Assessment of a 60-biomarker health surveillance panel (HSP) on whole blood from remote sampling devices by LC/MRM-MS and DIA-MS analysis

Cedars Sinai

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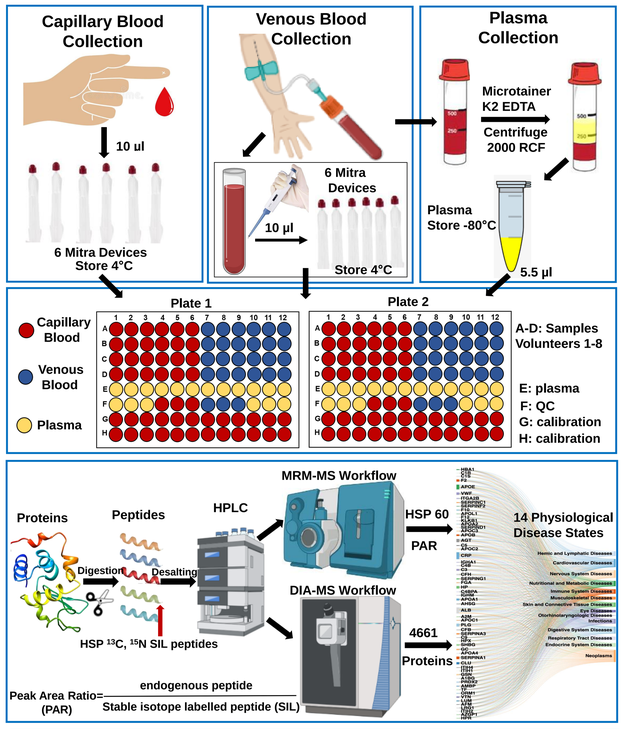
Introduction

An increase in chronic diseases from a diverse aging global population compounded by poor nutrition and sedentary lifestyles has accelerated interest in precision medicine to identify the mechanisms of an individual's disease state(s) so that an effective intervention or treatment can be prescribed to correct or alleviate phenotypic ailments. There has also been a rise in precision health, where individuals are focused on disease prevention and optimizing their health through strategies involving nutrition, physical fitness, microbiome health, wearable technology, and mental health wellbeing. The blood proteome is an informative source of biomarkers representing an individual's physiological biosignature where a remote collection device for blood tests coupled with a multiplex biomarker assay will facilitate access to both precision medicine and health.

Experimental Design



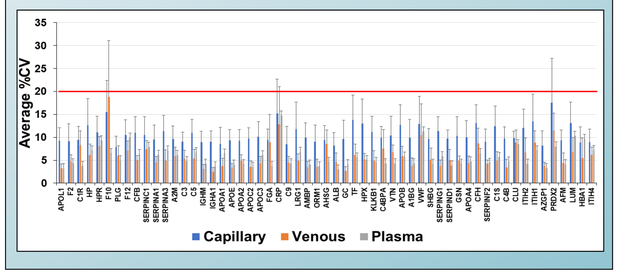
Methods

- Eight volunteers collected their own capillary whole blood from a lancet finger prick using six Mitra devices, which was compared to the traditional phlebotomy method of venous whole blood collection and plasma isolation.
- All samples were spiked with 114 stable-isotope-labelled (SIL) HSP peptides and 466 transitions targeted by LC/MRM-MS and analyzed by data-independent acquisition mass spectrometry (DIA-MS).
- Four sets of quality control (QC) groups were utilized at start, middle and end of analysis: pooled capillary blood, pooled venous blood, pooled plasma, and pooled commercial plasma.
- Compare the reproducibility of the LC/MRM-MS assay between all three biofluids.
- Determine the physiological concentrations of the HSP proteins.
- Analyze the 8 Volunteers biological replicate data from capillary blood (n=48), venous blood (n=48) and plasma (n=24) by hierarchical clustering, heat map analysis, and Pearson statistical analysis.
- Determine the proteomic depth and reproducibility of discovery DIA-MS analysis.

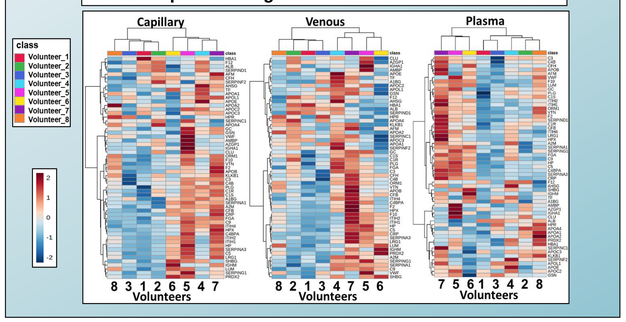
Results

Average HSP is <20%CV in Capillary, Venous and Plasma

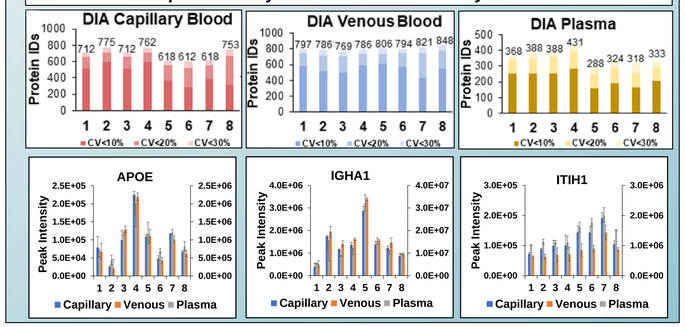
8 volunteers: capillary (n=48), venous (n=48) and plasma (n=24)



