Immune and Hemostatic Dysregulation in HIV: Theranostic Nanoparticles as a Dual-Function Therapeutic Strategy

Isabella Barrios, Sara Thomas, Joseph Budisak, & Saurabh Aggarwal MD PhD

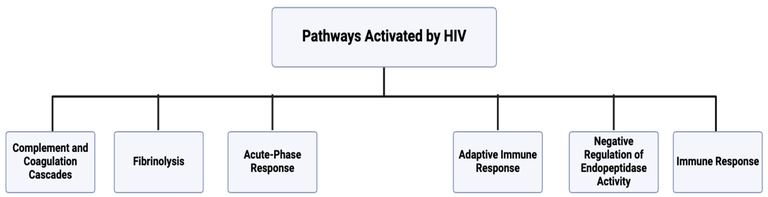
Department of Cellular and Molecular Medicine, Robert Stempel College of Public Health and Social Work, Howard Wertheim College of Medicine, Florida International University, Miami, FL 33174; Division of Infectious Diseases, School of Medicine, Emory University, Atlanta, GA, 30322; Division of Nephrology, University of Alabama at Birmingham, Birmingham, AL, 35294

PI name: Saurabh Aggarwal; PI email: saaggarw@fiu.edu

Abstract

HIV infection remains a significant global health challenge, leading to acquired immunodeficiency syndrome (AIDS) and an increased risk of cardiovascular and pulmonary diseases despite effective antiretroviral therapy (ART). This study investigates the proteomic profiles of HIV-infected individuals compared to healthy controls to identify potential biomarkers and therapeutic targets. Using mass spectrometry-based proteomics, we analysed samples from 20 HIV patients and 20 non-HIV controls. Differentially expressed proteins (DEPs) were identified through the “limma” R package and Wilcoxon test, revealing 22 DEPs. Gene Ontology and KEGG analyses showed significant enrichment in blood coagulation and fibrinolysis processes among the HIV DEPs, with gene set enrichment analysis highlighting the upregulation of the complement and coagulation cascades pathway. Chronic immune activation and inflammation contribute to a hypercoagulable state, increasing the risk of thrombotic events. This study underscores the critical proteomic changes associated with HIV infection and their implications for cardiovascular and pulmonary complications, suggesting avenues for novel therapeutic strategies to address the heightened risk of comorbidities in HIV-infected patients. In this regard, a theranostic nanoparticle system may be a valuable avenue for treating hypercoagulation and thrombosis as they can be simultaneously used to diagnose the presence of clots. These nanoparticles utilize materials like iron oxide nanoparticles (IONPs) and gold nanoparticles (AuNPs), which can be imaged with MRI and near-infrared fluorescence imaging.

Goal of the Study



Chronic immune activation in HIV patients leads to coagulation dysregulation and an increased thrombotic risk; therefore, theranostic nanoparticles, such as iron oxide nanoparticles (IONPs) and gold nanoparticles (AuNPs), could be utilized for simultaneous detection and treatment of hypercoagulation.

Materials and Methods

Study Population: Blood samples from **20 HIV-infected patients** and **20 non-HIV controls** were collected for proteomic analysis.
Proteomic Analysis: Protein extraction was performed using standard protocols, and **mass spectrometry-based proteomics** was used for protein identification and quantification.
Differential Expression Analysis: Differentially expressed proteins (DEPs) were identified using the **limma R package** and the **Wilcoxon test**, with significance set at **p < 0.05**.
Pathway Enrichment Analysis: Gene Ontology (GO) and KEGG pathway analysis were conducted to determine significantly enriched biological processes and pathways in HIV-infected individuals.
Gene Set Enrichment Analysis (GSEA): GSEA was performed using the **fgsea R package**, with an **FDR < 0.25** cutoff to validate enriched pathways.
Protein-Protein Interaction (PPI) Analysis: Interaction networks were constructed using **STRING database**, with significant enrichment set at **p < 1.0e-16**.
Data Visualization: Heatmaps, volcano plots, and enrichment maps were generated using **pheatmap (version 1.0.12) and ggplot2 (version 3.5.1) in R**.
Statistical Analyses: All statistical comparisons were conducted using **Two-way ANOVA and Tukey post hoc test**, with significance set at **p < 0.05**.

