Educational Case

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Educational Case: Histologic and Molecular Features of Diffuse Gliomas

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The following fictional cases are intended as a learning tool within the Pathology Competencies for Medical Education (PCME), a set of national standards for teaching pathology. These are divided into three basic competencies: Disease Mechanisms and Processes, Organ System Pathology, and Diagnostic Medicine and Therapeutic Pathology. For additional information, and a full list of learning objectives for all three competencies, see http://journals.sagepub.com/doi/10.1177/2374289517715040.[1]

Keywords
pathology competencies, organ system pathology, nervous system-central, brain tumor classification, astrocytoma, oligodendroglioma, tumor grading, molecular genetics

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Primary Objective

Objective NSC1.2: Classification of Brain Tumors. Compare and contrast the common types of brain tumors that affect the cerebrum, the cerebellum, the meninges, and the cranial nerves in adults and children, and outline their molecular basis and clinicopathologic features.

Competency 2: Organ System Pathology, Topic: Nervous System–Central Nervous System (NSC), Learning Goal 1: CNS Neoplasia

Secondary Objective

Objective NSC1.4: Grading of Brain Tumors. Explain the pathophysiologic basis for grading primary brain tumors and discuss how grading relates to prognosis and governs patient management.

Competency 2: Organ System Pathology, Topic: Nervous System–Central Nervous System (NSC), Learning Goal 1: CNS Neoplasia

Patient Presentation, 1

A 35-year-old woman presents to the emergency department after having awoken in the middle of the night with nausea, confusion, and an inability to recognize her husband. The patient developed a generalized seizure as she was being transported by ambulance to a hospital, where the patient had 2 additional generalized seizures. She has no significant past medical history. She had no history of exposure to radiation or chemotherapy.

Diagnostic Findings, Part 1

A neurologic examination demonstrates normal memory, eye movements, hearing, vision, gait, and sensation and strength throughout with no prosopagnosia or deficit in stereognosis. She denied headache, vomiting, fever, chills, or nuchal rigidity.

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Questions/Discussion Points, Part 1

Given the Patient's Clinical Presentation and Neurologic Examination, What Would Be Considered in the Differential Diagnosis?

The patient's confusion followed by a generalized seizure is consistent with a temporal lobe partial focal seizure that progressed to a generalized seizure. In a young adult presenting with a first seizure, the initial workup must determine whether the event was a true seizure or a seizure mimicker. The most common clinical events that can present like seizures include migraines, syncope, transient ischemic attacks (TIAs), psychogenic nonepileptic seizures, panic attacks, and paroxysmal movement disorders. True seizures, both focal and generalized, will show electroencephalogram (EEG) epileptiform abnormalities, are often less than 1 to 5 minutes in duration, and have an associated postictal state. Psychogenic nonepileptic seizures will not show any EEG abnormalities. The symptoms associated with focal seizures can vary depending on the location of activity in the brain and may or may not have associated loss of consciousness. Generalized seizures will have loss of consciousness with tonic–clonic jerks and/or staring and may have tongue-biting or urinary incontinence. Transient ischemic attack and migraine symptoms can last up to a few hours. Migraines present with a slower onset than TIAs or focal seizures. Panic attacks and migraines are both associated with classic symptoms; migraines often have visual and sensory symptoms associated with a headache and panic attacks frequently present with palpitations, dizziness, dyspnea, chest pain, and a sense of impending doom.

How Could This Patient Be Worked Up?

The next step in a seizure workup involves finding the underlying cause. Seizures can have environmental triggers such as lights, intense emotions, and physical exertion, or physiological triggers such as stress, sleep deprivation, and fevers. Intoxication or withdrawal from alcohol and other substances of abuse can also lower the seizure threshold. There are also many prescription and over-the-counter medications that are associated with seizures (eg, psychiatric medications, stimulants, sympathomimetics, antimicrobials, antidiabetic agents, and herbs/natural remedies). Aside from triggering or toxic insults to the brain, the brain itself can have an abnormality that causes seizures. This is best elucidated through neuroimaging.

Neuroimaging is indicated in all patients with suspected intracranial lesions, including patients presenting with their first seizures. Various imaging modalities can be used to demonstrate specific characteristics of intracranial lesions. Computed tomography (CT) has lower resolution compared to magnetic resonance imaging (MRI) and is more commonly used in emergencies or in patients with contraindications to MRI (ie, patients with implanted devices or claustrophobia). Magnetic resonance imaging of the brain employs multiple types of sequences that can help differentiate mass lesions from each other and rule out nonneoplastic etiologies, as described below.

Diagnostic Findings, Part 2

Imaging

A head CT angiogram with intravenous (IV) contrast demonstrates medial displacement of the left posterior cerebral artery (PCA) branches related to a left medial temporal hypodense tumor without significant arterial narrowing (Figure 1A). Magnetic resonance imaging of the brain with and without contrast demonstrates a 4.5 cm × 8.4 cm × 5.5 cm, T1-hypointense, nonenhancing mass centered in the posteromedial left temporal lobe with inferior extension into the left anterior posterior fossa (Figure 1B and C). The mass is centered in the left temporal lobe, with mass effect on the left posterolateral brain stem. A portion of the tumor wraps around the corpus callosum to involve the posterior cingulate gyrus. The tumor is hyperintense on T2 and fluid attenuation inversion recovery (FLAIR) images (Figure 1D and E). No diffusion restriction is observed on diffusion-weighted imaging (DWI), indicating that the tumor is not densely cellular (Figure 1F).

Discussion/Questions Points, Part 2

How Do the Imaging Findings Help Narrow Down the Differential?

The imaging is most consistent with a low-grade glioma. Gliomas are primary brain tumors derived from glial cells. In the tradition of Harvey and Cushing, these tumors are named for their resemblance to normal central nervous system (CNS) cells under the assumption that these tumors have common cells of origin.[2] Infiltrating gliomas are the most common type of glioma and they include both diffuse astrocytomas and oligodendrogliomas.[3] In contrast to a discrete lesion, an infiltrative lesion does not have a clearly defined border between the lesion and the uninvolved tissue. Diffuse astrocytomas and oligodendrogliomas are low-grade gliomas that are less clinically aggressive than their high-grade counterparts. High-grade gliomas, glioblastomas, and anaplastic astrocytomas are enhancing tumors (hypointense on T1-weighted images that enhance with contrast) that are commonly associated with vasogenic edema. Glioblastomas characteristically demonstrate rim enhancement along the margins with central clearing (indicative of necrosis or cystic change) with increased blood flow on perfusion imaging. Low-grade gliomas are usually nonenhancing, expansile lesions that involve both the cortex and the underlying white matter without associated edema. Diffuse astrocytomas and oligodendrogliomas can also be differentiated based on their location and appearances on MRI sequences.

