General Discussion

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In 2016 the World Health Organization (WHO) classification of tumours of the central nervous system was updated.[1] This update marks a historic moment in the glioma field as testing for molecular markers became central in the diagnosis of glioma subtypes. The update altered the composition of diagnostic subtypes, which led to more accurate prognosis estimation.[2] Current treatment strategies are based on several patient factors, of which the WHO subtype is a very important one which determines the treatment intensity. Therefore, due to the changes in diagnostic groups that occurred after the WHO 2016 revision, the clinical management of especially lower-grade gliomas requires reevaluation. Also, the WHO 2016 update does not mark the end of a search for an improved classification, but marks the start of a molecular era in the neuro-oncology field wherein one of the goals is to further refine the molecular classification in order to personalize and further improve clinical management of gliomas. In this thesis we aimed to evaluate surgical management of glioma in the light of the WHO 2016 classification, and to further refine molecular diagnostics.

SURGICAL MANAGEMENT OF (PRESUMED) LOW-GRADE GLIOMAS

Despite the "low-grade" prefix, and the fact that low-grade gliomas never metastasize, it is in principle a fatal disease. Complete eradication (surgical and with systemic therapy) is barely possible due to the infiltrative growth into, and eventually destruction of, vital brain areas, as the skull is a closed and non-compliant compartment.[3, 4] The optimal surgical management of (presumed) low-grade glioma remains a matter of controversy, as surgery comes with a risk of neurological sequelae, especially in low-grade gliomas as these tumors are often located in or near eloquent areas. As no randomized trials exist related to effect of timing and extent of surgery on outcome, surgical treatment strategy varied per treatment center and was mostly depending on local opinions in the past. For a long time, the general consensus on initial treatment for low-grade glioma was a wait and scan policy. With this strategy MR scans (and in the early days CT scans), were performed at regular intervals. Hereby a lesion suspected to be a low-grade glioma was followed over time, with the intention to start active treatment once significant growth, clinical deterioration, or new contrast enhancement (taken as a sign of malignant transformation) were present. This strategy was supported by limited data from some retrospective cohort studies performed in the 1990's that did not find an association between timing of surgery and prognosis.[5-7] In the last two decades the general opinion in the field shifted and nowadays early maximal safe resection is considered standard of care in symptomatic lesions suspected

to be a low-grade glioma. There is a growing bulk of evidence that more extensive resections are associated with a longer overall survival, but the optimal timing of surgery is hardly investigated and most evidence supporting early resections remains retrospective and circumstantial.[8-13] With randomized trails considered not feasible, an influential retrospective study in Norway that was published in 2012 showed that early surgery was associated with a longer overall survival compared to a diagnostic needle biopsy followed by a wait-and-scan approach.[14] In this study the outcome of a unique situation was studied, also labeled as 'postal code randomization'. In this study treatment outcome was compared between two neurosurgical centers in two different regions in Norway with different treatment strategies. One center favored a biopsy followed by a wait-and-scan policy as initial treatment, the other favored early maximal surgical resection; patients were treated in one of these centers depending on their region of residence. The authors concluded that patients treated in the center favoring early resections had a significant longer overall survival.[14] The drawback of this study, and indeed of all studies investigating extent of resection in low grade glioma, is the patient selection that is based on histology obtained during surgery. This is not reflecting the actual clinical situation in which surgery is decided upon preoperative patient characteristics, while the histology is unknown yet. Therefore these retrospective studies are subjected so selection bias due to exclusion of patients with a suspected low-grade gliomas that turn out to be high grade gliomas, and with inclusion of patients with histologically proven low-grade glioma with preoperative enhancement on imaging that was suggestive of a high grade glioma. In our study described in **chapter 6** we tried to address this issue by approaching this clinical issue from a preoperative and more clinically relevant perspective. We retrospectively included patients with a presumed low grade glioma that was eligible for an extensive resection by selection based on imaging and preoperative clinical characteristics and not on histological diagnosis. In this manner we eliminated selection bias by histology and avoided selection bias on indication as much as possible. We investigated three different treatment strategies (wait-and scan, early resection, (needle or open) biopsy) and compared overall survival between groups. We could not confirm superiority of early resection over wait-and-scan (HR 0.92; 95% CI 0.43-2.01; P = 0.85). These data suggest that a wait-and-scan strategy can be safely proposed until some evidence of progression occurs, and that the timing of surgery does not influence the prognosis as long as the patient is monitored and treatment is initiated when necessary. An explanation for this unexpected result could be that the intrinsic biological behavior of the tumor has more impact than the timing of surgery, especially in tumors with a relatively long overall survival. The median time till intervention in the wait-and-scan group was 35.4 months, which is still relatively early in the course of the disease (median overall survival of 11.9 years in the wait-and-scan group). As is showed in **chapter 5** (of which

