Gray Zones in Brain Tumor Classification: Evolving Concepts

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Whereas the WHO 2007 scheme\(^1\) is extremely useful, up to date, and user friendly, many “gray zones” remain and are likely to persist for some time to come, pending the development of more specific and reliable biomarkers. Given time constraints, I will focus on three of the most common areas of difficulty and confusion, including the topics that have been of primary focus in my own research over the last decade. Not surprisingly, these same issues are often the ‘bread and butter’ of my consult service and problematic cases that colleagues have graciously shared with me over the years have influenced many of my current approaches. In that respect, a disclaimer is probably needed in that “the opinions portrayed in the following presentation in no way reflect the views of the USCAP companion meeting organizers, who clearly cannot be held liable for the sometimes creative, though hopefully expert opinions of the presenter.” With that in mind, the topics for discussion are as follows:

1. Oligodendroglial features; the “grayest of the gray”.

   Minimal requirements for the diagnosis of an oligodendroglial component in mixed oligoastrocytomas (MOAs) remain amongst the most contentious and “grayest” of all issues in Neuropathology\(^2\). Given that this has significant clinical implications, it is a source of great frustration for pathologists and clinicians alike. Even in terms of grading for example, the finding of just 2 or 3 mitoses in the entire specimen of a pure astrocytoma is considered enough for an anaplastic (WHO grade III) designation. In
contrast, MOAs are graded similarly to pure oligodendrogliomas and require frequent mitoses or as vaguely defined by the WHO, “increased cellularity, nuclear atypia, pleomorphism and increased mitotic activity”\(^3\). Since Giannini and colleagues have shown that microvascular proliferation and/or the presence of \( \geq 6 \) mitoses per 10 HPF (even focally) are associated with statistically significant decreases in patient survival\(^4\), these are the criteria that we have adopted at Washington University for anaplasia in both pure oligodendrogliomas and oligoastrocytomas. Nevertheless, this means that a diffuse glioma with scattered mitoses (but \(<6/10\) HPF) would be considered WHO grade II if designated a MOA versus WHO grade III if diagnosed as a pure astrocytoma. If you now add microvascular proliferation, the differential is between anaplastic MOA, WHO grade III and GBM, WHO grade IV. Therefore, the definition of an oligodendroglial component becomes critical not only for cellular classification, but also for grading (and therefore, therapeutic decisions).

In my opinion, nuclear cytology is still the most important part of the definition. Low-grade oligodendroglioma cells (in pure form or in MOA) have round, uniform nuclei with crisp nuclear membranes, delicate (often “salt and pepper”) chromatin, and inconspicuous to small nucleoli. Unfortunately, this cytology is often distorted in frozen sections and poorly preserved specimens. Clear perinuclear haloes are useful, but not necessary. Anaplastic transformation is often recognized by more solid, vaguely nodular, cellular proliferations with enlarged epithelioid to plasmacytoid cells containing moderate eosinophilic cytoplasm. The nuclei become a bit more pleomorphic, but often retain an overall sense of regularity and roundness, typically with more vesicular chromatin and increased nucleolar prominence. GFAP is either negative or highlights
minigemistocytes and gliofibrillary oligodendrocytes. The cells are usually p53 negative, but this is not entirely reliable. Synaptophysin positivity is surprisingly common and a paranuclear dot-like pattern is often associated with chromosome 1p/19q codeletions by FISH or LOH. The latter “genetically favorable” pattern is strong evidence in favor of oligodendroglioma, but is only encountered in 15-20% of MOAs\(^5\). In other words, H&E remains the gold standard! Despite the subjective nature, MOAs display survival curves intermediate between pure astrocytomas and pure oligodendrogliomas, even after adjusting for grade and 1p/19q status\(^6\).

2. ‘New’ GBM variants and patterns: Small cell vs. GBM-O vs. GBM-PNET.

Although none of these histologic patterns are absolutely new, objective definitions and genetic features have only recently been published\(^6-10\). Given that they have not been in the literature that long and they overlap in terms of high cellularity, minimal cytoplasm, and marked proliferation, they have generated considerable confusion. Common distinguishing features are listed below. All of them also often show features of conventional glioblastoma at least focally. The small cell glioblastoma is most often mistaken for a high-grade oligodendroglial neoplasm, given its bland nuclear cytology and overlapping features, such as microcalcifications, “chicken wire” capillaries, perinuclear haloes, and perineuronal satellitosis; unlike oligodendrogliomas however, they lack mucin-filled microcystic spaces and frequently show EGFR amplification and 10q deletion, rather than 1p/19q codeletion\(^8,9\). Glioblastomas with oligodendroglial components (GBM-O) remain controversial, but are now accepted in the WHO 2007 scheme\(^1\) and are synonymous with “grade IV mixed oligoastrocytomas”, a
term that was preferred by a minority of the WHO participants. Based on a series of 1093 high-grade gliomas diagnosed at Washington University, we found that the MOAs containing necrosis did considerably worse than anaplastic MOA without necrosis, but better than conventional GBMs (i.e. purely astrocytic); the mean survival for GBM-O patients was estimated at just under 2 years, as compared with 10 months for GBM and >7 years for anaplastic MOA. Lastly, there have been rare case reports of GBM or gliosarcoma developing neuroblastic or PNET-like foci, often as discrete hypercellular nodules with medulloblastoma-like cytology, Homer Wright rosettes, extensive synaptophysin immunoreactivity, and/or MYC gene amplifications. Many of them additionally show increased cell size and pleomorphism, cell wrapping, and other features resembling anaplastic/large cell medulloblastomas. We have recently summarized our experience with 52 GBM-PNETs (submitted) and the main clinical implications are 1) a significantly increased risk of CSF dissemination and 2) the possibility of response to platinum based chemotherapy regimens.

