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# Biomarkers of environmental enteric dysfunction among children in rural Bangladesh

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## Abstract

**Objectives—**Environmental enteric dysfunction (EED) may inhibit growth and development in low- and middle-income countries, but available assessment methodologies limit its study. In rural Bangladesh, we measured EED using the widely-used lactulose mannitol ratio (L:M) test and a panel of intestinal and systemic health biomarkers to evaluate convergence among biomarkers and describe risk factors for EED.

**Methods—**In 539 18-month-old children finishing participation in a randomized food supplementation trial, serum, stool and urine collected after lactulose and mannitol dosing were analyzed for biomarkers of intestinal absorption, inflammation, permeability and repair, and systemic inflammation. EED scores for each participant were developed using principal component analysis (PCA) and partial least squares regression (PLS). Associations between scores and L:M and with child sociodemographic and health characteristics were evaluated using regression analysis.

**Results—**EED prevalence (L:M>0.07) was 39.0%; 60% had elevated acute phase proteins (CRP>5mg/L or AGP>100mg/dL). Correlations between intestinal biomarkers were low, with the highest between myeloperoxidase and α-1 antitrypsin ($r=0.33$, $p<0.01$), and biomarker values did not differ by supplementation history. A one-factor PLS model with L:M as the dependent variable explained only 8.6% of L:M variability. In adjusted models, L:M was associated with child sex and SES index, while systemic inflammation was predicted mainly by recent illness, not EED.

**Conclusions—**Impaired intestinal health is widespread in this setting of prevalent stunting, but a panel of serum and stool biomarkers demonstrated poor agreement with L:M. Etiologies of

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AUTHOR CONTRIBUTIONS

Designed research (RKC, KS, PC); conducted field research (RKC, KS, SS, SM, HA); conducted laboratory analyses (RKC, KS, RR, SB); field and data management (KS, SS, SM, HA, LW, AL, KPW, PC); analyzed data (RKC); wrote paper (RKC).

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intestinal and systemic inflammation are likely numerous and complex in resource-poor settings, underscoring the need for a better case definition with corresponding diagnostic methods to further the study of EED.

**Keywords**

undernutrition; stunting; inflammation; South Asia; biomarkers

## INTRODUCTION

Environmental enteric dysfunction (EED) may be an important cause of persistent stunting in low- and middle-income country (LMIC) children (1–4). EED is described as a subclinical condition of partial villous atrophy, crypt hyperplasia, leaky tight junctions and enteric immune cell proliferation (1, 5), thought to result from chronic environmental pathogen and toxin exposure (1, 3). Studies in some LMICs have reported nearly universal EED (6–9) and inverse associations between EED severity and linear growth (9–15). Idiopathic systemic inflammation is also ubiquitous in LMIC children and may be an important pathway whereby EED inhibits growth (1, 11, 16, 17).

Assessing the burden of EED and quantifying its contribution to stunting is currently inhibited by the assessment tools available. The lactulose mannitol ratio (L:M) test of intestinal permeability is widely used but suffers from several limitations: it measures permeability but not intestinal or systemic inflammation (2); it lacks formal validation studies (18, 19); and it is burdensome to implement in field settings. Proposed alternate markers of EED measured in serum and stool could address these shortcomings (2, 4, 13), but limited data exist to support their use for this purpose. Several proposed fecal markers, such as myeloperoxidase, neopterin, lactoferrin and calprotectin, markers of inflammation, and α-1 antitrypsin, a marker of protein-losing enteropathy, come from the study of inflammatory bowel disease (20–22). Endotoxin core antibodies measured in serum may indicate systemic exposure to endotoxin of intestinal origin and have been used previously for assessing EED (11, 15).

We aimed to assess a panel of EED biomarkers in 18-month-old Bangladeshi children to (1) describe intestinal and systemic health, (2) use statistical data reduction techniques to empirically identify an efficient subset of markers for diagnosing EED and (3) describe risk factors for EED.

## METHODS

This study was nested in a community-based randomized trial of complementary food supplements **(CFS)** for children 6–18 mo of age conducted from September 2012 through May 2014 in northwest Bangladesh, which demonstrated benefits for linear growth and prevention of stunting (23). Children were randomized based on their geographic sector of residence to receive child feeding counseling (CFC) for mothers only or CFC plus one of four formulations of CFS fed as a daily snack for one year. EED was assessed at age 18 mo with the L:M test and a panel of intestinal and systemic health biomarkers selected to broadly capture the EED condition.

