Biomarkers of environmental enteric dysfunction are differently associated with recovery and growth among children with moderate acute malnutrition in Sierra Leone

Akriti Singh,[1] *Shibani Ghosh,*[1] *Honorine Ward,*[2] *Mark J Manary,*[3] *Beatrice L Rogers,*[1] *and Irwin H Rosenberg*[1]

[1]Friedman School of Nutrition Science and Policy, Tufts University, Boston, MA, USA; [2]Tufts Medical Center and Tufts University School of Medicine, Boston, MA, USA; and [3]Department of Pediatrics, Washington University, St Louis, MO, USA

ABSTRACT

Background: Environmental enteric dysfunction (EED) may influence growth during and recovery from moderate acute malnutrition (MAM), however, biomarkers to assess these relations have yet to be identified.

Objectives: The objectives of this study were to: *1*) develop a score for EED based on host fecal mRNA transcripts, *2*) compare biomarkers of EED with each other, and *3*) examine associations between the EED biomarkers and recovery from MAM and growth outcomes.

Methods: In a cohort of 520 Sierra Leonean MAM children, biomarkers of EED included the lactulose: mannitol (L: M) test, 15 host fecal mRNA transcripts, and host fecal proteins [α-1-antitrypsin (AAT), myeloperoxidase (MPO), neopterin (NEO)]. Anthropometry data were also collected and z scores were computed for length-for-age (LAZ) and weight-for-length (WLZ). Recovery from MAM was defined as midupper arm circumference $\geq$12.5 cm. Factor analysis was used to identify EED scores using the mRNA transcripts, and mixed effects regression was conducted to test for associations.

Results: The 15 host fecal mRNA transcripts were clustered into 3 scores: gut inflammation (GI) score, gut structure (GS) score, and gut defense (GD) score. We found agreement between certain inflammation markers (GI score and MPO), and permeability markers (GS score and AAT; AAT and the L: M excretion ratio). Antimicrobial gut defense (GD score) was inversely associated with percent lactulose excreted, a measure of intestinal permeability. LAZ (β: –0.08; 95% CI: –0.14, –0.02) and WLZ (β: –0.03; 95% CI: –0.06, –0.01) were negatively associated with GI score. A high GD score (β: 0.36; 95% CI: 0.08, 0.64) and low AAT (β: –1.35; 95% CI: –2.35, –0.36) were associated with recovery from MAM.

Conclusions: Scores derived from host fecal mRNA transcript variably correlated with the L: M test and host fecal proteins. Markers of intestinal inflammation, permeability, and defense were associated with growth outcomes and recovery from MAM. *Am J Clin Nutr* 2021;113:1556–1564.

Keywords: environmental enteric dysfunction, biomarkers, intestinal inflammation, intestinal permeability, intestinal antimicrobial defense, lactulose: mannitol test, fecal host proteins, fecal host mRNA transcripts, growth outcomes, Sierra Leone

Introduction

There are an estimated 50 million acutely malnourished children in the world, with approximately two- thirds suffering

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Address correspondence to AS (e-mail: akriti.singh@tufts.edu).

Abbreviations used: AAT, α-1-antitrypsin; CSB + w/oil, Corn Soy Blend Plus with Fortified Vegetable Oil; CSWB w/oil, Corn Soy Whey Blend with Fortified Vegetable Oil; DEFA6, defensin α-6; ddPCR, droplet digital PCR; EED, environmental enteric dysfunction; GD, gut defense; GI, gut inflammation; GS, gut structure; LAZ, length-for-age z score; L: M, lactulose: mannitol; LMER, lactulose: mannitol excretion ratio; LYZ, lysozyme; MAL-ED, Etiology, Risk Factors and Interaction of Enteric Infections and Malnutrition and the Consequences for Child Health and Development; MAM, moderate acute malnutrition; MPO, myeloperoxidase; MUAC, midupper arm circumference; NEO, neopterin; PHU, peripheral health unit; RUSF, Ready to Use Supplementary Food; SAM, severe acute malnutrition; (SC + A), Super Cereal Plus with Amylase; SNF, specialized nutritious food; WLZ, weight-for-length z score; %L, percent lactulose excreted; %M, percent mannitol excreted.

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from moderate acute malnutrition (MAM) (1). MAM is defined as either weight-for-height or length (WLZ) –2 and ≥–3 SDs below the median of the 2006 WHO child growth standards or midupper arm circumference (MUAC) ≥11.5 cm and <12.5 cm (2). Children with MAM are at a 3-fold increased risk of mortality and intervening at this stage could prevent progression to severe acute malnutrition (SAM) (3). In food-insecure settings, children with MAM receive supplemental foods as treatment, but not all children (50–100%) who receive treatment recover (4). Environmental enteric dysfunction (EED) has been reported among children with SAM (5, 6). Given the much higher prevalence of MAM, understanding if EED influences treatment outcomes is of critical importance.

