



Overall research theme:

Clinical and biochemical aspects of the haemostatic balance in relation to development and prevention of cardiovascular diseases.

Latest update:

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Characteristics of the research group:

The research activities of the Department for Thrombosis Research are mostly related to the research areas defined by the Ministry of Research and the Ministry of Health as high priority areas. These are defined in the national strategy for health research (NASTRA), in "Dansk Sundhedsforskning - tilbud og muligheder", January 1996, and in the preventive programmes 1998-2007 given by the Danish Government. A major part of the activities, including "preventive research" is additionally within the area "genetic research and treatment modality research". The persistent goal of our activities is to achieve the best possible health promoting measures and prevention of thrombotic and atherosclerotic diseases, and to maintain a combination of basic research and patient-related research to improve the velocity of implication of new knowledge in the treatment of patients. Thus, we attempt to use health research as an instrument to improve treatment. In particular, our research is directed towards tracking down patients at high risk for developing thrombotic diseases and towards a more rational and hopefully preventive treatment. The cornerstone of our research is establishment of clinical biochemical assays among others techniques within molecular biology. This is related to the following areas of interest:

- Clinical, experimental research focussing on risk stratification of patients suffering from myocardial infarction at young age, diabetic patients, pregnant women, and women treated with oestrogen
- Experimental studies to improve the understanding of the basic mechanisms involved in the regulation of the processes of blood coagulation and fibrinolysis, and their correlation with other metabolic systems
- Research in prevention (intervention strategies) to reduce the risk of groups of patients at high risk and individuals - in particular focussing on the gene/lifestyle interplay
- Methodological and theoretical research on the standardization of analysis techniques and treatment strategies
- Elucidation of pathological disturbances in genes and proteins important for inflammation and coagulation, fibrinolysis and thrombosis

Running projects: Titles and abstracts:

Risk studies of cardiovascular diseases in post menopausal women with special reference to the effect of hormone replacement therapy (DOPS)

In 1991, a national prospective multicentre trial was initiated in Denmark to study the protective effects of HRT against osteoporosis (The Danish Osteoporosis Prevention Study). At study entry, 719 postmenopausal women were randomised to either hormone replacement therapy or no treatment. We collaborate on this study together with the Odense University Hospital (Dr. Jonna Skov Madsen). The aim is to analyse the genotype-specific effect of long-term hormone replacement therapy on haemostatic and inflammatory cardiovascular risk markers.

The Collaborative Danish Climacteric Study

The Collaborative Danish Climacteric Study generates integrated data on a variety of key variables obtained from comparative investigations performed in healthy postmenopausal women during administration of different types of hormone replacement therapy (HRT). The study was performed in co-operation with the Copenhagen University Hospital (Rigshospitalet and Frederiksberg Hospital) (Prof. Sven O. Skouby). The data comprise information from 12-month study periods in six groups of postmenopausal women with comparable clinical characteristics at baseline. The aim of the study was to compare the effect of various HRT-regimens on haemostatic and inflammatory markers, in order to perform a biochemical evaluation of the potential thrombogenicity of HRT.

Open-label, randomised study to evaluate the effects of seven monophasic oral contraceptive regimens on haemostatic variables (E-1658)

This international multicentre study focuses on the influence of genetic polymorphisms on the effect of 2nd and 3rd generation oral contraceptives on the haemostatic system.

The haemostatic system in patients with phlebographically verified deep venous thrombosis

The study is performed in collaboration with Department of Haematology and Department of Radiology, Ribe County Hospital, Esbjerg/Varde. The study includes more than 300 consecutive patients referred to the hospital with clinically suspected deep vein thrombosis (DVT). The aim was to evaluate the potency of a number of biochemical markers in separating patients with DVT from patient without DVT. The primary focus is put on D-Dimer, lupus anticoagulant, anti-cardiolipin antibodies and antibodies against β 2-glycoprotein I.

Insulin resistance in general practice

The study includes 1350 patients from a general practitioner's practice (Dr. Poul Erik Heldgaard, Tjele, Denmark). The aim is to establish the prevalence of insulin resistance in an unselected population and to investigate the relationship between insulin resistance and haemostatic and inflammatory cardiovascular risk markers.

