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EDITORIAL (BY INVITATION)

WHO 2016: Open questions and practical implications

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The WHO Classification of Tumors of the Central Nervous System has been a story of success over the past decades. There is general agreement that the WHO Classification represents the basis not only for neuropathological diagnosis but also for prognosis and therapy. The world-wide consensus of using this classification system is a remarkable achievement and may serve as a role model for communities working on other neurological diseases where heterogeneous and conflicting classification systems impair scientific and clinical progress.

The most recent WHO Classification has been issued in May 2016 and includes several changes, as detailed in the "Blue Book" [3] and summarized in an accompanying review article [4] as well as in the paper by Banan and Hartmann published in this issue of *Acta Neurochirurgica*. As in previous updates of the WHO Classification, a few newly recognized clinico-pathological tumor types have been added, such as diffuse leptomeningeal glioneuronal tumor, epithelioid glioblastoma, and anaplastic pleomorphic xanthoastrocytoma. A few others were deleted, such as protoplasmic astrocytoma, cellular ependymoma, and gliomatosis cerebri. Other tumors were renamed, including fibrillary astrocytoma as diffuse astrocytoma, hemangiopericytoma as solitary fibrous tumor/hemangiopericytoma, and primitive neuroectodermal tumor (PNET) as CNS embryonal tumor. Tumors that have been known (and diagnosed) for a long time before but somehow had been "forgotten" in previous WHO Classifications have been added in chapters on lymphomas, histiocytic tumors, tumors of the cranial and paraspinal nerves, and mesenchymal tumors. Finally, brain invasion has been clearly defined as being sufficient for making the diagnosis of atypical meningioma.

However, the most dramatic change, which is unparalleled by previous editions, is the inclusion of molecular data in the diagnosis of several tumor types (actually still being a minority), resulting in integrated histological/molecular diagnoses. Examples include "oligodendroglioma, IDH-mutant and 1p/19q-codeleted, grade II" and "classical medulloblastoma, SHH-activated and TP53 mutant, grade IV". Compared to the conventional, relatively broad tumor types solely defined by histology, the narrowly defined, combined histological/molecular newcomers will lead to more homogeneous tumor groups, which are expected to be advantageous for clinical studies and eventually for the management of individual patients. While the updated classification is straightforward, clinically relevant, and based on recent molecular insight, it provokes questions concerning practical application. A few of these questions are listed below, not necessarily complemented with an unequivocal answer.

Is 1p/19q analysis required for all cases of diffuse glioma?

Codeletion of the short arm of chromosome 1 and the long arm of chromosome 19 is currently considered a defining molecular feature of oligodendroglioma. While in cases of histologically classical oligodendroglioma 1p/19q analysis is essential for making the final (integrated) diagnosis, this is less clear for cases with less pronounced oligodendroglial differentiation or even for histologically astrocytic tumors. The WHO Classification states that the presence of an astrocytic *component* is compatible with the diagnosis of oligodendroglioma when molecular testing reveals the entity-defining combination of IDH mutation and 1p/19q codeletion. This means that histologically pure astrocytomas do

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not need to be analyzed for 1p19q codeletion. On the other hand, in the review article written by the editors of the WHO Classification, it is stated that "genotype trumps histological phenotype", i.e., a diffuse glioma that histologically appears astrocytic, but proves to have IDH mutation and 1p/19q codeletion necessitates a diagnosis of oligodendroglioma, IDH-mutant, and 1p/19q-codeleted [4]. This means that 1p/19q analysis would be required in *all* cases of diffuse glioma. The most appropriate practical approach may depend on the amount/representativeness of the material in the individual case as well as on systematic studies revealing the actual frequency of this kind of constellation, i.e., completely disparate genotype versus histotype. Some clarification and ideally consensus appears useful.

How can tumors with discrepant genotype and histological phenotype be diagnosed?

Combining histology and genetics in one diagnosis necessarily implicates the occurrence of occasional cases where genetics appears to contradict histology. No classification can encompass the totality of nature's breadth and diversity. For example, true oligodendroglioma without IDH mutation and without 1p/19q codeletion may exist in pediatric patients. These "pediatric-type oligodendrogliomas" are mentioned in the Blue Book [3] but they are not part of the WHO Classification, and occurrence in adult patients remains unclear. Similarly, while oligoastrocytoma has been practically eliminated from the WHO Classification (with only the Not Otherwise Specified category remaining, i.e., for tumors without complete molecular analysis), the presence of genetically true oligo-astrocytomas with dual genotype [2, 9] has been acknowledged in the Blue Book but not considered a distinct WHO entity. Finally, diffuse midline glioma, H3 K27M-mutant, represents a newly introduced, predominantly astrocytic tumor of pons, thalamus, or spinal cord with poor prognosis and a K27M mutation in one of three histone genes. While the H3 K27M mutation had been considered as being the defining mutation of this brain tumor group, it may also occur in other brain tumors, such as pilocytic astrocytoma [6] and ependymoma [1], without necessarily being associated with poor prognosis. Whether these tumors have to be classified as diffuse midline glioma (grade IV) with aberrant phenotype or as low-grade glioma with unusual H3 K27M mutation remains unclear to date. These cases certainly represent only a small minority of brain tumors, but their classification poses problems.