EED is an impairment of the structure and function of the small intestine, characterized by increased permeability, reduced absorptive capacity, and inflammation (7). Identifying field-appropriate biomarkers of EED is an area of active research as the most widely used lactulose: mannitol (L: M) test has many limitations (8). Researchers also recognize the need for biomarkers to measure different characteristics of EED (7, 9, 10). This may have been influenced by conflicting results of the association between L: M test markers and growth outcomes (8, 11). Host fecal proteins and host fecal mRNA transcripts are being considered as alternative biomarkers of EED (12).

α-1-antitrypsin (AAT), myeloperoxidase (MPO), and neopterin (NEO) are the most commonly measured fecal proteins as markers of EED (13). AAT is a measure of intestinal permeability, whereas MPO and NEO are measures of intestinal inflammation (14–16). Consistent correlations between L: M test markers and these proteins, and associations between the fecal proteins and growth outcomes have not been reported (11). Several studies from Malawi have explored host fecal mRNA transcripts as alternatives given the observed correlations with L: M markers (17, 18). Fifteen of these transcripts were included in this study (see **Supplemental Table 1** for description and functions) (18).

To our knowledge, there have been no comparative assessments of the L: M test, host fecal proteins, and mRNA transcripts on the same individual in examining the burden and consequences of EED, namely linear and ponderal growth, and recovery from MAM. Thus, the objectives of this study were to: *1*) develop a score for EED based on host fecal mRNA transcripts, *2*) compare biomarkers of EED with each other, and *3*) examine associations between the EED biomarkers and recovery from MAM and growth outcomes. We hypothesized that *1*) biomarkers of EED, measuring the same intestinal characteristics, would be associated with each other, and *2*) poorer EED characteristics would influence poor growth outcomes and recovery from MAM.

Methods

Study design

This observational study was nested within the Four Foods MAM Treatment Study, a prospective, cluster-randomized, controlled clinical effectiveness, and cost-effectiveness trial assessing 4 specialized nutritious foods (SNFs) to treat children aged 6–59 mo with MAM in Pujehun district of Sierra Leone (19). The Four Foods MAM Treatment Study was registered at clinicaltrials.gov as NCT03146897. Pujehun is a rural district in the Southern Province, ∼200 km from the capital city Freetown. The 4 SNFs used in the study were Super Cereal Plus with Amylase (SC + A), Corn Soy Blend Plus with Fortified Vegetable Oil (CSB + w/oil), Corn Soy Whey Blend with Fortified Vegetable Oil (CSWB w/oil), and Ready to Use Supplementary Food (RUSF). In summary, SC + A, CSB + w/oil, CSWB w/oil were cereal-based foods, whereas RUSF was a lipid-based supplement. In terms of animal protein, SC + A and RUSF contained skimmed milk, CSWB w/oil contained whey, and CSB + w/oil had no animal source ingredients. The daily ration for all 4 foods provided ∼550 kcal/d.

Participants were eligible to receive the study foods for a period of 12 wk or until they reached a MUAC value of ≥12.5 cm, whichever was sooner. Procedures for this study were conducted at 8 of the 29 study peripheral health units (PHUs), 2 per arm, selected based on logistical constraints. Biological samples were collected at enrollment only, from July 2017 to August 2018. Anthropometric measurements included in this study were collected at enrollment and 2 wk later. The outcome, recovery, was calculated from anthropometry collected ≤12 wk of MAM treatment.

The study was approved by the Tufts University Health Sciences Institutional Review Board and the Sierra Leone Ethics and Scientific Review Committee. Written informed consent was obtained from caregivers of all study participants.

Sample size and eligibility

The sample size for this study was calculated using G*Power software (20). With a power of 0.80 and α of 0.05, a sample size of 400 would allow us to detect a correlation between each of the 15 fecal host mRNA transcripts and lactulose: mannitol excretion ratio (LMER) of 0.13 or greater, assuming a design effect of 1.1. Previous studies have reported correlation coefficients between host fecal mRNA transcripts and the L: M ratio to be on average 0.16, and that between host fecal proteins and LMER to be on average 0.14 (18, 21). Therefore, a sample size of 400 would provide sufficient power to detect correlations between fecal host mRNA transcripts and LMER, and to detect correlations between the fecal protein markers and LMER at concentrations observed in the published literature (18, 21).

At the 8 PHUs, children who were aged 6–59 mo, had MAM defined as MUAC ≥11.5 cm and <12.5 cm, and exhibited no bipedal edema, were eligible for the Four Foods MAM Treatment Study. Children were excluded from this study if their caretaker reported diarrhea (defined as watery stool 1 or more times per day) the day before or on the day of sample collection.

Field methods

All study procedures took place at the PHUs. After an 8-h overnight fast, a 20 mL dose comprising 5 g of lactulose (McKesson) and 1 g of mannitol (Sigma Aldrich) were orally given to each participant using a medicine cup or syringe. Water was allowed as often as desired by the child before and after being dosed with the sugar solution. After dosing, a urine collection bag (Thermo Fisher Scientific) and a locally prepared, nonabsorbent diaper were attached to the study child.

