Precision medicine: The future of diagnostic approach to pulmonary hypertension?

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Abstract
Pulmonary hypertension (PH) is a common finding that can result from many different pathological conditions. Depending on the etiology, treatment may be quite different, but early diagnosis and correct classification of PH is difficult. With an aging population and recently suggested decreased pulmonary arterial pressure threshold defining PH, we are facing even more diagnostic uncertainties. A new approach to patients’ phenotyping is needed. Here we present available data and future perspectives on employing an in-depth analysis of the omics cascade to allow an earlier and more reliable diagnosis and classification of PH. Indeed, with the help of super-fast computing, it became possible to simultaneously consider the levels of potential biomarkers to find patterns specific for clinically suspected disease. The omics cascade is an invaluable source of information. However, while the genome can be perceived as providing possibilities, transcriptome—as carving them this is metabolome that may tell us “what is really going on” in an individual living organism. Metabolomics research requires blinded search for characteristic patterns of discreet changes in the levels of detectable metabolites. Since as many as 40,000 various substances are produced as a “side effect of staying alive”, metabolite profiling can be compared to fishing up for organized signals in a universe of chaos. Although difficult, such search for metabolic patterns that might lead to replacing the term biomarker by metabolic fingerprinting in the area of pulmonary circulation has already begun. (Anatol J Cardiol 2019; 22: 168-71)

Keywords: pulmonary hypertension, systems biology, metabolome, genome, transcriptome, proteome, epigenetics

Introduction
Despite a plethora of publications on candidate laboratory biomarkers, pulmonary hypertension (PH) cannot be diagnosed or properly classified by any single laboratory test. The DETECT trial is a good example of combining biomarkers including NT-proBNP in a stepwise approach to final diagnosis in a population at a moderately increased PAH risk (1). A relatively new concept, still in pre-clinical development, is based on systems biology, so-called systeomics (Fig. 1). This new holistic approach to living organisms is based on exploring their characteristics on several levels of organization. It has been recognized that although the genome determines hereditary or new mutation-dependent predispositions of an individual organism, it rarely fully determines the final phenotype (2). In pulmonary hypertension, such a strong influence of a single gene is exemplified by recently discovered EIF2AK4 biallelic mutation, leading to the PVOD phenotype (3).

However, in most instances, the structural and functional properties of an individual organism are less directly related to genome being modified by epigenetic and environmental influences. As an example predisposition, to develop PH in sarcoidosis seems to be related to a signature involving 18 different genes (4).

The role of epigenetics in pulmonary hypertension has been studied mostly by the assessment of microRNA (miRNA, miRs), small non-coding RNA that downregulates the gene expression and in this way influences phenotypes. miRNA operates intracellularly, but it can be also detected and quantified in plasma. Some of the circulating miR were found to be decreased in PAH (150, 26a, 23 a, 125a) (5-8), while other (miR 130/301, and miR210) were increased in pulmonary circulation of PAH (9, 10). The miR patterns derived from different studies of PH are not always the same. A number of other downregulated circulating (miR-451, miR-1246) and upregulated cmiRNAs (miR-23b, miR-130a, miR-191) were identified in another study, suggesting that observed patterns may not have a universal clinical value (11). Indeed, relying...
Metabolomics research requires blinded and not hypothesis-driven search for characteristic patterns of discrete modifications of levels of detectable metabolites. Since as many as 40,000 of various substances are produced as a side effect of “staying alive,” metabolite profiling can be compared to fishing up for organized signals in a universe of chaos. Search for metabolic patterns that might lead to replacing the term biomarker by metabolic fingerprinting is ready as a concept but still waiting for validation and implementation in the area of pulmonary circulation.

The existence of metabolic heterogeneity of pulmonary arterial hypertension has been elegantly documented in tissue samples from the lungs explanted from PAH recipients when those were compared to the lung tissue sampled from patients with lung cancer. Metabolites were identified by means of liquid and gas chromatography and mass spectrometry. The lung tissue in patients with PAH presented disrupted glycolysis, an increased TCA (Krebs) cycle, and fatty acid metabolites with abnormal oxidation, suggesting that there are specific metabolic pathways likely contributing to the vascular remodeling process (15).

