Published in final edited form as:

J Pediatr Gastroenterol Nutr. 2017 July ; 65(1): 4–5. doi:10.1097/MPG.0000000000001591.

Biomarkers of Environmental Enteric Dysfunction: The good, the bad and the ugly

Lissette Jimenez, MD and

Division of Gastroenterology, Hepatology and Nutrition, Boston Children's Hospital, Boston, Massachusetts

Christopher P. Duggan, MD, MPH

Division of Gastroenterology, Hepatology and Nutrition, Boston Children's Hospital, Boston, Massachusetts

Environmental enteric dysfunction (EED) has been described as altered enteric architecture and function resulting in poor absorption, increased permeability, and chronic mucosal inflammation among infants and children who live in low and middle income countries (LMIC) (1–3). EED has been hypothesized to underlie high rates of stunting and growth failure among these vulnerable children (4–7), as well as impaired immune response to vaccines (4) and suboptimal cognitive development (8). The etiology of the condition has been thought to be related to chronic gastrointestinal exposure to enteropathogens via an unhygienic environment, although other contributing factors may include altered mucosal and/or systemic immune function, preceding acute infectious illnesses, micronutrient deficiencies, both pre-and post-natal infant-maternal interactions, heavy metal exposures, and other as yet undiscovered factors.

Despite the wealth of evidence cited above about the important role of EED in child health, development and growth, there is currently no single gold-standard diagnostic test for EED. While early studies documented morphometric changes in the small bowel mucosa of residents of South Asia (9, 10), findings that are supported by more recent confocal microscopy data (11), routine endoscopic examination among children at risk of EED is thought to be untenable due to its cost, invasiveness, and need for technical expertise. Hence numerous investigators have pursued less invasive measures of EED, including tests of gastrointestinal permeability, absorption, enterocyte mass and function, inflammation, microbial translocation, and systemic and immune activation (1).

Chief among the proposed biomarkers of EED include urinary measures of gastrointestinal absorption and permeability, which are feasible via straightforward if laborious field tests, reflect important components of gastrointestinal barrier and absorptive function. After oral administration of two carbohydrates, the ratio of urinary lactulose (a sugar not usually absorbed by intact GI mucosa, whose concentration is therefore taken to correlate with

Address correspondence and requests for reprints to: Christopher P. Duggan, Division of Gastroenterology, Hepatology, and Nutrition, Boston Children's Hospital, 333 Longwood Ave, Boston, MA, 02115; christopher.duggan@childrens.harvard.edu; Phone: 617-355-7612; Fax: 617-730-4722.

Conflicts of interest: The authors have no conflicts of interest to declare.

intestinal permeability) and mannitol (a sugar usually passively absorbed, whose concentration is therefore thought related to mucosal surface area) are most commonly used. The non-invasive nature of these markers has facilitated their use as a measure of EED in numerous settings. Denno et al. summarized numerous studies of the urinary L:M ratio in a recent systematic review, noting that despite its many advantages (non-invasiveness, wide availability of subtrates, and face validity), variation in data presentation, substrate dosing, subject selection and fasting status were among several factors that need to be considered in efforts to improve the utility of this test (12).

In this volume of Journal of Pediatric Gastroenterology and Nutrition, two large and detailed studies of numerous markers of EED, including the urinary L:M ratio, are reported. In one, Kosek et al. (13) describe changes in GI absorption and permeability using the urinary L:M test in several large and diverse populations at risk of EED and its associated growth faltering, with the goal of defining a reference standard for this commonly used test of enteropathy. The authors hypothesized that their analyses would provide references across sites, ages, and sex and thus allow the creation of L:M standards for broader use. The study was part of a large parent study “The Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development” (the MAL-ED study), whose overall goal is to evaluate the association of childhood growth, gastrointestinal function (14) and infectious disease morbidity with standard tests of neurodevelopmental ability and vaccine response (http://mal-ed.fnih.org). The study utilized a common protocol to follow and collect samples among infants over time in eight distinct locations, including both rural and urban sites in several LMIC’s: Dhaka, Bangladesh; Vellore, India; Bhaktapur, Nepal; Naushero Feroze, Pakistan; Fortaleza, Brazil; Loreto, Peru; Venda, South Africa; and Haydom, Tanzania. Infants were followed over the course of 24 months with growth, dietary intake and morbidity patterns documented closely. For the current paper, nearly 2,000 infants underwent urinary L:M testing four times over the first two years of life. Consider the monumental efforts involved in studying this large number of young children in resource limited settings: at ages 3, 6, 9 and 15 months of age, infants were fasted for 2 hours, provided an oral dose of the dual sugars, and then underwent multiple five hour timed urine collections. The 7,260 urine collections completed therefore represent at a minimum 36,300 hours of research staff time for the timed collections only; additional time for contacting families, scheduling research visits, sample preparation and analysis were of course needed as well. In addition, the authors report an impressive rate of subject follow-up, given the complexity of the study.

