The comparative utility of metagenomic next-generation sequencing and universal PCR for pathogen detection from cerebrospinal fluid: a retrospective analysis from a tertiary care center

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Background

- Many neurologic syndromes are underpinned by infectious etiologies that are difficult to diagnose.[1]
- Broad-range universal PCR (uPCR) and metagenomic next-generation sequencing (mNGS) are emerging molecular techniques that may allow for enhanced pathogen detection in challenging cases.[2-5]
- The comparative clinical utility of uPCR and mNGS for pathogen detection from cerebrospinal fluid (CSF) has not previously been described.

Methods

- Electronic medical records at UCSF were searched for all patients who had mNGS and uPCR results available from the same CSF specimen.
- Using all available clinical information, patients' clinical episodes were assigned to one of four categories: 1) confirmed central nervous system (CNS) infection, 2) likely CNS infection, 3) confirmed or likely non-infectious etiology, 4) unknown etiology.
- It was also determined whether mNGS and/or uPCR results changed clinical management.

Results

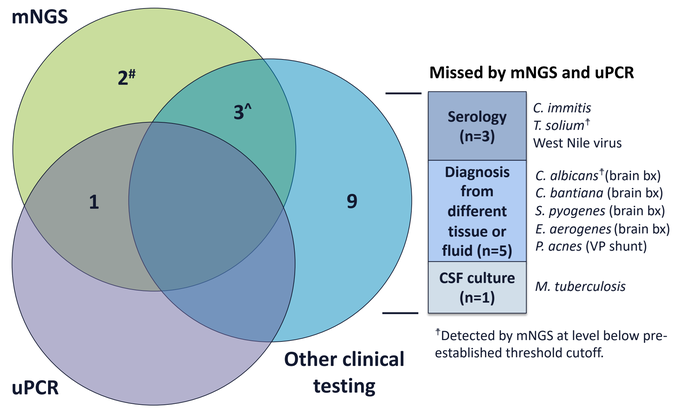
- We identified 75 patients with 77 paired mNGS and uPCR results from CSF.
- n=13/77 (16.9%) had a confirmed CNS infection underpinning their clinical presentation, n=11 (14.3%) had likely CNS infection, n=33 (42.9%) had a confirmed or likely non-infectious cause, and n=20 (26.0%) had etiologies that could not be determined.
- Of the 13 patients with confirmed CNS infection, n=4 (30.8%) were diagnosed by mNGS and n=1 (7.7%) by uPCR (**Figure 1)**.
- Most diagnoses missed by mNGS and uPCR were made from sites other than CSF or by CSF serology (**Figure 1).**
- After inclusion of sub-threshold positive results (n=2), mNGS detected a corresponding pathogen in n=6/13 (46.2%) of confirmed infections.
- Overall, mNGS detected a pathogen in n=10/77 (13.0%) cases, compared to n=4/77 (5.2%) using uPCR (**Table 1**). Among those with a positive mNGS result, n=6/10 represented a true or likely true positive result, while the remaining were likely contaminants. Of those with a positive uPCR result, n=1/4 represented a true positive result, while n=3/4 were likely contaminants.
- Clinical management was changed by the mNGS or uPCR result in two cases (**Table 1**).

