Molecular and Histologic Diagnosis of Central Nervous System Infections

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KEYWORDS

• Meningitis • Encephalitis • 16S rRNA • Internal transcribed spacer
• Metagenomic next-generation sequencing

Key points

- A wide range of microorganisms, including bacteria, mycobacteria, fungi, viruses, and parasites, can infect the central nervous system and cause significant morbidity and mortality.
- Diagnosis of CNS infections from surgical biopsies requires integration of histologic findings, including special stains and immunohistochemistry, with cultures, molecular diagnostics, and other laboratory and clinical findings.
- Molecular diagnostics performed on formalin-fixed paraffin-embedded tissue can be targeted at individual pathogens, classes of microorganisms, panels of organisms, or be completely unbiased.

ABSTRACT

Infections of the central nervous system cause significant morbidity and mortality in immunocompetent and immunocompromised individuals. A wide variety of microorganisms can cause infections, including bacteria, mycobacteria, fungi, viruses, and parasites. Although less invasive testing is preferred, surgical biopsy may be necessary to collect diagnostic tissue. Histologic findings, including special stains and immunohistochemistry, can provide a morphologic diagnosis in many cases, which can be further classified by molecular testing. Correlation of molecular, culture, and other laboratory results with histologic findings is essential for an accurate diagnosis, and to minimize false positives from microbial contamination.

OVERVIEW

The central nervous system (CNS) can be infected by a variety of microorganisms, resulting in significant morbidity and mortality. A variety of bacteria, mycobacteria, fungi, viruses, and parasites have been reported to cause disease, with significant variations in incidence associated with geography, age, and immune status. Some highly virulent pathogens cause disease in otherwise healthy individuals, whereas many others opportunistically infect people with compromised immune systems. People living with human immunodeficiency virus (HIV), organ transplant recipients, immunotherapy-treated individuals, and infants and the elderly are all at increased risk for a variety of infections. The most common route of CNS infection is hematogenous, with symptoms that are predominantly neurologic or manifest as part of a disseminated systemic disease. Infections can also be spread by local extension from adjacent structures (eg, paranasal sinuses), retrograde axonal transport, and introduced by trauma or surgery. Symptoms, which can range from mild headaches to herniation and death, depend on the specific areas of brain or spinal cord involved, acuity of the infection, and any prior immunity acquired by the patient.

Diagnosis of CNS infections largely relies on testing performed in the clinical laboratories. Examination of cerebrospinal fluid (CSF) collected via lumbar puncture can provide insight into

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Surgical Pathology 13 (2020) 277–289
https://doi.org/10.1016/j.path.2020.01.001

possible infectious etiologies based on opening pressure, color, protein, glucose, and cell counts. Further testing including cultures, serology, and molecular diagnostics can identify specific microorganisms, which is critical for the selection of appropriate antimicrobial therapy. When less invasive testing is nondiagnostic, surgical sampling of brain or spinal cord tissue is performed, either as a core biopsy, or as a larger resection in conjunction with efforts to relieve elevations of intracranial pressure. Standard histopathologic evaluation of tissue, including special stains, can provide a great deal of information about infections through identification of characteristic inflammatory patterns and by direct visualization of organisms. Further characterization is achieved by immunohistochemistry and the selective use of targeted and broad-spectrum molecular assays. This review highlights the utility of molecular testing for the diagnosis of CNS infections, presents recommendations for histologic screening to guide molecular assay selection, and suggests strategies for integration of unexpected molecular findings (**Table 1**). Although several examples are discussed, a comprehensive list of diagnostic features for all possible CNS infections is beyond the scope of this article, and consultation with infectious disease textbooks or pathologists is recommended.[1–3]

BACTERIAL INFECTIONS

EPIDEMIOLOGY AND LABORATORY DIAGNOSTICS

Bacterial meningitis is the most common CNS infection, and affects 0.7 to 40 per 100,000 people per year depending on geographic location.[4] In the absence of vaccination, Group B *Streptococcus*, *Streptococcus pneumoniae*, *Listeria monocytogenes*, *Escherichia coli*, *Neisseria meningitides*, and *Haemophilus influenza* are the most commonly identified organisms with varying frequency from newborns to young adults.

Table 1
Overview of histologic features and broad-spectrum molecular assays for diagnosis of CNS infections

Organism	H&E	Special Stains	Immunohistochemistry	Molecular Assays
Bacteria	Cocci and bacilli variably visible	Gram, Warthin-Starry, Steiner, PAS (*Tropheryma whipplei*)	*Treponema*	16S rRNA
Mycobacteria	Rarely detectable	Ziehl-Neelsen, Fite-Faraco	Not widely used	16S rRNA, *hsp65*, *rpoB*, IS6110 (*Mycobacterium tuberculosis*)
Fungi	Yeast and hyphae often visible as eosinophilic or negatively staining structures	GMS, PAS, Gram (*Candida*), Mucicarmine/ Fontana-Masson (*Cryptococcus*)	Not widely used	28S rRNA D1/D2, ITS
Viruses	Characteristic cytoplasmic or nuclear inclusions present for some viruses	Luxol fast blue (PML)	HSV, VZV, EBV (ISH), CMV, adenovirus, polyomavirus	mNGS
Parasites	Diagnosis based predominantly on morphologic features	Giemsa, acid fast (cestode hooklets), GMS/PAS (ameba cyst walls)	*Toxoplasma*	mNGS

Abbreviations: CMV, cytomegalovirus; EBV, Epstein-Barr virus; GMS, Grocott-Gomori methenamine–silver nitrate stain; H&E, hematoxylin and eosin; HSV, herpes simplex virus; ISH, in situ hybridization; ITS, internal transcribed spacer; mNGS, metagenomic next-generation sequencing; PAS, periodic acid–Schiff; PML, progressive multifocal leukoencephalopathy; VZV, varicella zoster virus.