A population-based study of 60-year individuals followed for 20 years. The Glostrup Population Study

This study is performed in collaboration with the Glostrup University Hospital (Dr. Thomas Drivsholm). A cohort of all men and women born in Glostrup in 1936 (n=1198) has been followed since 1976. In 1996, the cohort was re-examined (695 subjects), and we investigated the genotype-specific association between haemostatic and inflammatory cardiovascular risk markers and ultrasonographic measurements of carotid artery intima-media thickness (IMT) and plaques.

Treatment of acute respiratory distress syndrome with inhaled plasminogen activator

These studies have focused on the systemic and local effects of inhaled plasminogen activators in traumatised pigs. They include experimental studies on aerosolisation of a plasminogen activator, the effects of a standardised gunshot trauma on haemostasis in pigs, and the local and systemic effects of aerosolised plasminogen activators in pigs exposed to gunshot trauma. Further studies are in progress dealing with these aspects in humans.

Isolation and characterisation of the factor XII-dependent plasminogen activator

The aim of this study is to isolate and characterise a plasminogen activator putatively present in the surface-induced pathway of fibrinolysis. Chromatographical techniques such as ion exchange chromatography and gel filtration are used for isolation of proteins while the characterisation is performed by means of SDS-PAGE, native PAGE, Western blotting, fibrin clot-lysis and zymography.

The effect of surface binding on the plasminogen activating properties of the components of the surface-induced haemostatic pathway

Factor IIa, Factor XIa and kallikrein are involved in the surface-induced fibrinolytic pathway. Preliminary results have indicated that the plasminogen activating properties of these enzymes are dependent on their binding to surfaces. The aim of this study is to characterise the specificity of surface exposure on the plasminogen activating properties of "factor XIIa, factor XIa and kallikrein. The study involves various techniques such as SDS-PAGE, native PAGE, Western blotting, fibrin clot-lysis and zymography.

"Cost-effectiveness of Computer-Assisted Anticoagulant Dosage" under European Action on Anticoagulation (EAA) (2002-) – Programme no. QLG4-CT-2001-02175.

A randomised study for evaluation of the value of computer assistance for calculation of dosage of vitamin K antagonists. The efficacy of oral anticoagulant treatment is evaluated by the presence of clinical end-points such as thrombosis or bleeding.

Standardisation of fibrinolysis assays within the framework of IFCC/ISTH

There is a lack of well-established criteria for the specific measurement of fibrinolytic variables. On behalf of the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis the Subcommittee on Fibrinolysis started a process to develop such criteria. Senior staff members from the Department for Thrombosis Research are involved in a number of SSC-working groups dealing with establishment of reference methods for determination of fibrinolytic variables.

Genetic contribution to levels of haemostasis and inflammatory cardiovascular risk factors in the elderly. A twin study

In this study, we determined in elderly twins (73-94 years old) the contribution of genetic factors to the variation in plasma levels of haemostatic and inflammatory cardiovascular risk markers and the contribution of corresponding genetic promoter polymorphisms to this variation. The study is performed in collaboration with Professor Kaare Christensen, Institute of Public Health, University of Southern Denmark.

Impact of dietary antioxidant intervention on markers of oxidative stress and haemostasis

In this Ph.D-study (Ph.D-student Christine Dalgård) we investigate, how dietary antioxidants affect atherosclerotic risk markers among patients with peripheral atherosclerotic disease.

The Collaborative Climacteric Study - US/EU program

A multinational, multicentre, randomised, double-blinded, parallel, actively controlled, comparative study, aimed for evaluation of the endometrial, histological profile after treatment with tibolone (OD14) or conjugated oestrogen plus medroxyprogesterone acetate (MPA) among postmenopausal women. The study is conducted in co-operation with Prof. Sven O. Skouby.

Rehabilitation of patients with ischaemic heart disease

The effects of a multidisciplinary rehabilitation program (including dietary guidance, smoking cessation, and intensive cardiovascular exercise) on circulation, endothelial cell function, inflammatory markers and the haemostatic system.

Internal quality control of PCR-based genotyping methods in research studies and patient diagnostics

In this project, we focus on internal quality control aspects of PCR-based DNA polymorphism analyses used in thrombosis research.

The fibrin clot structure and risk of cardiovascular disease. Importance of environmental and genetic factors.

The aim of this project is to characterise the fibrin clot structure in groups of patients with atherothrombotic diseases and to look for environmental and genetic determinants of the fibrin clot structure (in fibrin clots made ex vivo from plasma samples).

Effects of different insulin regimens on postprandial coagulation activation in patients with type II diabetes.