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Within a geographically-designated area selected to contain ~15% of trial participants balanced by study arm, an enhanced data collection protocol including blood collection at age 18 mo was implemented (n=828). Among those consenting to the enhanced protocol, children born between March 2012 and September 2013 were eligible for the EED assessment (L:M test and stool collection), to achieve a target sample size of 500 based on ability to detect differences in L:M by supplementation group.

## Field Methods

Extensive child and household data were collected within the parent trial. Household socioeconomic status (SES), including parental education and occupation and household asset ownership, electricity and water and sanitation facilities, was assessed via questionnaire at enrollment. Length and weight were measured at enrollment (age 6 mo) and every 3 months thereafter by standardized anthropometrists.

Biospecimens were collected immediately following the child's 18-mo birthday. L:M urine tests and serum collection occurred at central field clinics, while stool samples were collected at home within a day of the clinic visit. Upon arrival at the field clinic, mothers completed a 7-day recall of the child's morbidity symptoms. Assessments were rescheduled for children with a current illness. Following a 2-hour fast, children were dosed by body weight (2 mL/kg weight up to 20 mL maximum) with 255 mg/mL lactulose and 50 mg/mL mannitol. Urine was subsequently collected for two hours (24, 25), weighed, mixed with chlorhexidine as a preservative and aliquoted for transport and storage. Blood was collected by venipuncture and allowed to clot. At the project field laboratory, blood was centrifuged, serum transferred to cryovials and urine and serum stored in liquid nitrogen. Stool was retrieved promptly from households by field workers and transported in cold boxes to the field laboratory, where the sample was homogenized by stirring, aliquoted into cryovials and stored in liquid nitrogen pending shipment.

## Laboratory Methods

Urine was analyzed for lactulose and mannitol concentrations by high pressure ion chromatography (Dionex, Thermo Fisher Scientific, Sunnyvale, CA) at the icddr,b in Dhaka, Bangladesh. Standard solutions with melibiose were run for quality control. Lactulose and mannitol recoveries were calculated from the measured amount of each in urine (concentration x total urine volume) relative to the initial dose of each sugar and expressed as the ratio of percent lactulose recovered to percent mannitol recovered.

Stool concentrations of myeloperoxidase (MPO), α-1 antitrypsin (AAT) and neopterin (NEO) and serum concentrations of endotoxin core antibody (EndoCAb), glucagon-like peptide-2 (GLP-2), total immunoglobulins (Igs), C-reactive protein (CRP) and α-1 acid glycoprotein (AGP) were assessed in the Johns Hopkins Center for Human Nutrition lab using commercial kits (Table S1, supplemental digital content). Commercial standards and controls and participant-derived control samples were run in duplicate for each run to monitor assay performance and reliability.

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## Statistical Analysis

SES indicators were collapsed into a living standards index (LSI) and dichotomized around the internal median value of the index (26). Improved household sanitation was defined as access to a slab, water-sealed or flush toilet, and improved drinking water as access to piped or tubewell water. Length-for-age (LAZ) and weight-for-length (WLZ) Z-scores were calculated relative to WHO standards (27), with stunting and wasting defined as LAZ and WLZ, respectively, < −2. LAZ and WLZ at enrollment (6 mo) and the change in each from 6–18 mo were used in this analysis.

Distributions of biomarker values were examined for outliers, normality and with respect to established cutoffs as available: L:M>0.07 (7, 28, 29); MPO>2000 ng/mL, AAT >270 μg/mL, NEO>70 nmol/L (13, 30); CRP>5 mg/L, AGP>1 g/L (31). Subsequently, all were natural log-transformed. To examine relationships between L:M and other biomarkers, Pearson correlation coefficients were explored and multivariable linear regression models developed with dependent variable log-L:M and log-transformed serum and stool biomarkers as independent variables. Supplementation effects on biomarker values were assessed in multilevel linear regression models with the log-transformed biomarker as the independent variable, indicator variables for assigned supplementation group, and random intercepts for sector of residence, the unit of randomization.

Two methods, principal component analysis (PCA) and partial least squared (PLS) regression, were used to generate EED scores, each with progressively more inclusive sets of log-transformed biomarkers as independent variables. PCA components were retained guided by scree plots and the Kaiser criteria (eigenvalues>1) (32). PLS models specified log-transformed L:M as the dependent variable (33). The PLSSAS command was used to call the PLS procedure in SAS from Stata. EED scores for each child were calculated from retained factors in the final PCA and PLS models by summing, for PCA, the product of each biomarker value and its model loading and, for PLS, the product of each biomarker, its loading and the absolute value of its weight. Scores were standardized around their means and standard deviations and shifted to have positive values. Scores were named empirically based on their highest loading biomarkers.