Diffuse astrocytomas commonly arise in the cerebral hemispheres. They usually do not have contrast enhancement on MRI.[4] They are typically confined to the white matter and cause mass effect on the adjacent cortex. Generally, low-grade astrocytomas are isointense to hypointense compared

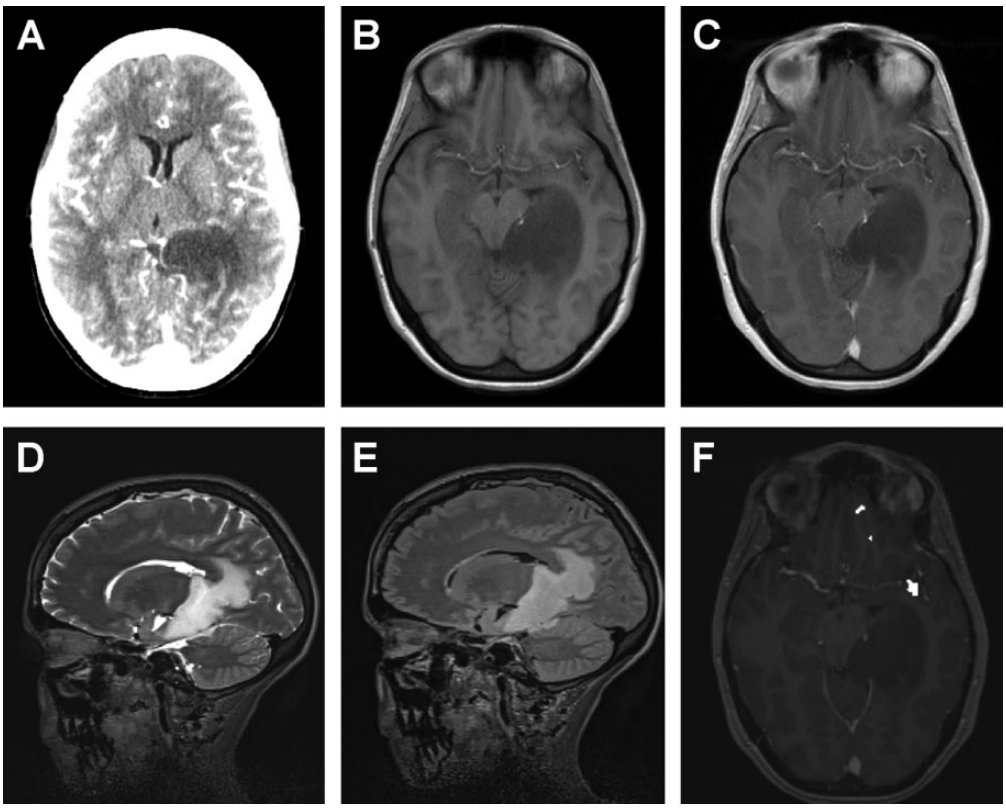

Figure 1. Patient 1 imaging studies. A, A right mediotemporal mass is observed on contrast computed tomography (CT) with midline shift. T1-weighted magnetic resonance imaging (MRI) demonstrates a hypointense mass (B; precontrast) that does not enhance following administration of contrast (C). Sagittal T2-weighted imaging demonstrates involvement of left temporal lobe by this hyperintense mass (D) that is less intense on fluid attenuation inversion recovery (FLAIR) imaging (E). F, The mass is hypointense on diffusion-weighted MRI.

to white matter on T1-weighted images and hyperintense on T2-weighted images. The lesion in this case showed a nonenhancing, T1-hypointense and T2-hyperintense signal.

Diffusion-weighted imaging is based on the principle that the ability of water molecules to diffuse within a tissue is a function of the density of that tissue. Typically, denser tissue, such as an area of infarction or a highly cellular tumor, results in less water molecule mobility. This is also known as diffusion restriction. The T2 sequence will demonstrate a less intense, dark signal within areas that water can diffuse easily. The apparent diffusion coefficient (ADC) value is another way to refer to diffusion restriction. Low ADC values signify abnormal restricted diffusion. Astrocytomas have lower cellularity and a greater hyaluronan proportion, giving them higher ADC values than oligodendrogliomas.[5] Therefore, DWI can help distinguish between the two. However, there was no diffusion restriction demonstrated in this case.

The FLAIR sequence is similar to T2-weighted images because the gray matter is bright and white matter is dark on imaging. However, FLAIR also cancels out the signal from cerebrospinal fluid, which is usually bright on T2-weighted sequences, thereby allowing the clinician to detect any changes around the periventricular region and hemisphere periphery. There is evidence that many isocitrate dehydrogenase (IDH) mutation and non-1p19q-codeleted gliomas display a T2-FLAIR mismatch sign—complete or near-complete T2-hyperintense signal throughout but relatively hypointense signal on FLAIR sequences.[6] This case did not demonstrate this finding. The significance of IDH mutations and 1p19q-codeleted gliomas will be discussed in part 3.

Oligodendrogliomas predominantly arise in the frontal lobes and rarely metastasize outside the CNS.[3] They have hyperintense or mixed signal compared to gray matter on T2-weighted images and hypointense or mixed signal compared to gray matter on T1-weighted images.[7] Oligodendrogliomas usually have more indistinct borders and nonuniform signal on T1- and T2-weighted images than diffuse astrocytomas.[8] Oligodendroglial calcifications can be seen as low-intensity signals on T2-weighted images and dark signal on susceptibility-weighted imaging (SWI) sequences.

On noncontrast CTs, oligodendroglial tumors have mixed density. Oligodendrogliomas usually have calcifications that are high attenuating.[9] High-attenuating areas may also be due to hemorrhage. About half of oligodendrogliomas enhance to a variable degree on contrast CTs. Low-grade astrocytomas appear as poorly defined, isodense or hypodense regions with mass effect. They usually do not have contrast enhancement or calcifications.

