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# What every neuropathologist needs to know: Update on neuro infectious disease workups and consultation resources

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## ABSTRACT

Efficient histopathological diagnosis of central nervous system infections can be challenging but is critical for therapeutic decision making in cases for which less invasive blood or cerebrospinal fluid testing has been unrevealing. A wide variety of bacteria, fungi, viruses, and parasites can cause infections, particularly in immunocompromised individuals. Histological findings may be nonspecific or overlap with noninfectious inflammatory conditions. To minimize wasted tissue and time, a systematic approach is recommended in which: (1) relevant patient history (eg, comorbidities, travel and other exposures, and immune status) and radiological findings are reviewed, (2) a preliminary differential diagnosis based on this information and on inflammatory patterns and visualization of potential microorganisms on hematoxylin and eosin stains is generated, (3) special stains, immunohistochemistry, in situ hybridization, or molecular testing (pathogen-specific or broad-spectrum) are used for confirmation and further classification, and (4) correlation with culture results and other laboratory testing is performed to arrive at a final integrated diagnosis. Discrepancies between molecular and histological findings are often due to contamination and require careful evaluation to avoid treatment of false positives. Consultation with infectious disease pathologists or public health reference laboratories may be needed to confirm diagnoses of unusual organisms or when specialized testing is required.

**KEYWORDS:** abscess; encephalitis; immunohistochemistry; infectious disease pathology; in situ hybridization; meningitis; metagenomic sequencing.

## INTRODUCTION

CNS infections are caused by a wide range of bacteria, fungi, viruses, and parasites and have the potential to cause significant morbidity and mortality.[1,2] Delays in diagnosis and initiation of appropriate treatment can result in permanent sequelae with a disastrous impact on patient quality of life. Incidence of specific infections varies with geography, season, and demographics, particularly age and immune status.[3] Microorganisms may infect both the immunocompetent and immunocompromised, with the elderly, infants, HIV-positive individuals, organ transplant recipients, and immunotherapy patients being the most at risk for opportunistic infections and greater disease burden.[4] Due to the large variety of causative organisms and relevant host factors, clinical presentations of CNS infections vary considerably both in acuity and severity.[5] CNS infections can be classified based on structures involved, including meningitis (meninges), encephalitis (brain parenchyma), and myelitis (spinal cord), and combinations including meningoencephalitis and encephalomyelitis when multiple compartments are involved. Necrotic or mass-forming lesions are referred to as abscesses (eg, epidural abscess) or "-omas" (eg, tuberculoma), respectively, regardless of location.

The diagnosis of CNS infections is challenging due to the variety of nonspecific or difficult-to-localize symptoms, including fever, neck stiffness, photophobia, headache, nausea/vomiting, seizures, weakness, coma, and altered mental status.[6] In addition to a thorough neurological exam and standard blood laboratory testing, diagnosis typically involves brain and/or spinal cord MRI followed by a lumbar puncture for CSF analysis. Similar to primary CNS tumors, MRI may show a range of patterns from diffusely infiltrating and noncontrast enhancing (eg, early cerebritis) to necrotic and ring-enhancing lesions (eg, abscess).[7,8] The presence of multifocal lesions may mimic metastatic tumors, particularly in the setting of newly identified extra-cranial masses (eg, unbiopsied lung lesions). Measurement of CSF opening pressure, cell counts, color, glucose, and protein content can help favor bacterial (leukocytosis, high protein, and decreased glucose), fungal, parasitic (increased eosinophils), or viral (lymphocytic pleocytosis with normal glucose) pathogens. Cultures, antigen and antibody assays, and molecular diagnostics can establish a definitive diagnosis.[9] Identifying a specific infectious etiology is vital for appropriate treatment, which can include antimicrobial drugs or therapy to reduce effects of inflammation and edema. When less invasive testing fails to identify a specific etiology

or when treatable noninfectious etiologies remain in the differential, brain (or spinal cord) biopsies may be obtained for diagnosis.[10,11] Larger resections may be performed for source control and to alleviate elevated intracranial pressure in cases with abscess or other mass forming lesions. When a diagnosis is not confirmed premortem, due to lack of sufficient time or access to tissue, autopsy may yield definitive results which can provide answers/closure to surviving family members,[12] and be reported to public health agencies for documentation if indicated.[13]

While neuropathologists hold the primary responsibility for evaluating surgical and autopsy brain tissue, diagnosis of CNS infections in these cases often depends on close communication with the clinical team(s), a high degree of suspicion, and familiarity with the strengths and limitations of ancillary testing. To maximize diagnostic yield while optimizing resource utilization, a systematic approach is recommended using patient history and hematoxylin and eosin (H&E) findings to generate a preliminary differential which can be confirmed by special stains, immunohistochemistry (IHC)/in situ hybridization (ISH), molecular diagnostics, and correlation with cultures and other laboratory testing (Figure 1). While it is beyond the scope of this article to describe the epidemiology, pathogenesis, and diagnostic features of every potential CNS infection, an overall framework for diagnosis will be described, including general strategies with specific examples. Additional details regarding specific microorganisms can be found in dedicated infectious disease pathology textbooks.[14–17]

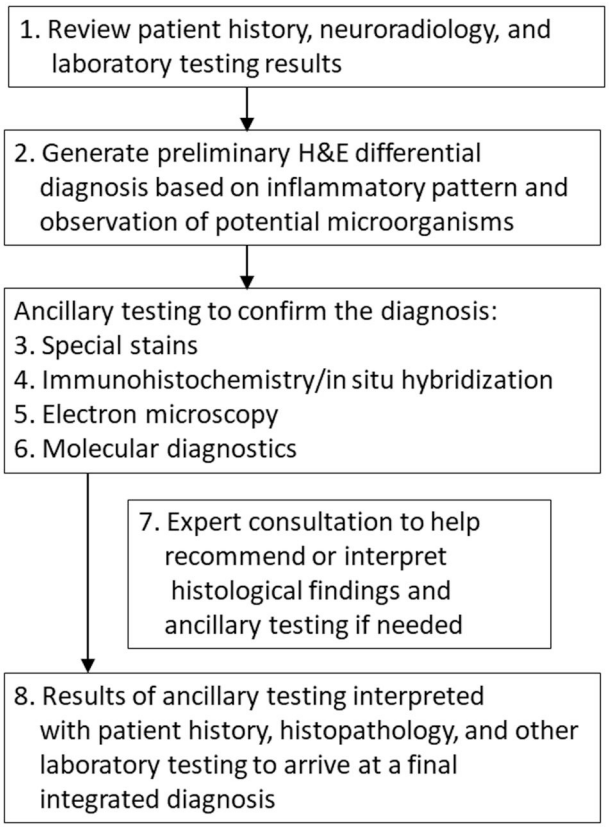


**Figure 1.** Systematic approach to diagnosis of central nervous system infections. Potential infectious etiologies can be efficiently diagnosed by utilizing all available clinical information, generating a preliminary diagnosis, confirming through appropriate ancillary testing, and interpreting all results (with expert consultation as needed) into a final integrated diagnosis.

