

Metagenomic Next-Generation Sequencing for Pathogen Detection and Transcriptomic Analysis in Pediatric Central Nervous System Infections

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Background. 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 and in characterizing prognosis. Usual care testing by culture and polymerase chain reaction is often unable to identify a pathogen. We examined the systematic application of metagenomic next-generation sequencing (mNGS) for detecting organisms and transcriptomic analysis of cerebrospinal fluid (CSF) in children with central nervous system (CNS) infections.

Methods. We conducted a prospective multisite study that aimed to enroll all children with a CSF pleocytosis and suspected CNS infection admitted to 1 of 3 tertiary pediatric hospitals during the study timeframe. After usual care testing had been performed, the remaining CSF was sent for mNGS and transcriptomic analysis.

Results. We screened 221 and enrolled 70 subjects over a 12-month recruitment period. A putative organism was isolated from CSF in 25 (35.7%) subjects by any diagnostic modality. Metagenomic next-generation sequencing of the CSF samples identified a pathogen in 20 (28.6%) subjects, which were also all identified by usual care testing. The median time to result was 38 hours.

Conclusions. Metagenomic sequencing of CSF has the potential to rapidly identify pathogens in children with CNS infections.

Keywords. encephalitis; meningitis; metagenomics; next-generation sequencing; pediatric.

Pediatric central nervous system (CNS) infections are potentially life-threatening. Mortality and morbidity occur in up to 28% and 56% of patients, respectively [1, 2]. Central nervous system infections encompass a range of manifestations that vary based on pathogen [3]. Identifying a pathogen is vital, both in terms of guiding therapy and in characterizing prognosis. Culture and polymerase chain reaction (PCR) assays are frequently unable to identify a pathogen. For encephalitis, in more than 40% of patients, usual care testing is unable to identify a pathogen [3–5]. In bacterial meningitis, culture may be negative after antibiotic pretreatment, a common occurrence [2, 6]. Polymerase chain reaction offers rapid and sensitive testing, but it is restricted to the

pathogens for which the platform is targeted. Given the limitations of available diagnostics, “Pan-omic” platforms offer promise [7, 8]. One such modality is metagenomic next-generation sequencing (mNGS) for pathogen deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) [9, 10]. This methodology poses the opportunity to broadly evaluate for pathogens by testing a single specimen. Turnaround times have shortened, making mNGS techniques more applicable as a method of achieving a timely diagnosis. We conducted a prospective, multisite, study to evaluate DNA sequencing (DNA-seq) and RNA sequencing (RNA-seq) of cerebrospinal fluid (CSF) for identification of pathogens in children with a suspected CNS infection.

METHODS

Trial Design and Oversight

Pediatric Infectious Disease Precision Medicine Using Sequencing Evaluation of CSF ([PIPSEC] ClinicalTrials.gov Identifier NCT03796546) was a prospective, multisite study to evaluate DNA and RNA sequencing of CSF for identification of pathogens in subjects who had undergone evaluation for a CNS infection. The members of the research team had final responsibility for the trial design and oversight.

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Patient Consent Statement

Written consent was obtained from subjects or their guardian. The Western Institutional Review Board and the Children's Hospital of Orange County Institutional Review Board provided human subject protection oversight.

Eligibility Criteria

Eligible subjects were less than 18 years of age undergoing evaluation for CNS infection and found to have a CSF pleocytosis. Suspicion for CNS infection was determined based on the clinical impression of the ordering provider. Cerebrospinal fluid pleocytosis was defined as a white blood cell count (WBC) >15 cells/ μ L with <5000 cells/ μ L red blood cells in the same sample [11]. A protocol deviation was approved for 2 subjects with 14 cells/ μ L WBCs in the CSF at the request of the study site principle investigator (PI).

