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The impact of the 2016 World Health Organization classification of tumours of the Central Nervous System upon diagnosis and prognosis

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EDITORIAL

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Introduction

Neoplasms of the central nervous system are a heterogeneous group of conditions which demonstrate remarkable diversity in their clinical behaviour, microscopic appearance and genetic profiles. The recently published 2016 World Health Organization (WHO) classification of Tumours of the Central Nervous System (CNS)[1] is an update of the 4th edition of the Blue Book series and introduces a number of significant changes from the previous edition, and in many respects represents a major change in the approach to glioma classification and diagnosis. For the first time the classification incorporates molecular findings into the formulation of CNS tumour diagnoses, breaking the long standing principle of classification based almost exclusively on light microscopic appearances. This brief and selective review will highlight this new approach, with specific emphasis on the family of diffuse gliomas. Other alterations introduced in the 2016 WHO CNS Classification are briefly outlined.

Discussion

Accurate diagnosis, classification and grading of CNS tumours are fundamental to both patient management and to the interpretation of clinical and basic research investigations.

Glioma type and grade, considered together with clinical factors such as patient age, performance status, extent of resection and tumour location, continue to form the basis upon which clinical decisions are largely made. Key factors which influence the evaluation of CNS biopsy specimens include biopsy size, morphologic preservation, tumour heterogeneity, and the need for meticulous correlation with the clinical history and neuroimaging findings. Regional heterogeneity is a well-recognised feature of many gliomas, and therefore the accuracy of glioma classification and grading is highly dependent on the extent and site of sampling. A final diagnosis should never be formulated until the findings have been carefully correlated with the results of neuroimaging studies. Neuroimaging has a role in defining the presence, recurrence or progression of a tumour and response to treatment. Accurate identification of anatomical attachments and any blood supply is of value pre-operative in selected cases as embolization may be undertaken at that time to assist the surgeon.

Historically, the classification and grading of CNS tumours has been based on light microscope examination using haematoxylin and eosin-stained sections. The classification adopts a histogenic approach; it attempts to link the morphological resemblance of CNS tumours at a microscopic level to a putative 'cell of origin' or with particular lines of differentiation. In recent decades immunohistochemistry and electron microscopic studies have been used to complement and refine these basic microscopic observations. Immunohistochemistry, detecting specific antigens in tissue sections, can aid in tumour characterisation.

The ***histologic grade***, as used by WHO, communicates a ''stage of malignancy'' that will predict biologic behaviour. Thus, by WHO criteria, grade I CNS tumours are generally well circumscribed, slowly progressing, and can often be cured by resection; grade II lesions are typically infiltrative with low proliferation, but have a higher likelihood of recurrence; grade III tumours are histologically malignant and generally require more aggressive adjuvant therapy; and grade IV tumours are highly malignant and can be rapidly fatal. Historically, glioma grading has been based on an assessment of microscopic features, and this remains unchanged, for the present at least, in the 2016 WHO system.

Morphology based glioma classification and grading schemes have been the 'back-bone' of diagnostic practice for decades. They have led to the definition and characterisation of well-defined entities, and as this process has continued to evolve, new glioma types have continued to be described. In recognition of the essentially subjective nature of tumour classification based on microscopic examination and issues of inter-observer variability in the classification and grading of gliomas, attempts to refine morphologic interpretation and provide strict and well defined criteria have resulted in improved diagnostic concordance.[2] Nonetheless, it has been apparent that the limits of glioma classification and grading based solely on morphology have been reached in some areas. For example, it is widely recognised that some gliomas may appear histologically identical, yet have widely differing clinical behaviours and responses to therapy and outcomes.

Difficulties in glioma diagnosis and grading, in prognostic stratification and the prediction of likely response to particular therapies in situations has prompted extensive evaluation of the utility of molecular markers in these areas.