## DIAGNOSTIC APPROACH

### 1. Clinical history

Clinical context is important for interpretation of all anatomic pathology cases, and a thorough review of patient history can provide critical clues to a specific infectious etiology and guide appropriate confirmatory testing. Sufficient details are typically not included on a pathology requisition form and review of electronic medical records is required. Neurosurgery, neurology, and infectious disease notes commonly contain helpful information regarding patient presentation, physical exam findings, and summary of laboratory testing performed at other institutions. Comorbidities leading to immunosuppression and increased susceptibility to infection should be noted, particularly human immunodeficiency virus infection, solid organ or bone marrow transplantation, and treatment with immunomodulatory drugs, for example, rituximab.[18,19] Prior surgeries or trauma may serve as potential sites of wound infection with normal flora or other environmental organisms (typically bacterial or fungal).[20] Potential sources of exposure are numerous, including sick contacts, contaminated food or water, and infected animals/insects. Vaccination status and prior infections, particularly against measles, mumps, rubella, varicella, *Haemophilus influenza* type b, meningococcus, pneumococcus, poliovirus, Japanese encephalitis virus, tick-borne encephalitis virus, and rabies virus, have a significant impact on potential infections.[21,22] Relative incidence of infections varies widely geographically, due in part to vaccination history, sanitary conditions, and animal (reservoir) and insect (vector) populations, and thus it is critical to determine where a patient has traveled and lived.

MRI findings can also help focus the infectious differential, and it is useful both to review the radiology reports as well as key images or sequences (eg, T2/FLAIR and T1-post contrast). Meningitis is typically represented by contrast enhancement. Mass forming lesions with central necrosis suggest an abscess, which can be caused by bacteria (eg, *Staphylococcus aureus*), fungi (eg, *Candida albicans*), or parasites (eg, *Toxoplasma gondii*) due to hematogenous or contiguous spread from adjacent structures (eg, from nasal sinuses).[23] Viral infections are frequently diffuse and symmetrical, and may preferentially involve structures including the temporal lobes (herpes simplex virus-1 [HSV-1] or human herpes virus 6), thalamus/basal ganglia (many arboviruses), anterior spinal cord (poliovirus), or subcortical white matter (JC polyomavirus).[24] Parasite lesions range from nodular enhancement (eg, granulomatous reaction to *Schistosoma* spp. ova) to multicentimeter cystic lesions (eg, cysticercosis).[25] In some cases, minimal or no abnormalities are identified radiologically.

Laboratory testing performed prior to biopsy frequently includes blood work and CSF analysis.[9] Results may be suggestive of infection but nonspecific (eg, elevated white blood cell count) or consistent with a systemic infection with

uncertain CNS involvement (eg, bacteremia). While bacterial cultures can be positive in as little as 1-2 days, anaerobic organisms, mycobacteria, and fungi may take weeks to months to grow. Since it is not uncommon for cultures collected before a brain biopsy to turn positive after tissue is collected, it is recommended to check culture results of prior and concurrently submitted specimens: (1) at the time the biopsy is performed (eg, at the time of intraoperative frozen section consultation), (2) prior to sign out of permanent sections (eg, when all special stains and IHC are completed), and (3) after molecular testing results or external consultation is performed (eg, before addendum for final integrated diagnosis). Specimens submitted to reference laboratories for testing (eg, serology panels for viral infections) may take 1-2 weeks to report, and a brain biopsy may be performed prior to receiving those results if the patient's condition fails to improve. In addition to prebiopsy testing, additional laboratory testing is often ordered by the clinical team once a preliminary differential is raised (eg, *Treponema pallidum* serology in setting of abundant plasma cells), and these results can help confirm the diagnosis suggested histologically.

## 2. Histopathology

Histopathologic findings in infectious disease pathology can be divided into host inflammatory reactions and direct evidence of microorganisms. The former is classified based on the type of inflammatory cells and regions or structures involved. Neutrophilic inflammation is associated with acute infection and is frequently present in cases of bacterial leptomeningitis, cerebritis, abscess (with necrosis) due to various organisms, and early viral meningoencephalitis. Mixed lymphoplasmahistiocytic inflammation is present in subacute to chronic abscesses and viral meningoencephalitis, the latter of which also includes microgliosis, microglial nodules, and neuronophagia. Increased plasma cells are also present in infections with *T pallidum*. Granulomatous inflammation with multinucleated giant cells is characteristic of pachymeningitis due to *Mycobacterium tuberculosis*, as well as fungal and parasitic infections involving brain parenchyma. Parasitic infections are also frequently accompanied by increased eosinophils. CNS vasculitis can be associated with a variety of infections, including rickettsiosis, syphilis, and varicella zoster virus (VZV) vasculopathy.[26]

While inflammation due to CNS infection can be dense and diffuse, immunosuppressed individuals may exhibit mild or in some cases no inflammation. In these situations, the diagnosis of infection relies on direct visualization of microorganisms. On H&E-stained sections, gram-positive and gram-negative bacteria can appear as eosinophilic to basophilic dot-like to rod-like structures individually or in large clusters. Bacteria are often present in the center or near the edge of a necrotic abscess and may be hard to distinguish from cellular debris. Bacteria can also be present intracellularly, such as *Tropheryma whipplei* bacilli in foamy histiocytes. Spirochetes, rickettsia, and *Mycoplasma* are not observable on H&E, while mycobacteria are generally not identifiable unless present in extremely high numbers.

In contrast to bacteria, fungi are frequently observable on H&E stains, often appearing as white "negatively" staining structures that do not pick up hematoxylin or eosin. One notable exception is phaeohyphomycosis in which intrinsic melanin pigment causes fungal elements to appear brown. Fungal wall components and internal structures may also appear eosinophilic or basophilic. Fungal hyphae may be found involving blood vessel lumens and walls, resulting in thrombosis and surrounding infarction. Yeast/yeast-like structures and pseudohyphae/hyphae may also be observed infiltrating brain parenchyma and leptomeninges and may be present within multinucleated giant cells. Size and budding patterns of yeast can often be appreciated on H&E stains, although organisms appear far less abundant than on special stains. Similarly, hyphal width, frequency of septations, and branching patterns can be analyzed for preliminary fungal identification.

