Microbial DNA testing for inflammatory diseases of the brain of uncertain etiology

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Summary

Neurologists may be confronted with patients who present with inflammatory brain lesions where the diagnosis cannot be made through history and physical examination alone. Molecular testing for bacterial infections, tuberculosis, and fungal infections may aid in the diagnosis. Since the treatments for these disorders are different and delays can result in permanent neurologic disability and death, rapid and accurate diagnoses are critical. This review provides the neurologist with testing options and recommends ways to enhance sensitivity and specificity.

We present a patient with an inflammatory brain lesion with unclear etiology. Universal bacterial PCR testing on a biopsy specimen allowed us to make the diagnosis of neurosyphilis.

Case report

A 27-year-old woman presented to a local hospital with complaints of headache for 3 weeks and an episode of right upper extremity weakness lasting about an hour. The outside facility obtained a brain MRI, which revealed large, diffuse, confluent, T2 hyperintense signals in the frontal lobes (figure 1). There was rim and nodular gadolinium enhancement with sparing of the gray matter. Given this unusual MRI scan, the patient was transferred to our facility.

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Figure 1 Brain MRI, with representative slices from different sequences

(A) Axial fluid-attenuated inversion recovery shows T2 hyperintensity that spares the gray matter. (B) Axial diffusion-weighted imaging, no restriction present. (C) Sagittal T1 postcontrast demonstrates meningeal enhancement. (D) Sagittal T1 postcontrast, lesion does not enhance, but there is nodular meningeal enhancement. (E) Sagittal T1 postcontrast, nodular meningeal enhancement.

The patient underwent 2 lumbar punctures. White blood cell counts were marginally elevated on both, with 5 and 7 white cells and lymphocytic predominance. Oligoclonal bands were positive and immunoglobulin G (IgG) index was elevated at 1.16. Notably, serum rapid plasma reagin (RPR) and fluorescent treponemal antibody absorption test (FTA-Abs) were positive. Her CSF Venereal Disease Research Laboratory (VDRL) was weakly positive. Since diagnostic workup was otherwise unrevealing, the patient had a brain biopsy. The biopsy showed mixed necrotizing granulomas as well as neutrophilic inflammation involving the dura and brain (figure 2).

While the initial neuropathology did not appear typical for syphilis, infectious disease consultants reported they had seen similar pathologic findings with hepatic syphilis. Brain tissue samples were sent to the University of Washington for further testing. Universal bacterial PCR assay of the 16S rRNA gene showed sequences consistent with *Treponema pallidum*. The patient was diagnosed with neurosyphilis and responded well to high-dose penicillin.

Availability and utility of universal bacterial PCR

Our case is an illustrative example of the utility of universal bacterial PCR. As described above, this technology was pivotal in securing a difficult diagnosis. There were many factors complicating this case: the initial brain MRI was unusual but not characteristic of any particular disease process, the patient was relatively young to have neurosyphilis considering its average 4- to 7-year latency period,¹ and the neuropathologic appearance was atypical, with granulomas but no gummas or direct visualization of spirochetes. While the positive serum RPR and FTA-Abs, CSF IgG abnormalities, and a weakly positive CSF VDRL suggested syphilis, we

Figure 2 Representative brain pathology slides, from surgical biopsy

(A) Dural granuloma with multinucleated giant cell. (B) Parenchymal granulomas. (C) Parenchymal necrosis. (D) Blood vessel damage with thrombosis. (E) Macrophages CD68 stain. (F) CD-3 stain T cells.

were unable to confirm that *T pallidum* was the definitive cause of her symptoms until the DNA test result was returned.

Neurologists are generally familiar with the use of PCR to diagnose specific viral infections using CSF, such as herpes simplex encephalitis and progressive multifocal leukoencephalopathy, but there is much less familiarity with the use of universal PCR to diagnose bacterial and fungal infections using brain tissue and CSF. Bacterial PCR technology screens for a range of pathogens rather than testing for specific, individual organisms.

