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EDITORIAL COMMENT

Metabolomics in Single Ventricle Heart Disease

Glimpsing the Pathobiology of Stage 2 Palliation*

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Palliation for single ventricle heart disease (SVHD) involves a series of staged operations designed to separate the right and left circulations. These include the Stage 1 Norwood procedure, the Stage 2 hemi-Fontan or bidirectional Glenn operation, and the Fontan operation, a procedure that provides passive systemic venous blood flow to the lungs without a sub-pulmonary pumping ventricle. Each of these steps has a unique and distinctive physiology. Numerous improvements in the surgical procedures over the past years have led to dramatic gains in survival at each stage and after the Fontan operation. However, despite analyses of variables including surgical techniques,[1] timing of operation,[2] anatomic substrate, single ventricle function,[3] respiratory and arrhythmia complications,[4] there remains a risk of mortality at each procedural stage, and particularly in the time between the first operation and second operation, known as the interstage period. There is an ongoing need to identify and understand which patients may be unable to progress to subsequent stages or who may have risk factors for long-term morbidity and mortality after the Fontan operation.[5]

Metabolomic analysis of circulating proteins and biomarkers has provided a remarkable glimpse into important pathways that are altered during these procedures, and may be associated with response to therapy and prognosis in patients with heart failure and pulmonary arterial hypertension.[6,7] The ability to use targeted metabolic profiling to study simultaneous measurements of distinct metabolites and their changes over time allows new ways to characterize disease states and patient outcomes. Metabolomics has emerged as an important tool to study pathobiology and physiologic changes in patients with congenital heart disease and, in particular, SVHD.

In this issue of *JACC: Advances*, Frank et al[8] report a prospective cohort study of circulating metabolic profiles in SVHD patients undergoing Stage 2 palliation. The authors enrolled 52 patients with interstage SVHD prior to Stage 2 operations and 42 similar aged control patients undergoing noncardiac surgical procedures. They obtained preoperative blood samples at the time of pre-Stage 2 catheterization from systemic venous and pulmonary venous catheters or from a central venous line prior to bypass at the time of Stage 2 operation. In addition, they obtained fixed time point postoperative Stage 2 samples from all SVHD patients. Control samples were drawn from patients at the time of noncardiac surgical procedures. Using liquid chromatography-tandem mass spectrometry, the authors performed targeted metabolomic phenotyping, characterizing 175 metabolites. They used multiple univariate regression analysis to examine metabolite levels between cases and controls and among clinical variables in subjects and employed the supervised machine learning algorithm random forest classifier to differentiate between groups. The primary clinical variable examined was postoperative hypoxia, defined as the percent of time a patient experienced systemic oxygen saturation below 70% in the first 48 hours after the Stage 2 procedure. Secondary variables included duration of endotracheal intubation, volume of chest tube drainage, and hemodynamic variables (mean

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pulmonary artery pressure and indexed pulmonary vascular resistance [PVRi]) from the pre-Stage 2 catheterization.

Study analysis showed distinctive metabolites and metabolic profiles between interstage and control groups. More than 40% (74 of the 175) measured metabolites differed between SVHD preoperative cases and control patients, with alteration in 27 of 39 metabolic pathways including the pentose phosphate pathway, arginine biosynthesis and metabolism, tryptophan metabolism, nicotinamide metabolism, and glutamate metabolism. Random forest analysis was able to differentiate between the groups with error rates suggesting appropriate classification of results. In addition, 71 of 174 metabolites differed between preoperative and postoperative Stage 2 samples with 33 of 39 metabolic pathways altered including arginine metabolism and biosynthesis, glycine metabolism, pyrimidine metabolism, and tryptophan metabolism pathways. Again, random forest analysis showed excellent discrimination between these groups. Finally, the authors noted preliminary associations of both altered preoperative metabolites in patients with elevated preoperative PVRi and as well as differences in postoperative metabolites with in patients with post-Stage 2 hypoxemia.

The limitations of this study are those typical of exploratory research projects with novel biomarkers. There is a relatively small number of patients, treated at a single center, with a heterogeneous cohort of interstage patients of variable ages, underlying ventricular morphology (80% right ventricle), Stage 1 operative technique and PVRi (10/52 subjects had PVRi above 3 WU). The control patients were also a heterogeneous cohort with median age significantly older than the study group and with underlying issues requiring a variety of noncardiac surgical procedures. Other clinical factors such as preoperative medical regimens and diets, not recorded, may have differed among interstage subjects and controls and could influence metabolic results. Finally, performance of the study at altitude (Denver, Colorado, USA) may contribute to metabolic alterations[9,10] and could limit the applicability of results. The authors note that postoperative discharge on oxygen and use of sildenafil in more than one-third of patients may differ from post-Stage 2 management in many centers, a number that far exceeds typical experience and suggests unique features of the patient population. It is interesting to note, however, that moderate altitude has not been associated with clinical outcomes after Stage 2 operation in prior studies.[11] Future multicenter studies will be important to provide sufficient study numbers, ensure appropriate comparisons between subjects and controls and allow for correlation with important clinical endpoints.

This manuscript builds on prior studies linking metabolic shifts in infants undergoing cardiopulmonary bypass to mortality and intensive care unit length of stay[12] and research identifying circulating biomarkers associated with heart failure in young SVHD patients.[13] These investigations are an important start in the endeavor to characterize varied physiologic stages in SVHD, identify patients at risk and predict future outcomes with molecular precision. The authors have begun some of this work with the demonstration of proteomic differences between interstage SVHD infants and controls[14] and association of circulating endothelin-1 levels with postoperative outcomes in SVHD patients after Stage 2 operation.[15] Additional genetic and epigenetic studies will be necessary in the future. At the same time, this is pioneering work helping to blaze a trail for the innumerable metabolomic studies that are sure to follow in cardiopulmonary medicine. By demonstrating the feasibility of this approach and crafting a framework for analytic strategies, the investigators are helping move us to an era when the great power and promise of 'omics will enhance our utilization of markers and measures. With that granular data, we move toward the goal of capturing a complete molecular definition of a complex SVHD patient at a precise stage in time, and improving their care.

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