The majority of CNS parasite identifications in anatomic pathology are accomplished through careful evaluation of H&E-stained sections[27] (Table 1). *Toxoplasma gondii* infection (toxoplasmosis) can be diagnosed by detection of bradyzoite-containing cysts (5-50 μm in diameter) or individual tachyzoites (4-8 μm in length). *Trypanosoma cruzi* infection (Chagas disease) is characterized by intracellular collections of amastigotes (1-5 μm in length), identified by the presence of a nucleus and kinetoplast commonly described as "dot-dash" morphology, which are indistinguishable from *Leishmania* amastigotes. Infections with free-living amoeba, including *Acanthamoeba*, *Balamuthia*, and *Naegleria* species, can be identified by the presence of multiwalled cysts or trophozoites (ranging in size from 10 to 60 μm in diameter) with central karyosome-containing nuclei. Helminth infections (ie, cestodes, trematodes, and nematodes) vary markedly in size depending on the involvement of ova, larvae, or adult worms. The most common cestode infection of the CNS, neurocysticercosis (due to *Taenia solium* larvae), can be identified by 1-cm cysts containing a single protoscolex with refractile hooklets, a wall with distinctive tegument surface, and underlying stroma with scattered calcareous corpuscles. Intact cysticerci illicit minimal immune response, while degenerating larvae are associated with marked inflammation and edema leading to seizure activity. CNS infections with *Schistosoma* spp. (schistosomiasis) cause symptoms primarily due to the granulomatous response to the trematode ova, which are circular to oval-shaped (50-240 μm in length depending on species but often appear shorter in tissue due to tangential sectioning) and contain distinctive lateral or terminal spines. Nematodes can be identified by their fluid-filled body cavity containing gastrointestinal and reproductive organs, and distinctive lateral alae. *Angiostrongylus cantonensis* larvae are associated with eosinophilic meningitis and can be identified in sections containing leptomeninges.[28]

In contrast to other microorganisms, viruses cannot be directly visualized by light microscopy, but in some cases may be confidently identified by characteristic viral cytopathic effects in infected cells (Table 2). In general, RNA viruses tend to produce cytoplasmic inclusions, such as the distinctive eosinophilic Negri bodies present in neurons infected with rabies virus. However, HIV encephalitis is frequently

**Table 1.** Parasite structures present in central nervous system infections.

| Parasite name or group | Structures seen in CNS tissue |
|---|---|
| **I. Protozoans** | |
| Free-living amoebas (eg, *Naegleria fowleri, Acanthamoeba* spp., *Balamuthia mandrillaris*) | Trophozoites and/or cysts |
| *Plasmodium falciparum* | Parasitized erythrocytes |
| *Toxoplasma gondii* | Bradyzoites and/or tachyzoites |
| *Trypanosoma* spp. (*T cruzi, T brucei* spp.) | Amastigotes |
| **II. Helminths** | |
| **A. Cestodes** | |
| *Taenia solium* (cysticercosis) | Larvae |
| *Taenia multiceps* (coenurosis) | Larvae |
| *Echinococcus* spp. (hydatid disease) | Larvae |
| *Spirometra* spp. (sparganosis) | Larvae |
| **B. Trematodes** | |
| *Schistosoma* spp. (*S mansoni, S japonicum, S haematobium, S mekongi*) | Ova |
| *Paragonimus westermani* | Ova |
| **C. Nematodes** | |
| *Strongyloides stercoralis* | Larvae |
| *Angiostrongylus cantonensis* | Larvae |
| *Gnathostoma spinigerum* | Larvae |
| *Toxocara* spp. | Larvae |
| Human filariases (eg, *Loa , Dracunculus medinensis, Onchocerca volvulus*) | Microfilariae |

**Table 2.** Diagnostic methods for viral infections of the central nervous system.

| Virus name or group | Diagnostic method in tissue |
|---|---|
| **I. DNA viruses** | |
| Adenovirus | VCPE, IHC |
| Herpes simplex virus 1 and 2 | VCPE, IHC |
| Varicella zoster virus | VCPE, IHC |
| Epstein–Barr virus | ISH |
| Cytomegalovirus | VCPE, IHC |
| Human herpesvirus 6 and 7 | Reference lab (IHC or PCR) |
| JC polyomavirus | VCPE, IHC |
| **II. RNA viruses** | |
| Rabies virus | VCPE, reference lab (IHC or PCR) |
| Measles virus | VCPE, reference lab (IHC/ISH or PCR) |
| Mumps virus | Reference lab (IHC or PCR) |
| Rubella virus | Reference lab (IHC or PCR) |
| Poliovirus | Reference lab (IHC or PCR) |
| Nonpolio enteroviruses (eg,. Enterovirus D68, Coxsackievirus B3) | Reference lab (IHC or PCR/sequencing) |
| HIV | VCPE, reference lab (IHC or PCR) |
| Human T-lymphotropic virus type 1 | Reference lab (IHC or PCR) |
| Influenza virus | Reference lab (IHC or PCR) |
| Parainfluenza virus | Reference lab (IHC or PCR) |
| Arboviruses (eg, West Nile virus, Eastern equine encephalitis virus, Powassan virus, Zika virus, Jamestown Canyon virus, Japanese encephalitis virus) | Reference lab (IHC/ISH or PCR/sequencing) |
| Hendra virus and Nipah virus | Reference lab (IHC or PCR) |
| Lymphocytic choriomeningitis virus | Reference lab (IHC or PCR) |

Abbreviations: IHC, immunohistochemistry; ISH, in situ hybridization; PCR, polymerase chain reaction; VCPE, viral cytopathic effect.

associated with multinucleated giant cells, while subacute sclerosing panencephalitis (due to defective measles virus) is associated with eosinophilic nuclear inclusions. The majority of meningoencephalitis-associated enteroviruses and arboviruses (including flaviviruses, bunyaviruses, and togaviruses) exhibit no distinguishing viral cytopathic effects. DNA viruses tend to produce nuclear inclusions, including glassy basophilic JC polyomavirus inclusions in oligodendrocytes in progressive multifocal leukoencephalopathy (also associated with demyelination). Adenovirus infection is associated with smudgy nuclear inclusions, while cytomegalovirus (CMV) is associated with enlarged endothelial cells containing both basophilic nuclear and eosinophilic cytoplasmic inclusions. In contrast to epithelial sites, identification of HSV-1/2 nuclear inclusions is rare in CNS due to sampling later in disease course.

### 3. Special stains

Once an inflammatory pattern compatible with infection is observed, the next step in identifying a specific infectious etiology is to use histochemical stains (commonly referred to as "special stains") to better highlight organism morphology. These include tissue Gram stains (Brown–Brenn, Lillie–Twort, and various modifications), which typically demonstrate high sensitivity for gram-positive cocci (eg, *Staphylococcus*, *Streptococcus*, and *Enterococcus* species) and gram-positive bacilli (eg, *Bacillus*, *Corynebacterium*, and *Listeria* species), which appear as dark purple (cresyl violet-positive) structures 1-2 μm wide and variable length[29] (Figure 2). Conversely, gram-negative cocci and bacilli (eg, *Neisseria*, *Klebsiella*, and *Pseudomonas* species) are frequently poorly stained by the red safranin counterstain and may blend into the background. Sensitivity of tissue Gram stains typically correlates with organism burden and may be negative in cases lacking visible bacteria on H&E-stained sections. In this context, positive stains provide additional information regarding the type of bacteria present (eg, gram-positive cocci morphologically compatible with *Streptococcus pneumoniae* isolated from blood cultures); negative stains do not rule out an infectious etiology due to potential for sampling error. Silver nitrate-based stains (eg, Steiner and Warthin–Starry) can be used to highlight gram-negative bacteria (including spirochetes such as *T pallidum*) in some scenarios, but may be difficult to interpret in areas with high density of axons, which are also highlighted by silver stains.[30] Mycobacteria can be highlighted by unmodified acid-fast bacilli (AFB) stains as well as modified AFB (mAFB) stains, including the Fite-Faraco method.[31] *Mycobacterium* species including *Mycobacterium tuberculosis* and *Mycobacterium avium* will appear as thin red bacilli on AFB and mAFB stains due to carbol fuchsin affinity for mycolic acids. *Mycobacterium leprae* (short bacilli) and *Nocardia* spp. (filamentous and partially acid fast) are typically only highlighted by the gentler mAFB stains, which utilize mineral oil and a lower concentration of acid for decolorization. Acid-fast bacilli stains have also been used to highlight cestode hooklets, most commonly in neurocysticercosis (eg, *T solium* larvae).[32]

