Aus dem Institut für Neuropathologie
(Prof. Dr. med. C. Stadelmann-Nessler)
der Medizinischen Fakultät der Universität Göttingen

From the morphological to the integrated diagnosis of diffuse gliomas – a retrospective study

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Dominik Adl Amini
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Dekan: Prof. Dr. med. W. Brück

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Betreuer: Prof. Dr. med. W. Brück...……

Ko-Betreuerin: Prof. Dr. med. D. Mielke, MBA……

Prüfungskommission

Referent/in Prof. Dr. med. W. Brück ……

Ko-Referent/in: Prof. Dr. med. D. Mielke, MBA…….

Drittreferent/in: Prof. Dr. M. Schön ……

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Hiermit erkläre ich, die Dissertation mit dem Titel "From the morphological to the integrated diagnosis of diffuse gliomas – a retrospective study" eigenständig angefertigt und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

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List of Abbreviations

CNS	Central nervous system
SEER	National Cancer Institute's Surveillance Epidemiology and End Results program
MRI	Magnetic resonance imaging
FLAIR	Fluid-attenuated inversion recovery
CT	Computer-assisted tomography
TCGA	The Caner Genome Atlas project
IDH	Isocitrate dehydrogenase
alpha-KG	Alpha-ketoglutarate
NADPH	Nicotinamide adenine dinucleotide
2-HG	2-hydroxyglutarate
G-CIMP	Glioma CpG island methylator phenotype
FUSE	Far-upstream element
FUBP	Far-upstream element binding protein
TERT	Telomerase reverse transcriptase
FISH	Fluorescence in situ hybridization
ATRX	Alpha-thalassemia/ mental retardation syndrome X-linked gene
ALT	Alternative lengthening of telomeres
MGMT	O^6-methylguanine-DNA methyltransferase
PCR	Polymerase chain reaction
p53	Tumor protein 53
EGFR	Epidermal Growth Factor Receptor
PTEN	Phosphatase and Tensin homolog
RTOG	Radiation Therapy Oncology Group
EORTC	European Organization for Research and Treatment of Cancer

1 Introduction

1.1 Classification of diffuse gliomas

1.1.1 Historical overview of CNS Tumors

The German pathologist Rudolf Virchow, who practiced at the Charité in Berlin, was the first person publishing a report about brain tumors and their classification in 1863. (Virchow 1863) After a period of more than fifty years, in 1926, Harvey Cushing and Percival Bailey introduced terms for tumor entities that are still used even now. (Bailey 1926) Their work on brain tumors also lead to displaying the first theory about the potential relationship between different types of brain tumors and the general development of the human brain. Baileys' and Cushings' concept contained the theory that brain tumors arise by dedifferentiation of mature cells or from glial or neuronal precursors that remained in a certain developmental stage. Nearly one century later, these ideas are still apparent in our modern World Health Organization classification scheme. (Huse et al. 2011)

With ongoing research, James Watson Kernohan and colleagues (1949) finalized a whole different concept of classifying brain tumors in 1949. (Kernohan and Mabon 1949) In their new concept, they drastically reduced the numbers of brain tumor entities and led the focus towards the hypothesis of tumor grading for the first time. (Banan and Hartmann 2017)

Previous attempts to establish a systemic method on classifying brain tumors with a general nomenclature such as the Union Internationale Contre le Cancer (UICC), Atlas of Histology of Brain Tumors and the Atlas of Gross Neurosurgical Pathology, failed. Astonishingly, the revolutionary AFIP (Armed Forces Institute of Pathology) Fascicle Tumors of Central Nervous System that was designed by Kernohan and Sayre (1952) did not become established in Europe. (Kernohan and Sayre 1952)

In 1952, the WHO Expert Committee on Health Statistics predefined general principles on classifying human tumors, which was strongly influenced by the Armed Forces Institute of Pathology (AFIP). In these general principles, there were three columns such as histologic tumor type, consideration of anatomic site and the degree or grade of malignancy, to assure ease and flexibility. (Scheithauer 2009)

In 1979, based on the terminology of Cushing and Bailey from 1926, combined with the concept of grading brain tumors according to Kernohan from 1949, the first WHO-based classification of brain tumors was published by Zülch and colleagues. (Bailey 1926; Banan and Hartmann 2017; Johnson et al. 2017; Zülch et al. 1979) Other researchers like Catherine Daumas-Duport and Bernd Scheithauer published an alternative grading system, known as the St Anne-Mayo grading scheme in 1988 that reached a definite amount of acceptance, but never enough to overcome the first WHO classification. (Daumas-Duport et al. 1988) Within the following years the first WHO Classification has been developed continuously leading to the second edition in 1993, a third edition in the year 2000 followed by a fourth edition that was released in 2007 and updated in 2016. (Banan and Hartmann 2017)

Studies, made within the past two decades, have described several genetic bases of tumorigenesis in most entities of brain tumors whether common or relatively rare and therefore pointing out that these findings may contribute to reclassifying tumors of the CNS. (Louis 2012) It is to mention that some of these genetic alterations have been known back in 2007 but were not considered significant enough for including them in the CNS WHO Classification, rather they were considered providing prognostic and predictive data within the categories based on conventional histology. (Louis et al. 2016)

Nevertheless, a meeting under the auspices of the International Society of Neuropathology held in Haarlem, the Netherlands in 2014 regarding a major revision of the 2007 CNS WHO Classification, established guidelines on how to incorporate the genetic alterations and molecular findings into brain tumor

diagnosis. As a result of that meeting, the most recent update on CNS Classification, called the 2016 World Health Organization Classification of brain tumors, breaks down the old principle of diagnosis based entirely on microscopy and integrates molecular parameters into the classification of CNS tumor entities. (Louis et al. 2016)

1.1.2 The 2016 WHO Classification of CNS Tumors focused on diffuse gliomas (Haarlem Consensus)

Just like in many other neoplasms, in the past years, there have been major achievements in the understanding of diffuse gliomas and therefore calling into question whether the traditional grading, using exclusively morphological criteria for classifying glial tumors, like in the WHO 2007 classification, is still up to date. (Louis 2012)

Brain tumors, especially diffuse gliomas, have been part of the molecular revolution due to greater insights into molecular basis of human tumors regarding (1) biological understanding of neoplasm, (2) the ability to diagnose tumors and estimate their prognosis, (3) as well as predicting response to specific therapies. (Louis 2012) On top of that a periodic revision of tumor classifications has huge impact on our individual and population health. (Louis et al. 2014) Consequently, the Word Health Organization has supported consistently new assessments on the classification of tumors. That also accounts for brain tumors and their previous WHO 2007 Classification, which included over 100 entities, many without molecular characteristics.

In order to review and perhaps renew the above-mentioned WHO 2007 Classification, a meeting titled “WHO’s Next?: A Colloquium to Guide Next Steps in Brain Tumor Classification and Grading” was held from May 1st through May 3rd, 2014 in Haarlem, the Netherlands. (Louis et al. 2014) The meeting was enabled with

support from the STOPbraintumors Foundation (the Netherlands) and sponsored by the International Society of Neuropathology. (Louis et al. 2014)

Prior to the meeting, every single one of the twenty-seven participating neuropathologists from ten different countries, were recommended to survey their colleagues on how to improve classification of brain tumors. As a result, information from over 150 neurooncological specialists including neurooncologists, neurosurgeons, neuropathologists, medical oncologists, neuroradiologists and radiation oncologists, were carried together, building a solid foundation for discussing several new approaches. (Louis et al. 2014)

The debates were mostly focused on diffuse gliomas and embryonal tumors, which showed the greatest progress in decrypting molecular aberrations in the past years and hence illustrate on how molecular information could be incorporated in a diagnosis. (Louis et al. 2014) The major question that had to be challenged was how non-histological criteria can be used to enhance typing and grading of human brain tumors and how to incorporate these non-histological data into a new WHO Classification. (Louis et al. 2014; Banan and Hartmann 2017)

1.1.2.1 General principles

To battle the above-mentioned question, the specialists present agreed on the usage of an “Integrated Diagnosis” just like hematopathologists handled similar questions in their field as explained by Daphne de Jong in her opening speech at the meeting (Louis et al. 2014; Louis et al. 2016) The usage of that integrated diagnosis, including phenotypic and genotypic parameters, adds a greater level of objectivity that has not been there in the past.

Due to the more objective way of classifying, more homogeneous and narrowly defined entities will be produced and therefore lead to way higher accuracy in diagnosing different diffuse gliomas. Additionally, patients will benefit with a more

accurate determination of prognosis and treatment as well as an improved patient management. (Louis et al. 2016) Moreover, researchers can use the more exact defined groups and focus on further prognostic and diagnostic markers, which will help shifting the way of diagnosing diffuse gliomas towards an even more molecular, therefore more objective and less histological, way. (Louis et al. 2016)

More narrowly outlined groups will also increase the risk of creating groups including tumors that do not fit into any entity. These tumors will now be included in a so-called not otherwise specified (= NOS) group. The WHO 2016 Classification recommends, that the NOS group should only be used with the absence of diagnostic molecular testing or in the rare instance of dual genotype. (Louis et al. 2016) It is hoped that in the future, scientists will be able to further classify the NOS group and therefore establish newer entities of diffuse gliomas.

As an example, the diagnosis of oligoastrocytoma that has always been difficult to define on morphological grounds and therefore had a great interobserver discordance, will now be either pushed towards the diagnosis of astrocytoma or oligodendroglioma based on the more precise classification using both phenotype and genotype. (Giannini et al. 2001; Van Den Bent 2010) Only rare cases of molecularly defined, true oligoastrocytomas will remain within the subtype of “Oligoastrocytoma, NOS”. Therefore more homogeneous groups of the more common subtypes of astrocytomas and oligodendrogliomas are defined. (Huse et al. 2015; Wilcox et al. 2015; Louis et al. 2016)

Another general principal that was set, is that genotype trumps the histological phenotype. (Louis et al. 2016) Using genotype and phenotype can result in contradictory results, meaning that a diffuse glioma, that histologically appears to be astrocytic but shows IDH mutation and 1p19q codeletion, cannot be diagnosed as an astrocytoma because the molecular genetic features supersede the light microscopic features. (Louis et al. 2016)

In the future, it might be possible to make a diagnosis without any histology but as of yet, a histological diagnosis of a diffuse glioma still has to be made. Besides, the WHO grade is still based on the histology and on top of that, there are still tumors that do not fit any of the narrowly defined groups, however a diagnosis still has to be made.

