Innovative Coagulation Diagnostics
The blood clotting system serves a vital role in the protection against bleeding in case of injury. However, the blood clotting process can also be activated in response to internal injuries of the vascular system (for example, inflammation or rupture of an atheromatous plaque), which can result in excess clotting, i.e. thrombosis. Current diagnostic tests to assess the risk of bleeding or thrombosis, are laboratory based (expensive, cumbersome) and lack sensitivity. The main aim of the INCOAG program was to develop a set of state-of-the-art diagnostic tests that ultimately can be used in a near-patient setting to estimate, to estimate the risk of bleeding, or thrombosis. By developing either overall assays, or methods that assess specific pathways from the coagulation cascade, or novel markers linked to coagulation (miRNA), a set of new laboratory methods has now been developed that have the potential for further clinical development. These assays include:

1. A new thrombin generation based FXIa assay demonstrates a significantly increased FXIa plasma concentration in acute myocardial infarct patients (MUMC+);
2. A method for the accurate measurement of thrombogram parameters in a point-of-care setting with a single drop of blood from a fingerstick (Synapse and MUMC+). This prototype model is currently optimized towards a point of care device in the PREBAT validation program, funded by CTMM and NHS.
3. An enzyme-linked immunosorbent (ELISA) assay that specifically recognizes FVIIa without recognizing its zymogen FVII (10,000 time more specific) (UMCU). Other nanobody based ELISAs, including assays that detect complexes between coagulation proteases and antithrombin, are added (LUMC, MUMC) to establish a panel that can be converted onto a miniaturized detection device that is developed by Philips. Application of an associated systems biology module will address combinations of testing to be included into clinical prediction rules (RAM) (Philips);
4. A microspot-based whole-blood perfusion test for assessment of shear-dependent thrombus formation combined with a systematic analysis of a panel of output has been developed. Deficiency in formation of type III thrombi in this measurement system may be a good indicator of major platelet function deficiency, associated with an increased risk of bleeding (MUMC+).
5. Normalization procedure miRNA (AMC) and characterization of altered miRNA profiles, especially predict aspirin resistance as measured by whole blood aggregation (AMC).

The total package of assays has been validated in a nested case control study in patients with systemic atherosclerosis, while separate studies towards the predictive value of combinations of the above technologies are either completed or underway (funded through the DUCODIS NHS grant). Finally, MTA is involved in developing models for calculating cost-efficacy for these new as compared to conventional laboratory assays, related to cardiovascular disease (Kemta, MUMC+). In the course of the next 2 years the clinical utility and economic value of each assay and combinations of above assays, will become evident. Similar to the current clinical validation of the whole blood thrombin generation assay, we hope and expect to be able to further develop a substantial number of the prototypic assays for commercialization and ultimate application in practice!

Prof.dr. H. Ten Cate

“This is the first time that a concerted collaborative effort involving top Dutch researchers and industrial players is being undertaken to improve coagulation diagnostics! The combination of up to date translational research and state-of-the-art industrial technology provides a strong basis for establishing new diagnostic tests that may better detect the risk of thrombosis and may also be suited to monitoring a range of new antithrombotic drugs.”
Thrombosis

Thrombosis is a consequence of aberrant interactions of the three elements of the triad of Virchow, that is the blood flow, the (damaged) vessel wall and the blood constituents. Blood coagulation and platelet activation are the two principal processes that in an interactive way establish formation of a blood clot and thrombus. Blood flow controls the delivery of thrombus constituents, but also limits the size of the formed clot.

Already decades ago, kinetic tests (clotting times, bleeding times) have been devised that allow to detect haemostatic defects due to a lack of a particular coagulation factor (e.g., haemophilia) or a lack of platelet function (e.g., subtypes of von Willebrand’s disease). Although such tests can identify clinically relevant haemorrhagic diatheses, they are inadequate to detect an increased tendency to clotting. Also, many of the newly developed tests are too expensive to be applied in general practice (outside the research laboratory).

Aim of INCOAG

Our goal is to develop a set of state-of-the-art diagnostic tests that can be used to estimate -with more ease and sensitivity than now possible-, the risk of bleeding as well as venous or arterial thrombosis. The same tests will also be more useful to monitor the efficacy of preventive and curative antithrombotic medication of any type, even at home.

The development of three different but complementary types of test systems:

- methods that estimate the clotted activity in the circulating blood (real time assays).
- methods that measure the capacity of the blood to clot (capacity assays).
- methods that assess miRNA’s involved in blood coagulation protein synthesis and activity

The outcome will be implemented on miniature platforms, where the thrombin-generating and clot-forming potential of blood can be assessed in a test tube-like manner.

