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Alteration in Indole Metabolites After Cardiopulmonary Bypass Surgery in Neonates and Infants

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IMPORTANCE: Cardiopulmonary bypass (CPB) surgery is associated with changes in the intestinal microbiome. Metabolism of tryptophan into the indole pathway is entirely facilitated by the intestinal microbiome, and indole metabolites play a critical role in intestinal epithelial integrity, intestinal and systemic vascular tone, and intestinal and systemic immune response.

OBJECTIVES: To evaluate the impact of CPB on microbial-derived indole metabolites and their association with clinical outcomes.

DESIGN, SETTING, AND PARTICIPANTS: Prospective cohort study of neonates and infants younger than 6 months of age undergoing CPB at a quaternary children's hospital.

MAIN OUTCOMES AND MEASURES: Serum samples underwent quantitative pathway mapping via mass spectroscopy. Clinical outcomes of interest included cardiac ICU (CICU) length of stay and Vasoactive-Inotropic Score (VIS) at 48 hours.

RESULTS: Ninety patients between 2 and 169 days old were enrolled. Patients showed significant postoperative changes in seven of eight indole metabolites. A two-fold increase in preoperative levels of indole-3-carboxylic acid was associated with 0.63 odds of requiring vasoactive medications at 48 hours ($p = 0.023$) and among those subjects still requiring vasoactives at 48 hours, they had an average 7.1% decrease in VIS at 48 hours ($p = 0.005$), and a 12.25% reduction in CICU length of stay ($p = 0.001$). Higher levels of indole-3-carboxylic acid preoperatively and at 24 and 48 hours postoperatively were also significantly associated with decreased CICU length of stay. Conversely, increased levels of several metabolites, including indole-3-lactic acid, indole-3-carbaldhyde, indole-3-propionic acid, tryptamine, and tryptophol, in the preoperative and postoperative period were associated with higher VIS at 48 hours and increased CICU length of stay.

CONCLUSIONS AND RELEVANCE: CPB was associated with significant changes in indole metabolite levels postoperatively. Indole-3-carboxylic acid, which suppresses T-regulatory (Treg) differentiation, is associated with improved patient outcomes, whereas other metabolites, that promote Treg differentiation, were associated with worse outcomes.

KEYWORDS: cardiopulmonary bypass; enteral nutrition; indole-3-carboxylic acid; indoles; tryptophan

Cardiothoracic surgery with cardiopulmonary bypass (CPB) for the correction of congenital heart disease (CHD) leads to physiologic stress and alterations in substrate metabolism, including the amino acid tryptophan (1, 2). Tryptophan is metabolized through three distinct pathways: kynurenine, indole, and serotonin pathways. Metabolites of the indole pathway play a critical role in intestinal epithelial integrity, intestinal and systemic vascular tone, intestinal

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KEY POINTS

Question: Is cardiopulmonary bypass (CPB) surgery in children with congenital heart disease associated with changes in circulating indole levels, and are indole metabolite levels associated with cardiac ICU length of stay and Vasoactive-Inotropic Score at 48 hours?

Findings: We found changes in seven circulating indole metabolites. Indole-3-carboxylic acid, which suppresses T-regulatory (Treg) cell differentiation, was associated with improved clinical outcomes. Other metabolites promoting Treg cell differentiation were associated with worse outcomes.

Meaning: Indole metabolites are associated with clinical outcomes in children undergoing CPB surgery, which may be related to their impact on Treg cell differentiation.

motility, and intestinal and systemic immune response (3, 4). Catabolism of tryptophan through the indole pathway is unique in that it is facilitated by bacteria of the intestinal microbiota, including *Peptostreptococcus*, *Clostridia*, *Bacteroides*, and *Bifidobacterium* species (5, 6). There are specific bacterial species that contain different enzymes that generate individual indole metabolites (5). CPB alters the intestinal microbiota (7), but to date, no studies have evaluated the impact of CPB on indole metabolism and its association with clinical outcomes. The aim of this study was to evaluate the impact of CPB surgery on indole metabolism and determine the association of preoperative and postoperative indole levels with postoperative outcomes. We assessed whether preoperative method of nutrition administration was associated with indole levels. Our hypothesis was that cardiothoracic surgery with CPB would be associated with significant changes in indole levels compared with preoperative baseline and that those changes would be associated with preoperative nutrition delivery. We also hypothesized that patients with higher levels of indole metabolites would have decreased vasoactive support at 48 hours and decreased cardiac ICU (CICU) length of stay.