All urine excreted over the next 4 h was collected in a cup with 10 mg thimerosal (Sigma Aldrich) to prevent bacterial degradation of the sugars. The urine was mixed with a pipette, transferred to plastic cryovials, and flash frozen in liquid nitrogen at the PHU. The total urine volume was recorded using a graduated cylinder. Participants and caregivers were provided lunch 3 h after dosing when breastfeeding was also allowed. Lunch for caregivers consisted of typical Sierra Leonean food such as rice and cassava leaves, whereas lunch for the study participants consisted of rice porridge. Caregivers were also provided transport reimbursement worth $3 for returning to the PHU. Every month, samples placed in liquid nitrogen tanks at the PHU were transferred to a –20°C freezer at the University of Makeni and shipped on dry ice to Baylor College of Medicine, Texas, USA, for analysis.

Stool samples were collected at any point before, during, or after the 4-h wait period for the L: M test. Once a participant had a bowel movement, the diaper was removed, and all stool collected was mixed with a plastic spatula and transferred into plastic cryovials without any fixative. If a child had diarrhea, a stool sample was not collected. The cryovials with stool were flash frozen in liquid nitrogen at the PHU. Every month, samples were transferred to a –80°C freezer at the University of Makeni and shipped on dry ice to labs for analysis as described.

Laboratory methods

The concentration of the sugars in the urine was analyzed by HPLC at Baylor College of Medicine, Texas, USA (22). The concentrations of the 2 sugars (mg/mL) enumerated in the collected samples were used to calculate the L: M markers. To calculate the percentage of each sugar excreted [percent lactulose excreted (%L) and percent mannitol excreted (%M)], the concentration of the sugar was multiplied by the 4-h volume of urine (v) and divided by the amount of sugar dosed (d): (s*v)/d. The LMER was calculated as the ratio of %L divided by %M. The L: M ratio was calculated as the concentration of lactulose divided by the concentration of mannitol. The LMER and L: M ratio measure intestinal permeability along with %L; %M is a measure of absorptive surface area.

Fifteen host fecal mRNA transcripts were analyzed by droplet digital PCR (ddPCR) at the University of Washington School of Medicine at St Louis, Missouri, USA. Briefly, the fecal mRNAs were extracted from stool samples using the NucliSENS easyMAG system (bioMerieux) as per the protocol of Stauber et al. (23). The extracted mRNA transcripts were assayed in a ddPCR system (QX100; Bio-Rad Laboratories, Inc.). The concentration of each transcript was normalized to the mRNA transcript for GAPDH. Fecal proteins were analyzed using commercially available ELISA kits: AAT (R&D Systems), MPO (R&D Systems), and NEO (GenWay Biotech) at the USDA Human Nutrition Research Center on Aging/Tufts University, Massachusetts, USA (13). The concentration of AAT, MPO, and NEO were reported as ng/mL, ng/mL, and nmol/L, respectively.

Anthropometry and covariates

Data on background characteristics (age, gender, SAM status, breastfeeding, diarrhea, household food insecurity) of study participants, and anthropometry (recovery, MUAC, length and weight at enrollment and 2 wk into the feeding program) were extracted from the Four Foods MAM Treatment Study main dataset. Length was measured in duplicate to the nearest 0.1 cm using a portable length board (SECA 417). Weight was measured in duplicate to the nearest 0.1 kg using an electronic scale (SECA334). MUAC was measured in triplicate on the left arm using an insertion tape.

Statistical analysis

All statistical analysis was conducted using Stata 15 software (Stata Corps). Biomarker distributions were examined for outliers and normality. Due to skewed distribution, values were ln transformed for all biomarkers. One outlier for the host fecal mRNA transcript, lysozyme (LYZ), which was 140 times higher than values for all other participants, was excluded.

Scores were constructed using the fecal host mRNA transcripts and fecal proteins. For the fecal protein score the method described previously by the Etiology, Risk Factors and Interaction of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) study was adopted (13). The host fecal mRNA transcript EED scores were constructed using factor analysis; similar techniques have been used to construct EED scores previously (13, 21). As per the Kaiser rule, factors with Eigen values >1 were retained (24). The factors were assigned names based on the known function of the highest loading mRNA transcripts (correlation coefficient >0.5).

The primary outcome, recovery, was defined as achieving a MUAC $\geq$12.5 cm within 12 wk. The secondary outcomes were length-for-age z score (LAZ) and WLZ at enrollment, and change in length and change in weight over 2 wk. LAZ and WLZ were calculated using WHO child growth standards (25). Outliers defined as $-6 >$LAZ >6 and $-5 >$WLZ >5 were excluded from analysis. By these definitions, 7 outliers were removed from analysis for LAZ, and no outliers were detected for WLZ. Change in length and weight over 2 wk were calculated in centimeters and kilograms, respectively.