Parenchymal involvement, most often as cerebral abscess or cerebritis, is caused by *Staphylococcus aureus*, *Streptococcus* spp., Enterobacteriaceae, anaerobes, and may be polymicrobial.[5] CSF or brain tissue cultures are typically the test of choice for diagnosis, allowing for species-level identification and phenotypic information on antibiotic susceptibilities. The recent integration of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry into routine clinical laboratory use has markedly improved the accuracy and speed of diagnosis once an organism has been isolated, and assays to directly test CSF are currently under development.[6] In addition, individual organisms or panels of common pathogens can be selectively targeted for rapid diagnosis (eg, Biofire ME panel, Salt Lake City, Utah).[7]

HISTOLOGIC FEATURES

Compared with autopsy series, surgical tissue is rarely obtained for the diagnosis of bacterial meningitis. Characteristic histologic features include abundant neutrophilic infiltration of the leptomeninges, and infarction of cortex because of compromised blood flow through surface vessels may be present. Abscess tissue may be collected during surgery to relieve intracranial pressure caused by mass effect, and to rule out neoplastic processes or other noninfectious etiologies. Lesions vary from millimeter-sized microabscesses to multi-centimeter-sized lesions. A necrotic center is typically surrounded by a rim of neutrophils and dying tissue, although inflammation may be limited in immunocompromised patients (**Fig. 1**A). Early lesions may only show neutrophilic inflammation in the form of cerebritis, which may be biopsied to diagnose a radiologic abnormality.[8] Depending on the acuity of the infection and extent of preceding antibiotic treatment, organisms may not be observed, even in the setting of positive cultures or molecular testing. Some bacteria (cocci and bacilli) are seen on routine hematoxylin and eosin (H&E) stains as eosinophilic or basophilic structures, but must be distinguished from necrosis and apoptotic debris. Gram-positive organisms including staphylococci and streptococci are best visualized with tissue Gram stains, such as Brown-Brenn and Lillie-Twort, which can also highlight Gram-negative organisms with varying success (**Fig. 1**B). Silver stains including Warthin-Starry and Steiner can identify a wide variety of organisms, but are particularly useful for Gram-negatives and spirochetes (**Fig. 1**C). Grocott-Gomori methenamine–silver nitrate stain (GMS), primarily used to identify fungi, can also stain bacteria, including Gram-positive organisms that have stained Gram-negative because of antibiotic treatment effects. Periodic acid–Schiff (PAS) can highlight *Tropheryma whipplei* bacilli within macrophages. Although numerous antibodies for bacteria are available, the large degree of cross-reactivity between species and genera limits widespread adoption because of limited clinical utility. One notable exception is antitreponemal antibodies for the diagnosis of syphilis and other spirochete-associated diseases with increased sensitivity compared with silver stains.[9] *In situ* hybridization for the bacterial 16S rRNA gene has been investigated as a research tool, but not yet implemented as a diagnostic test for CNS bacterial infections.[10] Morphologic descriptions including shape, Gram status, and arrangement (eg, Gram-negative diplococci) can help guide empiric antibiotic therapy, and should be correlated with available culture and molecular results (eg, morphologically compatible with *N. meningitides* isolated in culture). Given the frequency of polymicrobial or multiple concurrent infections, caution should be used in making definitive statements regarding organism identifications in most circumstances.

MOLECULAR DIAGNOSTICS

When cultures are negative or not attempted, molecular testing of formalin-fixed paraffin-embedded (FFPE) tissue is performed to identify potential pathogens including bacteria. Unless a specific organism is suspected, the broad range test of choice is sequencing of the 16S rRNA gene, an approximately 1500 base pair gene that contains multiple conserved and variable regions useful for phylogenetic classification and clinical diagnosis.[11] Although sequencing the full gene produces the greatest amount of information, this is difficult to achieve in formalin-treated tissue because of DNA fragmentation and shorter reads.[12] A large proportion of pathogenic species are distinguished by sequencing the V1 and V2 regions consisting of an approximately 250 base pair sequence.[13] Clinical and Laboratory Standards Institute guidelines have been developed for genus and species identifications made by 16S sequencing, which specify percentage agreement with reference sequences and difference from next closest species (eg, 98.5% with 1.0% to next closest species).[14] False positives with 16S sequencing may occur because of contamination at multiple points in the gross pathology, histology, and molecular laboratories.[15] Therefore, extreme caution should be used in interpreting molecular results of unusual organisms, particularly when there is a discrepancy with histologic findings. All such cases should be rereviewed,

