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Extracellular vesicles as a marker of vascular disease activity in PAH

INTRODUCTION:

Pulmonary arterial hypertension (PAH) is a progressive, fatal disease characterized by disorganized vascular proliferation, inflammation, and thrombosis. Extracellular vesicles (ECV) are 30 nm – 1000 nm in size and influence thrombosis, inflammation, and angiogenesis. Although extensively studied in other systemic vascular disease, ECV research in PAH is in its infancy.

BACKGROUND:

Extracellular vesicles (ECV) consist of exosomes (30-100 nm), microparticles (100-1000 nm), and apoptotic bodies (>1000 nm). ECV are released during normal cellular homeostasis, cellular injury and activation, and apoptosis. ECV cargo includes nucleic acids, proteins, and enzymes to facilitate intercellular communication. Platelets produce the majority of ECV in healthy people with some released by leukocytes (monocytes) and endothelium. ECV have been studied in cardiovascular disease, malignancy, and sepsis. There have been limited data on ECV in patients with pulmonary arterial hypertension (PAH). Procoagulant microparticles shed from endothelial cells have been identified in the circulating blood of PAH patients. Other studies have concluded that larger microparticles are increased relative to healthy controls. Unfortunately, it is hard to draw conclusions or make comparisons between studies as they used different blood sample preparation techniques and different flow cytometers (typically limited in identifying ECV <400 nm); studies were generally isolated to a single blood draws. NanoSight is a technology complimentary to flow cytometry which can identify and count particles between 10 nm – 1000 nm analyzing Brownian motion. The ISTH published a consensus statement in 2012 (Lacrois et al., J Thromb Haemost, 10: 437-446) to help standardize processing of ECV and therefore allow for multi-site clinical research and cross laboratory comparison. Using Malvern Nanosight N300 and NTA software we have established a standardized protocol (camera and NTA settings) using samples from humans to allow for consistent analysis.

HYPOTHESIS AND OBJECTIVES:

We hypothesize that serial evaluation of ECV could be used as a biomarker of pulmonary vascular disease activity:
1) ECV changes (total number and origin) reflect disease activity and treatment response in the pulmonary vasculature;
2) ECV cargo, specifically miRNA, will be a marker of vascular health and response to therapy.

SPECIFIC AIM 1:

Characterize ECV concentration and stability/change over time with the NanoSight in male and female PAH research participants (10 treatment naïve PAH initiating therapy; 20 treated PAH with a low-risk phenotype, no change in therapy; 10 treated PAH with high-risk phenotype intensifying therapy; 20 matched healthy controls).

SPECIFIC AIM 2:

Characterize ECV cellular origin using Immunodepletion to isolate various fractions of ECV from blood already collected in Aim 1. Determine whether this distribution changes with therapy or disease activity.

SPECIFIC AIM 3:

Evaluate ECV miRNA from blood already collected in Aim 1. Determine whether vasodilator therapy changes miRNA in ECV. Correlate miRNA from ECV with PAH risk profiles.