Proteomics of Arterial Thrombi in Acute Limb Ischemia

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Abstract

This study compares the proteomic composition of ALI vs in vitro clots, identifying 141 upregulated proteins in ALI clots with Vitronectin being most abundant (1067-fold ↑). Findings show a fibrinolysis-resistant phenotype driven by the VTN-PAI1 axis and thromboinflammation, revealing potential therapeutic targets.

Background

- **Acute limb ischemia (ALI)**: vascular emergency with sudden occlusion of arterial blood flow due to thrombosis or thromboembolism.
- High rates of **amputation (11-37%)** and **mortality (16-42%)**.[1]
- Resistance to thrombolysis limits treatment success.
- Proteomic composition of arterial thrombi poorly characterized.
- Molecular drivers of fibrinolysis resistance unclear.

SFA Occlusion with absent popliteal runoff[2]

Thrombectomy (Endovascular or Open Surgical technique)

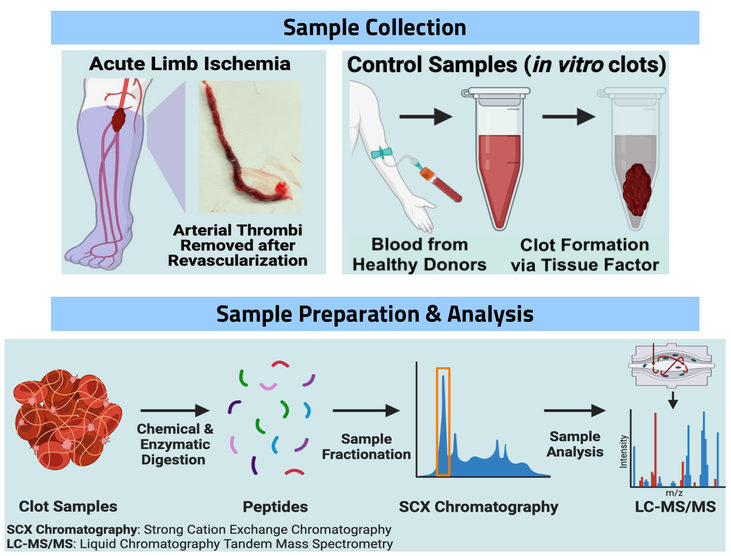
Patent SFA with restoration of popliteal/tibial flow[2]

Objectives

- Quantify proteomic composition of ALI clots (n=12) vs in vitro clots (n=10).
- Identify differentially abundant proteins that contribute to clot durability.
- Categorize protein roles in fibrinolysis, platelet activation, complement function, and RBC degradation.

Methods

- ALI thrombi (n=12) collected post-revascularization (IRB 19-2998/17-1286).
- Controls: in vitro TF-induced clots (n=10 healthy donors).
- Bottom-up proteomics: guanidine wash, digestion, SCX fractionation.
- LC-MS/MS (Orbitrap Fusion); label-free quant (AUC, Proteome Discoverer).
- Stats: unpaired t-test (p<0.05); literature review for categorization.



Results

- **651 proteins identified**:
  - **141** proteins with **increased abundance** in ALI clots (p<0.05).
  - **38** proteins with **decreased abundance** in ALI clots (p<0.05).
- **17 Fibrinolysis regulators**: **VTN (1067-fold ↑)**, PLG (79-fold ↑), ENO1 (61-fold ↑).
- **8 RBC proteins**: HPX (7.7↑), HP (6.6↑); Hgb subunits ↓.
- **6 Complement**: CFB (107↑), C4BPA (51↑), C3 (13.5↑).
- **36 Platelet regulators**: GP9 (65↑), PDIA3 (77↑), VWF (11.6↑).