How Do the Radiologic Findings Correlate With the Patient's Presentation? What Other Neurologic Deficits Can Be Associated With a Tumor in This Location?

Left PCA displacement is indicative of the tumor's slow-growing nature, as the brain is able to accommodate vessel displacement with collateral circulation over time. Rapid growth and/or acute displacement of the left PCA in the dominant hemisphere may cause stroke-like symptoms (contralateral homonymous hemianopsia, visual agnosia, prosopagnosia, dyslexia, receptive aphasia, and memory defects) due to ischemia of regions that receive blood from the PCA including the inferomedial temporal lobes, occipital lobes, midbrain, thalamus, and cerebellum.

The tumor is located within the posteromedial left temporal lobe and extends into the posterior cingulate gyrus. The medial temporal lobe contains the hippocampus, critical for long-term memory consolidation and storage. If affected, the patient could experience anterograde amnesia. The inferomedial temporal lobe is composed of the fusiform gyrus and parahippocampal gyrus, which are responsible for higher levels of visual processing of faces and scenes, respectively. Therefore, damage to the inferior temporal lobe could result in prosopagnosia. The cingulate gyrus and hippocampus are both part of the limbic system, which is involved in emotion formation and processing, learning, and memory.

The patient's initial confusion likely represents a partial seizure focused in the left hippocampus that subsequently generalized to involve the entire brain.

This tumor also demonstrates mass effect onto the left posterolateral brain stem, most notably at the dorsal midbrain. Involvement of the superior and inferior colliculi can impair eye movement and hearing. Eye movement can also be affected by oculomotor and trochlear nerve involvement. Cerebral aqueduct stenosis can cause cerebrospinal fluid obstruction and hydrocephalus.

Based on the Clinical History, What Are the Most Likely CNS Neoplasms This Patient Might Have?

Diffuse astrocytomas commonly present in the third and fourth decades. They are slow-growing tumors, developing over years before producing symptoms. Similar to oligodendrogliomas, diffuse astrocytomas typically arise in the frontal lobes and initially present with seizures. Symptoms such as speech difficulties, sensory changes, vision disturbances, and motor changes can present earlier. Given its predilection for the frontal lobes, diffuse astrocytomas can also present with behavioral changes. Astrocytomas can cause increased intracranial pressure via mass effect with subsequent compression of the ventricular system, although this is less common in low-grade tumors.

Oligodendrogliomas typically present between the second to fourth decades of life, and patients are usually asymptomatic for many years due to the slow growth of these tumors.[3] Low-grade tumors and smaller lesions without surrounding edema are more likely to be clinically silent. Oligodendrogliomas can present as incidental findings on imaging obtained for other reasons. Due to cortical involvement, the most common presenting symptom is a seizure. The seizures can be focal or evolve to a generalized seizure. Oligodendrogliomas arise predominantly in the frontal lobes, often causing focal tonic–clonic seizures involving one extremity.[10] Other less common presenting symptoms include headache, signs of elevated intracranial pressure, focal neurological deficits, and mental status changes.

Based on the clinical presentation, the differential includes infiltrating gliomas, though other CNS malignancies and non-neoplastic processes would also be included.

What Risk Factors Predispose a Patient to Develop CNS Neoplasms?

Ionizing radiation is a dose-dependent risk factor for the development of a CNS neoplasm.[11] The latency period between irradiation and brain tumor development ranges from 5 years to decades later. Receiving irradiation treatment for other primary tumors and acute leukemia before the age of 5 confers the greatest risk for developing gliomas later in life.[12]

Several familial syndromes are associated with CNS tumor development. Patients with neurofibromatosis type 1 have an increased risk for developing optic pathway gliomas.[13] Familial adenomatous polyposis (*APC* gene mutations) and Lynch syndrome (mismatch repair gene mutations) are also associated with medulloblastomas and gliomas, respectively.[14-16] Patients with Li-Fraumeni syndrome (*TP53* germ line mutations) can develop diffuse astrocytomas.[17] Many sporadic low-grade infiltrating gliomas have mutations in the *IDH-1* or *IDH-2* genes (see below); low-grade astrocytomas are also associated with somatic mosaic mutations in the *IDH-1* or *IDH-2* genes in patients with Ollier-type multiple enchondromatosis or with Maffucci syndrome.[18,19]

Additional genomic loci have been associated with familial glioma syndromes. Isocitrate dehydrogenase–mutant oligodendrogliomas and astrocytomas are associated with a low-frequency single-nucleotide polymorphism (SNP) at 8p24.21.[20] An SNP is a nucleotide variant at a specific base position within the genome. Each nucleotide variant is present in differing percentages within different populations. Oligodendrogliomas are also associated with GSTT1 null genotype (glutathione S-transferase theta 1), SNPs in *GLTSCR1* (gliomas tumor suppressor candidate region gene 2 protein) and *ERCC2* genes, and germ line mutations in shelterin complex genes.[21-23] GSTT1 is part of a family of proteins involved in catalyzing glutathione reduction. The *ERCC2* gene encodes a protein involved in nucleotide excision repair. Shelterin is a protein complex that safeguards telomeres.

In Addition to Neuroimaging, What Other Diagnostic Testing Is Available for This Patient?

The definitive diagnosis of infiltrative glioma requires an adequate tissue sample for histopathologic and molecular studies.