Associations between child and household characteristics and EED were evaluated in regression models with L:M and the PCA- and PLS-generated scores as continuous dependent variables and sociodemographic (sex, LSI, mother’s education), morbidity (past 7-day diarrhea, fever, cough) and anthropometric (6 mo LAZ and WLZ, change in LAZ and WLZ from 6–18 mo) measures as independent variables. Analyses were repeated in the subset of participants with no reported morbidity in the seven days preceding the biospecimen collection to confirm that observed associations among biomarkers were not driven by symptomatic morbidities.

Study protocols were approved by the Institutional Review Boards of the Johns Hopkins Bloomberg School of Public Health and icddr,b. Parental written consent was required for participation. Analyses were conducted in Stata 14 (StataCorp, College Station, TX) and SAS 9.3 (SAS Institute Inc., Cary, NC).

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## RESULTS

Of all eligible children (n=566), parents of 27 children (4.8%) refused the EED assessment, yielding a consenting sample size of 539. The mean (SD) age of enrolled children was 18.3 (0.3) mo. Children lived in households with access to an improved source of drinking water (100%) and most (82.5%) had access to improved sanitation facilities (Table 1). Percent of mothers with some schooling was 78.4%. Baseline characteristics of children enrolled in the EED assessment were similar to those enrolled in the main trial, though some socioeconomic status indicators were higher in children with EED assessment, likely due to the geographic designation of the enrollment area (results not shown). Baseline characteristics were also similar across assigned supplementation groups within the substudy sample (results not shown). Prevalence of stunting and wasting in children was high at enrollment (age 6 mo) at 27.8% and 5.0%, respectively, and increased to 45.0% and 14.6% at age 18 mo. The vast majority of children (97%) were still breastfeeding at 18 months of age.

Serum, stool and urine samples were successfully collected from 509 (94.4%), 515 (95.6%) and 434 (80.5%) children, respectively. The geometric mean of L:M was 0.06 (95% CI 0.05, 0.06) and was elevated in 39% of children (Table 2). Fecal enteric inflammation markers were widely elevated: 84%, 56% and 100% for MPO, AAT and NEO, respectively. Systemic inflammation was also common, with 56% having elevated AGP and 20% having elevated CRP. None of the intestinal or systemic health biomarkers differed by assigned supplementation group (Table S2, supplemental digital content), and data from all groups were combined in subsequent analyses.

Correlations between continuous log-transformed L:M and log-transformed biomarkers were modest (Table 2). The highest correlation coefficient between intestinal health biomarkers was for MPO and AAT ($r=0.33$, $p<0.01$), and most others were 0.10 to 0.15 or less. EndoCAb and total immunoglobulin types were consistently inter-correlated, while CRP and AGP were highly correlated with each other ($r=0.55$, $p<0.01$) and moderately correlated with other markers. In a multivariable model with all biomarkers included as independent variables, the panel of biomarkers explained only 10.8% of the observed variability in L:M. In the subset of participants with no reported recent morbidity symptoms (n=302), correlations between biomarkers were only marginally greater and results of subsequent analyses were conserved in that subgroup (results not shown).

Principal component analysis (PCA) on three sets of biomarkers are presented in Table 3. The first model, including intestinal health biomarkers MPO, AAT, NEO, GLP-2 and EndoCAb IgG, produced a 2-component solution with MPO and AAT loading on the first component and EndoCAb IgG, GLP-2 and NEO loading on the second component. Subsequent models with additional biomarkers suggested little joint variability with the previously generated components. The second model, with gut biomarkers plus CRP and AGP loading on three components, was retained to generate EED scores for subsequent analyses. Each score was named according to its strongest loading variables: “systemic inflammation (SI) score” for component 1 with high loadings by CRP and AGP, “gut inflammation (GI) score” for component 2 with negative MPO and AAT loadings and

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moderate positive NEO loading and “gut permeability (GP) score” for component 3 with GLP-2 and EndoCAb IgG loading strongly in opposite directions.

In the PLS models, one factor was retained for each model, as additional factors provided minimal further predictive value for the dependent variable (Table 3). In the PLS model with all biomarkers included, MPO, AAT, total IgA, CRP and AGP loaded highly. That factor explained only 12.7% of variability in the biomarker panel and 8.6% of L:M variance. This most inclusive model was used to generate a score for use in subsequent analyses, calculated for each child from the factor loading and weight values and named the “PLS score”.

