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Glioblastoma: Changing concepts in the WHO CNS5 classification

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Abstract

Glioblastoma is the most common malignant central nervous system (CNS) tumor in adults. Acute common clinical symptoms include headache, seizure, behavior changes, focal neurological deficits, and signs of increased intracranial pressure. The classic MRI finding of glioblastoma is an irregularly shaped, rim-enhancing or ring-enhancing lesion with a central dark area of necrosis. This constellation of features correlates with microscopic findings of tumor necrosis and microvascular proliferation. Besides these common features, several well-recognized histological subtypes include giant cell glioblastoma, granular cell glioblastoma, gliosarcoma, glioblastoma with a primitive neuronal component, small cell glioblastoma, and epithelioid glioblastoma. While glioblastoma was historically classified as isocitrate dehydrogenase (IDH)-wildtype and IDH-mutant groups, the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy (cIMPACT-NOW) and the fifth edition of the WHO Classification of Tumors of the Central Nervous System clearly updated the nomenclature to reflect glioblastoma to be compatible with wildtype IDH status only. Therefore, glioblastoma is now defined as “a diffuse, astrocytic glioma that is IDH-wildtype and H3-wildtype and has one or more of the following histological or genetic features: microvascular proliferation, necrosis, Telomerase reverse transcriptase promoter mutation, Epidermal growth factor receptor gene amplification, +7/−10 chromosome copy-number changes (CNS WHO grade 4).”

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Full Text

Introduction

Glioblastoma is the most common malignant central nervous system (CNS) tumor in adults. It accounts for approximately 14.5% of all CNS neoplasms, 48.6% of all primary malignant CNS tumors, and 57.7% of gliomas in the United States.[1] The annual incidence of glioblastoma in the United States, adjusted to the standard population, is 3.23 per 100,000 population.[1] Glioblastoma can occur in patients of any age, but more frequently in older adults, with the highest rates occurring among those aged 75 to 84 years.[2] Rates in non-Hispanic Whites adults are >3-fold the rates in those who are Asian or Pacific Islander. There is a slight male predominance (1.6:1 in the United States).

While the pathological diagnosis of glioblastoma had been historically based on morphological features (i.e., a high-grade diffuse glioma with the presence of microvascular proliferation and/or tumor necrosis), specific molecular features are included in the diagnosis criteria according to cIMPACT-NOW and the fifth edition of the WHO Classification of Tumors of the Central Nervous System (WHO CNS5). In this short review, we will summarize clinical presentation, imaging, histological features including variant morphological subtypes, and several molecular tests that are essential for diagnosing glioblastoma. We hope this concise summary helps surgical pathologists in their daily practice.

Tumor localization and clinical presentations

Glioblastoma most often occurs in the subcortical white matter of the cerebral hemispheres, including the frontal (28.6%), temporal (25%), parietal (15.3%), and less frequently, occipital (3.9%) lobes.[2] High-grade gliomas occurring in a midline structure (thalamus, brainstem, cerebellum, spinal cord, etc.) may represent a diffuse midline glioma, H3K27-altered, therefore additional molecular testing is warrantied. The patients usually present with acute symptoms (<3 months), and the clinical symptoms depend largely on the tumor location. The most common presenting symptom is seizure, followed by behavior changes, focal neurological deficits, and signs of increased intracranial pressure.

Imaging

The classic MRI finding of glioblastoma is an irregularly shaped, rim-enhancing or ring-enhancing lesion with a central dark area of necrosis, although exceptions are present in some subtypes (see below). There is often a T2/FLAIR hyperintensity surrounding the enhancing area representing the diffuse infiltrating portion of the tumor. The tumor may extend across the corpus callosum into the opposite hemisphere, forming a “butterfly lesion.” Sometimes the tumor may present as multiple separate foci of contrast enhancement, although these areas are often contiguous histologically. Radiation necrosis (“pseudo-progression”) may show similar imaging findings in glioblastoma patients who have received radiation, which causes a diagnostic challenge clinically and often results in a biopsy.

Histology

Glioblastoma is a high-grade glioma with predominantly astrocytic differentiation. The classic glioblastoma has “fibrillary” morphology. On cytological smears, the tumors show irregular hyperchromatic nuclei and elongated fibrillary processes. On histological sections, the cytoplasm is often inconspicuous, and the tumor cells are mostly present as “naked nuclei” in a dense fibrillary background. The cellularity is usually very high, with brisk mitotic activity. The morphology of tumor cells varies significantly, which in the past led to its name “glioblastoma multiforme,” which aptly encompassed its morphologic heterogeneity. Most tumors show significant nuclear pleomorphism with variable multinucleated giant cells, while some tumors have relatively uniform tumor cells (e.g., small cell). The intratumoral heterogeneity is sometimes remarkable, making histological diagnosis challenging. The other diagnostic hallmarks of glioblastoma include microvascular proliferation [Figure 1]a and/or necrosis. Microvascular proliferation is also called microvascular hyperplasia, endothelial cell hyperplasia, or endothelial proliferation. It refers to the multilayered appearance of the vessel caused by proliferating endothelial cells. Microvascular proliferation is frequent in the areas next to necrosis or in the infiltrating edge of glioblastoma. When the endothelial cells proliferate to form multiple lumens similar to renal glomeruli, they are called as “glomeruloid.” Necrosis in

