Clostridioides difficile infection (CDI) is associated with considerable morbidity, mortality, and healthcare costs globally. CDI is preceded by asymptomatic C. difficile carriage and antibiotic-induced modulation of the intestinal microbiota is thought to initiate infection. However, there are currently no predictive markers for CDI development. Using high-resolution 16S rRNA gene-profiling we characterized longitudinally collected fecal samples from 1,007 hospitalized patients receiving broad-spectrum antibiotic treatment and identified microbiota-based markers associated with a 5-fold higher risk of CDI development. These markers may be used to develop microbiota-based diagnostics for clinical management of patients at risk of CDI.

METHODS

1,007 patients receiving treatment with broad-spectrum antibiotics were enrolled in this study, where 1,002 provided fecal samples at baseline (D1), 848 after antibiotic treatment (D6), and 33 at occurrence of a first diarrheal episode (S1). All samples underwent targeted 16S rRNA gene paired-end sequencing on a MiSeq instrument (Illumina Inc., USA). Data analysis with OCToPUS (1) rendered 945, 775, and 32 successfully sequenced samples at D1, D6, and S1, respectively.

Definitions:

- AAD: Patients with C. difficile-negative antibiotic-associated diarrhea
- CDI: Patients with clinically confirmed CDI
- ND: Non-diarrheic patients

Sample collection:

1,007 enrolled patients analyzed with 16S rRNA metagenome sequencing at 1,007 timepoints.

Data analysis:

Microbiota-based biomarkers predictive of CDI were identified by comparing microbial diversity and composition of the human intestinal microbiota at D1 in patients who later during the 90-day study period developed CDI and AAD with ND patients. Among patients who developed diarrhea within 90 days, those with CDI (n=14) exhibited significantly lower diversity (p=0.016) and a distinctly different microbial composition at D1 compared to those with non-C. difficile AAD (n=64) and no diarrhea (n=669, 198 lost to follow-up). At D1, the microbiota was enriched for Enterococcus spp. in patients who later developed CDI, for Clostridiales Incertae Sedis XI, Blautia and Ruminococcus spp. in patients developing non-C. difficile AAD, and for Blautia luti, Porphyromonas, Prevotella, and Bifidobacterium spp. in non-diarrheic patients (Fig. 1).

RESULTS

Microbiota-based biomarkers predictive of CDI were identified by comparing microbial diversity and composition of the human intestinal microbiota at D1 in patients who later during the 90-day study period developed CDI and AAD with ND patients. Among patients who developed diarrhea within 90 days, those with CDI (n=14) exhibited significantly lower diversity (p=0.016) and a distinctly different microbial composition at D1 compared to those with non-C. difficile AAD (n=64) and no diarrhea (n=669, 198 lost to follow-up). At D1, the microbiota was enriched for Enterococcus spp. in patients who later developed CDI, for Clostridiales Incertae Sedis XI, Blautia and Ruminococcus spp. in patients developing non-C. difficile AAD, and for Blautia luti, Porphyromonas, Prevotella, and Bifidobacterium spp. in non-diarrheic patients (Fig. 1).

Microbial composition was compared a baseline (D1), after treatment with broad-spectrum antibiotics (D6), and at occurrence of the first diarrheal episode (S1) in patients developing AAD (n = 26). A severely disrupted microbiota is observed in patients with diarrhea (Fig. 2, AMOVA, p<0.001).

CONCLUSIONS

Here, we have identified biomarkers predictive of the development of CDI. Future applications include enrichment of high-risk patients in prospective clinical trials, development of predictive, microbiota-based diagnostics to tailor antibiotic therapy or biobanking stools from high-risk patients prior to antibiotic therapy, exemplifying a precision medicine approach.

REFERENCES