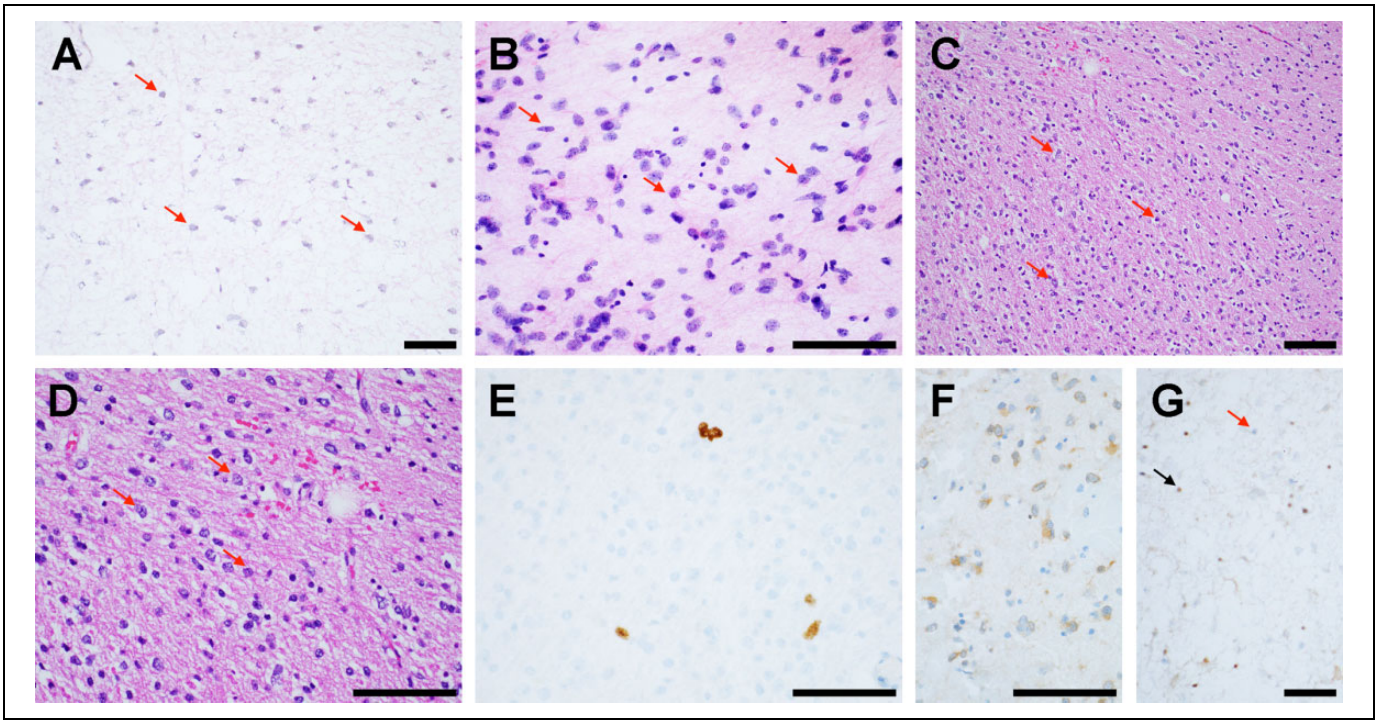

Figure 2. Patient 1 histology. Intraoperative analysis slides. A, The frozen section demonstrates tissue splayed apart by edema and infiltrated by atypical nuclei (arrows; ×20, hematoxylin and eosin [H&E] stain). B, A smear demonstrates numerous tumor cells (arrows) with mildly pleomorphic nuclei and fine glial processes (×40, H&E). Formalin-fixed, paraffin-embedded (FFPE) tissue analysis. H&E stains demonstrate white matter infiltrated by tumor cells (arrows) with mildly pleomorphic nuclei (C, ×20; D, ×40). E, The percentage of cells that express Ki-67 is low, here less than 2% (×40). F, An isocitrate dehydrogenase-1 (IDH-1) R132H immunostain demonstrates expression of the mutant protein by tumor cells (×40). G, Tumor cells do not express ATRX (blue counterstained nuclei, red arrow). Inflammatory cells (black arrow) serve as an internal control for detection of protein expression (×20). Scale bar = 100 μm.

Tissue can be obtained via stereotactic biopsy for deep-seated tumors or at the time of surgical resection for more accessible tumors.

Diagnostic Findings, Part 3

The patient underwent a stereotactic partial resection of the anterior portion of the left temporal glioma 1 month after initial presentation. An intraoperative frozen section was performed (Figure 2A and B).

Questions/Discussion Points, Part 3

The frozen section demonstrated hypercellular white matter with many atypical nuclei (Figure 2A). A small portion of the biopsy was smeared to reveal that many of the atypical nuclei in the tissue belonged to cells with fibrillar, astrocytic processes (Figure 2B). Permanent sections confirmed the intraoperative impression, revealing white matter infiltrated by the atypical nuclei of an astrocytoma (Figure 2C and D). No mitoses were identified. An additional immunohistochemical workup demonstrated tumor expression of R132H-mutant IDH-1, loss of *ATRX* expression, and a relatively low Ki-67 proliferation index (Figure 2E-G).

Postoperatively, the patient demonstrated right-sided neglect, difficulty naming objects, and a right superior quadrantanopsia (loss of vision in a quarter of the field of vision). The first surgery achieved a subtotal gross resection; because it was subsequently felt that more tumor could be removed safely and extent of glioma resection correlates strongly with overall survival,[24,25] a second surgery was performed 5 months after the first with no new deficits. Over the next year, the patient's visual and memory symptoms improved. The patient underwent partial brain radiotherapy and is undergoing procarbazine, lomustine, and vincristine (PCV) chemotherapy with stable disease 4 years after presentation.

What Is the Purpose of Performing an Intraoperative Consultation? How Would You Approach an Intraoperative Consultation for a Brain Specimen?

The main goals in performing intraoperative frozen sections are to guide intraoperative and postoperative management, to confirm the presence of lesional tissue for subsequent ancillary studies, and to process tissue optimally for diagnosis, treatment, or research. Neuropathology frozen consultations usually include a cytologic smear along with the frozen section slides. Edematous CNS tissue is particularly susceptible to freeze

artifact, which must be taken into account when assessing the specimen cellularity.

When assessing the frozen specimen, the first distinction is whether the tissue is normal or abnormal. Abnormal tissue falls under 2 broad categories: nonneoplastic and neoplastic. Neoplastic features include cytologic atypia and hypercellularity. Nonneoplastic entities include inflammatory and infectious processes. The neoplastic processes can be further differentiated by assessing the architecture. Well-circumscribed tumors can be metastases or other neuropathologic entities: gangliogliomas, ependymomas, pilocytic astrocytomas, and pleomorphic xanthoastrocytomas.

What Histologic Features Distinguish Between Diffuse Oligodendroglioma and Astrocytoma?