With advances in molecular and genetic techniques, much has been learned of the events underpinning the development and progression of many gliomas. Efforts have been made to identify distinctive molecular markers useful in diagnosis, prognostication and prediction of likely response to therapy for various CNS tumour types. As a result, molecular markers have become increasingly incorporated into practice, to refine classification and improve diagnosis, to provide better prognostication, and to allow for patient-specific therapeutic decisions.[3]

The **Haarlem Consensus guidelines**[4] proposed a new approach which was adopted for the revised WHO classification of tumours of the CNS that is based on the integration of 'phenotype' (microscopic appearances) and 'genotype' (genetic biomarkers). The 2016 WHO Classification is predicated on the basis of combined morphologic and genotypic classification and on the generation of **"integrated" diagnoses**. Some CNS tumour entities now require specific molecular information to render a specific "integrated" diagnosis. A less specific not otherwise specified 'NOS' designation is used in the absence of requisite genotypic and molecular results. It should also be noted that some entities remain defined by histology alone.

It should be noted the update is aimed at providing integrated genotypic/phenotypic diagnoses, and does not mandate inclusion of valuable prognostic and/or predictive markers in the formulation of a final integrated diagnosis. 06-methylguanine-DNA methyltransferase (MGMT) promoter methylation status is an example of this principle. MGMT methylation status influences clinical decisions, but is of no specific diagnostic value. Nonetheless, MGMT methylation status remains an important component of pathology reports. MGMT is an epigenetic marker of prognostic importance, particularly in elderly patients with glioblastoma multiforme (GBM).[5]

2016 Legend and Nomenclature: the International Classification of Diseases for Oncology (ICD-O) assigns a 4 digit code e.g., diffuse astrocytoma WHO grade II, IDH-mutant is 9400/3.

The number that follows after the slash refers to biological behaviour and is not the WHO grade which is always in Roman numerals and upper case.

To avoid several hyphens *wildtype* is used without a hyphen. An en-dash is used in some designations e.g., *RELA fusion*—positive. For tumours in which a specific genetic alteration is present or absent, the term 'positive' can be used if the molecular characteristic is present e.g., *Ependymoma, RELA fusion*—positive.

CNS integrated diagnoses consist of a histopathological name followed by the genetic features, with the genetic features following a comma and as adjectives. e.g., diffuse astrocytoma, IDH-mutant or Medulloblastoma, WNT-activated. Some tumours have more than one genetic determinant so these are included in the name e.g., Oligodendroglioma, IDH-*mutant* and 1p/19q co-deleted.
If a tumour does not have a genetic mutation the term *wildtype* is used provided an official *wildtype* entity exists e.g., glioblastoma, IDH-*wildtype*. When a formal *wildtype* is not available and the tumour under investigation lacks a diagnostic mutation, it is given an NOS label (Not

otherwise specified). The use of NOS also permits some regions without suitable laboratories to continue to provide diagnoses in the new framework.

Italics are used for specific gene symbols e.g., *ATRX* but not for gene families e.g., IDH.

The approach adopted in the 2016 WHO classification has resulted in a **major restructuring of the classification of diffuse gliomas, medulloblastomas, other embryonal malignancies, with incorporation of genetically defined entities**.

Restructuring of the classification of diffuse gliomas is a prototypic example of this new approach. The family of diffuse gliomas, which include astrocytomas, oligodendrogliomas, and rare mixed oligoastrocytomas, represent the most common primary CNS tumours. All share a tendency for local recurrence and anaplastic degeneration, and they rarely, if ever, can be completely resected, due in part to their infiltrative growth patterns. Historically, typing and grading of diffuse gliomas has been one of the most problematic areas in diagnostic surgical neuropathology. It is critical to distinguish between diffuse astrocytic and oligodendroglial gliomas, since these two tumour subtypes have considerably different clinical courses and optimal therapeutic approaches. Aside from morphologically 'classic' forms of diffuse astrocytoma and oligodendroglioma, there is a large group of intermediate or hybrid lesions in which the distinction between astrocytic and oligodendroglial features, and the relative importance of each, has been controversial and difficult, with problems related to both typing and grading.

Utilising the approach outlined in the 2016 WHO Classification, using a combination of genotype (IDH and 1p/19q co-deletion status) and phenotype (microscopic morphology) will allow almost all of these problematic cases to be resolved as being either astrocytoma or oligodendroglioma. Thus, determination of Isocitrate dehydrogenase 1 (IDH 1) and isocitrate dehydrogenase 2 (IDH 2) gene status and_1p/19q co-deletion status thus become central to the diagnosis of the diffuse glioma family.

