Plasma Microbial Cell-Free DNA Metagenomic Sequencing for the Diagnosis of Severe Pneumonia in Mechanically-Ventilated Patients

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Background: Metagenomic sequencing of respiratory microbial communities may overcome the limitations of culture-based pneumonia diagnostics. Nonetheless, respiratory metagenomics require high-quality specimens, may miss deep-seated infections and cannot distinguish colonization from infection. Plasma microbial cell-free DNA (mcfDNA) sequencing may offer a non-invasive alternative for culture-independent diagnosis in hospitalized patients with pneumonia. To examine the feasibility and clinical validity of sequencing diagnostics of pneumonia, we conducted the Rapid Pneumonia Pathogen Identification with DNA Sequencing (RaPPID-Seq) study in a cohort of mechanically-ventilated patients with and without clinical diagnosis of pneumonia. Methods: We obtained concurrent plasma and endo-tracheal aspirate (ETA) samples from 64 mechanically-ventilated patients (26 culture-positive, 24 culture-negative pneumonia, 14 uninfected control patients). We performed plasma mcfDNA sequencing (Karius® Test, Redwood City, CA) and ETA metagenomics (MiNION, Oxford Nanopore Technologies). We compared sequencing results with clinical microbiologic cultures for identified DNA pathogens and examined the evolution of mcfDNA quantities overtime in patients with available follow-up plasma samples. Results: Among uninfected control patients, 11/14 (79%) had negative signal for mcfDNA in plasma samples, whereas 3/14 (21%) had low mcfDNA quantities (median 150 molecules per microliter [MPMs]). Plasma sample sequencing in culture-positive pneumonia had higher mcfDNA quantities (median[IQR] 3,160[36,703] MPMs vs. 385[4,839], p=0.005) and detected a median of 3[1-5] vs. 1[0-3] (p=0.02) organisms per sample compared to culture-negative pneumonia. Plasma and ETA sequencing were concordant in pathogen identification with clinical microbiologic cultures in 21/26 cases (80%). In culture-negative cases, plasma sequencing was negative in 10 (42%) with ETA sequencing revealing typical oral bacteria abundance, thus both technologies not supporting pneumonia diagnosis from a DNA pathogen. In nine culture-negative cases (38%) both sequencing approaches detected plausible pathogens. In 13 cases with follow-up plasma samples on day 5, a significant decline of mcfDNA quantities was demonstrated (median 4,015[56,289] on day 1 to 604[1,425]
on day 5, p<0.001). Conclusions: Metagenomic sequencing in plasma and ETA samples showed good concordance between the blood and lung compartments as well as with culture results in pneumonia patients. Metagenomics revealed potential pathogens missed by cultures in 38% of culture-negative pneumonias and suggested polymicrobial infections in several cases of culture-positive pneumonias. Further research is needed to evaluate the clinical utility of real-time metagenomics for pneumonia diagnosis in mechanically ventilated patients for guidance of targeted antibiotic prescriptions.

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