Deep Sequencing of 16S RNA Gene Amplicons to Screen Umbilical Cord Blood of Preterm Infants

Leena B. Mithal, MD,1 Michael Malczynski, BS,2 Stefan J. Green PhD3, Chao Qi, PhD4, Ram Yoge, MD1, and Karen Mestan, MD1,4

1Pediatrics, Northwestern University Feinberg School of Medicine; 2Clinical Microbiology Laboratory, Northwestern Memorial Hospital; 3Center for Genomic Research, University of Illinois at Chicago; 4Pathology, Northwestern University Feinberg School of Medicine. Chicago, IL, United States.

ABSTRACT

Background: Early onset sepsis (EOS) causes morbidity and mortality in preterm infants, yet diagnosis is inadequate. 16S RNA gene sequencing on cord blood can be a culture-independent method of pathogen detection that may improve EOS diagnosis. Uniprot and Moptapso spp. have been isolated from CB with severe clinical significance, and POM is a more sensitive. Cord blood normally sterile? Our objective was to compare Sanger (S) and next generation (NGS) 16S RNA sequencing results of CB from preterm infants with confirmed sepsis (cEOS), presumed sepsis (PS) and no sepsis (control).

Methods: This is a nested case-control study within a prospective cohort with archived CB collected by sterile venipuncture at birth. Infants (31-33 weeks gestation) were selected - antibiotics,ULT, and intrauterine infection and chorioamnionitis. For Sanger sequencing (S), a positive band was defined, after DNA extraction, 16S RNA sequencing using S and NGS. BLAST and QIME were used for annotation of sequences and microbe identification.

RESULTS: Postnatal EOS cases were E. coli and S. epidermidis. S identified 4 of 8 EOS cases in CB; NGS identified 7 of 8 EOS cases. One EOS case did not identify S, but identified elevated levels of Ureaplasma, Gardnerella, Neisseria, and Leptotrichia. NGS identified 10 of 22 patients as having a predominant bacterial taxon. Ureaplasma, Neisseria, Acholeplasma, and Thaxtomonas were the most common taxa. Alpha diversity analyses were performed using the Greenine 3.1 and reference database22. Principal Component Analysis and nMDS did not separate the two groups; in contrast, beta diversity was determined using the “R” software.

Alpha diversity analyses

- Shannon index: S16S (kg) genus level values are significantly different across groups (Kruskal-Wallis test, p < 0.001)
- Pair-wise comparison of M between SERS and controls is also significant (Mann-Whitney test, p < 0.001)

CONCLUSIONS

- Cord blood 16S RNA gene amplification from genomic DNA coupled with deep sequencing (NGS) detected the pathogenic organism in all cases of EOS. A complex microbiota was often detected, which may indicate that other organisms (not just the primary pathogen) are present when infections occur.
- Patience were detected in some EOS patients with negative postnatal blood cultures.
- Sanger and NGS aligned in these cases.
- Alpha diversity was significantly higher in infants with infection than controls, and low diversity is a daily indicator of the presence of a dominent pathogen.
- Bacterial taxa associated with vaginal, oral, and gastrointestinal flora were detected in cord blood of controls. This may represent the presence of low or non-pathogenic and perhaps commensal bacteria.

in summary, NGS detected bacterial taxa that were not identified through cultivation or with cultivation-independent PCR and Sanger sequencing. Direct Sanger sequencing (without cloning) can be negatively impacted when elevated microbial diversity is present. Deep sequencing can be used to delineate the presence and role of rare or novel organisms in EOS, or to better characterize mixed microbial communities.

Limitations of this technology for identification of infection include:
- High sensitivity, potential for contamination
- Unclear significance of levels of potentially pathogenic organisms
- Inability for species level identification
- Inability to detect viruses and microaerophilic organisms

According to this preliminary data, cord blood does not appear to be sterile. The significant of bacterial DNA in these cord blood samples is unclear. Further investigation is warranted to study maternal microbial community composition of samples at gestational age and birth weight.

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