Diffuse astrocytomas and oligodendrogliomas have distinct histopathological features. Diffuse astrocytomas have increased nuclear pleomorphism (variability in size and shape) and elongated, hyperchromatic nuclei (excessive, dark nuclear staining). The cytoplasm of astrocytomas is eosinophilic (pink-red) and glial fibrillary acidic protein positive.[3] Microcystic changes—an architectural pattern of scattered small cystic spaces—can be observed.

Oligodendroglioma tumor cells classically have a "fried egg" appearance of round nuclei surrounded by a clear cytoplasmic halo with a background of thin, branching "chicken-wire" blood vessels. Oligodendrogliomas are well-differentiated (similar in appearance to normal tissue), diffusely infiltrating tumor cells that resemble oligodendrocytes. Oligodendrogliomas commonly have calcifications. Although the majority of low-grade astrocytomas and oligodendrogliomas have distinctive "classic" morphologic features, some tumors have histologic features that overlap those of the 2 entities.

How Are Diffuse Gliomas Graded?

The tumor grade provides important prognostic information based on the tumor's microscopic features. Diffuse glioma grading uses the World Health Organization (WHO) grading system.[26] Diffuse gliomas are by definition at least grade II because a gross total resection will result in recurrence due to residual tumor that infiltrated into the surrounding brain. Important histologic features for grading include nuclear atypia, mitotic activity, microvascular proliferation (MVP), and necrosis. Nuclear atypia is a term used to describe nuclei with variation in shape and size (nuclear pleomorphism) and abnormally dense chromatin (hyperchromasia). Low-grade gliomas (grade II), diffuse astrocytomas, and oligodendrogliomas have mild nuclear atypia, and mitotic activity is rare if observed at all. Anaplastic astrocytomas and oligodendrogliomas (grade III) demonstrate increased nuclear pleomorphism, cellularity, and mitotic activity. An astrocytoma with increased mitotic activity that also has evidence of necrosis and MVP meets criteria for a grade IV diagnosis of glioblastoma. There is no grade IV equivalent for oligodendroglial tumors; an oligodendroglioma with increased mitotic activity and necrosis or MVP is still classified as an anaplastic oligodendroglioma (grade III).

The Ki-67 proliferation index is an immunohistochemical stain that serves as an adjunct to histologic grading. Ki-67 is a protein that is expressed by cells at all stages of the cell cycle except G_0, and the percentage of cells that express the protein (the proliferation index) can be used to estimate a tumor's growth rate. A higher proliferation index typically correlates with more aggressive tumor growth and a higher histologic grade.

What Role Does Molecular Diagnostics Play in the Classification of CNS Tumors?

The WHO regularly sponsors consensus meetings of expert brain tumor pathologists to update and revise the classification and grading of CNS neoplasms; an update in 2016 has now included the mutational status of IDH-1 or IDH-2 as an integral component of the diagnosis.[26] Recent studies have revealed that a tumor's molecular genetic profile can be more accurate in predicting prognosis and response to treatment than histopathology alone. There is now published data that in infiltrating gliomas, IDH-1 and IDH-2 mutation status can provide a better indication of prognosis than histologic grading.[27-29] For this reason, the 2016 revision emphasizes the importance of determination of *IDH* gene mutation status in the classification of infiltrating gliomas.[26] The WHO group has subsequently gone even further in incorporating molecular diagnostics into the classification of IDH wild-type tumors; IDH wild-type gliomas that lack histologic features of glioblastoma yet have epidermal growth factor receptor gene amplification, telomerase reverse transcriptase (TERT) promoter mutation, or a combination of whole chromosome 7 gain and whole chromosome 10 loss now meet criteria for a diagnosis of "diffuse astrocytic glioma, IDH wild type, with molecular features of glioblastoma, WHO grade IV.[30]" The significance of *TERT* will be discussed in the subsequent case (patient 2).

Methylation of the *O*-6-methylguanine DNA methyltransferase (MGMT) gene promoter is associated with improved response to chemotherapy and has become a standard assay that informs therapeutic decisions. *O*-6-methylguanine DNA methyltransferase promoter methylation is thought to result in lower expression of the protein that confers cellular resistance against alkylating chemotherapy agents such as temozolomide, one of the mainstays of glioma chemotherapy.[31,32] Evidence also suggests that MGMT promoter methylation may be a reflection of global DNA hypermethylation that ultimately predicts better prognosis and response to therapy.[33,34]

How Do IDH Mutations Contribute to Tumorigenesis?

Isocitrate dehydrogenases are NADP-dependent enzymes that regulate normal cellular metabolism and are more active in high citrate synthesizing cells such as astrocytes.[35] Isocitrate dehydrogenase gene mutation is an early event in the sequence to glioma development for many tumors.[27] Arginine (R) is

substituted at specific active site positions in IDH-1 (R132) or IDH-2 (R172) in 90% of adult grade II and III gliomas and secondary glioblastomas.[35] These gain-of-function mutations result in the production of an oncometabolite, (D) 2-hydroxyglutarate, that competitively inhibits key enzymes involved in cell division and regulation of DNA methylation, resulting in hypermethylation of the tumor genome.[35] The MGMT promoter is one of many foci in the genome that is methylated secondary to IDH gene mutations.[36]

How Does IDH R132H Immunohistochemistry Aid in the Evaluation of Infiltrating Gliomas?

Ninety percent of IDH mutant gliomas harbor the IDH-1 R132H missense mutation and antibodies have been developed to identify the mutant protein in glioma specimens and are now in widespread use.[35,37] In addition to being used to identify the presence of the R132H IDH1 mutation in infiltrating gliomas, when the mutation is present, this immunostain enables the identification of tumor cells in even mildly infiltrated brain and, when positive, can aid in distinguishing gliomas from gliosis.[38]

Based on the Histologic and Molecular Findings, What Is the Patient's Diagnosis?

The histologic findings show an infiltrating glial tumor with atypical, mildly pleomorphic nuclei (Figure 2C and D). The tumor has low-grade features: absent mitotic figures and a low Ki-67 labeling index (Figure 2E). The tumor is IDH-1 R132H-mutant with a loss of *ATRX* expression (Figure 2F and G). These diagnostic findings support a final diagnosis of a diffuse astrocytoma, IDH1 mutant, WHO grade II. The significance of *ATRX* in gliomas will be discussed in the subsequent case (patient 2).

