Utility of Next generation sequencing cell free DNA testing for diagnostic utility in children

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Introduction

- Next-generation sequencing of microbial cell-free DNA (NGS cfDNA) testing has the potential to quickly diagnose multiple pathogens.
- Negative predictive value of NGS cfDNA is not well characterized, but has been described as helpful in excluding infection diagnosis.

Study Aims:

- Describe utility of NGS cfDNA testing in healthy cases vs cases with specific underlying illnesses.
- Characterize utility of NGS cfDNA testing in quick and accurate diagnosis in children.
- Provide information on utility of NGS cfDNA to exclude infection.

Methods

Study Design & Setting: Retrospective chart review of 93 consecutive pediatric patients with first-time NGS cfDNA testing at a free-standing 240-bed children's hospital and affiliated clinic in Central Texas.

Variables: Detailed chart review included the following: demographics, patient medical history, underlying illness, diagnosis at the time of testing, conventional laboratory testing and timing, medical treatment, and NGS cfDNA test results for clinical relevance or incongruent negative results compared to conventional testing.

Chart Review Methods:

Step 1: Retrospective chart review and preliminary determination of utility of NGS cfDNA testing to plan of care. Determination of utility was selected as "Clinically useful, likely useful, unlikely useful or not useful" by pediatric infectious diseases RN research coordinator and pre-med student.

Step 2: Pediatric infectious disease nurse practitioner involved in the care of these patients reviewed the initial assessment for agreement with step 1.

Step 3: Detailed chart review to clarify cases initially determined to be "likely or unlikely useful" by pediatric infectious diseases nurse practitioner and RN research coordinator.

Step 4: Pediatric infectious diseases MD provider review of select cases unable to be categorized as clearly "useful" or "not useful" to the plan of care through steps 1-3.

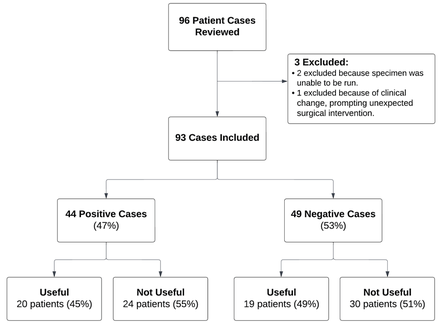

Figure 1. Case inclusion and overall categorization.

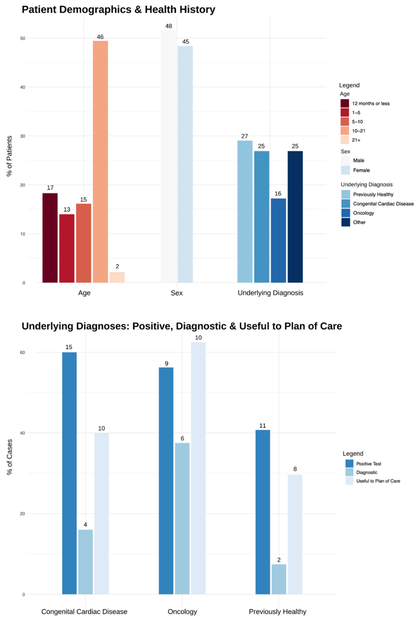

Figure 2: (2A) Demographics (ages, sex, underlying diagnosis) (2B) Underlying diagnosis and % positive and useful.

Results

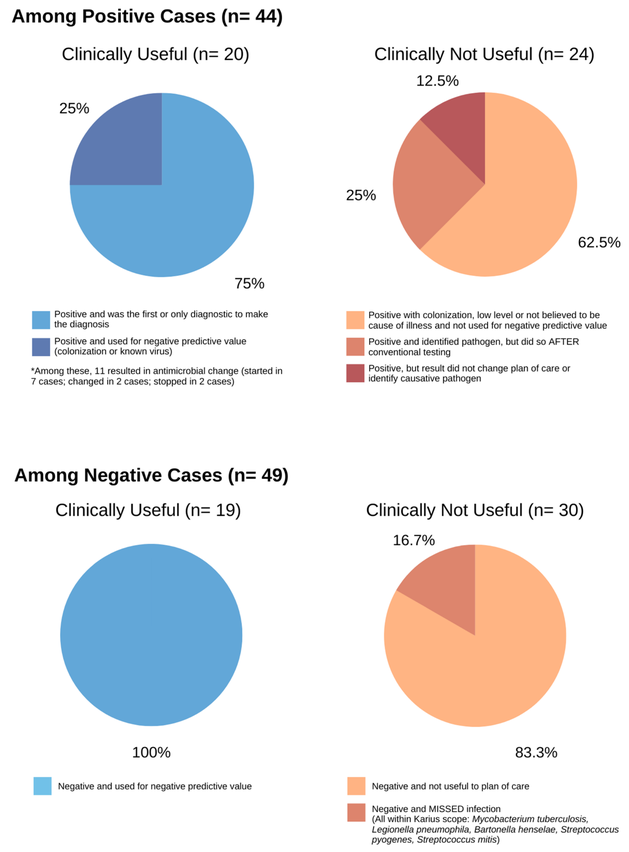

Figure 3: Clinical utility of positive and negative tests.

Results

- **15 (16%) cases:** NGS cfDNA testing identified a pathogen responsible for illness exclusively, or before conventional testing.
  - **10 (67%) of above cases:** Patient had underlying oncology or congenital cardiac disease.
- **11 (12%) cases:** NGS cfDNA testing used to change antibiotics (new antibiotic started in 7 cases; changed or de-escalated in 2 cases; stopped in 2 cases).
- **24 (26%) cases:** NGS cfDNA helped exclude infection as a diagnosis (5 positive for low level pathogens; 19 negative).
- **5 (5%) cases:** NGS cfDNA test was negative, but conventional testing identified causative pathogens, which are detectable in this test.

Missed Diagnosis	Description of testing
Symptomatic Probable *Legionella* Pneumonia	NGS cfDNA negative. On the same date *Legionella* IgG 1:1024. IgG decreased to 1:12 1 month later after treatment. Indicative of current or recent past infection.
Streptococcus pyogenes meningitis	NGS cfDNA negative. Within 1 day *S. pyogenes* + on CSF by broad range PCR. CSF culture from 10 days prior to NGS cf DNA test positive for *S. pyogenes*
Bartonella henselae	NGS cfDNA negative. One the same day, *Bartonella henselae* serology positive (IgM and IgG)
Streptococcus mitis infection r/t baclofen pump (in CSF and Abdominal fluid)	NGS cfDNA negative. CSF + for *S. mitis* by broad range PCR collected 2 days prior; *S. mitis* + on abdominal fluid by 16S testing 2 days prior.
MTB meningitis/disseminated TB	NGS cfDNA negative. 9/14; MTB PCR + from CSF 4 days later and from lymph node collected one day after.
ESBL-producing *E.coli* infection	NGS cfDNA correctly identified *E.coli*, but unable to detect drug resistant patterns at this time.

Conclusions

- NSG cfDNA testing identified pathogens quickly and correctly in 16% of cases.
- Most often diagnostic in cases with underlying cardiac or oncology diagnosis.
- NGS cfDNA testing was helpful to exclude infection.
- In 5% of cases, the diagnosis was missed by NGS cfDNA testing.
- NGS cfDNA testing can provide valuable information regarding diagnosis.
- We caution against using this test as a single evaluative measure to exclude infection, particularly in cases where disease is suspected at sequestered sites outside the bloodstream.