The markers differed in their associations with child and household characteristics (Table 4). In multivariable models females had significantly higher L:M than males (geometric mean (95% CI): 0.06 (0.06, 0.07) in females and 0.05 (0.05, 0.06) in males, p=0.05), and L:M was lower in those of high versus low SES (0.05 (0.05, 0.06) vs. 0.07 (0.06, 0.08), p=0.04). The gut permeability score also differed by household economic status, with mean (SE) values lower by 0.22 (0.10) SDs in those with high LSI relative to low. Neither of the other PCA-generated scores nor the PLS score differed by sociodemographic characteristics. L:M was not associated with recent morbidity history, but several EED scores were. The systemic inflammation score was higher by 0.67 (0.17) and 0.31 (0.10) standard deviations in those with reported fever and cough, respectively, in the prior week. The gut inflammation score was greater by 0.52 (0.18) SDs in those with reported fever, and the PLS score was 0.24 (0.10) SDs higher in those with reported cough versus not. Similar trends by reported fever and cough in the prior week were observed in CRP and AGP values (Tables S3 and S4, supplemental digital content). The gut permeability score was not associated with past 7-day morbidity history. LAZ and WLZ at 6 months and their change from 6 to 18 months were associated only with the gut permeability score; each unit greater LAZ at age 6 months was associated with 0.14 (0.05) SD reduction in gut permeability score.

## DISCUSSION

In 18-month-old Bangladeshi children living in a setting of prevalent stunting and enrolled in a randomized controlled food supplementation trial, enteropathy and systemic inflammation were pervasive. Contrary to expectations, serum and stool biomarkers demonstrated low agreement internally and with the widely used L:M test of EED, and principal component analysis (PCA) and partial least squares (PLS) regression did not identify a subset of markers that closely approximated L:M. Further, biomarkers of intestinal and systemic health were not improved in children receiving daily complementary food supplementation. The biomarker scores and L:M differed in their associations with child sociodemographic characteristics, recent morbidities and prior anthropometry, further suggesting that the measured biomarkers reflect multiple underlying biological processes within a context where children face numerous and repeated insults to their intestinal and systemic health.

The inclusion of the L:M ratio in this study gives structure to the evaluation of the more novel biomarkers and enables comparison to prior studies. Though nearly 40%, the observed prevalence of EED based on elevated L:M was less than half of that reported in other studies

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in young children in South Asia (6, 7). Widespread access to improved sanitation facilities and tubewell drinking water in this rural Bangladesh setting may reduce prevalence of EED, as may exposure among mothers in all arms of the study to messaging about food handling and hygiene. Variable findings from prior studies using L:M cast some doubt, however, on the extent to which it can be considered a “gold standard” diagnostic test for EED (2, 19). At best L:M measures only two aspects of the intestinal pathology characteristic of EED: increased permeability and reduced absorptive capacity, while the panel of serum and stool biomarkers in this study was selected deliberately to assess the EED condition more broadly. Concerns have also been raised that mannitol can be present naturally in the urine (2, 34), and that the HPIC method may lack sensitivity for determining typically low lactulose concentrations (35). In addition to underscoring the need for a better diagnostic test for EED, these limitations suggest that the alternate biomarkers presented here may be capturing aspects of the EED condition despite low agreement with L:M.

L:M has been widely used to define EED despite its limitations, hence our aim of evaluating the biomarker panel relative to it. The PLS method is well-suited to that purpose, as it identifies patterns of joint variability between specified independent and dependent variables (33). The PLS-generated score inclusive of all of the biomarkers did explain the most variance in L:M of the models investigated, but that maximum was less than 10% of the total variability in L:M. Further, five of twelve biomarkers across the intestinal and systemic domains loaded with moderate and approximately equal strength in that model, supporting the conclusion that no concise subset of the measured markers approximates L:M.

The PCA-generated scores offer more insight than the one-factor PLS score does into the joint variability and common underlying factors within the set of serum and stool biomarkers. The gut inflammation (GI) score, on which MPO, AAT and NEO loaded, was not associated with any of the examined child and household factors aside from past 7-day fever, underscoring uncertainty about the factors underlying those biomarkers and their common variability. In two other studies that took a similar PCA approach to stool markers of EED, similar magnitudes of loadings for MPO, AAT and NEO were reported (13, 36), but only in our study did NEO load in the opposite direction of MPO and AAT. A score of MPO, AAT and NEO as a stand-alone marker of EED has been proposed by investigators from the Mal-ED study (13), but our findings suggest more research is needed into the physiology underlying those markers and into the processes driving their joint variability. In particular, the inverse relationship we observed between NEO and MPO and NEO and AAT concurs to some extent with a recent study in Brazil suggesting the relationship between NEO and growth may be modified by MPO or, said differently, that high NEO in the absence of high MPO may be indicative of normal intestinal immune function while high NEO and MPO together indicate excessive inflammation, as in EED, that constrains growth (37). A diagnostic measure that uses these markers in combination may, then, be appropriate, but more normative data and validation studies in subclinical disease, ideally in pediatric populations, are needed to fully understand the biology underlying these markers.