Study Outcomes

The primary aim of this study was to investigate the diagnosis rate of mNGS for pathogen detection in CSF samples compared with usual care testing performed on CSF samples from the same subject [12]. Usual care testing consisted of all CSF diagnostic testing performed on the case in a CAP/CLIA certified laboratory (see Supplement Table 1). Usual care testing was done at the discretion of the clinical team and thus varied by subject. Data were extracted from the electronic medical record. Site PIs were additionally sent clinician surveys to assess clinical utility of mNGS results in relation to usual care. The treating physician, site PIs, and study PI then reviewed the CSF mNGS results in comparison to usual care testing from CSF and clinical presentation to determine the likelihood that the identified organism was indeed putative. Each mNGS result was identified as a true positive (TP), false positive (FP), true negative (TN), or false negative (FN) relative to usual care testing from CSF (composite of PCR, culture, and other CSF testing) as the reference standard. Determination of TP or TN was made when mNGS and usual care testing were concordant. In the event of discordant results, mNGS was compared with the reference standard composite of usual care testing. If mNGS identified an organism that was not identified by usual care testing, this was considered an FP. If mNGS failed to identify the organism identified by usual care testing, this was considered an FN. The *P* values for comparison of means were calculated using the Student *t* test.

Sequencing

Cerebrospinal fluid samples were collected from the laboratory after usual care testing had been performed. The minimum volume for mNGS testing was 0.5 mL. Samples for mNGS testing were stored at -80°C until they were shipped to the reference laboratory. Samples were shipped via express courier to achieve a less than 24-hour transit time. The CSF samples were

tested with research use only next-generation shotgun DNA-seq and RNA-seq protocols; the resulting data were analyzed with the Explify Platform (IDbyDNA Inc., Salt Lake City, UT) as previously described [10, 13, 14]. The DNA (after host depletion) and RNA were extracted separately from residual samples. The DNA and RNA sequencing libraries were prepared with the Nextera DNA Flex Library Prep Kit (Illumina, San Diego, CA) and sequenced on a NextSeq550 instrument (Illumina) using mid-output kits. In this study, a range of 8–18 total libraries, including external controls, were sequenced per run for a median depth of 9.98×10^6 and 1.1×10^7 single-end, 150-base pair sequencing reads per sample-derived DNA and RNA library, respectively. Sequencing reads were adapter-trimmed and quality-filtered as part of the Explify analysis. The assay was quality controlled using internal control organisms spiked into each sample at the lysis step and the inclusion of 3 external controls (positive, negative, and blank) in each sample batch. Sample results were released after performance metrics for internal and external controls were evaluated for data quality and quantity. Sequencing data analysis was performed with the Explify platform. The RNA-seq and DNA-seq data were analyzed together, and the final result for a given sample was based on evidence from either.

Transcriptomic Analysis

Host transcriptomic counts were analyzed using Rosalind (OnRamp Bioinformatics, San Diego, CA). Sample transcript counts were generated with Kallisto (v.0.46.0) normalized by relative log expression using DESeq2 R library [15]. Heatmaps generation and clustering were performed using the Partitioning Around Medoids methods using the fpc R library [16]. Differential gene expression (DEG) was considered as a fold change of more than 1.5 and a false discovery rate cutoff of 0.05 [17]. Hypergeometric distribution was utilized to analyze pathway enrichment, gene ontology, domain structure, and other ontologies. The topGO R library was used to determine local similarities and dependencies between gene ontology terms to perform Elim pruning correction [18]. Several databases were utilized for enrichment analysis including REACTOME, HOMER, and molecular signatures database (MSigDB) [19–21]. Weighted correlation network analysis (WGCNA) was performed using filtered \log_2 -transformed RNA-seq data [22]. This was applied to construct scale-free networks that specify gene modules [22]. To explore the modular structures of the coexpression network, the adjacency matrix was transformed into a topological overlap matrix [22]. The WGCNA parameters were as follows: minimum module size equal to 30 genes, tree-cut height set to 0.99. Significant modules were defined as those with a strong correlation ($r > 0.4$) and a $P < .05$. Interacting genes were visualized as a network using String version 11.0 (<http://string-db.org>). K-means clustering was used to determine gene clusters of interest and/

or highly connected genes within this isolated M25 network [23]. The mNGS data were pseudo-aligned to the human genome (assembly hg38) using Kallisto to yield host response transcriptomic data and then filtered for ribosomal RNA reads before downstream analysis. The sequencing depth for mapped reads varied from 30 000 reads to 5 million reads (mean = 1.5 million).

