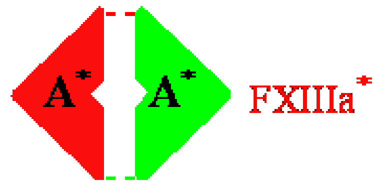
Intermediates and endproducts of the activation process:
Thrombin cleaved inactive form of plasma and cellular factor XIII:

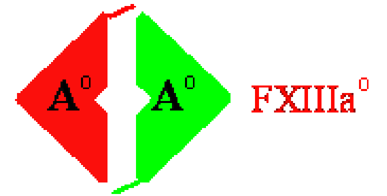


Thrombin-cleaved active factor XIII:



Non-cleaved active factor XIII:


A*
A*
FXIIIa*


A⁰
A⁰
FXIIIa⁰

Factor XIII

Chair: R. Ariens
Co-Chairs: A. Ichinose, H. Kohler, M. Maurer, R. Seitz.
Active Members: L. Muszbek, A. Inbal, V. Ivaskevicius

Session I: Vascular biology. *Ikuro Maruyama* (Japan) discussed the role of nuclear DNA binding protein HMGB1, which is found intracellularly as well as in the circulation, in inflammation and multiple organ failure during sepsis. Recent data show and effect of HMGB1 on several coagulation parameters, amongst which FXIII. HMGB1 competes with thrombin for thrombomodulin binding and increases FXIII activation. *Aida Inbal* (Israel) presented data on the role of FXIII in angiogenesis and wound healing. Experimental models in FXIII knockout mice show reduced angiogenesis and wound healing. Reconstitution with FXIII restores these defects effectively.

Session II: Registry and standardisation. *Vystas Ivaskevicius* (Germany) presented the first International Registry on FXIII mutations and deficiency. The registry currently lists data from 105 patients with FXIII deficiency, including mutations in both FXIII A- and B-subunit, and details on phenotypic presentation. A website for the registry that includes submission forms and where information on the mutations can be found has been set up at www.f13-database.de. Currently most data are from patients from Europe , and submissions from the rest of the world including less developed countries are encouraged. *Sanj Raut* (UK) presented an update on current standardisation activities of FXIII. Data from the addendum report on assignment of antigen level to the 1 st International plasma Standard for FXIII was presented. The report has been approved by the SSC, FXIII SWG (standard working group) and expert reviewers. Approval will be sought from the Business meeting to submit the report to the WHO. Protocols for an International collaborative study for the development of a standard for FXIII concentrate were presented. It is currently discussed which assays should be included in this study. *Akitada Ichinose* (Japan) presented an overview of the history of the FXIII standard working group, which he chairs. Issues regarding formation of rFXIII-A complexes with B in plasma were discussed in light of eventual need for a standard for rFXIII-A. Dr Ichinose expressed concerns regarding acknowledgement and financial support for the contribution of academics and scientists (who are funded by research and government grants) involved in the standardisation processes.

Session III: Measurements. *Muriel Maurer* (USA) presented an overview of current methodologies available to transglutaminase scientists for the measurement if these enzymes. Sensitivity and specificity of the various assays, including spectrophotometric methods, radioactive labelling, biotin/streptavidin systems, fluorescence and phage displays were discussed. Suitability for high-throughput strategies (low volume, suitability to multiwell plates) were also discussed. Dr Maurer suggested that future assay systems may be developed that make use of mass spectrometric or NMR technologies. *Janos Kappelmayer* (Hungary) discussed data using flow-cytometry that showed the presence of FXIII-A in the blast cells of monocytic and myelomonocytic origin in patients with acute myeloid leukemia and acute lymphoblastic leukaemia (ALL). Sixty percent of ALL cells stained positive for FXIII-A. Data were confirmed by western blots and ELISA and suggest that FXIII-A may be a useful marker in the diagnosis of

ALL. *Rainer Seitz* (Germany) discussed data from a fluorescent isopeptidase assay. The assay is based on the release of a quencher by transamidase activity. The quencher is released from a synthetic peptide that is based on the sequence of alpha2-antiplasmin, a specific substrate for FXIII. Data were shown on the Km and kcat values for FXIII in plasma and FXIII/fibrinogen mixtures. The assay has a low detection limit and shows linearity with other activity assays.

Session IV: Regulation of FXIII. *Hans Kohler* (Switzerland) discussed preliminary data from a novel ELISA assay designed to measure the activation peptide of FXIII. In plasma, the FXIII AP signal increases within minutes from the addition of thrombin with concomitant decrease of the A2B2 tetramer signal. FXIII AP was also detected in serum. The assay system will be further characterised with regards to specificity and sensitivity. *Laszlo Muszbek* (Hungary) discussed novel data on the degradation of FXIII by proteases (cathepsin G, elastase) released by polymorphonuclear cells in the clot. Elastases degraded FXIII within 3 hours, alomg with fibrin degradation which also occurs by the same enzymes. The degradation was reversed by specific inhibitors of these enzymes. *Helen Philippou* (UK) showed novel data on the degradation of FXIII by plasmin. This degradation was found to be dose- and time-dependant, reversed by alpha2-antiplasmin, occurred mainly with activated FXIII, was enhanced by fibrin and was shown to occur in plasma clots. The degradation of FXIII by plasmin preceded lysis of the clots. Clots from plasminogen depleted plasma did not show FXIII degradation.