TRANSCRIPTOMIC ANALYSIS OF EXTRACELLULAR VESICLES FOR BIOMARKER DISCOVERY IN PERIPHERAL ARTERY DISEASE (PAD)

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INTRODUCTION AND OBJECTIVES

Peripheral artery disease

- Major cause of chronic vascular pathologies in developed countries.
- High risk of developing cardiovascular (CV) events.
- Need of new molecular targets and/or prognostic tools.
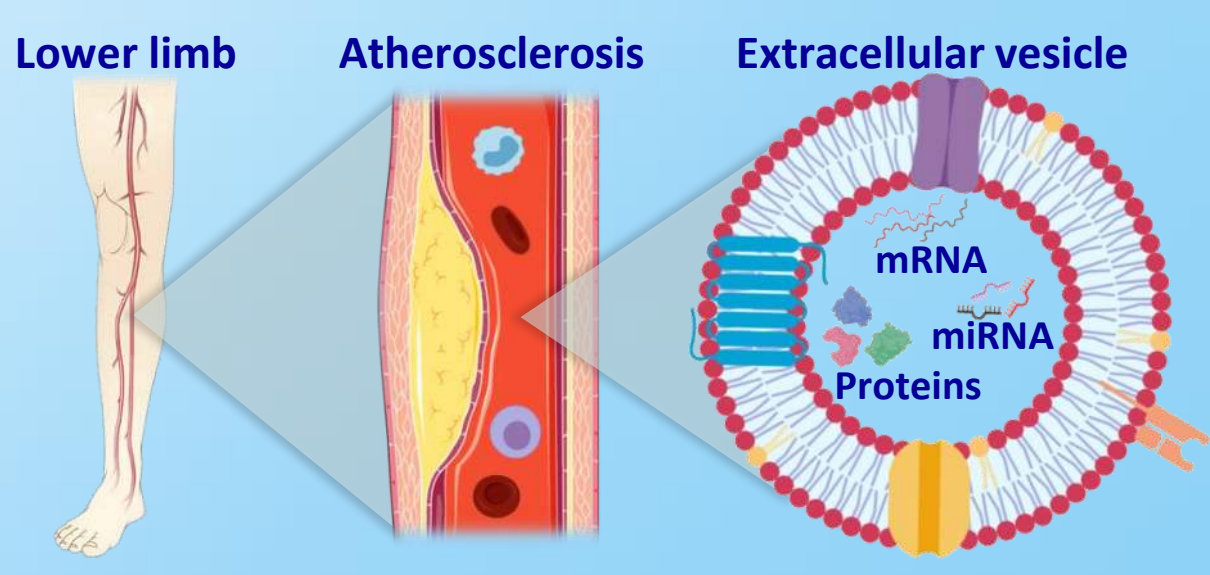
Extracellular vesicles

- Known as messengers for intercellular communication.
- Are augmented in subjects with CV risk factors.
- Their composition (liquid biopsy) could represent a valuable biomarker.

METHODS

1 Isolation of EVs

Liquid biopsy → PFP → High speed centrifugation → Isolated EVs

2 scRNA-seq library generation

| Adapter | mRNA fragment | Barcode + UMI | Adapter | → Illumina sequencing

3 Bioinformatic analysis

Raw 3' scRNAseq data → STAR → Mapped reads → UMI-tools quant3p → Read counts → Low count genes filtering and normalization

Differential expression:
- LimmaVoom
- Kruskal-Wallis

Gene set enrichment analysis ← mdGSA

4 Validation cohort

Control (n=100) and PAD (n=317)