Clinical Need

An optimal set of complementary assays to assess thrombosis and/or bleeding risk in blood samples aimed at small scale devices for application in routine clinical laboratory or in general practitioner’s setting:

Tools

1) coagulation activation markers, with an associated systems biology module,
2) coagulant capacity under flow conditions
3) micro-RNA as new markers associated with thrombosis risk.
Public-Private Partnership

New devices for risk assessment and monitoring of Thrombosis
Organization and Partners

**Organizational Structure**

**Advisory Board**
- ISAC CTMM

**Steering Cie**
- Partner Representatives
- CTMM

**Project Team**
- PI: Prof. H. Ten Cate (MUMC+)
- Co-PI: Prof. P. Reitsma (LUMC)
- WP-leaders
- Industrial Partners
  - Dr. E. Buitenhuis (Dutch Heart Foundation)
  - Dr. E. Erdtsieck-Ernste (CTMM)

**Workpackage Leaders**
- WP1: Prof. P. Reitsma (LUMC)
- WP2: Dr. B. de Laat (Synapse)
- WP3: Prof. J. Heemskerk (MUMC+)
- WP4: Prof. J. Meijers, Dr. S-J. Pinto-Sietsma (AMC), Prof. J. Voorberg (Sanquin Blood Supply)
- WP5: Dr. M. Joore (MUMC+)
- WP6: Prof. H. Ten Cate (MUMC+)

**Partners**
- Coordination
- Finance
- Publications

**Operations**
- CTMM

**Coordination**
- Finance
- Publications
Budget: CTMM manages the flow of funds

**Funding:**
- 25% Academia
- 25% Industrial
- 50% Government Subsidy

**Project costs:**
- Personnel
- Materials
- Use of existing equipment
- Investments
- Third parties
- Management (5%)
Facts & Figures

Distribution of the INCOAG consortium budgets to perform the R&D activities

**Budget**: 14,1 M€
**Start**: 2009
**End**: 2015
**Partners**: 8

**CASH COSTS**

- **PhD**
- **PostDoc**
- **Sen. Staff**
- **Supp. Staff**
- **M&S**
- **Investments**

**KIND COSTS**

- **Academic cash costs**
- **Industrial cash costs**
- **Academic in kind costs**
- **Industrial in kind costs**

**Legend**

- Academic
- Industrial Large
- Industrial SME
- CTMM investments
# Facts & Figures

<table>
<thead>
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<th>Category</th>
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<td><strong>Budget</strong></td>
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<td><strong>End</strong></td>
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<td><strong>FTE</strong></td>
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</table>

| Papers                  | 29                                                                      | 23 papers in submission - mean impact factor all published INCOAG papers: 6.9 |
| Theses                  | 4                                                                       | 10 in preparation                                                           |
| Personal grants         | 4                                                                       | LSBR Research Grant 2010 (0219) Prof. Dr. J. Heemskerk  ZonMW 2013 Research Grant (114021004) Prof. Dr. J. Heemskerk Senior Dr. Dekker Grant 2012 R. van Oort Dr. Dekker Grant NHS 2011 Dr. J. Cosemans |
| Patent Applications     | 3                                                                       | Dr. B. Bakker (Philips) Dr. SJ Pinto-Sietsma (AMC) Dr. SJ Pinto-Sietsma (AMC) |
| Licenses                | 0                                                                       | None                                                                      |
| Spin-off Companies      | 0                                                                       | None                                                                      |
| Raising Capital (> 1 M€)| 1                                                                       | PREBAT, Valorization grant CTMM – Dr. B. de Laat (Synapse), Prof. Dr. H. Ten Cate (MUMC), Dr. E. Buitenhuis (NHS) |
| Awards                  | 8                                                                       | Dr. M. Bos (LUMC) – Bayer Early Career Investigator Award 2011 & Ulla Hedner Award 2013 Prof. dr. J. Heemskerk - BACH Investigator Recognition award and Willy van Heumen Prize 2011 Dr. M. Roest – ISTH Science Award 2012 Dr. E. Creemers – Circulation Research Best Paper 2010 Dr. J. Cosemans - Mannucci award 2010 Drs. S. de Witt - Kootstra award/fellowship 2014 Dr. J. Cosemans - Edmond Hustinx award 2014 Prof. dr. Ph.G. de Groot: BACH Established Investigator Award 201 |
2011: A method for the accurate measurement of thrombogram parameters in a point-of-care setting with a single drop of blood from a fingerstick (Synapse)