Grocott's or Gomori's methenamine silver (GMS) and periodic-acid Schiff with diastase (PAS-D) stains are the most common histochemical stains used to highlight fungal morphology and distribution throughout a tissue section, including perineurial and angioinvasion.[33–35] Filamentous fungi can be divided into narrow hyphae with frequent septations and acute-angle branching (eg, *Aspergillus*, *Fusarium*, and *Scedosporium* species) vs broad ribbon-like pauciseptate hyphae with 90-degree angle branching (eg, Mucorales order species) (Figure 3A). Yeast can be divided by size and budding pattern, from small (3-5 μm in diameter) with narrow-based budding (eg, *Histoplasma* species) to large (>20 μm in diameter) with broad-based budding (eg, *Blastomyces* species) (Figure 3B). Additional histochemical stains including mucicarmine and Alcian blue can be used to highlight the residual capsule in *Cryptococcus* species (medium-sized yeast with narrow-based budding). Fontana-Masson can also be used to highlight *Cryptococcus* yeast forms. In these cases, it is critical to correlate with organism size as Fontana-Masson also reacts with melanin pigments in other yeast and yeast-like structures including *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, and *Coccidioides immitis* endospores.[36] *Candida* species may appear as small- to medium-sized yeast highlighted by tissue Gram stains, pseudohyphae with variable Gram positivity, and occasionally true hyphae that lack any Gram staining. Grocott's or Gomori's methenamine silver and PAS-D are not specific for fungi, and will variably stain bacteria when present, including filamentous bacilli of *Actinomyces* and *Nocardia*, which are easily differentiated from fungal hyphae by their narrow width (1-2 μm). Periodic-acid Schiff with diastase highlights the intracellular bacilli of *T whipplei*, which despite being gram-positive are not readily highlighted by Gram stains. Grocott's or Gomori's methenamine silver and PAS-D can also highlight cysts of free-living amoeba and other parasite structures, as well as various normal brain structures (eg, corpora amylacea

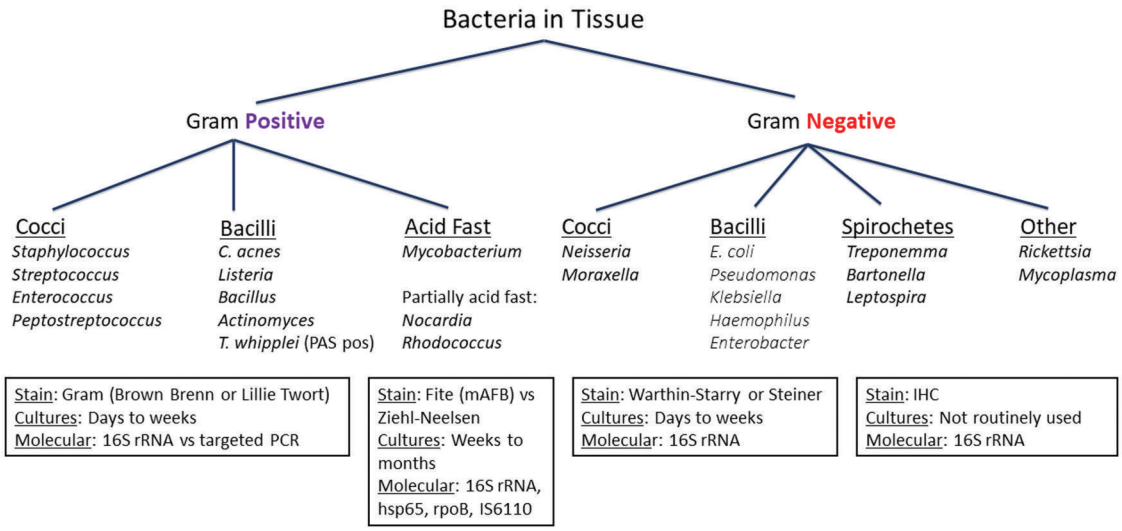


**Figure 2.** Diagnosis of central nervous system bacterial infections. Bacteria are classified based on positive or negative Gram staining and organism morphology. Special stains, typical time for culture growth, and molecular testing targets are listed for each group of organisms.