The GP score, on which EndoCAb IgG and GLP-2 loaded strongly in opposite directions, was inversely associated with the wealth index and LAZ at age 6 months. Accounting for the directionality of the loading for EndoCAb on that score, this finding means children from

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wealthier households and with greater LAZ at baseline had higher EndoCAb values, generally indicative of greater intestinal permeability to pathogens, a finding that contradicts expectations. It is possible that healthier (larger) children had more mature immune systems that were better able to mount a response to insults, reflected in higher EndoCAb IgG values. Evidence supporting the use of EndoCAb for EED assessment is mixed and, to our knowledge, no normative data for the marker exists in the literature. Prior studies have used EndoCAb alone to measure EED (14, 38), but of three studies known to us that measured EndoCAb and L:M concurrently, one reported a strong association between the two markers (11), one found no association (39) and a third did not report agreement between the two, but did find divergent associations with child height (15). Our findings further support the need for more normative and mechanistic research into EndoCAb as a marker of EED. Lower GLP-2 values in children who were taller at baseline and in those of higher SES suggest less need for intestinal repair in line with better intestinal health overall. Lower GLP-2 may also indicate slower whole body growth, which again may be expected in relatively healthier children with better growth prior to age 18 months. The inverse associations we see with prior anthropometric measures suggest a potential bi-directionality between EED and growth that will be important to investigate in planned future analyses of the EED biomarkers in relation to longitudinal growth.

The acute phase proteins and total immunoglobulins, aspects of systemic inflammation, loaded on their own components when added to the PCA model rather than combining with the gut markers, which is counter to a hypothesized cascade of effects from subclinical intestinal inflammation and permeability to stunting via activation of systemic inflammatory processes (3, 17). Further, the SI score, on which only the acute phase proteins loaded, was associated with recent respiratory symptoms and fever but not diarrhea, suggesting that intestinal health may be less responsible for systemic inflammation in this age group than originally hypothesized.

Strengths of this study include the large sample size and the broad panel of candidate biomarkers assessed in combination with L:M. Nesting the study within a large randomized trial allowed for enrollment of participants from an enumerated source population, which, along with the very high participation rate, suggests a low risk of bias and good generalizability to rural Bangladesh and perhaps to other rural South Asian populations. In addition to limitations of L:M as a reference method, other caveats include that biospecimens were collected at a single time point rather than serially, a tradeoff we accepted for a larger sample in accordance with a primary aim of investigating interrelationships and agreement among the biomarkers, and a lack of normative data in children for several of the serum and stool biomarkers. Much remains unknown about the performance of these markers under various conditions, such that this study represents a contribution to the literature in that they were explored simultaneously in a well-described population. Future analyses are planned to explore the dynamics between growth and the EED and systemic health biomarkers using longitudinal models to incorporate serial anthropometric measures and to account for our finding of an inverse relationship between baseline length and some gut health markers.

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In a large sample of 18-month-old children in rural Bangladesh, a panel of biomarkers measuring different aspects of the EED syndrome suggested widespread perturbed intestinal health. The low internal agreement among markers and unknown accuracy and likely poor precision of the L:M test for EED underscores the urgent need for developing validated biomarkers that can advance this field. Our investigation of 12 biomarkers, split between widely used and novel markers, contributes to this effort. However, this study also highlights the complexities of the biology underlying each of these proposed biomarkers and the difficulty of pinpointing EED in a context of widespread infectious exposures and immune activation.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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**What is known**

- Subclinical impairment to intestinal structure and function, termed EED, may be pervasive in low-income countries and contribute to young children's poor linear growth via intestinal and systemic inflammation.
- Methods for assessing EED are difficult to implement in field settings and may not fully capture the condition.

**What is new**

- Proposed alternate markers of EED demonstrated low agreement with the widely used lactulose mannitol ratio (L:M) test.
- Limitations of the L:M test make it an inadequate gold-standard measure for assessing alternate markers.
- In this South Asian setting with prevalent stunting, systemic inflammation was widely observed but not associated with poor intestinal health.