2012: INCOAG researchers developed an enzyme-linked immunosorbent (ELISA) assay that specifically recognizes FVIIa without recognizing its zymogen FVII (10,000 time more specific) (UMCU)

2013: A new thrombin generation based FXIa assay demonstrate a significantly increased FXIa plasma concentration in AMI patients during the acute thrombotic event compared to the levels at 6 months post event and compared to healthy controls (MUMC+)

2013: A microspot-based whole-blood perfusion test for assessment of shear-dependent thrombus formation combined with a systematic analysis of a panel of output has been developed. Deficiency in formation of type III thrombi in this measurement system may be a good indicator of major platelet function deficiency, associated with an increased risk of bleeding (MUMC+)

2013: ELISA-based assays that measure activation markers of coagulation (FIXa and FXa in complex with AT) have been developed (LUMC)

2013: Cultures of blood outgrowth endothelial cells (BOECs) from peripheral blood of patients (Sanquin)

2013: Completion of the Peripheral Arterial Disease (PAD) cohort with 280 PAD patients and 140 controls. This cohort is one of the disease focus areas of this consortium (MUMC+)

2014: An miRNA identified that potentially can predict aspirin resistance (AMC)

2014: Analysis of coagulation parameters (procoagulant and thrombin generation markers) in a liver cirrhosis cohort (73 patients) was the first to reveal that even though these patients have an overall procoagulant plasma milieu, their whole blood clot formation capacity is reduced (MUMC+)

2014: Normalization procedure to characterization altered miRNA profiles in various atherothrombotic patient samples.

Highest Impact Papers – mean 13,3

1. Versteeg HH et al., Physiol Rev. 2013 Jan;93(1):327-58
3. van Kruchten R et al., Blood. 2013 Mar 7;121(10):1850-7
5. De Cuyper IM et al., Blood. 2013 Mar 7;121(10):e70-80

Mean Impact Factor

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<th>International - cardiovascular</th>
<th>CTMM - cardiovascular</th>
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2 - Mean impact factor based on 106 papers from the CTMM cardiovascular first call projects.
### Scientific Value Creation - Theses

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<th>Thesis</th>
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<tr>
<td>Brigitte Sondermeijer</td>
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<tr>
<td>Karen Gilio</td>
<td>MUMC+</td>
<td>2012&lt;sup&gt;cum laude&lt;/sup&gt;</td>
</tr>
<tr>
<td>Susanne de Witt</td>
<td>MUMC+</td>
<td>2014&lt;sup&gt;cum laude&lt;/sup&gt;</td>
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<td>Roger van Kruchten</td>
<td>MUMC+</td>
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<td>Marisa Ninivaggi</td>
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<td>2014</td>
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<td>Argon Hyseni</td>
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<td>Maayke Kok</td>
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<td>Nienke van Rein</td>
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<td>Marjolein Meinders</td>
<td>Sanquin</td>
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<tr>
<td>Simone Huijberts</td>
<td>UMCU</td>
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</table>
**Scientific Value Creation - Infrastructure**

- **Immuno- and coagulation assays**
  - Enzyme-linked immunosorbent (ELISA) assay that specifically recognizes FVIIa without recognizing its zymogen FVII (UMCU)
  - Enzyme-linked immunosorbent (ELISA) assays that specifically recognize activation markers of coagulation (FIXa and FXa in complex with AT) (LUMC)
  - Modified thrombin generation based assay was developed to quantify plasma levels of FXIa (MUMC+)
  - Novel flowcytometry test of platelet aggregation, in which 10- to 25-fold lower platelet counts or sample volumes can be used, either of platelet-rich plasma or whole blood from human subjects or small animal models (Sanquin)

- **Data-driven Methods**
  - Bioinformatics platform based on statistical and machine learning methods and computational biology methods (the latter developed outside INCOAG, partly under an STW project) for data integration purposes (Philips)
  - HTA Methods

