Value of Prognostic and Diagnostic Histological and Molecular Glioma Markers

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This review will highlight how molecular tests help improving the diagnosis of gliomas and inform about prognosis and therapeutic options. Relevant molecular markers in glioma diagnostics are chromosomal losses 1p and 19q, and mutations of the IDH and ATRX genes in oligodendroglial and astrocytic tumours of WHO grades II (low grade gliomas) and III (anaplastic or high grade gliomas). In glioblastoma, the most malignant glial brain tumour, the value of testing the methylation of the promoter of the DNA repair enzyme MGMT will be discussed. The following questions, focussing on diagnostic and predictive/prognostic values of the tests, will be addressed:

1) What is the benefit of 1p/19q and IDH molecular testing for the patient?
2) Is there a benefit to use the marker ATRX in glioma diagnostics?
3) What is the predictive and prognostic value of the MGMT promoter methylation?

Epidemiology of gliomas

Astrocytic and oligodendroglial tumours have an annual incidence of 10.1 new tumours per million population in Western countries, and glioblastomas have an incidence of 35.5 per million and that of all intrinsic brain tumours is 52.7 per million [1-3]. Extrapolated for the UK (population 63.7 million), this equates to approximately 640 new oligodendroglial and astrocytic tumours, and more than 2000 GBM annually.

IDH gene mutations

Mutations in the isocitrate dehydrogenase (IDH) 1 or 2 genes occur in approximately 75% of astrocytomas and oligodendrogliomas [4], Figure 1. The majority of the mutations occur in the IDH1 gene, mostly being the R132H mutation. Less commonly, the IDH2 gene is mutated, mostly resulting in the amino acid change R172K. IDH mutations in gliomas are thought to be early pathogenic events, and are associated with several clinically relevant parameters including patient age, histopathological diagnosis, combined 1p/19q deletion, TP53 mutation, ATRX mutation, MGMT promoter hypermethylation and patient survival [5-9]. As a consequence, these mutations are also present in so-called secondary glioblastomas, which developed in situ from pre-existing diffuse or anaplastic astrocytomas.

Testing of the IDH status is relevant for diagnostic and prognostic considerations in primary brain tumours. An antibody, specific for the IDH1 (R132H) mutation was developed in 2009 and is commercially available for diagnostic testing on paraffin sections (10). This antibody detects 90% of IDH mutations, present in 74% of astrocytic and oligodendroglial gliomas (Figure 2 [Figure 3 – IHC image]). However, all other IDH1 and all IDH2 mutations would be missed and it is recommended to test all IDH immunonegative cases (i.e. 8.2% false negatives and 26% true negatives) by sequencing [7,11]. IDH immunostaining is a simple and cost effective test, which can be implemented in all routine pathology laboratories and thus it is debated if IDH sequencing needs to be considered an essential routine test. It is suggested that selected low grade gliomas in a patient group where IDH mutation status would impact on treatment decisions, should be additionally tested. Performing these tests on DNA extracted from paraffin sections is relatively straightforward and can be done in selected referral centres.

High-grade gliomas with IDH mutations show a better prognosis [9,12], whilst the prognostic role of IDH mutations in low grade gliomas is less well established and conflicting results have been reported [13,14].

LOH 1p/19q

The combined loss of the chromosomal arms (LOH, loss of heterozygosity) 1p and 19q is a significant predictor of outcome for patients with tumours of oligodendroglial and oligoastrocytic histology. LOH 1p/19q is associated longer progression free survival and for chemotherapy response. This was reported and validated in multiple studies [15-22].