glioblastoma has two forms: palisading necrosis [densely packed tumor cells form palisades around a necrotic center with their long axis perpendicular to necrosis, [Figure 1]b and geographic necrosis. The rapidly growing tumor cells outgrow the limited oxygen supply, resulting in tumor necrosis. Meanwhile, angiogenic factors (i.e., Vascular endothelial growth factor (VEGF)) secreted by tumor cells induce additional microvascular proliferation. Indeed, therapies targeting VEGF and VEGF receptors have been extensively explored and remain a promising treatment although current outcomes of benefit are inconclusive.[3] The proangiogenic microenvironment is coupled with various pro-inflammatory cytokines, promoting lymphocytic infiltration,[4] although very often glioblastomas are considered "immune-cold."{Figure 1}

Immunohistochemical features

Glioblastoma cells are often positive for glial fibrillary acidic protein (GFAP), although the degree of reactivity varies significantly even within the same tumor. In addition, the tumor cells are frequently positive for S100 and Olig2 (a nuclear transcription factor that is highly specific for the glial lineage). Positivity for cytokeratin, in particular AE1/AE3, primarily represents cross-reactivity with GFAP, but reactivity with CK5/6 and CAM5.2 may indicate epithelial metaplasia. By definition, glioblastoma is negative for Isocitrate dehydrogenase-1 (IDH1) (R132H) and H3 K27M. Nuclear reactivity for ATRX is mostly retained and p53 overexpression may be frequently seen in the giant cell glioblastoma. Mutant V-Raf Murine Sarcoma Viral Oncogene Homolog B (BRAF) (V600E) positivity is observed in a subset of epithelioid glioblastoma.

Molecular features

IDH mutation

Based on the 2016 WHO classification system, IDH-mutant glioblastoma accounts for approximately 10% of all glioblastomas. In WHO CNS5, glioblastoma refers only to IDH-wildtype gliomas. IDH-mutant glioma does not qualify as glioblastoma, regardless of high-grade features. To make a diagnosis of glioblastoma, therefore, it is crucial to determine the wild-type IDH status. The commercially available monoclonal antibody can only detect the IDH1 R132H mutation,[5],[6] which accounts for up to 90% of all IDH mutations, but it cannot detect the other non-canonical mutations in IDH1 or IDH2 genes. When the IDH1 R132H immunostaining pans out negative, it is advisable to reflex to gene sequencing to test for the other non-canonical IDH1/2 mutations in younger (<55 years old) patients group.[7]

In addition, genetic landscape other than IDH has been identified in glioblastoma.[8] Among these, three recurrent molecular alterations, as described below, emerge as the defining molecular features of glioblastoma.

Telomerase reverse transcriptase (TERT) promotor mutation

TERT encodes the catalytic subunit of the telomerase complex. High frequency of TERT promoter mutation (pTERTmut) is detected in glioblastoma and in oligodendroglioma, as well as multiple other tumors (e.g., melanoma, myxoid liposarcoma, urothelial carcinoma, etc., reviewed in Bell et al.[9]). Although not specific for glioblastoma, pTERTmut is deemed one of the defining molecular features for glioblastoma in the appropriate context. While this review is focused on glioblastoma (IDH-wildtype by definition), pTERT mutation can be found in IDH-mutant astrocytoma and oligodendroglioma. In grades 2 and 3 IDH-mutant glioma, however, the presence of pTERT mutant confers a slightly better prognosis compared with IDH-mutant/pTERT-wildtype subgroup.[10]

TERT is the gene encoding the functional component of telomerase. Telomerase is normally expressed in embryonic stem cells, or transit-amplifying stem-like cells or germ cells. In the post-mitotic and differentiated cell where telomerase is absent, the telomeres underwent continuous erosion, resulting in cellular senescence. In multiple tumor cells, however, TERT protomer mutation leads to upregulation of telomerase activity and cellular immortalization.[11]

The most frequently reported mutation sites are cytosine to thymidine transition at −124 bp and −146 bp upstream of the translation start site (−124C >T/−146C >T). These mutations create a long-range chromatin interaction between the TERT promoter and a 300-kb upstream region, and permit recruitment of the transcription factor GABPA in mutant TERT promoters, thus promoting overexpression of TERT.[12]

Epidermal growth factor receptor (EGFR) gene amplification

EGFR is the most frequently amplified gene in glioblastoma and is often associated with overexpression.[8] EGFR amplification occurs in approximately 35% to 45% of glioblastomas and constitutes one of the three defining molecular features of glioblastoma. Of note, EGFR amplification refers to focal high-level copy number gains of the EGFR gene, as defined by validated techniques in clinical use, such as fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR), methylome profiling, and next-generation sequencing (NGS). Immunohistochemistry for EGFR protein does not provide adequate specificity for detecting EGFR amplification. Very frequently, EGFR amplification is associated with secondary EGFR alterations, which include an in-frame deletion of exon 2-7 encoding EGFRvIII.