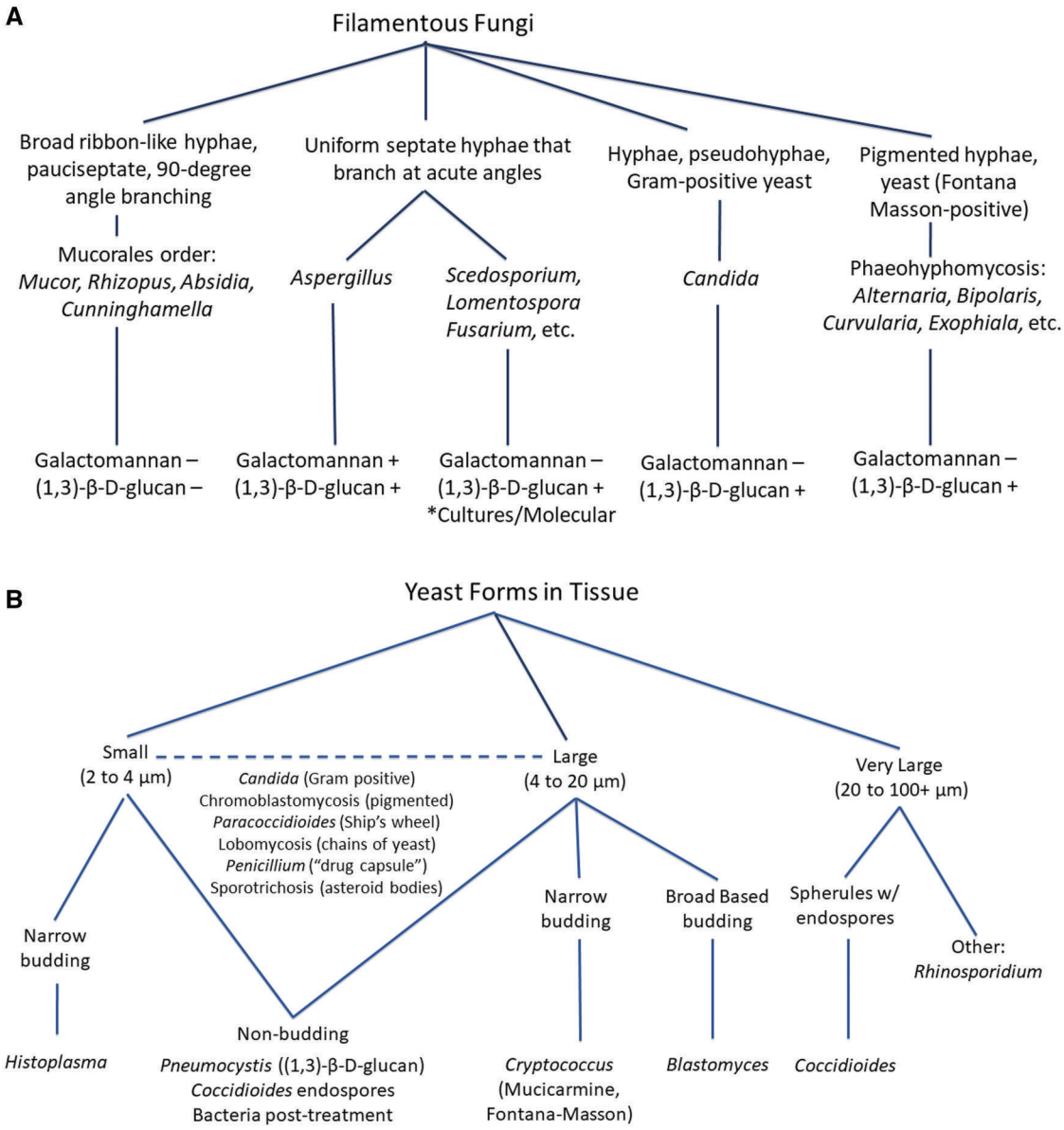


**Figure 3.** Diagnosis of central nervous system fungal infections. (A) Filamentous fungi are identified by hyphae size, shape, and pigmentation in the context of serum or cerebrospinal fluid galactomannan and (1-3)-β-D-glucan levels. (B) Yeast and yeast-like organisms are identified by size, shape, and budding pattern.

and intraneuronal lipofuscin). Correlation with H&E slides can help distinguish between special stain artifacts and microorganisms.

While there are no special stains routinely used for diagnosis of viral infections, Luxol fast blue in conjunction with PAS, cresyl violet, or H&E is commonly used to assess for demyelination in cases of progressive multifocal leukoencephalopathy due to JC polyomavirus reactivation. Giemsa stain can be used in tissue sections to assist with evaluation of parasite morphology but generally yields limited additional information beyond H&E or hematoxylin counter-stained IHC slides.

When choosing an initial panel of special stains, it is generally prudent to start broadly, due to the lack of specificity of inflammatory patterns and potential for polymicrobial infections. This could include a Gram stain for bacteria, an mAFB stain for mycobacteria, and GMS stain for fungi. If the patient's history, laboratory data (eg, CSF glucose and protein levels), or H&E findings (eg, bacteria or fungal elements observed) increase suspicion for a particular group of organisms, then additional stains for bacteria (eg, Warthin–Starry), mycobacteria (unmodified AFB), and fungus (PAS-D) can be added on as appropriate. For evaluation of yeast, Gram, mucicarmine, and/or Fontana-Masson can be added on to rule in or out *Candida* and *Cryptococcus*, respectively.

### 4. Immunohistochemistry/in situ hybridization

Immunohistochemistry has many applications for infectious disease diagnostics, with a variety of monoclonal and polyclonal antibodies in widespread use.[37] Most IHC assays are designated as laboratory developed tests, and specific protocols for

antigen retrieval, primary antibody concentration and incubation time, and secondary antibodies and detection method vary slightly between labs, introducing numerous opportunities for differences in sensitivity and specificity. It is therefore important to review positive and negative control slides to ensure stains are performing appropriately with each run.

Immunohistochemistry has had the greatest impact for improving diagnosis of viral infections, and most clinical laboratories have ready access to HSV-1/2, VZV, CMV, adenovirus, and JC polyomavirus assays (Table 2). For viruses with readily identifiable nuclear or cytoplasmic inclusions (eg, CMV, HSV-1/2, JC polyomavirus), IHC is used as a confirmatory test, and can be omitted if the patient has classic H&E findings in the setting of positive PCR or serological testing. For cases with equivocal viral cytopathic effects (eg, VZV, adenovirus, and some cases of HSV-1/2), IHC can effectively clinch the diagnosis. For less common infections (eg, due to measles virus, lymphocytic choriomeningitis virus, and arboviruses), IHC assays may be available from commercial or public health reference laboratories but are not practical for individual laboratories to validate and maintain active protocols. Nonspecific viral stains such as dsRNA IHC have been evaluated to assist in these types of cases.[38]

Immunohistochemistry is less commonly used for bacterial infections due to antigen cross-reactivity between genera and species.[39] In addition, commonly encountered bacteria such as staphylococci and streptococci can be isolated from cultures in 1 to 2 days, with more specific species-level identification than could be accomplished by IHC in a similar amount of time. Notable exceptions include IHC for spirochetes (eg, *T pallidum*), which is widely available and preferred to silver nitrate-based histochemical stains.[40] *Mycobacterium* spp. IHC is increasingly used in academic laboratories and has similar sensitivity and specificity as modified AFB stains.[41] Other less common bacteria, including difficult-to-culture species such as *T whipplei*, *Mycoplasma/Ureaplasma* spp., and *Rickettsia* spp., can also be identified by IHC at reference laboratories.[42–44]

Due to similar cross-reactivity issues as bacteria and the abundance of information gained from morphology alone, IHC is not routinely used for fungal identification. However, antibodies for Mucorales order, *Aspergillus*, *Candida*, *Fusarium*, and other genera have been studied, and may be available for diagnostic use in some academic or reference laboratories.[45–47]

As parasites are largely identified based on morphology, IHC is typically unnecessary for diagnosis.[27] One notable exception is *Toxoplasma* IHC, which can help identify tachyzoites amongst necrotic debris when more distinctive bradyzoites are not present.[48] Other applications include confirmation of free-living amoeba vs histiocytes, with distinction between *Acanthamoeba*, *Balamuthia*, and *Naegleria* species, and definitive identification of *Leishmania* and *Trypanosoma* amastigotes.[49–51]

In contrast to the lack of specificity of IHC due to antigen cross-reactivity, ISH is sensitive enough to distinguish different strains of the same species if sufficient nucleic acid sequence diversity is present. Epstein–Barr virus encoded RNAs (EBER) ISH is widely used to identify infections and neoplastic/lymphoproliferative cells.[52] Probe sets targeting a wide variety of organisms are commercially available and automatable using IHC staining platforms. While not widely used for diagnostics, RNAScope-based ISH has been used to confirm unexpected sequencing results with viral infections and may play a useful role in the diagnosis of fungal infections.[53,54] In situ hybridization is an attractive rapidly deployable option for detection of emerging infections prior to the availability of less expensive IHC assays.