the results will be discussed later in this chapter), extent of resection is associated with overall survival. We did not have information on extent of resection in the study in **chapter 6**, and that is an important limitation. As low-grade gliomas grow slowly (~4mm per year), it is to be expected that total resections are more achievable when surgery is performed early in the natural course of the disease, especially in larger lesions that are not superficially located. As there is no evidence available that timing of surgery is indeed correlated with extent of resection however, it remains speculative how this might have influenced the survival curves of the cohort of **chapter 5**. There is however a plausible explanation for another observation we made: a worse outcome in the biopsy group, as in **chapter 5** we showed that the percentage of biopsies was significantly higher in patients with *IDH* wildtype low-grade gliomas compared to *IDH* mutated reflecting a difference in location of IDHwt versus IDHmt tumors (which we also show in **chapter 7**). We think it is therefore likely that the shorter overall survival observed in the biopsy group in **chapter 6** is explained by a similar higher proportion of patients with *IDH* wildtype tumors. This does not explain the poor results of the biopsy patients in the Jakola cohort. In 2017 Jakola et al. published an survival update on their 2012 cohort and now added molecular classification as well: after adjustment for molecular markers the positive effect on overall survival with early surgery persisted.[15]

In **chapter 5** we investigated the association between extent of resection and overall survival in low-grade glioma in the light of the WHO 2016 classification. We were the first to report on a relatively large series of molecularly classified low-grade glioma patients that were investigated for extent of resection with a quantitative measure. We could validate current standard of care, as we showed that postoperative tumor volume was inversely correlated with outcome irrespective of molecular subtype. The effect of even small postoperative tumor volumes was particularly strong in *IDH* mutated astrocytomas, as any residual tumor >0 cm^3 already negatively impacted overall survival. This finding argues for second-look surgeries when this is safely possible, when a small residue remains in this subtype. Similar findings were reported for anaplastic glioma, although not in a volumetric manner as in our study. Kawaguchi et al. showed that gross total resection (GTR) was associated with longer overall survival when lumping together all 3 molecular subtypes of anaplastic glioma. However, when looking at molecular subtypes individually, GTR had a positive significant impact on overall survival only in *IDH* mutated astrocytoma, and not in *IDH* wildtype astrocytoma and oligodendroglioma.[16] Another study in 2016 by Wahl et al. reported on clinical outcomes by molecular subtype of a phase II study of adjuvant chemotherapy for low-grade glioma. Although it was not the primary objective of the study, in an exploratory analysis it was found that postoperative tumor volumes were associated with outcome irrespective of molecular subtype.[17]

How to interpret the data form these studies and translate this into treatment guidelines for glioma patients? At first glance the results in **chapter 5 and 6** seem conflicting. However, we have to realize both cohorts are differently selected and try to answer different questions: first, when to resect and second, if one decided to resect how much should be resected. Uniform treatment recommendations that apply to all patients are impossible to give, but one thing is clear: a biopsy should not be part of standard care if a resection is possible. It should only be used in patients wherein a resection is not possible, but with a need for active treatment, to establish an accurate diagnosis. With **chapter 5** we provided the evidence that also for molecularly defined low-grade glioma, lower postoperative tumor volumes are associated with longer overall survival. Therefore a resection should be as extensive as safely possible in all newly diagnosed lower grade glioma patients. That introduces another important element; the question when a lesion is considered eligible for a meaningful extensive resection that is also safely possible (thus with low risk of complications). Resectability is dependent on tumor size, delineation, location, eloquency, and patient condition. Although a total resection is the ultimate aim, this is not possible in most patients, leaving the question what cut-offs for a minimal extent of resection is meaningful in terms of survival benefit. Although the results obtained in **chapter 6** do not show a survival benefit from early surgery, all other presently available data suggests that when a safe and extensive resection is possible, it should be performed early in the course of the disease, once the radiological diagnosis of a presumably low grade glioma is established. We also think this is in patients' best interest as a resection in a later stage will be technically more challenging and may be associated with a less extensive resection. However, when a meaningful resection is considered not possible and a patient has no symptoms other than well controlled seizures, a wait-and-scan period ('active surveillance') can be considered until treatment is necessary. Ideally, in the future we would have prospective data generated from a randomized trial that provides us unbiased evidence on optimal timing of surgery, as well as on the impact of extent of resection, and if so, the minimum extent of resection to aim for. However, it is unlikely that a trial like this will ever be performed and early surgery is now in general accepted as standard of care, despite the lack of prospective data.

