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Title: Intestinal microbiome changes in an infant with right atrial isomerism and recurrent necrotizing enterocolitis: A case report

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Dear Editor,

Thank you very much for having considered our manuscript "Intestinal microbiome changes in an infant with right atrial isomerism and recurrent necrotizing enterocolitis: A case report". We would like to express our appreciation to you and reviewers for the thoughtful comments.

Our responses to the reviewers' comments are given below

Reviewer #1:

Reviewer's code: 05038685

We highly appreciate the reviewer's helpful comments on our manuscript. We have modified the text according to the reviewer's remarks.

Answers to comments

1. "Materials and Methods" section is not necessary for Case Report. The detection method of fecal microbiota profiles can be described in "Laboratory examinations" part. Other laboratory testing methods such as Fecal culture or Blood culture are routine and therefore do not require a detailed description of the procedure

Response: The description of detection method of fecal microbiota profiles moved to the "Further diagnostic work-up: 16S rRNA gene-based fecal microbiota profiling" part (page 12). The description of Other microbiological laboratory testing methods was removed.

2. The discussion part of this article is tedious and should be simplified. This article focuses on intestinal microbiome changes in an infant with right atrial isomerism and recurrent necrotizing enterocolitis, so the cardiopulmonary bypass (Paragraphs 11 and 12 in the Discussion) -- should not be discussed alone here.

Response: Paragraphs 11 and 12 in the Discussion part with the cardiopulmonary bypass description were removed.

Reviewer #2:

Reviewer's code: 05208463

We highly appreciate and thank the reviewer for very interesting and thoughtful comments on the article.

Answers to comments

1. Page 10, on the section of “history of the first episode of NEC”, why did this baby receive the epinephrine (α and β receptor), non-epinephrine (α receptor), and dobutamine (β receptor) simultaneously? The action of these inotropic agents overlaps at some extent.

Response: After admission from the operating room, the hemodynamics of the baby was unstable (blood pressure dropped to 58/28 mmHg, SpO₂ 50%, heart rate 176 min⁻¹) while inotropic therapy with a maximal allowable dose of epinephrine 0.5 mcg·kg⁻¹·min⁻¹, while dobutamine dose was 5 mcg·kg⁻¹·min⁻¹. Considering the ineffectiveness of the therapy, norepinephrine was added with 0.2 -> 0.4 mcg·kg⁻¹·min⁻¹, the dose of epinephrine was decreased to 0.3 mcg·kg⁻¹·min⁻¹. This led to a blood pressure increase to 88/40 mmHg, SpO₂ 71%.

Epinephrine acts on α and β receptors. At low doses, it increases myocardial contractility and peripheral vasodilatation (β_1 and β_2 effects). High doses of epinephrine (> 0.1 mcg·kg⁻¹·min⁻¹) predominantly act on α_1 receptors and cause vasoconstriction (Klabunde R. E., 2011; Ruoss J. L., 2015). However, while on the maximal allowable dose of epinephrine the hemodynamics of the patient was unstable (arterial hypotension, low SpO₂). Therefore, norepinephrine was added to increase perfusion pressure.

Norepinephrine mainly acts on α_1 -receptors and causes α_1 -mediated vasoconstriction (Ruoss J. L., 2015) and increases systolic and mean arterial blood pressure to a greater extent than epinephrine (Klabunde R. E., 2011).

Dobutamine mainly acts on β_1 receptors, has a greater inotropic effect and causes an increase of cardiac output by increasing myocardial contractility (Ruoss J. L., 2015). Dobutamine was used to increase myocardial contractility and cardiac output.

2. Page 12, on the section of “final diagnosis”. The cardiogenic NEC is a special definition? If available, please adopt guidelines or expert consensus to clarify the criterion for diagnosing cardiogenic NEC.

Response: The definition of cardiogenic NEC was suggested by Siano E. et al in 2018 based on a systematic review of the characteristics of infants with NEC and congenital heart diseases (CHD). Authors define cardiogenic NEC as NEC developed in infants with CHD. Infants with CHD have a cardiogenic cause of NEC that results in mesenteric hypoperfusion and bowel ischemia. Infants with CHD and NEC had a higher gestational age and greater birth weight in comparison with non-CHD infants with NEC (Siano E., 2018).

Bubberman J.M. et al noted a different anatomic localization of NEC in infants with CHD - the colon was significantly more often involved in infants with CHD versus preterm neonates (Bubberman J. M., 2019).

A literature review of NEC in infants with CHD was published by Kelleher S. T. et al. in 2021. Authors note, that, unlike premature neonates, infants with CHD could have multiple pre-operative and post-operative risk factors of NEC including co-morbidities, hemodynamic instability, cardiopulmonary bypass surgery and mode of enteral feeding (Kelleher S. T., 2021)

3. After both episodes of NEC, enteral feeding was initialized with hydrolyzed formula. Why is breast milk not the first choice?