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## Table 1

Characteristics of children participating in an environmental enteric dysfunction (EED) assessment in northwest Bangladesh (n=539)

| Characteristic | n | Mean (SD)/%[1] |
|---|---|---|
| Sex, female | 269 | 49.9 |
| Age (mo) | | 18.27 (0.33) |
| Stunting (LAZ<−2), 6 mo | 149 | 27.8 |
| Wasting (WLZ<−2), 6 mo | 27 | 5.0 |
| Stunting (LAZ<−2), 18 mo | 239 | 45.0 |
| Wasting (WLZ<−2), 18 mo | 77 | 14.6 |
| Continued breastfeeding, 18 mo | 525 | 97.4 |
| Father's Employment | | |
| None | 6 | 1.1 |
| Farmer/owns business | 254 | 48.2 |
| Day laborer/fisherman | 182 | 34.5 |
| Private/gov't. service | 85 | 16.1 |
| Mother's Education | | |
| None | 116 | 21.6 |
| 1–9 years | 348 | 64.8 |
| SSC passed | 33 | 6.1 |
| 11+ years | 40 | 7.4 |
| Electricity | 165 | 30.7 |
| Improved water | 537 | 100.0 |
| Improved toilet[2] | 443 | 82.5 |
| LSI, Mean (SD) | | 0.11 (1.05) |

[1]Values are the percent of children in the specified category out of all with complete data for that question or the mean and standard deviation of values for that item.

[2]Water sealed or slab toilet.

Abbreviations: EED, environmental enteric dysfunction; LAZ, length-for-age z-score; LSI, living standards index; SSC, secondary school completion exam; WLZ, weight-for-length z-score.

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**Table 2**

Biomarker summary statistics and correlations in 18-month-old environmental enteric dysfunction (EED) study participants

| Biomarker | n | GM (95% CI) | Elevated, n (%)[1] | Pearson Correlation Coefficients[2] | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | L:M | MPO | AAT | NEO | GLP-2 | Endo IgG | Endo IgM | Endo IgA | Total IgG | Total IgM | Total IgA | CRP | AGP |
| L:M | 446 | 0.06 (0.05, 0.06) | 174 (39.0) | 1.00 | | | | | | | | | | | | |
| MPO (ng/mL) | 498 | 4460.3 (4145.0, 4799.5) | 420 (84.3) | 0.15 | 1.00 | | | | | | | | | | | |
| AAT (μg/mL) | 501 | 326.9 (303.1, 352.5) | 278 (55.5) | 0.15 | 0.33 | 1.00 | | | | | | | | | | |
| NEO (nmol/L) | 502 | 767.4 (716.5, 821.8) | 502 (100) | 0.11 | 0.01 | −0.12 | 1.00 | | | | | | | | | |
| GLP-2 (ng/mL) | 490 | 3.0 (2.9, 3.1) | | 0.03 | −0.05 | −0.12 | 0.02 | 1.00 | | | | | | | | |
| Endo IgG (mu/mL) | 501 | 45.4 (41.7, 49.4) | | −0.05 | −0.06 | −0.04 | 0.08 | −0.05 | 1.00 | | | | | | | |
| Endo IgM (mu/mL) | 505 | 72.6 (69.6, 75.8) | | −0.10 | −0.03 | −0.06 | −0.00 | −0.04 | 0.13 | 1.00 | | | | | | |
| Endo IgA (mu/mL) | 463 | 9.7 (8.8, 10.8) | | 0.00 | −0.02 | 0.00 | −0.01 | 0.08 | 0.19 | 0.18 | 1.00 | | | | | |
| Total IgG (g/L) | 502 | 16.6 (15.9, 17.4) | | −0.03 | −0.01 | −0.01 | 0.01 | 0.15 | −0.01 | 0.04 | 0.14 | 1.00 | | | | |
| Total IgM (g/L) | 503 | 1.0 (1.0, 1.1) | | −0.08 | 0.03 | −0.05 | −0.00 | −0.07 | 0.07 | 0.49 | 0.15 | 0.13 | 1.00 | | | |
| Total IgA (g/L) | 503 | 0.7 (0.7, 0.8) | | 0.10 | −0.03 | −0.06 | 0.08 | 0.03 | 0.01 | 0.02 | 0.25 | 0.10 | 0.39 | 1.00 | | |
| CRP (mg/L) | 505 | 1.20 (1.03, 1.38) | 103 (20.4) | 0.12 | 0.18 | 0.12 | 0.06 | −0.08 | 0.02 | −0.11 | −0.05 | 0.03 | 0.09 | 0.11 | 1.00 | |
| AGP (mg/dL) | 505 | 104.8 (101.9, 107.7) | 281 (55.6) | 0.09 | 0.13 | 0.07 | 0.11 | 0.02 | 0.01 | −0.10 | 0.04 | 0.08 | 0.15 | 0.25 | 0.55 | 1.00 |

[1] Cutoffs for elevated biomarker values: L:M > 0.07 (1); MPO > 2000 ng/mL (2, 3); AAT > 270 μg/mL (2, 3); NEO > 70 nmol/L (2); CRP > 5 mg/L (4); AGP > 100 mg/dL (4).

