appropriate therapy. Rapid DNA diagnostics will be an important step forward, but they are not the holy grail. For example, in many patients (41%), no mutation was detected. Outcome in these patients was intermediate (5-year ESKD-free survival, 65%), suggesting that this group is heterogeneous and may include patients with CaHUS and those with other causes of a CaHUS-like condition. The recently proposed ex vivo endothelial complement activation assay, although not specific, might improve sensitivity of our diagnostic algorithms.[4]

2. Outcome in patients with CaHUS is not optimal, even in the era of eculizumab. Although eculizumab therapy is effective, many patients in this study had persistent severe chronic kidney disease (CKD), with 46% of patients with CKD stage ≥3 and 21% of patients needing renal replacement therapy. In a multivariate analysis, older age at presentation, lower estimated glomerular filtration rate at presentation, high systolic blood pressure, less severe thrombocytopenia, and a longer interval between diagnosis and start of eculizumab therapy were all associated with persistent kidney injury. It would have been interesting to analyze the association between the initial changes in laboratory parameters after the start of eculizumab therapy and persistent kidney injury. Increasing awareness of CaHUS, and earlier referral, may be a way to improve outcome.
3. Uncertainty of the relevance of variants of uncertain significance (VUSs). Although genetic analysis is pivotal in evaluating patients with suspected CaHUS, the pathogenicity of discovered variants is often unclear. These variants are categorized as VUSs. Because the 5-year ESKD-free survival in treated patients with CaHUS and with VUSs is comparable to the survival of those with pathogenic complement variants (with a similar relapse rate), many VUSs may be pathogenic. Functional analysis of VUSs will add to a better characterization of CaHUS.
4. Uncertainty about the efficacy of eculizumab in non-CaHUS (secondary thrombotic microangiopathies). Complement activation is observed in many patients with secondary TMA. In many patients, this is a physiological process, well regulated by the intrinsic complement inhibitors. Still, ongoing debate exists whether complement inhibition with eculizumab could benefit some or all of these patients. Unfortunately, this study does not provide an answer. By chance, the study included 51 patients treated with eculizumab, who were later shown not to have CaHUS. The 5-year kidney survival in this group was worse (57%), suggesting limited efficacy of eculizumab. However, to draw conclusions, a comparison with untreated controls, matched for secondary TMA diagnosis and the absence of genetic variants, would be needed.
5. Relapse rate after eculizumab withdrawal. This study demonstrated a low relapse rate of 1 per 9.5 patient-years (py) in patients with a pathogenic genetic variant, 1 in 10.8 py in patients with VUSs, and no relapse in 67 py in patients with no variant detected. These data should be interpreted with some caution, as the distribution of patients stopping therapy did not match the overall patient population. For example, of the 14 patients stopping therapy with a pathogenic variant, 9 had a variant in the *membrane cofactor protein* (*MCP*) gene, which is associated with overall good outcome, even when untreated. Still, the data add to other recent studies that support considering eculizumab withdrawal in patients with CaHUS, especially in patients with no pathogenic variants.[2,3,5,6]

Brocklebank and colleagues have provided a wealth of data, improving our understanding of CaHUS. Still, many questions remain, and further work is needed to improve the management of patients with TMA and suspected CaHUS.

Conflict-of-interest disclosure: N.C.A.J.v.d.K. has received consultancy and lecture fees from Novartis, Roche, and Alexion. J.F.M.W. has received consultancy fees from Novartis and Alexion. ■

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https://doi.org/10.1182/blood.2023021474

TRANSPLANTATION

Comment on Masetti et al, page 1387

Microbes matter in pediatric allo-HSCT

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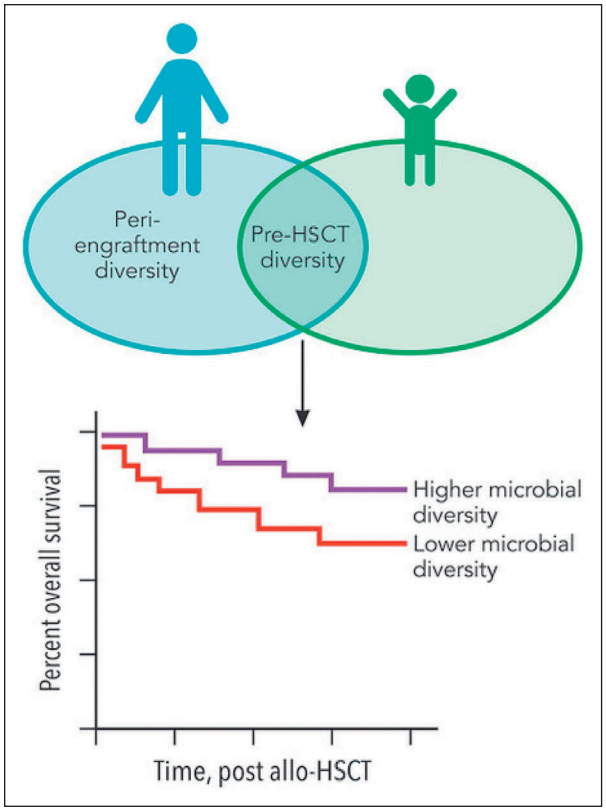
In this issue of *Blood*, Masetti et al[1] report on the findings of a multicenter observational study that explored the role of the microbiome in pediatric allogeneic hematopoietic stem cell transplantation (allo-HSCT). They studied 90 children and found that patients with high fecal microbial diversity prior to allo-HSCT had longer overall survival and a lower incidence of acute