RESULTS

We screened 221 subjects at 3 study sites from January 2019 to January 2020 and enrolled 70 subjects (Supplement Figure 1). See Table 1 for demographic information. The mNGS of the CSF samples identified a pathogen in 24 subjects. Twenty of these were deemed to be putative (TP) versus 25 by usual care testing of CSF (Table 2 and Table 3). Two subjects, one with acute disseminated encephalomyelitis and another with autoimmune encephalitis, received a noninfectious diagnosis. Nine subjects had enterovirus meningitis. Discordant results that the treating physician deemed to be FP results by mNGS were identified in 4 subjects. The organisms identified

for these subjects included *Cryptococcus* spp, *Staphylococcus warneri*, *Mucor circinelloides*, and *Streptococcus bovis*. In these 4 cases, usual care testing, including gold standard CSF culture, did not identify an organism, and the treating clinician did not deem the mNGS result to be clinically relevant. In 5 subjects, CSF mNGS was deemed falsely negative with relation to usual care CSF testing. Two involved an infected ventriculoperitoneal shunt, one due to *Staphylococcus epidermidis* and the other to *Staphylococcus aureus* (both identified on culture). Upon chart review, the site of CSF sampling was not clear for either case (the sample may have been obtained proximally or distally from the shunt). Upon review of the mNGS data, both organisms were detected, but they did not meet prespecified thresholds for reporting. In the third case, PCR for Epstein-Barr virus (EBV) was positive from the CSF at just 151 IU/L. The subject improved without antiviral therapy. In the fourth case, CSF culture from a subject with chronic meningoencephalitis recovered a single colony of *Cryptococcus neoformans* and CSF cryptococcal antigen was positive. A prolonged antifungal course resulted in a full recovery. In the fifth case, human herpes virus 6 (HHV-6) was identified on a CSF multiplex PCR panel. This patient had full clinical recovery and antiviral therapy was not initiated. Of note, the HHV-6 target from the multiplex PCR panel was positive for 2 other subjects. In one subject, described above (Subject 2016), the CSF culture was also positive for *S aureus* and was accordingly adjudicated as an mNGS false negative. In the other subject (Subject 1004), a dedicated HHV-6 PCR was negative, and this subject was adjudicated as “concordant CSF mNGS and CSF usual care” and therefore an mNGS TN. Dedicated HHV-6 PCR was not sent for any other subjects.

Cerebrospinal fluid culture identified a putative organism in 12 cases (17.1%). A CSF PCR multiplex panel was utilized for 51 subjects, 15 (29.4%) of which were positive. In one subject with a negative culture and multiplex PCR panel, 16S broad-range PCR and mNGS both detected *Streptococcus dysgalactiae*. Median time to result from the time sample was received for CSF mNGS was 38.0 hours (interquartile range [IQR], 32.6–60.5 hours). The mean CSF nucleated cells was higher in the subjects for whom a pathogen was detected by mNGS in comparison to subjects for whom no organism was detected (1499.6 vs 410.7 cells/ μ L, $P = .05$). Mean CSF protein value (228.3 vs 184.4 mg/dL, $P = .02$) and mean glucose level (38.2 vs 52.6 mg/dL, $P = .004$) were also both higher in subjects for whom an organism was detected by mNGS versus subjects for whom no organism was detected. Based on survey results, clinicians indicated that CSF mNGS helped in the management in 17.1% of cases. In 10 of these cases, the negative mNGS results were used to buttress the clinical impression that a treatable infectious etiology was not missed by CSF testing. In one case, the clinician explicitly stated that the mNGS result was used to provide clearance for surgical intervention. In another case, the clinician was