Response: Considering the higher incidence of gastrointestinal complications in infants who underwent palliative cardiac surgery, internal protocol considers the start of enteral feeding with hydrolyzed (semi-elemental) formula in infants with mesenteric hypoperfusion. A semi-elemental diet could be better tolerated by the intestinal mucosa in the case of intestinal injuries (Rooze S., 2019).

Semi-elemental formulas have been shown to reduce the degree of regurgitation, gastric emptying times, and gagging while improving tolerance (Alexander D. D., 2016). Such formulas are well-tolerated, digested, and absorbed among various patient groups, including those with Crohn's disease, acute and chronic pancreatitis, stroke, HIV, and critically ill (Alexander D. D., 2016).

Nevertheless, there are no clear guidelines for initiating substrate for enteral nutrition after NEC. The choice of substrate for refeeding infants after NEC depends on many factors such as gestational age, the availability of human breast milk, risk of the short gut syndrome and/or malabsorption, as well as the risk of cow's milk allergy (Lapillonne A., 2016).

However, mother's own milk, if tolerated, could be better for feeding infants.

A number of protocols recommend to start enteral feeding after cardiac surgery with breast milk (Furlong-Dillard J., 2018; Scahill C. J., 2017; Schwalbe-Terilli C. R., 2009). However, enteral feeding strategies of infants with CHD who underwent palliative cardiac surgery and have mesenteric hypoperfusion are diverse. del Castillo S. L. (2010), Raymond T. T. (2020) suggest starting enteral feeding in infants with single ventricle with electrolyte solution in the early postoperative period, if feeding is tolerated - advance feeding to breast milk / high-calorie formula (20 kcal/oz) (Raymond T. T., 2020), breast milk / hydrolyzed formula (del Castillo S. L., 2010).

Rooze S. et al. (2019) have not revealed significant differences between tolerance of semi-elemental (hydrolyzed) formula and polymeric formula in children with CHD, there were not cases of NEC in children. However, the authors did not evaluate the

influence of type of cardiac surgery (radical or palliative) mesenteric hypoperfusion on enteral feeding tolerance.

Martini S. et al (2021) reviewed studies of feeding strategies in infants with CHD-feeding with mother's own milk could be considered in these infants.

The studies of enteral feeding in infants with persistent mesenteric hypoperfusion are few. Enteral feeding strategies in infants who underwent palliative cardiac surgery need further research.

4. Aspirin, as one representative of NSAIDs group, acts by inhibiting the Cox, which could theoretically increase the risk of NEC. So, what is the role of aspirin on the pathogenesis of the second episode of NEC?

Response: Infants, who underwent Blalock-Taussig shunt placement, in early postoperative period may be anticoagulated with heparin for 24-72 h, then (after enteral feeding was started) they need long-term aspirin therapy for shunt patency maintenance (Kiran U., 2017). Aspirin could be a cause of haematochezia and feeding intolerance in children (Muller M., 2019). However, we could not find studies estimating the influence of aspirin doses on NEC incidence in term infants with CHD.

The risk of spontaneous intestinal perforation in preterm neonates treated with NSAIDs (Ibuprofen) is described by Rao R. et al. (2011). Mitra S. et al (2018) in a systematic review have not revealed a significant difference in the odds of mortality, necrotizing enterocolitis, or intraventricular hemorrhage with the use of placebo or no treatment compared with Indomethacin, Ibuprofen, and Acetaminophen used for closure of patent ductus arteriosus in preterm infants.

Lu H. et al. (2014) suggest the role of COX-2 in the ability of the gastrointestinal mucosa to respond to injury. Authors revealed an increased expression of ileal COX-2 mRNA resulted in a significant increase in the incidence and severity of NEC. However, COX-2 mRNA expression was significantly upregulated with the repair of intestinal injury at 24 h after LPS injection. Authors suggest the induction of COX-2 activity participates in the exacerbation of the injury and resolution of inflammation (Lu H., 2014).

According to recent experimental studies, the role of Cox inhibition in the pathogenesis of NEC could be controversial. Golden J. et al. (2020), Sun L. (2021) in experimental studies showed a possible protective role of low-dose cyclooxygenase-2 (COX-2) inhibitor celecoxib in the rat model of neonatal necrotizing enterocolitis.

We cannot exclude the impact of aspirin on NEC development in the patient. However, we also cannot evaluate the aspirin influence on NEC development in this case.

Science editor:

We thank the Science editor for the positive comments and for the opportunity to revise the article.

1. The figure legends should be properly annotated and easy to read and interpret so that readers can understand what is being expressed without reading the full text.

Response: We have modified the figure legends

2. The form of the table in the article should adopt the form of three-line table.

Response: The form of the table was adopted.

3. The content of the article needs to be simplified to avoid being boring.

Response: The description of Other microbiological laboratory testing methods was removed. Paragraphs 11 and 12 in the Discussion part with the cardiopulmonary bypass description were removed.

4. Please provide documents following the requirements in the journal's Guidelines for manuscript type and related ethics: Copyright License Agreement.

Response: We provided the Copyright License Agreement

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