Demographical and clinical parameters

CV risk factors
ABI
Treatment
Mortality
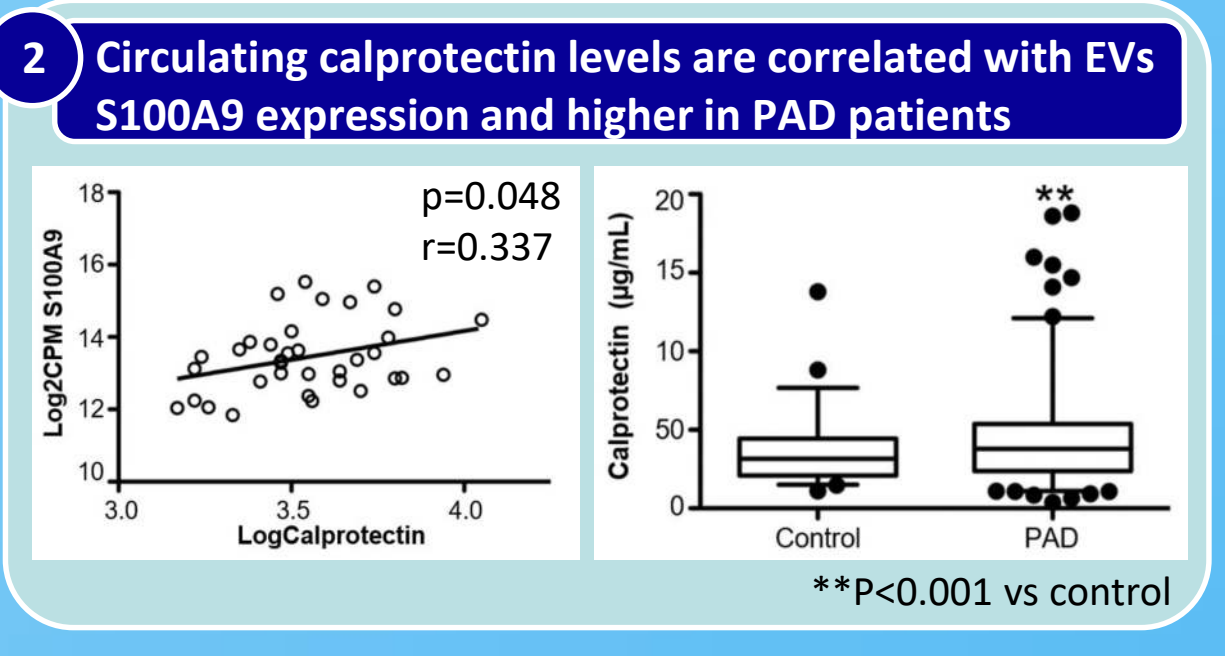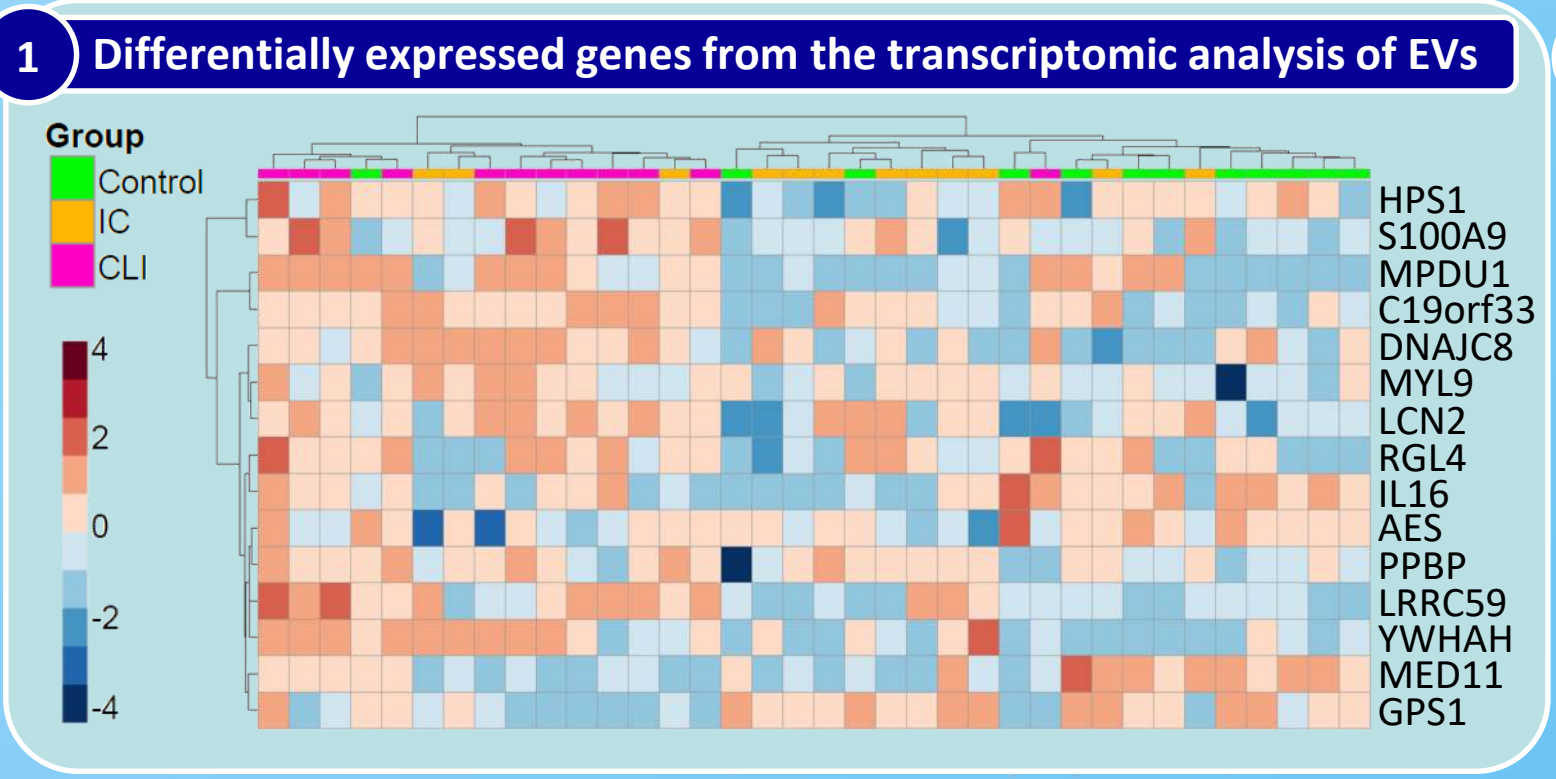CV events
Inflammatory markers