Is molecular diagnostics more reliable than histology?

It has been reiterated many times that molecular typing of (brain) tumors is more reliable and precise than histological classification, but data confirming this belief are largely missing. While it appears intuitive that searching for absence versus presence of a mutation is more straightforward and afflicted with less inter-rater variability than a diagnosis based on the bewildering variety of histological pictures, it still remains a hypothesis that needs to be tested in systematic inter-rater reliability studies. Preliminary endeavors have revealed surprisingly high inter-rater variability of molecular neuropathology. In an unpublished German study involving 22 neuropathology institutions, 20 gliomas were examined for MGMT promoter methylation. Uniform results of methylation versus non-methylation among all institutions were obtained in only four of 20 cases (20%), which is most probably lower than reliability expected for microscopical diagnosis. MGMT analysis may predispose to relatively high variability due to heterogeneous techniques and molecular targets, while assays for hotspot point mutations (IDH) or deletion (1p/19q) are expected to be more reliable, but this remains to be demonstrated and urgently calls for inter-laboratory studies and consensus protocols to guarantee reliable molecular and integrated diagnoses.

Are there valid and convenient surrogate markers?

In general, molecular classification is performed using appropriate molecular methods, such as sequencing or methylome analysis. These techniques tend to be expensive and need to be well controlled. Existence of reliable and valid surrogate markers using more convenient and standard methods such as immunohistochemistry would be advantageous. While current agreement indicates that surrogate markers for 1p/19q codeletion do not exist, a few neuropathologists (including Banan and Hartmann, who are authors of the corresponding review article in this issue) believe that molecular classification of medulloblastoma (WNT, SHH, non-WNT/SHH) and ependymoma, *RELA* fusion-positive, can be performed using appropriate immunohistochemical markers. The problem is that the spectrum of markers as suggested by different experts is variable, and sensitivity and specificity of these markers is less than ideal. Furthermore, in any institution, immunohistochemical markers need to be validated against molecular methods in a large series of tumors before diagnostic application, because immunohistochemical methods and their evaluation may vary widely among institutions.

For example, immunohistochemistry for L1CAM has been suggested as a potential surrogate marker for the diagnosis of ependymoma, *RELA* fusion-positive. Unfortunately, L1CAM may also be expressed by other ependymoma subtypes and other brain tumors, and only 82% of *RELA* fusion-positive ependymomas have been shown to be positive for L1CAM in a systematic, well-controlled study [8]. It appears reasonable to assume that sensitivity and specificity will be even

lower in a routine setting when immunostaining is performed in a single case every few weeks or months. In a similar vein, a variety of immunohistochemical markers have been recommended for the molecular classification of medulloblastoma, but their sensitivity and specificity are currently less clear than neuropathologists occasionally believe, who otherwise would be unable to classify medulloblastoma according to WHO 2016 or make only a NOS ("not otherwise specified") diagnosis. Since molecularly defined entities of the WHO Classification are clinically, prognostically and potentially therapeutically relevant, the exclusive use of surrogate markers with 50, 80, or even 98% sensitivity does not appear diagnostically, scientifically, and ethically appropriate, if exact molecular methods are available elsewhere and neuropathology should still be considered the gold standard of diagnosis. Much more work remains to be done.

Economics or ethics?

Undoubtedly exact neuropathological diagnosis of brain tumors has become more expensive with WHO 2016. In an ideal world without financial constraints, every brain tumor would be comprehensively genetically characterized, including whole genomic sequencing and methylome analysis. In general, a classification system does not include statements about what is affordable, in part because there are huge differences between and within nations. However, the Blue Book makes an interesting point with respect to the molecular diagnosis of glioblastoma. The 2016 Classification includes glioblastoma, IDH wild type (also referred to as primary glioblastoma, about 90%) and glioblastoma, IDH mutant (also referred to as secondary glioblastoma, about 10%). The two tumor types differ with respect to age, length of clinical history, and prognosis, making a correct diagnosis clinically relevant. About 90% of IDH mutations are represented by *IDH1* R132H, which can be reliably detected using an antibody specific for the mutant protein, whereas the other 10% mutations (*IDH1* non-R132H, *IDH2*) can only be revealed by sequencing *IDH1* and *IDH2* genes. The proportion of glioblastomas with IDH mutation substantially decreases with age. Accordingly, the Blue Book states that it may be sufficient or "safe" in older patients to rely solely on negative immunohistochemistry for making the diagnosis of glioblastoma, IDH wild type, because in an immunohistochemically negative glioblastoma from a patient without prior lower-grade glioma, the probability of an alternative IDH mutation is <6% in a 50-year-old patient and decreases to <1% in patients aged >54 years. It is debatable whether saving cost and workload by refraining from sequencing *IDH1*/*IDH2* genes in all glioblastomas justifies molecular misclassification in <5% of patients.