METHODS

This was a prospective, single center cohort study of patients younger than 6 months undergoing CPB for treatment of CHD. This study was approved by the Colorado Multiple Institution Review Board (No. 20-3059, Approval: February 23, 2023, Title: Metabolic Profiling and Comprehensive Metabolic Pathway Mapping: A Systems Biology Approach to Cardiovascular Failure and Organ Injury Following Infant Congenital Heart Disease Surgery). Informed consent was obtained from a patient's parents or legal authorized representative. All study procedures followed were in concordance with the ethical standards for human subjects research at the University of Colorado and with the Helsinki Declaration of 1975. We excluded patients less than 2 kg. Serum samples were drawn preoperatively, after the induction of anesthesia, prior to the start of surgery, and at 2, 24, and 48 hours postoperatively. Clinical data were collected until hospital discharge and managed using Research Electronic Data Capture tools hosted at the University of Colorado (8, 9).

The following indole pathway metabolites were measured in 60 µL of serum using liquid chromatography/tandem mass spectrometry: 3-indole acetic acid (IAA), tryptamine, indole-3-lactic acid (ILA), indole-3-carboxylic acid (ICA), tryptophol, indole-3-propionic acid (IPA), indole-3-carboxaldehyde, and 3-indoxylsulfate (3-IS). An isotope labeled internal standard mix containing IAA-d2, 3-IS-d5, ILA-d5, and tryptamine-d4 was used to generate calibration curves and to calculate endogenous concentrations in serum.

Indole metabolite levels were log-2 transformed to account for nonnormal distribution and reduce influence of outliers. Repeated-measures analysis of variance was used to compare indole metabolite levels preoperatively and postoperatively. When performing outcomes analyses, metabolite levels were compared at the time they were drawn, not relative to preoperative levels. Zero-inflated negative binomial regression models were used to identify associations of metabolites at each timepoint with Vasoactive-Inotropic Score (VIS) (10) at 48 hours. Multivariable linear regression was used to identify the association between indole metabolites at each time point and CICU length of stay. For patients 29 days or less, multivariable linear regression was used to evaluate the relationship between method of nutrition delivery (enteral nutrition only, parenteral nutrition only, or both) and indole metabolites. Multivariable models included the considered covariates of sex, age, CPB time, cross-clamp time, and Society of Thoracic Surgeons and the

European Association for Cardio-Thoracic Surgery (STAT) category (11). Final multivariable linear and logistic models (CICU length of stay) used stepwise selection to minimize Bayesian Information Criterion, and zero-inflated negative binomial models (VIS) used backward stepwise variable elimination with an α threshold of 0.05.

RESULTS

Ninety patients between 2 and 169 days old were included in this cohort. Full patient characteristics are described in **Table 1**.

Patients demonstrated significant postoperative changes in seven of eight indole metabolites compared with their preoperative baseline (**Fig. 1**). Most metabolites increased in the immediate postoperative period. IAA and ILA decreased. Indole-3-carbaldehyde was significantly altered after CPB.

Outcomes: VIS

For every two-fold increase in preoperative levels of ICA, there were 0.63 (95% CI, 0.43–0.92) odds of requiring vasoactive medications at 48 hours. A two-fold increase in tryptophol preoperatively, at 24 and 48 hours postoperatively had a 1.88 (95% CI, 1.14–3.16), 2.05 (95% CI, 1.08–3.88), and 2.30 (95% CI, 1.19–4.43) odds of requiring vasoactive medications at 48 hours. Similarly, a two-fold increase in ILA at 2 and 24 hours postoperatively had 2.36 (95% CI, 1.19–4.67) and 2.27 (95% CI, 1.08–4.74) odds of requiring vasoactive medications at 48 hours.