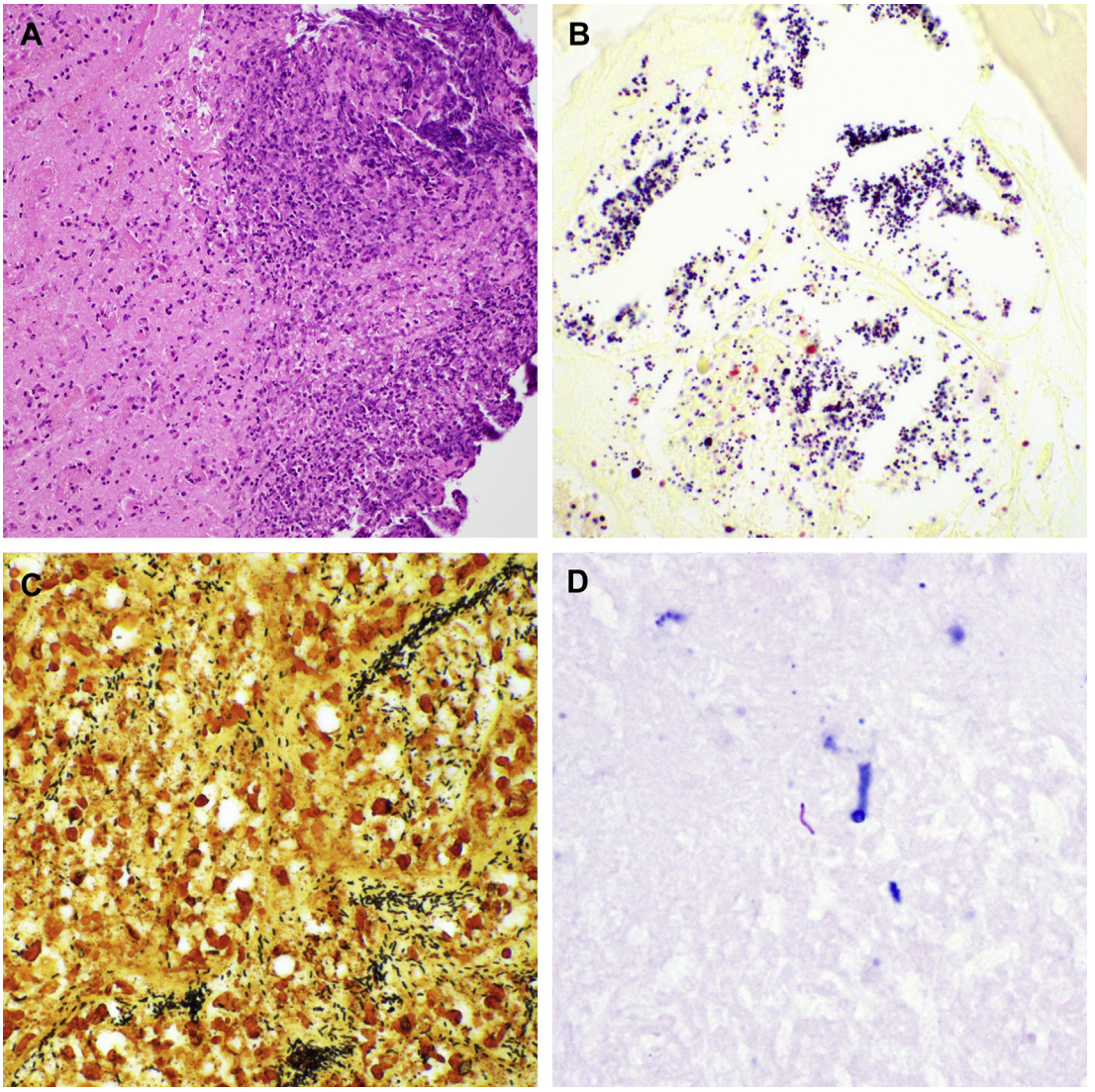

Fig. 1. Bacterial and mycobacterial infections. (*A*) This frontal lobe biopsy shows brain with reactive changes and abscess formation, findings suggestive of an infectious process. Correlation with special stains, concurrent culture results, or testing by 16S rRNA gene sequencing is necessary for confirmation and further classification of a bacterial infection (hematoxylin-eosin, original magnification ×200x). (*B*) Gram stain (original magnification ×400x) of a bone flap from a prior craniotomy highlights clusters of Gram-positive cocci, morphologically compatible with *Staphylococcus aureus* isolated in culture. (*C*) Warthin-Starry stain (original magnification ×400x) of subdural material shows multiple bacilli that are negative on Gram stain (not shown), morphologically compatible with *Pseudomonas aeruginosa* isolated in culture. (*D*) Ziehl-Neelsen staining (original magnification ×1000x) of a right frontal dural-based mass with necrotizing granulomas highlights rare acid-fast bacilli, consistent with *Mycobacterium tuberculosis*.

and an interpretative comment stating the possibility of contamination is added in an addendum (eg, the molecular findings of *Meiothermus silvanus* [a Gram-negative bacillus] is discordant with the presence of Gram-positive cocci observed histologically, raising the possibility of a false-positive molecular finding caused by laboratory contamination).

MYCOBACTERIAL INFECTIONS

EPIDEMIOLOGY AND LABORATORY DIAGNOSTICS

Mycobacterial infections of the CNS are typically caused by *Mycobacterium tuberculosis*, which can manifest as tuberculous meningitis,

tuberculoma, or abscess.[16] Less commonly, nontuberculous mycobacteria (NTM) is involved, and *Mycobacterium bovis* bacillus Calmette-Guérin vaccine strain is a rare cause of disseminated disease.[17] *Mycobacterium leprae* affects peripheral nerves, but has not been reported to have a significant impact on the CNS.[18] Many *Mycobacterium* spp. are slow-growing, taking weeks to months for culture isolation from CSF or brain tissue.[19] Treatment typically requires greater than 6 months with multiple antibiotics, which is determined empirically by species identification or through phenotypic testing.[20] CNS involvement, although rare, can often be suspected in the setting of active pulmonary or disseminated disease, which is rapidly diagnosed by polymerase chain reaction (PCR) of sputum, bronchoalveolar lavage fluid, or other tissue samples.[21]

HISTOLOGIC FEATURES

Biopsies of dural-based or parenchymal lesions typically show necrotizing granulomas, comprised of a necrotic center surrounded by a rim of multinucleated giant cells, lymphocytes, and plasma cells. Lesions early in development may show a neutrophilic predominance. Some NTMs exhibit a large number of histiocytes containing numerous organisms. The presence of mycobacteria is demonstrated by acid-fast staining methods, such as Ziehl-Neelsen and Fite-Faraco, in which carbol fuchsin is retained in the cell walls after acid decolorization (**Fig. 1**D). Although mycobacteria show some variations in length and width, these features, particularly in tissue sections that contain transected organisms, are not reliable for speciation. Mycobacterial immunostains are commercially available, but have limited specificity and must be interpreted in the appropriate histopathologic context.[22] Partially acid-fast organisms including *Nocarida* spp. can also stain with modified acid-fast stains, a distinguishing feature from *Actinomyces* spp., both of which are filamentous Gram-positive bacteria that are detected by Gram and GMS stains.

MOLECULAR DIAGNOSTICS

Because of the slow-growing nature of many mycobacteria, molecular testing of FFPE has been used to provide a more rapid species-level diagnosis to help guide antibiotic therapy. Similar to other bacteria, 16S rRNA sequencing is used to distinguish many *Mycobacterium* spp. Additional targets with increased interspecies variability include *heat shock protein 65* (*hsp65*) and beta subunit of RNA polymerase (*rpoB*), the latter of which can also detect common rifampin resistance mutations.[23] IS6110 is an 81-base pair insertion sequence present in multiple copies in many *M. tuberculosis* strains.[24] Although the yield of molecular testing is lower when organisms are not identified histologically, the sensitivity of some assays is high enough to detect rare organisms that may not be present on the examined sections.[22] In these cases, molecular testing must be guided by the overall histologic findings and clinical suspicion.[25] Numerous NTMs are present in the environment and can cause false positives if FFPE samples are contaminated, highlighting the need for caution in interpreting unusual organisms, particularly in immunocompetent individuals.

