Blood Outgrowth Endothelial Cells (BOECs) and Megakaryocytes (MKs) for in vitro studies of hemostatic and secretory function.

Published: 12-08-2020
Last updated: 04-07-2024

1. To establish optimized approaches for the isolation and characterization of BOECs, CD34+-derived MKs, iPSC- ECs and iPSC-MKs.2. Dissect the cellular mechanisms that control endothelial and platelet (secretory) function, thereby understanding the...

Ethical review	Approved WMO
Status	Pending
Health condition type	Other condition
Study type	Observational non invasive

Summary

ID

NL-OMON56840

Source
ToetsingOnline

Brief title
Cellular function in BOECs and MKs

Condition

- Other condition
- Coagulopathies and bleeding diatheses (excl thrombocytopenic)
- Blood and lymphatic system disorders congenital

Synonym
hemostatic defects ; secretory function defects

Health condition

inherited platelet function disorders

Research involving
Human

Sponsors and support

Primary sponsor: Erasmus MC, Universitair Medisch Centrum Rotterdam
Source(s) of monetary or material Support: Ministerie van OC&W,NWO Nationale Wetenschapsagenda,Landsteiner Stichting voor Bloedtransfusie Research

Intervention

Keyword: endothelial cells, hemostasis, megakaryocytes, Von Willebrand factor

Outcome measures

Primary outcome

A maximum of seventy milliliters of blood will be drawn for the isolation of

PBMCs, platelets, plasma and DNA. Several parameters will be measured in plasma

to characterize the hemostatic and angiogenic profile. BOECs, iPSC-ECs/MKs and

CD34+-derived primary MKs will be characterized in vitro. Protein expression

profiles will be determined by whole proteome mass spectrometry. DNA will be

genotyped for SNPs and mutations in VWF and VWF associated genes.

Secondary outcome

n/a

Study description

Background summary

Rationale:
Hemostasis is critically dependent on Von Willebrand factor (VWF), a multimeric adhesive plasma protein that is crucial for mediating platelet adhesion to sites of vascular damage and that acts as a chaperone for coagulation factor VIII (FVIII) in plasma. The bulk of plasma VWF is synthesized by endothelial cells (ECs) where it is stored in and released from Weibel-Palade bodies (WPBs). Platelets also store and secrete VWF from alpha-granules after

activation, which contributes to thrombus formation. The molecular mechanisms that control secretion of VWF from ECs and platelets are poorly understood. Abnormalities in the (secretory) function of platelets and endothelial cells can lead to bleeding, such as observed in platelet storage pool disorders (SPD), Von Willebrand disease (VWD) or in individuals with *low VWF*.

Problem definition:
In ~30% of type 1 VWD patients and individuals with low VWF (defined as VWF levels <50 IU/dL) no VWF mutations are found. Another problem is that in around 50% of bleeding patients referred to tertiary centers the underlying mechanisms that cause clinically relevant bleeding problems cannot yet be identified, so-called bleeding of unknown cause (BUC). Desmopressin is administered therapeutically to improve platelet function and raise VWF in plasma by inducing WPB exocytosis from endothelial cells. In ~25% of patients desmopressin fails to trigger (sufficient) release of VWF/FVIII for reasons largely unknown.

Research hypothesis:
The main hypothesis of this study is that defects in components of the secretory machinery of VWF are causative for reduced VWF levels and lack of desmopressin response. Also, defects in this machinery may be causative in patients with BUC. To identify new determinants of VWF levels and vascular health we will study secretory mechanisms in ECs and platelets of individuals with abnormalities in hemostatic and/or secretory function. For this we will establish ex vivo (patient-derived) cellular model systems of endothelial and platelet secretion using blood outgrowth endothelial cells (BOECs), platelets, CD34+-derived megakaryocytes and induced pluripotent stem cell (iPSC)-derived endothelial cells (iPSC-ECs) and megakaryocytes (iPSC-MKs).

Study objective

1. To establish optimized approaches for the isolation and characterization of BOECs, CD34+-derived MKs, iPSC- ECs and iPSC-MKs.
2. Dissect the cellular mechanisms that control endothelial and platelet (secretory) function, thereby understanding the pathophysiology of bleeding disorders and the genetic control of VWF secretion using BOECs, platelets, CD34+-derived MKs, iPSC- ECs and iPSC-MKs.
3. To develop in vitro methods to introduce or correct (disease-causing) mutations in regulators of endothelial or platelet function in BOECs or iPSC-derived ECs/MK using CRISPR-mediated genome engineering.

Study design

The study is a basic science, experimental in vitro study that requires the sampling of venous peripheral blood of the participants.

Study burden and risks

The objectives of this study can only be reached by studying patients and individuals with hemostatic and/or secretory abnormalities, their family members and healthy controls. The participants do not directly benefit from this research, however the burden is minimal as it is restricted to a venepuncture of negligible risk. Participants may be requested for repeated sampling, however this will be limited to three times.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adolescents (12-15 years)
Adolescents (16-17 years)
Adults (18-64 years)
Children (2-11 years)
Elderly (65 years and older)

Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

Patients
• Prior diagnosis of VWD, low VWF, inherited platelet function defects, bleeding of unknown cause or genetic disorders in which dysfunction of the secretory pathway has been implicated in disease pathogenesis.
• For patients >=16 years old; written informed consent
• For patients 12-15 years old; written informed consent from both the patient and their parent(s)/legal guardian(s)
• For patients <12 years old; written informed consent from their parent(s)/legal guardian(s)

Family members
• Age 18 years or older
• Family member of a patient with VWD, low VWF, inherited platelet function defects, bleeding of unknown cause or genetic disorders in which dysfunction of the secretory pathway has been implicated in disease pathogenesis.
• Family members may or may not be affected.

Healthy controls
• Age 18 years or older
• Not previously diagnosed with VWD, low VWF, inherited platelet function defects, bleeding of unknown cause or genetic disorders in which dysfunction of the secretory pathway has been implicated in disease pathogenesis.

Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:
• Unable to give written informed consent.
• Use of medication that can compromise platelet function or hemostasis.

Study design

Design

Study type:	Observational non invasive
Intervention model:	Other

Allocation:	Non-randomized controlled trial
Masking:	Open (masking not used)
Control:	Active
Primary purpose:	Other

Recruitment

NL

Recruitment status:	Pending
Start date (anticipated):	01-04-2020
Enrollment:	700
Type:	Anticipated

Ethics review

Approved WMO

Date:	12-08-2020
Application type:	First submission
Review commission:	METC Erasmus MC, Universitair Medisch Centrum Rotterdam (Rotterdam)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL72564.078.20