There is a transition of morphological features between oligodendrogliomas and astrocytomas, resulting in considerable interobserver variability to diagnose astrocytomas, oligoastrocytomas and

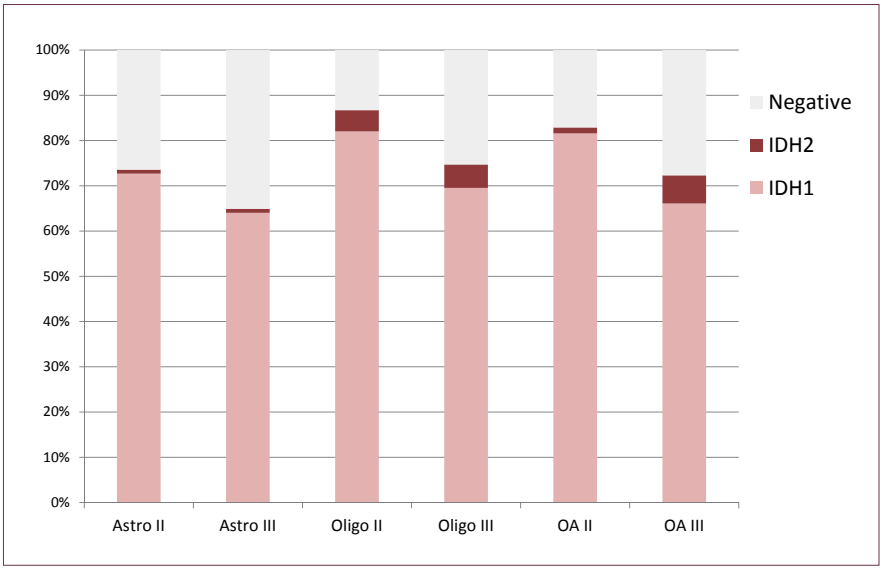

Figure 1: Frequency of IDH1 and IDH2 mutations in astrocytomas, oligoastrocytomas and oligodendrogliomas (WHO Grade II) and their anaplastic forms (WHO Grade III). The majority of IDH mutations are in the IDH1 gene, and a much smaller number in the IDH2 gene. IDH1 and 2 mutations are mutually exclusive. As a general rule the IDH mutation frequency is higher in oligodendroglial tumours than in astrocytic tumours, is higher in low grade than the respective high grade forms, and IDH 2 mutations occur more often in oligodendroglial tumours than in astrocytomas. The data are based on frequencies published in a large scale study on 1010 tumours (4). Other studies showed similar frequencies.