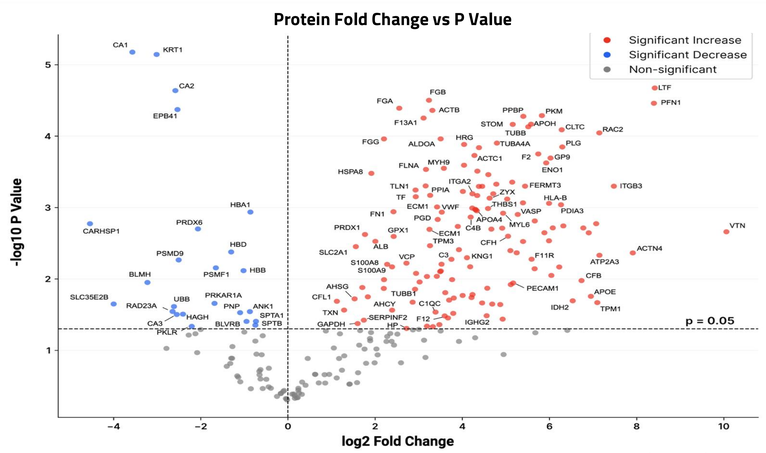


Fig. 1: Volcano plot. Log_2 fold change vs. $-log_{10}$ p-value for all detected proteins (p<0.05).

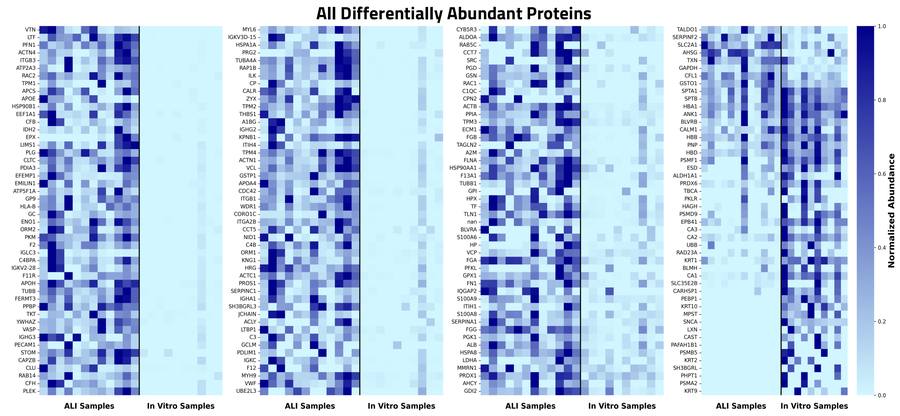


Fig. 2: Heatmap of all 179 differentially abundant proteins (intensity normalized across ALI vs in vitro samples).

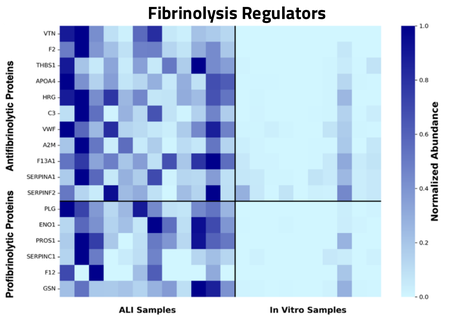


Fig. 3: Heatmap of fibrinolysis regulators (VTN, PLG, ENO1 most abundant in ALI thrombi).

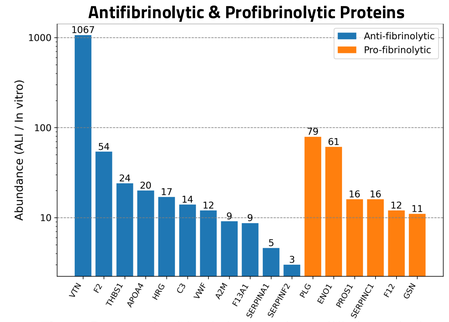


Fig. 4: Bar graph of fold changes in antifibrinolytic (blue) vs. profibrinolytic (orange) proteins.