1.1.2.2 Meeting Conclusions

At the end of the main meeting in Haarlem and after a second meeting in Heidelberg, in which the consensus was finally decided, all present specialists reached agreement of a broad set of conclusions for future classifications of nervous system tumors. (Louis et al. 2014; Komori 2017) Some of them can now be found in the newest WHO Classification. Nevertheless, there are still many questions unacknowledged such as recommendations about individual entities. (Louis et al. 2014)

As mentioned above, the major question of the meeting was how to incorporate non-histological information such as molecular findings, imaging, clinical information etc. into a diagnosis and how to use these data to enhance typing and grading of human brain tumors. To do so, the experts, taking part in the 2014 meeting, agreed on the following conclusions:

> Tumor entities should be defined as accurately as possible to establish biologically and clinically highly uniform groups. As already stated, that can lead to tumors which will not fit into one specific category and can be seen as “grey zones”. These need further analysis and further research in order to be placed in their exact position of the classification. (Louis et al. 2014)
>
> The definition of some diagnostic entities such as astrocytomas and oligodendrogliomas should include molecular information. To be more

precise, the molecular information is required in order to provide a fully integrated diagnosis with all layers {see 3) and chapter Nomenclature} for these entities and only in the case of no availability for molecular testing, it is allowed to make a diagnosis based on only descriptive histological data. (Louis et al. 2014) For other entities, histological criteria alone will remain the basis of the diagnosis or in case of no molecular testing, the formal NOS (not otherwise specified) can be applied. To do so, it is a recommendation to refine the definition of some tumor entities and add information to the definition of other entities. (Louis et al. 2014) However, the specialists did not give any advice on how the molecular tests have to be carried out. It is in the hand of the pathologist/treatment team to choose whether FISH, MPLA, arrayCGH or other available technologies within the given facility are used to get molecular information. (Banan and Hartmann 2017)

The general concept of the new classification is that the diagnosis should be “layered”. On top of that, the usage of a layered diagnosis simplifies standardization of diagnosis. (Louis et al. 2014) Further details of the layered diagnosis are explained in the next chapter.

Concerning molecular testing, the experts agreed on the fact that such molecular tests have to be based on histological evidence of the tumor, thus, after histological confirmation of a tumor entity by a pathologist. Regarding the report and documentation of the tumor, it is important to annotate that a molecular test was ordered and is still in progress or if the test was not performed. The latter should give a short explanation about the reason for not performing. Additionally, methods and results of the tests should be included in reports providing a better multi-institutional patient care. (Louis et al. 2014)

> The present specialists also agreed on using the current WHO standard of grading astrocytomas and oligodendrogliomas. Only in the four circumstances of (1) the tumor being not clearly astrocytic nor oligodendroglial, (2) a biopsy that is too small to carry out molecular testing, (3) a discrepancy in the results of molecular test and morphology as well as (4) molecular pattern which does not fit into one specific tumor entity, the WHO grade may be left off completely or may be defined as low or high grade. The term “anaplastic” can be used for any astrocytic or oligodendroglial tumor of grade III, whereas the word “glioblastoma” may only be used for astrocytic neoplasm Grad IV. (Louis et al. 2014)
>
> Information that is not-tissue-based such as radiological or clinical data is not mandatory for the layered diagnosis but if these informations lead to a greater advantage in reaching a solid final diagnosis, they can be added in a comment section within the diagnosis. (Louis et al. 2014)

1.1.2.3 Nomenclature

With a new concept of classifying tumors, a new practical and standardized nomenclature needed to be worked out. (Louis et al. 2014) Since the concept of the 2016 edition of the WHO brain tumor classification is based on the way hematopoietic/lymphoid pathology renewed their classification, it is more than reasonable to also apply their nomenclature system.

The skeleton of the “Integrated Diagnosis” is the principle of a “layered diagnosis” which has 4 different layers that all need to be filled out in order to make a complete diagnosis. Layer 1 represents the final “integrated diagnosis” which can only be used if all lower layers (Layer 2 to 4) are available, meaning layer 1 can be seen as a summary of all lower layers. Layer 2 represents the histological classification such

as oligodendroglioma or astrocytoma and layer 3 illustrates the WHO grade of the neoplasm (Grade I, II, III, IV). In previous classification, layer 2 and 3 alone would have been enough to make a diagnosis. (Johnson et al. 2017) With the new 2016 Classification layer 4, as the lowest layer, gives information about the specific molecular characteristics such as IDH-mutant, 1p/19q-codeleted. That layer 4 now has an enormous importance in making a diagnosis (Figures 1 and 2). (Louis et al. 2014)

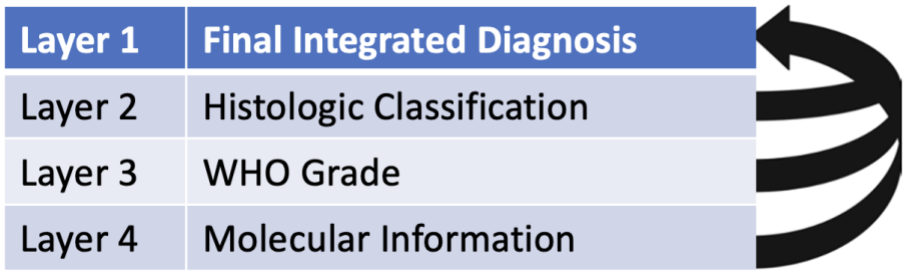

Figure 1 Layered diagnosis of CNS tumors according to the 2016 WHO Classification

Using such layered diagnosis leads to the question of what happens if one layer cannot be filled out for example due to lack of availability of molecular data. In this case the pathologist is allowed to generate a diagnosis based on only layer 2 and 3 as it was in the 2007 WHO Classification, however, the lack of layer 1 indicates that the diagnosis is not fully completed in the concept of a layered diagnosis. (Banan and Hartmann 2017)

As explained above, not only the basic structure of the international standardized nomenclature for CNS tumors was defined, the specialists also agreed on homogeneous directives on how to format the integrated diagnosis. In this way, (1) the histopathological name (layer 2) is followed by (2) the genetic features. These 2

main parts are separated with a comma e.g. diffuse astrocytoma, IDH-mutant. (Louis et al. 2016)

In the case of entities having more than one genetic determinant, all of those need to be listed e.g. oligodendroglioma, IDH-mutant and 1p/19q-codeleted, whereas tumors with an aberration of genetic mutation, the term “wildtype” for example “diffuse astrocytoma IDH-wildtype” can be used. At that point it is important to mention, that most likely those tumors lacking specific genetic information will be pushed towards the diagnosis “NOS” (not otherwise specified) rather than using the term of wildtype. (Louis et al. 2016)

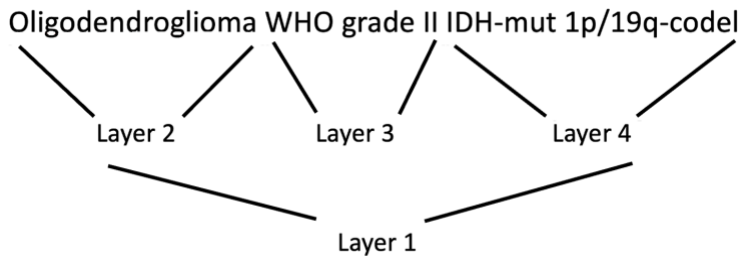

Figure 2 Syntactical structure of the diagnostic phrase of an oligodendroglioma

1.2 Necessary molecular biomarkers and genetic determinants

In the new 2016 WHO Classification two characteristics need to be assessed in order to make a diagnosis of low-grade diffuse glioma following the integrated diagnosis. These are the isocitrate dehydrogenase (IDH) status and the 1p/19q chromosomal deletion. (Louis et al. 2016) Other molecular markers and genetic determinants can help making a diagnosis and guide the pathologist towards the right entity of glioma but are not explicitly required. (Louis et al. 2016)

1.2.1 Isocitrate dehydrogenase (IDH)

With the new way of classifying gliomas and the new diagnostic approach, IDH became one of the most important molecular markers for diffuse gliomas. Furthermore, IDH functions as a possible prognostic marker. (Louis et al. 2016)

In 2008, The Cancer Genome Atlas (TCGA) project, that evaluated glioblastomas, discovered new mutations in the cytosolic NADPH-dependent isocitrate dehydrogenase 1 (IDH1) gene. (Wesseling et al. 2011) This mutation was located in the R132 position of the gene positioned at chromosome locus 2p33 and was observed in 12% of glioblastoma patients. Additionally, it was enhanced in patients with secondary glioblastomas. During further analysis, it was shown that nearly all mutations were heterozygous somatic point mutations where single nucleotides changed at codon 132 for IDH1 and codon 172 for isocitrate dehydrogenase 1 (IDH2). In cases of IDH1, in about 90%, a substitution from arginine to histidine was found leading to amino acid change compared to the wild-type. (Labussiere et al. 2010a; Siegal 2015)

Soon after the initial study with glioblastomas, further studies revealed that the cytosolic IDH1 mutation, and less frequent the mitochondrial IDH2 mutation are present in a vast majority of diffuse low grade gliomas, involving approximately 80% of grade II and grade III gliomas. (Wesseling et al. 2011; Cohen et al. 2013)