What Are the Treatment Options for the Patient?

Low-grade gliomas are both treated and diagnosed by surgical resection.[39] Total resection is preferred but not always possible due to the infiltrative nature of diffuse gliomas. Hence, all patients must ultimately be treated with adjuvant radiation therapy and chemotherapy. Regarding the timing of adjuvant radiation, earlier radiotherapy following surgery lengthened progression-free survival but did not affect overall survival.[40] All adults with newly diagnosed low-grade gliomas are recommended to undergo radiotherapy regardless of the extent of resection.[41] As an alternative to higher dose immediate postoperative radiotherapy, a lower dose is considered an equivalent alternative in the management of low-grade gliomas to reduce the risk of therapy-associated toxicity.[41]

These patients will eventually have tumor recurrence and require additional therapy at that time. Temozolomide and nitrosourea chemotherapy agents are the mainstay in glioma recurrence treatment.[42,43] Patients who have symptomatic residual tumors or progression of disease after surgical resection are usually immediately treated with radiotherapy and/or chemotherapy. For tumors that have a glioblastoma-like molecular profile (eg, IDH wild type with a *TERT* promoter mutation, as described above), resection with immediate postoperative temozolomide and radiotherapy has been demonstrated to improve survival.[44]

What Is the Expected Outcome After This Procedure?

The completeness of initial resections of low-grade gliomas is an independent predictor of progression-free survival and overall survival.[39] Poor prognostic factors include older age, poor functional status, baseline neurological deficits, nonepileptic presentation, tumor location other than frontal and parietal lobes, and tumor size greater than 4 to 5 cm.[45,46] Most low-grade gliomas recur as a low-grade glioma or transform into a high-grade glioma.

Patient Presentation, 2

A 61-year-old woman presents to the emergency department with a 2-week history of recurrent, chronic, dull, severe, frontal headaches. She reports a 45-year history of occasional headaches that increased in frequency over the past 7 years but were managed with ibuprofen. Her current headaches are not manageable by over-the-counter analgesics and are exacerbated by sound and activity. She is left handed. She has never been exposed to radiation or chemotherapy.

Diagnostic Findings, Patient 2, Part 1

A neurologic examination demonstrates normal speech, cognition, vision, strength, coordination, graphesthesia (recognition of writing on skin by touch), and stereognosis (recognition of objects by touch).

Questions/Discussion Points, Patient 2, Part 1

Given the Patient's Presentation and Initial Neurologic Examination, What Would Her Initial Differential Diagnosis Include and Her Workup Involve?

The severity and chronicity of the patient's headaches are concerning signs for a more serious underlying etiology of her headaches. A change in pattern from a previous headache can be a presenting symptom of an intracranial mass, infection, systemic disorder, or metabolic disturbance. As stated previously, neuroimaging should be performed in all patients with suspected intracranial lesions. Noncontrast head CTs are usually the initial studies performed. Systemic symptoms—myalgias, fever, night sweats, and weight loss—often accompany infectious and hematologic disorders. Clinicians should perform lumbar punctures if the presentation is suggestive of any acute infectious process involving the CNS. This patient did not have any symptoms that were suspicious for acute meningitis or encephalitis (ie, neck stiffness, fever, lethargy, preceding viral infection). She did not have night sweats or weight loss, making lymphoma a less likely etiology. A normal laboratory workup also made a metabolic disturbance less likely.