## 5. Electron microscopy

In the current era of IHC and molecular testing, electron microscopy (EM) is rarely used diagnostically for infectious diseases. This is due in part to the focal nature of many microorganisms and the limitations of biopsying brain tissue. Higher quality images are obtained when fresh tissue is placed in glutaraldehyde, but tissue from formalin-fixed paraffin-embedded (FFPE) blocks can be deparaffinized and processed for EM if needed; this also allows for correlation with specific areas of interest. When present, virions are assessed for size, shape, capsid morphology, and presence or absence of an envelope, which can allow for identification to a family or genus (eg, poxviruses, herpesviruses, and coronaviruses).[55] More specific identification typically requires antibody targeting such as immunogold labeling. Electron microscopy studies are extremely important in emerging infections to study the distribution of microorganisms in different cell types and subcellular localization and to confirm that unexpected molecular results correspond to tissue invasive pathogens and are not artifactual reads or environmental contaminants introduced through reagents.[56,57] As normal cellular organelles can resemble some viruses in size and shape, it is critical that the microscopist have sufficient expertise in viral identification to avoid misdiagnosis.[58] While less common than for viruses, various protozoans can also be identified by EM, including microspordia.[59]

## 6. Molecular diagnostics

Molecular testing has become increasingly available to aid in infectious disease diagnosis and can rapidly generate clinically actionable information when utilized appropriately. Specimen selection is important, as there are tradeoffs between analyzing frozen tissue allocated at the time of specimen collection vs routinely processed FFPE material. If allocated in a sterile manner, either in the operating room or microbiology laboratory, the risk of contaminating nucleic acids can be minimized. In addition, the lack of formalin cross-linking allows for longer and higher quality PCR sequences. In contrast, FFPE tissue may be contaminated by microbial nucleic acids from any of the reagents used, including paraffin wax, or from concurrently processed specimens.[60,61] Histology water baths are also a potential source of contamination, and samples for molecular testing should be collected directly into sterile plastic tubes when possible, rather than scraped from intermediary glass slides used to pick up floating sections. The yield of molecular testing increases when organisms are known to be present in the sample tested,[62,63] which can be confirmed in many FFPE cases by histochemical stains but not in blindly sampled fresh

tissue specimens; this may sufficiently offset the decrease in yield following formalin fixation.

After collecting the sample to be tested, an appropriate molecular assay should be selected that can be targeted to a specific organism, group of organisms, or completely nontargeted. When a high degree of suspicion is present for a specific organism due to clinical history or histological findings, RT-PCR with or without sequencing can be used to confirm the diagnosis. Examples of when this may be useful include confirmation of PAS-D-positive intracellular bacilli as *T whipplei*, identification of free-living amoeba as *Acanthamoeba* or *Balamuthia* species, and identification of JC polyomavirus in samples with equivocal viral cytopathic effects and demyelination. In cases with wider differentials, broad-spectrum sequencing of conserved microbial genes that are used for taxonomic classification can also be useful for diagnosis. Specific bacterial species can be identified by sequencing the hypervariable V1/V2 region of the 16S rRNA gene,[64] while mycobacterial sequencing assays often target a combination of 16S rRNA, hsp65, and rpoB genes[65] (Figure 2). Fungal sequencing targets the D1/D2 region of the 28S rRNA gene, as well as the internal transcribed spacer region encompassing the 5.8S rRNA gene.[66,67] As there are no universally conserved viral genes, the primary method for broad spectrum molecular testing is metagenomic next-generation sequencing (mNGS).[68] This can be performed in a nontargeted fashion, in which all non-human RNAs or DNAs are identified, or using a targeted primer set approach (eg, hybrid capture) enhancing for known disease-associated microorganisms. Metagenomic next-generation sequencing is generally slower and more costly than other molecular assays due to additional steps in sample preparation and sequencing interpretation but has high sensitivity due to the potential to detect any region of microbial genome without a risk of primer incompatibility. While not widely available for FFPE samples outside of research studies, CSF mNGS is available at multiple reference laboratories.[69]

Similar to molecular testing with brain tumors, it is always recommended that results of molecular testing for infections be correlated with histology, as well as cultures and other laboratory results.[70] Bacterial and mycobacterial sequencing can yield positive results in cases with negative special stains, due to poorly staining intracellular species or extremely low organism burden. However, molecular detection of readily detectable gram-positive cocci (eg, *Staphylococcus epidermidis*) with negative tissue Gram stains should raise the possibility of contamination. Similarly, the detection of fungal DNA in the absence of histologically identified fungal elements is almost guaranteed to be contamination, and sequencing is not recommended in this setting.[62] When a specific organism is suspected and both targeted and broad-spectrum molecular tests are available (eg, *T whipplei*), it is generally recommended to perform the targeted test first, and if negative, reflex to the broader test (eg, 16S rRNA sequencing).

### 7. Expert consultation

While many CNS infections are straightforward to diagnose, there are situations in which consultation can be useful. Infectious disease pathology expertise may be found in the clinical microbiology laboratory or from anatomic pathologists with different organ subspecialty expertise who have also developed a subinterest in infections.[71] These individuals can assist in the recommendation and interpretation of ancillary testing including special stains, IHC, and molecular testing. Referral of cases to public health reference laboratories such as the CDC Infectious Diseases Pathology Branch may be useful for suspected infections of unknown etiology, infection with suspected pathogens for which no commercial testing options are available, and for emerging or other infections with public health implications which may require confirmation in the postmortem setting. Diagnostic assistance for parasite infections can also be obtained through DPDx, a website maintained by the CDC Division of Parasitic Diseases and Malaria.

## INTEGRATED DIAGNOSIS

As discussed above, the histological findings, special stains, IHC/ISH, and molecular findings should be correlated with patient history, culture results, and other laboratory findings into a final integrated diagnosis, analogous to the World Health Organization classification of brain tumors. Several examples of this are as follows:

### Case 1

*History:* A 73-year-old man with a history of severe Crohn's disease, status post multiple resections on chronic total parenteral nutrition, adalimumab, methotrexate, and prednisone was admitted with subacute altered mental status. MRI identified a nodular heterogeneously enhancing lesion in the left insula (2.6 cm) suspicious for high-grade glioma, metastasis, or infection (Figure 4A). *Histopathology:* Fragments of necroinflammatory debris and reactive brain tissue, consistent with abscess (Figure 4B). Gram, GMS (Figure 4C), and mAFB (Figure 4D) stains highlight long filamentous bacteria, which are best seen on GMS and are only focally positive on Gram and mAFB stains. *Laboratory testing:* Cultures positive for *Nocardia* sp. and 16S rRNA sequencing positive for *Nocardia puris. Final integrated diagnosis: N puris* brain abscess.

### Case 2

*History:* A 76-year-old man with a history of renal transplant and diabetes presenting with left facial pain. MRI showed multiple peripherally enhancing lesions (0.4-1.5 cm) involving the right parietal lobe, bilateral frontal lobes, left posterior cerebral hemispheres, and left putamen (Figure 4E). *Histopathology:* Abscess containing negatively staining round structures with surrounding halo (Figure 4F). Grocott's methenamine silver highlights medium-sized irregularly shaped yeast forms with narrow-based budding, positive for Fontana-Masson, and capsule positive by mucicarmine (Figure 4G). *Laboratory testing:* Cultures negative for growth after 28 days. Serum (1-3)-β-D-glucan <31 pg/mL (normal range 0-80 pg/mL), and CSF *Cryptococcus* antigen negative. *Integrated diagnosis: Cryptococcus* sp. brain abscess (Cryptococcoma).