Looking at the function of IDH it is to say that the normal function of the IDH1 enzyme is to catalyze the oxidative decarboxylation of isocitrate into alpha-ketoglutarate (alpha-KG) and nicotinamide adenine dinucleotide (NADPH) in the cytosol, whereas the IDH2 enzyme carries out the same reaction in mitochondria. With a mutation in the IDH1 or IDH2 genes, these enzymes become unable to produce alpha-KG as mentioned above and alternately convert isocitrate to 2-hydroxyglutarate (2-HG). This 2-HG is recognized as a potential oncometabolite and accumulates substantially in gliomas. (Cohen et al. 2013; Siegal 2015)

Several studies suggest that IDH1 mutations are considered early events in the development of gliomas and also play a unique role in pathogenesis. IDH1 mutations can be found in a high percentage of low-grade diffuse gliomas meaning in about 71% of grade II and 64% of grade III. Regarding glioblastomas, IDH1 mutations are rarely detected in primary glioblastomas (6%) but it is interesting to see that secondary glioblastomas, that derived from low grade gliomas, show a 71% mutation rate. IDH2 mutations have been found in less that 3% of gliomas and therefore do not play a huge role in first line diagnostics, but in the case of a negative IDH1 status, IDH2 has to be investigated. (Labussiere et al. 2010a; Cohen et al. 2013)

Up to now, three pathogenetic pathways can be differentiated based on the IDH mutation status in gliomas. (Cohen et al. 2013) The first one starts with an IDH mutation followed by Tumor protein 53 (TP53) mutation and is considered to lead to an astrocytic tumor. These tumors start as WHO grade II astrocytomas, show the evolution of glioma CpG island methylator phenotype (G-CIMP) and then progress into high grade tumors, as for instance, secondary glioblastomas. (Cohen et al. 2013) The second pathway also starts with the mutation of IDH and is then followed by codeletion of 1p and 19q and consequently results in the development of grade II oligodendrogliomas. These can then gain other genetic alterations and become an anaplastic oligodendroglioma. (Cohen et al. 2013) Oligodendrogliomas rarely demonstrate TP53 mutations, therefore the differentiation between oligodendrogliomas and astrocytomas is possible using p53 and 1p19q testing. The third pathway includes gliomas with non-mutated, or wild-type *IDH* genes. These tumors develop very rapidly and gain multiple complex genetic alterations such as amplification of Epidermal Growth Factor Receptor (EGFR) and loss of the Phosphatase and Tensin homolog (PTEN) gene. As a result, they often become glioblastomas in a very early stage of their differentiation. (Cohen et al. 2013)

IDH mutations are favorable prognostic markers in adult low grade, as well as high grade gliomas. Studies have shown that tumors with WHO grade II, WHO grade III together with secondary glioblastoma that carry IDH mutation have a better prognosis than tumors of the same grade with IDH wild-type. (Yan et al. 2009; Siegal 2015) Additionally, patients with mutated *IDH* are significantly younger than people with IDH wild-type gliomas across all grades. Important to note is that in pediatric gliomas IDH mutations are rare. (Cohen et al. 2013; Siegal 2015) Moreover, prospective analyses displayed that patients with *IDH* + gliomas grade II to IV had significantly longer overall survival than patients without IDH mutation. Radical surgical resection beyond tumor margins resulted in a survival benefit only in the case of IDH1 mutation. (Cohen et al. 2013; Siegal 2015) Interestingly, prognostic importance of IDH mutation is independent of other prognostic factors such as the patients age, WHO grade of the tumor and 0^6-methylguanine-DNA methyltransferase methylation (MGMT) status. (Cohen et al. 2013)

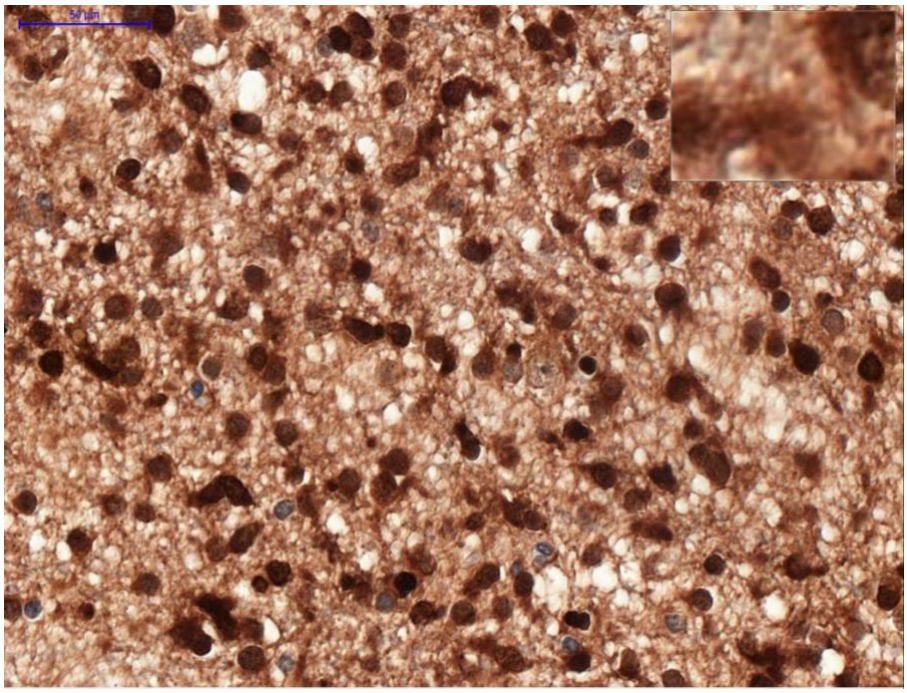

Figure 3 Example of a positive (= brown nuclei) IDH1 immunohistochemistry

The testing for IDH is performed on tissue samples and can be done either with immunohistochemistry or by sequencing. Sequencing is considered as the gold standard, whereas immunohistochemistry is used as the routine diagnostic approach because it is simple and less costly. (Cohen et al. 2013) Therefore, monoclonal antibodies directed against the product of the most frequently occurring IDH1 mutation (IDH1-R132H) were created. This isoform represents 85 up to more than 90% of all IDH mutations in gliomas and consequently, immunohistochemistry is used in routine diagnostics. Sequencing on the other hand is used in the case of a negative IDH mutation status with immunohistochemistry in order to detect the infrequent mutations. (Wesseling et al. 2011; Siegal 2015) Up till now, non-invasive methods using imaging to detect tumors with IDH1 mutation are under development. In the future, it is hoped that the abnormal accumulation of the oncometabolite 2-HG can be detected by magnet resonance spectroscopy. (Siegal 2015)

1.2.2 1p19q codeletion

In addition to IDH mutations, 1p19q chromosomal codeletion is another genetic abnormality that is necessary to test for, in order to make a diagnosis in low grade diffuse gliomas.

1p19q codeletion is based on an unbalanced translocation between the chromosome arms 1p (short arm of chromosome 1) and 19q (long arm of chromosome 19), resulting in the loss of the derivative chromosome 19 and thereby in loss of heterozygosity. (Bromberg and van den Bent 2009; Labussiere et al. 2010b; Capper and Reifenberger 2015; Siegal 2015)

This genetic abnormality is present in the majority (up to 60 – 80%) of oligodendrogliomas and shows association with classic histological appearance just like perinuclear halos and chicken-wire vascular pattern. (Bromberg and van den

Bent 2009; Kim et al. 2010; Labussiere et al. 2010b; Wesseling et al. 2011; Goodenberger and Jenkins 2012; Theeler et al. 2012; Capper and Reifenberger 2015) Due to the fact that oligodendrogliomas, just like other diffuse gliomas, are difficult to reproducibly diagnose morphologically, 1p19q codeletion is considered as the most objective molecular definition of the oligodendroglial lineage. (Theeler et al. 2012) According to the new WHO 2016 Classification, tumors that lack 1p19q codeletion cannot be described as oligodendrogliomas and are therefore considered astrocytic. (Theeler et al. 2012)

Just like IDH1 mutations, 1p19q codeletion is also considered as an early genetic event in oligodendroglial tumorigenesis and can occur with other molecular findings. (Labussiere et al. 2010b) Gliomas with 1p19q codeletion nearly always have mutations in the homolog of the Drosophila gene capicua (CIC)-Gene on Chromosome 19 and partially mutations of the far-upstream element (FUSE) binding protein (FUBP)1-gene on chromosome 1p. (Goodenberger and Jenkins 2012; Capper and Reifenberger 2015) 1p19q codeletion is only seen in IDH mutated gliomas and is associated with activating mutations in the telomerase reverse transcriptase (TERT)-promoter. (Goodenberger and Jenkins 2012; Capper and Reifenberger 2015) Furthermore, it is to mention that 1p19q codeletion is mutually exclusive with TP53 mutation and EGFR amplification but is associated with MGMT promoter methylation, implicating that MGMT promoter hypermethylation and methylated CpG sites were seen more frequently in 1p19q codeleted than in intact tumors. (Labussiere et al. 2010b)

Looking at the prognostic and predictive nature of this marker it is to say that patients with 1p19q codeleted tumors have a better prognosis than patients with tumors of the same grade that are not codeleted. This might be due to a better response to genotoxic stress in cytotoxic therapies. (Senetta et al. 2013; Capper and Reifenberger 2015; Siegal 2015) Long-term follow up data from both the Radiation Therapy Oncology Group (RTOG) and the European Organization for Research and

Treatment of Cancer (EORTC) displayed that 1p19q codeleted tumors have better outcome with Procarbazine, Lomustine and Vincristine (PCV) chemotherapy. (Labussiere et al. 2010b; Theeler et al. 2012) Moreover it was demonstrated that the codeletion shows better outcome when treated with radiotherapy. (Labussiere et al. 2010)

The median survival of patients with 1p19q codeletion doubles when the above-mentioned treatment is carried out in comparison to non-codeleted tumors. To be more precise, the median survival with 1p19q codeletion is 12 – 15 years in low grade oligodendrogliomas and more than 7 years in anaplastic oligodendrogliomas whereas its only 5 – 8 years and 2 – 3 years in cases when there is no codeletion, respectively. (Labussiere et al. 2010)