MOLECULAR TESTING FOR GLIOMAS

Even though prognosis prediction for patients with glioma improved significantly with the WHO 2016 update, there is still variation in outcome within specific subtypes, and therefore further refinement of this classification would be valuable. Historically, the grade of glioma used to be a strong prognostic marker and grading is still part of the

WHO criteria. Grading is however purely based on microscopic features examined on hematoxylin and eosin stained slides, and currently no molecular markers are available to discriminate outcome within grade II (low-grade) and grade III (anaplastic) gliomas. In **chapter 2** we analyzed the publically available *TCGA* dataset (*The Cancer Genome Atlas*) and found that also for molecular glioma subtypes the histological tumor grade was inversely correlated with patient overall survival. We could not find in this dataset specific single molecular markers that were associated with tumor grade, but we did found that tumor grade was correlated with the mutational load (total number of genetic changes within one sample). This is an interesting observation, as it may partially explain the increased aggressiveness of gliomas with higher tumor grades. However, in the WHO 2016 classification the impact of tumor grade on prognosis seems to be more subtle and not as distinct as in the WHO 2007 classification. Reuss et al performed a study wherein they combined multiple datasets of *IDH* mutated astrocytoma (including the *TCGA* dataset) and found that both low-grade and anaplastic *IDH* mutated astrocytoma present at a similar age and that the difference in overall survival between grade II and III IDHmt astrocytoma is minimal. Partially this may be explained because *IDH* wildtype astrocytomas are now a separate entity and the differences between grades in the WHO 2007 classification were probably predominantly dependent on this subgroup.[18] In **chapter 2** we also showed that within *IDH* mutated astrocytomas, mutations in *PI3* kinase genes *PIK3CA* and *PIK3R1*, are associated with poorer prognosis, and we could confirm this finding in two independent datasets. The prevalence of these mutations in low-grade glioma patients is low however, and does not explain all variation in prognosis within subgroups. Several large efforts have aimed to identify molecular markers that correlate with tumor grade and could replace the current histological grading system. Most studies in the past focused on anaplastic glioma, with different studies showing conflicting results. For example loss of entire chromosomal arm 9p or loss of 9p21.3 region was reported as a marker of poor prognosis, mainly in grade III and grade IV glioma, but results are conflicting.[19-22] It is also unclear which region should be tested for: the entire 9p arm, the 9p21.3 region or the CDKN2A gene only. In **chapter 3** we used a targeted next generation sequencing panel to evaluate the prognostic relevance of frequently reported prognostic glioma markers in a consecutive treated series of grade II glioma. We analyzed 207 *IDH* mutated glioma samples and investigated the impact of loss of 9p21.3 and entire 9p on outcome in both oligodendroglioma and *IDH* mutated astrocytoma. In both groups of our cohort loss of 9p21.3 of entire 9p was not associated with overall survival, although a trend towards shorter overall survival was visible in grade II oligodendrogliomas. Therefore, longer follow-up and expansion of our dataset is necessary for more definitive final conclusions. We did not find homozygous deletions of CDKN2A/B in our dataset, which is in line with a recently published cohort

of grade II,III, and IV *IDH* mutated 1p19q intact tumors. The authors describing that cohort found that CDKN2A/B homozygous deletions and total number of copy number variations were strong predictors of worse outcome in a cohort of grade II,III, and IV *IDH* mutated 1p19q intact tumors. In line with our grade II cohort, they did not find CDKN2A/B homozygous deletions in WHO 2016 grade II gliomas.[23]