The lung tissue or intracellular matrix are not attractive sampling options for clinical medicine, but metabolome can also be assessed by sampling blood, urine, saliva, or even exhaled air. Drawing conclusions from plasma levels of metabolites seems more reasonable than doing so for microRNAs, which predominantly operate intra-cellularly. Several studies found distinct metabolic patterns in patients with PH using this approach. Lewis et al. (16) found that 21 out of 107 metabolites obtained from blood samples using of a multiplexed liquid chromatography mass spectrometry (LC-MS) system identified a characteristic pattern significantly associated with hemodynamic abnormalities assessed during RHC at rest and during exercise.

Bujak et al. (17) assessed metabolic fingerprints of 20 patients with PAH and compared them with matched healthy volunteers (n=20) using multiplatform metabolomics approach. Liquid chromatography and gas chromatography provided 21 and 9 metabolites, respectively, to form a PAH fingerprint. Some of those metabolites were related to energy imbalance, particularly glycolysis, but also to fatty acid, lipid, and amino acid metabolism. Interestingly, a profile consisting of 16 metabolites was confirmed as statistically significant in the validation study (17). Recently a much larger study found 53 circulating metabolites to distinguish patients with PAH (n=365) from healthy control subjects (n=121). Moreover, 20 out of 53 metabolites also discriminated patients with PAH from symptomatic patients in whom PH was excluded (n=139) (18). Sixty-two metabolites were related to the prognosis in PAH, majority acting independently from established prognostic markers. Also, in this study, metabolites related to bioenergetics were found to be of importance, which can be related to modified functional preferences of mitochondria in cells of the pulmonary arteries and right ventricle in patients with PAH (19).

The authors concluded that such deep phenotypic characterization of patients should be decisive for selecting and monitoring...
Characteristics of metabolic modifications linked to deranged pathophysiologic pathways operating in PAH would allow individualized treatment decisions based on deep phenotyping rather than on uncertain clinical and hemodynamic classification. Moreover, such treatment could consist of interventions ultimately modifying metabolome toward patterns compatible with health and good outcome.

However, many problems remain to be solved before systems biology will provide diagnostic tools for routine clinical practice in PH. While several teams have found characteristic metabolic patterns in PAH, the "fingerprints" reported by different research teams are not identical, and sometimes, they are quite different. The blame is placed on differences in analytical methods or environmental influences. Still, we are quite far from a validated, universally approved PAH metabolome. Furthermore, for obvious reasons, metabolomes were assessed in patients with unequivocal phenotypes, thus usually in late phases of PAH. Some of the modifications in metabolome profiles could be actually due to the nonspecific consequences of low cardiac output, hypoxemia, and venous stasis. Such a metabolome will be helpful neither for diagnosis nor differential diagnosis of PAH. Serial metabolic profiling of patients at risk for PAH (e.g., with a family history and/or carrying mutations, patients with scleroderma) could shed light on specific patterns that can predict development of clinically unequivocal phenotypes. Such monitoring of the metabolome could provide also information useful "a rebours", that is, in assessing the effects of treatment targeting pulmonary vascular disease. Finally, the presence or absence of specific PAH metabolome and its modifications during treatment would be of great value in patients with comorbidities to objectively assess whether specific treatment targeting pulmonary vascular disease is justified and effective.

**Conclusion**

A large number of candidate laboratory biomarkers derived from PH or PAH pathophysiology were tested for their clinical value. Individually, none was found useful for diagnostic purposes, except for the EIF2AK4 gene and NT-proBNP, the latter, however, as a part of stepwise diagnostic score. The future of laboratory biomarkers belongs to their contribution to "deep phenotyping" consisting of search for characteristic patterns in the genome, transcriptome, proteome, and/or metabolome of the patient (2, 23-26). Without neglecting the importance of genetic and epigenetic signatures, currently metabolomics emerges as the most informative area of systems biology research. Metabolome may provide a cross-reference confirming differential diagnosis made by standard phenotyping. In the future, metabolome may even provide information directly driving treatment decisions, while its modification over time may be considered as "deep monitoring" of treatment results.

So far, however, metabolic signatures have been assessed in well-defined, homogenous study populations. New research paradigms are now necessary to prove their value for early detection and differential diagnosis of PAH in real life.

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