In the case of universal bacterial PCR, the region identified by the probe, 16S rDNA, codes for a small ribosomal RNA. This region is unique because it is present in all bacteria (archaeobacteria and eubacteria) but divergent between species as well, since mutations have accumulated in this region between different bacterial species. The entire region is about 1,500 base pairs, but typically only 500 base pairs are needed to identify a particular species. Hence, clinicians do not need to order tests with a specific infectious bacterium in mind; rather, if their suspicion is high for bacterial infection (e.g., brain abscess, nonspecific inflammatory change), this test may be performed and a bacterial species identified.²

Microbial identification by sequence analysis of 16s DNA is accurate as well. Currently, laboratory errors in DNA interpretation occur in about 1/5,000–1/10,000 base pairs, which is infrequent enough that these errors should not have an effect upon species identification.² Another advantage to this technology is its relatively fast turnaround time: results can be procured in 1–2 days, as compared to culturing tissue, which can take many days and, depending on the bacterial species and prior antibiotic therapy, cultures may be negative. Novel species and their molecular similarities to other bacteria may also be identified by this technology.² The costs of these tests are summarized in table 1.³

There are limitations to PCR testing. Since PCR technology amplifies DNA regions of all bacteria in a sample, it does not work well for samples with mixed flora. In addition, DNA in the sample must also be stable and stored properly.⁴

Besides assessing brain tissue for bacterial infection, similar PCR technology may be pivotal in the identification of bacterial species causing meningitis. PCR has been compared to culture results in their ability to procure diagnoses. PCR often can identify the causative bacterial species, including tuberculosis, in patients with meningitis where cultures failed to produce any results.⁵

Table 1 Costs of testing for inflammatory infections, as quoted by the University of Washington (Hee Jin Oh, personal communication to acquire specific test costs, 2013)

Test name	Estimated cost
16s ribosomal testing on brain parenchyma or CSF (BCTPCR)	\$275.96
Fungal PCR testing on brain parenchyma or CSF (FUNPCR)	\$450.28
Tuberculosis identification/PCR on brain parenchyma or CSF (TBPCR)	\$202.65

PCR testing for *Mycobacterium tuberculosis* and fungal infections

PCR testing is now useful in diagnosing tuberculosis. Left untreated, tuberculosis meningitis is nearly 100% fatal, and delay in diagnosis can cause irreversible neurologic injury (table 2).⁶ Since conventional microbiologic methods for the detection of *M. tuberculosis* are slow and often negative, there has been considerable research to discover PCR probes that can be performed on CSF and produce a diagnosis. Current gene targets include *IS6110*, *groEL2*, *pstS1*, *devR*, *mpt64*, and *PPE*.⁷ The accuracy of PCR testing has been improving significantly as well. Newer assays, particularly nested, real-time PCR assays, are able to identify tuberculosis with as few as 2–3 bacilli, with sensitivities of 95.8% and specificities of 100%.^{6,8} Tests with this type of high sensitivity may decrease the need for high-volume CSF samples when testing for tuberculous meningitis.⁸

Fungal infections of the brain and meninges occur in both immunocompetent and immunocompromised hosts; the presentation may be cryptic and delay in diagnosis may result in poor function and even death.⁹ The most common pathogens, from most common to least common, were mucormycosis, cryptococcus, and aspergillosis.⁹ The diagnosis may be challenging because symptoms are nonspecific, the most common symptoms being headache, fevers, and decreased vision. While these infections are relatively uncommon, it is important for clinicians to be aware of them because they result in devastating morbidity and mortality; 28% of immunocompromised and 14% of immunocompetent patients died, even when appropriate treatments were rendered.⁹ Hence, we emphasize the gravity of this disease and the need for prompt and accurate diagnosis.

