Microfluidic assay of hemophilic blood clotting: Distinct deficits in platelet and fibrin deposition at low factor levels.

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Background

Hemophilia

- Normal PT (activated by TF)
- Prolonged PTT (activated by kaolin, silica, etc.)

IIA

↓ Fibrin Deposition
↓ Aggregate Stability
↓ Platelet Adhesion

Can we develop a model of hemophilia using human whole blood which incorporates physiologically relevant hemodynamics?
Platelet adhesion and fibrin generation is mediated by factor Xll

Whole Blood (4 μg/mL CTI)
Venous shear (100 s⁻¹) [20 min]
Collagen type 1 (No tissue factor)
Healthy Donor (4 μg/mL CTI)
Venous shear rate (100 s\(^{-1}\))
8.5 min perfusion
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Collection/Preparation of Patient Samples

Patient blood drawn:
4 μg/mL CTI
- factor XIIa inhibitor
- approx. 30-40 min clotting time

Incubate whole blood samples:
anti-CD41a
- platelet label
anti-fibrin
- detection of thrombin generation

Patient data collected:
- diagnosis (Hem A/B/C [severity] or VWD)
- residual critical factor level (%)
- platelet count
- time since last therapy, name of therapy
- viral status
- Activated Partial Thromboplastin Time / Prothrombin Time
Patients (n=27)

- Severe Hemophilia A (13)
  - No therapy (6)
  - Therapy <48 hrs (7)
- Moderate Hemophilia A (3)
- Mild Hemophilia A (3)
- Severe Hemophilia B (1)
- Hemophilia C (1)
- Von Willebrand Disease Type 1 (4)
- Von Willebrand Disease Type 2A (1)
- Von Willebrand Disease Type 3 (1)

**Graph:**
- PTT (sec) vs Residual Activity (%)
- R² = 0.71
- >40% normal
- >5% mild
- >1% but <5% moderate
- <1% severe
Healthy clotting events proceed to full occlusion while patient samples embolize

Healthy Donor

Severe Hemophilia A
Healthy clotting events proceed to full occlusion while patient samples embolize
Platelet and fibrin deposition are measured in real-time.

Healthy Donor

Severe Hemophilia A
Thrombin does not drive platelet adhesion alone during the first 2.5 min of perfusion.
Model – Hemophilia

- Collagen/GPVI mediated platelet adhesion
- Thrombin plays only partial role in plt. deposition

0-6 min

- Collagen/GPVI mediated platelet adhesion
- Thrombin plays only partial role in plt. deposition

6-10 min

- No fibrin when <13% factor
- Defect in thrombin production results in slowly growing platelet aggregates

>10 min

- Full channel occlusion absent (severe-moderate)
- Platelet washout
Conclusions

• We have developed a microfluidic model of platelet aggregation/coagulation under flow driven by collagen and thrombin derived from the intrinsic pathway of coagulation.

• Platelet adhesion and fibrin generation in this assay were sensitive to defects in the activity of essential intrinsic pathway proteins (Factor VIII/Factor IX/Factor XI).

• The PTT was a good predictor of platelet adhesion in this assay and fibrin generation showed all or nothing behavior around the high-normal clotting time.
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Arterial shear rates potentiate platelet adhesion but not fibrin generation
WB incubation with low CTI does not lead to platelet activation

Whole Blood Incubation Assay (4 μg/mL CTI)

- Fluorescent Fibrinogen
- P-Selectin
- Phosphatidyl Serine

- PPACK
- 30 min (CTI)
- CVX - 5 min
- CVX – 30 min