Clinical Utility of a Next Generation Sequencing Test for Pathogen Detection in Pediatric Central Nervous System Infections

Nanda Ramchandar, MD, MPH,[1] Nicole G. Coufal, MD, PhD,[1,2,3] Anna S. Warden, PhD[7], Benjamin Briggs, MD, PhD,[4] Rita Stinnett, PhD,[4] Robert Schlaberg, MD, PhD,[4] Jennifer Foley, BSN,[3] Christina Clarke, RN,[1] Bryce Waldeman, BA,[1] Claudia Enriquez,[5] Stephanie Osborne,[5] Antonio Arietta, MD,[5] Daria Salyakina, PhD,[6] Michelin Janvier,[6] Prithvi Keshavamurthy, MD,[6] Balagangadhar R. Totapally, MD,[6] David Dimmock, MD,[1] Charlotte Hobbs, MD, PhD,[1] Stephen F. Kingsmore, MB[1], Lauge Farnaes, MD, PhD[1*]

[1]Rady Children's Institute for Genomic Medicine, [2]Department of Pediatrics, University of California, San Diego, [3]Rady Children's Hospital San Diego,[4]IDbyDNA, Salt Lake City, UT, [5]Children's Hospital of Orange County, [6]Nicklaus Children's Hospital[7-] Department of Cellular and Molecular Medicine, University of California, San Diego

Nanda Ramchandar,
nramchandar@rchsd.org,
858 576 1700, ext 5331

Introduction

- Pediatric central nervous system (CNS) infections are potentially life-threatening and may incur significant morbidity.
- Identifying a pathogen is important, both in terms of guiding therapeutic management, but also in characterizing prognosis.
- Standard care testing by culture, serology, and PCR is often unable to identify a pathogen. For encephalitis specifically, in over 50% of patients where an infectious etiology is suspected, standard care testing is unable to identify a pathogen.
- We examined use of next generation sequencing (NGS) of cerebrospinal fluid (CSF) in detecting an organism in children with CNS infections.

Methods

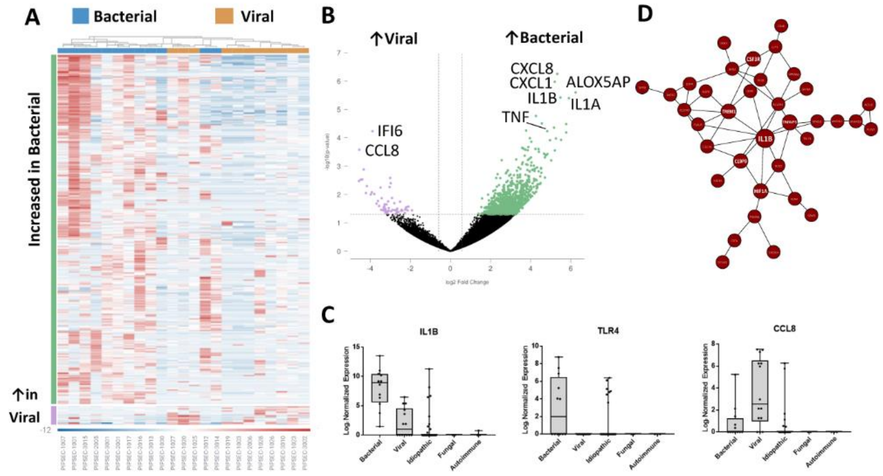
- This is a prospective multi-site study that enrolled children with a CSF pleocytosis and suspected CNS infection admitted to three tertiary pediatric hospitals.
- After standard care testing had been performed, the remaining CSF was sent for mNGS.

Results

- We enrolled 70 subjects over a 12-month recruitment period.
- A putative organism was isolated from CSF in 24 (34.3%) subjects by any diagnostic modality.
- NGS of the CSF samples identified a pathogen in 20 (28.6%) subjects.
- Using a reference composite of standard care testing, we determined the sensitivity and specificity of CSF NGS to be 83.3% (95% CI, 62.6-95.3%) and 91.3% (95% CI, 79.2-97.6%) respectively.