The agreement among the biomarkers was explored through Spearman correlation on the untransformed values. Associations among the biomarkers were examined using unadjusted and adjusted mixed effects linear regression models to control for PHU-level clustering. The associations between the 3 biomarkers and recovery from MAM were examined using unadjusted and adjusted mixed effects logistic regression models.

Adjusted models included child age, gender, and whether the child had been transferred to the MAM treatment program after recovering from SAM. These covariates were chosen because previous studies have reported that LMER increases with age and varies by gender (26, 27). Previous SAM status was chosen because these children only consumed ready-to-use therapeutic food prior to enrollment in the MAM treatment program.

Adjusted models for change in length and weight included baseline length and weight, respectively, as covariates instead of previous SAM status. Adjusted models for recovery, change in length, and change in weight also included study arm as an independent predictor because the children had already begun consuming 1 of the 4 study foods.

For the mixed linear regression models, diagnostic tests for normality of residuals, influential observations, and variance inflation factor for collinearity among independent variables were assessed. Since regression residuals were not normally distributed, robust SEs to correct for this violation were reported. For the mixed effects logistic models, diagnostic tests for influential observation, collinearity, Hosmer-Lemeshow's goodness-of-fit test, and intracluster correlation coefficients were examined. Subjects with negative length values >0.1 cm between enrollment and 2 wk, and those with missing outcome or covariate data were excluded from analysis. The results remained the same even when the models for change in length were run using multiple imputations. Statistical significance was set at $P < 0.05$ for all analyses. Since multiple tests of association were made with the same dependent variable, the Benjamini–Hochberg correction with a false discovery rate of 15% was applied (28).

Results

Study population

Characteristics of study participants are presented in **Table 1**. Of the 601 participants enrolled in the study, 520 provided samples that were successfully analyzed for ≥1 of the 3 types of biomarkers (L: M test, fecal host mRNA transcripts, and fecal proteins) (see **Supplemental Figure 1** for the participant flow diagram). The participants' mean age ± SD was 13.86 ± 8.49 mo; 57% were female; 23% had transferred from the SAM treatment program; 77% were currently breastfed; 6% had diarrhea in the past 7 d; and 54% reported severe household food insecurity (29). At enrollment, the average LAZ was –2.76 ± 1.23, and WLZ was –1.83 ± 0.75; 68% of participants recovered from MAM within 12 wk of supplementation. In the first 2 wk, participants gained on average 0.58 ± 0.46 cm length and 0.17 ± 0.29 kg weight. At enrollment, 1 child was excluded due to missing age data. At 2 wk, 89 children were excluded from analysis for implausible length data, 19 children were excluded from analysis for missing length data, and 17 children were excluded from analysis for missing weight data. No recovery data was available for 36 children as they were lost to follow-up.

TABLE 1 Study participant characteristics at enrollment and recovery from MAM within 12 wk[1]

	n	Mean ± SD or *n* (%)
Age, mo	519	13.86 ± 8.49
Female	520	298 (57%)
Transferred from SAM	520	118 (23%)
Currently breastfed	516	396 (77%)
Diarrhea in past 7 d	518	32 (6%)
Household food security		
Food secure	520	172 (33%)
Moderately food insecure	520	67 (13%)
Severely food insecure	520	281 (54%)
Anthropometry		
MUAC, cm	520	11.97 ± 0.27
LAZ	512	−2.76 ± 1.23
WLZ	519	−1.83 ± 0.75
Change in length, 2 wk, cm	412	0.58 ± 0.46
Change in weight, 2 wk, kg	503	0.17 ± 0.29
Recovery from MAM	484	327 (68%)

[1]Note: household food security was defined using the Household Food Insecurity Access Scale (29). LAZ, length-for-age z score; MAM, moderate acute malnutrition; MUAC, midupper arm circumference; SAM, severe acute malnutrition; WLZ, weight-for-age z score.

TABLE 2 Distribution of EED biomarkers at enrollment[1]

	Median (25th, 75th percentile)
L: M test ($n = 422$)	
LMER	0.09 (0.06, 0.15)
%Lactulose	0.34 (0.21, 0.61)
%Mannitol	3.87 (2.46, 5.67)
Fecal protein ($n = 200$)	
AAT, ng/mL	2217.49 (1756.43, 2947.04)
MPO, ng/mL	42,172.51 (19,253.97, 87,815.40)
NEO, nmol/L	977.91 (469.45, 1878.22)
Protein score	5.00 (4.00, 6.00)

[1]AAT, α-1-antitrypsin; EED, environmental enteric dysfunction; LMER, lactulose: mannitol excretion ratio; L: M test, lactulose: mannitol test; MPO, myeloperoxidase; NEO, neopterin; %L, percent lactulose excreted; %M, percent mannitol excreted.