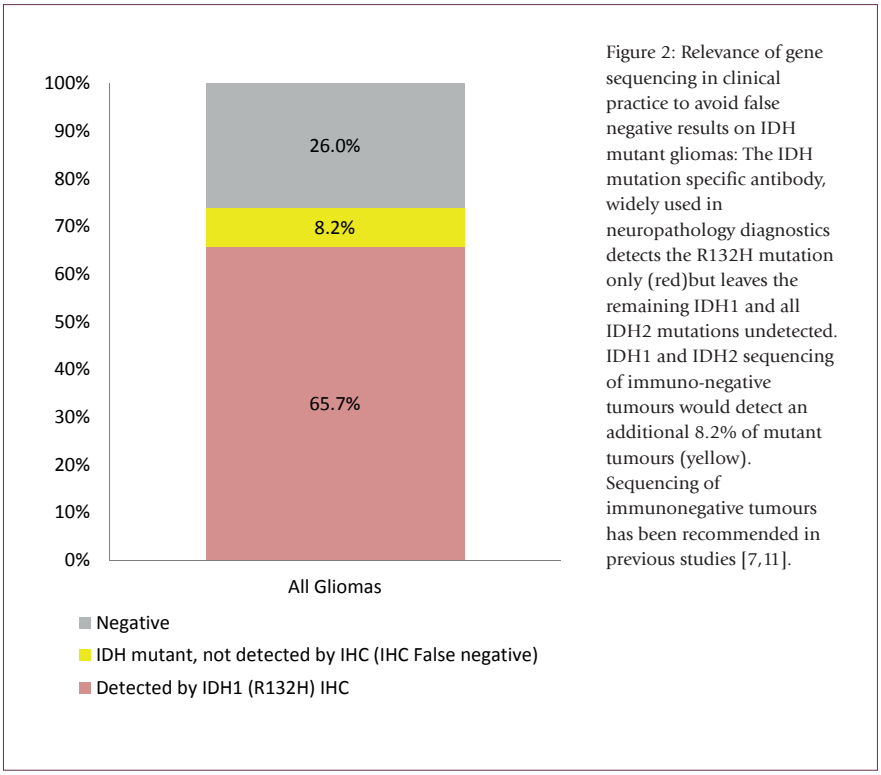

Figure 2: Relevance of gene sequencing in clinical practice to avoid false negative results on IDH mutant gliomas: The IDH mutation specific antibody, widely used in neuropathology diagnostics detects the R132H mutation only (red)but leaves the remaining IDH1 and all IDH2 mutations undetected. IDH1 and IDH2 sequencing of immuno-negative tumours would detect an additional 8.2% of mutant tumours (yellow). Sequencing of immunonegative tumours has been recommended in previous studies [7,11].

oligodendrogliomas [23,24].

In current clinical practice patients with gliomas (WHO grade II/III) carrying 1p19q co-deletions will now usually be treated with first line chemotherapy with a good clinical response. Therefore, the need for radiotherapy with its adverse side-effect can be delayed particularly in this group of patients with predicted long-term survival. In contrast, patients without 1p19q deletions will be usually offered first line radiotherapy due to the limited chance of response to chemotherapy. There is no other way of reliably determining best treatment for these patients.

Role of ATRX

The ATRX (alpha-thalassemia/mental retardation syndrome X-linked) protein [25] is an essential member of a multiprotein complex with a role in regulating chromatin remodelling, nucleosome assembly, telomere maintenance and deposition of histone H3.3 at transcriptionally silent regions of the genome. ATRX loss has been described in pancreatic neuroendocrine tumours, [26], neuroblastoma [27], and in paediatric glioblastoma [28]. More recently, a strong diagnostic and prognostic value of ATRX loss in IDH mutant gliomas has been described, in that ATRX loss occurs almost exclusively in IDH mutant tumours, and that ATRX loss and 1p/19q co-deletion are almost mutually exclusive (Figure 3). ATRX loss is a favourable prognostic marker [29] in the "biomarker cohort" of the NOA-04 clinical trial. The NOA-04 trial compared the efficacy and safety of radiotherapy versus chemotherapy with either PCV or temozolomide (TMZ) as initial therapy in patients with newly diagnosed, supratentorial anaplastic gliomas (WHO grade III) and examined the clinical relevance of 1p/19q LOH, O6-methylguanine DNA-methyltransferase (MGMT) promoter methylation, and IDH1 mutations in these tumours [30].
Based on the molecular profiles and the clinical outcome, the authors [29] suggested a stratified diagnostic algorithm to distinguish "molecular" astrocytomas, oligodendrogliomas and glioblastomas. Importantly, the positive effect of ATRX loss on survival applies to anaplastic gliomas where patient underwent chemo-radiation. Instead the histological value of the ATRX test goes beyond this limitation, as is helps eliminating the ambiguity of the rather vaguely defined group of oligoastrocytomas.

MGMT promoter methylation

MGMT (The O(6)-Methylguanine-DNA Methyl Transferase) is a DNA repair protein that reverts the naturally occurring mutagenic O6-methylguanine back to guanine. This prevents errors during DNA replication. In the context of chemotherapy with alkylating agents (e.g. temozolamide, TMZ) it removes a cytotoxic lesion, thus counteracting the chemotherapeutic effects of the drug.