- **Cohorts**
  - PAD cohort: 280 multicenter patients diagnosed with PAD and 140 healthy individuals
  - QUA-VKA cohort: >20,000 patients treated with Vitamin K antagonists. Blood and DNA collected. 90 bleeding cases and 360 controls will be selected to identify risk factors for bleeding complications
  - LIVER cohort: 73 patients with liver cirrhosis and 20 matched healthy controls
  - TRACS cohort: 120 ACS cases, 120 non-ACS controls selected in the MUMC+
  - PAS-PEDIGREE: sub-cohort of 500 premature atherosclerosis subjects
  - PREBAT Surgery cohort: 1000 surgery patients in which TG-POC is tested as part of the pre-surgical haematological screening
  - PREBAT Surgery cohort: 100 patient in which the hemostatic state is monitored during surgery

- **Biobanks**
  - VHH nanobody libraries which can be used to select new VHHs against antigens from various activated coagulation markers and platelets (UMCU)
  - ELISA robot for large scale sample testing (UMCU)
  - Microscope facility for measurement of coagulation under flow conditions (MUMC)
  - Proteomics facility for analysis of isolated Weibel Palade Bodies (Sanquin)
  - Advanced PCR miRNA screening with normalization procedure for high throughput measurements of selective miRNA panels (AMC)
Clinical and Economic Value Creation of the INCOAG Consortium

New ‘products’ for clinical care
Main Product Pipelines

Selection of coagulation biomarkers

- Production antigen
- Lama Immunisations
- Isolation & selection VHH
- VHH-based ELISA
- Commercial antibody based ELISA

Assay development

- Liver cirrhosis patients
- Selected assays for clinical validation

Assay verification in extreme phenotypic patients

- Thrombosis Service Patients
- Small groups of 8 different patient types with known coagulation deficiencies
- Glanzmann thrombasthenia & LAD-III patients

Clinical Verification & Validation

- Thrombosis Service Patients
- DVT PAD
- Not yet financed

- Thrombosis Service Patients
- PAD DVT
- Surgery

- Thrombosis Service Patients
- PAD
- Not yet financed

- Thrombosis Service Patients
- PAD
- Not yet financed

- Thrombosis Service Patients
- PAD re-in stent trombosis
- High risk atherosclerosis cohort

- Thrombosis Service Patients
- LETS cohort – algorithm
  5 patient characteristics & 3 laboratory measurements
- MEGA cohort
  DVT (cancer & recurrent)

Added value of the combination of activity, capacity and miRNA markers
The INCOAG project aims to identify a new panel of protein biomarkers for measurement on a new point-of-care biosensor that can be used to assess the level of thrombosis and bleeding risk of patients with vascular risks (e.g., during chemotherapy or after an earlier thrombotic event) and coagulation tendency.

**Hand held device**
A surface sensitive technique involving frustrated total internal reflection is used to detect the presence of magnetic particle labels attached to the sensor surface via a sandwich immunoassay. The optics and actuating electromagnets are integrated in a compact portable device. Fully integrated, plastic disposable cartridge allow for easy use.

**Leading Company**
Royal Philips

**Current Diagnostics**
A sensitive measurement of thrombosis and bleeding risk is currently not available.

**Expected impact for the patient**
A fast and easy-to-use blood test indicating thrombosis risk enables optimization of (prophylactic) anti-coagulant treatment. Ensuring reduction of (recurrent) thrombotic events and unnecessary treatment will improve patient's lives.

The handheld and easy-to-use system aims to be suitable for application outside of the clinical laboratory, e.g., peri-operative, in general practitioners offices and even by patients at home. The cost of testing is estimated to be substantially lower than the savings resulting from avoided thrombotic events and complications.

Proteomics of Weibel Palade Bodies

Weibel-Palade bodies (WPBs) are secretory organelles present in endothelial cells. The main component of these organelles is VWF, an important mediator of primary hemostasis. In addition, WPBs contain vaso-active, inflammatory and hemostatic proteins implying that release of bioactive components from WPBs is crucial for maintaining vascular hemostasis. We anticipate that the content of WPBs provides a reservoir of potential novel biomarkers for cardiovascular disorders. We perform mass spectrometry analysis of WPB-enriched endothelial subcellular fractions. A novel WPB candidate identified is insulin-like growth factor binding protein-7 (IGFBP7), which is reported to be involved in angiogenesis. Optimized technology is expected to reveal more novel biomarkers.

**Main results in CTMM**

**Assay development:**

**Experimental verification:**
Mass Spectrometry, Confocal Microscopy.

**Clinical Validation:**
Candidate biomarkers can be evaluated in different cohort-studies.

**Future Outlook:**
Biomarkers for Cardiovascular Disease.

**Expected impact for the patient**

**Quality of Life:** We anticipate that this project will provide us with a set of novel biomarkers for cardiovascular disease.