Heat Map Clustering of 8 Volunteers in Each Biofluid

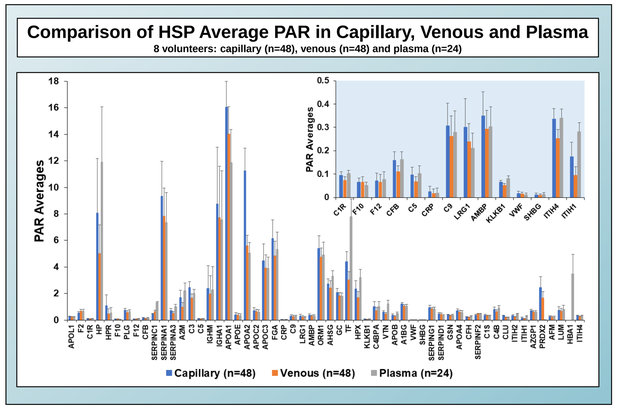


Reproducibility of Proteins Identified by DIA-MS



Comparison of HSP Average PAR in Capillary, Venous and Plasma

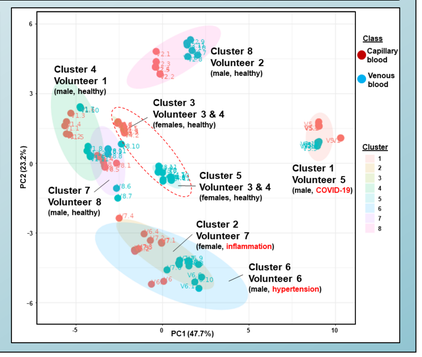
8 volunteers: capillary (n=48), venous (n=48) and plasma (n=24)



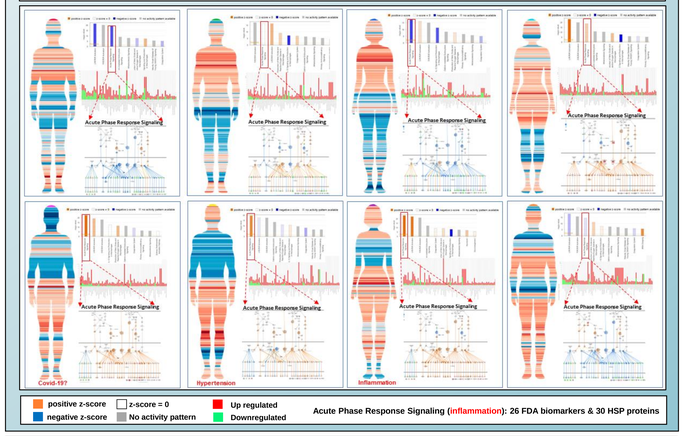
Clustering of Capillary Blood and Venous Blood Replicates

Cluster	Volunteers	Gender and Health
Cluster 1	Volunteer 5 Volunteer 5	male, covid 19
Cluster 2	Volunteer 7 Volunteer 7	female, inflammation
Cluster 3	Volunteer 3 Volunteer 4	females, healthy
Cluster 4	Volunteer 1 Volunteer 1	male, healthy
Cluster 5	Volunteer 3 Volunteer 4	females, healthy
Cluster 6	Volunteer 6 Volunteer 6	male, hypertension
Cluster 7	Volunteer 8 Volunteer 8	male, healthy
Cluster 8	Volunteer 2 Volunteer 2	male, healthy

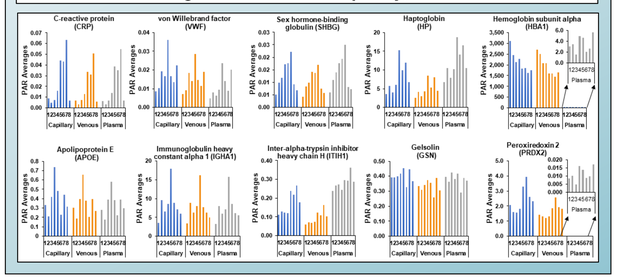
Blood Draw Date	Qual. IgG Nucleocapsid (N) protein	Quant. IgG Spike (S) protein (AU/mL)
Blood Draw Date	Qual. IgG Nucleocapsid (N) protein	Quant. IgG Spike (S) protein (AU/mL)
03-09-2022	2.52	34,118
04-13-2022	2.33	27,661
05-17-2022	2.57	16,505
07-15-2022	2.22	11,594
Qualitative IgG against N Positive >= 1.40		
Quantitative IgG against S Positive >= 50 AU/mL		