In **chapter 4** we showed that *IDH* wildtype low-grade gliomas, in contrast to the current WHO 2016 classification, are not a single entity, but in fact a clinical and molecular heterogeneous group of tumors. We found that *IDH* wildtype gliomas with trisomy of chromosome 7, loss of chromosome 10 (+7/-10q) and *TERTp* mutations have a dismal prognosis almost similar to glioblastoma. We also identified a group of *IDH* wildtype tumors with only a *TERTp* mutation without presence of +7/-10q. These patients even had a worse prognosis than the patients with +7/-10q. More importantly, we found *IDH* wildtype glioma patients without these molecular aberrations, and observed they had a significant better outcome. Our results were confirmed in independent studies.[24-26] This implies that more extensive molecular testing should be performed in *IDH* wildtype gliomas, in order to accurately estimate prognosis and guide treatment decisions. At least assessment of *TERT* promoter status or +7/-10q status is necessary on case of *IDH1/2* wildtype low-grade or anaplastic glioma. When these markers are absent, then further testing for at least *BRAF* and *H3F3A* mutations is necessary. Ideally, however, all makers are included in a single panel for routine diagnostics, to avoid diagnostic delay.

In **chapter 7** we aimed to correlate WHO 2016 molecular subtypes with anatomic location of grade II glioma. Several studies reported a correlation between anatomic location of gliomas and the genetic background of the tumor. [27-29]As such, tumor location might contribute to pre-surgical decision-making and non-invasive prediction of molecular diagnosis, which would be particularly helpful for lesions wherein there is doubt if it is actually a glioma or in patients wherein surgery has too high risk of morbidities but where systemic treatment is eligible. We reported on a large series of WHO 2016 classified tumors of which we created anatomic location heatmaps. Despite relevant overlap between molecular subgroups, we still found unique locations for *IDH* mutated grade II gliomas in the anterior extensions of the lateral ventricles. Interestingly, *IDH* wildtype astrocytomas were predominantly located in the midline and basal ganglia. This explains the relatively high percentage of biopsies in this group (**chapter 5**). Our data shows that location is a potentially important parameter for radiogenomics, but also emphasizes that other parameters are necessary to accurately predict molecular subtype, as there is significant overlap between groups.

In conclusion, we provide several insights that further refine the WHO classification. We suggest testing for mutations in PI3K genes to be added to clinical routine diagnostics, and to further stratify *IDH* mutated astrocytoma. Furthermore, stratifica-

tion of *IDH* wildtype gliomas is essential, as this group of tumors turns out to be very heterogeneous molecularly, and even more important, clinically (strong differences in overall survival), which has direct implications for clinical management. In **Figure 1** we summarize our findings and propose an updated diagnostic scheme. Still, not all variation in clinical outcome is explained by this scheme. To unravel this we need even larger glioma datasets than in *TCGA*, including matched primary and recurrent tumor samples to find markers of progression and of poor outcome. Just as important as sample size, or maybe even more important, these datasets need a more detailed and accurate clinical annotation than is provided in *TCGA*. Including MR imaging with standardized protocols is also important, as this might provide us with imaging correlates of molecular markers and/or prognostic subgroups. This can only be achieved with large international collaborations, as glioma is a rare disease.

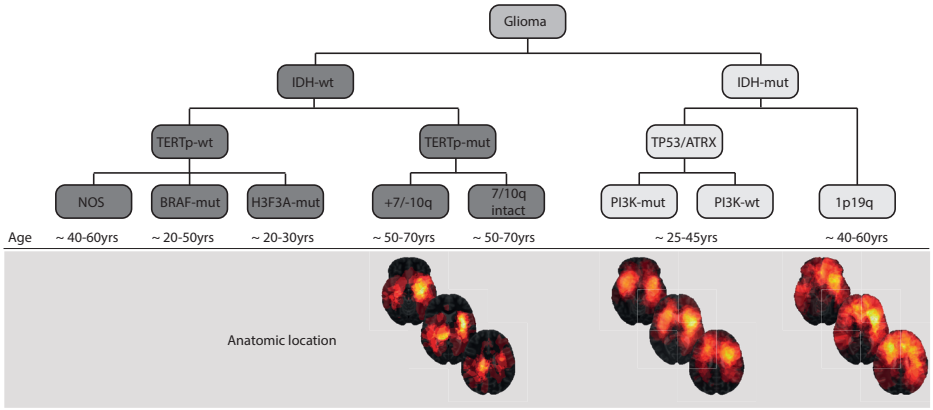

Figure 1. This figure forms a summary of our findings and proposes an updated classification scheme.

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