Discussion

- Marked **VTN enrichment**, together with other antifibrinolytic proteins, supports a state of **fibrinolysis shutdown** that may underlie thrombolysis resistance in ALI.
- Concurrent enrichment of **platelet activators and thrombogenic complement proteins** suggests a thromboinflammatory environment that promotes and stabilizes ALI thrombi.
- **Heme/hemoglobin scavengers** (HPX, HP) indicate ongoing RBC breakdown that may further fuel thrombosis and inflammation.
- **Therapeutic Targets**: findings highlight fibrinolytic regulators (VTN–PAI-1 axis) and complement/platelet pathways as potential targets to enhance thrombolysis and prevent recurrent ALI.
  - **Example Targets**: PAI-1 inhibition (TM5275), complement blockade (eculizumab), and anti-platelet therapies (abciximab).
- **Future studies** should pair proteomics with functional clot-lysis assays and larger, etiologically homogeneous ALI cohorts.
- **Limitations**: single-center, small sample size; mixed ALI etiologies; in vitro clots may not fully recapitulate arterial shear conditions.

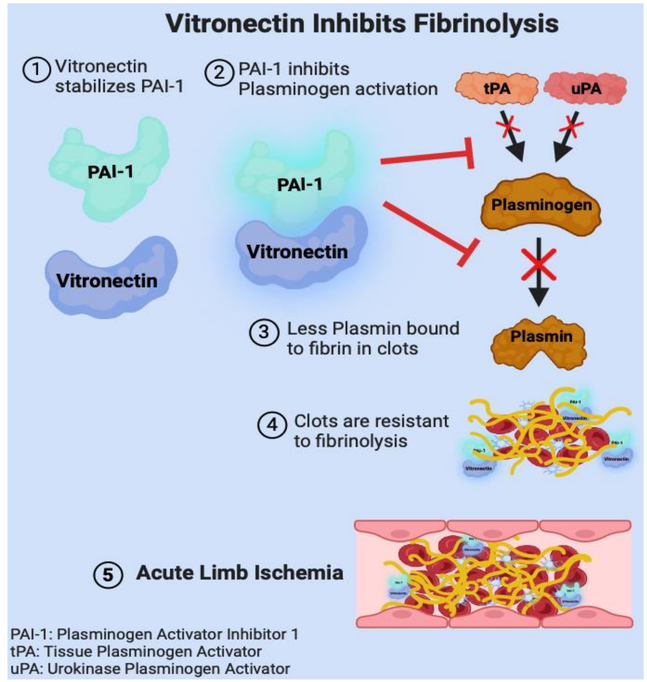


Fig. 5: Vitronectin mechanism: stabilizes PAI-1 to inhibit plasminogen activation & fibrinolysis.

Conclusion

- ALI thrombi show a proteomic signature enriched in antifibrinolytic proteins, platelet activators, and thrombogenic complement factors.
- Extreme vitronectin abundance supports a fibrinolysis-shutdown phenotype that may explain thrombolysis resistance.
- These data validate a bottom-up proteomic workflow for insoluble arterial thrombi and reveal candidate biomarkers of clot durability.
- Targeting fibrinolytic regulators and complement/platelet pathways may improve thrombolytic efficacy and long-term ALI outcomes.

Citations:
1. Genovese EA, et. al. Risk factors for long-term mortality and amputation after open and endovascular treatment of acute limb ischemia. Ann Vasc Surg. 2016; 30:82–92.
2. Lind B, et. al., Endovascular Strategies in the Management of Acute Limb Ischemia. Vasc Specialist Int. 2019 Mar;35(1):4-9. doi: 10.5758/vsi.2019.35.1.4.

Funding: NIH NIGMS RM1GM131968; CU Cancer Center P30CA046934.
Conflicts of Interest: No competing financial interests.
Acknowledgments: Thank you to my mentor Dr. Wohlauer & co-authors for their support and the CU Vascular Surgery team for clot sample collection.

Article: Stegman RD et al. J Thromb Thrombolysis. 2025.
DOI: 10.1007/s11239-025-03123-0.
Link: https://doi.org/10.1007/s11239-025-03123-0