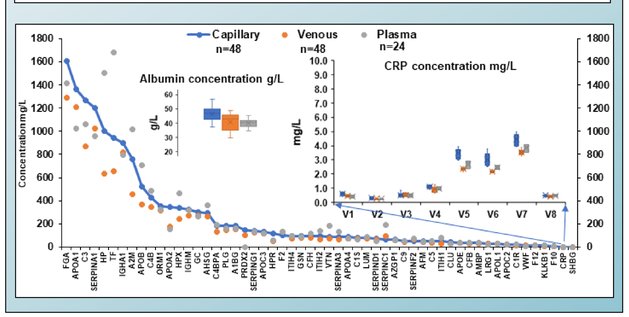
DIA-MS Informs Physiological Biosignatures in Canonical Pathways



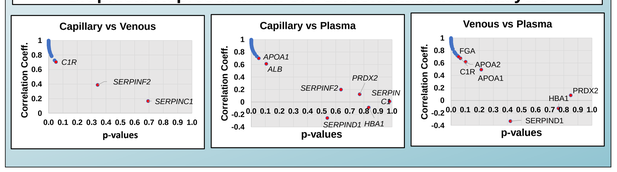
Similar PAR Biosignatures Across Capillary, Venous and Plasma



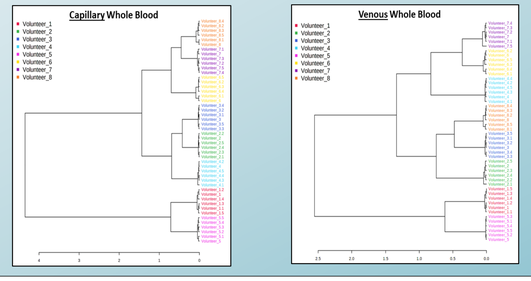
HSP Concentration in Capillary Blood, Venous Blood and Plasma



Graphical Representation of Pearson Statistical Analysis

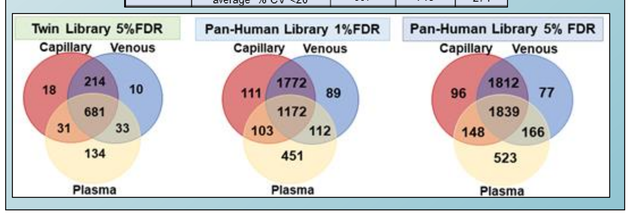


Dendrogram Hierarchical Clustering

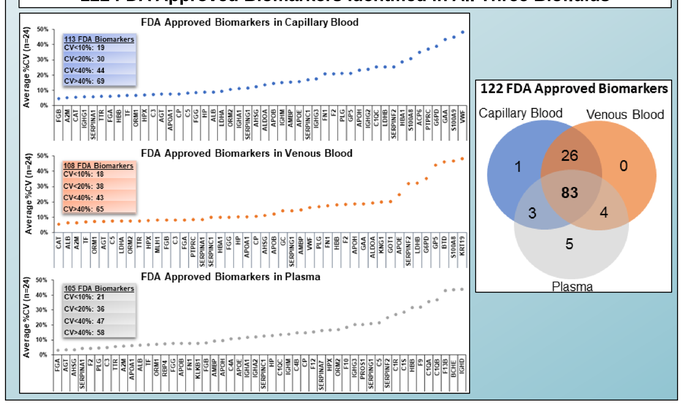


DIA-MS Discovery Analysis of All 8 Volunteers (n=3)

Spectral Libraries	60 minute gradient	Proteins: Capillary Blood	Proteins: Venous Blood	Proteins: Plasma
Twin Plasma (5% FDR)	All Identifications (n=24)	1121 proteins & 5175 peptides		
	observed in >66% samples	396	399	388
	average % CV <40	332	333	305
	average % CV <20	249	257	214
Pan-Human (1% FDR)	All Identifications (n=24)	3811 proteins & 7953 peptides		
	observed in >66% samples	877	1029	364
	average % CV <40	655	815	283
	average % CV <20	469	609	217
Pan-Human (5% FDR)	All Identifications (n=24)	4661 Proteins & 10788 peptides		
	observed in >66% samples	1229	1345	573
	average % CV <40	922	1035	416
	average % CV <20	607	718	274



122 FDA Approved Biomarkers Identified in All Three Biofluids



Conclusions

- QC and sample data was reproducible (CV<20%) for all biofluids.
- Graphical statistical analysis indicates that replicates group within each biofluid.
- HSP protein levels are within published physiological ranges.
- Each "normal/healthy" individual volunteer has a distinct proteomic biosignature.
- Discovery DIA-MS analysis quantifiably captures hundreds of proteins including 122 FDA approved biomarkers and 37 proteins used in LDT assays.
- CRP indicates inflammation in volunteers 5, 6, and 7 while DIA-MS analysis indicates detailed protein changes in canonical pathways (30 HSP and 26 FDA biomarkers) .
- HSP can be adapted to capillary blood collected onto a Mitra Device.