The authors evaluated sex, age and site specific rates of mannitol and lactulose recovery, as well as the urinary L:M ratio. While the wide range of geographic locations was a strength of their study, this feature also introduced some methodologic variability in specimen analysis. To aid in the interpretation of these sex, age and locale-specific findings, the investigators used the raw data to create Z scores, using the Brazil site as the reference group due to low rates of clinically apparent diarrhea episodes as well as normal growth patterns. The main findings included significantly higher percent mannitol and lactulose recovery in the youngest age groups and for boys compared to girls at all ages, suggesting sex differences in intestinal permeability. Measured L:M ratios were noted to have a highly variable pattern across site locations and over time. Specifically, in three sites (Bangladesh,

Nepal, and Pakistan) L:M declined with age, in two sites (India and Peru) it increased, and in two sites (South Africa and Tanzania) it remained the same. The sex differences seen in the individual sugar data were not seen in the ratio data, leading the authors to surmise that evaluating the individual components (lactulose and mannitol recovery) of the test might have more utility than the ratio per se in interpreting permeability across sex, time and location. The data obtained in this study enabled the authors to create Z score reference data (normalized to age and sex) that can be used in future studies.

Also in this volume of JPGN, Campbell et al. (15) report their findings of a cross-sectional study among 539 Bangladeshi children who underwent numerous measures of potential biomarkers for EED at age 18 months. Among these participants of a randomized trial of food supplementation, the authors collected numerous additional samples for the analysis of stool myeloperoxidase (MPO), alpha-1 antitrypsin (AAT), and neopterin; serum endotoxin core antibody (EndoCAb), glucagon like peptide 2 (GLP-2), total immunoglobulins (Igs), C-reactive protein (CRP), and alpaha-1 acid glycoprotein (AGP)); and urine (L:M test). These markers were chosen due to recent data that have identified fecal (7) and systemic measures of inflammation (16) as correlates of stunting in children living in LMIC's. The findings were quite striking. First, using standard cut-offs, most of the children studied had elevated concentrations of stool, urine or serum markers of EED, seemingly confirming that in a population with high rates of stunting (45% at age 18 months), these markers hold promise as biomarkers of EED. Second, there was little to no correlation among these various proposed markers of EED, possibly suggesting that that some of these may reflect elevations in inflammatory markers that are not specific to GI disease. Finally, multivariable modeling using all of the biomarkers as independent variables explained only 10.8% of the variability in urinary L:M ratio, suggesting that either L:M ratio is an imperfect test of EED itself (see Kosek et al.) and/or that EED is not a uniform entity in which a single marker or groups of markers will be able to accurately diagnose.

The utility of these two papers to the global pediatric gastroenterology and nutrition community is substantial. The paper by Kosek et al. describes an impressively large and well collected data set with broad geographic boundaries concerning a common test of GI intestinal permeability and absorption, highlighting the need to consider methodologic and geographic factors in interpreting this test. In addition, the finding of age and sex-specific normative data informs our knowledge of gastrointestinal function over sex and time. Similarly, the paper by Campbell et al. emphasizes the low correlation observed among several purported tests of EED, even in a population with highly prevalent stunting and elevations of numerous biomarkers.

The ultimate value in these data set will of course be whether and how these biomarkers of EED are related to important health outcomes, including growth, neurodevelopment, vaccine response and perhaps other non-communicable diseases (17). Indeed in neither of these papers was the relationship of these EED biomarkers with growth outcomes presented. The completion of additional analyses, wherein these biomarkers are related with these and other outcomes, as well as ongoing studies of endoscopic and histologic measures of EED, are awaited with interest.