Male Sex, n (%)	40 (57.1)
Age (years), average (SD)	5.7 (5.9)
Immunocompromised, n (%)	4 (5.7)
Hispanic ethnicity, n (%)	41 (58.6)
Caucasian/White, n (%)	46 (65.7)
African-American/Black, n (%)	2 (2.9)
Asian, n (%)	2 (2.9)
Other, n (%)	12 (28.6)
Presenting Symptoms	
Fever, n (%)	51 (72.9)
Vomiting, n (%)	28 (40.0)
Seizures, n (%)	16 (22.9)
Lethargy, n (%)	30 (42.9)
Altered Mental Status, n (%)	26 (37.1)
VP shunt, n (%)	6 (8.6)
Received antibitoics prior, n (%)	28 (40.0)
CSF Parameters	
Nucleated cells cells/µL, median (IQR)	8.85 (35.5-513.5)
Erythrocytes cells/µL,median(IQR)	12 (6.0-368.0)
Protein mg/dL, median(IQR)	3 (41.8-168.0)
Glucose mg/dL, median(IQR)	43 (40.0-56.0)
Length of Stay, median days (IQR)	6 (3.0-18.5)
Death, n (%)	3 (4.3)
Total Patients	70

Demographics



A. Heat map comparing PIPSEC patients with bacterial and viral diagnoses identified 409 differentially expressed genes with FDR > 0.05 and fold change ≥ 1.5. B. Volcano plot identifying differentially expressed genes between bacterial and viral meningitis. C. Box and whisker plot (10-90%) of expression. D.STRING-based visualization of highly connected genes within the WCGNA co-expression module 25 that was upregulated in bacterial meningitis patients.

	% positivity	sensitivity	specificity
CSF culture	17.1%	50.0 (95% CI, 29.1-70.9%)	100 (95% CI, 92.3-100.0%)
CSF standard care	34.3%	100 (95% CI, 85.7-100%)	93.5(95% CI, 82.1-98.6%)
CSF mNGS	28.6%	83.3 (95% CI, 62.6-95.3%)	91.3 (95% CI, 79.2-97.6%)

Percent (%) positivity, sensitivity, and specificity of CSF diagnostic modalities

standard of care work up	standard care and mNGS both TN*	standard care and mNGS both TP**	results discordant: standard care TP, mNGS FN†	results discordant: standard care FP, mNGS TN‡
PCR only	0	11	1	3
culture only	0	4	3	0
PCR and culture	0	5	0	0

* true negative, ** true positive, †false negative, ‡true negative

Comparison of cerebrospinal fluid (CSF) culture, CSF standard care testing, and CSF mNGS results

Conclusions

- In this multi-site site prospective study of pediatric CNS infections, mNGS testing of the CSF was similar to standard care in identifying a putative pathogen.
- While certain standard care tests may remain more sensitive depending on the etiologic pathogen and the specific disease process, mNGS may have value as an adjunctive diagnostic tool and may even supplant standard care testing in some situations.
- Further studies are required to clarify the best use of mNGS in the evaluation of pediatric CNS infections.

Funding Source

- Funding Source: This work was supported by IDbyDNA, Salt Lake City, UT (NCT03796546)

References

1. Tunkel AR, Hartman BJ, Kaplan SL, et al. Practice guidelines for the management of bacterial meningitis. Clin Infect Dis Off Publ Infect Dis Soc Am **2004**; 39:1267–1284.
2. Britton PN, Dale RC, Blyth CC, et al. Causes and Clinical Features of Childhood Encephalitis: A Multicenter, Prospective Cohort Study. Clin Infect Dis Off Publ Infect Dis Soc Am **2019**;
3. Tunkel AR, Glaser CA, Bloch KC, et al. The management of encephalitis: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis Off Publ Infect Dis Soc Am **2008**; 47:303–327.
4. Wilson MR, Sample HA, Zorn KC, et al. Clinical metagenomic sequencing for diagnosis of meningitis and encephalitis. N Engl J Med **2019**; 380:2327–2340.
5. Meningitis/Encephalitis Panel. Clin Infect Dis **2018**; 67:1125–1128.
6. Hong NTT, Anh NT, Mai NTH, et al. Performance of Metagenomic Next-Generation Sequencing for the Diagnosis of Viral Meningoencephalitis in a Resource-Limited Setting. Open Forum Infect Dis **2020**; 7:ofaa046.
7. Lepennetier G, Hracsko Z, Unger M, et al. Cytokine and immune cell profiling in the cerebrospinal fluid of patients with neuro-inflammatory diseases. J Neuroinflammation **2019**; 16:219.