Table 1. Research Subject Demographic Characteristics^a

Male Sex, n (%)	40 (57.1)
Age (years), median (IQR)	3.8 (0.2–11.8)
Rady Children’s Hospital San Diego, CA, n (%)	31 (44.3)
Children’s Hospital Orange County, CA, n (%)	24 (34.3)
Nicklaus Children’s Hospital, FL, n (%)	15 (21.4)
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 antibiotics prior, n (%)	28 (40.0)
CSF Parameters	
Nucleated cells cells/ μ L, median (IQR)	109 (35.5–513.5)
Erythrocytes cells/ μ L, median (IQR)	16 (6.0–368.0)
Protein mg/dL, median (IQR)	97 (41.8–168.0)
Glucose mg/dL, median (IQR)	47.5 (40.0–56.0)
Length of stay, median days (IQR)	6 (3.0–18.5)
Admitted to ICU, n (%)	37 (52.9)
Death, n (%)	3 (4.3)
Total Patients	70

Abbreviations: CSF, cerebrospinal fluid; ICU, intensive care unit; IQR, interquartile range; VP, ventriculoperitoneal.

^aNOTE: Two unique tubes of residual CSF were received for 1027. Each was processed and analyzed separately by the reference laboratory; both samples produced the same result. The read counts listed in the table represent the mean sequencing reads for these 2 samples.

Table 2. Comparison of Subjects for Whom CSF Testing Was Positive by Any Method

Subject	CSF Culture	HSV PCR	EV PCR	CSF Multiplex PCR	CSF Other Test (Positive Result)	CSF mNGS	Total Sequencing Reads	Number of Reads for Identified Organism
Concordant CSF mNGS and CSF Usual Care Results								
1001	<i>Haemophilus influenzae</i>	negative	not done	not done		<i>H influenzae</i>	2.85E + 07	5.17E + 04
1003	no organisms	positive	not done	HSV		HSV	2.38E + 07	9.80E + 01
1007	<i>Enterococcus faecalis</i>	not done	not done	not done		<i>E faecalis</i>	2.63E + 07	2.05E + 04
1017	<i>Streptococcus agalactiae</i>	not done	not done	<i>S agalactiae</i>		<i>S agalactiae</i>	1.81E + 07	5.61E + 05
1019	no organisms	not done	positive	not done		Enterovirus	1.54E + 07	2.26E + 02
1020	no organisms	negative	positive	Enterovirus		Enterovirus	1.84E + 07	1.43E + 03
1023	no organisms	negative	positive	not done		Enterovirus	2.27E + 07	3.34E + 02
1025	no organisms	not done	positive	Enterovirus		Enterovirus	1.60E + 07	9.90E + 01
1026	no organisms	negative	positive	Enterovirus		Enterovirus	1.72E + 07	8.70E + 01
1027	no organisms	negative	positive	not done		Enterovirus	1.60E + 07	1.27E + 04
1028	no organisms	not done	positive	not done		Enterovirus	1.67E + 07	1.30E + 01
1030	<i>Streptococcus pneumoniae</i>	negative	not done	<i>S pneumoniae</i>		<i>S pneumoniae</i>	2.22E + 07	5.53E + 03
2001	<i>S agalactiae</i>	not done	not done	not done		<i>S agalactiae</i>	2.26E + 07	1.58E + 05
2005	<i>S pneumoniae</i>	not done	negative	<i>S pneumoniae</i>		<i>S pneumoniae</i>	3.17E + 07	5.73E + 05
2006	no organisms	not done	not done	Enterovirus		Enterovirus	2.41E + 07	1.98E + 03
3001	<i>Neisseria meningitidis</i>	not done	not done	<i>N meningitidis</i>		<i>N meningitidis</i>	2.11E + 07	3.71E + 02
3002	no organisms	not done	not done	Enterovirus		Enterovirus	1.48E + 07	3.40E + 01
3012	no organisms	not done	not done	negative	16S PCR <i>Streptococcus dysgalactiae</i>	<i>S dysgalactiae</i>	2.11E + 07	8.29E + 05
3013	<i>S agalactiae</i>	not done	not done	negative		<i>S agalactiae</i>	1.16E + 07	8.44E + 02
3015	<i>S pneumoniae</i>	not done	not done	<i>S pneumoniae</i>		<i>S pneumoniae</i>	1.34E + 07	1.19E + 05
False-Positive CSF mNGS Results								
1009	no organisms	not done	not done	not done		<i>Cryptococcus</i> spp	2.25E + 07	5.54E + 02
2003	no organisms	not done	not done	not done		<i>Staphylococcus warneri</i>	3.06E + 07	1.17E + 03
2011	no organisms	not done	not done	not done		<i>Mucor circinelloides</i>	2.27E + 07	6.77E + 04
2022	no organisms	not done	not done	negative		<i>Streptococcus bovis</i>	2.20E + 07	1.41E + 02
False-Negative CSF mNGS Results								
1016	<i>Cryptococcus neoformans</i>	not done	not done	negative		no pathogens detected	2.37E+07	-
2015	no organisms	not done	not done	HHV-6		no pathogens detected	1.33E + 07	-
2016	<i>Staphylococcus aureus</i>	not done	not done	HHV-6		no pathogens detected	1.83E + 07	-
3010	no organisms	not done	not done	negative	EBV (PCR)	no pathogens detected	1.85E + 07	-
3014	<i>Staphylococcus epidermidis</i>	not done	not done	Negative		no pathogens detected	1.25E + 07	-

