JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 6, NO. 4, 2021

EDITORIAL COMMENT

The Microbiome is a Welcome Addition to the Growing "Omes" of Cardiopulmonary Bypass in Congenital Heart Disease Surgery*

John D. Watson, MD, Jesse A. Davidson, MD, MPH, MSc

Pediatric cardiac surgery with cardiopulmonary bypass (CPB) results in broad physiologic changes, the full impact of which continues to be defined. Neonates and infants with congenital heart disease (CHD) may be at particular risk for metabolic disruption due to a combination of complex surgeries, use of deep hypothermic circulatory arrest/selective cerebral perfusion, residual heart failure and cyanosis, repetitive injury from staged surgeries, and suboptimal nutritional intake during critical developmental periods. Biomarker studies in this population traditionally focused on single or small panels of molecules. Although single biomarker strategies are appealing for their simplicity, low cost, and minimal sample volume requirements, these strategies do not address the complex physiologic interactions inherent to CHD surgery. Fortunately, recent advances in biochemical analysis allow simultaneous measurement of large numbers of circulating molecules (proteins, metabolites, and microRNAs) from small biologic samples, making it possible to map changes across multiple molecular pathways in a comprehensive manner.

As an example, targeted metabolomic and proteomic approaches have recently been applied to assess changes in the circulating metabolome and proteome following infant CHD surgery (1,2). The breadth of postoperative biochemical disruption in these patients was striking. Significant postoperative changes occurred in >40% of measured proteins and >80% of measured metabolites after correction for multiple comparisons. Interstage single-ventricle heart disease patients also showed significant differences in their preoperative circulating proteome compared to healthy controls (2). These findings highlight the physiologic stress inherent to unrepaired/palliated CHD and the added strain of surgery. Molecular profiling studies, however, represent only a first step in omics research, and should be followed by focused studies to quantitatively map key pathways and determine pathway interactions. These clinical studies can be combined with relevant translational models to allow organ-/cell-specific study and pathway manipulation, identifying novel molecular drivers that represent future diagnostic and therapeutic targets. Based on the early molecular profiling studies, CHD is likely a high value area for omics-based techniques and warrants dedicated research.

In this issue of *JACC: Basic to Translational Science*, Salomon et al. (3) remind us that the microbiome represents an important additional "ome" that could significantly impact CHD patients, both in the perioperative period and throughout childhood. This prospective study assesses the effect of CHD surgery with CPB on the intestinal microbiome, intestinal barrier function, and stool eicosanoid production. Controls included patients without CHD undergoing surgery without CPB. Intestinal microbiome richness, α-diversity, taxonomies (phylum and genus), and β-diversity were measured. Dysbiosis was found

*Editorials published in *JACC: Basic to Translational Science* reflect the views of the authors and do not necessarily represent the views of *JACC: Basic to Translational Science* or the American College of Cardiology.

From the Department of Pediatrics, University of Colorado, Aurora, Colorado, USA.

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2021.02.003

preoperatively in CHD patients and was exacerbated by surgery with CPB, with average proinflammatory bacteria increasing from 17% to 27% postoperatively. In contrast, controls showed few postoperative changes in the microbiome. Furthermore, this study confirmed intestinal barrier dysfunction following CPB (significant elevation of circulating FABP2, claudin-3, and reduction in citrulline). Stool eicosanoids were also elevated following CPB, providing preliminary evidence that the postoperative gut microbiome may produce proinflammatory metabolites that could be released into circulation in the setting of compromised intestinal permeability.

The impact of the gut microbiome on cardiovascular disease is beginning to be recognized. A recent review by Witkowski et al. (4) highlights the growing body of literature linking the gut microbiome to a range of adult cardiovascular diseases including heart failure, atherosclerosis, and metabolic syndrome. Many of these studies are associative, identifying dysbiosis of the gut microbiome in affected patients compared to controls. Similar to metabolomic and proteomic profiling studies, associative microbiome studies identify differences among groups of patients, providing valuable preliminary data for hypothesis generation. Unlike metabolites and proteins, which typically have direct biologic activities, microbial community composition and microbial gene abundance only suggest differences in functional capacity rather than establishing potential functional links.

The gut microbiome may, however, be directly linked to host outcomes through the circulating metabolome. Newer evidence points towards bacterial metabolites as drivers of cardiovascular disease and systemic inflammation (4). These metabolites enter the circulation either through intestinal barrier breakdown or metaorganismal processes, where bacterial metabolites are normally absorbed and then processed by the host into unique biologically active metabolites. Probably the best-defined example is trimethylamine N-oxide (TMAO), which is converted from bacterial-derived trimethylamine in the liver. In animal models, TMAO increases platelet activation, atherosclerosis, and vascular inflammation, and exacerbates heart failure and chronic kidney disease (4). Circulating TMAO is strongly associated with adult cardiovascular disease risk and mortality across multiple observational studies (4). Other microbial-derived metabolites thought to modulate cardiovascular disease risk or systemic inflammation in adults include tryptophan metabolites, bile acids, phenylacetylglutamine, and short chain fatty acids. Much less is known about the link between the gut microbiome and circulating metabolites in children with CHD. The findings of increased proinflammatory bacterial populations combined with increased stool eicosanoid concentrations and intestinal permeability in the current study imply a potential new mechanism for post-bypass inflammation in the CHD population. However, as the authors state, these findings remain associative and further study is needed to establish causality.