Prior to the availability of PCR-based detection, fungal species were difficult to identify. Fungal cultures would take weeks to grow and occasionally results were inconclusive. Hence, there was a clear need for the development of DNA-based PCR methods of identification. The internal transcribed spacer 2 (ITS2) region of fungal DNA is a reliable sequence that can be used for the identification of fungal species. A study at the University of Washington demonstrated that when both the ITS1 and ITS2 regions are utilized, 98% of fungal species may be identified.^{10,11} These studies clearly show that similar to bacteria and tuberculosis, fungal identification through PCR technology is accurate and clinically useful.

Proper specimen handling

In practical terms, clinicians can help to ensure the highest yield possible from brain tissue samples by ensuring proper handling and timely shipment to the testing laboratory.

When a sample is being tested for bacterial or fungal DNA, unfixed, frozen tissue shipped on dry ice is the best way to ensure high specificity and sensitivity during laboratory interpretation. Formalin-fixed tissue can be used for PCR testing, but the DNA is often of poor quality, which lowers the sensitivity and specificity of the assay. If specimens are immediately frozen after biopsy, both sensitivity and specificity approach 100% (Karen Stephens, PhD, personal communication). In contrast, cases stored in the nonbuffered formalin and embedded paraffin had highly degraded, poor DNA samples.¹¹ Hence, when obtaining

Table 2 Tests for the detection of tuberculosis

Test ⁶⁻⁹	Sensitivity/specificity	Drawbacks
Smear microscopy	Sensitivity 0%–40%, depending on the laboratory	Unable to differentiate between <i>Mycobacterium tuberculosis</i> and nontuberculous bacteria
Culture identification	Sensitivity 0%–80%, depending on the laboratory	A total of 4–8 weeks to produce results, requires skilled technicians
Pathologic examination		Unable to differentiate between other diseases that produce granulomas, such as sarcoid, leprosy, or systemic lupus erythematosus, unless there are acid-fast bacteria present
Tuberculosis skin testing		False-positive results if patient has received BCG vaccine, false-negatives in immunocompromised patients and the elderly
Interferon-γ release assay		Unable to differentiate between latent and active tuberculosis

biopsy material on which PCR testing may be wanted, it is important that neurologists communicate the need for frozen tissue to neurosurgeons and pathologists prior to the biopsy.

RNA testing is presently not useful for diagnostic purposes but may become useful in the future. The possible applications of RNA quantification and analysis are presented here. RNA integrity is also affected by postbiopsy or postmortem preservation techniques.¹³ RNA quantification and analysis may be important in studies of gene expression profiles of postmortem, human brain tissues. In many neurodegenerative disorders, such as Parkinson disease and Alzheimer disease, RNA analysis may give a better idea of cellular trafficking.¹² RNA extracted from frozen tissue shipped on dry ice shows better preservation when compared to nonfrozen tissue.¹⁴ While there is some speculation that RNA degradation may occur at different rates depending on the transcript type or gene family, studies of the 28S rRNA (an important region that identifies fungus) demonstrate similar rates of degradation throughout brain tissue.¹⁵

RNA integrity may be quickly compromised when tissue is thawed. Prior studies demonstrate that while RNA remains relatively stable 1 hour post thaw, it quickly degrades.^{14,16} After 8 hours post thaw, RNA is severely compromised; RNA is almost completely degraded 24 hours post thaw.¹⁴ Current recommendations maintain that RNA must be used 1–2 hours post thaw to ensure RNA stability. Therefore, frozen sections are the best way to preserve DNA integrity and RNA stability, provided that studies to quantify and qualify RNA are well planned and executed soon after brain tissue is thawed.

In summary, bacterial and fungal DNA and RNA sequences may be reliably identified from brain tissue specimens. The practicing neurologist should be aware of the following recommendations when ordering microbial studies and interpreting the results of diagnostic testing.