Combined whole chromosome 7 gain and whole chromosome 10 loss

In the absence of endothelial proliferation and/or necrosis, the detection of EGFR amplification is a very strong surrogate marker for the diagnosis of glioblastoma in IDH-wildtype diffuse astrocytic tumors. The + 7/−10 signature is also an independent strong surrogate marker, although these alterations can be found in pleomorphic xanthoastrocytoma (PXA).[13] The EGFR gene described above is located on chromosome 7p. Phosphatase and tensin homolog (PTEN) is located on chromosome 10 and PTEN mutation or loss is frequently seen in glioblastoma. Nevertheless, chromosome 7 and chromosome 10 harbor multiple gene groups that are functionally critical for cell growth and differentiation, and involved in a variety of hematopoietic, epithelial, and mesenchymal neoplasms. A comprehensive list of candidate cancer genes on chromosome 7 and 10 can be found in Cancer Genetic Web resource (http://www.cancerindex.org/geneweb). However, the details regarding relative contribution of these genes to glioblastoma tumor genesis remains to be elucidated.

O6-methylguanine-DNA methyltransferase promoter

O6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair enzyme that repairs damaged guanine nucleotides by transferring the methyl at O6 site of guanine to its cysteine residues. Loss of function in MGMT is more likely to result in DNA damage and cell death induced by chemotherapy, including alkylating agents such as temozolomide.[14] The expression of MGMT gene is regulated by epigenetic modification–methylation of CpG island of MGMT promoter. CpG island methylation leads to heterochromatinization and obscuring the transcription initiation sites, thus reducing MGMT transcription.[15],[16]

MGMT promoter methylation has been associated with longer survival in patients treated with temozolomide. In a randomized clinical trial, comparing radiotherapy alone versus radiotherapy combined with temozolomide, patients with MGMT promoter methylated tumor had a significant survival benefit when they were treated with temozolomide and radiation, as compared with those whose tumor was unmethylated and were assigned to only radiotherapy (21.7 vs 15.3 months).[17] Even in the elderly patients (>60 years), patients treated with temozolomide who had a tumor with MGMT promoter methylation had significantly longer survival than those without MGMT promoter methylation (9.7 months vs 6.8 months).[18] Therefore, MGMT promoter methylation status is a useful predictive marker for benefit from temozolomide in patients with newly diagnosed or recurrent glioblastomas.[19] This is especially important in the elderly patients who cannot tolerate standard radiotherapy.[20]

In addition, it is worth noting that MGMT promoter may change unpredictably after chemotherapy and/or radiotherapy.[21] This is likely due to clonal selection of cancer stem cell during tumor evolution and treatment.[22],[23] Therefore, it is mandatory to retest MGMT methylation status in recurrent glioblastoma.

BRAF mutation

BRAF V600E mutation has been identified in papillary craniopharyngioma, PXA, ganglioglioma, pilocytic astrocytoma, histiocytic tumors, melanoma, papillary thyroid carcinoma, a subset of colorectal carcinoma, and other CNS and non-CNS neoplasms. This mutation creates a hyperactive biological effect and thus a therapeutic target for BRAF kinase inhibitor. In general, BRAF V600E mutation is more frequently associated with low-grade glioma/glioneuronal tumors and rarely seen in glioblastoma, but is relatively enriched in epithelioid glioblastoma and younger patients. Nevertheless, glioblastoma harboring BRAF V600E may be a distinct subtype and this patient group may survive longer than those with EGFR mutations.[24] A large-scale meta-analysis indicated that BRAF V600E was associated with an improved overall survival in glioma patients (HR = 0.60; 95% CI = 0.44–0.80), but it is only associated with improved survival in pediatric and young adult gliomas (under 35 years of age) and did not have prognostic value in old adults. In addition, BRAF V600E was only associated with a favorable prognosis in lower grade glioma.[25] However, it is a negative prognosticator in pediatric ganglioglioma and childhood gliomas, particularly in conjunction with Cyclin-dependent kinase inhibitor 2A (CDKN2A) deletion, latter group frequently transforms to secondary high-grade gliomas.[26],[27]

FGFR3-TACC3 fusion

Fibroblast growth factor receptor (FGFR)-transforming acidic coiled-coil (TACC) fusion was identified initially in a small subset of glioblastoma,[28] and subsequently found in various cancers including but not limited to urothelial carcinoma, non-small cell lung cancer, and squamous cell carcinoma of head and neck regions.[29],[30] Within the CNS, FGFR-TACC fusion (specifically FGFR1-TACC1) is also present in low-grade neuroepithelial tumors including extraventricular neurocytoma.[31],[32]

FGFR-TACC fusions appear to be clonal tumor-initiating events that confer strong sensitivity to FGFR tyrosine kinase inhibitors, which induce partial response or stable disease in approximately one-third of patients with recurrent glioblastoma and/or other glioma subtypes harboring FGFR alterations (https://clinicaltrials.gov/ct2/show/NCT01975701). Given this therapeutic target, identifying this fusion in glioblastoma is important for prognostic value, although this fusion event is relatively rare in glioblastoma.