FUNGAL INFECTIONS

EPIDEMIOLOGY AND LABORATORY DIAGNOSTICS

Fungal infections of the CNS are typically spread hematogenously through dissemination of angioinvasive skin or lung infections.[26] Less commonly, infections may occur through local invasion, such as from the paranasal sinuses into the frontal lobes. A wide variety of organisms can cause disease, some as true pathogens, but most opportunistically in immunocompromised individuals.[27] Clinical manifestations tend to correlate with size of the organisms, such that yeast are associated with meningitis, pseudohyphae with microabscess and focal infarcts, and true hyphae with arterial thrombosis and large strokes. Cultures of CSF and brain tissue are used to isolate most infections for identification and antibiotic susceptibility testing. However, many organisms require weeks to grow, or may not readily exhibit diagnostic features precluding morphologic speciation. Because of the urgent need for treatment, rapid testing options have been developed including India ink for cryptococcal meningitis, and a variety of antigen tests (eg, cryptococcal antigen).[28] Markers of fungal wall components, such as (1,3)-beta-D-glucan, are useful to support a suspected fungal infection and to help rule out species that produce little or no amounts (eg, *Cryptococcus* spp. and Mucorales).[29]

HISTOLOGIC FEATURES

Surgical tissue from fungal infections is frequently collected from large abscesses or space-occupying lesions for diagnosis and treatment of symptoms. Smaller lesions may also be biopsied

in the case of microabscesses, focal infarcts, or leptomeningitis, when a radiologic abnormality is identified and other less-invasive testing is nondiagnostic. The type and degree of inflammation vary with patient immune status, and can range from minimal inflammation with rare lymphocytes and histiocytes to large necrotizing granulomas or abscesses. Fungal forms, including yeast, pseudohyphae, and hyphae, can usually be seen on H&E sections, including intraoperative frozen sections, which typically show minimal staining and refractile cell walls (**Fig. 2**A).[30] Further

