

Analyzing the endothelium's metabolome in the context of pulmonary hypertension

Clarissa Erica*, David Jones

Erica C, Jones D. Analyzing the endothelium's metabolome in the context of pulmonary hypertension. *Clin Cardiol J* 2023;7(1):1-2.

ABSTRACT

Group 1 Idiopathic Pulmonary Arterial Hypertension (IPAH) and connective tissue disease-associated PAH (CTD-aPAH) and group 4 Chronic Thromboembolic Pulmonary Hypertension (CTEPH) are two of the five clinical diagnostic categories for Pulmonary Hypertension (PH). The disease PH is progressive, fatal, and incurable. Recent research has shown that aberrant metabolic processes in the endothelium may play a key part in the pathogenic mechanisms producing PH, but these pathways are still poorly understood. In order to examine intracellular metabolism, this study

provides a novel method for analyzing PH endothelium function. It builds on the genome scale metabolic reconstruction of the EC. We show that, regardless of the PH diagnosis, the intracellular metabolic activities of ECs in PH patients cluster into four phenotypes. Notably, the clinical importance of the metabolic phenotypes is suggested by the large differences in illness severity amongst them. The PH phenotypes' considerable metabolic variations suggest that several treatment approaches may be necessary. Additionally, research into whether this creates a brand new field of precision medicine is necessary to enable their identification through diagnostic capacities.

Keywords: Thromboembolic; Hypertension; Ketogenesis; Pathophenotype

INTRODUCTION

Despite recent developments in pharmaceutical therapy, the 5 years survival rate for Pulmonary Hypertension (PH), a progressive condition with no effective curative medication, is still as low as 65-70%. Studies on the plasma metabolome of patients with Idiopathic PAH (IPAH) have shown that worse outcomes are associated with changes in lipid metabolism, glucose homeostasis, and bioenergetics. Widespread metabolic reprogramming was discovered by metabolomics analysis of bone morphogenetic protein receptor type 2 mutations in the pulmonary endothelium. More recently, a phase 2 trial showed that the drug sotatercept, which inhibits dysfunctional bone morphogenetic protein pathway signaling, is effective in treating PAH. Chronic Thromboembolic Pulmonary Hypertension (CTEPH) is characterized by abnormal lipid metabolism, as indicated by increased lipolysis, fatty acid oxidation, and ketogenesis in the plasma metabolomics profile. Under typical circumstances, the endothelium is in a quiescent state. In order to regain homeostasis, the endothelium secretes a variety of growth factors and cytokines that influence EC proliferation, apoptosis, and coagulation, draw inflammatory cells, and/or change vasoactivity. Chronic endothelium activation causes EC dysfunction, which causes pathological alterations like those seen in PH. Many factors, including shear stress, hypoxia, inflammation, cilia length, and heredity, have been proposed as potential causes of EC dysfunction in PH. Vasoconstrictive and proliferative factors are secreted by the overactive endothelium in PH, while vasodilators are secreted less frequently. This suggests that EC dysfunction may be a key element in the pathophysiology of PH [1].

Using a genome scale metabolic reconstruction of the microvascular EC, this study presents a novel method for examining intracellular metabolism. This EC model is relevant for studying the PH population because it was built using human metabolic models (RECON₁), metabolomics data from human umbilical vein endothelial cells, a manual literature review of endothelial metabolism, and finally transcriptomic data from three subtypes of endothelial cells (human umbilical vein, human mammary vascular, and human pulmonary artery) [2].

LITERATURE REVIEW

Setting and patients

The group includes adult (>18 years) PH patients who were admitted to the Department of Cardiology at the Rigshospitalet in Copenhagen, Denmark. The study was carried out in accordance with the declaration of Helsinki and received approval from the ethics committees in the capital region of Denmark (H-17021130). Upon arriving at the ambulatory clinic, blood samples were taken from a peripheral vein, and each patient gave their informed consent [3].

Patient selection

From a biorepository of more than 450 patients, patients were randomly chosen retrospectively for enrolment in this study based on their PH diagnosis. With right heart catheterizations, including measurements of pulmonary pressures and cardiac output/index using the thermo dilution method, the PH diagnosis was confirmed in all patients. Twenty previously published healthy volunteers and a total of 12 patients with IPAH, 12 patients with CTD-aPAH, and 11 patients with CTEPH were chosen. Electronic health records and a research database were used to locate the patients' clinical data [4].