IDH 1 and IDH 2 gene mutations in glioma were first identified in large scale sequencing analysis of a group of 22 glioblastomas and were found in codons 132 and 172 of IDH1 and IDH2 respectively.[6] Mutations were preferentially identified in younger patients, in "secondary" forms of glioblastoma, and were associated with a better prognosis. Further studies revealed that somatic IDH 1 mutations are present in the vast majority of diffuse WHO grade II and WHO grade III gliomas. IDH 1 and IDH 2 mutations are effectively restricted to diffuse gliomas, and are found in approximately 80 per cent of diffuse astrocytomas and oligodendrogliomas, and in approximately 15 per cent of glioblastoma [practically all of which are secondary in type.

Mutations are very early events in gliomagenesis, presumably affecting some form of glial precursor cell, and always appear before the acquisition of p53 gene mutation or 1p/19q co-deletion.[7] IDH mutation is a strong, independent and favourable prognostic marker in high grade glioma, and in certain instances is a more powerful prognostic than WHO grade. Diffuse gliomas with mutation have a far better prognosis than their type and grade matched wild type counterparts. WHO grade III anaplastic astrocytomas lacking a mutation fare just as badly as wild type grade IV GBM. In contrast, gliomas of similar morphology with IDH mutations progress more slowly. Approximately 80 to 90 per cent of IDH mutant gliomas contain the arginine to histidine R132H variant. Mutation specific antibodies applicable to the study of formalin fixed, paraffin embedded tissue sections are available. The R132H IDH 1 antibody is virtually 100 per cent specific,[8] but will miss the approximately 10 per cent of gliomas carrying rarer IDH1 or IDH2 mutations. A negative R132H IDH 1 immunohistochemical result therefore does not exclude the possibility of an underlying mutation. Screening immunonegative gliomas by sequencing will identify gliomas with rarer IDH1 or IDH2 mutations.

Accumulation of the oncometabolite 2HG which results from IDH mutation not only results in methylation phenomenon which can drive mutagenesis/oncogenesis but can now be detected in vivo using MR Spectroscopy.[9] This is likely to result in changes to future imaging approaches to diffuse glioma diagnosis and monitoring.

IDH mutations can also occur in non CNS tumours such as acute myeloblastic leukaemia.[10]

The **1p/19q co-deletions** recognised as the molecular signature of oligodendroglioma result from an unbalanced translocation t(1;19)(q10;p10), in which the 1p19q derivative is lost, resulting in whole arm 1p and 19q deletions. The association between 1p/19q co-deletion and improved response to chemotherapy and longer survival in patients with [recurrent] anaplastic oligodendrogliomas was first reported in 1998.[11] This

observation has been confirmed in numerous subsequent studies, which have demonstrated that co-deletion is a powerful prognostic marker in WHO grade III gliomas. The survival difference is much stronger in WHO grade III anaplastic than WHO grade II oligodendrogliomas. Gliomas with the co-deletion are more sensitive to adjuvant therapies in general, and when they are treated, have a significantly longer survival. Determination of 1p and 19q status can also help differentiate oligodendroglioma from morphologically similar lesions. Perhaps the most important use is in the distinction between WHO grade III anaplastic oligodendroglioma and the small cell variant of astrocytoma / glioblastoma (GBM).

Whereas all astrocytic neoplasms were previously grouped together, the 2016 WHO Classification **distinguishes diffuse astrocytomas from specific circumscribed astrocytoma subtypes** (such as pilocytic astrocytoma and pleomorphic xanthoastrocytoma) which lack IDH mutation, and often have BRAF alterations. Also subependymal giant cell astrocytoma (SEGA) with *TSC1/TCS2* mutations. Each of these circumscribed astrocytoma subtypes generally have a much more favourable prognosis than diffuse astrocytoma. Another change of note regards the approach to grading for pilomyxoid astrocytomas. Previously assigned as WHO grade II, it is no longer clear that the pilomyxoid variant follows a more aggressive clinical course than conventional pilocytic astrocytoma. Considerable overlap in morphology and genotype exists between the two. For these reasons the pilomyxoid variant is not automatically assigned WHO grade II and formal grading suppressed until this matter is further resolved. Also of note brain invasion in meningioma has now been added as one criterium for WHO grade II Atypical Meningioma.