Tumor mutational burden in glioblastoma

Tumor mutational burden (TMB) is a quantitative parameter defined as the number of mutants/Mb, which can be detected and calculated using sequencing approach. Tumors with high TMB are present in subsets of multiple solid tumors, particularly in colonic and endometrial cancer. TMB-high tumors tend to develop more immunogenic neoantigens and attract prominent tumor infiltrating T cells.[33] This mechanism provides an opportunity to unlock the anti-tumor potential of T cells using anti-PD-1 drugs. In general, TMB-high tumors appear to be more sensitive to immunotherapy compared with TMB-low tumors. Patients with POLE mutation, albeit a rare occurrence, have ultra-mutant glioblastoma and respond well to such therapy.[34]

Recent changes in diagnostic criteria

Historically, diagnosis of glioblastoma required the presence of either microvascular proliferation or necrosis in a diffuse astrocytic glioma. However, in recent years, diagnostic criteria for glioblastoma have changed dramatically due to advances in our understanding of the molecular underpinnings and biological behavior of these tumors. In the 2016 WHO classification of tumors of the CNS system,[35] glioblastoma was divided into IDH-mutant and IDH-wildtype with significant differences in patients' survival. In 2018, the cIMPACT-NOW working group published the recommended diagnostic criteria for “diffuse astrocytic glioma, IDHwildtype, with molecular features of glioblastoma, WHO grade IV.”[36] This recommendation proposed three molecular criteria (pTERTmut, EGFR gene amplification, combined whole chromosome 7 gain, and whole chromosome 10 loss) for identifying an IDH-wildtype diffuse astrocytic glioma. Despite histological appearance of a WHO grade 2 or 3 neoplasm (i.e., lacking microvascular proliferation and necrosis), this group of glioma would follow an aggressive clinical course more closely resembling that of an IDH-wildtype glioblastoma. In 2020, the cIMPACT-NOW working group published another updated diagnostic criteria for glioblastoma, IDH-wildtype, and suggested that an IDH-wildtype diffuse astrocytic glioma could be diagnosed as “glioblastoma, IDH-wildtype, WHO grade 4” if there is microvascular proliferation or necrosis or one (or more) of the three genetic alterations (pTERTmut, EGFR gene amplification, +7/−10 chromosome copy number changes).

WHO CNS5[37] continues to incorporate these 3 genetic parameters as criteria for a diagnosis of glioblastoma, IDH-wildtype. As a result, glioblastoma, IDH-wildtype, is currently diagnosed in the setting of “IDH-wildtype diffuse astrocytic glioma in adults” if there is microvascular proliferation or necrosis or pTERTmut or EGFR gene amplification or + 7/−10 chromosome copy number changes.

In addition, WHO CNS5 takes a new approach to classify diffuse gliomas by dividing them into three different families: adult-type diffuse gliomas, pediatric-type diffuse low-grade gliomas, and pediatric-type diffuse high-grade gliomas, given the fact that adult-type and pediatric-type diffuse gliomas are prognostically and biologically distinct groups. The adult-type diffuse gliomas are further divided into three types: astrocytoma, IDH-mutant; oligodendroglioma, IDH-mutant, and 1p/19q-codeleted; and glioblastoma, IDH-wildtype. Therefore, the term glioblastoma is reserved only for adult-type, IDH-wildtype astrocytic glioma, and cannot be used for pediatric-type diffuse high-grade gliomas.

Histological subtypes

There are several well-recognized histological subtypes. A diagnosis of specific subtype is warranted when the corresponding histologic feature predominates. More often, however, we see some components exhibiting regionally some of these special features yet not constituting a major proportion. In this situation, a final diagnosis of generic glioblastoma is rendered, albeit with a mention of the observed special components in the “microscopic description and comment” section.

Giant cell glioblastoma

Giant cell glioblastoma is rare, accounting for <1% of all glioblastomas. It tends to occur in younger patients. Radiologically, it often presents as a well-circumscribed mass in the temporal and parietal lobes. Histologically, giant cell glioblastoma is characterized by numerous bizarre, multinucleated giant cells and frequent atypical mitoses [Figure 2]a,[Figure 2]b, [Figure 2]c, [Figure 2]d. In some cases, an abundant reticulin network may be observed. Genetically, giant cell glioblastoma has a high frequency of harboring a TP53 mutation (>80%) and/or mismatch repair defect, but typically lacks EGFR amplification, pTERTmut, and CDKN2A homozygous deletion.[38] Despite the high degree of anaplasia observed histologically, giant cell glioblastoma has a somewhat favorable prognosis compared with classic glioblastoma, which may be related to a higher possibility of gross total resection.[39] Giant cells can also be seen in patients with constitutional mismatch repair deficiency (CMMRD) but they tend to be generally focal in such instances.[40]{Figure 2}

Granular cell glioblastoma

Granular cell glioblastoma is composed of large cells with abundant coarse, eosinophilic granules in the cytoplasm [Figure 2]e, [Figure 2]f. The granules correspond to lysosomes and are positive for periodic acid-Schiff. Due to the bland cytology, this type may be misinterpreted as a macrophages-rich lesion such as demyelinating disease, although granular cells are larger and more coarsely granular than macrophages. In addition, granular cells are usually positive for glial markers such as GFAP and Olig2, and negative for CD163 (some granular cells may be positive for CD68).