For those patients who continued to require vasoactive medications at 48 hours, a two-fold increase in preoperative levels of ICA and indoxyl sulfate were associated with a decrease in VIS at 48 hours (**Table 2**). Increased ILA, indole-3-carbaldhyde, tryptophol, and tryptamine levels at various time points were associated with increased VIS at 48 hours.

TABLE 1.
Patient Characteristics

Characteristics	Overall, *n* = 90	Infant, *n* = 45	Neonate, *n* = 45	*p*[a]
Gender				
Male	49 (54%)	19 (43%)	30 (67%)	0.020
Female	41 (46%)	26 (58%)	15 (44%)	
Ethnicity				0.3
Non-Hispanic or Latino	52 (58%)	27 (60%)	25 (56%)	
Age (d) on day of surgery	27 (6–16)	106 (82–129)	6 (4–9)	< 0.001
Number of subjects with single ventricle physiology	20 (22%)	3 (7%)	17 (38%)	0.016
Society of Thoracic Surgeons and the European Association for Cardio-Thoracic Surgery score				< 0.001
1	14 (16%)	13 (29%)	1 (2.2%)	
2	17 (19%)	13 (29%)	4 (8.9%)	
3	14 (16%)	7 (16%)	7 (16%)	
4	24 (27%)	11 (24%)	13 (29%)	
5	21 (23%)	1 (2.2%)	20 (44%)	
Bypass time (min)	146.5 (110.0–186.0)	129.0 (108.0–188.0)	153.0 (133.00–181.0)	0.2
Cross-clamp time (min)	73.0 (55.0–92.0)	76.0 (49.0–103.0)	69.0 (59.0–86.0)	0.5
Vasoactive-Inotropic Score at 48 hr	5.0 (0.0–11.0)	3.0 (0.0–5.0)	10.0 (7.0–13.0)	< 0.001
ICU length of stay (d)	4.85 (2.15–9.88)	2.66 (2.02–4.17)	8.13 (4.90–10.82)	< 0.001
Peak lactate 24 hr postoperatively	2.50 (1.55–4.17)	1.55 (1.33–2.06)	4.04 (3.17–5.34)	< 0.001

[a]*p* value calculated using Fisher exact test, Pearson χ^2 test, and Wilcoxon rank-sum test as appropriate.
Data are expressed as *n* (%) or median (interquartile range).