Abbreviations: CSF, cerebrospinal fluid; EBV, Epstein-Barr virus; EV, enterovirus; HHV-6, human herpes virus 6; HSV, herpes simplex virus; mNGS, metagenomic next-generation sequencing; PCR, polymerase chain reaction.

reassured by concordance between the positive mNGS and usual care result both identifying the same viral pathogen.

Transcriptome Analysis

In addition to pathogen data, mNGS also generated incidental host transcriptomic data. Data were compared for all subjects using multidimensional scaling and did not globally cluster by diagnosis gender, age, CSF white blood cell count, or neutrophil predominance (Supplemental Figure 2A). A comparison of subjects diagnosed with viral (N = 12) compared with bacterial (N = 11) meningitis identified 409 differentially expressed genes (DEGs) (Figure 1) and overall clustered by infectious diagnosis. The DEGs included up-regulation of genes identified in host response to viral and bacterial infection, including interleukin 1A/B and interleukin 8 in bacterial meningitis. Gene ontology analysis identified pathways that included immune response and both leukocyte and neutrophil activation (Supplemental Figure 2). Interleukin 1B ([IL1B] *P*-adjusted value [p-adj] = .004) and CXCL8 (p-adj = .0002) were most associated with bacterial meningitis, and CCL8 (p-adj = .007) was most associated with viral meningitis compared with all other patient samples (Figure 1C). The WGCNA pathway analysis identified a statistically significant module of 68 coexpressed genes (Dark red module, Supplementary Figure 3). Quantitative trait analysis was not significant for any other patient features. Taken together, gene ontology and WGCNA analysis suggest that IL1B may be a key regulator of the proinflammatory response observed in bacterial meningitis (Supplement Figure 4). Diagnostic performance was assessed using receiver operator characteristic curves with a corresponding area under the curve of 0.95 (95% confidence interval, 0.89–0.998) for IL1B (Figure 1D). A subanalysis of the FN mNGS results suggested infection based on host response in 2 of these 4 patients.

Post Hoc Cost Analysis

The median cost of usual care testing was \$531.09 (IQR, \$439.00–\$721.09). An average of 3 usual care CSF tests (including culture) were sent per subject. Per-sample direct cost of CSF mNGS laboratory testing is dependent on the number of samples being run at a time and is estimated to range between \$390.00 and \$2000.00. In this study, no more than 5 CSF samples were sequenced per run to expedite the turnaround time. Per-sample direct cost for shotgun sequencing can be decreased by an order of magnitude through optimization of the balance between analytical sensitivity, test volume, and turnaround time considerations.

DISCUSSION

We describe application of metagenomics in the diagnosis of pediatric CNS infections that included all consenting subjects meeting enrollment criteria rather than only a referred subset.

Table 3. Evaluation of a Metagenomic Assay for Detection of CSF Pathogens Against a Composite Reference of Usual Care Testing^a

CSF mNGS % Positivity	28.6%
CSF mNGS positive predictive agreement	80.0 (95%, 59.3–93.2%)
CSF mNGS negative predictive agreement	91.1 (95%, 78.8–97.5%)
% agreement (kappa)	87.1 (0.80)

Abbreviations: CSF, cerebrospinal fluid; mNGS, metagenomic next-generation sequencing.