- Universal DNA sequence testing for bacteria and fungi testing may be performed on all brain specimens; in fact, it can be performed on any tissue from a normally sterile site
- Specimens placed in formalin, paraffin, or refrigeration are less usable and have lower yield of DNA because these methods tend to degrade DNA
- The best specimens are frozen specimens; they yield the highest specificity and sensitivity
- RNA analysis may be performed on frozen samples, ideally 1–2 hours post thaw

Since PCR technology amplifies DNA regions of all bacteria in a sample, it does not work well for samples with mixed flora.

- If a specimen is fixed, pathologists must indicate in their report how long a specimen was fixed; this will aid the laboratory personnel performing the PCR in determining the specificity and sensitivity of the results
- Fungal and bacterial PCR testing are available at the University of Washington, Knight Diagnostic Labs, and the Mayo Clinic (Zach Zaret, personal communication, 2013).

REFERENCES

1. Kent ME, Romanelli F. Reexamining syphilis: an update on epidemiology, clinical manifestations, and management. *Ann Pharmacother* 2008;42:226–236.
2. Clarridge JE. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clin Microbiol Rev* 2004;17:840–862.
3. Oh H, J Hee. Question about costs. 2013. http://depts.washington.edu/molmicdx/mdx/available_tests.shtml. Accessed September 10, 2013.
4. James G. PCR for Clinical Microbiology. Dordrecht: Springer Netherlands; 2010:209–214.
5. Pandit L. Diagnosis of partially treated culture-negative bacterial meningitis using 16S rRNA universal primers and restriction endonuclease digestion. *J Med Microbiol* 2005;54:539–542.
6. Takahashi T, Tamura M, Asami Y, et al. Novel wide-range quantitative nested real-time PCR assay for *Mycobacterium tuberculosis* DNA: clinical application for diagnosis of tuberculous meningitis. *J Clin Microbiol* 2008;46:1698–1707.
7. Mehta PK, Raj A, Singh N, Khuller GK. Diagnosis of extrapulmonary tuberculosis by PCR. *FEMS Immunol Med Microbiol* 2012;66:20–36.
8. Kulkarni SP, Jaleel MA, Kadival GV. Evaluation of an in-house-developed PCR for the diagnosis of tuberculous meningitis in Indian children. *J Med Microbiol* 2005;54:369–373.
9. Sethi PK, Khanna L, Batra A, et al. Central nervous system fungal infections: observations from a large tertiary hospital in northern India. *Clin Neurol Neurosurg* 2012;114:1232–1237.
10. Traboulsi RS, Kattar MM, Dbouni O, Araj GF, Kanj SS. Fatal brain infection caused by *Aspergillus glaucus* in an immunocompetent patient identified by sequencing of the ribosomal 18S–28S internal transcribed spacer. *Eur J Clin Microbiol Infect Dis* 2007;26:747–750.
11. Chen Y-C, Eisner JD, Kattar MM, et al. Polymorphic internal transcribed spacer region 1 DNA sequences identify medically important yeasts. *J Clin Microbiol* 2001;39:4042–4051.
12. Ferrer I, Armstrong J, Capellari S, et al. Effects of formalin fixation, paraffin embedding, and time of storage on DNA preservation in brain tissue: a BrainNet Europe study. *Brain Pathol* 2007;17:297–303.
13. Popova T, Mennerich D, Weith A, Quast K. Effect of RNA quality on transcript intensity levels in microarray analysis of human post-mortem brain tissues. *BMC Genomics* 2008;9:91.
14. Ross J. Messenger RNA turnover in eukaryotic cells. *Mol Biol Med* 1988;5:1–14.
15. Ross BM, Knowler JT, McCulloch J. On the stability of messenger RNA and ribosomal RNA in the brains of control human subjects and patients with Alzheimer's disease. *J Neurochem* 1992;58:1810–1819.
16. Yasojima K, McGeer EG, McGeer PL. High stability of mRNAs postmortem and protocols for their assessment by RT-PCR. *Brain Res Brain Res Protoc* 2001;8:212–218.

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