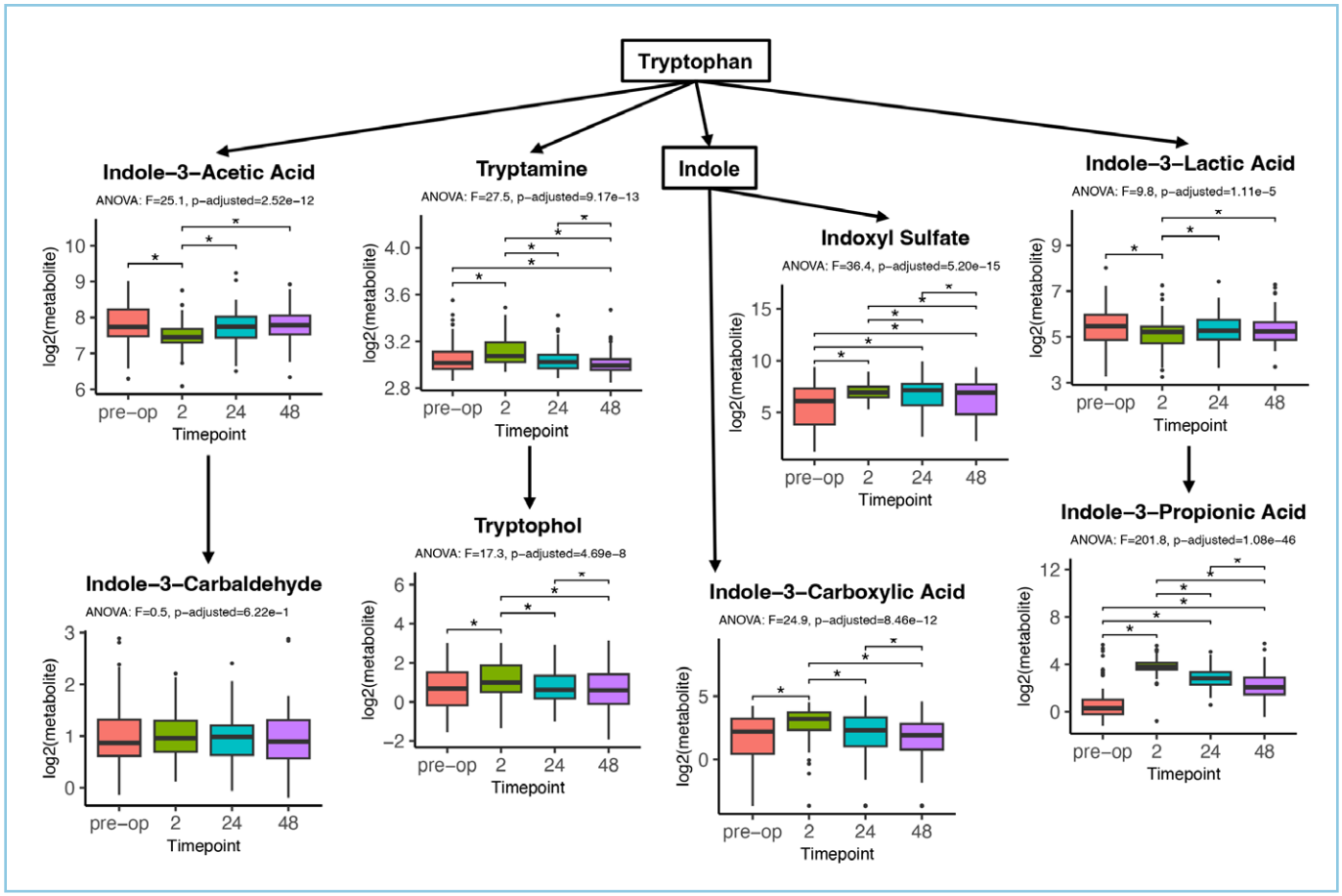

Figure 1. A majority (7/8) of indole metabolites demonstrated significant changes from preoperative baseline except for indole-3-carbaldehyde. Tryptamine, tryptophol, indoxyl sulfate, indole-3-carboxylic acid, and indole-3-propionic acid were significantly increased. Indole-3-lactic acid and indole-3-acetic acid both decreased significantly postoperatively. For each analysis of variance (ANOVA) test, *F* is the *F* test value and *p*_adjusted is the false discovery rate (FDR) adjusted *p* value. Each *bracket* with *asterisk* represents a significant change in metabolite levels between the two indicated time points (FDR adjusted $p < 0.05$).

Outcomes: CICU Length of Stay

Preoperative levels of ICA were associated with decreased CICU length of stay (Table 2). ICA levels at 24 and 48 hours postoperatively were also associated with decreased CICU length of stay. IPA and tryptophol at 24 hours postoperatively; indole-3-cabaldehyde, tryptamine, and IPA at 48 hours postoperatively were associated with increased CICU length of stay.