^aEach mNGS result was identified as a true positive, false positive, true negative, or false negative relative to usual care testing from CSF (composite of polymerase chain reaction [PCR], culture, and serologic testing for each subject) as the reference standard. A culture was sent for every subject. Dedicated enterovirus PCR was sent for 19 subjects, and dedicated herpes simplex virus PCR was sent for 13 subjects. A multiplex PCR was sent for 52 subjects. A dedicated Epstein-Barr virus PCR was sent for 1 subject. Broad-range universal PCR was sent for 1 subject.

As described in previous studies, an infectious etiology was not identified for most subjects with suspected CNS infection [5]. A putative organism was isolated from CSF in 25 (35.7%) subjects, and, of these, mNGS of the CSF samples identified a pathogen in 20 (28.6%) subjects (Table 3). Using the above-described adjudication scheme, FP results by mNGS were identified in 4 subjects, and, in these subjects, usual care testing did not identify a pathogen. In 5 cases, a putative organism was recovered by usual care testing of the CSF, but not by CSF mNGS. Two of these cases involved hardware infection, and, in another subject with chronic meningoencephalitis, a single colony of *C neoformans* was isolated from CSF. In the fourth subject, although EBV was detected by PCR, the viral load was just 151 IU/L. The fifth case was a positive result for HHV-6 identified by CSF PCR multiplex panel, which is of unclear clinical significance (previous studies have described FP results for this target) [24]. In these cases, burden of organism was likely low even before initiation of antimicrobial therapy, which may indicate that, for some organisms and in certain circumstances, culture and PCR remain more sensitive [8]. Upon review of the subjects with hardware infections (Subjects 2016 and 3014) deemed as mNGS FNs, a signal for *S aureus* and *S epidermidis* was detected but below the reporting threshold (Supplement Figures 5 and 6). Limitations in the application of this study, including imperfect sampling, may have further degraded the sensitivity of mNGS.

Cerebrospinal fluid sampling is important for management of CNS infections. Sensitivity of culture in bacterial meningitis is high, but it is diminished if CSF is obtained after initiation of antibiotics [6]. Culture is less sensitive for fungi and mycobacteria. Polymerase chain reaction may demonstrate high sensitivity for certain targets, but it requires clinicians to suspect the organism before testing, which may lead to missed diagnoses and to broad and potentially unnecessary testing. Metagenomic next-generation sequencing may be used to evaluate for the presence of many pathogens with a single test. Wilson et al [9] examined the use of mNGS in 204 subjects with suspected CNS infections. Among 58 subjects in whom an organism was identified, mNGS identified 13 (22%) that were not identified