Plasma & serum → ELISA → Calprotectin

Mean follow-up of 4 years (min 1 month max 8 years)

RESULTS

1 Differentially expressed genes from the transcriptomic analysis of EVs

Group: Control, IC, CLI

Genes: HPS1, S100A9, MPDU1, C19orf33, DNAJC8, MYL9, LCN2, RGL4, IL16, AES, PPBP, LRRC59, YWHAH, MED11, GPS1

2 Circulating calprotectin levels are correlated with EVs S100A9 expression and higher in PAD patients

$p=0.048$
$r=0.337$

**P<0.001 vs control

3 Calprotectin levels are associated to amputation in the follow-up

	Calprotectin (µg/mL)		
	SHR	95% CI	P
Amputation			
Unadjusted	2.49	1.54-4.04	<0.001
Model 1	2.56	1.56-4.19	<0.001
Model 2	2.62	1.58-4.34	<0.001
Model 3	2.57	1.58-4.17	<0.001

Model 1: sex, age. Model 2: diabetes mellitus, dyslipidemia. Model 3: HT, eGFR.

CONCLUSIONS & FUTURE PERSPECTIVES

1. A protocol for studying the transcriptome of circulating EVs has been established.
2. 15 differentially expressed genes have been identified in EVs of PAD patients.
3. An association between calprotectin and amputation in PAD has been observed.

Sorting of EVs by cellular origin → Sequencing (scRNA-seq) → Identify and validate new prognostic biomarkers

References
[1] Fowkes FG et al. *Nat Rev Cardiol*. 2017;14:156-170.
[2] Jansen et al. *Circulation Research*. 2017;120:1649–1657.