graft-versus-host disease (GVHD) compared to patients with low microbial diversity. In adult allo-HSCT recipients, high pretransplant fecal diversity has also been positively correlated with overall survival. Importantly, a key difference between this pediatric study and prior analyses in adults is that in this work, the perineutrophil-engraftment microbiome was not associated with outcome, whereas intestinal microbial features during this time window have been shown to predict outcome in adults (see figure).[2,4]

In adult HSCT recipients, outcomes linked with intestinal bacteria include overall survival,[2,3] acute and chronic GVHD,[5,6] infection,[7] relapse,[8] and immune reconstitution.[4] Data regarding children undergoing allo-HSCT have thus far been lacking, and, therefore, the results from this study represent an important addition to the existing allo-HSCT literature.

Several factors may explain the differences between this pediatric study and previous work in the adult population. Firstly, the intestinal microbiome is likely to be more dynamic during childhood than in adulthood. Early studies suggested that the intestinal microbiome of children approximated the adult state early in life, at around the time children begin eating solid food.[9] However, more recent data suggest that the microbiome of children continues to evolve and does not become comparable to that of adults until later in childhood. In addition, although Masetti et al's study is very important for the field and was rigorously and carefully analyzed, the cohort is smaller than many of the adult transplant cohorts in which the microbiome has been analyzed. A larger pediatric cohort, although undoubtedly challenging to recruit, may reveal further relationships and help to untangle confounders like dietary habits, antibiotic stewardship, or medication use that impact microbial diversity and community composition. Finally, the disease indications for allo-HSCT as well as allo-HSCT conditioning approaches in pediatric and adult populations differ. The incorporation of adult and pediatric patients in future studies may aid in clarifying the most important microbial contributors to transplant outcomes at any stage of life as well as precisely when their presence is required.



The Venn diagram shows the overlapping features in pediatric[1] and adult[2] allo-HSCT recipients. In adults, diversity of the pretransplant and peri-engraftment intestinal microbial communities has been associated with overall survival (OS): high diversity correlates with longer OS. In this pediatric study, the diversity of the pretransplant microbiome was correlated with OS, but the peri-engraftment time point was not. The survival curve denotes the common finding between pediatric[1] and adult[2,3] allo-HSCT recipients: ie, higher microbial diversity pretransplant is associated with improved OS. Professional illustration by Patrick Lane, ScEYEnce Studios.

The specific mechanisms underpinning the relationship between intestinal bacteria and allo-HSCT outcomes remain unclear. One hypothesis is that a healthy, diverse intestinal microbiome is linked with favorable clinical outcomes owing to microbe-derived metabolites, such as the short-chain fatty acid butyrate. In this study, the "high diversity" stool samples were enriched for genera that are known short-chain fatty acid producers, including *Blautia*, *Faecalibacterium*, and *Bacteroides*. This finding aligns with the view that short-chain fatty acid production is immunomodulatory and promotes favorable outcomes in the transplant setting.[10] This suggests that despite differences in the predictive time window between children and adults, there may be a common microbiome-associated "protective pathway" for allo-HSCT patients.

In addition, interventions aimed at mitigating or repairing microbial damage during the peri-transplant period are currently being studied in preclinical models and in active clinical trials. This important study from Masetti and colleagues suggests that these strategies are also likely to benefit children undergoing allo-HSCT.

Conflict-of-interest disclosure: M.S. reports an advisory role in A28 Therapeutics, an advisory board role in BMS, and consulting for Novartis. K.A.M. holds equity in and is on the advisory board of PostBiotics Plus and has consulted for Incyte. ■

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https://doi.org/10.1182/blood.2023021608