Biomarker estimates at enrollment are presented in **Table 2** (see **Supplemental Figure 2** for individual mRNA transcript distributions). Using %L ≥0.2 as the cut-off, we found that 78% of participants had an increased intestinal permeability. To our knowledge, no cut-offs have been established for the fecal host mRNA transcripts. Previous studies have used the following normal cut-offs for the proteins among children under the age of 2 y: AAT <0.27 mg/g, MPO <2000 ng/mL, and NEO <70 nmol/L (13, 21, 30). Using these cut-offs, we found that 99% of participants had elevated concentrations of MPO and NEO, but none had elevated concentrations of AAT.

mRNA transcript-based scores

Factor analysis resulted in 3 factors with Eigen values >1: Factor 1 (Eigen value = 5.55), Factor 2 (Eigen value = 2.48), and Factor 3 (Eigen value = 2.22) (see **Supplemental Table 2** for factor loadings). The mRNA transcripts aquaporin9 (AQP9), CD53 molecule (CD53), lysosomal thiol reductase (IFI30), LYZ, phosphoinositide3-kinase adaptor protein1 (PIK3AP1), S100 calcium binding protein a8 (S100A8), and selectin L (SELL) loaded high on Factor 1. These mRNA transcripts encode proteins involved in the inflammatory response, thus Factor 1 was named the gut inflammation (GI) score. Baculoviral IAP repeat containing3 (BIRC3), caudal type homeobox1 (CDX1), 2,4-dienoyl-coa reductase1 (DECR1), major histocompatibility complex class II (HLA-DRA), and mucin12 (MUC12) loaded high on Factor 2. These mRNA transcripts encode proteins involved in maintaining the structure of the intestinal epithelium, thus Factor 2 was named the gut structure (GS) score. Defensin α-6 (DEFA6), regenerating islet-derived 1-α (REG1A), and regenerating islet-derived 3-α (REG3A) loaded high on Factor3. These mRNA transcripts encode proteins involved in gut repair or have antimicrobial properties; thus Factor 3 was named the gut defense (GD) score.

Comparison of host fecal mRNA transcripts and fecal proteins against L: M markers

In adjusted models, LMER, a measure of intestinal permeability, was positively associated with the intestinal permeability protein AAT (**Table 3**). However, LMER was not associated with any of the mRNA transcript scores. %L, also a marker of intestinal permeability, was negatively associated with the GD score, as was %M, a marker of absorptive surface area. The Spearman correlations among nontransformed biomarkers are tabulated in **Supplemental Table 3**.

Comparison of host fecal mRNA transcripts and fecal proteins

After adjusting for covariates, a high GI score was associated with high inflammation protein MPO, and high inflammation protein score (**Table 4**). A high GS (gut structural integrity) score was associated with high permeability protein AAT, but low inflammation protein MPO. A high GD score was associated with a low inflammation protein score.

Association between biomarkers, and growth and recovery

In adjusted models, high LAZ and high WLZ were both associated with a low GI score (**Table 5**). High WLZ was also associated with a low GS score, but high inflammation protein MPO. After correcting for multiple comparisons, only the association between WLZ and MPO was no longer statistically significant (**Supplemental Tables 4** and **5**).

After adjusting for covariates, the log odds of recovery increased by 0.36 but decreased by 1.35 for 1 unit increase in GD score and intestinal permeability protein AAT, respectively (**Table 6**). Lower length gain over 2 wk was associated with higher absorptive surface area marker %M and higher permeability protein AAT. Lower weight gain over 2 wk was associated with a lower GD score, but higher inflammation protein MPO and inflammation protein score.

Discussion

In this study of children with MAM enrolled in a supplementary feeding program in Sierra Leone, we found high prevalence of intestinal permeability (%L, LMER) and inflammation (MPO, NEO). Using host fecal mRNA transcripts, we identified 3 novel markers: GI score, GS score, and GD score. When comparing these scores with other biomarkers of EED, there was agreement between certain inflammation (GI score and MPO) and permeability (GS score and AAT; AAT and LMER) markers. High antimicrobial defense, measured by the GD score, was negatively associated with the permeability marker %L, suggesting that higher levels of the score indicate better gut health. Examining the associations between these biomarkers and growth outcomes revealed that both lower LAZ and WLZ were associated with higher intestinal inflammation using GI score. WLZ was also negatively associated with the GS score. A lower length gain was associated with higher intestinal permeability using AAT. A lower weight gain was associated with a lower GD score and higher intestinal inflammation captured by MPO. A high GD score and low intestinal

TABLE 3 Association between L: M test variables and fecal host mRNA transcripts and fecal proteins[1]