Results

A

HIV-non-HIV	No	ID	Genes	logFC	P.Value
	1	P01701	Ig lambda chain V-I region NEW	0.49506458	5.19E-07
	2	Q96PM9	Zinc finger protein 385A	-0.4748603	8.44E-07
	3	Q96PE2	Rho guanine nucleotide exchange factor 17	-0.3772086	1.51E-06
	4	P02743	Serum amyloid P-component	-0.1987435	5.03E-06
	5	P05452	Tetranectin	-0.3436625	8.22E-06
	6	P05090	Apolipoprotein D	-0.264321	2.00E-05
	7	P02765	Alpha-2-HS-glycoprotein	-0.1529769	2.41E-05
	8	O43866	CD5 antigen-like	-0.2773438	5.86E-05
	9	P06276	Cholinesterase	0.2217956	0.00028606
	10	P02675	Fibrinogen beta chain	-0.1604547	0.00033925
	11	P01593	Ig kappa chain V-I region AG	-0.146064	0.00050823
	12	B7ZLE5	FN1 protein	0.66657919	0.00063656
	13	P01625	Ig kappa chain V-IV region Len	-0.1489978	0.00066811
	14	P04196	Histidine-rich glycoprotein	-0.1471878	0.00417443
	15	P05160	Coagulation factor XIII B chain	-0.199591	0.00466942
	16	P01591	Immunoglobulin J chain	-0.1747472	0.00521047
	17	P02652	Apolipoprotein A-II	0.19771336	0.00598366
	18	Q9UK55	Protein Z-dependent protease inhibitor	0.15820824	0.00653511
	19	O60309	Leucine-rich repeat-containing protein 37A3	-0.1647813	0.00692744
	20	P01700	Ig lambda chain V-I region HA	-0.1465424	0.01741952
	21	P06331	Ig heavy chain V-II region ARH-77	-0.2034591	0.03566748
	22	P04264	Keratin, type II cytoskeletal 1	0.20439102	0.04862958

B

KEGG analysis

Category	Term	Benjamini
KEGG_PATHWAY	Complement and coagulation cascades	7.40E-01

C

GO analysis

Category	Term	Protein	FDR
GOTERM_BP_DIRECT		P05160, P04196, P02675,	
CT	blood coagulation	Q9UK55	0.0191
GOTERM_BP_DIRECT			
CT	fibrinolysis	P04196, P04264, P02675	0.0191

Figure 1: Blood coagulation and fibrinolysis is the process that prevents blood clots from growing, are enriched in HIV patients.
(A) KEGG and GO analyses were performed on differentially expressed proteins (DEPs) in HIV vs. non-HIV samples. (B) KEGG analysis identified significant enrichment of the complement and coagulation cascades. (C) GO analysis showed up regulation of proteins involved in blood coagulation (P05160, P04196, P02675, Q9UK55) and fibrinolysis (P04196, P04264, P02675), suggesting increased thrombotic risk in HIV patients. Genes with a p-value < 0.05 and |fold change| > 1.1 were considered significant.

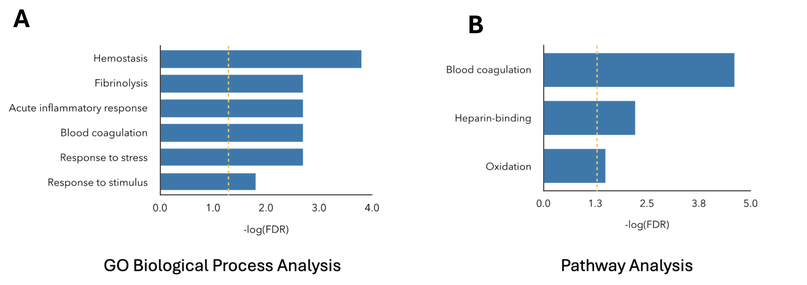


Figure 2: Pathway Enrichment Analysis for the Differentially Expressed Proteins.
Gene Ontology (GO) and pathway analyses of differentially expressed proteins (DEPs) reveal significant enrichment in hemostasis, fibrinolysis, blood coagulation, and inflammatory response. (A) GO Biological Process analysis shows increased coagulation and immune response activation. (B) Pathway analysis confirms enrichment in blood coagulation, heparin-binding, and oxidation, suggesting heightened thrombotic risk and oxidative stress in HIV patients. *FDR < 0.05 considered significant.*