Analysis of clinical characteristics

Utilizing Rstudio, a statistical analysis was carried out. Descriptive information about patients is shown as percentages or medians with Interquartile Ranges (IQRs). The four metabolic phenotypes were compared using the nonparametric Kruskal-Willis test to determine whether there were any variations in the primary cellular metabolic processes.

Mass spectrometry analysis

Thermo fisher scientific's vanquish UHPLC system in conjunction with a Q executive mass spectrometer were used to conduct the Ultra High Performance Liquid Chromatography-Mass Spectrometry (UHPLC-MS) analysis, as it was previously reported. The ionisation source was an electrospray ionisation interface. Both negative and positive ionisation types

Department of Cardiology, University of Copenhagen, Denmark

Correspondence: Clarissa Erica, Department of Cardiology, University of Copenhagen, Denmark; E-mail: clarissa_e@gmail.com

Received: 08-Aug-2022, Manuscript No. PULCJ-22-5245; **Editor assigned:** 10-Aug-2022, PreQC No. PULCJ-22-5245 (PQ); **Reviewed:** 24-Aug-2022, QC No. PULCJ-22-5245; **Revised:** 09-Jan-2023, Manuscript No. PULCJ-22-5245 (R); **Published:** 17-Jan-2023, DOI: 10.37532/PULCJ.22.7(1)1-2.



This open-access article is distributed under the terms of the Creative Commons Attribution Non-Commercial License (CC BY-NC) (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits reuse, distribution and reproduction of the article, provided that the original work is properly cited and the reuse is restricted to noncommercial purposes. For commercial reuse, contact reprints@pulsus.com

of analysis were used. For the purpose of identifying the chemicals, a QC sample was analysed in MS/MS mode. Amino and non-amino organic acids were detected using quadrupole mass spectrometry and Gas Chromatography-Mass Spectrometry (GC-MS) (5977B, Agilent). Chemstation was in charge of the system (Agilent). Prior to being imported and analysed in Matlab R2018b using the PARADiSe software, raw data were transformed to netCDF format using chemstation (Agilent).

DISCUSSION

The primary conclusion of the current study was that the intracellular metabolic activity of ECs from patients with PH clustered into four phenotypes (A-D), which were unrelated to the patients' PH diagnoses. Notably, the metabolic phenotypes' clinical relevance was shown by the significant differences in illness severity (as measured by NT-proBNP levels); phenotype D had the highest levels while phenotype B had the lowest values. Our findings are consistent with those of Kariotis and colleagues, who categorised IPAH patients into three subgroups based on whole-blood transcriptome analysis, accounting for more than 90% of the cohorts. These subgroups were linked to prognoses that were low, moderate, and good. Our research includes patients with CTEPH, indicating that the metabolic characteristics are not unique to PAH. Making direct comparisons with the literature is challenging because the current study is, to the best of our knowledge, the first to investigate the inferred intracellular endothelium metabolism in PH patients in the context of a genome scale metabolic model (GEM). Cell culture experiments using ECs from PH patients receiving lung transplants or from biorepositories are the foundation of the majority of EC research in PH. A direct comparison with the findings of metabolic systems analysis is challenging due to the fact that these studies were carried out under static conditions without hemodynamic shear stress and the manipulated cells were cultured on plastic, which is five orders of magnitude stiffer than *in vivo* conditions. Similar to human disease, complicated animal models of PH necessitate substantial cell manipulation. This could account for why there haven't been any prior reports on the discovery of clinically significant EC phenotypes with distinctly diverse intracellular metabolic activity. In our exploratory modelling analyses, phenotype B showed the highest ATP production from glycolysis but the lowest overall ATP generation. ECs may be able to boost lactate production and so support angiogenesis by giving glycolysis the upper hand over oxidative phosphorylation, which has previously been identified as a crucial aspect in studies of general metabolism in PAH vascular cells. The TCA cycle produces Reactive Oxygen Species (ROS) during oxidative phosphorylation, which can be reduced by using the glycolytic pathway. The glycolytic pathway can also produce ATP more quickly than the TCA cycle [5].