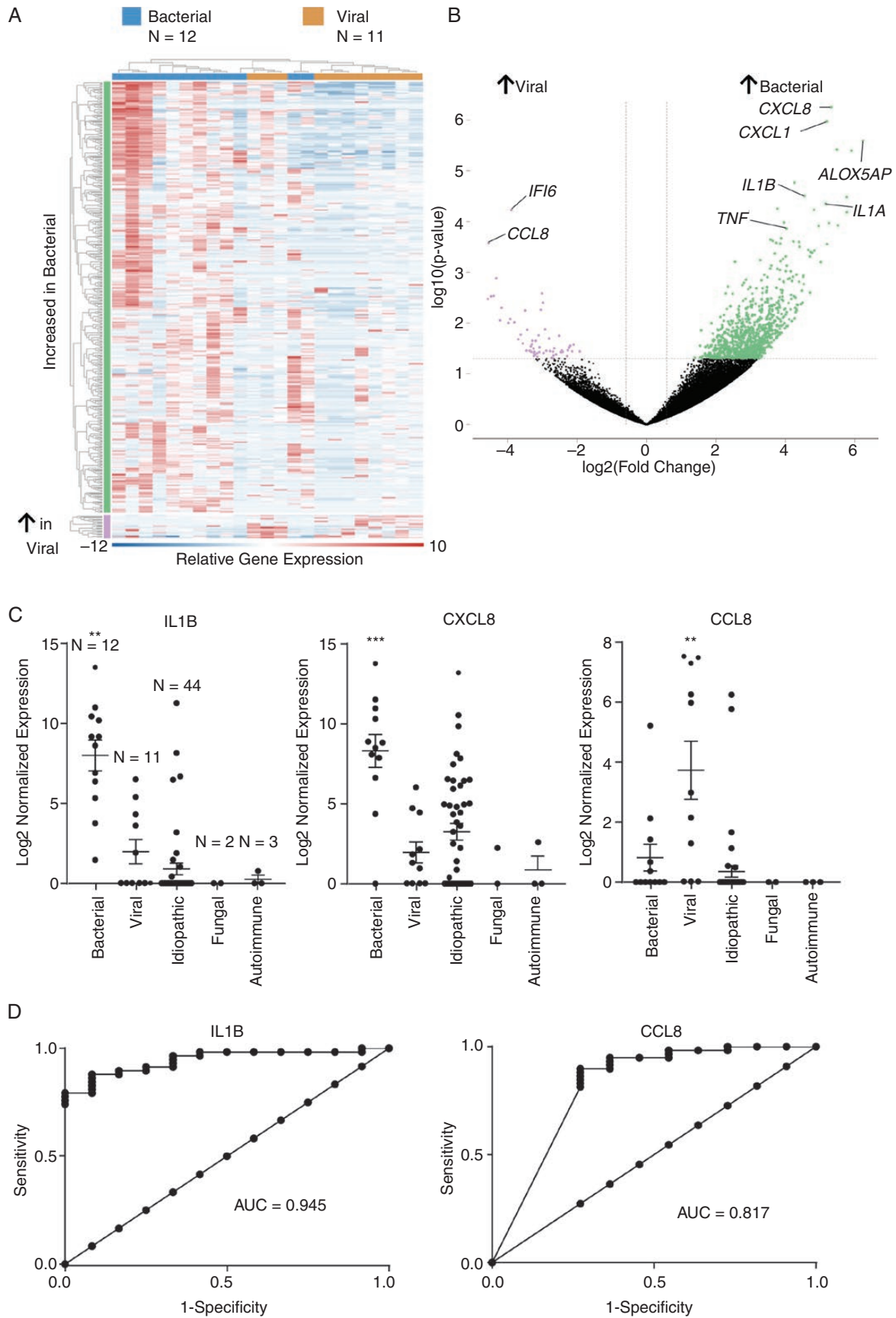


Figure 1. (A) A heat map comparing Pediatric Infectious Disease Precision Medicine Using Sequencing Evaluation of CSF (PIPSEC) subjects with bacterial and viral diagnoses identified 409 differentially expressed genes with false discovery rate >0.05 and fold change ≥ 1.5 . (B) Volcano plot identifying differentially expressed genes between

by usual care testing. A notable difference in this cohort from our study is the older study population, many of whom were immunocompromised. In addition, many of these subjects were recruited after an extensive evaluation, which increased the likelihood that mNGS would recover an uncommon pathogen where usual care had already failed to discover an etiology. The subjects recruited in our study were more often previously healthy, and therefore they were more likely to have infection with common pathogens. Metagenomic next-generation sequencing was additionally used as a first-tier test in conjunction with usual care. This decreased the likelihood that mNGS would detect an organism not identified by usual care testing. In a cohort more similar to our study, Hong et al [25] evaluated mNGS for CNS infections in a resource-limited setting, and they found that mNGS of the CSF identified a pathogen in 14 of 19 CSF samples that were positive by PCR. Using PCR as the reference assay, they calculated a sensitivity and specificity of 74% and 66%, respectively. Our data show that mNGS offers similar diagnostic yield, although to fully understand the limit of detection and the importance for clinical care, larger studies will be needed, especially as mNGS techniques involving enrichment and depletion technologies are refined. Metagenomic next-generation sequencing may be better utilized as an adjunct to current usual care testing, especially when usual care testing has already failed to recover a pathogen. In addition, although cost has been a limiting factor in the adoption of mNGS, if mNGS is ultimately shown to provide similar results to usual care in a shorter time at a similar price point, mNGS may prove to be more attractive [26]. Turnaround time can additionally be improved by local implementation of mNGS testing, a model that has become more common in tertiary care centers.