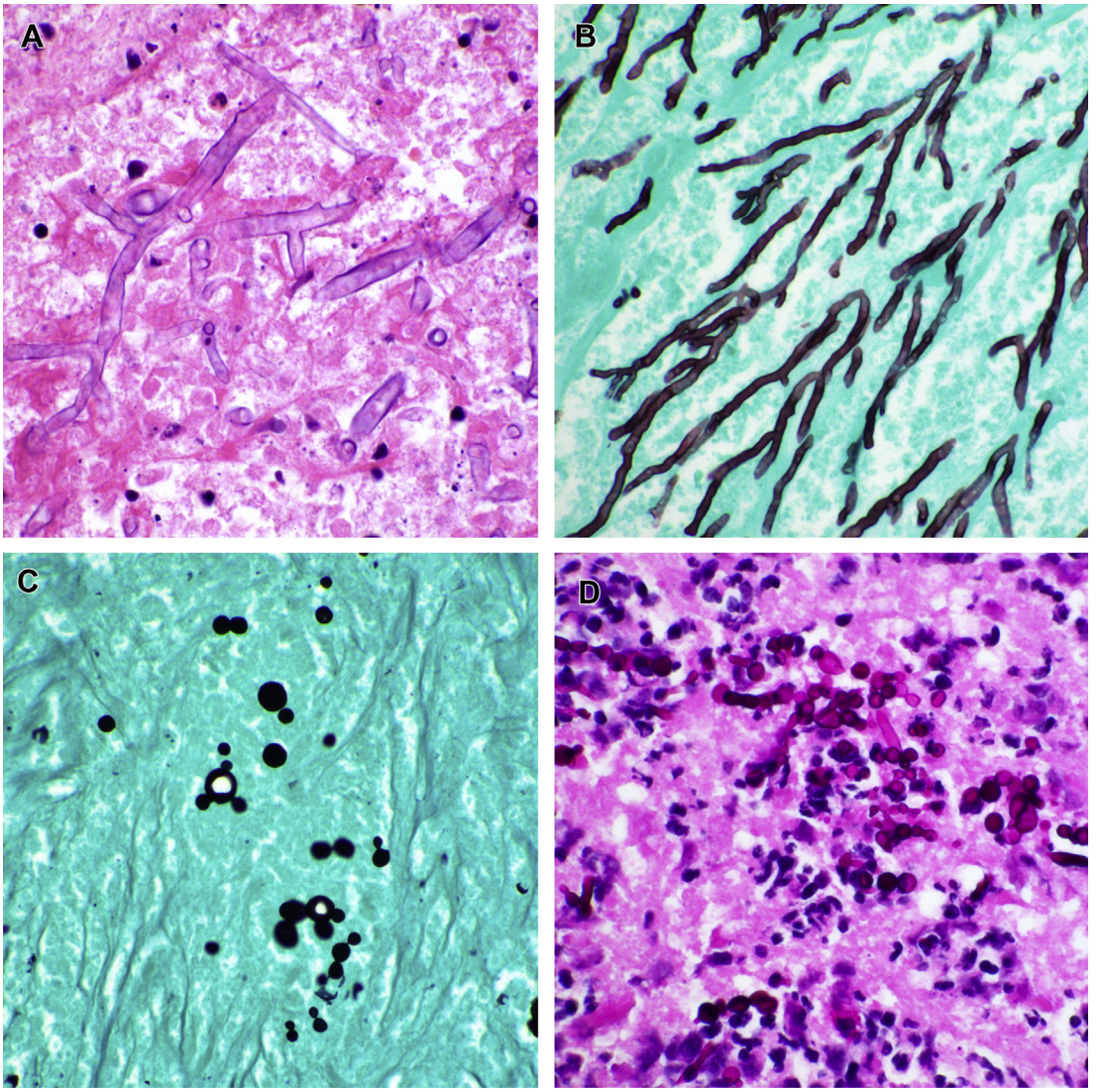

Fig. 2. Fungal infections. (*A*) Fungal forms can often be visualized on standard hematoxylin and eosin stains (original magnification ×400x), as shown in this biopsy of an occipital lobe mass. Wide ribbon-like, pauciseptate hyphae with 90-degree angle branching are present in a necrotic background, consistent with Mucorales infection. Subsequent sequencing of the FFPE tissue targeting the internal transcribed spacer region was positive for *Lichtheimia corymbifera*. (*B*) GMS staining (original magnification ×400x) of tissue from a temporal lobe abscess highlights narrow hyphae with acute-angle branching and frequent septations, consistent with *Aspergillus* spp., further classified as *Aspergillus fumigatus* by culture. (*C*) GMS staining (original magnification ×400x) of a cerebellar abscess biopsy highlights yeast forms with peripheral budding reminiscent of a ship's steering wheel, diagnostic of *Paracoccidioides brasiliensis*. (*D*) Periodic acid–Schiff staining (original magnification ×400x) of a right lobectomy specimen targeting multiple small, radiologically ring-enhancing lesions identified microabscesses containing scattered fungal elements. Medium-to-large, irregular yeast-forms with pseudohypahe are present, suggestive of *Candida* spp. Subsequent sequencing of FFPE using internal transcribed spacer primer sets was positive for *Candida albicans*.

classification is aided by GMS and PAS-D stains, which highlight organism size and morphology (**Fig. 2**B–D). Histologic classification of yeast is based on size and budding pattern (eg, variable-sized yeast with multiple buds of *Paracoccidioides brasiliensis*) (see **Fig. 2**C), and presence of pseudohyphae (eg, *Candida* spp.) (see **Fig. 2**D). Additional staining properties including mucicarmine and Fontana-Masson positivity in *Cryptococcus* spp. and Gram positivity of *Candida* spp. yeast can aid diagnosis, but are not specific and must be interpreted in histologic context.[31] Fungal hyphae are classified by width, branching patterns, frequency of septations, and presence of pigmentation.[32] Mucorales (eg, *Rhizopus* spp. and *Mucor* spp.) exhibit wide, irregular ribbon-like, pauciseptate hyphae with 90-degree angle branching (see **Fig. 2**A). *Aspergillus* spp. exhibit narrow, regular hyphae with frequent septations and 45-degree angle branching (see **Fig. 2**B); however, these features are not specific, and in the absence of supportive laboratory findings, it is recommended to report a morphologic description with a differential including *Fusarium* spp., *Scedosporium* spp., and other hyaline molds. Immunohistochemistry can be performed for fungal identification, but because of substantial cross-reactivity between species offers limited additional information beyond morphologic identification.[33]

MOLECULAR DIAGNOSTICS

Species-level identification of fungi from FFPE is accomplished by sequencing of rRNA genes.[34] Unlike bacterial 16S rRNA sequencing of the small subunit, fungal identification typically relies on the D1/D2 region of the large subunit (28S rRNA gene), and the internal transcribed spacer region, because of increased variation between species. Clinical and Laboratory Standards Institute guidelines have been published for fungal sequencing identification.[14] Additional targets may be necessary to classify beyond complexes, including β-tubulin, calmodulin, or actin for *Aspergillus* spp. In addition to the challenges of sequencing formalin-fixed tissue, fungal cell walls provide an additional barrier and challenge to sequencing, which requires additional steps for adequate lysis and release of fungal nucleic acids.[35] Various physical (eg, beads), chemical/enzymatic, and temperature-based methods have been used to increase yield of fungal DNA extraction. Because even small yeast forms are identified by GMS or PAS-D stains, there is little utility in fungal sequencing of tissue that lacks visible organism. Even in the setting of compatible inflammatory patterns, molecular testing results are more likely to be false positives because of contamination of reagents or nonsterile collection sites than true infections.[36]

VIRAL INFECTIONS

EPIDEMIOLOGY AND LABORATORY DIAGNOSTICS

Numerous RNA and DNA viruses can infect the CNS of immunocompromised and healthy individuals, manifesting as meningitis, encephalitis, or myelitis.[37] Symptoms are predominantly neurologic or part of a disseminated systemic infection, and can occur either as an acute infection or as a reactivation event because of decreased immune system function. Infections frequently diffusely involve the entire brain via hematogenous spread, but may also exhibit some anatomic predilections, including temporal lobes for herpes simplex virus and thalami/basal ganglia for West Nile virus.[38] Viruses can also exhibit varying levels of tropism for different cell types, such as JC virus in oligodendrocytes, causing white matter–predominant disease as progressive multifocal leukoencephalopathy, and poliovirus infecting anterior horn cells of the spinal cord as poliomyelitis.[39] CSF testing showing a lymphocyte predominance is typical for CNS viral infections, and a specific pathogen may be diagnosed by the presence of CSF antibodies or viral nucleic acids.[40] Detection of IgM in serum or CSF is often the test of choice for viruses with short viremic windows, including most arboviruses; however, immunomodulatory drugs, such as rituximab, an anti-CD20 monocolonal antibody, can decrease the ability to produce antibodies resulting in false-negative testing.[40] Cultures can detect some viruses, but are less sensitive and more time consuming to perform compared with molecular methods, limiting overall utility.[41]

HISTOLOGIC FEATURES

Surgical biopsies are rarely undertaken for the diagnosis of CNS viral infections, because of the high yield of CSF testing with substantially lower costs and morbidity.[42] Biopsy tissue may be obtained to shed light on the cause of a radiologic abnormality for which all less-invasive testing has been unrevealing, and typically encompasses a broad differential, such as glioma, lymphoma, infections, demyelinating, or toxic/metabolic etiologies. In cases with severe edema and risk of death caused by herniation, tissue may be obtained during decompressive surgery. On rare occasions, biopsies of “nonlesional” tissue are

obtained, most often from the frontal lobes or other accessible locations, with the goal of identifying a diffuse process, such as vasculitis.[43] General histologic features include varying amounts of perivascular, parenchymal, and leptomeningeal chronic inflammatory infiltrates; microgliosis with microglial nodules and neuronophagia; and neuronal loss (Fig. 3A, B). Inflammatory infiltrates