[2] Cell values are Pearson correlation coefficients between log-transformed biomarkers with outliers removed. Correlations of absolute value ≥ 0.121 are significant at the p≤0.01 level; correlations of absolute value 0.097 < r ≤ 0.116 are significant at the p≤0.05 level; correlations of absolute value 0.086 ≤ r ≤ 0.077 are significant at the p≤0.1 level.

Abbreviations: AGP, α-1 acid glycoprotein; AAT, α-1 antitrypsin; CI, confidence interval; CRP, C-reactive protein; EED, environmental enteric dysfunction; Endo, endotoxin core antibody; GLP-2, glucagon-like peptide-2; GM, geometric mean; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; L:M, lactulose:mannitol ratio; MPO, myeloperoxidase; NEO, neopterin.



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**Table 3**

Principal component analysis (PCA) and partial least squares (PLS) regression models for environmental enteric dysfunction (EED) score development

| Biomarker[1] | PCA Component Loadings | | | | | | | | | | PLS Component Loadings | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Model 1 | | Model 2[2] | | | Model 3 | | | | | Model 1 | Model 2 | Model 3[2] |
| | Comp. 1 | Comp. 2 | Comp. 1 | Comp. 2 | Comp. 3 | Comp. 1 | Comp. 2 | Comp. 3 | Comp. 4 | Comp. 5 | Comp. 1 | Comp. 1 | Comp. 1 |
| MPO | 0.58 | 0.10 | 0.36 | −0.42 | 0.10 | 0.08 | 0.36 | −0.34 | 0.26 | 0.22 | 0.71 | 0.54 | 0.48 |
| AAT | 0.68 | 0.12 | 0.29 | −0.60 | −0.12 | 0.06 | 0.32 | −0.47 | 0.34 | 0.21 | 0.62 | 0.46 | 0.45 |
| NEO | −0.28 | 0.40 | 0.10 | 0.46 | −0.05 | 0.08 | 0.05 | 0.35 | −0.34 | 0.19 | 0.32 | 0.26 | 0.25 |
| GLP-2 | −0.24 | −0.57 | −0.13 | 0.20 | 0.73 | −0.09 | −0.08 | 0.46 | 0.51 | 0.01 | 0.01 | −0.01 | 0.05 |
| EndoCAb IgG | −0.23 | 0.70 | −0.01 | 0.30 | −0.65 | 0.12 | −0.17 | 0.04 | −0.30 | 0.72 | −0.10 | −0.07 | −0.08 |
| CRP | | | 0.63 | 0.21 | 0.02 | 0.38 | 0.45 | 0.09 | −0.16 | −0.01 | | 0.48 | 0.41 |
| AGP | | | 0.60 | 0.29 | 0.13 | 0.43 | 0.40 | 0.23 | −0.11 | −0.06 | | 0.43 | 0.38 |
| EndoCAb IgM | | | | | | 0.22 | −0.43 | −0.36 | −0.07 | −0.06 | | | −0.27 |
| EndoCAb IgA | | | | | | 0.27 | −0.28 | 0.04 | 0.27 | 0.49 | | | 0.08 |
| Total IgG | | | | | | 0.22 | −0.08 | 0.27 | 0.48 | −0.02 | | | 0.07 |
| Total IgM | | | | | | 0.49 | −0.29 | −0.22 | −0.07 | −0.29 | | | −0.08 |
| Total IgA | | | | | | 0.47 | −0.12 | 0.11 | 0.09 | −0.15 | | | 0.30 |
| **% of X variance**[3] | 27.82 | 21.46 | 24.67 | 19.07 | 15.18 | 16.69 | 14.41 | 10.99 | 9.85 | 9.08 | 23.77 | 22.08 | 12.88 |
| **% of Y variance**[4] | 2.26 | | 3.42 | | | 4.99 | | | | | 5.21 | 5.44 | 8.64 |

[1] Biomarkers were log-transformed and outliers removed prior to analysis. Gray shaded boxes indicated biomarkers not included in the model presented in that column.