Preoperative Nutrition and Postoperative Metabolite Levels

Among our patients younger than 29 days, six subjects were exclusively enterally fed, 22 received a combination of enteral and parenteral nutrition, and 17 received exclusive parenteral nutrition. Subjects who received a combination of enteral and parenteral nutrition or parenteral nutrition exclusively had a 45.83% (95% CI, 5.18–102.19%) and 53.57% (95% CI, 9.55–115.26%) increase in IAA at 2 hours postoperatively. At 48 hours postoperatively, IPA and indoxyl sulfate were decreased by 68.21% (95% CI, –88.71% to –10.50%) and 91.34% (95% CI, –99.07% to –19.83%) in those who received a combination of enteral and parenteral nutrition compared with those who received enteral nutrition exclusively. There were no significant changes in other indole metabolites based on feeding type.

DISCUSSION

In this single-center, prospective study, we sought to evaluate the impact of cardiothoracic surgery with CPB on circulating indole metabolites and the association between preoperative/postoperative indole levels and clinical outcomes. We found that infants with CHD exhibited significant changes in circulating

TABLE 2.

Percent Change in Cardiac ICU Length of Stay or Vasoactive-Inotropic Score at 48 Hours for a Two-Fold Increase in Level of Metabolite at Each Time Point

Metabolite	Preoperative	2-hr Postoperative	24-hr Postoperative	48-hr Postoperative
Percent change in cardiac ICU length of stay (95% CI)				
Indole-3-acetic acid	−5.41 (−28.65 to 25.40)	−19.01 (−42.81 to 14.70)	0.29 (−23.47 to 31.41)	15.03 (−12.04 to 50.43)
Indole-3-carbaldehyde	9.82 (−10.40 to 34.61)	14.69 (−13.15 to 51.45)	10.17 (−13.66 to 40.60)	**25.72 (1.34–55.98)**
Tryptamine	57.65 (−43.77 to 341.96)	120.76 (−17.19 to 488.54)	192.76 (−4.72 to 799.53)	**316.86 (14.00–1424.34)**
Tryptophol	−1.50 (−13.37 to 11.99)	8.47 (−5.39 to 24.36)	**14.81 (0.06–31.73)**	10.01 (−3.59 to 25.52)
Indole-3-carboxylic acid	**−12.25 (−18.35 to −5.68)**	−9.58 (−18.89 to 0.81)	**−9.03 (−15.43 to −2.14)**	**−6.46 (−11.59 to −1.03)**
Indoxyl sulfate	−0.04 (−5.74 to 6.01)	9.18 (−5.55 to 26.19)	2.10 (−5.66 to 10.50)	−2.20 (−8.37 to 4.38)
Indole-3-lactic acid	−0.44 (−13.25 to 14.25)	−8.12 (−21.67 to 7.78)	−2.79 (−16.81 to 13.58)	8.57 (−10.59 to 31.84)
Indole-3-propionic acid	3.54 (−4.25 to 11.96)	9.73 (−5.21 to 27.03)	**16.26 (1.68–32.94)**	**14.22 (3.43–26.13)**
Percent change in Vasoactive-Inotropic Score at 48 hr (95% CI)				
Indole-3-acetic acid	14.47 (−2.61 to 31.52)	0.73 (−18.56 to 24.60)	14.18 (−3.06 to 34.48)	5.40 (−10.01 to 23.44)
Indole-3-carbaldehyde	**17.81 (5.52–31.52)**	12.20 (−5.83 to 33.69)	**26.18 (8.91–46.20)**	5.99 (−6.44 to 20.06)
Tryptamine	50.47 (−14.34 to 164.32)	43.31 (−19.64 to 155.56)	67.98 (−7.90 to 206.36)	**110.34 (5.77–318.28)**
Tryptophol	6.69 (−1.09 to 15.09)	6.42 (−2.38 to 16.02)	**9.55 (1.23–18.56)**	6.33 (−2.10 to 15.47)
Indole-3-carboxylic acid	**−7.12 (−12.04 to −1.93)**	−4.27 (−11.60 to 3.67)	1.10 (−4.26 to 6.78)	0.88 (−3.12 to 5.04)
Indoxyl sulfate	**−3.99 (−7.19 to −0.68)**	−8.41 (−17.27 to 1.40)	−4.85 (−10.01 to 0.61)	−3.73 (−7.59 to 0.33)
Indole-3-lactic acid	**13.54 (3.88–24.11)**	4.74 (−5.62 to 16.25)	**18.91 (7.39–31.67)**	11.82 (−0.47 to 25.63)
Indole-3-propionic acid	−1.21 (−7.72 to 5.76)	0.55 (−10.30 to 12.71)	2.34 (−7.90 to 13.72)	3.63 (−4.65 to 12.62)

