Stool consistency as a major confounding factor affecting microbiota composition: an ignored variable?

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In this age of the microbiome, the one request most oft heard is 'so what is the biomarker for this disease?' In a world of 'one-size-fits-all' solutions, and a one pathogen-one disease framework of investigation, most clinicians and health-care industrialists just want the punchline - what is the bacterium that is causing that disease? The problem with this approach is that it remains a challenge to fully understand whether a given microbiome has any causative influence on a given condition, or whether identified associations have any relevance outside of a given case study. Unlike monomicrobial diseases where Koch's postulates can be applied, the interaction between the microbial ecology of the body, and health and disease is extremely complex, leading to fuzzy concepts of organismal ratios and phylogenetic groups of organisms being associated with a specific condition. In their recent paper, Vandeputte and colleagues [1] seek to determine whether gut-transit time has an influence on microbial community profiles in stool, and by what mechanism this occurs, as well as considering whether this influence could have an impact on clinical studies.

Clinicians and microbiome researchers continue to struggle with the delicate balance that exists between elucidating the pathogenesis of a disease and defining the role of a particular organism, or group of organisms in this process. This can be a slippery slope; on the one hand we want a microbiome-based diagnosis and a discriminative biomarker. Naturally the promise of this approach is that we will be able to analyze the patient's microbiome, most likely from the stool, and tailor therapy accordingly. Although big pharmaceutical companies are ready and able to invest in this approach, they remain wedded to the paradigm that novel compositions of drugs that hit a single target offer the most 'bang for their buck'. However the problem with microbes is their plasticity. We need to be able to determine if the microbe in question is responding in the same way under different conditions. This has proven to be the most difficult part. Not everyone that has methicillin resistant *Staphylococcus aureus* gets sick [2], similarly, members of your own commensal gut microbiota can turn virulent under the appropriate conditions [3,4]. Thus drug targets, when it comes to microbes, are truly moving targets. Microbial

consortia start out as a heterogeneous population and local conditions select for, or transformed to, the causative phenotype. Once the microbe or microbial community is isolated and screened for drug targets in vitro, it may not express the target most important to the *in vivo* condition. Therein lies the ruse.

Vandeputte and colleagues [1] highlight the importance of understanding transit time through the gut when assessing the microbial profile of an individual. Virtually every aspect of human life is likely to have some influence on the microbial community profile in the gut. Where you grew up, whether you were on a farm or in a city, where you work, who you live with, your diet, recreational and clinical drug use, activity level, immune and inflammatory state, prior disease history, etc., can all have a profound influence on the composition and structure of the bacteria living inside you as an adult. That ecosystem can be perturbed, leading to profound changes in the composition and function of the now remaining and stable microbial community. Recent evidence in mice has shown that the microbial community undergoes significant shifts in structure with changes in the timing of food delivery [5], so 'flow rate' of material through the gut will also influence microbial structure. Microbial community profiles are most likely influenced by the growth rate and ability to adhere to the gut mucosal layer of different taxa [6].

The selection pressure of decreased transit time affects the ecology of the system. Microbial organisms can be partitioned into r-selected and k-selected types, which describe how environmental pressure selects for life-strategy traits for a species. A significant decrease in transit time would likely lead to more r-selected organisms that are able to grow fast when nutrients are available in unstable ecosystems, compared to k-selected organisms who are strong competitors in crowded, more stable ecological niches. In Vandeputte's study, Ruminococcaceae and Bacteroides as a taxonomic group showed an increase in growth potential with increased transit time, but watery stool was dominated by Bacteroides, not Ruminococcaceae, which suggests that Bacteroides are r-selected organisms, able to multiply rapidly in the unstable ecosystem of diarrhea. Interestingly, Prevotella showed no significant correlation between growth potential and transit time, and while they are more abundant in stool with a slow transit time, this is again a weak relationship. This suggests that the Prevotella are potentially using enhanced attachment strategies to avoid being washed out during period of high transience. Indeed Vandeputte and colleagues hypothesize that Prevotella's ability to bind collagen and degrade mucin [7,8] could be a valuable mechanism to help it maintain high relative abundance in an unstable ecosystem.

How do we use this information to identify biomarkers of disease and potentially to design more effective therapeutics? Well, for one, it is essential to understand as much about the patient as possible to determine the ecological relevance of a given microbial profile. As most microbiome sequencing analyses are based on relative estimates of abundance, the same individual could show dramatic changes in their microbiome profile depending on their diet, antibiotic usage, or GI transit time. Therefore, if you found a patient enriched for Bacteroides, and compared them to a large database of microbiome profiles (e.g. American Gut; www.americangut.org) and observed a correlation with a

particular disease, you would then have to determine whether the observed correlation was in fact a true correlation to the disease process or merely a function of the patient's physiological or environmental context at that time. This has huge possibilities to confound microbiome-based diagnoses. Finally, considering an organisms' ability to resist being flushed from the GI tract due to either rapid growth or an adherence strategy might be useful when deciding on a therapeutic approach (i.e probiotics, anti-virulence agents, etc). By examining stool samples longitudinally, as patients experience variations in transit times, it should be possible to determine the mechanisms by which different bacteria develop specialized niche domains, how they maintain their population density and how they shape into stable or unstable communities. Considering all these possibilities could lead to a more global ecosystem engineering approach to the gut microbiome that might prevent the diarrhea that invariably develops when use of powerful and broad spectrum antibiotics are needed to treat serious infections.

The gut microbiome is a complex ecosystem, and every study that examines the factors that influence its structure and function find a myriad of confounding factors. Examining these influences through time, and across patient populations will have a profound influence on our understanding of the ecological stability and resilience of this environment, and help us to develop more effective therapies and treatment strategies to deal with complex disease states in individual patients.

Competing Interest: None declared.

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