**Accessibility:** Mass spectrometry analysis is a powerful approach for proteomic analysis of WPBs. Candidate biomarkers can be evaluated in different cohort-studies.

This work was supported by CTMM, the Netherlands Organization for Scientific Research and the Netherlands Thrombosis Foundation.

An imbalance in the haemostatic system can lead to an increased bleeding tendency. The cause of a bleeding tendency can be either inherited or acquired, for example due to drugs or diseases. During surgery or as a result of (serious) accidents an increased bleeding tendency can lead to excessive, life-threatening blood loss. It is a challenge for the clinician to identify patients with an increased bleeding risk. At this moment there is no laboratory test to assess hemostasis in a quick and reliable fashion.

**Device**
The point-of-care Thrombin Generation Assay measures the haemostatic state at a fast and accurate manner in whole blood sample in a small volume, for example from a fingerpuncture. This new device can help in monitoring patients before and during surgery.

**Leading Company:** Synapse BV

**Current diagnostics:** aPTT, PT-INR, FPA Rotem

**Progress obtained in translational pipeline**

<table>
<thead>
<tr>
<th>Discovery Pathways biomarkers</th>
<th>Selection Pathways biomarkers</th>
<th>Demonstrator Development device</th>
<th>Clinical Evaluation cohorts</th>
<th>Market access</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td></td>
<td>2015</td>
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**Main results in CTMM**

**Development of the demonstrator:** The prototype of a point-of-care (POC) device for measuring thrombin generation in blood has been miniaturized. It consists of a microfluidic chip, micro dual channel fluorometer, micro heater and mobile phone. Lateral flow method was developed to determine Thrombin generation in a drop of whole blood, measured by LED illumination and detected by a CCD Firewire camera or microconfocal fluorescent detector.

**Experimental Verification:** Technical validation of the POC device showed intra- and inter-assay CV values of less than 10% for all thrombin generation parameters comparable to the results with the TG laboratory instruments.

**Clinical Validation:** In several small cohorts the first validation experiment have been performed. Currently a validation study has been started in 1000 surgery patients in which TG-POC is tested as part of the pre-surgical haematological screening.

**Future Outlook:** Currently we are testing the prototype in several diseases and clinical situations to evaluate in which situation the POC-TG device can be effectively used.

**Expected impact for the patient**

A fast and sensitive marker measurement enabling a more accurate prognosis. Improving patient safety by developing peri-procedural diagnostic and therapeutic interventions protocols (e.g. peri-operative bleeding risk management).

This test is fast and can be performed near the patient and can predict and thereby prevent excessive blood. This can save transfusion products which makes this method cost-effective.

Multispot Flow Chamber Assay – Thrombus Forming Capacity

**Development of parallel-plate microfluidic flow chambers with an array of platelet-adhesive surfaces for assessment of thrombus formation in whole blood under flow.**

The microscopic-based multivariate flow-chamber setup should be able to detect:

- insufficient hemostasis and bleeding risk,
- prethrombotic state and activated platelets,
- efficacy of established and novel antithrombotic medication,
- efficacy of blood component transfusion treatment during surgery.

**Device**

PDMS-based, small-volume microfluidics parallel-plate flow chamber with array of microspotted thrombogenic surfaces, operating with a table-top microscope.

**Leading Partner**

MUMC+, Sanquin

**Current Diagnostics**

Conventional platelet function tests (LTA, PFA).

**Main results in INCOAG (CTMM)**

**Development of the demonstrator.** The Maastricht chamber was used as prototype to develop a multiparameter micro spot assay for analysis of human and mouse blood samples. Based on this experience, various PDMS flow-chip devices have been developed with various channel numbers and diverse geometries.

The third generation device in combination with micro spots gave acceptable thrombus formation. Different fluorescence probes and microscopic image analysis techniques have been optimized for proper detection to obtain a standardized assay.

**Experimental Verification:** The various flow devices were used in numerous research studies with human blood (controls, patients with rare diseases, new antithrombotic agents) and mouse blood (genetic modifications of platelet and plasma proteins), the latter in comparison to in vivo thrombosis studies.

**Clinical Validation** with blood from patients with bleeding symptoms or peripheral arterial disease.

**Future Outlook:**


**Expected impact for the patient**

Early recognition of bleeding risk or prethrombotic state. Adequate control of (combined) antithrombotic medication directed against platelets and coagulation.

Test is fast and can be developed to a point-of-care method, used near the patient.