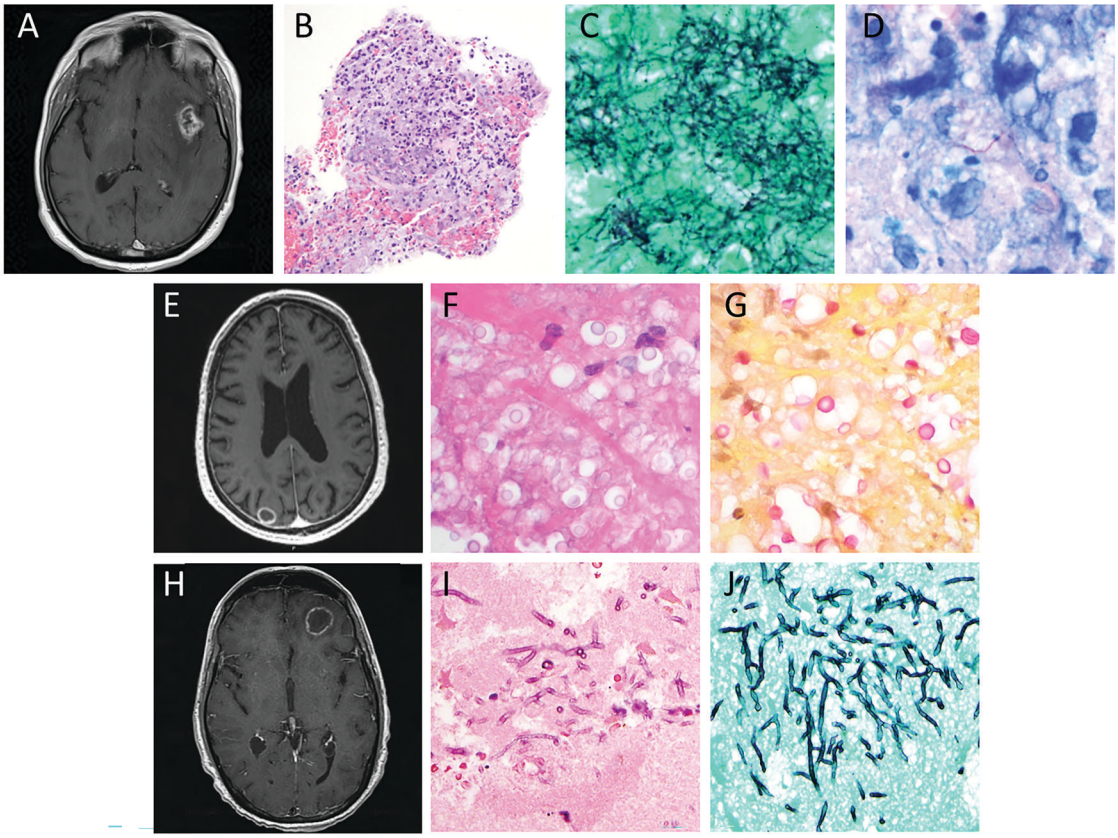


**Figure 4.** Neuroimaging and histopathology of central nervous system infections: Cases 1-3. (A-C) *Nocardia puris* brain abscess appears as a 2.6-cm heterogeneously contrast-enhancing lesion in the left insula on MRI (A) and consists of neutrophilic inflammation with necrosis on H&E stain (B) with abundant filamentous bacteria highlighted by Grocott's methenamine silver stain (black) (C) that are only focally highlighted by modified acid-fast bacilli stain (red) (D). (E-G) *Cryptococcus neoformans* abscess appears as a 1.5-cm peripherally enhancing mass in the right parietal lobe on MRI (E) and contains yeast that are round negatively staining structures on H&E stain (F) with capsules highlighted by mucicarmine stain (red) (G). (H-J) *Scedosporium apiospermum* abscess appears as a 2.4-cm ring-enhancing mass in the left frontal lobe on MRI (H) and contains narrow hyphae with abundant septations infiltrating brain parenchyma highlighted by H&E stain (I) and Grocott's methenamine silver stain (black) (J).

### Case 3

*History:* A 51-year-old man presented with relapsed acute myeloid leukemia, status postbone marrow transplant and was found on brain MRI to have a left frontal lobe ring-enhancing mass (2.4 cm) with surrounding edema, suspicious for abscess (Figure 4H). *Histopathology:* Fungal abscess with narrow non-pigmented hyphal forms visible on H&E sections (Figure 4I) and highlighted by GMS (Figure 4J) and PAS-D stains. *Laboratory testing:* Serum (1-3)-β-D-glucan elevated to 166 pg/mL (normal range 0-80 pg/mL) and normal levels of galactomannan (0.24; normal range 0-0.49 index). Cultures positive for *Scedosporium apiospermum*. *Integrated diagnosis*: *S apiospermum* brain abscess.

### Case 4

*History:* A 70-year-old man with autoimmune polyendocrine syndrome type I on chronic corticosteroids presented with altered mental status after an unwitnessed fall followed by 2 witnessed seizures. MRI identified edema in the left temporal lobe and hippocampus (Figure 5A). *Histopathology:* Autopsy sections from the temporal lobe show lymphohistiocytic inflammatory infiltrates involving the leptomeninges with diffuse parenchymal involvement and perivascular collections, consistent with chronic meningoencephalitis (Figure 5B). HSV-1/2 IHC highlights numerous ghost cells and neuropil with relative sparing of blood vessels (Figure 5C). *Laboratory testing:* Lumbar puncture for CSF evaluation yielded 4 white blood cells per cubic millimeter and mildly elevated protein. Premortem CSF positive for HSV-1 by PCR, and HSV-1 isolated from postmortem temporal lobe tissue cultures. *Integrated diagnosis:* Herpes simplex virus 1 meningoencephalitis.

