SSC Subcommittee Project/Collaborative Project

NAME OF PROJECT

COSYNE COntact SYstem NEtwork

Subcommittee
Factor XI and the Contact System

Person responsible (Chair / Principal Investigator):
Coen Maas, PhD

Description Abstract
State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Suggested length is 2-3 paragraphs.

The contact system is considered redundant for hemostasis but contributes to thrombosis in preclinical studies in a variety of settings.
Although there are a multitude of emerging therapeutic strategies to block the contact system, there is a lack of direct clinical evidence for contact system activation in the patient plasma of thrombosis patients. This is for a large part attributable to the absence of generally available and standardized detection methods. Rather than comparing the efforts of various methodologies that are used in different labs, we propose a concerted methodological program. For our research, we have recently developed nanobody-based ELISA strategies to detect contact system activation in human plasma (Table 1). The aim of the proposed project is to disseminate our reagents and protocols of these assays for external validation; paying particular attention to the assay performance in relation to operator-dependency, (preanalytical) artefacts and preparation of assay standards for quantitation. We expect that this knowledge transfer will ultimately enable investigation of the contact system in human prothrombotic states.

Contact Factor	***Application / Validation***	***Reference***
Activated Factor XII (only detects free FXIIa)	In *vitro* studies	de Maat *Thromb Haemost* 2013 de Maat J Thromb Haemost 2018
	Bronchalveolar fluid in lung injury	Hess et al. *Thromb Haemost* 2017
		Wygrecka et al. *American journal of respiratory and critical care medicine* 2017
	Plasma (triggered) of HAE patients	de Maat et al. *J Allergy Clin Immunol* 2016
PKa-C1INH complexes *FXIa-C1INH complexes* *FXIIa-C1INH complexes*	Mechanistic + clinical studies on hereditary angioedema	de Maat et al. *J Allergy Clin Immunol* 2016
	In *vitro* studies on polyphosphate	Verhoef *Blood* 2017
Cleaved HK	A clinical study on hereditary angioedema	Hofman *J Allergy Clin Immunol* 2017

Table 1. Available assays and their published applications

Design and methodology (Data expected to collect, sample size and statistical analysis):
We will mass-produce nanobody sets indicated in Table 2 and perform quality control (i.e. coomassie and functional validation), prior to distribution. Furthermore, we will prepare well-characterized blinded samples with varying amounts of activated contact factor levels for external validation. We request budget for these activities.

Target	***Capture***	***Detect***	***Reagents in sample diluent***	***Assay standard + PPACK***
Free FXIIa	B7	E2	PPACK	Purified FXIIa in citrated plasma
C1INH-enzyme complex	1B12	FXII: Polyclonal VhH PK: Cedarlane FXI: Affinity Bio	PPACK	(dilutions of) Contact-activated plasma
cHK	D1	H4	PPACK+ DXS500k	(dilutions of) Contact-activated plasma

Table 2. Specific reagents that will be distributed amongst participants

The participants (Table 3) will receive between 1-5 mg of each nanobody. Typically, 1 mg is sufficient for 40 ELISA plates (96-wells). Should more be required, new productions will be performed. Participants will receive comprehensive written instructions on how to perform COSYNE ELISAs at their own facilities.

	Name (alphabetical)	*Institute*	*Expertise*
PI	Dr. Coen Maas	Utrecht University Medical Center	Biochemistry
1	Prof. Heiko Herwald	Lund University	Microbiology
2	Prof. Owen J. McCarty	OHSU	Flow models
3	Prof. Keith McCrae	Cleveland Clinic	Oncology
4	Prof. Joost C.M. Meijers	Amsterdam Medical Center / Sanquin	Clinical studies
5	Prof. Helen Philippou	LIGHT Labs, University of Leeds	Biochemistry
6	Prof. Thomas Renné	University Hamburg	Laboratory Hematology
7	Prof. Malgorzata Wygrecka	Giessen University	Pulmonary disorders

Table 3. List of confirmed participants

Phasing

1) Setup phase (6 months). Participants receive nanobodies, instructions for their use (including a list of essential reagents and example data) and a protocol for development of assay standards. We will also prepare a citrated pooled plasma of 20+ healthy volunteers at the University Medical Center Utrecht, that will be shipped along to serve as reference material for comparison to other plasma sources. Furthermore, blinded plasma samples with known amounts of analytes, as well as normal pooled plasma from three individual sources, will be provided for determination of inter- and intra assay variability.

2) Validation phase (12 Months). Participating labs perform the assays and compare their outcomes to the provided example data. There will be regular (i.e. every 6 months)

discussion via Skype or Conference calls, organized by the principal investigator to identify key parameters that influence the outcomes. We will circulate a feedback form for optimization of the provided protocols. Blinded reference samples will be measured and results

3) Exploration phase (6 months). The assay procedures will be deployed in specific areas of participants' expertise for validation of general applicability.

Expected timeline:

Project stage/set up	1/3/2019
Launch	1/9/2019
Duration	2 years
Finalization/analysis	1/3/2021
Reporting	1/6/2021

Study population (Inclusion, exclusion, eligibility) (patient population; recruitment of participating institutions/physicians and subjects; minimum number needed; expected number):

Suggested length 2-3 paragraphs

In the third phase, we will explore various settings in which standardized assays for contact activation should prove valuable.

Dr. McCarty's lab has an interest to study the plasma downstream from sites of thrombus formation in flow systems for contact activation markers (Zilberman-Rudenko et al. *ATVB* 2016, Verhoef et al. *Blood* 2017, Zilberman-Rudenko et al. *ATVB* 2018).
Dr. Meijers will study base-line contact system activation levels by comparing various sources of normal pooled plasma, and will also determine effects of procoagulant agents on contact activation.
Dr. Renné will investigate whether the Hemolysis-Icterus-Lipemia (HIL) phenomenon, a common cause of diagnostic error, influences the levels- and detection of contact activation markers in plasma. He has access and ethical approval to study plasma samples that are routinely analyzed for diagnostics in the Hamburg University Medical Center (18000/day) and will a select a sample set (initially ~100) with varying HIL scores.
Dr. Wygrecka will study levels of activated contact factors in 20-25 bronchoalveolar fluid (BALF) samples obtained from healthy volunteers and 40 well-characterised BALF samples from patients with ARDS/pneumonia.
Dr. Philippou will investigate whether COSYNE ELISAs can be used to accompany the development of therapeutic contact factor antagonists.

Expected outcomes (i.e. publications):

We will prepare a joint report on the outcomes of the project for *J Thromb Haemost*. Spinout research on assay performance in specific applications (if applicable) will be reported separately.

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Publication type (SSC Communication, Guidance document or original article):
SSC recommendation and guideline: we take the recent SSC publication (recommendations and guidelines) on clot lysis assays by Pieters et al. *J Thromb Haemost* 2018 as an example. The shared experiences of the working party and identified experimental variables have been carefully described in this paper.

Description of project set/up and management, needed infrastructure and resources (summary):
The required nanobodies have been developed in the Maas laboratory (ISO9003-certified) and can be produced on large scale in a dedicated bioreactor. Protein purification is performed by affinity chromatography and, where needed, followed by gel filtration on an AKTA Pure system.
Proteins are shipped in aliquotted portions on dry ice by courier. In the Set-up stage of the project, we will prepare ready-to-go protocols to accompany reagents. These protocols with encompass 1) Application in ELISAs 2) Preparation of assay standards and optionally: 3) Mild plasma contact activation (required for drug development monitoring).

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