The impact of oxygen tension on neutrophil biomechanical profiles in Pulmonary Arterial Hypertension

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Background: Pulmonary Arterial Hypertension (PAH) is a progressive and destructive disease of the cardiopulmonary vasculature characterised by uncontrolled cell proliferation and inflammation that leads to extensive occlusion of the pulmonary arterioles. Patients with PAH have increased peripheral blood neutrophil counts and higher circulating neutrophil elastase activity that correlates with disease severity and mortality. The increase in circulating neutrophil secretion products suggest a heightened activation phenotype that may contribute to vascular remodelling. Upon activation, neutrophils become stiffer, reflecting a pronounced actin cortex. These cytoskeletal changes can affect the transit time of neutrophils through the lung micro-vasculature network. The effect of oxygen tension on neutrophil biomechanics and its impact on the pathophysiology of PAH remain unknown.

Real-Time Deformability Cytometry (RT-DC) is a novel, sensitive high-throughput approach that measures quantitative biomechanical parameters from single cells without manipulation and within a physiological environment, thus enabling indirect determination of basal cellular activity. A greater understanding of the dynamic biomechanical processes that modulate neutrophil biophysical form during transits through diseased pulmonary micro-vasculature beds has the potential to identify new therapeutic targets and reduce neutrophil driven vascular remodelling. Here, we investigated the impact of physiological re-oxygenation on neutrophil biomechanical properties as they pass through the PAH diseased lung microvasculature.

Methods: Pulmonary arterial (PA, 5% oxygen) and radial arterial (10% oxygen) blood was taken from patients with PAH (N = 14) during a right heart catheter procedure. Cellular biomechanical properties were assessed by RT-DC: size (area), elasticity/stiffness (Young's Modulus/Deformation) and cell roughness (porosity). Whole blood was perfused through a microfluidic chip at a flow rate of 0.03uL/s sample and 0.09uL/s buffer.

Results: Arterial neutrophils (10% O2) were smaller ($p < 0.05$) and stiffer ($p < 0.01$) than PA neutrophils (5% O2). There was a larger standard deviation in arterial neutrophil porosity ($p < 0.005$) and elasticity ($p < 0.005$) compared with PA neutrophils, indicating more variability within the arterial population. Arterial lymphocytes were smaller than PA lymphocytes ($p < 0.005$) but stiffness was unchanged between the two groups.

Conclusion: The diseased PH lung may induce a pro-inflammatory neutrophil phenotype. Increased variability in cell roughness and deformability suggests an activated neutrophil population after passing through the diseased pulmonary vasculature which may be related to the change in oxygen tension. Increased neutrophil stiffness in PH may lead to increased neutrophil retention in pulmonary microvascular, increasing the potential for neutrophil-mediated inflammation and microvascular damage.