Multi-spot and multi-parameter testing of platelet (and coagulant) function provides a many-in-one test, which in principle can replace extensive and time-consuming platelet aggregation testing (LTA). The test operates with smaller blood samples than PFA-100.

**Flow Cytometry-Based Platelet Aggregation Assay**

The main function of platelets is to maintain normal hemostasis. Inefficient platelet production and/or defective platelet function results in bleeding disorders resulting from a wide range of genetic traits and acquired pathologies. Several platelet function tests have been developed for use in the clinic and in experimental animal models. In particular, platelet aggregation is routinely measured in an aggregometer, which requires normal platelet counts and significant blood sample volumes. For analysis of platelet in thrombocytopenic patients, infants, and animal models a more sensitive assay is needed.

<table>
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<tr>
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<th>B</th>
<th>PMA (t = 10 min)</th>
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</table>

Figure: human platelet rich plasma

**Device** flow cytometer

**Leading Company**: Sanquin

**Curent Diagnostics:**
Conventional platelet function tests (LTA, PFA).

This work was supported by the Landsteiner Foundation for Blood Transfusion Research, Sanquin Blood Supply, the Center for Translational Molecular Medicine, NWO & Deutsche Forschungsgemeinschaft

**Main results in CTMM**

**Assay development:**
A novel flowcytometry test of platelet aggregation, named FCA, has been developed, in which 10- to 25-fold lower platelet counts or sample volumes can be used, either of platelet-rich plasma or whole blood from human subjects or small rodents.

**Technical validation:**
The methods was validated in whole blood samples from patients with Glanzmann’s thrombasthenia and LAD-III patients as well in plasma of patients with auto-antibodies against platelet membrane antigens

This test is suitable for screening of chemical libraries or drugs, in small volumes of multiple samples on a large scale. The test is potentially very relevant for screening studies for the development of novel hemostatic drugs, but also for patients-directed antiplatelet therapy monitoring

**Future Outlook:**
FCA stands as a promising tool for the diagnosis of platelet defects and the for screening of drug effects on platelet function.

**Expected impact for the patient**
A novel promising tool, which allows analysis of platelet aggregation in thrombocytopenic patients or infants.

This set-up can be applied for samples containing low platelet numbers. The newly developed assay is also useful to test the effect of a variety of stimuli, drugs or antibodies on platelet aggregation without the need of special devices but a flow cytometer.

Key reference: De Cuyper et al Blood 121, 2013
In the search for new biomarkers in complex diseases such as venous and arterial thrombosis, miRNAs are promising candidates, since their expression is highly tissue specific during development and disease. Proof-of-principle for the use of miRNAs as biomarkers comes from cancer and heart research, where expression analysis studies reveal altered miRNA expression in tumors and diseased hearts as compared to normal tissues. A cohort of subjects with inherited premature cardiovascular disease will be used for discovery of a specific panel of miRNA, which will subsequently be validated in various clinical conditions.

Figure: heat map with hierarchical clustering to find disease-specific miRNA clusters.

**Device** Development of a device was not intended in this consortium. A PCR-based method will be used.

**Leading partners** AMC, Sanquin

**Current Diagnostics** Proteins and enzymes of the coagulation pathways

**Main results within CTMM**

- **Biomarker to detect aspirin insensitivity**
  
  We could show that a low expression of platelet miR-A after aspirin use was associated with aspirin insensitivity on aggregation.

- **New normalization method for circulating miRNAs.**
  
  A standardized normalization method was developed for the normalization of circulating miRNAs analyzed by qPCR, for whole blood, serum, and platelets.

- **Biomarker to detect smoking related atherosclerosis.**
  
  We could show that an up regulation of miR-B in healthy smoking individuals was associated with higher coronary calcium on CT scanning.

**Future Outlook:**

- Assessing the predictive value of miR-A for acute arterial thrombotic event in subjects on aspirin.

- Assessing the predictive value of miR-B for peripheral artery disease (PAD) in subjects whom smoke.

**Expected impact for the patient**

Observations that miRNAs displayed high stability in paraffin-embedded tissues from clinical samples or in human plasma raised the possibility that miRNA expression analysis may be a useful tool to define disease state.

The current discovered microRNA expression patterns can potentially identify subjects at risk of coronary artery disease in an early phase, so that preventative measures can be taken.