	LMER		%L		%M	
	Unadjusted β (95% CI)	Adjusted β (95% CI)	Unadjusted β (95% CI)	Adjusted β (95% CI)	Unadjusted β (95% CI)	Adjusted β (95% CI)
Fecal host mRNA transcripts ($n = 331$)						
GI score	0.04 (−0.08, 0.16)	0.04 (−0.08, 0.16)	−0.10 (−0.32, 0.11)	−0.10 (−0.33, 0.13)	−0.51 (−1.13, 0.11)	−0.48 (−1.05, 0.08)
GS score	0.0005 (−0.08, 0.08)	−0.004 (−0.08, 0.07)	0.03 (−0.08, 0.14)	0.003 (−0.12, 0.12)	−0.21 (−0.53, 0.12)	−0.21 (−0.60, 0.17)
GD score	0.03 (−0.02, 0.08)	0.02 (−0.03, 0.06)	−0.11 (−0.20, −0.03)**	−0.19 (−0.29, −0.10)***	−0.39 (−0.85, 0.08)	−0.50 (−0.94,−0.06)*
Fecal proteins ($n = 143$)						
AAT	0.18 (−0.03, 0.38)	0.26 (0.02, 0.49)*	0.78 (0.17, 1.39)*	0.54 (−0.18, 1.27)	0.73 (−0.42, 1.87)	−0.54 (−2.51, 1.44)
MPO	0.05 (−0.05, 0.15)	0.04 (−0.07, 0.16)	0.03 (−0.18, 0.25)	0.08 (−0.14, 0.29)	−0.22 (−1.10, 0.66)	−0.06 (−0.81, 0.69)
NEO	−0.02 (−0.12, 0.09)	−0.03 (−0.12, 0.07)	−0.45 (−1.02, 0.12)	−0.33 (−0.86, 0.19)	−0.96 (−2.01, 0.09)	−0.56 (−1.51, 0.39)
Protein Score	0.03 (−0.03, 0.10)	0.03 (−0.03, 0.10)	0.05 (−0.04, 0.14)	0.06 (−0.03, 0.14)	−0.12 (−0.57, 0.34)	−0.09 (−0.59, 0.41)

[1] Results for mixed linear regression models adjusted for PHU-level clustering. Adjusted model includes age, gender, and previous SAM status as independent variables. The L: M test variables were multiplied by 10 prior to regression analysis. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. AAT, α-1-antitrypsin; GD, gut defense; GI, gut inflammation; GS, gut structure; LMER, lactulose: mannitol excretion ratio; L: M test, lactulose: mannitol test; MPO, myeloperoxidase; NEO, neopterin; PHU, peripheral health unit; SAM, severe acute malnutrition; %L, percent lactulose excreted; %M, percent mannitol excreted.

TABLE 4 Association between fecal host mRNA scores and fecal proteins ($n = 179$)[1]

	Unadjusted β (95% CI)	Adjusted β (95% CI)
Gut inflammation score		
AAT	− 0.11 (−0.37, 0.15)	− 0.00 (−0.26, 0.26)
MPO	0.20 (0.13, 0.28)***	0.18 (0.11, 0.26)***
NEO	− 0.00 (−0.04, 0.04)	− 0.05 (−0.10, 0.00)
Protein score	0.07 (0.01, 0.12)*	0.06 (0.01, 0.11)*
Gut structure score		
AAT	0.24 (0.10, 0.38)**	0.18 (0.05, 0.30)**
MPO	− 0.14 (−0.21, −0.07)***	− 0.13 (−0.21, −0.05)**
NEO	− 0.05 (−0.13, 0.02)	− 0.03 (−0.09, 0.04)
Protein Score	− 0.04 (−0.07, 0.00)	− 0.04 (−0.07, 0.00)
Gut defense score		
AAT	− 0.14 (−0.36, 0.07)	− 0.17 (−0.36, 0.01)
MPO	− 0.06 (−0.17, 0.06)	− 0.05 (−0.16, 0.06)
NEO	− 0.14 (−0.27, −0.02)*	− 0.15 (−0.32, 0.01)
Protein Score	− 0.05 (−0.09, −0.00)*	− 0.04 (−0.08, −0.00)*

[1]Results for mixed linear regression models adjusted for PHU-level clustering. Adjusted model includes age, gender, and previous SAM status as independent variables. One influential observation excluded from models with AAT as outcome. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. AAT, α-1-antitrypsin; MPO, myeloperoxidase; NEO, neopterin; PHU, peripheral health unit.

permeability protein AAT were associated with recovery from MAM.

Our study adds to the growing body of literature on field appropriate biomarkers of EED. The clusters of host fecal mRNA transcripts identified by this study have also been reported previously (31). Agreement between the clusters of inflammation and permeability with other more established EED biomarkers gives us confidence that they are measuring changes to the intestine characteristic of EED. Additionally, the association between the GD score and recovery from MAM is a novel finding. In support of our speculation that a high GD score is a sign of better gut health, low expression of DEFA6 (a transcript that loaded high on the GD score) was reported among Zambian participants with elevated enteropathy compared with their UK counterparts (32).