Gliosarcoma

Gliosarcoma is characterized by a biphasic pattern composed of different areas demonstrating malignant glial and mesenchymal differentiation. The glial component is usually astrocytic, with typical histological features of glioblastoma. Gliosarcoma can also arise in ependymoma (ependymosarcoma)[41] and oligodendroglioma (oligosarcoma).[42],[43] The sarcomatous component is often composed of densely packed pleomorphic, malignant spindle cells with abundant collagen deposition [Figure 3]a, [Figure 3]b, [Figure 3]c. Additional mesenchymal differentiation, such as osteoid, chondroid, muscular, and lipomatous formation, can also be observed. A malignant mesenchymal component that is rich in reticulin and negative for GFAP is crucial for the diagnosis of gliosarcoma. Gliosarcoma harbors frequent TP53 mutations, PTEN mutations, CDKN2A deletions, but infrequent EGFR amplification.[44],[45] Gliosarcoma is more frequent to metastasize to extracranial locations compared with classic glioblastoma.{Figure 3}

Glioblastoma with a primitive neuronal component

An otherwise classic glioblastoma sometimes can have one or several well-demarcated nodules of primitive neuronal cells [Figure 3]d, [Figure 3]e, [Figure 3]f. These primitive neuronal nodules are morphologically reminiscent of medulloblastoma with extreme hypercellularity, high nuclear-to-cytoplasmic ratio, Homer Wright rosettes, and frequent mitoses and karyorrhexes. By immunohistochemistry, the primitive neuronal cells show positivity for synaptophysin, reduced GFAP, and a high Ki-67 labelling index. MYCN or MYC amplification can be detected. Glioblastoma with a primitive neuronal component has a high tendency (30% to 40%) of cerebrospinal fluid dissemination,[46] and therefore spinal MRI should be considered. Such cases may also benefit from platinum-based chemotherapy.

Small cell glioblastoma

Small cell glioblastoma is composed of relatively monomorphic, small-to-medium sized tumor cells with round to oval, hyperchromatic nuclei, and minimal cytoplasm [Figure 4]a and [Figure 4]b. In contrast to the relatively bland cytology, there is usually brisk mitotic activity. Small cell glioblastoma may morphologically mimic grade 3 (anaplastic) oligodendroglioma but the nuclear features on high-power help distinguish the two. Further, in contrast to oligodendroglioma, small cell glioblastoma is IDH-wildtype, and frequently demonstrates EGFR amplification [Figure 4]c and/or PTEN/10q loss, and more often occurs in the elderly.{Figure 4}

Epithelioid glioblastoma

Epithelioid glioblastoma is a rare and aggressive subtype with frequent leptomeningeal dissemination and poor outcome. This subtype is most frequently associated with BRAF V600E

mutation, pTERTmuts, and homozygous deletions of CDKN2A/2B.[47] Characteristic morphology is presence of epithelioid to rhabdoid cells with abundant cytoplasm, eccentrically placed nuclei, and conspicuous nucleoli. This morphology along with BRAF V600E mutation share similarity with CNS WHO grade 3 PXAs. Based on unsupervised hierarchical clustering analysis of epithelioid glioblastoma methylation profile, epithelioid cases do not form a discrete cluster. Instead, 60% (38/64) cases clustered with PXA (CNS WHO grades 2 and 3), 26% (17/64) with conventional IDH-wildtype glioblastoma, and the remaining minority (9/64) cluster with pediatric RTK1 glioblastoma in one of the published studies.[48] Therefore, epithelioid glioblastoma may represent a hybrid or continuum of the malignant transformation that is closely related to anaplastic PXA.

Molecular glioblastoma

As previously mentioned in “recent changes in diagnostic criteria,” WHO CNS5 will continue to use the three molecular features (EGFR amplification, +7/-10, or pTERTmut) to define glioblastoma in the absence of microvascular proliferation and necrosis. Such tumors conform to “molecular glioblastoma.” On morphology alone, this subtype cannot be distinguished from grade 2/3 diffuse gliomas. Microvascular proliferation may or may not be prominent. Correspondingly, MRI may show non-enhancing lesion, instead of an enhancing mass that is typically seen in conventional glioblastoma. Necrosis is also notably inconspicuous.

Practical approach for diagnosis

In patients older than 55 years of age, glioblastoma can be confidently diagnosed based on the classic morphology (hypercellular diffuse astrocytoma with significant pleomorphism, brisk mitotic activity, and microvascular proliferation or necrosis) and negative immunostain for IDH1-R132H in a tumor that is distant from the midline structures, and lacks prior history of lower grade glioma. If the IDH1-R132H immunostain is negative in this elder population, the likelihood of the tumor harboring a non-canonical IDH mutation is minimal (<1%).Therefore, reflex sequencing is not mandatory in our opinion, although it is covered in various NGS panels that are increasingly performed in clinical practice worldwide including ours. In IDH-wildtype, histologically lower grade diffuse glioma, however, additional molecular testing is mandatory to search for EGFR amplification, +7/−10, or pTERTmut in order to confirm the diagnosis of molecular glioblastoma. EGFR amplification and + 7/−10 can be conveniently assessed by fluorescence in situ hybridization, while pTERTmut can be detected by Sanger or NGS.