### Case 5

*History:* A 56-year-old man with history of mantle cell lymphoma in remission on maintenance rituximab presented with rapidly progressing dementia of unknown etiology in the setting of persistently inflammatory CSF unresponsive to high-dose steroids. MRI showed slight ventriculomegaly attributed to atrophy but was otherwise normal (Figure 5D). *Histopathology*: Autopsy sections from multiple brain sections show diffuse infiltration of lymphocytes involving leptomeninges

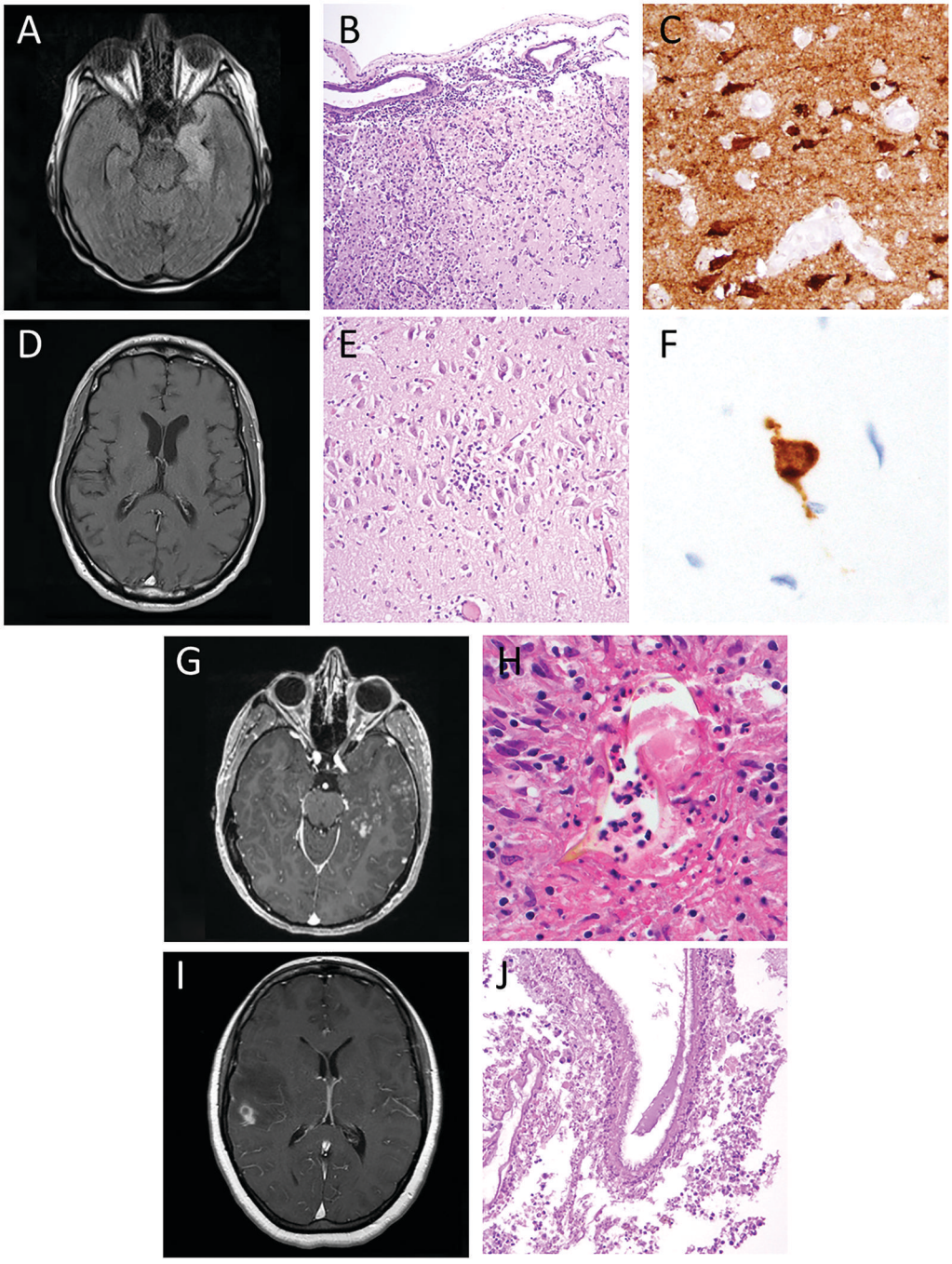


**Figure 5.** Neuroimaging and histopathology of central nervous system infections: Cases 4-7. (A-C) Herpes simplex virus 1 meningoencephalitis exhibits left temporal lobe hyperintensity on MRI (A) and contains leptomeningeal and parenchymal lymphohistiocytic inflammatory infiltrates on H&E stain (B) with presence of viral antigen confirmed by HSV-1/2 immunohistochemistry (brown) (C). (D-F) Jamestown Canyon virus encephalitis lacks focal abnormalities on MRI (D) and contains microglial nodules on H&E stain (E) with viral infected cells confirmed by JCV RNA in situ hybridization (brown) (F). (G, H) Neuroschistosomiasis due to *Schistosoma mansoni* appears as multifocal nodular linear enhancement on MRI (G) and contains ova with lateral spines engulfed by granulomatous inflammation on H&E stain (H). (I, J) Degenerating *Taenia solium* cysticercus appears as a 1.5-cm ring-enhancing mass in the right supramarginal gyrus (I) and contains strands of tegument on H&E stain (J).

and brain parenchyma and microgliosis with microglial nodules and neuronophagia, consistent with chronic meningoencephalitis (Figure 5E). Jamestown Canyon virus RNA ISH highlights scattered neurons (Figure 5F). *Laboratory testing:* CSF mNGS positive for Jamestown Canyon virus, confirmed by serum and brain tissue PCR. Serum and CSF negative for Jamestown Canyon virus IgM. *Integrated diagnosis:* Jamestown Canyon virus meningoencephalitis.

### Case 6

*History:* A 25-year-old woman presented to the emergency department following a generalized seizure, who previously

spent a semester living in Ghana 4 years prior. MRI showed multiple left temporal, right temporoparietal, and right cerebellar areas of abnormality with nodular linear enhancement, suspicious for vasculitis, lymphomatoid granulomatosis, granulomatous disease, or tumor (Figure 5G). *Histopathology:* Reactive brain tissue with necrotizing granulomatous inflammation surrounding refractile structures up to 100 μm in length with lateral spines, morphologically compatible with trematode ova (Figure 5H). *Laboratory testing:* Schistosoma IgG antibody positive (1.37; normal range 0-0.99 titer). *Integrated diagnosis*: Cerebral schistosomiasis (reaction to *Schistosoma mansoni* ova).

### Case 7

*History*: A 49-year-old woman with no significant past medical history who traveled yearly to the Dominican Republic presented with double vision, facial twitching, and bilateral upper extremity weakness. CT revealed brain and lung nodules concerning for malignancy, and MRI showed a 1.5-cm ring-enhancing mass at the right supramarginal gyrus (Figure 5I). *Histopathology*: Fragment of brain with partially encapsulated lesion with associated chronic inflammation, fibrosis, and macrophages surrounding a foreign organism, composed of epithelial-like layer with tegument and calcareous corpuscles, consistent with degenerating cestode larvae (Figure 5J). *Laboratory testing*: Cysticercosis IgG antibody negative by Western blot. *Integrated diagnosis*: Neurocysticercosis (degenerating *T solium* larvae).

## CONCLUSIONS

Efficient diagnosis of CNS infections is critical for patient care, and the neuropathologist serves an important role in brain tissue evaluation. A systematic approach is recommended in which relevant patient history and radiological findings are reviewed, followed by H&E evaluation to generate a preliminary differential diagnosis, which is confirmed by special stains, IHC/ISH, or molecular testing and correlated with culture results and other laboratory testing into a final integrated diagnosis. When necessary, expert consultation can be obtained to recommend or assist in interpretation of stains and molecular testing.

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## CONFLICTS OF INTEREST

The authors declare no relevant conflicts of interest.

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