CLASS of DIFFUSE GLIOMAS

The class of diffuse gliomas, with incorporation of genetically defined entities in the 2016 WHO Classification, now includes WHO grade II, III and IV astrocytic tumours, WHO grade II and III oligodendrogliomas, and diffuse midline glioma of childhood.

Diffuse astrocytomas represent a morphological and biological continuum ranging from well differentiated to highly anaplastic lesions. The current WHO classification distinguishes three grades, and separates (well differentiated) WHO grade II diffuse astrocytoma from its higher grade counterparts WHO grade III anaplastic astrocytoma (AA) and WHO grade IV astrocytoma - (GBM). The **grading of diffuse astrocytomas** involves an assessment of the presence or absence of certain histological factors (which tend to be acquired in sequence as diffuse astrocytomas become progressively anaplastic) - the parameters (in their usual order of acquisition) are pleomorphism, mitotic activity, endothelial proliferation and / or necrosis.

WHO grade II diffuse astrocytomas, WHO grade III anaplastic astrocytomas and WHO grade IV astrocytoma (GBM) are further divided into **IDH-mutant, IDH-wildtype and NOS** (not otherwise specified). For grade II and III diffuse astrocytomas, in laboratories where IDH testing is available, most are shown to belong to the IDH-mutant group. IDH-wildtype examples, which are rare, can be diagnosed provided both immunohistochemistry for mutant R132H IDH1 protein and sequencing for *IDH1* and IDH2 gene mutations are negative. In a situation where IDH testing is not available the diagnosis is diffuse astrocytoma NOS (not otherwise specified). Some, but not all, recent reports suggest the prognostic differences between IDH-mutant WHO grade II diffuse astrocytomas and IDH-mutant WHO grade III anaplastic astrocytoma are not as great as previously thought. WHO grade III anaplastic astrocytomas lacking a mutation, however, fares just as badly as grade IV GBM wildtype, and often have a genotypic profile resembling primary GBM.

WHO grade IV astrocytoma (GBM) is a highly malignant form of glioma, with an exceedingly poor prognosis**.** Glioblastomas comprise 60 per cent of diffuse gliomas. WHO grade IV astrocytoma/GBM are divided into IDH-wildtype (90 per cent), corresponding to 'primary' GBM which affects the adult population; especially aged more than 55 years and IDH-mutant (10 per cent) corresponding to 'secondary' GBM which tend to occur in younger patients, have a slightly better prognosis and are associated with anaplastic degeneration of a pre-existing lower grade astrocytoma.

The current WHO classification recognizes two grades of oligodendroglioma; WHO grade II oligodendrogliomas and WHO grade III anaplastic oligodendrogliomas, though the criteria for grading are not as clearly defined as for diffuse astrocytoma.

Diagnosis of both **oligodendrogliomas and anaplastic oligodendrogliomas** in the 2016 Classification require the demonstration of both an IDH gene family mutation and combined whole-arm losses of 1p and 19q (1p/19q co-deletion). If such testing is not available, a tumour recognised by histopathology alone is diagnosed as NOS (not otherwise specified).

Diffuse midline glioma, H3K27M-mutant is a new entity in the 2016 classification.[12]
It has K27M mutations in the histone H3 gene, *H3F3A* and sometimes in the *HIST1H3B* gene. It shows a diffuse growth pattern and favours the midline such as thalamus, brain stem and spinal cord. It comprises most of what has traditionally been referred to as intrinsic diffuse pontine gliomas. One unique molecular alteration that has been identified in paediatric gliomas is K27M missense mutation in **histone H3 variants** and the presence of this mutation **correlates with poor prognosis**. K27M means the 27th amino acid in the translated polypeptide/protein is changed from a lysine (single letter code K) to a methionine (single letter code M) by a substitution mutation.