The most widely used technique for investigating 1p19q codeletion is fluorescence in situ hybridization (FISH) followed by polymerase chain reaction based microsatellite analysis that displays the loss of heterozygosity. (Senetta et al. 2013; Siegal 2015) The problem with FISH is that currently there is no standardization for defining 1p19q status. (Senetta et al. 2013) Additionally, FISH probes used in assessing 1p loss are often located on the 1p36.6 position and therefore also react positive if a deletion occurs involving that region. (Bromberg and van den Bent 2009) This may lead to a false positive 1p19q result. In order to prevent that, other techniques such as genomic arrays like array comparative genomic hybridization or single nucleotide polymorphism array as well as chromosome arm painting should be preferred in the future. (Labussiere et al. 2010) Important to mention is also when to look for the codeletion. Two rules should be followed. Tumors without *IDH* mutation don’t have to be checked for 1p19q and the testing for 1p19q is not only recommended for histological appearing oligodendrogliomas, but for every *IDH* mutated glioma that does not show a loss of nuclear ATRX expression. (Capper and Reifenberger 2015)

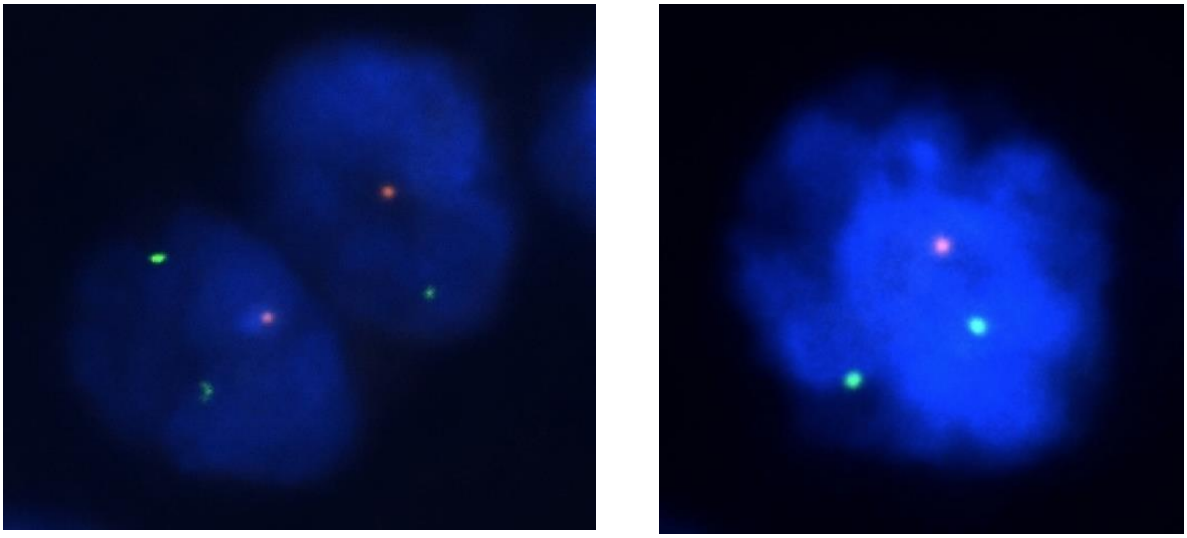

Figure 4 Characteristic fluorescence in situ hybridization (FISH) findings in an oligodendroglioma; Left: 1p deletion, Right: 19q delection = loss of red signal; combined known as 1p19q codeletion

1.3 Diagnostic procedure of the different types of low grade diffuse gliomas

In the previous WHO 2007 classification for brain tumors, the gold standard for diagnosis was the histology and the morphological appearance of tumor cells and their correspondence to regular brain cells. (Van Den Bent et al. 2017; Van Den Bent et al. 2018) Due to a high inter- and intraobserver variability and the different clinical outcome of allegedly comparable tumors, this classification left many pathologist unsatisfied. As mentioned in the beginning, with the new WHO 2016 classification, the focus is no longer on the histology alone but because of great development and research, on genetics. (Van Den Bent et al. 2018) Therefore, all diffuse infiltrating gliomas, whether of astrocytic or oligodendroglial origin, are now grouped together while in the past, astrocytic and oligodendroglial tumors were strictly seen separately. (Louis et al. 2016)

1.3.1 Astrocytoma

In the new WHO 2016 classification the following entities can be found:

Diffuse astrocytoma WHO grade II, IDH mutant

Diffuse astrocytoma WHO grade II, IDH wild type

Diffuse astrocytoma WHO grade II, NOS

Anaplastic astrocytoma WHO grade III, IDH mutant

Anaplastic astrocytoma WHO grade III, IDH wild type

Anaplastic astrocytoma WHO grade III, NOS

Diffuse astrocytomas can be found in the CNS and as the name suggests, these tumors infiltrate widely throughout the parenchyma of the brain. They can mostly be found in the frontal or parietal lobe and the lesions are often harbored in the subcortical white matter. Infiltration by these tumors infest the cerebral cortex, deep grey structures and the contralateral hemisphere. (Brat and Perry 2010; Van Den Bent et al. 2018)

Looking at the radiological features, magnetic resonance imaging (MRI) is the most helpful diagnostic tool. Diffuse astrocytoma and anaplastic astrocytomas show ill-defined, deep-seated or predominantly subcortical cerebral hemispheric masses. In anaplastic astrocytomas, faint, punctate or irregular contrast enhancement can additionally be found. (Brat and Perry 2010) In contrast to oligodendrogliomas, astrocytomas typically do not calcify, don’t involve the cortex and have a distinct border. (Van Den Bent et al. 2018) Perfusion MRI scans have a high accuracy (>90%) in distinguishing high grade from low grade astrocytomas. In those scans, the relative blood volume can be demonstrated which is increased in high grade

astrocytomas. (Van Den Bent et al. 2018) Midline shift, ventricular compression and sulcal effacement represent secondary signs of mass effect. (Brat and Perry 2010)

The first step of the diagnostic algorithm in the WHO 2016 classification remains assessing the histology. Observing the histopathology of astrocytomas, elongate, irregular, hyperchromatic nuclei often without recognizable cytoplasm, so called naked nuclei, can be found. (Brat and Perry 2010) In order to document infiltration, recognition of the low to high cellularity gradient from regions of non-neoplastic brain tissue to regions of the tumor may help. In the case of high density of tumor cells in the biopsy material, infiltration might be difficult to assess due to the adumbration of the background CNS microarchitecture. (Brat and Perry 2010) Then immunohistochemistry or staining with silver can highlight the axons and therefore help the observer. Diffuse astrocytomas WHO grade II have modestly increased cellularity, the nuclei are enlarged, have an oblong shape, are hyperchromatic and have irregular contours compared with normal astrocytes. Moreover, microcyst formation, distortion of CNS parenchyma because of edematous splaying of neuropil, as well as occasional microcalcification can be seen. (Brat and Perry 2010) Anaplastic astrocytoma WHO grade III represents the transition from grade II infiltrative tumors to glioblastomas and therefore shows an increased cellularity, a presence of mitotic activity and a higher degree of nuclear atypia than diffuse astrocytoma. (Brat and Perry 2010)

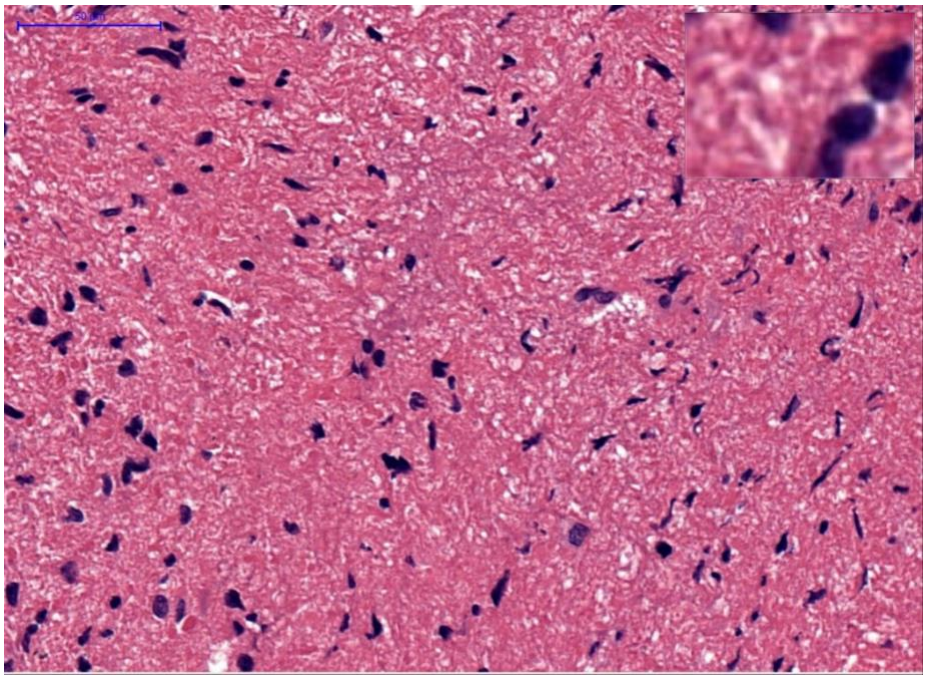

Figure 5 Typical characteristics of a diffuse astrocytoma WHO GRADE II: elongate, irregular, hyperchromatic nuclei.