Although the biomarkers in our study did not all agree with each other, this may be a sign that they are measuring different aspects and stages of the complex condition that is EED. Similar to our findings, other studies have found conflicting results for associations between the biomarkers examined in this study (11, 18, 31). Differences in age, study design, and selection of biomarkers among studies muddy the evidence for the association

TABLE 5 Association between EED biomarkers and growth outcomes at enrollment[1]

	LAZ at enrollment		WLZ at enrollment	
	Unadjusted β (95% CI)	Adjusted β (95% CI)	Unadjusted β (95% CI)	Adjusted β (95% CI)
L: M test				
LMER	0.83 (−0.26, 1.92)	0.39 (−0.68, 1.46)	0.31 (−0.57, 1.18)	0.41 (−0.11, 0.94)
%L	− 0.32 (−0.76, 0.13)	− 0.04 (−0.57, 0.50)	− 0.16 (−0.48, 0.17)	0.16 (−0.09, 0.42)
%M	− 0.12 (−0.32, 0.08)	0.06 (−0.16, 0.28)	− 0.13 (−0.28, 0.02)	0.01 (−0.14, 0.16)
Fecal host mRNAs				
GI score	− 0.01 (−0.09, 0.07)	− 0.08 (−0.14, −0.02)*	− 0.00 (−0.04, 0.03)	− 0.03 (−0.06, −0.01)*
GS score	0.02 (−0.06, 0.09)	0.03 (−0.01, 0.07)	− 0.05 (−0.09, −0.01)*	− 0.06 (−0.09, −0.02)**
GD score	− 0.02 (−0.12, 0.08)	0.02 (−0.10, 0.13)	− 0.01 (−0.10, 0.08)	0.04 (−0.05, 0.12)
Fecal proteins				
AAT	− 0.48 (−0.86, −0.10)*	0.16 (−0.19, 0.51)	− 0.40 (−0.59, −0.20)***	0.02 (−0.27, 0.31)
MPO	0.14 (0.00, 0.28)	0.04 (−0.15, 0.23)	0.08 (0.03, 0.14)**	0.06 (0.00, 0.12)*†
NEO	0.21 (0.02, 0.40)*	0.00 (−0.17, 0.17)	0.19 (0.08, 0.29)**	0.03 (−0.06, 0.11)
Protein score	0.04 (−0.05, 0.13)	0.02 (−0.06, 0.10)	0.03 (−0.00, 0.07)	0.04 (−0.01, 0.09)

[1]Results for mixed linear regression model adjusted for PHU-level clustering. Adjusted model includes age, gender, and previous SAM status as independent variables. All biomarkers except for the score were ln transformed prior to analysis. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. †not statistically significant after correcting for multiple comparisons. Sample size for LAZ: L: M test ($n = 413$), mRNA ($n = 410$), and proteins ($n = 193$); for WLZ: L: M test ($n = 419$), mRNA ($n = 415$), proteins ($n = 198$). AAT, α-1-antitrypsin; EED, environmental enteric dysfunction; GD, gut defense; GI, gut inflammation; GS, gut structure; LAZ, length-for-age z score; LMER, lactulose: mannitol excretion ratio; L: M test, lactulose: mannitol test; MPO, myeloperoxidase; NEO, neopterin; PHU, peripheral health unit; WLZ, weight-for-length z score; %L, percent lactulose excreted; %M, percent mannitol excreted.

TABLE 6 Association between EED biomarkers and recovery from MAM within 12 wk, and change in length and weight within 2 wk of supplementation[1]