A further question is how to classify IDH-wildtype astrocytoma/glioma that does not have necrosis and microvascular proliferation and is also negative for molecular features for a GBM. This is still an evolving topic. Nevertheless, in this scenario, it is important to (a) distinguish if the tumor is indeed diffusely infiltrative and not a well-circumscribed glioma/glioneuronal tumor that is demonstrating focal invasion and (b) to make sure whether the tumor has anaplastic features (i.e., increased mitotic activity, pleomorphism, and densecellularity). If the second criterion is fulfilled, it would be appropriate to label it as a high-grade glioma with a comment and describe the pertinent histologic features. Once that has been established, it would be critical to take ATRX (IHC), BRAF (IHC and FISH), and CDKN2A/B (FISH) data into account to rule out the possibility of high-grade astrocytoma with piloid features (HGAP), although other MAPK pathway alterations such as FGFR1 and NF1 may only be available once the NGS results are back. Also pertinent for this scenario would be if there is any (primitive) neuronal differentiation because H3G34V mutant gliomas may not always have microvascular proliferation or necrosis or diffuse infiltration and should be considered if the tumor is superficially located in the temporal lobe and there is concurrent ATRX and/or p53 mutant staining pattern. Finally, correlation with NGS data would be essential to understand the molecular underpinnings and for its appropriate stratification (e.g., whether it is a pediatric type glioma, particularly in younger patients). After exhausting all the testing (immunohistochemistry, FISH, sequencing and methylome) possibilities, “not elsewhere classified (NEC)” designation maybe used if the tumor still fails to conform to a (thus far) known diagnostic category. The Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy (cIMPACT-NOW update 1)[49] has recommended that, not elsewhere classified (NEC), be used for situations in which testing was performed but in which the results were not diagnostic for a particular type. In some instances, this will be caused by a mismatch between clinical, histological, immunohistological, and genetic features; in others, the results may support a new or emerging entity that is not yet included in the WHO classification.

Prognosis

Due to the widely infiltrative nature, glioblastomas cannot be completely resected and they almost invariably recur and progress. On average, glioblastoma patients survive only 15 to 18 months after the initial diagnosis. The prognosis is much worse than IDH-mutant WHO grade 4 astrocytomas.[50] Younger age (<50 years), high Karnofsky performance status, gross total resection, and MGMT promotor methylation status seem to be associated with longer survival. On the other hand, high-level EGFR amplification[51] and pTERTmut[52] seem to be poor prognostic factors.

Special considerations for differential diagnosis

Discussed above but worth reiteration, some morphological subtypes of glioblastoma may resemble other tumor types. Primitive neuronal component may mimic CNS embryonalneoplasm; small cell glioblastoma may look like anaplastic oligodendrogliomas; epithelioid glioblastoma as well as giant cell glioblastoma may regionally appear similar to PXA; molecular glioblastoma is morphologically indistinguishable from lower grade diffuse glioma; H3 K27 altered diffuse glioma can be very similar except for its midline location. Nevertheless, most cases can be differentiated after factoring in clinical information, tumor location, and ancillary testing results. In addition, it is worth ruling out the possibility of “high-grade astrocytoma with piloid features” in appropriate context.

Diffuse midline glioma bears the molecular hallmark of H3 K27 alteration and is usually (but not always) a high-grade glioma in morphology. It is generally located in the proximity to the midline structures (i.e., corpus callosum, thalamus, basal ganglia, brainstem, and spinal cord) but rare reports outside the midline have been reported.[53] While this tumor is frequently seen in younger patients, it can occur in elderly population. And therefore, in our opinion, it is worth adding H3 K27M immunostain to the diagnostic panel whenever the tumor location involves the midline structures, regardless of the patient's age.

High-grade astrocytoma with piloid features (HGAP) is a new tumor type included in WHO CNS5. HGAP is IDH-wildtype, featuring CDKN2A/B loss, MAPK pathway alteration (NF1, BRAF, or FGFR1), and/or loss of ATRX, and notably conforming to a distinct methylation class. The presence of piloid features and BRAF alteration frequently raise differential possibility of pilocytic astrocytoma. However, high-grade morphology, in combination with CDKN2A/B loss, may appear to overlap with glioblastoma but concurrent ATRX loss would be unusual.

Conclusion

In summary, current diagnosis of glioblastoma depends on multidimensional evaluation and integration of imaging, morphology, immunohistochemistry, and molecular data. This combined information provides solid basis for classification, prognosis, and helps guide clinical treatment. Notably, this field is constantly evolving as more and more scientific discovery gets incorporated in the diagnosis and patient management, and as such, this review should be used as a preface only.

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Conflicts of interest

There are no conflicts of interest.

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Figure 1: (a): Characteristic morphological features of microvascular proliferation (highlighted by an arrow) within an infiltrating glioma, and (b): palisading necrosis