Table 1. Overview of CSF mNGS and uPCR positive specimens

#	Age	Sex	Presentation	CSF culture results	CSF mNGS result	CSF uPCR result	Additional positive CNS microbiology	Final Diagnosis	How was diagnosis made?	mNGS Classification	uPCR Classification	Did mNGS or uPCR change management?
mNGS and uPCR positive for same organism												
1	82	M	Acute febrile illness, new brain brain lesion	Bact/AFB/ Fung neg	*Nocardia farcinica,* Cytomegalovirus	*Nocardia farcinica*	N/A	*Nocardia* brain abscess	uPCR and mNGS (CSF)	True positive (confirms diagnosis)	True positive (makes diagnosis)	Yes – tailored antibiotics after uPCR result
mNGS and uPCR positive for different organisms												
2	6	M	Encephalopathy, acute retinal necrosis	Bact/AFB/ Fung neg	*Bacteroides* sp., MW polyomavirus	*Aspergillus sp.* (not fumigatus	VZV-specific PCR (CSF)	VZV meningo-encephalitis with ocular involvement	Varicella zoster virus-specific PCR (CSF)	MW polyomavirus likely true positive (unclear clinical significance)*	Likely contaminant	No
3	72	F	Focal status epilepticus	N/A	*Staphylococcus epidermidis*	*Eurotium sp.*	N/A	Unknown	N/A	Likely contaminant	Likely contaminant	No
4	23	F	Recurrent encephalopathy	Bact neg	*Staphylococcus lugdunensis, Finegoldia, Corynebacterium, Anaerococcus, Streptococcus*	*Rhizobacter sp.*	N/A	Unknown	N/A	Likely contaminant	Likely contaminant	No
mNGS positive only												
5	36	M	Sub-acute, progressive left-hand weakness	N/A	JC virus, Epstein-Barr virus, Human immunodeficiency virus	Neg	JC virus specific PCR (CSF)	Progressive multifocal leuko-encephalopathy	JC virus-specific PCR (CSF)	True positive (confirms diagnosis)	N/A	No
6	54	F	Acute febrile illness, progressive weakness	Bact/Fung neg	West Nile virus	Neg	West Nile virus-specific PCR (CSF)	West Nile virus encephalitis	mNGS (CSF)	True positive (makes diagnosis)	N/A	Yes – stopped all antimicrobials after mNGS result
7	7	M	Acute Encephalopathy	Bact/AFB/ Fung neg	Varicella-zoster virus	Neg	Varicella-zoster virus-specific PCR (CSF)	Recurrent VZV meningo-encephalitis	Varicella-zoster virus-specific PCR (CSF)	True positive (confirms diagnosis)	N/A	No
8	32	M	Acute transverse myelitis	Bact/AFB neg	Human herpesvirus 7	Neg	N/A	Unknown	N/A	Likely true positive (unclear clinical significance)	N/A	No
9	39	F	New seizure disorder, persistent encephalopathy	Bact/AFB/ Fung neg	*Geobacillus sp., Enterobacter sp., Leuconostoc sp., Debaryomyces hansenii*	Neg	N/A	Unknown	N/A	Likely contaminant	N/A	No
10	16	F	Acute encephalopathy with respiratory failure	Bact neg	*Klebsiella pneumoniae, Enterobacter cloacae, and Citrobacter freundii*	Neg	N/A	Unknown	N/A	Likely contaminant	N/A	No

Abbreviations: 'AFB'= mycobacterial culture; 'Bact' = bacterial culture; CSF = cerebrospinal fluid; 'F' = female; 'Fung' = fungal culture; 'M'= male; mNGS = metagenomics next generation sequencing; TB= tuberculosis; uPCR = universal polymerase chain reaction. *MW polyomavirus isolated twice by mNGS on CSF and also confirmed by orthogonal sequencing

Figure 1. Overview of mNGS and uPCR results among patients with true-positive CNS infection (n=15)



†Detected by mNGS at level below pre-established threshold cutoff.

#Human herpesvirus 7 (HHV-7) and MW polyomavirus (MWPyV) confirmed by orthogonal PCR testing but of unclear clinical significance

^mNGS testing made diagnosis in 1 case (West Nile virus, WNV), confirmed diagnosis in 2 cases (JC virus (JCV) and varicella-zoster virus (VZV))

Conclusions

- mNGS had superior clinical utility to that of uPCR for pathogen detection in CSF samples, in large part because of additional ability to detect DNA and RNA viruses.
- mNGS and uPCR molecular testing alone are insufficient to replace all conventional microbiological testing for neurological infections, as some cases are diagnosable only by serology or analysis of a tissue or body fluid type other than CSF.
- Communication of sub-threshold pathogen detection results to treating physicians may enhance the utility of mNGS testing.
- Further studies are required to determine the clinical scenarios in which mNGS and/or uPCR testing is likely to have maximal diagnostic yield.

References

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