	Recovery		Change in length		Change in weight	
	Unadjusted β (95% CI)	Adjusted β (95% CI)	Unadjusted β (95% CI)	Adjusted β (95% CI)	Unadjusted β (95% CI)	Adjusted β (95% CI)
L: M test						
LMER	0.43 (−2.43, 3.29)	− 1.03 (−4.02, 1.95)	0.23(−0.64, 1.11)	0.34 (−0.39, 1.08)	− 0.01 (−0.43, 0.42)	0.10 (−0.33, 0.53)
%L	0.81 (−0.13, 1.75)	− 0.01 (−1.02, 1.01)	− 0.11 (−0.32, 0.11)	− 0.02 (−0.19, 0.14)	0.09 (−0.06, 0.25)	0.11 (−0.06, 0.28)
%M	0.44 (0.02, 0.86)*	0.24 (−0.20, 0.69)	− 0.12 (−0.17, −0.08)***	− 0.09 (−0.13, −0.06)***	0.05 (0.00, 0.09)*	0.04 (−0.01, 0.08)
Fecal host mRNAs						
GI score	− 0.11 (−0.32, 0.10)	− 0.16 (−0.38, 0.06)	0.01 (−0.05, 0.07)	0.00 (−0.06, 0.06)	− 0.01 (−0.04, 0.03)	− 0.01 (−0.04, 0.03)
GS score	− 0.09 (−0.29, 0.11)	− 0.10 (−0.31, 0.10)	− 0.03 (−0.07, 0.01)	− 0.02 (−0.06, 0.01)	− 0.01 (−0.04, 0.02)	− 0.01 (−0.04, 0.02)
GD score	0.39 (0.12, 0.66)**	0.36 (0.08, 0.64)*	0.02 (−0.04, 0.08)	0.02 (−0.03, 0.08)	0.02 (0.00, 0.03)*	0.02 (0.00, 0.03)*
Fecal proteins						
AAT	− 1.05 (−1.80, −0.29)**	− 1.35 (−2.35, −0.36)**	− 0.25 (−0.35, −0.15)***	− 0.19 (−0.31, −0.08)**	− 0.08 (−0.20, 0.04)	− 0.11 (−0.22, 0.00)
MPO	− 0.18 (−0.46, 0.11)	− 0.28 (−0.60, 0.04)	0.04 (−0.02, 0.09)	0.04 (−0.02, 0.10)	− 0.05 (−0.08, −0.02)**	− 0.04 (−0.07, −0.02)**
NEO	0.26 (−0.06, 0.58)	0.34 (−0.05, 0.72)	0.08 (0.03, 0.14)**	0.04 (−0.02, 0.09)	0.01 (−0.03, 0.05)	0.02 (−0.02, 0.06)
Protein score	− 0.14 (−0.30, 0.01)	− 0.16 (−0.32, 0.01)	− 0.00 (−0.04, 0.03)	− 0.00 (−0.03, 0.03)	− 0.03 (−0.04, −0.01)**	− 0.03 (−0.04, −0.01)***

[1]Results for logistic regression model (recovery) and mixed linear regression model (change in length and weight) adjusted for PHU-level clustering. Adjusted model (recovery) includes age, gender, previous SAM status, and study arm as independent variables. Adjusted model (change in length and weight) includes study arm, age, gender, and baseline length/weight as independent variables. All biomarkers except for the scores were ln transformed prior to analysis. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. Sample size for recovery: L: M test ($n = 388$), mRNA ($n = 389$), and proteins ($n = 190$); change in length: L: M test ($n = 323$), mRNA ($n = 323$), proteins ($n = 152$); and change in weight: L: M test ($n = 405$), mRNA ($n = 402$), proteins ($n = 195$). AAT, α-1-antitrypsin; EED, environmental enteric dysfunction; GD, gut defense; GI, gut inflammation; GS, gut structure; LMER, lactulose: mannitol excretion ratio; L: M test, lactulose: mannitol test; MAM, moderate acute malnutrition; MPO, myeloperoxidase; NEO, neopterin; PHU, peripheral health unit; %L, percent lactulose excreted; %M, percent mannitol excreted.

between EED and growth outcomes. In our study, the GI score was inversely associated with concurrent linear (LAZ) and ponderal (WLZ) growth, suggesting that this biomarker may measure current growth status quite effectively. Surprisingly, WLZ was positively associated with MPO, and we are unsure why inflammation would be associated with better ponderal growth. Our results support the idea that studies examining EED should employ biomarkers that capture a range of intestinal characteristics rather than a single measure, with the host fecal mRNA transcripts a likely contender.

There are limitations to our study; first, conducting the L: M test on the same day as stool collection may have affected the AAT protein concentrations. Despite the rather low AAT concentration in this study, the values were higher than the mean AAT concentration of 597 ng/mL reported by the MAL-ED Peru birth cohort (33). Second, due to logistical constraints we were only able to collect all biomarker samples at 1 time point. Although we examined whether biomarker concentrations at enrollment were associated with future recovery and growth longitudinally, this does not take into account the possibility that values for these biomarkers could change over time. Third, 2 wk is a very short period to examine changes in length, however, we wanted to assess the utility of the novel mRNA-based scores in measuring growth longitudinally.

Our findings add to the growing consensus that potential biomarkers of EED measure specific characteristics of this condition and are not always in complete agreement with the L: M test. Since the study was conducted among MAM children in Sierra Leone, these biomarkers should be examined in other settings and among children of different nutritional status. The group of fecal host mRNA transcripts associated with recovery from MAM is a promising finding. This suggests that children who start the MAM supplemental feeding program with a "healthier" intestine (including a robust antimicrobial system), are able to respond better to treatment. Also, more children who had less intestinal permeability measured using AAT recovered. These biomarkers could provide insight to how intestinal dysfunction may influence the risk of malnutrition in children and how malnourished children may be expected to respond to interventions, nutritional and environmental. Future studies should assess these biomarkers longitudinally through repeated measurements in the first 2 y of life among children with and without malnutrition.

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The authors' responsibilities were as follows—IHR, BLR, and AS: conducted the research; AS: analyzed the data; and all authors: were involved in the design of the research and in the writing of the manuscript, and read and approved the final manuscript. The authors report no conflicts of interest.

Data Availability

Data described in the manuscript, code book, and analytic code will be made publicly and freely available without restriction at https://data.usaid.gov/.

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