Multivariable linear regression was used to evaluate the percent change in cardiac ICU length of stay for a log-2-fold increase in an individual metabolite above the median value at each measured time point. Zero-inflated negative binomial models were used to evaluate percent change of Vasoactive-Inotropic Score at 48 hr for a log-2-fold increase in an individual metabolite above the median value at each time point. Bolded values have a $p < 0.05$.

indoles after CPB surgery and indole levels were associated with clinical outcomes.

Increased levels of ICA were consistently associated with decreased CICU length of stay and decreased duration and need for vasoactive inotropic medications. There are limited data describing the role of ICA in human health. ICA competes with binding at the aryl hydrocarbon receptor (AhR) and inhibits differentiation of CD4 T cells into T-regulatory (Treg) cells (12). ICA may serve as a counterbalance to AhR ligands, helping prevent an overly robust anti-inflammatory response to CPB. Further research is needed to further delineate these relationships and the role of ICA on T cell differentiation. ICA and AhR could represent potential therapeutic targets.

Tryptamine, tryptophol, ILA, and indole-3-carbaldhyde were associated with worse clinical outcomes, including increased VIS score at 48 hours and increased CICU length of stay. These metabolites are ligands for the AhR (13–16). They have been shown to promote antioxidation and anti-inflammatory pathways, including differentiation of CD4 T cells into Treg. Elevated levels of IPA at 24 and 48 hours postoperatively were associated with increased CICU length of stay. IPA also promotes an anti-inflammatory milieu through its binding of pregnane X receptor (PXR) (17).

This is the first study to demonstrate an association between microbe-derived indole metabolites that serve as AhR and PXR ligands and worse clinical outcomes. These findings are similar to those that describe worse outcomes in patients with sepsis who have persistently elevated levels of the tryptophan metabolite, kynurenine, another potent AhR agonist (18). It is hypothesized that increased Treg differentiation after AhR binding may lead to a state of immunoparalysis, contributing to poor outcomes. Since catabolism of tryptophan into indole metabolites is dependent on bacteria with the required enzymes to facilitate metabolism, it is likely that changes in microbial diversity and relative abundance drive differential indole metabolite levels.

Mode of nutrition delivery was associated with changes in IAA at 2 hours postoperatively and IPA and indoxyl sulfate at 48 hours postoperatively. Nutrition may play a role in altering indole levels through augmentation of the intestinal microbiome (19).

There are several limitations to this study that will inform the direction for future research. While variables sex, age, CPB time, cross-clamp time, and STAT category were considered in our multivariable models, our sample size limited the ability to assess changes in indole metabolites based on type of cardiac lesion. Evaluating the impact of feeding on indole metabolism is limited given the small sample size in each group. In addition, preoperative feeding data were limited to neonates only. Other factors, such as gestational age and delivery modality, likely impact the intestinal microbiome and should be considered in future studies.

In conclusion, cardiothoracic surgery with CPB is associated with changes in indole metabolism postoperatively. ICA, which suppresses Treg differentiation, is associated with improved patient outcomes, whereas other metabolites, that promote Treg differentiation, were associated with worse outcomes. Evaluation of the immune response to indole metabolites is an opportunity for future investigation. Metabolism of tryptophan into the indole pathway is completely facilitated by the intestinal microbiome and dependent on available tryptophan. Further research is needed to evaluate alterations in indole metabolism in the context of the intestinal microbiome. To have a more complete picture, research should evaluate whether the changes in indole levels are related to alterations in the constituents in the intestinal microbiota, changes in tryptophan available to enter the indole pathway, or combination of the two.