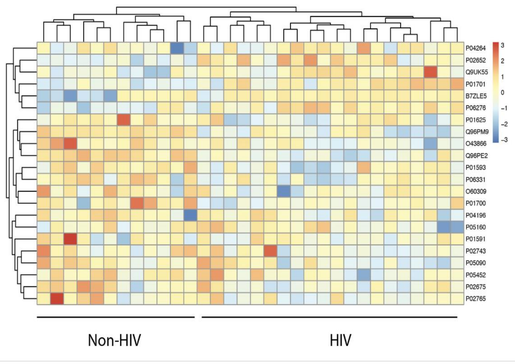


Figure 3: Differential expressed protein pattern between HIV and non-HIV infected samples.
Hierarchical clustering of differentially expressed proteins (DEPs) reveals distinct expression patterns between HIV and non-HIV samples. The heatmap shows upregulated (red) and downregulated (blue) proteins, highlighting proteomic alterations in coagulation, immune response, and metabolism in HIV patients.

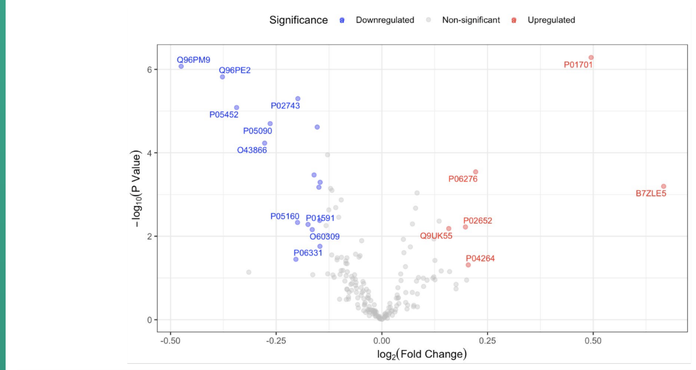
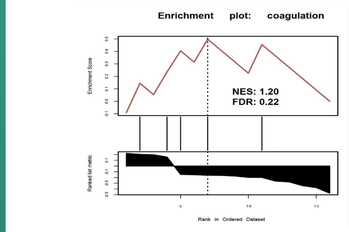


Figure 4: Volcano Plot of Differentially Expressed Proteins in HIV vs. Non-HIV
The volcano plot visualizes upregulated (red) and downregulated (blue) proteins in HIV patients. Key dysregulated proteins include those involved in coagulation, immune response, and metabolism. Proteins with log2(Fold Change) > 0.2 and p < 0.05 are considered significant.



Category	Term	Benjamini
KEGG_PATHWAY	Complement and coagulation cascades	7.40E-01
Category	Term	Benjamini
GOTERM_BP_DIRECT	fibrinolysis	3.70E-02
GOTERM_BP_DIRECT	acute-phase response	6.40E-02
GOTERM_BP_DIRECT	adaptive immune response	6.40E-02
GOTERM_BP_DIRECT	negative regulation of endopeptidase activity	6.40E-02
GOTERM_BP_DIRECT	immune response	6.40E-02

Figure 5: Gene Set Enrichment Analysis (GSEA) of Coagulation Pathways in HIV.
GSEA identified enrichment of the complement and coagulation cascades, with a normalized enrichment score (NES) of 1.20 and false discovery rate (FDR) of 0.22. (A) The enrichment plot highlights upregulated coagulation-related proteins in HIV. (B) GO analysis confirms significant enrichment in fibrinolysis, immune response, and acute-phase processes, indicating increased thrombotic and inflammatory activity in HIV patients.

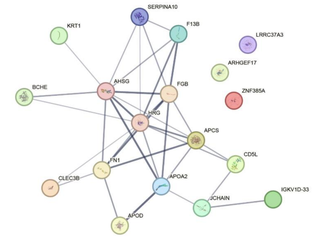


Figure 6: Protein-Protein Interaction (PPI) network of differentially expressed proteins in HIV.
PPI analysis reveals strong interactions among proteins involved in coagulation (FGB, F13B), immune response (CD5L, JCHAIN), and lipid metabolism (APOA2, APOD). Highly connected nodes suggest key regulatory roles in HIV-related thrombotic and inflammatory pathways. Data sourced from the STRING database (p < 1.0e-16).

Conclusion

Proteomic dysregulation in HIV, highlighting coagulation, immune, and inflammatory alterations. Enrichment in complement and coagulation cascades suggests a hypercoagulable state, increasing thrombotic risk. PPI analysis reveals key interactions among dysregulated proteins. Theranostic nanoparticles offer a promising approach for detecting and mitigating hypercoagulability in HIV patients, warranting further investigation.