Acknowledgments

Sources of support: NIH (K24 DK104676 - Dr. Duggan) (T32 DK007477 - Dr. Jimenez)

References

1. Keusch GT, Denno DM, Black RE, et al. Environmental enteric dysfunction: pathogenesis, diagnosis, and clinical consequences. Clin Infect Dis. 2014; 59(Suppl 4):S207–12. [PubMed: 25305288]
2. Syed S, Ali A, Duggan C. Environmental Enteric Dysfunction in Children: A Review. J Pediatr Gastroenterol Nutr. 2016
3. Crane R, Jones K, Berkeley J. Environmental enteric dysfunction: an overview. Food Nutr Bull. 2015; 36:S76–S87. [PubMed: 25902619]
4. Naylor C, Lu M, Haque R, et al. Environmental Enteropathy, Oral Vaccine Failure and Growth Faltering in Infants in Bangladesh. EBioMedicine. 2015
5. Campbell DI, Elia M, Lunn PG. Growth faltering in rural Gambian infants is associated with impaired small intestinal barrier function, leading to endotoxemia and systemic inflammation.[see comment]. Journal of Nutrition. 2003; 133(5):1332–8. [PubMed: 12730419]
6. Lunn PG, Northrop-Clewes CA, Downes RM. Intestinal permeability, mucosal injury, and growth faltering in Gambian infants. Lancet. 1991; 338(8772):907–10. [PubMed: 1681266]
7. Kosek M, Haque R, Lima A, et al. Fecal markers of intestinal inflammation and permeability associated with the subsequent acquisition of linear growth deficits in infants. Am J Trop Med Hyg. 2013; 88(2):390–6. [PubMed: 23185075]
8. Sudfeld CR, McCoy DC, Fink G, et al. Malnutrition and Its Determinants Are Associated with Suboptimal Cognitive, Communication, and Motor Development in Tanzanian Children. J Nutr. 2015; 145(12):2705–14. [PubMed: 26446481]
9. Lindenbaum J, Alam AK, Kent TH. Subclinical small-intestinal disease in East Pakistan. Br Med J. 1966; 2(5530):1616–9. [PubMed: 5926127]
10. Lindenbaum J, Kent TH, Sprinz H. Malabsorption and jejunitis in American Peace Corps volunteers in Pakistan. Ann Intern Med. 1966; 65(6):1201–9. [PubMed: 5928480]
11. Kelly P, Besa E, Zyambo K, et al. Endomicroscopic and Transcriptomic Analysis of Impaired Barrier Function and Malabsorption in Environmental Enteropathy. PLoS Negl Trop Dis. 2016; 10(4):e0004600. [PubMed: 27050312]
12. Denno DM, VanBuskirk K, Nelson ZC, et al. Use of the lactulose to mannitol ratio to evaluate childhood environmental enteric dysfunction: a systematic review. Clin Infect Dis. 2014; 59(Suppl 4):S213–9. [PubMed: 25305289]
13. Margaret Kosek M, Gwenyth O, Lee P, Richard L, Guerrant M, et al. The MAL-ED study: Age and sex normalixation of intestinal permeability for the assessment of enteropathy in infancy. Journal of Pediatric Gastroenterology & Nutrition. 2017
14. Kosek M, Guerrant RL, Kang G, et al. Assessment of environmental enteropathy in the MAL-ED cohort study: theoretical and analytic framework. Clin Infect Dis. 2014; 59(Suppl 4):S239–47. [PubMed: 25305293]
15. Rebecca Kramer Campbell PD, Kerry Schulze PD, Saijuddin Shaikh M, et al. Biomarkers of environmental enteric dysfunction among children in rural Bangladesh. Journal of Pediatric Gastroenterology & Nutrition. 2017
16. Prendergast AJ, Rukobo S, Chasekwa B, et al. Stunting is characterized by chronic inflammation in Zimbabwean infants. PLoS One. 2014; 9(2):e86928. [PubMed: 24558364]
17. Deboer MD, Lima AA, Oria RB, et al. Early childhood growth failure and the developmental origins of adult disease: do enteric infections and malnutrition increase risk for the metabolic syndrome? Nutrition reviews. 2012; 70(11):642–53. [PubMed: 23110643]