After the radiological and histopathological features suggested the diagnosis of diffuse glioma, molecular markers help the pathologist towards the final diagnosis. Diffuse astrocytoma WHO grade II and anaplastic astrocytoma WHO grade III can now be divided into three groups: IDH mutant, IDH wild type and the NOS (not otherwise specified). (Louis et al. 2016) This means that those two entities are defined by the presence, like in most cases, or absence of IDH mutation combined with intact 1p and 19q chromosomes. (Oberheim Bush and Chang 2016; Banan and Hartmann 2017) Furthermore, in nearly all cases (95%) TP53 alterations and (70-90%) ATRX mutations can be found in astrocytomas. (Oberheim Bush and Chang 2016; Banan and Hartmann 2017; Van Den Bent et al. 2018) In the case of a negative IDH1 R132H immunohistochemistry, sequencing for IDH1 codon 132 and IDH2 codon 172 gene has to be performed. Only in the rare case of both being negative, the lesion can be diagnosed as IDH wild type. (Louis et al. 2016; Banan and Hartmann 2017) Important to note is that the diagnosis diffuse astrocytoma IDH

wild type or anaplastic astrocytoma IDH wild type are uncommon diagnoses and their biologic characteristics remain unclear, even though some studies presume that there might be no difference between IDH mutated and wild type tumors. (Louis et al. 2016; Banan and Hartmann 2017) In those IDH wild type tumors, other mutations like those involving the EGFR and PTEN gene as well as polysomy of chromosome 7, loss of heterozygosity of chromosome 10p and TERTp mutations can be found. Lastly, if IDH testing cannot be performed or is not available, the diagnosis will be diffuse or anaplastic astrocytoma, NOS. (Louis et al. 2016; Banan and Hartmann 2017)

1.3.2 Oligodendroglioma

For oligodendrogliomas the following entities can be found:

Oligodendroglioma WHO grade II, IDH mutant and 1p19q codeleted

Oligodendroglioma WHO grade II, NOS

Anaplastic oligodendroglioma WHO grade III, IDH mutant and 1p19q codeleted

Anaplastic oligodendroglioma WHO grade III, NOS

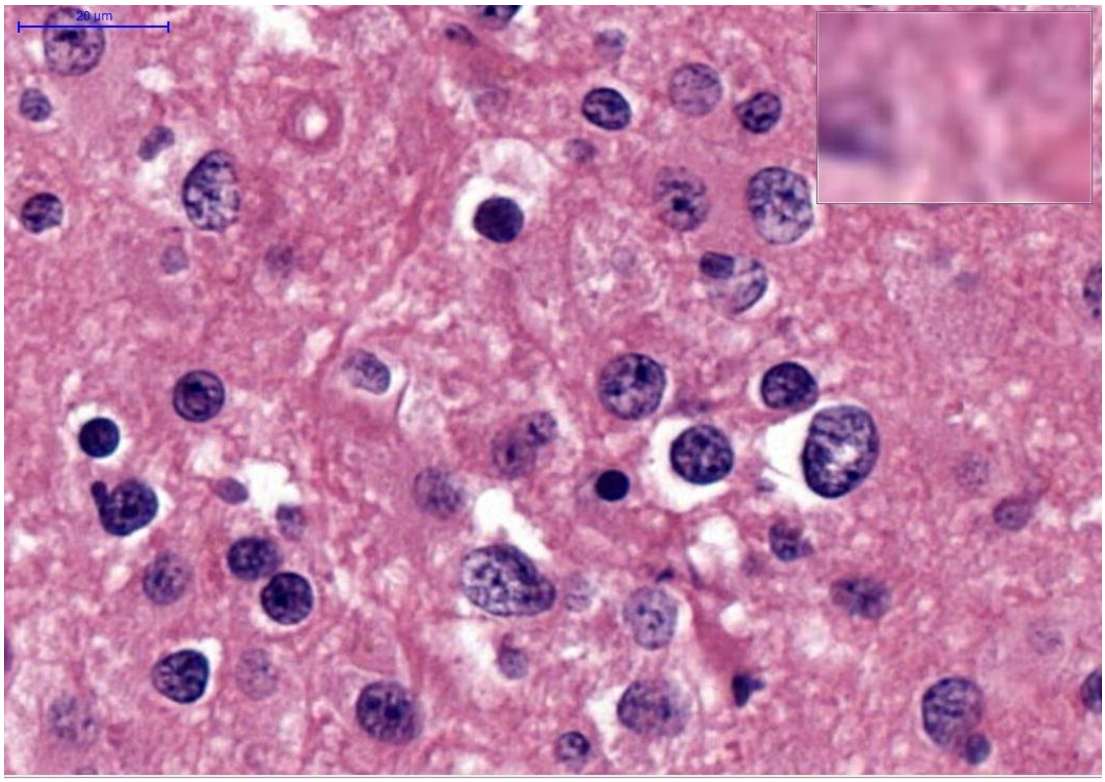

Figure 6 Typical characteristics of an oligodendroglioma: (A) round, regular, monotonous nuclei, (B) sharply defined nuclear membranes, (C) modest cell to cell variability, (D) Fixation artefact "fried egg and woody plant"

Oligodendrogliomas are masses in the cerebral hemispheres that most of the time involve the frontal lobe, followed by the parietal and temporal lobe. Often they are centered superficially in the brain, have cortical involvement and show indistinct borders of the tumor. (Brat and Perry 2010; Van Den Bent et al. 2018)

Radiologically grade II and grade III show different signs. Low grade oligodendrogliomas (grade II) are non-enhancing intra-axial masses with hypointense T1-weighted MRI scans but show hyperintensity on T2-weighted and FLAIR MRI sequences. Anaplastic oligodendrogliomas (grade III) in comparison are nearly always contrast enhancing. (Brat and Perry 2010) The hallmark feature of

oligodendrogliomas in general are the presence of calcification that is best appreciated on computed tomography (CT) and follow the pattern of an expanded cortical ribbon, indistinct borders and the above mentioned heterogeneous signal intensity on T2-weighted MRI scans. (Brat and Perry 2010; Van Den Bent et al. 2018) After contrast administration, oligodendrogliomas show up to moderate patchy, multifocal enhancement with dot-like pattern. Regarding perfusion MRI scans, it is to say that the perfusion in oligodendrogliomas is commonly moderately increased. (Van Den Bent et al. 2018)

The histopathology of oligodendrogliomas is characterized by infiltrating glioma cells with round, regular and monotonous nuclei with sharply defined nuclear membranes and only modest cell to cell variability. (Brat and Perry 2010) The cytoplasm of tumor cells tends to swell during fixation with formalin and the paraffin-embedding. This results in the fixation artefact that is called “fried egg” or “honeycomb” and “woody plant” histology with well-defined cell membranes, clearing of the cytoplasm and central spherical nucleus. (Brat and Perry 2010) Important to note is that this phenomenon is neither seen in frozen tissue samples nor is it required for a diagnosis. (Brat and Perry 2010) It merely helps the observer towards the right diagnosis. Cells in an oligodendroglioma show few cellular processes. Thus, they can be differentiated from astrocytomas due to the paucity of glial processes. (Brat and Perry 2010) Another histological characteristic for oligodendrogliomas is the so called “chicken wires” which represents a branching capillary network. (Brat and Perry 2010) Moreover, microcalcification, microcyst filled with mucin, perineuronal satellitosis and perivascular aggregation of tumor cells may be found. (Brat and Perry 2010)

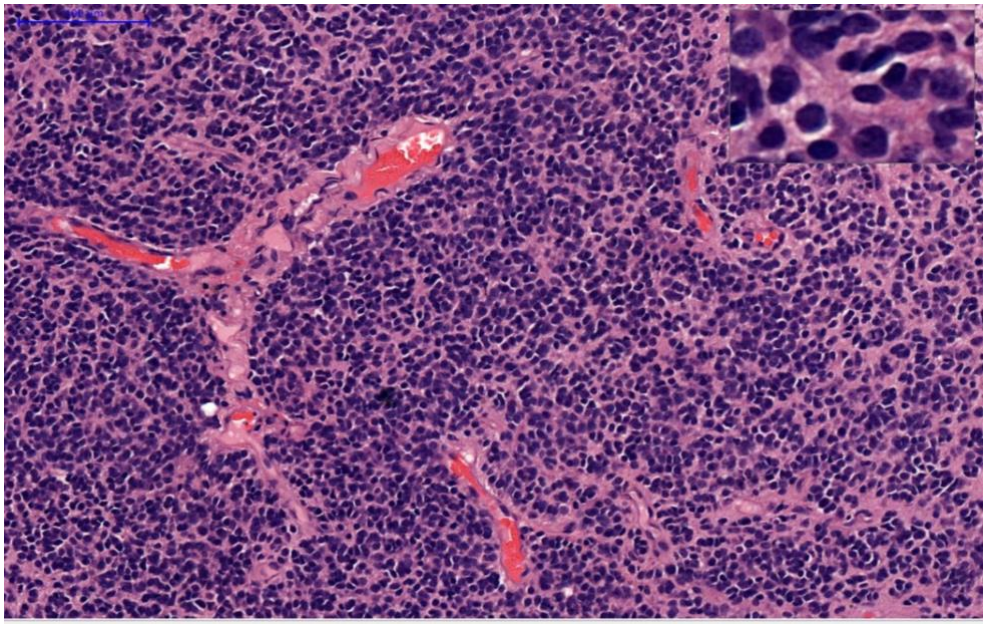

Figure 7 Typical characteristics of an oligodendroglioma: branching capillary network known as “chicken wire”

As for molecular markers, the diagnosis of an oligodendroglioma requires to have IDH1 or IDH2 mutations combined with 1p19q codeletion. (Louis et al. 2016; Oberheim Bush and Chang 2016; Banan and Hartmann 2017) In comparison to the previous 2007 WHO classification, now also astrocytic-like phenotypes that show the two above characteristics are considered as oligodendrogliomas. (Banan and Hartmann 2017) Further features like inactivating CIC mutations, FUBP mutations, NOTCH1 or NOTCH2 mutations and TERT promoter (TERTp) mutations can be found in oligodendrogliomas. (Banan and Hartmann 2017; Van Den Bent et al. 2018) Mutually exclusive on the other hand is the mutation of ATRX, which is only seen in astrocytomas and not in oligodendrogliomas. (Van Den Bent et al. 2018) Just like with astrocytomas, it is obligatory to determine the IDH status by

sequencing in case of a negative IDH1 R132H protein expression with immunohistochemistry. If both immunohistochemistry and sequencing are negative, the tumor can be described as IDH wild type. Furthermore, oligodendrogliomas can be labeled as NOS in the absence of testing capabilities or the failure/impossibility of determining the IDH and/or 1p19q status. (Louis et al. 2016; Banan and Hartmann 2017)