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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. Sabapathy D, Klawitter J, Silveira L, et al: Activation of kynurenine pathway of tryptophan metabolism after infant cardiac surgery with cardiopulmonary bypass: A prospective cohort study. *Metabolomics* 2020; 16:93
3. Roager HM, Licht TR: Microbial tryptophan catabolites in health and disease. *Nat Commun* 2018; 9:3294
4. Scott SA, Fu J, Chang PV: Microbial tryptophan metabolites regulate gut barrier function via the aryl hydrocarbon receptor. *Proc Natl Acad Sci U S A* 2020; 117:19376–19387
5. Su X, Gao Y, Yang R: Gut microbiota-derived tryptophan metabolites maintain gut and systemic homeostasis. *Cells* 2022; 11:2296
6. Gao J, Xu K, Liu H, et al: Impact of the gut microbiota on intestinal immunity mediated by tryptophan metabolism. *Front Cell Infect Microbiol* 2018; 8:13
7. Salomon J, Ericsson A, Price A, et al: Dysbiosis and intestinal barrier dysfunction in pediatric congenital heart disease is exacerbated following cardiopulmonary bypass. *JACC Basic Transl Sci* 2021; 6:311–327
8. Harris PA, Taylor R, Thielke R, et al: Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009; 42:377–381
9. Harris PA, Taylor R, Minor BL, et al; REDCap Consortium: The REDCap consortium: Building an international community of software platform partners. *J Biomed Inform* 2019; 95:103208
10. Davidson J, Tong S, Hancock H, et al: Prospective validation of the vasoactive-inotropic score and correlation to short-term outcomes in neonates and infants after cardiothoracic surgery. *Intensive Care Med* 2012; 38:1184–1190
11. Jacobs ML, O'Brien SM, Jacobs JP, et al: An empirically based tool for analyzing morbidity associated with operations for congenital heart disease. *J Thorac Cardiovasc Surg* 2013; 145:1046–1057.e1
12. Fong W, Li Q, Ji F, et al: Lactobacillus gallinarum-derived metabolites boost anti-PD1 efficacy in colorectal cancer by inhibiting regulatory T cells through modulating IDO1/Kyn/AHR axis. *Gut* 2023; 72:2272–2285
13. Lu C, Chen J, Yi C, et al: Gut microbiota mediated the protective effects of tuna oil on collagen-induced arthritis in mice. *Food Funct* 2021; 12:5387–5398
14. Yu K, Li Q, Sun X, et al: Bacterial indole-3-lactic acid affects epithelium-macrophage crosstalk to regulate intestinal homeostasis. *Proc Natl Acad Sci U S A* 2023; 120:e2309032120
15. Meynier M, Baudu E, Rolhion N, et al: AhR/IL-22 pathway as new target for the treatment of post-infectious irritable bowel syndrome symptoms. *Gut Microbes* 2022; 14:2022997
16. Agista AZ, Tanuseputero SA, Koseki T, et al: Tryptamine, a microbial metabolite in fermented rice bran suppressed lipopolysaccharide-induced inflammation in a murine macrophage model. *Int J Mol Sci* 2022; 23:11209
17. Flannigan KL, Nieves KM, Szczepanski HE, et al: The pregnane X receptor and indole-3-propionic acid shape the intestinal mesenchyme to restrain inflammation and fibrosis. *Cell Mol Gastroenterol Hepatol* 2023; 15:765–795
18. Wirthgen E, Hoeflich A: Endotoxin-induced tryptophan degradation along the kynurenine pathway: The role of indolamine 2,3-dioxygenase and aryl hydrocarbon receptor-mediated immunosuppressive effects in endotoxin tolerance and cancer and its implications for immunoparalysis. *J Amino Acids* 2015; 2015:973548
19. Nolan LS, Rimer JM, Good M: The role of human milk oligosaccharides and probiotics on the neonatal microbiome and risk of necrotizing enterocolitis: A narrative review. *Nutrients* 2020; 12:3052