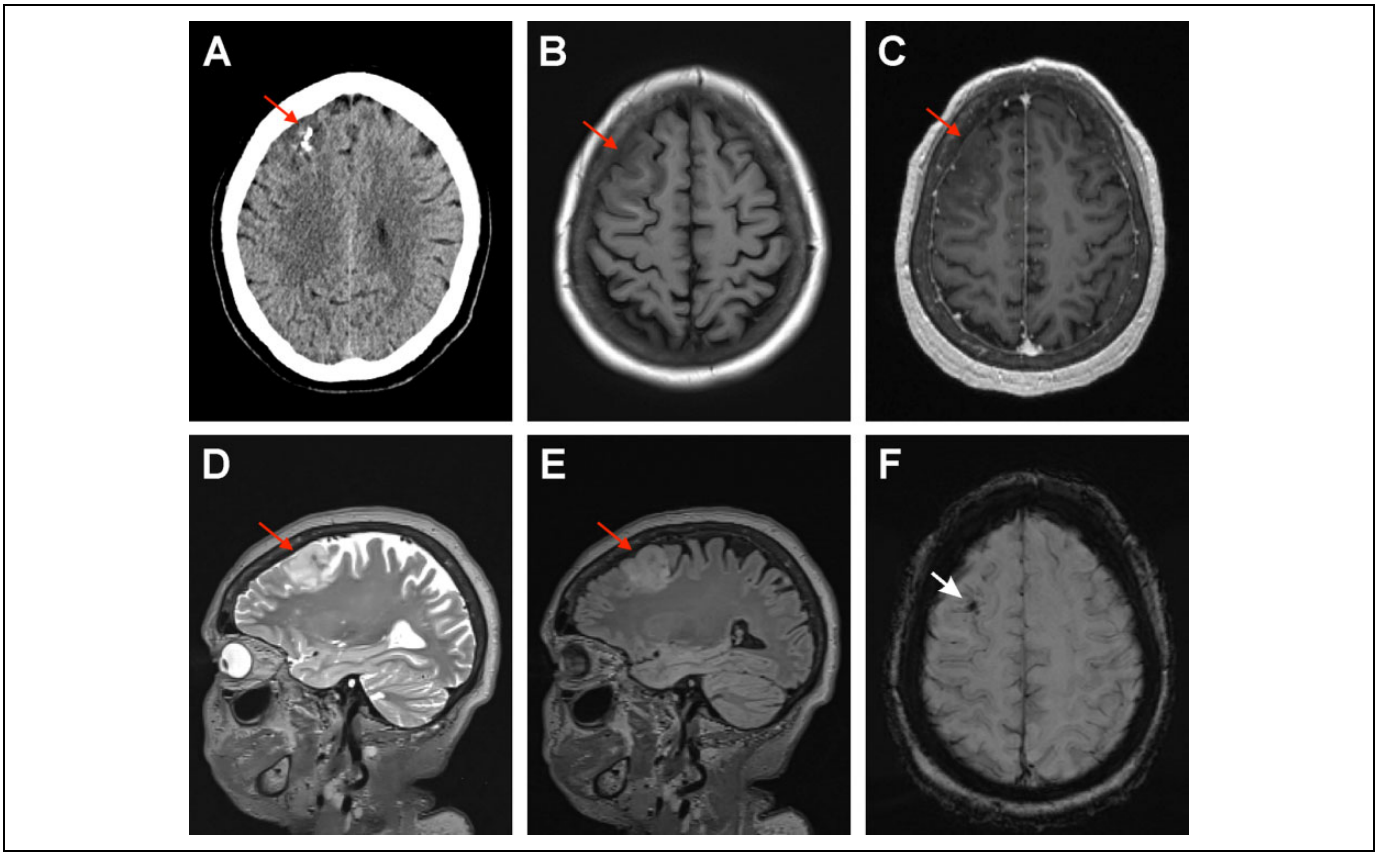

Figure 3. Patient 2 imaging. A, Mild expansion of gyri, blurring of gray-white boundary, and calcifications (red arrow) is observed in the right frontal cortex on axial, noncontrast computed tomography (CT). A hypointense mass (B; red arrow) that does not enhance with the addition of contrast (C; red arrow) is observed on T1-weighted magnetic resonance imaging (MRI). The mass is hyperintense on T2-weighted imaging (D; red arrow) and on fluid attenuation inversion recovery (FLAIR; E; red arrow). F, Mineral deposition, consistent with calcification, is identified on susceptibility-weighted imaging (white arrow).

Diagnostic Findings, Patient 2, Part 2

A head CT without IV contrast demonstrates a 4.1 cm × 3 cm × 2.5 cm right frontal, partially calcified lesion with mass effect (Figure 3A). There is focal expansion of the T1-hypointense lesion in the left frontal cortex involving the gray and white matter with blurring of the gray-white matter boundary. Magnetic resonance imaging of the brain with and without contrast demonstrates an expansile, nonenhancing mass that is hyperintense on T2 and T2/FLAIR imaging (Figure 3B-E). Susceptibility-weighted imaging demonstrates punctate, dark foci within the mass that are most consistent with calcifications (Figure 1F). No diffusion restriction is observed (data not shown).

Questions/Discussion Points, Patient 2, Part 2

How Does the Imaging Data Inform Your Differential Diagnosis?

Expansion of the cortex and white matter with blurring of the gray-white boundary are findings seen in MRI of infiltrating gliomas. The lesion had a hypointense T1 signal and a hyperintense T2 signal. Calcifications, frequently seen in oligodendrogliomas, can be seen as low-intensity signals on T2-weighted images and dark signal on SWI sequences. No T2-FLAIR mismatch sign was observed. These findings favor a diagnosis of oligodendroglioma.

Diagnostic Findings, Patient 2, Part 3

After consultation with a neuro-oncologist, the patient opted to postpone surgery but subsequently consented and underwent a gross total resection 8 months after initial presentation. An intraoperative frozen section was performed (Figure 4A and B). Multiple irregular fragments of soft tan tissue were received. Histology demonstrated an infiltrative lesion with round nuclei and fine processes on smear, cytoplasmic clearing on permanent sections, and thin, branching vasculature (Figure 4A-E). The tumor was positive for an IDH-1 R132H mutation (not shown). *ATRX* expression was preserved (Figure 4F). The tumor had relatively low Ki-67 of 2% to 3% (not shown). Loss of heterozygosity polymerase chain reaction–based genomic analysis demonstrated allelic loss of the short arm of chromosome 1p and the long arm of chromosome 19q (not shown). The final diagnosis was oligodendroglioma, IDH mutant, and WHO grade II.