[2] PCA Model 2 is the source of the PCA scores used in subsequent analyses: component 1 is the systemic inflammation (SI) score, component 2 is the gut inflammation (GI) score and component 3 is the gut permeability (GP) score. PLS model 3 is the source of the PLS score used subsequently.

[3] Percent of total variance in independent variables (biomarkers) explained by each factor.

[4] Percent of variance in L:M explained by each score model (generated by PLS command; for PCA, based on $R^2$ from regression of log-transformed L:M on PCA components).

Abbreviations: AAT, α-1 antitrypsin; AGP, α-1 acid glycoprotein; Comp, component; CRP, C-reactive protein; EED, environmental enteric dysfunction; EndoCAb, endotoxin core antibody; GLP-2, glucagon-like peptide-2; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; L:M, lactulose:mannitol ratio; MPO, myeloperoxidase; NEO, neopterin; PCA, principal component analysis; PLS, partial least squares.

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**Table 4**

Associations between environmental enteric dysfunction (EED) markers and child sociodemographic, morbidity and anthropometric characteristics in 18-month-old study participants[1]

| Characteristics by domain | n (%)/mean (SD) | L:M, β (SE) | PCA scores | | | PLS score |
|---|---|---|---|---|---|---|
| | | | Systemic inflammation, β (SE) | Gut inflammation, β (SE) | Gut permeability, β (SE) | β (SE) |
| **Sociodemographic** | | | | | | |
| Sex, female | 269 (49.9) | 0.16 (0.07) ** | 0.03 (0.10) | 0.00 (0.10) | 0.11 (0.10) | 0.04 (0.10) |
| LSI, high | 300 (55.9) | −0.26 (0.08) *** | 0.13 (0.10) | −0.04 (0.10) | −0.22 (0.10) ** | 0.08 (0.10) |
| Mother's education | | | | | | |
| 1–9 years | 348 (64.8) | −0.10 (0.09) | −0.17 (0.12) | −0.20 (0.12) | −0.08 (0.12) | −0.11 (0.12) |
| SSC passed[2] | 33 (6.1) | −0.32 (0.17) * | −0.31 (0.23) | −0.11 (0.23) | 0.36 (0.23) | −0.20 (0.23) |
| 11+ years | 40 (7.4) | −0.25 (0.17) | −0.17 (0.23) | 0.19 (0.23) | −0.10 (0.23) | −0.27 (0.23) |
| **Morbidity history** | | | | | | |
| Diarrhea[3] | 20 (3.8) | 0.27 (0.19) | −0.06 (0.25) | 0.37 (0.25) | 0.42 (0.26) | −0.00 (0.25) |
| Fever[3] | 47 (9.0) | −0.29 (0.14) | 0.67 (0.17) *** | 0.52 (0.18) *** | −0.21 (0.18) | 0.27 (0.18) |
| Cough[3] | 194 (37.1) | 0.05 (0.08) | 0.31 (0.10) *** | 0.09 (0.10) | 0.19 (0.10) | 0.24 (0.10) ** |
| **Anthropometry** | | | | | | |
| LAZ, 6 mo | −1.43 (1.05) | −0.00 (0.04) | −0.06 (0.05) | 0.05 (0.05) | −0.14 (0.05) *** | −0.09 (0.05) |
| WLZ, 6 mo | −0.26 (1.01) | −0.05 (0.05) | 0.01 (0.06) | −0.04 (0.06) | −0.08 (0.06) | 0.03 (0.06) |
| ΔLAZ, 6–18 mo | −0.25 (0.34) | −0.06 (0.13) | −0.07 (0.16) | −0.00 (0.16) | −0.04 (0.16) | −0.09 (0.16) |
| ΔWLZ, 6–18 mo | −0.40 (0.42) | −0.15 (0.12) | −0.17 (0.14) | −0.07 (0.15) | 0.01 (0.14) | −0.13 (0.14) |

[1] Coefficients and p-values are from multivariable linear regression models with dependent variable log-transformed L:M or standardized EED score and child characteristics as independent variables in separate models for each domain (i.e., sociodemographic, morbidity, anthropometry).

* 0.05<p≤0.1

** 0.01<p≤0.5

*** p≤0.01

[2] SSC is the secondary school completion exam, taken at the end of year 10 of schooling.

[3] Dichotomous variable for whether symptoms of the listed morbidity were reported by the mother for the 7 days prior to the EED assessment.



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Abbreviations: EED, environmental enteric dysfunction; LAZ, length-for-age z-score; L:M, lactulose:mannitol ratio; LSI, living standards index; PCA, principal component analysis; PLS, partial least squares regression; SSC, secondary school completion exam; WLZ, weight-for-length z-score.