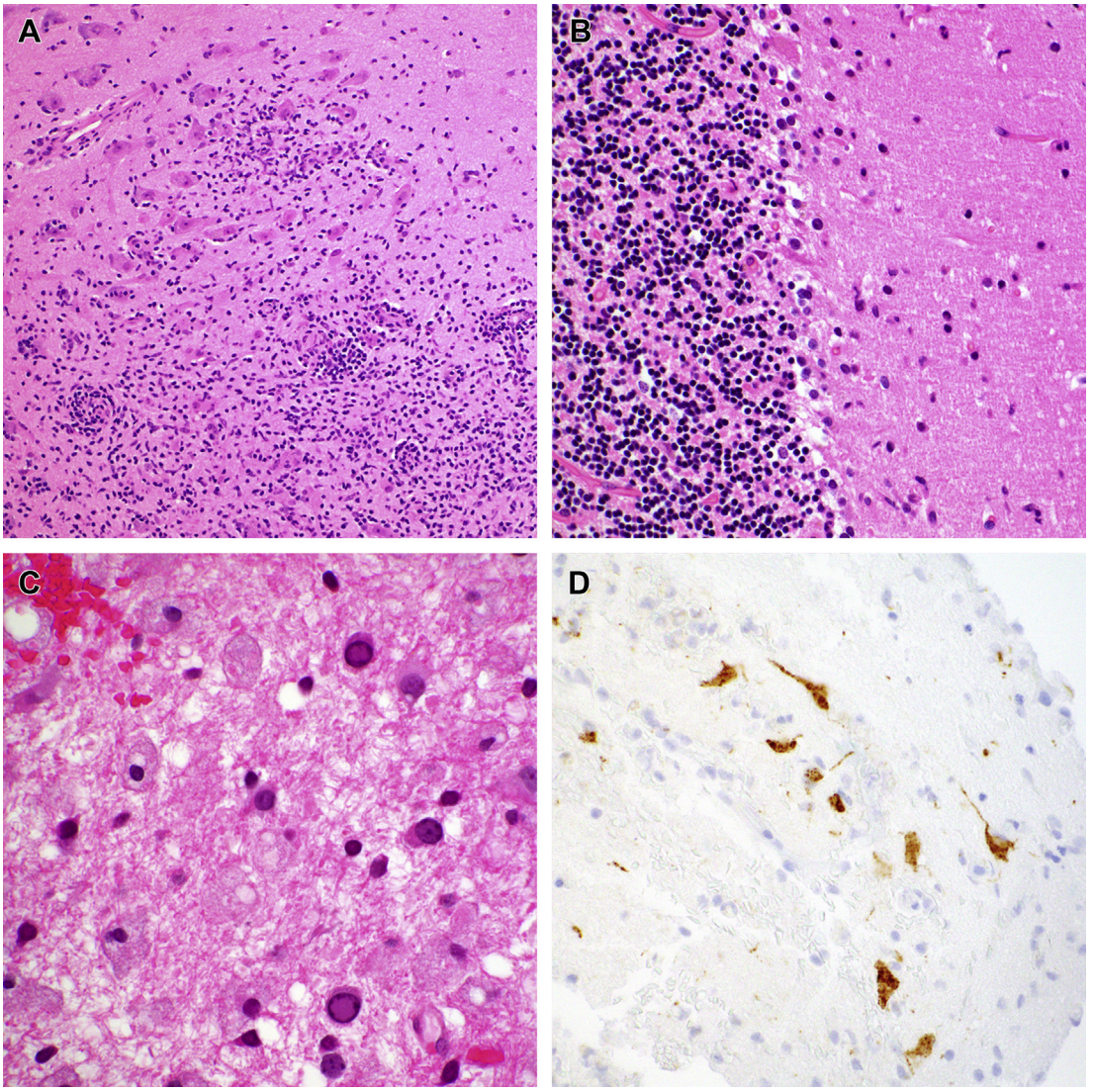

Fig. 3. Viral infections. Many viral infections of the central nervous system show overlapping histologic features with autoimmune encephalitis, precluding further classification without immunohistochemistry or molecular testing. (*A*) This temporal lobe biopsy shows a dense lymphoplasmacytic infiltrate with microglial nodules and neuronophagia of unclear cause (original magnification ×200x). (*B*) Extensive neuronal loss, as in this cerebellar biopsy showing Purkinje cell depletion and Bergmann gliosis, is another nonspecific finding that is seen in viral meningoencephalitis. Metagenomic next-generation sequencing of cerebrospinal fluid and formalin-fixed paraffin-embedded brain tissue was positive for Powassan virus (original magnification ×400x). (*C*) Some viral infections show characteristic viral cytopathic effects that can aid in diagnosis, including JC virus intranuclear inclusions present in oligodendrocytes in this left parietal lesion biopsy. Immunohistochemistry can help distinguish inclusions from reactive nuclei, and confirm specificity for polyomaviruses. Combined with myelin pallor and extensive macrophage infiltration (not shown), these findings are diagnostic of progressive multifocal leukoencephalopathy (original magnification ×400x). (*D*) Herpes simplex virus immunohistochemistry of a temporal lobe biopsy highlights scattered cells containing viral antigen, including neurons, confirming the diagnosis of herpes simplex virus-1 meningoencephalitis (original magnification ×200x).

are generally mixed and include lymphocytes (enriched for $CD8^+$ T cells), plasma cells, histiocytes, microglia, and may include neutrophils. In severely immunosuppressed individuals, inflammation may be minimal. Demyelination, confirmed by loss of Luxol fast blue staining, is a prominent feature of progressive multifocal leukoencephalopathy, but is also observed in noninfectious (ie, multiple sclerosis) or postinfectious (ie, acute disseminated encephalomyelitis) settings.[44] Vasculitis or ischemic lesions may be present in varicella zoster virus infections.[45] For practical considerations, viruses are often divided into ones that are diagnosed based on histology alone and ones that require ancillary testing in the form of immunohistochemistry or molecular assays. Viral cytopathic effects including nuclear or cytoplasmic inclusions are pathognomonic for certain infections, such as the oligodendrocyte nuclear inclusions of JC virus (**Fig. 3**C), and neuronal cytoplasmic Negri body inclusions of rabies virus. Less specific cytopathic effects, such as multinucleated cells of HIV encephalitis and measles virus, may require confirmation by immunohistochemistry. Similarly, inclusions of herpes simplex virus-1/2, varicella zoster virus, cytomegalovirus, and adenovirus are seen on routine H&E sections, but are easily highlighted by immunohistochemistry (**Fig. 3**D).[46] A large number of other viruses, including human herpesvirus-6/7, enteroviruses, and arboviruses require immunohistochemistry or molecular testing for diagnosis.