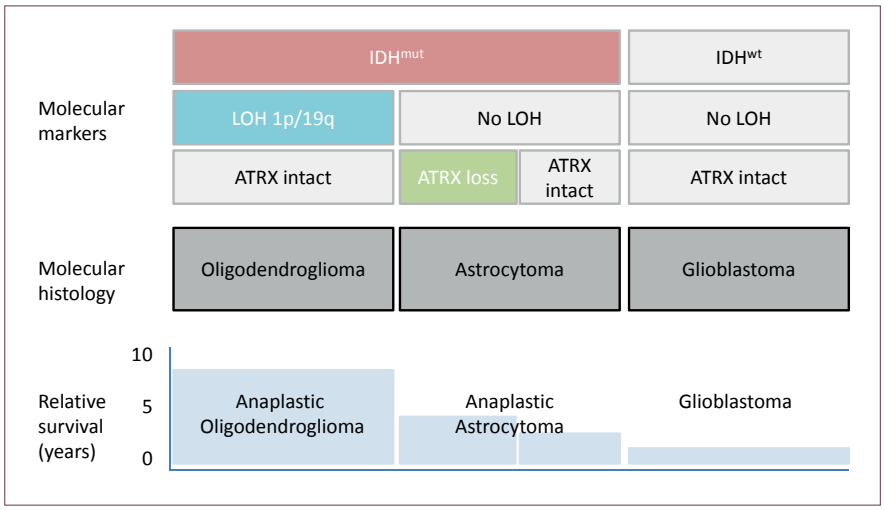

Figure 3: Simplified diagnostic algorithm for oligodendroglial and astrocytic tumours. This study is based on results of the biomarker cohort of the NOA-04 trial (29), which analysed ATRX and IDH mutations and 1p/19q status in relation to treatment response. The trial was carried out in patients with WHO Grade III tumours and the survival data in the graph refer to this cohort. These data suggest that 1p/19q LOH and ATRX mutations are almost completely mutually exclusive and in practical terms the combination of LOH1p/19q, IDH and ATRX tests allow a more accurate and reproducible histological diagnosis, eventually minimising the diagnosis of oligoastrocytomas.

Aberrant, cancer-related methylation of the MGMT promoter region leads to its silencing, a reduction of the MGMT enzyme expression and subsequently to less repair activity of DNA damage, including that induced by TMZ.

A landmark clinical trial of the effect of TMZ on newly diagnosed glioblastoma [31,32] showed that MGMT promoter methylation was an independent favourable prognostic factor. Patients with tumours with a methylated MGMT promoter had a survival benefit when treated with temozolomide and radiotherapy, compared to those who received radiotherapy only, whilst absence of MGMT promoter methylation resulted in a smaller and statistically insignificant difference in survival between the treatment groups. Further studies showed that patients with MGMT promoter-unmethylated tumours had no survival benefit from chemotherapy, regardless of whether given at diagnosis together with RT or as a salvage treatment [33,34]. Two prospective randomised trials, (NOA-08 [30] and the Nordic trial [35] concluded that MGMT promoter methylation is a useful predictive biomarker to stratify elderly glioblastoma patients for RT versus alkylating agent chemotherapy. Accordingly, these consistent trial results suggest that elderly glioblastoma patients eligible for either RT or TMZ should undergo MGMT testing prior to clinical decision making.

The MGMT status can be reliably tested by a standardised methylation-specific PCR [36]. Previous attempts to simplify the tests by detecting MGMT protein by imunohistochemistry had failed, in that it showed a poor inter-observer agreement and thus no correlation to molecular test results [37]. The use of imunohistochemistry to inform about MGMT promoter methylation status is therefore not advised.

In contrast to the promising predictive and prognostic values of the MGMT promoter methylation; there is no diagnostic value in testing for the MGMT methylation status.

Conclusion

The landscape of glioma diagnostics is rapidly changing. Large scale comparative whole genome expression [38] or methylation studies [39,40] helped identifying and biomarkers with diagnostic, predictive and prognostic value. These markers, further validated against survival and treatment responses are now gradually complementing morphological diagnosis. The greatest advantage for the clinical teams, and ultimately the patients will be an increasing accuracy and consistency of histological diagnoses. This will facilitate the clinical decision making process of adjuvant treatments, and stratification of patients to clinical trials. ●

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