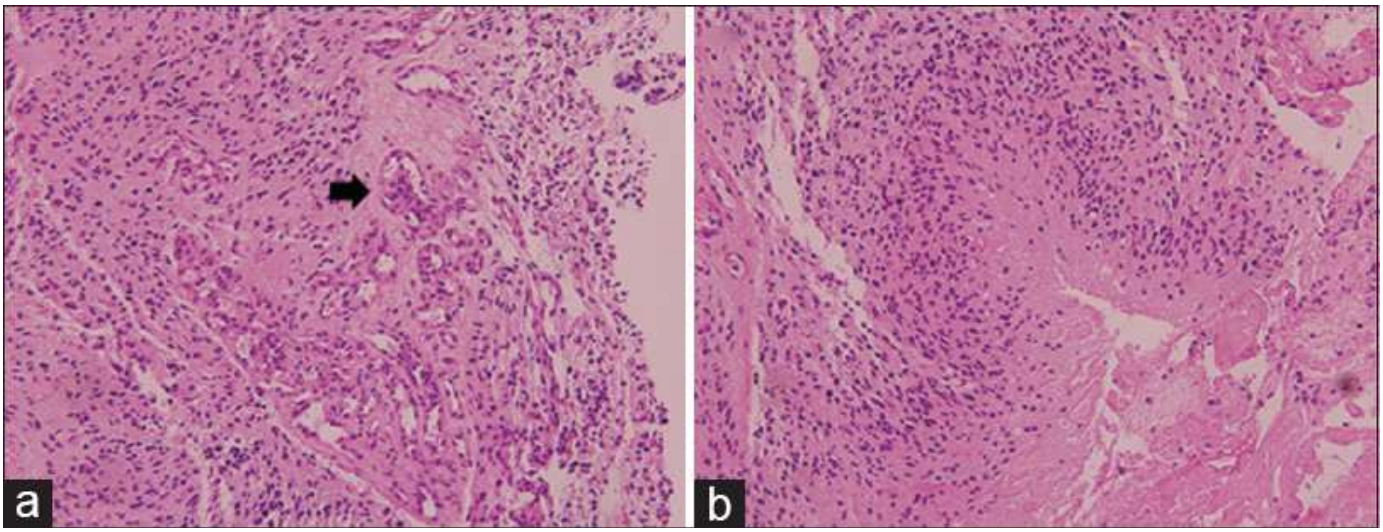

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Figure 2: (a–d): Giant cell glioblastoma (a, b) with large multinucleate and bizarre cells. The giant tumor cells are positive for GFAP (c, arrow) and demonstrate p53 nuclear overexpressionin >50% of tumor cells, consistent with p53 "mutant" staining pattern, (d) *TP53* R248W mutation was later confirmed by sequencing. (e, f): Granular cell glioblastomawith abundant granular cytoplasm and focal vacuolization (e). Focal perivascular aggregation (f)

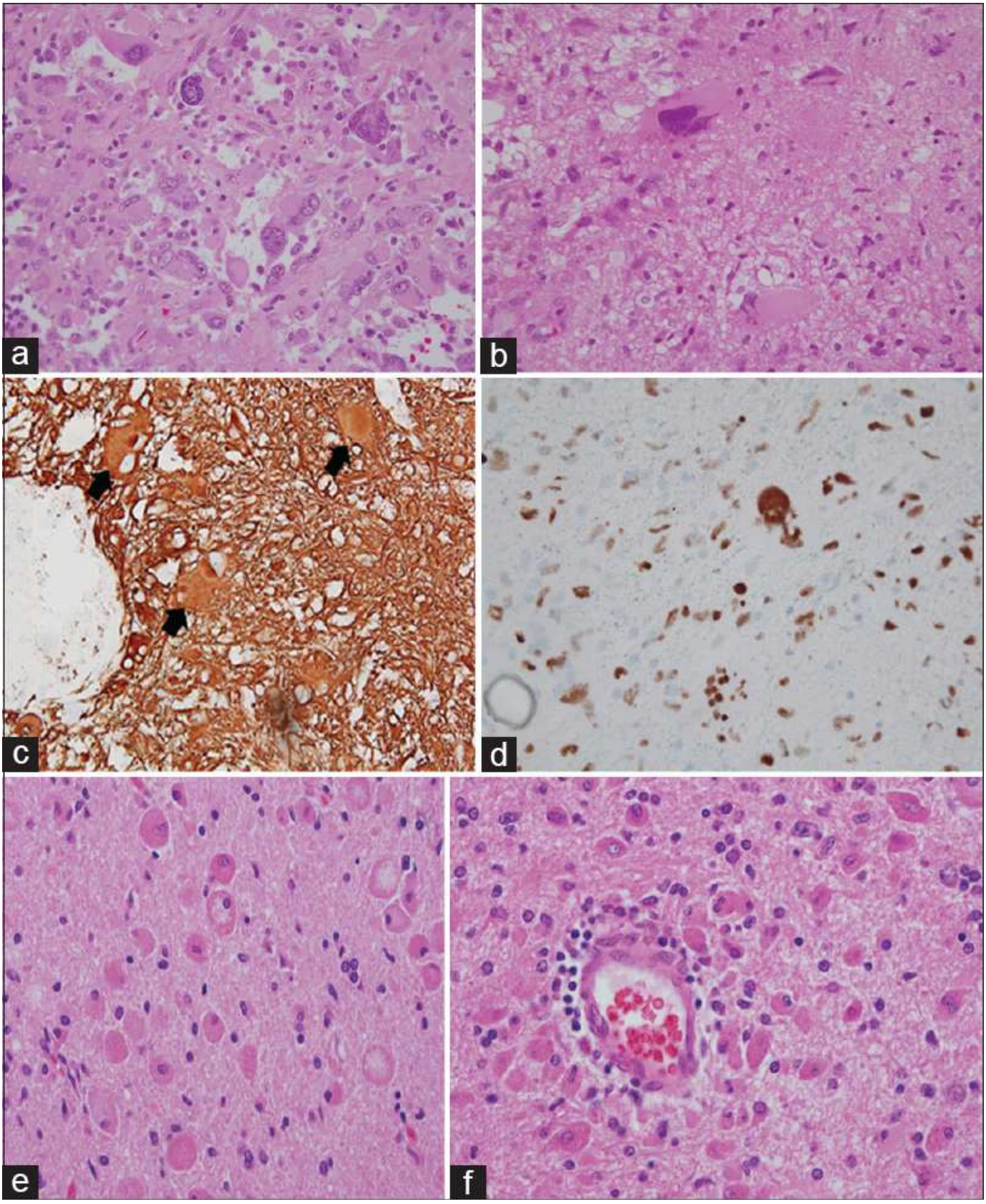
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Figure 3: (a–c): Gliosarcoma with spindle-cell morphology (a), rich pericellular reticulin deposition (b) which is in contrast to relatively reticulin-poor tumor stroma in conventional glioblastoma and its other subtypes, and paucity or loss of GFAP expression (c) corresponding to sarcomatous areas. (d–f): Glioblastoma with primitive neuronal component (d), harboring overexpression of p53 protein (e), and a very high Ki-67 proliferation rate (f)