How long does it take to make the final diagnosis?

As molecular diagnostics is performed following histological and immunohistochemical analysis, time to final diagnosis inevitably increases for brain tumors with integrated diagnosis. For example, since oligodendroglioma requires molecular pathology, and criteria of anaplasia differ for astrocytic versus oligodendroglial neoplasms, it is not unusual that in a glioma with ambiguous histology and a few mitoses a preliminary diagnosis of diffuse glioma (without type and grade) has to be made for a week or so. Diagnostic turnaround times mainly depend on types of methods and frequency of assays in individual labs.

How can we move forward between WHO classification updates?

Most probably, the number of molecularly defined brain tumor types will soon increase. Examples may include meningioma, atypical teratoid/rhabdoid tumor, diffuse astrocytoma IDH wild type, and pilocytic astrocytoma. Other molecular tumor types have not yet been introduced into the WHO Classification system, although they have been already included in consensus suggestions on clinical management, such as ependymoma with YAP fusion or infratentorial ependymoma types A and B [7]. Furthermore, new molecular or surrogate markers that are important for classification and diagnosis will be developed. The current intervals of 7–9 years between WHO Classification updates are certainly too long in this era of rapid progress. In order to provide prompt suggestions for the neuro-oncology community, members of the WHO Working Group and an associated Clinical Advisory Panel have recently constituted cIMPACT-NOW (Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy) [5]. As the suffix NOW indicates, this is Not Official WHO. Suggestions will be solicited from the neuro-oncology community, evaluated in working groups, and guidelines for diagnostics and suggestions for possible WHO updates will be regularly published.

The 2016 WHO Classification reflects a paradigm shift and transitional stage to a combined histological/molecular approach. While the new classification has been quickly adopted all over the world, some practical issues need to be resolved and new developments implemented by regular discussions, conferences, and updates. Genetic data and insight into molecular pathogenesis continue to be revealed at a rapid pace and soon will find their way into diagnosis and clinical management. This is an exciting time for neuropathological brain tumor classification and for understanding brain tumor biology. Progress will be even more successful with critical input from neurosurgeons.

References

1. Gessi M, Capper D, Sahm F, Huang K, von Deimling A, Tippelt S, Fleischhack G, Scherbaum D, Alfer J, Juhnke BO, von Hoff K, Rutkowski S, Warmuth-Metz M, Chavez L, Pfister SM, Pietsch T, Jones DT, Sturm D (2016) Evidence of H3 K27M mutations in posterior fossa ependymomas. Acta Neuropathol 132:635–637. doi:10.1007/s00401-016-1608-3
2. Huse JT, Diamond EL, Wang L, Rosenblum WK (2015) Mixed glioma with molecular features of composite oligodendroglioma and astrocytoma: a true "oligoastrocytoma"? Acta Neuropathol 129:151–153. doi:10.1007/s00401-014-1359-y
3. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (2016) World Health Organization histological classification of tumours of the central nervous system. International Agency for Research on Cancer, Lyon
4. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P, Ellison DW (2016) The 2016 World Health Organization classification of tumors of the central nervous system: a summary. Acta Neuropathol 131:803–820. doi:10.1007/s00401-016-1545-1
5. Louis DN, Aldape K, Brat DJ, Capper D, Ellison DW, Hawkins C, Paulus W, Perry A, Reifenberger G, Figarella-Branger D, Wesseling P, Batchelor TT, Cairncross JG, Pfister SM, Rutkowski S, Weller M, Wick W, von Deimling A (2017) Announcing cIMPACT-NOW: the consortium to inform molecular and practical approaches to CNS tumor taxonomy. Acta Neuropathol 133:1–3. doi:10.1007/s00401-016-1646-x
6. Orillac C, Thomas C, Dastagirzada Y, Hidalgo ET, Golfinos JG, Zagzag D, Wisoff JH, Karajannis MA, Snuderl M (2016) Pilocytic astrocytoma and glioneuronal tumor with histone H3 K27M mutation. Acta Neuropathol Commun 4:84. doi:10.1186/s40478-016-0361-0
7. Pajtler KW, Mack SC, Ramaswamy V et al (2017) The current consensus on the clinical management of intracranial ependymoma and its distinct molecular variants. Acta Neuropathol 133:5-12. doi:10.1007/s00401-016-1643-0
8. Parker M, Mohankumar KM, Punchihewa C et al (2014) C11orf95-RELA fusions drive oncogenic NF-κB signalling in ependymoma. Nature 506:451–455. doi:10.1038/nature13109
9. Wilcox P, Li CC, Lee M, Shivalingam B, Brennan J, Suter CM, Kaufman K, Lum T, Buckland ME (2015) Oligoastrocytomas: throwing the baby out with the bathwater? Acta Neuropathol 129: 147–149. doi:10.1007/s00401-014-1353-4