1.3.3 Oligoastrocytoma

The WHO 2016 classification recommends to strongly avoid using the diagnosis oligoastrocytoma. (Louis et al. 2016; Banan and Hartmann 2017) In the rare case of a true oligoastrocytoma, these entities can be used:

Oligoastrocytoma WHO grade II, NOS

Anaplastic oligoastrocytoma WHO grade III, NOS

Oligoastrocytomas are diffusely infiltrating gliomas with oligodendroglial and astrocytic components in either geographically distinct zones or, more commonly, intermixed. They can occur in the frontal, parietal and temporal lobe, while the temporal manifestation is more rapid. (Brat and Perry 2010)

There are no radiological features that are specific for oligoastrocytomas alone and could distinguish them from oligodendrogliomas or astrocytomas. Hence, grade II tumors show T1-weighted hypointensity and T2- or FLAIR hyperintensity. Anaplastic oligoastrocytomas show a larger mass effect than grade II tumors as well as some degree of contrast enhancement. (Brat and Perry 2010)

The histopathological criteria are comparable to those of oligodendrogliomas or astrocytomas with both elements being represented in the same tumor. Two types can be differentiated: The biphasic (compact) variant with separate areas of oligodendroglial and astrocytic structures and the more common intermixed (diffuse) variant in which the features are mixed. (Brat and Perry 2010)

As mentioned above, the diagnosis oligoastrocytoma is strongly discouraged because looking at the molecular markers, nearly all tumors can be classified as either an astrocytoma with IDH mutation, wild type or NOS and an intact 1p19q status or an oligodendroglioma with IDH mutation, wild type or NOS with a 1p19q codeletion. (Louis et al. 2016) Only tumors with microscopic true astrocytic and oligodendroglial features and an absence of appropriate molecular testing can be diagnosed as oligoastrocytoma or anaplastic oligoastrocytoma. To be absolutely sure in such cases, misinterpretation of regional heterogeneity due to technical problems, such as false-negative immunohistochemistry or false-positive FISH results for 1p19q codeletion, have to be ruled out. (Louis et al. 2016)

1.4 Objective of the present thesis

As indicated previously, with the update of the WHO 2007 to the WHO 2016 Classification, a whole new concept of classifying brain tumors has been established. (Louis et al. 2014; Louis et al. 2016) Traditional grading, using morphology alone, is not capable of predicting biological behavior accurately, several molecular factors overwrite the assumptions. (Louis et al. 2014)

As a result, morphology is no longer the gold standard for making a correct glioma diagnosis, more over it is molecular genetics. Therefore, histological classification and grading of glial neoplasms requires an update in light of recent molecular findings. (Louis et al. 2014)

The goal of this project is:

Retrospectively analyze non-glioblastoma glial neoplasms following the most recent guidelines (WHO 2016 Classification) and to compare the reclassification with the original diagnoses made by using the WHO 2007 Classification. To be more precise, comparison between the diagnoses made with the WHO 2007 to the newest WHO 2016 Classification should be carried out while focussing on what amount of diagnoses might change or if even changes can be demonstrated. Moreover, if changes in the diagnosis are present, we want to ascertain the cause of change.

Furthermore, potential immunohistochemical markers should be assessed which might help the observer to more precisely diagnose patients with the right tumor.

2 Material and Methods

2.1 Tumor Samples and demographic data

Fifty paraffin-embedded surgical human biopsies and their corresponding histological samples of non-glioblastoma diffuse low grade gliomas were taken out of the archives (tumor tissue bank) of the Department of Pathology, University of Pécs - Medical Faculty, Pécs, Hungary. Only true adult low grade (WHO grade II and III) diffuse gliomas from the years 2000 till 2017 were included in the study. High grade, such as primary and secondary glioblastomas, and pediatric tumors were not part of this project.

Demographic data, like the patient's gender, age at diagnosis and previous WHO 2007 diagnosis were obtained from written and electronic medical records.

The neuropathological, immunohistochemical and the genetical examinations were performed following to the national ethical guidelines and legal regulation at the Department of Pathology, University of Pécs - Medical Faculty, Pécs, Hungary and the Department of Neuropathology of the University Medical Center Göttingen. All detailed analyses were performed at the Department of Neuropathology of the University Medical Center Göttingen, approved by the local ethics committee (3/10/14).

2.2 Pathological review

As a first step, from the existing hematoxylin and eosin (HE) stained slides, used for primarily diagnosing the tumor at the date of diagnosis, comprehensive and only true tumor specimens of the tissue block were defined. Moreover, merely cases with a minimum of six millimeter tumor tissue sample were considered in order to sufficiently process tissue microarrays (TMA).

Afterwards, each of the fifty tumors has been reevaluated following the former WHO 2007 Classification regarding cytology, morphology of blood vessels, degree of nuclear atypia as well as mitotic index by one doctoral student and one board-certified neuropathologist (D.AA., B.K.). Afterwards the diagnosis was compared with the original diagnosis (WHO 2007) of the patient from medical records.

2.3 Sample Preparation

After reevaluating all cases and selecting suitable tumor regions in the paraffin block, tissue microarrays (TMA) have been manually created using a manual TMA punch-extractor pen (Histopathology Ltd., Hungary) which is capable of extracting a 2 mm diameter tissue core. In order to prevent loss of one TMA cylinder, two biopsy punches were extracted and transferred to a 4x6 2 mm TMA Block (Histopathology Ltd., Hungary) from each case, according to the manufacturer's instructions. In each TMA Block, two samples non-tumorous brain tissue were additionally inserted as a negative control.

Subsequently, from the establisched paraffin-embedded TMA blocks, 4µm thick slides were created using a microtome (Leica SM 2000R, Leica Germany) and then first stained with HE to check the correct transfer of the TMA cylinders and corroborate true tumor regions.

2.4 Immunohistochemistry

In order to immunohistologically assess all fifty cases, further sections of a thickness of 4µm were cut from each formalin-fixed, paraffin embedded TMA tissue block. They were then stained with the antibodies mentioned in Table 1 according to the manufacturer's instructions by an automated Immunostainer (DAKO Autostainer

Link 48) using standard histological and immunohistological techniques. First, the slides were deparaffinized using xylol for 2 × 10 min and afterwards they were rehydrated. For GFAP, pre-treatment with Tris/EDTA buffer (Merck KGaA, Germany), pH 6.0 was used for twenty minutes for antigen retrieval. For Nogo-A, Olig2 and pHH3 microwave pretreatment with Citrat 3x5 minutes or Tris/EDTA buffer for 3x5 minutes was performed. In all other cases, Tris/EDTA buffer (Merck KGaA, Germany), pH 9.0 was used for twenty or thirty (CyclinD1) minutes. The reactions were visualized using the EnVision System (Agilent Technologies Inc., USA) and EnVision FLEX visualization system (Agilent Technologies Inc., USA). To visualize nuclei, all slides were counterstained with hematoxylin-eosin.

Table 1: Used Antibodies; IDH1 = Isocitrate dehydrogenase; ATRX = Alpha-thalassemia/mental retardation syndrome X-linked gene; Olig 2 = Oligodendrocyte lineage gene, pHH3 = Phospho-Histone H3, GFAP = glial fibrillary acidic protein, EZH2= Enhancer of Zeste 2

Antibody	Clone	Manufacturer	Dilution	Pretreatment
IDH1-R132H	HO9	Dianova GmbH, GER	1/25	pH 9, 20 min
ATRX	Polyclonal	Abcam plc, UK	1/200	pH 9, 20 min
p53	DO7	Histopathology Ltd. HU	1/6000	pH 9, 20 min
Nogo-A	Polyclonal	SantaCruz Biotechnology Inc., GER	1/500	Microwave Citrat 3x5 min
Olig 2		Immuno-Biological Laboratories Co., Ltd., JPN	1/150	Microwave Tris/EDTA Buffer 3x5 min
pHH3	Polyclonal	BIOCARE Medical LLC., USA	1/200	Microwave Citrat 3x5 min
GFAP	GA_5	Biogenex Inc, USA	1/2000	pH 6, 20 min
CyclinD1	SP4	Histopathology Ltd., HU	1/20	pH 9, 30 min
EZH2	6A10	Leica/Novocastra GmbH, GER	1/200	pH 9, 20 min
Ki67/MIB1	B56	Histopathology Ltd., HU	1/400	pH 9, 20 min
WT1	6F-H2	DAKO-Agilent, USA	1/100	pH 9, 20 min

All fifty diffuse low grade gliomas were investigated for IDH1, ATRX, p53, GFAP and Ki67/MIB1. In addition to that, thirty-two of the fifty samples were further analyzed for Olig 2, Nogo-A, pHH3, CyclinD1, EZH2 and WT1.

The assessment of the immunohistochemistry was done manually for IDH1, ATRX, p53, Ki67/MIB1, GAFP, EZH2 and WT1 using a Nikon Alphaphot YS Binocular Microscope. All TMAs were furthermore digitalized with the Pannoramic MIDI slide scanner (3DHistech, Hungary) and subsequently all nuclear markers (ATRX, p53, Ki67/MIB1, CyclinD1 and EZH2) were also investigated using automating image analysis (NuclearQuant Analysis Module, QuantCenter, 3DHistech, Hungary).

In order to analyze the immunohistological stainings, the following categorizations was performed. For IDH1, GFAP and WT1, “strong”, “moderate” and “weak” positivity was manually categorized with a light microscope. The positivity score of ATRX, p53, Ki67/MIB1 and EZH2 was acquired manually using percentage scores (0% – 100%) via light microscope. Olig 2 and Nogo-A both were scored either “negative” (= no positive cells at all), “positive cells under 50% of all cells” and “positive cells over 50% of all cells”. For pHH3 the total amount of cells in the cell cycle were counted just like it was done for the mitotic activity. For nuclear immunohistological markers (ATRX, CyclinD1, p53, Ki67/MIB1, and EZH2), evaluated with automated image analysis (NuclearQuant Analysis Module, QuantCenter, 3DHistech, Hungary), the total amount of positive or negative cells was gathered. The scoring system for the automated image analysis was made based on the staining intensity on a 0 – 256 scale by the company’s quantification algorithm and is displayed in Table 2.