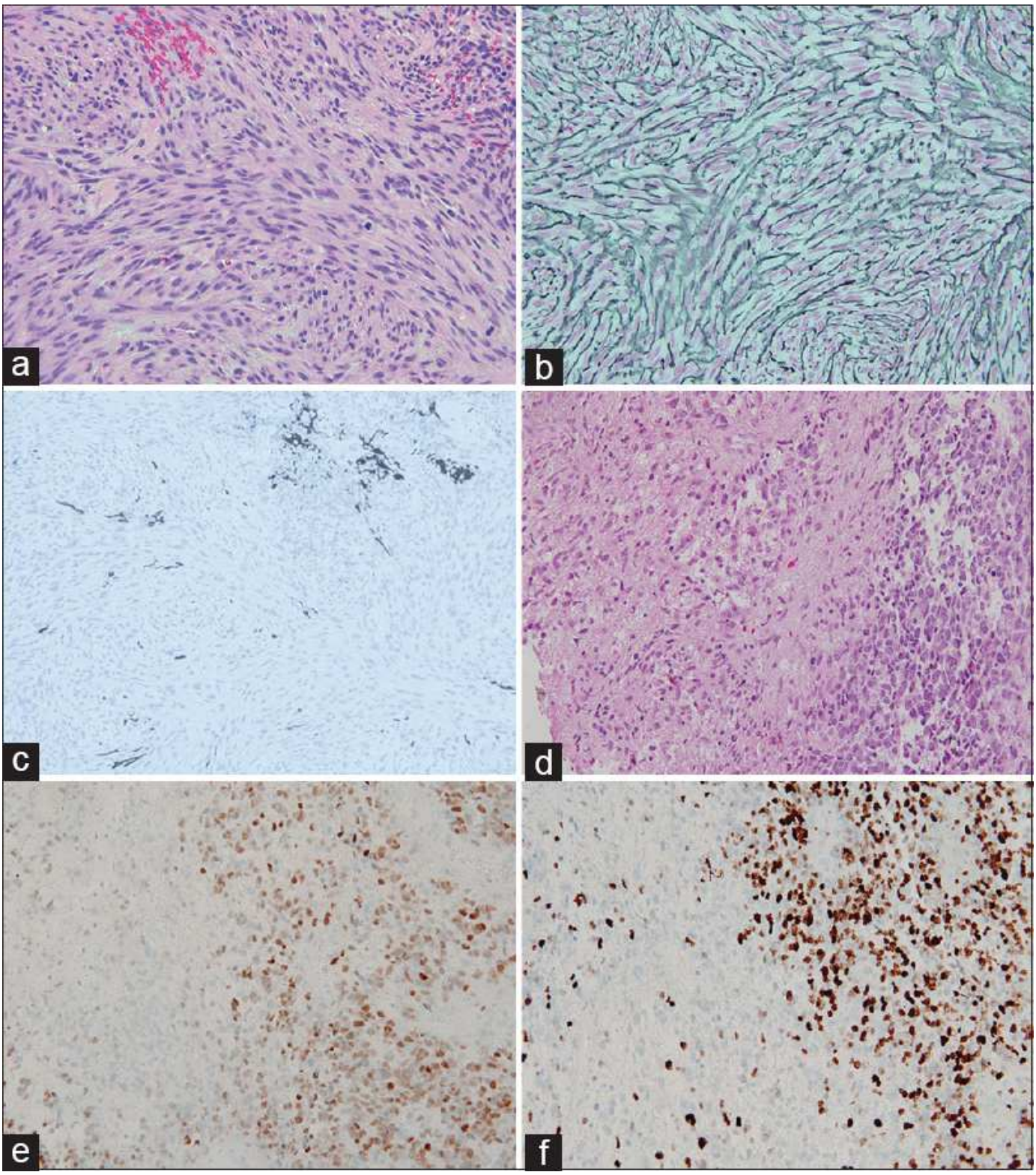

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Figure 4: (a-b): Small cell glioblastoma with relatively "uniform" cell population and "chicken-wire" vasculature in low power image (a) that mimics oligodendroglioma. However, high power usually demonstrates the irregular nuclear contours and brisk mitotic activity (b; 40×). (c) Fluorescence *in situ* hybridization (FISH) shows high-level *EGFR* gene amplification (red: *EGFR*; green: centromere probe for chromosome 7)