Similar metabolic reprogramming in cancer has also been documented, which gives ECs a survival advantage. It is possible that these ECs experience the least cellular stress because they had the lowest NT-proBNP levels and, thus, the mildest disease severity among the PH patients studied in this study. Phenotype D had the most severe illness, as determined by NT-proBNP, which may be related to increased cellular stress, forcing the cell to enhance amino acid breakdown through elevated intracellular reactive oxygen species. This is in line with our study's findings that 19 of the top 20 pathways for differentiating the phenotypes involved amino acid breakdown, which was significantly elevated in phenotype D. The highest levels of circulating catecholamine's were also found in phenotypic D. In agreement with this, new research has suggested that neurohumoral signaling, especially sympathetic activation, contributes to the PAH pathophenotype. Additionally, Nagaya and colleagues looked at 60 patients with PH and found that functional class IV patients' plasma norepinephrine levels were considerably higher than those of functional classes II and III. In contrast, much less amino acid degradation and more oxidation were seen in phenotypic A, which had many times lower NT-proBNP levels than phenotype D. In addition, phenotype C showed inverse ATP generation compared to phenotype B, with the lowest ATP generation from glycolysis and the highest oxidative phosphorylation, fueling the TCA

cycle from high amino acid degradation and beta-oxidation of fatty acids induced by low malonyl-CoA synthesis. Phenotype C had the second-highest disease severity as measured by NT-proBNP levels. It is still unknown if the variations in the intracellular metabolic activity of ECs between the various PH symptoms are caused by genetic variation or by variations in the severity of the diseases.

Due to the observational methodology of the current study's limitations, no causal effect can be deduced. The generalizability of the results may also be hampered by the study's small sample size of PH patients from a single center. It is significant that the PH patients received a variety of pharmaceutical treatments that may have agents that affect the endothelium, and this may have had an impact on the reported metabolic disturbances. However, sample sizes will need to be taken into account, and bigger multicenter studies will be necessary to validate the effect of the treatment on phenotypic. Expanding the metabolic coverage with an emphasis on membrane lipids and glycan metabolism is necessary, even if the iEC3006 is the most thorough metabolic reconstruction of the EC to date. In addition, despite encompassing all aspects of the central metabolism, the exploratory modelling analyses were based on a small number of extracellular metabolites in the plasma, which might have an impact on the measurement of intracellular metabolic cellular activity. As a result, it is necessary to validate the studies of the assumed intracellular pathways *in vitro* in healthy conditions. Additionally, because the iEC3006 is a single-cell metabolic model at the genome size, metabolites from other cell types in plasma cannot currently be included into it. Our theory, however, that the alteration in EC is predominantly reflected in plasma metabolism, is supported by the fact that 1 trillion EC are in continual touch with the blood in circulation. Last but not least, because SNP data were not available, we were unable to determine if the phenotype would represent a continuum of PH progression severity or genetic variations [6].

CONCLUSION

The results of the current exploratory study reveal that regardless of the patient's PH diagnosis, the endothelium exhibits four clearly unique intracellular metabolic phenotypes. The phenotypes are distinguished by variations in ATP synthesis linked to varied degrees of illness severity as determined by NT-proBNP. Significant metabolic variations among the PH phenotypes imply that they may require various therapeutic approaches; also, the development of diagnostic tools that allow for their identification is necessary to determine whether this opens up a brand new field of precision medicine.

REFERENCES

1. Panteghini M. Role and importance of biochemical markers in clinical cardiology. *Eur Heart J.* 2004;25(14):1187-96.
2. Flynn MR, Barrett C, Cosio FG, et al. The Cardiology Audit and Registration Data Standards (CARDS), European data standards for clinical cardiology practice. *Eur Heart J.* 2005;26(3):308-13.
3. Goldstein DS. Plasma norepinephrine as an indicator of sympathetic neural activity in clinical cardiology. *Am J Card.* 1981;48(6):1147-54.
4. Yellon DM, Downey JM. Preconditioning the myocardium: from cellular physiology to clinical cardiology. *Physiol Rev.* 2003; 83(4): 1113-51.
5. Harizi RC, Bianco JA, Alpert JS, et al. Diastolic function of the heart in clinical cardiology. *Arch Intern Med.* 1988;148(1):99-109.
6. Bristow MR. Treatment of chronic heart failure with β -adrenergic receptor antagonists: a convergence of receptor pharmacology and clinical cardiology. *Circ Res.* 2011;109(10):1176-94.