Table 2: Immunohistochemical scoring categories for automated image analysis

	Score			
	0	1	2	3
Staining intensity of the antibody	0%	1 – 33%	34 – 66%	67 – 100%

2.5 Fluorescence in situ hybridization (FISH)

Fluorescence in situ hybridization (FISH) was performed using the dual-color probes listed in Table 3. The 1p36/1q25 probe contains the LSI 1p36 Spectrum Red and 1q25 Spectrum Green as the control probe. Probe 19q13/19p13 encloses LSI 19p13 Spectrum Red and 19p13 Spectrum Green as control.

4µm thick tissue sections of FFPE tissue blocks were deparaffinized using xylol, 2 × 10 min, rehydrated and pretreated with Zymed pretreatment solution (Invitrogen, Carlsbad, CA) at 100 ºC for 15 minutes; afterwards in Zymed pepsin solution (Invitrogen, Carlsbad, CA) at room temperature for 20 minutes. The slides and probes were denatured on a heating plate together at 90 ºC for 5 minutes. Hybridization was performed overnight at 37 ºC. Posthybridization was performed in 2×SSC containing 50% formamide at 42 ºC for 3 × 5 minutes. The slides were covered by Vectashield (Vector Laboratories, Burlingame, CA) containing 0.5 µg/ml DAPI.

Due to the fact that there are no official international guidelines on evaluating FISH in gliomas, a minimum of one hundred cells were counted in every case for 1p deletion and afterwards further one hundred cells were counted for 19q deletion.

Only non-overlapping nuclei with a minimum of one green signal were incorporated. Assessment was done with 100x magnification and immersion oil using a Nikon Eclipse Ni-U Epifluorescence microscope (Nikon Instruments, Tokyo, Japan) with a L200NI 200W fluorescence illuminator (Nikon Instruments, Tokyo, Japan) and a Triple band pass filter for DAPI, Spectrum Green and Spectrum Red (Nikon Instruments, Tokyo, Japan).

The ratio of 1p36/1q25 and 19q13/19p13 was calculated by dividing the number of red and green signals. For each case, FISH data were investigated by the following. In case the mean of Red/Green value of a sample was equal or below 0.9, the sample was considered as positive. Several difficult to analyze cases were first counted once more for 1p and 19p deletion and then calculated again.

Table 3: Used FISH Probes

Probe	Manufacturer	Composition
1p36/1q25	Abbott-Vysis, Abbott Park, IL, USA	LSI 1p36 (Spectrum Red), LSI 1q25 (Spectrum Green)
19q13/19p13	Abbott-Vysis, Abbott Park, IL, USA	LSI 19q13 (Spectrum Red), LSI 19p13 (Spectrum Red)

2.6 Statistical analysis

Simple analysis was performed using Microsoft Excel, Version 2019 (Microsoft Corporation, Redmond, Washington, USA) in order to calculate percentages and mean values.

3 Results

In total, 50 specimens of 50 patients were part of this study. The mean age at diagnosis in years was 44 ranging from 20-68 years. (Table 4)

Table 4: Patient cohort characteristics

	n (%)
	50 (100)
Gender	
Male	27 (54)
Female	23 (46)
Age at diagnosis	
Mean (years)	44.5
Median (years)	44
Range (years)	20-68

After reevaluation of the former WHO 2007 classification, twenty-three tumors were astrocytomas (thirteen WHO grade II, ten WHO grade III), twenty tumors were oligodendrogliomas (eleven WHO grade II, nine WHO grade III) and eight were oligoastrocytomas (two WHO grade II, six WHO grade III).

In this project we first addressed the question to which extent diagnoses were changed when comparing the previous WHO 2007 Classification of diffuse gliomas with the most recent WHO 2016 Classification.

After the assessment of all compulsory immunohistological stainings and genetic determinants in order to make a diagnosis based on the WHO 2016 Classification,

thirty-fife cases were astrocytomas (twenty WHO grade II, fifteen WHO grade III), fifteen tumors were oligodendrogliomas (nine WHO grade II, six WHO grade III) and none of the seven cases of oligoastrocytomas could be replicated.

Therefore, the original diagnosis was altered in 32.0% (16/50 cases) following the WHO 2016 Classification. 40.0% (8/20 cases) were changed from a previous oligodendroglioma (light and dark orange Figure 8) to a now astrocytoma (light and dark blue figure 8), 4.3% (1/23 cases) changed from an astrocytoma to an oligodendroglioma and all seven oligoastrocytomas (light and dark red figure 8) were either pushed towards the diagnosis of an astrocytoma (71.4%, 5/7 cases) or an oligodendroglioma (28.5%, 2/7 cases). (Figure 8)

Observing more precisely, three different groups of changes could be demonstrated. In 24.0% (12 cases) solely the diagnosis changed (Table 5), in 6.0% (3 cases) only the WHO grade changed (Table 5) and in 8.0% (4 cases) the diagnosis and the WHO grade changed (Table 5).

Not only did we want to ascertain how many cases changed their tumor entity but also what the main reason of the change is. The major cause of change in the diagnosis was either the lack of 1p19q codeletion, as given in the 40.0% (8 cases) of oligodendrogliomas and 71.4% (5 cases) of oligoastrocytoma changed to astrocytomas or the positive 1p19q codeletion in the 4.0% (1 case) of astrocytomas and 28.6% (2 cases) of oligoastrocytoma changed to oligodendrogliomas.

Table 5: Total amount of cases including the WHO 2007 Classification diagnosis and WHO 2016 Classification diagnosis as well as the Reason of change in the diagnosis

Case	Original Diagnosis (WHO 2007)	New Diagnosis (WHO 2016)	Reason of change			Comment
			IDH	FISH		
				1p	19q	
1	Oligoastrocytoma II	Astrocytoma II	+	-	-	D
2	Astrocytoma II	Astrocytoma II	+	-	-	
3	Astrocytoma II	Astrocytoma II	+	-	-	
4	Oligodendroglioma II	Oligodendroglioma II	-	+	+	
5	Oligodendroglioma II	Oligodendroglioma II	+	+	+	
6	Oligodendroglioma III	Oligodendroglioma III	+	+	+	
7	Oligoastrocytoma II	Astrocytoma II	+	-	-	D
8	Oligodendroglioma III	Oligodendroglioma III	+	+	+	
9	Oligodendroglioma II	Astrocytoma III	+	-	-	D + G
10	Oligodendroglioma II	Astrocytoma II	+	-	-	D
11	Oligoastrocytoma III	Oligodendroglioma III	+	+	+	D
12	Astrocytoma III	Astrocytoma III	-	+	-	
13	Astrocytoma III	Astrocytoma III	+	**N/A**	**N/A**	
14	Astrocytoma II	Astrocytoma III	+	-	-	G
15	Astrocytoma III	Astrocytoma III	+	-	-	
16	Oligodendroglioma II	Oligodendroglioma II	+	+	+	
17	Oligodendroglioma III	Oligodendroglioma III	+	+	+	
18	Oligodendroglioma III	Oligodendroglioma II	+	+	+	G
19	Oligodendroglioma II	Oligodendroglioma II	+	+	+	
20	Astrocytoma II	Astrocytoma II	+	-	-	
21	Astrocytoma II	Astrocytoma II	+	-	-	
22	Oligodendroglioma II	Astrocytoma II	+	+	-	D
23	Oligoastrocytoma III	Astrocytoma II	-	-	-	D + G

Continuing table 5: Total amount of cases including the WHO 2007 Classification diagnosis and WHO 2016 Classification diagnosis as well as the Reason of change in the diagnosis

24	Oligoastrocytoma III	Oligodendroglioma III	+	+	+	D
25	Oligoastrocytoma III	Astrocytoma III	+	+	-	D
26	Oligodendroglioma II	Astrocytoma II	+	-	+	D
27	Oligoastrocytoma III	Astrocytoma III	-	-	-	D
28	Oligodendroglioma II	Astrocytoma II	+	-	+	D
29	Oligodendroglioma III	Oligodendroglioma II	+	+	+	G
30	Oligodendroglioma III	Astrocytoma II	+	-	+	D + G
31	Oligodendroglioma III	Astrocytoma III	-	-	-	D
32	Oligodendroglioma III	Astrocytoma III	-	+	-	D
33	Astrocytoma III	Astrocytoma III	+	+	+	
34	Astrocytoma II	Astrocytoma II	+	-	-	
35	Oligodendroglioma II	Oligodendroglioma II	+	+	+	
36	Astrocytoma II	Astrocytoma II	+	-	+	
37	Astrocytoma III	Astrocytoma III	-	-	-	
38	Astrocytoma II	Astrocytoma II	+	+	-	
39	Astrocytoma II	Astrocytoma II	+	-	+	
40	Astrocytoma III	Oligodendroglioma II	-	+	+	D + G
41	Astrocytoma II	Astrocytoma II	+	-	+	
42	Astrocytoma II	Astrocytoma II	+	-	-	
43	Astrocytoma III	Astrocytoma III	-	-	-	
44	Astrocytoma II	Astrocytoma II	+	-	-	
45	Oligodendroglioma III	Oligodendroglioma III	+	+	+	
46	Astrocytoma III	Astrocytoma III	+	-	-	
47	Astrocytoma II	Astrocytoma II	-	-	-	
48	Astrocytoma III	Astrocytoma III	+	+	+	
49	Astrocytoma III	Astrocytoma III	+	-	+	
50	Oligodendroglioma II	Oligodendroglioma II	+	+	+	
Total:	**12x the diagnosis, 3x the grade and 4x the diagnosis and grade has changed**					