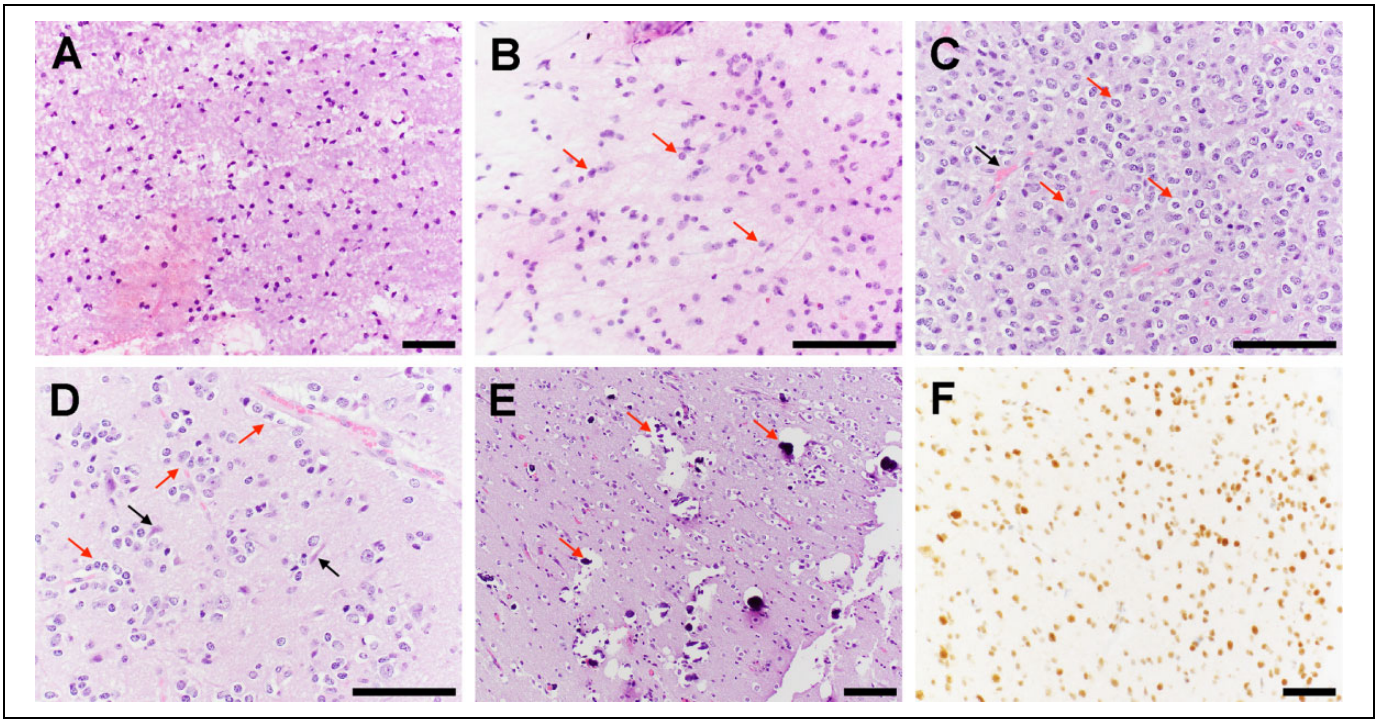

Figure 4. Patient 2 histology. Intraoperative analysis slides. A, Analysis of frozen tissue demonstrates hypercellular white matter (×20). B, A smear demonstrates that most tumor cells (arrows) have round nuclei with fine granular chromatin and fine processes (×40). Formalin-fixed, paraffin-embedded (FFPE) tissue analysis. C, Tumor cells (red arrows) demonstrate round nuclei and cytoplasmic clearing with a thin, branching vasculature (black arrow), characteristic of oligodendroglioma (×40). D, Tumor cells aggregate around neurons (black arrows) and along vessels (red arrows) with infiltration of the cortex (×40). E, Calcifications (arrows) are observed in infiltrated brain (×20). F, Tumor cells demonstrate retained expression of ATRX by immunohistochemistry (×20). Scale bar = 100 μm.

The patient's headaches resolved postoperatively with no deficits. Two months after surgery, the patient developed behavioral changes with agitation, delusion, and mania, and the patient required hospitalization for 1 month. The patient's psychiatric symptoms have been well controlled since that hospitalization and the patient is currently undergoing interval scans every 6 months for evidence of tumor progression 2 years following initial presentation.

Questions/Discussion Points, Patient 2, Part 3

What Molecular Features Distinguish Between Diffuse Oligodendroglioma and Astrocytoma?

Before the incorporation of molecular criteria into the diagnosis of oligodendroglioma, it had been recognized that oligodendrogliomas were associated with a better prognosis than astrocytomas, due at least in part to a more significant response to PCV chemotherapy, though temozolomide can also be used.[31,47] The increased sensitivity to chemotherapy attributed to oligodendroglial tumors is restricted to tumors with codeletion of chromosomal arms 1p and 19q.[28,29] We now know that IDH-mutated tumors with 1p/19q codeletion have better outcomes than IDH-mutated tumors with intact 1p/19q.[48] Due to the clinical significance of identifying 1p/19q codeletion, this finding is now a requirement along with IDH mutation in the establishment of a diagnosis of oligodendroglioma.[26]

All oligodendrogliomas and many diffuse astrocytomas have IDH mutations,[49] though other mutations can be used to distinguish between the 2 tumor types. Isocitrate dehydrogenase–mutant astrocytomas commonly have genetic alterations in *ATRX* and *TP53* and retain both copies of chromosomal arms 1p and 19q.[50] The *ATRX* gene is involved in regulating telomere length by encoding an essential chromatin-binding protein. *ATRX* mutations induce a phenomenon known as alternative lengthening of telomeres.[50,51] The *TP53* gene codes for the p53 tumor suppressor protein. Oligodendrogliomas have *TERT* mutations in conjunction with 1p/19q codeletion.[50] The *TERT* gene encodes for the catalytic part of telomerase. *TERT* and *ATRX* mutations are mutually exclusive in diffuse gliomas.[50] In astrocytic tumors, *ATRX* expression is also associated with DNA methylation; tumors with low expression of *ATRX*, as seen with loss of *ATRX*, harbor a higher DNA methylation level than tumors with higher *ATRX* expression.[52]

The histologic appearance of infiltrating gliomas can vary considerably from either idealized oligodendroglioma or astrocytoma morphologies, and, in the past, infiltrating gliomas with even focal resemblance to oligodendroglioma had been classified as oligoastrocytomas.[48] The 2016 WHO no longer recognizes "mixed oligoastrocytomas" as distinct entities, which is

consistent with the fact that true mixed tumors exist only as rare case reports of tumors containing molecularly distinct astrocytic and oligodendroglioma components.[53,54] The vast majority of tumors previously described as mixed gliomas have been demonstrated to be either IDH mutated and 1p/19q codeleted and better described as oligodendrogliomas or IDH mutated with intact 1p/19q and better described as IDH-mutant astrocytomas.[48,49] This distinction is important for giving the patient the correct diagnosis and anticipating the response to treatment.

What Is the Expected Outcome After This Procedure?

Estimates based on histopathological diagnoses made prior to the revised molecular diagnosis of oligodendrogliomas state that the median overall survival for low-grade oligodendrogliomas is 10 to 15 years.[55] More recent evidence has shown 1p/19q codeleted oligodendrogliomas treated with radiation and PCV may have a median survival closer to 20 and 15 years for grade II and III tumors, respectively.[56] The time to recurrence is longer in oligodendroglial tumors than in astrocytic tumors.

Teaching Points

- The diagnosis of infiltrating gliomas now requires integration of molecular diagnostic findings with histologic grading.
- Histologic grading of diffuse gliomas is based on 4 features: nuclear atypia, mitotic activity, MVP, and necrosis.
- Isocitrate dehydrogenase–mutant gliomas have a better prognosis than IDH-wild type gliomas. This is thought to be due to less aggressive growth and better responsiveness to alkylating agents.
- An IDH-mutant tumor with oligodendroglial morphology but lacking 1p/19q codeletion is now classified as an astrocytoma, IDH mutated.
- Oligodendrogliomas require both *IDH* gene mutation and 1p/19q codeletion for diagnosis.
- Low-grade IDH-mutant infiltrating gliomas with 1p/19q codeletion (oligodendrogliomas) have better outcomes than non-codeleted, IDH-mutant tumors.
- Isocitrate dehydrogenase immunohistochemistry can help distinguish infiltrating diffuse gliomas from reactive gliosis.

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