This study is a dietary cross-over study on patients with type II diabetes. The aim of the study is to investigate the effect of different insulin treatment regimes on postprandial coagulation activation, lipids, and glucose metabolism. Furthermore, the effects on other atherothrombotic risk markers, e.g. fibrinolysis, clot structure, inflammation, and endothelial function, will be investigated.

Chemometric data analysis (Unscrambler) of cardiovascular disease studies.

Multivariate (chemometric) data analysis is applied on cardiovascular studies already analysed with traditional statistics. The aim is to compare and combine the statistical methods in order to understand new relations and mechanisms and hopefully simplify prediction of cardiovascular risk. The study is performed in co-operation with Prof. Kim Esbensen PhD, Dep. for Chemical and Applied Engineering Sciences, Ålborg University Esbjerg.

Intraperitoneal LMW heparin in peritoneal dialysis. The MesoHep II study.

Patients with end-stage renal disease suffer from chronic low-grade inflammation in addition to marked atherosclerosis. We have previously demonstrated the adventurous effects of intraperitoneal (IP) LMWH heparin on peritoneal transport kinetics and local inflammation in patients on peritoneal dialysis (PD). In the present MesoHep II study the effect of IP heparin on markers of atherosclerosis and systemic inflammation in addition to further studies on peritoneal transport kinetics are to be investigated. The study is a double-blinded, randomized, place-controlled crossover study of IP tinzaparin versus placebo in patients on long-term PD.

The institutions involved in the project are Dep. of Clinical Biochemistry and Dep. of Nephrology, Ribe County Hospital in Esbjerg/Varde, Denmark, Dep. for Thrombosis Research, University of Southern Denmark. The participants are: Mikkel Brabrand (M.D.), Jonas Angel Sjøland, Robert Smith Pedersen (M.D.) and Jørgen Gram.

Inflammation and fibrin structure in patients with end-stage renal disease

The ph.d.-study comprises three clinical substudies:

The fibrin structure in peritoneal dialysis patients. A cross-section study.

The fibrin structure in hemodialysis patients. A cross-section study.

The influence of heparin on fibrin structure in patients on peritoneal dialysis.

Patients with end-stage renal disease suffer from increased mortality rates of cardiovascular disease in addition to their chronic low-grade inflammation. The aim of present ph.d-study is to test the hypothesis, that the fibrin structure in patients with chronic low-grade inflammation is more rigid and more resistant to fibrinolysis than in normal controls, and that these properties can be reversed by heparin.

The institutions involved in the ph.d.-study are Dep. of Clinical Biochemistry and Dep. of Nephrology, Ribe County Hospital in Esbjerg/Varde, Denmark, Dep. for Thrombosis Research, University of Southern Denmark, and Dep. for Chemical and Applied Engineering Sciences, Aalborg University Esbjerg, Denmark. The participants are: Jonas Angel Sjøland ; Jørgen Gram; Kim Esbensen; Robert Smith Pedersen (M.D.); and Johannes Sidelmann.

Recent publications related to the projects described above:

1. The Writing Group for the third European Conference on Sex Steroids and Cardiovascular Diseases. *The European Consensus Development 2002: Sex Steroids and Cardiovascular Diseases. On the route to combined evidence from OC and HRT/ERT, Monte Carlo, November 2002. Maturitas 2003;44:69-82.*
2. Poller L, Keown M, Chauhan N, van den Besselaar AMHP, Tripodi A, Shiach C, Jespersen J. Minimum numbers of ECAA lyophilized plasmas for ISI calibration of CoaguChek and TAS point-of-care whole blood prothrombin time monitors *Am J Clin Pathol 2003; 119: 232-40.*
3. Poller L, Keown M, Chauhan N, van den Besselaar AMHP, Tripodi A, Shiach C, Jespersen J. European Concerted Action on Anticoagulation (ECAA). Correction of displayed INR on two point-of-care-test (POCT) whole blood prothrombin time monitors (CoaguCheck Mini and TAS PT-NC) by International Sensitivity Index (ISI) calibration. *Br J Haematol 2003;112:944-9.*
4. Poller L, Keown M, Chauhan N, van den Besselaar AMHP, Tripodi A, Shiach C, Jespersen J. An assessment of a method for ISI calibration of two whole blood point-of-care PT monitor systems based on lyophilised plasmas using whole blood equivalent PT. *J Thromb Haemost 2003; 1: 766-72.*
5. Poller L, Keown M, Chauhan N, Shiach C, van den Besselaar AMHP, Tripodi A, Shiach C, Jespersen J. European Concerted Action on Anticoagulation (ECAA). Reliability of international normalised ratios from two point of care test systems: comparison with conventional methods. *Br Med J 2003; 327: 30-2.*
6. Bladbjerg EM, Skouby, SO, Andersen LF, Jespersen J. Effects of different progestin regimens in hormone replacement therapy on blood coagulation factor VII and tissue factor pathway inhibitor. *Human Reprod 2002;17:3235-41.*
7. Bladbjerg EM, Gram J, Jespersen J, de Maat MPM. Internal quality control of PCR-based genotyping methods in research studies and patient diagnostics. *Thromb Haemost 2002; 87:812-6.*
8. Madsen JS, Kristensen SR, Klitgaard NA, Bladbjerg E-M, Gram J, Abrahamsen B, Stilgren L, Jespersen J. Effect of hormone replacement therapy on plasma homocysteine in postmenopausal women: A randomized controlled study. *Am J Obstet Gynecol 2002;187:33-9.*
9. Abrahamsen B, Madsen JS, Tofteng CL, Stilgren L, Bladbjerg EM, Kristensen SR, Brixen K, Mosekilde L. A common methylenedihydrofolate reductase (C677T) polymorphism is associated with low bone mineral density and increased fracture incidence following the menopause: Longitudinal data from the Danish Osteoporosis Prevention Study. *J Bone Min Res 2003;18:723-9.*
10. Bladbjerg EM, Gram J, Jespersen J, De Maat MPM. Internal quality control of PCR-based genotyping methods: Practical experiences. *Vascular Pharmacology 2002;39:127-9.*
11. de Maat MPM, Bladbjerg EM, Drivsholm T, Johnsen KB, Møller L, Jespersen J. Inflammation, thrombosis, and atherosclerosis: results of the Glostrup study. *J Thromb Haemost 2003;1:950-7.*
12. Bladbjerg EM, Madsen JS, Kristensen SR, Abrahamsen B, Brixen K, Mosekilde L, Jespersen J. Effect of long-term hormone replacement therapy on tissue factor pathway inhibitor and thrombin activatable fibrinolysis inhibitor in healthy postmenopausal women. A randomised controlled study. *J Thromb Haemost 2003;1:1208-14.*
13. Madsen JS, Kristensen SR, Gram J, Bladbjerg EM, Henriksen FL, Gram J, Christensen K, Jespersen J. Positive impact of hormone replacement therapy on the fibrinolytic system: A long-term randomised controlled study in healthy postmenopausal women. *J Thromb Haemost 2003;1:1984-91.*
14. Gram J, Münster A-M, Dilling-Hansen B, Lavassani HA, Lahoz AX, Jespersen J. Inhalation/ intravenous recombinant tissue plasminogen activator and inhaled heparin in a patient with acute respiratory distress syndrome. *Fibrinolysis & Proteolysis 1999;13:209-12.*
15. Münster AMB, Bendstrup E, Jensen JI, Gram J. Jet and ultrasonic nebulization of single chain urokinase plasminogen activator (scu-PA). *J Aerosol Med 2000;13:325-33.*
16. Münster A-MB, Jensen JI, Bech B, Gram J. Activation of blood coagulation in pigs following lower limb gunshot trauma. *Blood Coagul Fibrinol 2001;12:477-85.*
17. Münster A-MB, Rasmussen L, Sidelmann J, Jensen JI, Bech B, Gram J. Effects of inhaled plasminogen activator on the balance between coagulation and fibrinolysis in traumatized pigs. *Blood Coagul Fibrinolysis 2002;13:591-601.*
18. Sidelmann JJ, Jespersen J, Andersen LF, Skouby SO. Hormone replacement therapy and hypercoagulability. Results from the Prospective Danish Climacteric Study. *Br J Obstet Gynecol. 2003;110:541-7.*
19. Sidelmann JJ, Booth NA, Hoffmann J, Nesheim ME, Rosén S. Criteria for specific measurement of plasminogen (enzymatic; procedure) in human plasma. *eJIFCC, vol 12 No 3, 2000.*
<http://www.ifcc.org/ejifcc/vol12no3/plasminogen.htm>
20. Sidelmann JJ, Gram J, Jespersen J, Klufft C. Fibrin clot formation and lysis: basic mechanisms. *Semin Thromb Hemost 2000;26:605-18.*