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<th>Small Cell GBM</th>
<th>GBM-O</th>
<th>GBM-PNET</th>
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<tbody>
<tr>
<td><strong>Nuclear cytology</strong></td>
<td>Oval bland nuclei resembling LGG, but many mitoses</td>
<td>Round bland nuclei or large epithelioid cells with nucleoli</td>
<td>Dark oval to carrot shaped nuclei or large cell/anaplastic</td>
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<td><strong>Architectural clues</strong></td>
<td>Invasive, “chicken wire” capillaries</td>
<td>Invasive, mucin-rich microcystic spaces</td>
<td>Discrete cellular nodules, Homer Wright rosettes, desmoplasia</td>
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<td><strong>IHC</strong></td>
<td>GFAP+ processes, EGFR-vIII+, SYN-</td>
<td>GFAP+ gliofibrillary oligodendrocytes and minigemistocytes, SYN- or dot-like+</td>
<td>PNET = SYN+, minor GFAP+, often diffusely p53+</td>
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<td><strong>FISH</strong></td>
<td>EGFR-AMP (70%), -10q (95%)</td>
<td>Nonspecific in most, 1p/19q codeletion (15-20%)</td>
<td>-10q (50%); in PNET: N-myc or c-myc AMP (40%)</td>
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3. Meningioma Classification and Grading

Although there were only a few changes in the meningioma chapter in the 2007 WHO scheme\textsuperscript{12} compared with the 2000 version (which was extensively revised from the 1993 scheme), there are a few “gray zones” worth discussing. The most significant change is that brain invasive meningiomas are now defined as WHO grade II, even when appearing otherwise benign. This has been a long debated issue, since for many years (going all the way back to Cushing and Eisenhardt), brain invasion was considered the most reliable sign of malignancy. In contrast, many of the European pathologists had disregarded this feature altogether arguing that some of these tumors appear benign, they don’t always do poorly, and they often don’t show the typical genetic features of higher grade meningiomas. In two large Mayo Clinic series, brain invasion in the absence of frank anaplasia was statistically shown to be associated with recurrence and death rates virtually identical to those of atypical meningiomas (WHO grade II)\textsuperscript{13,14}. Brain invasion is defined as the presence of tongue-like protrusions of tumor that breach the pial surface of the adjacent brain. Perivascular spread along Virchow-Robin spaces is insufficient, though fortunately a rare finding. In borderline examples of brain invasion, GFAP is extremely helpful to highlight entrapped glial elements within the substance of the tumor.

Only a few rare new patterns of meningioma have been reported since the 2000 edition (e.g. oncocytic), but it was not felt that there was sufficient experience with these to warrant the inclusion of new variants in the 2007 WHO scheme. The rare lymphoplasmacyte-rich variant remains enigmatic, since some of the cases described behave more like inflammatory processes than true tumors (e.g. regressing in one area and recurring in another). One possibility highlighted in the new WHO is that some of
these may in fact be inflammatory conditions that have induced meningothelial hyperplasia, rather than the other way around, wherein a meningioma has elicited an intense inflammatory response. In terms of other variants, the most difficult question is: how much of high-grade variant histologies (chordoid, clear cell, rhabdoid, papillary) are needed before automatically using the recommended WHO grade? For example, if a benign-appearing conventional meningioma shows focal rhabdoid features, is that sufficient for a grade III designation? All of the participants in the WHO Meningeal Tumours subgroup felt that the answer to that for now is ‘No’. Until such cases are studied in greater detail, the presence of these variant morphologies only focally is considered insufficient. In my own practice, I have only utilized the recommended WHO grades when the majority of the tumor (>50%) shows chordoid, clear cell, rhabdoid, or papillary features.

Other Gray Zones (to be discussed some other day)

1. Criteria for anaplasia in favorable variant tumors, such as pleomorphic xanthoastrocytoma, ganglioglioma, and pilocytic astrocytoma

2. Grading of ependymomas

3. Neuronal features, new glioneuronal tumors, and synaptophysin positivity in an otherwise classic glioma
4. Low grade tumors with high Ki-67 labeling indices

5. Why is pineocytoma a WHO grade I tumor, while histologically analogous tumors, such as central neurocytoma and extraventricular neurocytoma are considered WHO grade II?

6. Is there really sufficient data to stratify pineal parenchymal tumors (PPTs) into 4 WHO grades? If so, how does one separate intermediate differentiation PPT grade II from grade III?

7. If central neurocytoma is WHO grade II, what do you do with “atypical neurocytomas”?

8. How nodular does extensively nodular medulloblastoma need to be before it is diagnosed that way? How anaplastic does anaplastic medulloblastoma need to be?
References