Transcriptome analysis was used to evaluate host expression of genes associated with the immunologic response. Subjects with bacterial meningitis were found to have a distinct pattern of gene expression compared with those with a viral etiology. It is notable that CXCL8, CXCL1, tumor necrosis factor (TNF), and interleukin 1A/B have been found to be expressed in bacterial meningitis [27–29]. In contrast, CCL8 has been found to be associated with viral infections [27, 30, 31]. The results of our analysis show increased expression of CXCL8, CXCL1, and TNF in the subjects with a bacterial infection and increased expression of both CCL8 and IFI6 in subjects with viral infection (Figure 1). The WGCNA independently identified interleukin 1B as a hub gene in the host response to bacterial meningitis, which suggests that it may regulate proinflammatory gene expression. Interleukin 1 genotype has been implicated as contributing to the risk of fatal meningococcal meningitis [32]. The clinical utility of these findings needs to be clarified with further

studies, but, potentially, host response transcriptome analysis may prove useful in characterizing inflammatory response associated with specific pathogens and in attributing pathogenicity when an organism is identified [28, 33–37]. Furthermore, characterizing host response may additionally inform the differential diagnosis when an organism is not found.

This study has several limitations. The epidemiology of CNS infections may reflect geographic distribution, limiting generalizability. The number of enrollees is also likely too small to adequately capture rarer pathogens associated with CNS infections. Adjudication of results as putative or not with respect to a composite of usual care tests used to create a reference standard was hampered by the variability in the types of testing sent. Although every subject had CSF culture sent, the choice to use serologic- or molecular-based assays was purely at the discretion of the clinical team, and the validity of comparing mNGS results to a standard reference that is not uniform throughout the cohort is limited. In addition, characterization of mNGS results as FP are based on published epidemiology of CNS infection in children, which are biased toward historic norms and limited by the sensitivity of usual care testing modalities (including CSF culture). Furthermore, we were unable to utilize adjunctive orthogonal testing such as targeted or broad-range PCR due to unavailability of additional CSF sample. False-positive mNGS results may be attributable to sample misidentification, contamination introduced during sample collection or processing, biases within reference databases, or the analysis tools used. For the determination of a TN specifically, by using the criteria of no organism detected by any testing method, we may have incorrectly adjudicated subjects with organisms that were either in low abundance or difficult to isolate. In addition, subjects with suspected infection may instead have autoinflammatory or autoimmune phenomenon that may be challenging to differentiate from an infection [3, 9, 38, 39]. Cerebrospinal fluid parameters vary based on age, and the low cutoff used in the inclusion criteria may have allowed for enrollment of some subjects with physiologically normal CSF [11, 40]. In contrast, more indolent CNS infections may not generate a significant CSF pleocytosis. Finally, the protocol stipulated that only residual CSF be used for mNGS, which likely limited diagnostic yield, especially in cases in which burden of organism was lower [8].

CONCLUSIONS

In this prospective study of pediatric CNS infections, mNGS of the CSF identified an organism in 28.6% of subjects. Although certain usual care tests remain more sensitive for some targets, mNGS may have value as an adjunctive diagnostic tool, especially

bacterial and viral meningitis. (C) Scatter plot depicting mean, standard error of the mean, and individual values. ***P*-adjusted value (*p*-adj) < .01, ****p*-adj < .0001 compared with all other groups with a fold change of 10.0 for interleukin (IL)1B and 18.0 for CXCL8 in bacterial meningitis, and 14.0 for CCL8 in viral meningitis compared with all other samples. (D) Receiver operator characteristic (ROC) for IL1B and CCL8. AUC, area under curve.

in situations in which standard testing is known to have limited yield. Furthermore, this is specific to the sequencing and analysis methods used in this study, because sensitivity and specificity of mNGS vary depending on choice of wet laboratory methodology and analysis tools. Further studies are required to clarify the best use of mNGS in the evaluation of pediatric CNS infections.

Supplementary Data

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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