CLASSES of NON-GLIOMAS
Major restructuring of the **embryonal tumours** has occurred.[1] A detailed discussion is beyond the limits of this paper, but to briefly summarise: It is now generally accepted that ***medulloblastoma*, as well as having clinically significant histological variants (classic, nodular/desmoplastic, large cell/anaplastic and medulloblastoma with extensive nodularity)** may also be divided into four genetic groups with varying prognosis. These are WNT-activated (wingless-activated), SHH-activated (sonic hedgehog –activated) and the non-WNT/non-SHH, group 3 and group 4. Where appropriate testing is possible, medulloblastoma may be defined by both histology and molecular means.

Other embryonal tumours have also undergone considerable reorganisation of their classification, **with deletion of the term primitive neuroectodermal tumour (PNET)**. Many of these rare tumours have amplification of the C19MC region on chromosome 19 (19q13.42).[13] These include tumours previously known as embryonal tumours with abundant neuropil and true rosettes **– (also called embryonal tumours with multilayered rosettes), ependymoblastoma and medulloepithelioma.** In the 2016 WHO classification of CNS tumours, if C19MC amplification is present the diagnosis is **embryonal tumour with multilayered rosettes (*ETMR*), *C19MC*-altered** but if it is absent one should refer to embryonal tumour with multilayered rosettes, *NOS.*

Atypical teratoid/rhabdoid tumour (*AT/RT*), needs to have alterations of either *INII tumour suppressor gene* or rarely *BRG1 (Brahma-related gene-1)* to receive that diagnosis. Otherwise it can only be described as *CNS embryonal tumour with rhabdoid features.*

Briefly other important developments include incorporation of one genetically defined ependymoma variant:
Ependymoma, RELA fusion-positive which accounts for most of this type of tumour in the supratentorial compartment in children.

There has also been *addition* of newly recognized entities, variants and patterns:
Entities: Diffuse leptomeningeal glioneuronal tumour, anaplastic pleomorphic xanthoastrocytoma (PXA) and a **variant** epithelioid glioblastoma.
New Patterns: Glioblastoma with primitive neuronal component and Multinodular and vacuolated pattern of ganglion cell tumour

***Deletion* of gliomatosis cerebri** and of **protoplasmic and fibrillary astrocytoma** variants has occurred, as they are no longer felt to have specific clinical or biological relevance. Gliomatosis cerebri is not formally diagnosed in a radiology report. Instead the diffuse involvement is described and then a line added saying 'previously referred to as gliomatosis cerebri'.

Restructuring of solitary fibrous tumour and haemangiopericytoma (SFT/HPC) as one entity took place and a soft tissue type grading system was adapted to accommodate this change. Radiologically the two can be quite different on imaging and have to be considered in the differential diagnosis of extra-axial masses.[14]

Another new feature is ***expansion and clarification of entities included in nerve sheath tumours***, with addition of hybrid nerve sheath tumours and separation of melanotic schwannoma from other schwannomas. ***Expansion of entities included in haematopoietic/lymphoid tumours*** of the CNS (lymphomas and histiocytic tumours) has also occurred.

Impact of the 2016 WHO classification
Pathologists and radiologists will require additional co-operation because there is a gap in radiological practice in matching the extensive molecular markers available to the histopathologist.

Radiology complements the histopathology. Progressing beyond the imaging available over past decades will have implications for novel treatment trials as well as improving current practice.

Conclusions

The 2016 World Health Organization (WHO) Classification of Tumours of the CNS[1] represents a major advance in glioma classification and diagnosis, incorporating molecular findings into the formulation of some CNS tumour diagnoses. This new approach will no doubt continue to evolve. Some flexibility is maintained for institutions which do not have access to requisite molecular tests, but it is clear that the inclusion of molecular results in pathology reports should allow treatment to be planned in a more customized fashion and patient outcomes hopefully improved. Bodies funding laboratories in this new molecular era should be encouraged to provide suitable financial support for the molecular assessment of specimens, as in the long-term money would be expected to be saved by reducing financial losses resulting from inappropriate treatment. Molecular assays will need to be relatively inexpensive, sensitive, reproducible, and easily transferred laboratory to laboratory. Standardisation of techniques and interpretation will also be essential. Morphological assessment still remains central to diagnosis, and the principles of biopsy assessment i.e., meticulous correlation with the clinical history and neuroimaging findings remain unchanged.

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PEER REVIEW

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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