Why might the CHD population be at particular risk for pathologic consequences from gut dysbiosis? Preoperative dysbiosis with higher prevalence of proinflammatory bacterial populations raises the possibility that children with CHD could be primed for an increase in postoperative inflammation. Whether this dysbiosis is a modifiable risk factor remains to be seen and may rely in part on the underlying cause. Multiple factors could contribute to this dysbiosis including reduced gut perfusion, chronic cyanosis, altered immunity from genetic disorders or intraoperative thymectomy, and environmental considerations such as increased antibiotic use and poor enteral nutrition. Each represents different challenges and potentials for preoperative risk stratification and intervention. In the immediate postoperative period, multiple studies have confirmed decreased intestinal barrier function in children undergoing cardiac surgery with CPB (3). Postoperative endotoxemia occurs frequently in this population and if large molecules similar to endotoxin (>100,000 Daltons) escape the confines of the intestine, then almost certainly metabolites (<1,500 Daltons) are also released into circulation. Studies addressing this question are lacking, but recent findings of increased circulating eicosanoids and tryptophan metabolites after CHD surgery raise the question of what portion of these metabolites could be gut derived (1,3). Also unique to CHD surgery, the lungs may not serve their normal role as a filter of lymph-delivered gut metabolites (gut-lymph-lung axis) due to the presence of right-to-left shunts, potentially exposing these children to increased systemic effects from gut metabolites. In the late postoperative period, children undergoing neonatal repair remain at risk for poor tolerance of enteral nutrition and necrotizing enterocolitis. Necrotizing enterocolitis in premature infants is associated with gut dysbiosis and it would be important to assess if early perioperative dysbiosis persists or worsens in these high-risk neonates (5).

For these reasons, the novel findings by Salomon et al. (3) are intriguing and deserve significant consideration in the CHD field. The study has some limitations. Because the controls lacked both CHD

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 6, NO. 4, 2021
APRIL 2021:328–30

and CPB, it cannot be determined how much of the observed differences can be attributed to CPB versus underlying CHD. Additionally, although perioperative variables were not significantly different, many of them approached significance, which could represent type II error given the small sample size. A multicenter study with a larger sample size and longer study period would better account for these potential differences in groups and allow evaluation of generalizability across centers. From an epidemiologic standpoint, age-specific prevalence and features of dysbiosis remain important gaps in our understanding, as do specific risk factors for the development of dysbiosis. Embracing the big data approach, multiomics studies simultaneously evaluating the microbiome and the circulating metabolome would help evaluate the full scope of potential interactions that could be missed in studies with a narrower focus. Also, moving from bacterial species and genetic identification as the primary outcomes to understanding functional dysregulation of the microbiome is critical (4).

The long-term impact of these associative findings depends heavily on the scientific community's interest in pursuing the more challenging studies required to confirm causality, determine mechanisms, and test novel therapeutic approaches. Some of this work could be accomplished using clinical studies of stable isotope-labeled metabolites delivered enterally and sampled systemically to explore metabolic flux. These would then be paired with relevant translational models of CHD surgery, allowing more detailed sampling (fecal, intestinal, portal venous, and lymphatic samples, as well as target organ sampling), ultimately leading to identification of target pathways and experimental modulation of the gut microbiome, metabolic pathways, or both. Therapeutic strategies could include options as simple as promotion of healthy intestinal flora through increased use of breastmilk in infancy, judicious use of antibiotics, dietary interventions, and use of prebiotics and probiotics (5). These interventions could be introduced into models and clinical trials in a relatively straightforward fashion. More complex interventions such as focal modulation of gut microbial metabolism with small molecule inhibitors and alteration of host enzyme functions to control metaorganismal metabolite production offer the possibility of targeted therapies but require more extensive preclinical and clinical studies (4). Despite these challenges, it is clear that big data approaches are needed to account for the immense complexity of this pathophysiology and will increasingly become the norm as we continue to look for novel strategies to improve the lives of children born with CHD.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

ADDRESS FOR CORRESPONDENCE: Dr. Jesse A. Davidson, Department of Pediatrics, University of Colorado, 13123 East 16th Avenue, Box 100, Aurora, Colorado 80045, USA. E-mail: jesse.davidson@childrenscolorado.org.

REFERENCES

1. Davidson JA, Pfeifer Z, Frank B, et al. Metabolomic fingerprinting of infants undergoing cardiopulmonary bypass: changes in metabolic pathways and association with mortality and cardiac intensive care unit length of stay. J Am Heart Assoc 2018;7:e010711.

2. Frank BS, Khailova L, Silveira L, et al. Proteomic profiling identifies key differences between inter-stage infants with single ventricle heart disease and healthy controls. Transl Res 2021;229:24–37.

3. Salomon J, Ericsson A, Price A, et al. Dysbiosis and intestinal barrier dysfunction in pediatric congenital heart disease is exacerbated following cardiopulmonary bypass. J Am Coll Cardiol Basic Trans Science 2021;6:311–27.

4. Witkowski M, Weeks TL, Hazen SL. Gut microbiota and cardiovascular disease. Circ Res 2020;127:553–70.

5. Denning NL, Prince JM. Neonatal intestinal dysbiosis in necrotizing enterocolitis. Mol Med 2018;24:4.

KEY WORDS cardiopulmonary bypass, congenital heart disease, microbiome, pediatric