MOLECULAR DIAGNOSTICS

Because of the large diversity of virus genomes and lack of common genes across all families, no universal targeted gene sequencing for viruses exists that would be analogous to 16S rRNA for bacteria. Instead, individual or panels of real-time PCR assays have been developed, with or without a sequencing step, for a wide range of viral infections. Specific testing is guided by epidemiologic factors including immune status, age, geography, and exposures. Although these tests are reasonably sensitive and specific, they lack the ability to detect novel viruses, or unusual viruses that were not specifically targeted because of low probability and limited tissue availability. Metagenomic next-generation sequencing (mNGS) has recently become available as a clinical assay for CSF specimens, and has resulted in diagnoses of unexpected pathogens that would otherwise have left the cases unsolved.[47] Sensitivity of mNGS depends on acuity of infection, extent of prior antiviral treatment, and levels of virus shed into the CSF. mNGS assays for fresh/frozen or FFPE brain tissue are currently being developed, and are likely to have higher yield for ongoing infections and for viruses that exist predominantly intraparenchymaly in the brain.[48] Compared with CSF, brain tissue has a much higher percentage of human to nonhuman nucleic acids, requiring additional purification or bioinformatics steps to identify sequences from potential pathogens.[49] As for other pathogen types, formalin-fixation decreases the length of sequencing reads, although the ability to screen tissue for histologic signs of viral infection may offset this effect. Caution should be used in attributing low levels of human herpesvirus-6 or other viruses that retain latency as causes of acute meningoencephalitis, because these may reflect limited reactivation secondary to other immunosuppressive factors.[50] Although few targeted antiviral therapies are currently available, identification of a specific virus can help limit unnecessary further testing and provide evidence against immunosuppressive treatment of a possible autoimmune encephalitis.

PARASITIC INFECTIONS

EPIDEMIOLOGY AND LABORATORY DIAGNOSTICS

Several parasites can infect the CNS, variably manifesting as benign space-occupying masses to rapidly progressive destructive lesions.[51] Parasites differ widely in geographic distribution, which is heavily influenced by susceptible hosts and vectors, many of which can infect humans as part of a normal or aberrant life cycle. Basic organizational schemes split parasites into single-celled protozoans, including *Toxoplasma* spp., *Plasmodium* spp., and free-living amebas, and macroscopic helminths, which include nematodes (roundworms), cestodes (tapeworms), and trematodes (flukes). As with other types of infections, immunosuppression is associated with more severe infections and infections with unusual organisms.[52] Toxoplasmosis commonly involves the brain in HIV-positive individuals and can present as ring-enhancing lesions on MRI.[53] *Plasmodium falciparum* causes cerebral malaria, particularly in children, because of sequestration in CNS blood vessels.[54] Cysticerci, larval stage of pork tapeworm *Taeni solium*, can present as single or numerous cystic lesions on imaging, and are a major source of seizures worldwide.[55] Free-living amebas, including *Acanthamoeba* spp., *Balamuthia mandrillaris*, and *Naegleria fowleri*, are associated with exposure to fresh, stagnant water, and present with rapid clinical deterioration.[56] Diagnosis is often accomplished using serology, based

on exposures to travel, food, and animals/insects. Serum and CSF may show a marked increase in eosinophils, and organisms may be directly observed on peripheral blood smears or detectable by targeted PCR (eg, *Toxoplasma* spp. and *Plasmodium* spp.). Organisms are diagnosed morphologically in the microbiology/hematology laboratories, although cultures do not play a routine role in diagnosis. Treatments range from innocuous trimethoprim-sulfamethoxazole for