Key reference: in submission
The INCOAG strategy is based on the conviction that a panel of tests can make more accurate and reliable predictions than any one test separately. One reason underlying this idea is that the coagulation system is a highly complex and heterogeneous system. It is a vast network of interactions between many enzymes and other proteins, platelets and endothelial cells, and therefore hemostatic risk is expected to present on many different levels. No single parameter diagnostic test will be able to cover all these aspects of the coagulation system by itself and will therefore be too limited for complex diagnoses such as thrombosis or bleeding event prediction.

**Main results in CTMM**

**Development**: Software and analysis methods to interpret a combination of multiple risk estimators (levels of coagulation factors and clinical risk factors) to ultimately predict thrombosis risk have been developed and optimized. Employing a data-driven method (support vector machine approach), thrombosis risk can be estimated with high accuracy (AUC 0.79 on the LUMC LETS study) using a combination of three clinical risk factors and five protein concentrations, compared to an AUC of 0.70 without the use of protein concentrations.

**Clinical validation**: These results have been validated and improved on the LUMC MEGA study in a parallel project under the STW flag, and further improvements are ongoing.

**Added value**: More accurate identification of patients at high risk of thrombosis and subsequent choice of anticoagulant treatment prevents thrombotic events without increase in serious bleeding complications.

**Expected impact for the patient**

Multi-parameter tests, covering several aspects of the coagulation system will provide a much more complete assessment of a patient’s coagulation system and will therefore have much more predictive power compared to the single parameter approaches. Thus, although the patient will not directly notice a difference in clinical practice, the results of our study will enable a much more targeted and therefore safer preventive treatment of thrombosis. The patient will therefore have a much lower risk of developing a thrombosis or bleeding incident.

**Future Outlook:**

The methods developed will be used to target specific applications with a high need for better thrombosis risk assessment such as cancer patients and recurrent thrombosis, and implemented in clinical decision support software.

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**Device** algorithm

**Leading Company** Philips

**Current Diagnostics** Single assay parameters

*This work was supported by both STW and CTMM*

Key reference: Patent WO/2014/064585:
Early HTA: Key Elements and Findings

Three key elements of early HTA

Health economic modeling
In early stages of technology development health economic modeling is a powerful tool to assess the (potential) impact of the new health technology on future costs and health outcomes. It enables the use of different sources of information to answer this question. Also, it allows the exploration of the costs and outcomes of alternative options of using the new technology in clinical practice.

Cost-effectiveness analysis
Cost-effectiveness analysis is the comparative analysis of alternative courses of action, taking into account all relevant costs and health consequences. It tells us the extra cost per extra unit of benefit achieved when comparing one health technology against another. The extra unit of benefit is often expressed in Quality Adjusted Life Years (QALYs): one life year in perfect health. Decisions are made by comparing the additional costs for an additional QALY to a threshold. This threshold puts a limit on society’s willingness to pay for a QALY. Cost-effectiveness analysis inform the adoption a health care technologies by assessing the value for money.

Head Room Analysis
A headroom analysis determines the societal value and commercial viability of innovations in health care by using a threshold approach. It looks at the potential value of the use of a new health technology in a specific clinical setting and population. It asks the question whether the new health technology would be cost-effective if it would work as well as one would hope. It gives the maximum potential cost of the new technology factoring in any health service savings. If this cost [the headroom] is too low - then investments are not worthwhile.

Key findings INCOAG - PAD
There is a need for transparent, methodologically comparable and scientifically credible model-based economic evaluations in the field of Peripheral Arterial Disease (PAD).

A model-based health economic decision analysis suggests that targeted ABI screening and consequent secondary prevention of cardiovascular events using low dose aspirin or clopidogrel in the identified patients is a cost-effective strategy. Implementation of targeted PAD screening and subsequent treatment in primary care practices and in public health programs is likely to improve the health and to save health care costs by reducing catastrophic cardiovascular events.

Biomarker based PAD risk assessment and treatment tailoring is cost-effective. Identification of high-risk PAD patients and prescription of Oral Anti-Coagulants could save substantial health care costs and improve survival in high risk PAD patients. There is significant commercial headroom available for the development of more accurate risk stratifying biomarkers. Further research of risk stratifying biomarkers test accuracy is needed to support and strengthen the results of this modelling study.
Early HTA: Potential Impact of New Technologies

Health economic model

A probabilistic state transition model with the Markov property was used to represent the natural history of PAD. Health states used in our Markov model were PAD, post lower limb amputation (a local consequence of the PAD), post myocardial infarction (post MI) and post stroke (systemic consequences of generalized athero-thrombosis), post bleed (adverse consequence of preventive treatment) and an absorbing state of death.