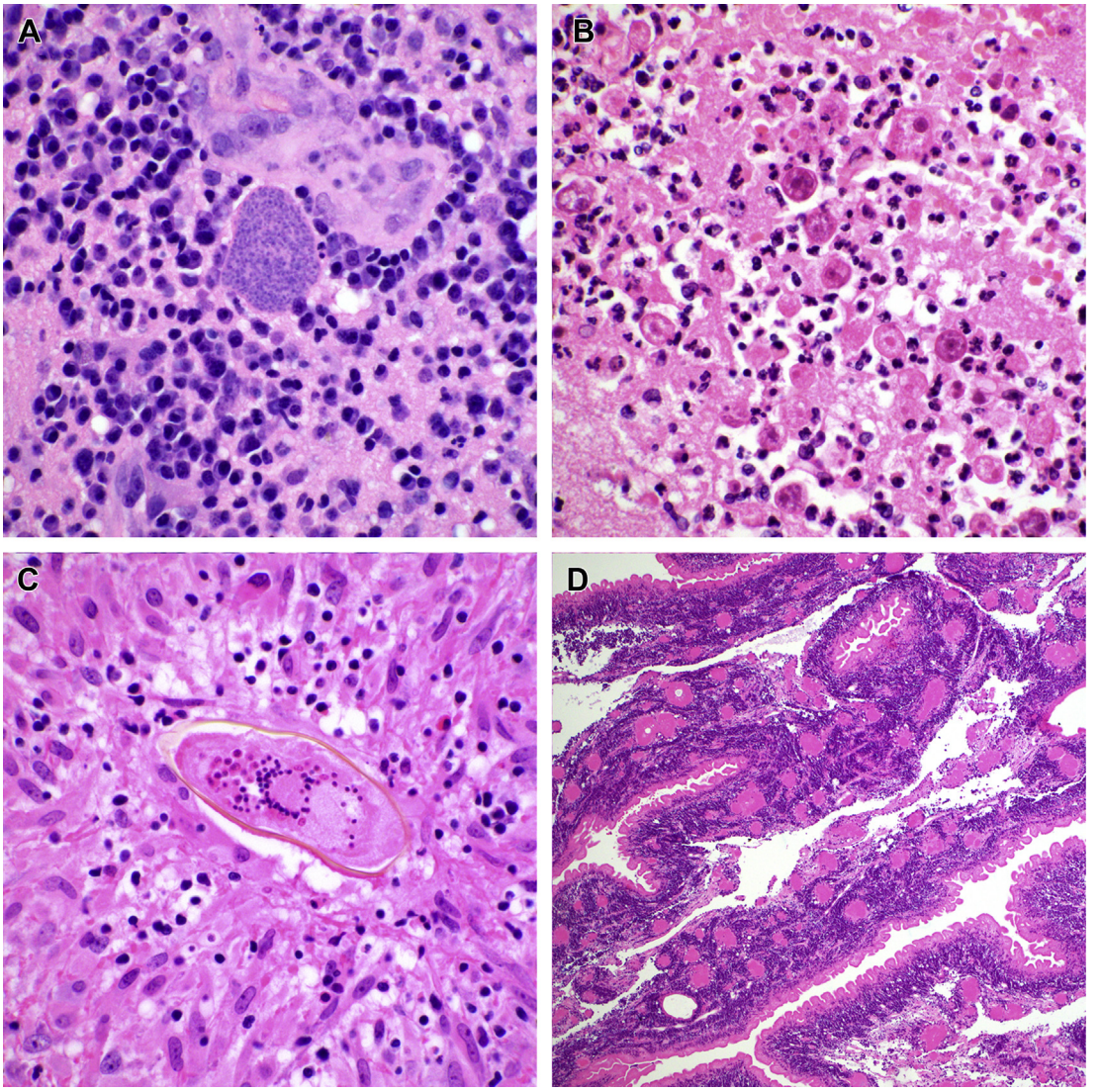

Fig. 4. Parasitic infections. Most parasitic infections of the central nervous system are diagnosed by morphologic features on hematoxylin and eosin sections, with occasional confirmation by serology or molecular testing. (*A*) This biopsy of a temporal lobe mass shows a dense lymphoplasmacytic infiltrate surrounding a thin-walled cyst containing numerous *Toxoplasma gondii* bradyzoites. Immunohistochemistry is used to highlight individual tachyzoites when bradyzoites are not readily identified, and to distinguish from other similarly appearing parasites, such as in trypanosomiasis (original magnification ×400x). (*B*) Autopsy sections from a fatal case of granulomatous amebic encephalitis show numerous trophozoites of *Balamuthia mandrillaris*, which resemble macrophages but contain a large nucleus with distinctive central karyosome (original magnification ×400x). (*C*) Temporal lobe biopsy sections show a marked granulomatous and mixed inflammatory reaction to *Schistosoma mansoni* eggs, which are characterized by oval shape and refractory wall. The location of spines, useful for differentiating among *Schistosoma* spp., are difficult to assess in FFPE sections, which may not contain an optimally oriented egg (original magnification ×400x). (*D*) Tissue from the resection of a large cerebellar cyst shows tegument, stroma, and occasional calcareous bodies without an observable protoscolex, findings consistent with the racemose form of neurocysticercosis (metacestode form of *Taenia solium*) (original magnification ×100x).

toxoplasmosis, to highly toxic and occasionally deadly melarsoprol for trypanosomiasis.[57]

HISTOLOGIC FEATURES

Surgical biopsies or resections may be undertaken for diagnosis and treatment of various radiologic abnormalities. Ring-enhancing lesions encompass a broad infectious and noninfectious differential, including toxoplasmosis.[58] Cystic lesions, such as echinococcosis and cysticercosis, may consist predominantly of fluid and contain extensively degenerated, but distinctly nonhuman appearing tissue. Inflammatory patterns depend on immune status, and range from minimal acute inflammation, to granulomatous inflammation and large areas of necrosis and abscess. Organisms may be found in brain parenchyma, blood vessels, or leptomeninges. Protozoan parasites are frequently recognized on H&E stains; may be intracellular (eg, *Toxoplasma* bradyzoites) (**Fig. 4**A) or extracellular (eg, *Acanthamoeba* spp. trophozoites and cysts) (**Fig. 4**B); and are distinguished based on morphologic features including size, shape, and subcellular organelles. Helminths can be present as eggs (**Fig. 4**C) or larvae/adult worms (**Fig. 4**D), and are distinguished based on size, shape, and presence of various structures including protoscoleces. Special stains, such as Giemsa, may be used to highlight protozoan morphology, acid-fast stains are used to stain the polarizable hooklets present in cestodes, and PAS and GMS stains are used to stain the cyst walls of amebas. Immunohistochemistry for *Toxoplasma* spp. is widely available, and can help highlight tachyzoites in a necrotic background, whereas amebas are distinguished from histiocytes by lack of CD68 staining. Other immunostains may be available at reference laboratories, including assays for specific ameba species.[59] Although no longer a frequent tool for diagnosis, electron microscopy is used to confirm the presence of small, intracellular parasites, such as in microsporidiosis.[60]

MOLECULAR DIAGNOSTICS

Molecular testing is not routinely used for the diagnosis of parasitic infections because of the characteristic histologic features seen in many cases. When only degenerating worm fragments are present precluding further classification (eg, features most consistent with nematode), molecular testing may provide a more specific diagnosis. In such cases, antiparasitic drugs are not indicated unless viable organisms are suspected elsewhere. Some situations for which targeted molecular testing is used include detection of *Toxoplasma gondii* by B1 gene and distinguishing between species of free living amebas by 18S rRNA real-time PCR.[61,62] mNGS has been successful in the diagnosis of parasitic infections from CSF in rare cases, and is likely to be extended to frozen or FFPE brain tissue in the future.[63,64]

SUMMARY

A wide range of organisms can infect the CNS and can cause minimal to lethal symptoms. Although less-invasive testing including CSF examination is preferred whenever possible, surgical biopsy or resection may be used to obtain diagnostic tissue, particularly when neoplastic diagnoses remain in the differential. Histologic evaluation including the use of special stains and immunohistochemistry can identify broad categories of infection (ie, bacterial, mycobacterial, fungal, viral, or parasitic), and can further classify to genus or species in many instances. Targeted molecular testing is used for confirmation in certain settings, whereas broad-spectrum and unbiased metagenomics testing are being increasingly used. Careful histologic review of slides is essential for selecting the ideal molecular test to make a diagnosis and support clinical decision making while minimizing resource overutilization.

ACKNOWLEDGMENTS

No conflicts of interest to declare. No sources of funding supported this work.

DISCLOSURE

The author has nothing to disclose.

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