The outcomes were life years, QALYs, and costs. The time horizon was life time and the cycle duration one year. This study was conducted using a societal perspective in the Dutch setting.

Two types of new interventions were assessed against usual care using the model:
- Screening for PAD and subsequent early anti platelet preventive treatment,
- stratification of PAD patients (d-dimer and subsequent intensified treatment of high risk patients with APT + OAC as opposed to APT alone).

Cost-effectiveness analyses: Screening

For the PAD screening strategy, life years and QALYs gained were 21.79 and 15.66 respectively at a lifetime cost of 26,548 Euros. Compared to no screening and treatment (usual care; 20.69 life years, 15.58 Quality Adjusted Life Years, 28,052 Euros), these results indicate that PAD screening and treatment is a dominant strategy. The probability of PAD screening being cost effective was 88% at a threshold of 40,000 Euros per QALY.

Cost-effectiveness analyses: Risk stratification

Usual care of all diagnosed PAD patients yielded on average 11.59 QALYs. Use of D-dimer for risk stratification followed by treatment of high-risk patients with OAC yielded on average 11.68 and 13.68 QALYs, respectively. The total mean costs per patient were 38,866 Euros for the usual care strategy, 38,056 Euros for the D-dimer strategy. Since the use of d-dimer for risk stratification and tailored treatment yields more QALYs against lower costs, this intervention is cost-effective. At a threshold of 40,000 Euros per QALY, the D-dimer strategy had a 99.3% probability of being cost-effective.

Headroom analysis

The headroom available for a perfect biomarker for risk stratification (100% accurate and zero costs) is computed to be 83,877 Euros per patient (test). This is the maximum additional cost of the biomarker test over the current care to be deemed cost effective.

In the base case analysis the proportion of high risk patients was assumed to be 20%. This assumption was changed in sensitivity analyses (see figure below). The results indicate that the headroom is sensitive to this input.
Partners

- Academic Medical Center (AMC) - Amsterdam
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- University Utrecht Medical Center (UMCU) - Utrecht
- Royal Philips - Eindhoven
- Sanquin Blood Supply - Amsterdam
- Synapse BV - Maastricht
- Dutch Heart Foundation - Den Haag
List of Publications

List of Publications


28. Martin-Ramirez J, Kok MG, Hofman M, Bierings R, Creemers EE, Meijers JC, Voorberg J, Pinto-Sietsma SJ. Individual with subclinical atherosclerosis have impaired proliferation of blood outgrowth endothelial cells, which can be restored by statin therapy. PLoS One. 2014 Jun 23;9(6).

29. Kok MG, Meijers JC, Pinto-Sietsma SJ. Individuals with coronary artery disease at a young age and features of the metabolic syndrome have an increased prothrombotic potential. Thromb Haemost. 2014 Mar 3;111(3):458-64.

Other Sources

- Samenvatting Medische Jaarverslagen van de Federatie van Nederlandse Trombosediensten (FNT) 2010.
- Bloedkatern, Jaargang 11, 2011
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ABI</td>
<td>Ankle Brachial Index</td>
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<tr>
<td>aPTT</td>
<td>activated Partial Thromboplastin Time</td>
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<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
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<td>BOECs</td>
<td>Blood Outgrowth Endothelial Cells</td>
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<td>CTMM</td>
<td>Center for Translational Molecular Medicine</td>
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<tr>
<td>CV</td>
<td>Cardio Vascular</td>
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<tr>
<td>DVT</td>
<td>Deep Venous Thrombosis</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked Immunosorbent Assay</td>
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<tr>
<td>FPA</td>
<td>Fibrinopeptide A</td>
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<tr>
<td>LTA</td>
<td>Light Transmittance Aggregometry</td>
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<tr>
<td>MI</td>
<td>Myocardial Infarction</td>
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<tr>
<td>PAD</td>
<td>Peripheral Arterial Disease</td>
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<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
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<tr>
<td>PFA</td>
<td>Platelet Function Analysis</td>
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<tr>
<td>POC</td>
<td>Point of Care</td>
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<tr>
<td>PT-INR</td>
<td>Prothrombin Time – International Normalized Ratio</td>
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<tr>
<td>STW</td>
<td>Stichting voor de Technische Wetenschappen</td>
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<tr>
<td>TG</td>
<td>Thrombine Generation</td>
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<tr>
<td>VHH</td>
<td>Variable domain of heavy chain of camelid antibodies</td>
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