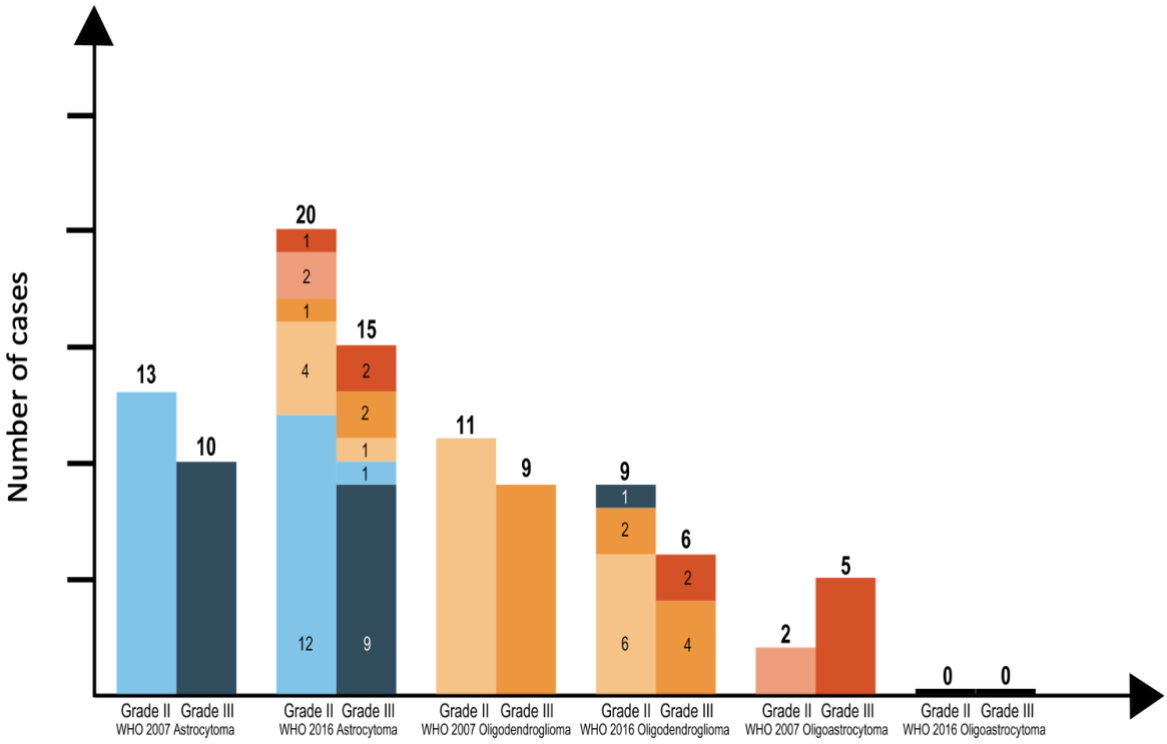

Figure 8: Total amount of cases segmented in the different entities and WHO grades and how the diagnosis shifts between the WHO 2007 and the WHO 2016 Classification's diagnosis

*Light blue: Grade II Astrocytoma, dark blue: Grade III Astrocytoma; light orange: Grade II Oligodendroglioma, dark orange: Grade III Oligodendroglioma, light red: Grade II Oligoastrocytoma, dark red: Grade III Oligoastrocytoma

The second part of this project was to assess meaningful immunohistological markers which might help the observer to diagnose patients more precisely with the right tumor entity.

In order to do so, we first evaluated all markers according to the prescription in the Material and Methods part and then a semiquantitative score was applied. For p53, Ki/67/MIB1, EZH2, Cyclin D1 and ATRX the scores were set as Score 1: 0% = negative, Score 2: 1-33%, Score 3: 34-66%, Score 4: 67-100%. For Nogo A and Olig 2 the scores were set as Score 1: 0% = no positive cells, Score 2: <50% positive cells and Score 3: >50% positive cells and for IDH 1 & GFAP Score 1: positive, Score 2: negative was applied. The scores for WT1 were Score 1: negative, Score 2: weak positive, Score 3: moderate positive, Score 4: strong positive and for pHH3 & the mitosis counted in HE staining the scores were Score 1: 0 total mitosis, Score 2: 1-5 total Mitosis, Score 3: 6-10 total Mitosis Score 4: >10 total Mitosis. (Figure 9)

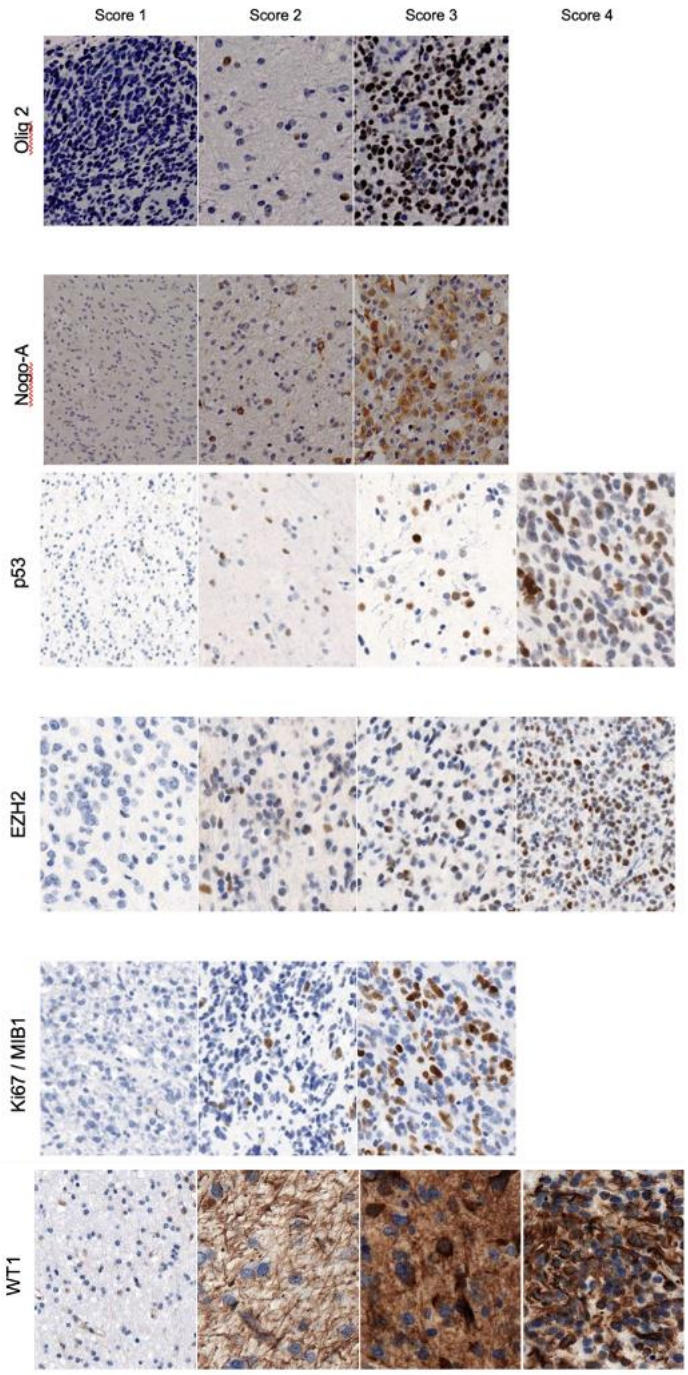

Figure 9: Scoring system for the different immunohistochemical markers

Analyzing the lineage marker Olig 2 regarding oligodendrogliomas, for grade II and III only strong positive cases could be evaluated (Score 3: >50% positive cells) (grade II 6/6 and grade III 5/5) and therefore giving a first suggestion on the tumors' entity. (Figure 10) For Olig 2 expression in astrocytic tumors the picture was not as clear, meaning in both grades (II and III) Score 3 cases were found even though they were the minority (3/12 grade II and 3/9 grade III). The majority of grade II (75%) and grade III (44%) astrocytomas showed scattered or marginalized oligodendrocytes which were ranked in Score 2.

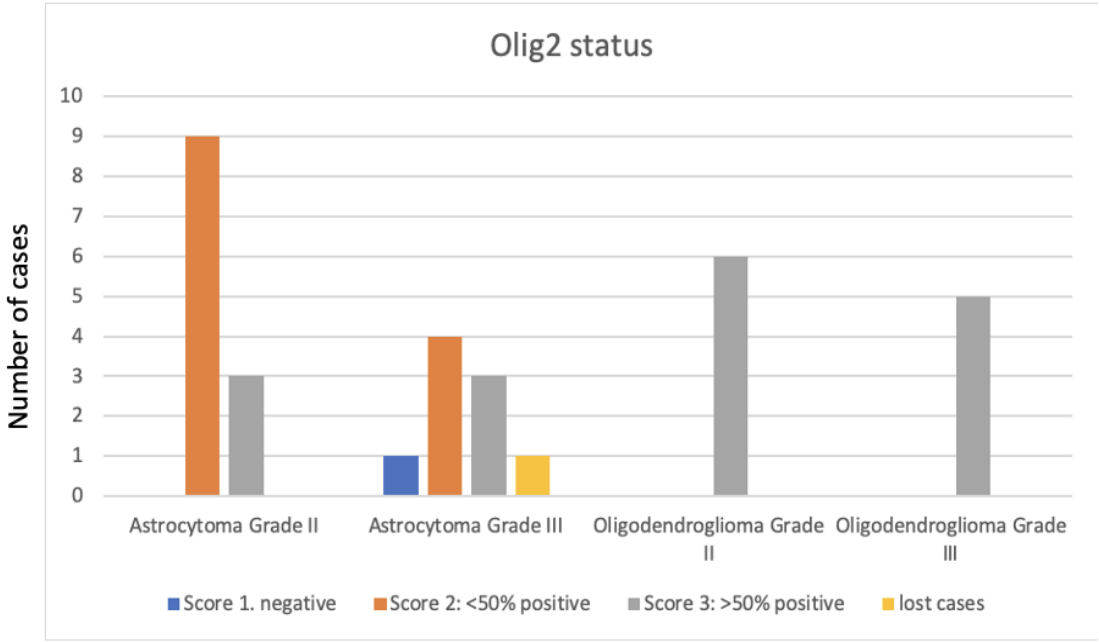

Figure 10: Olig 2 status

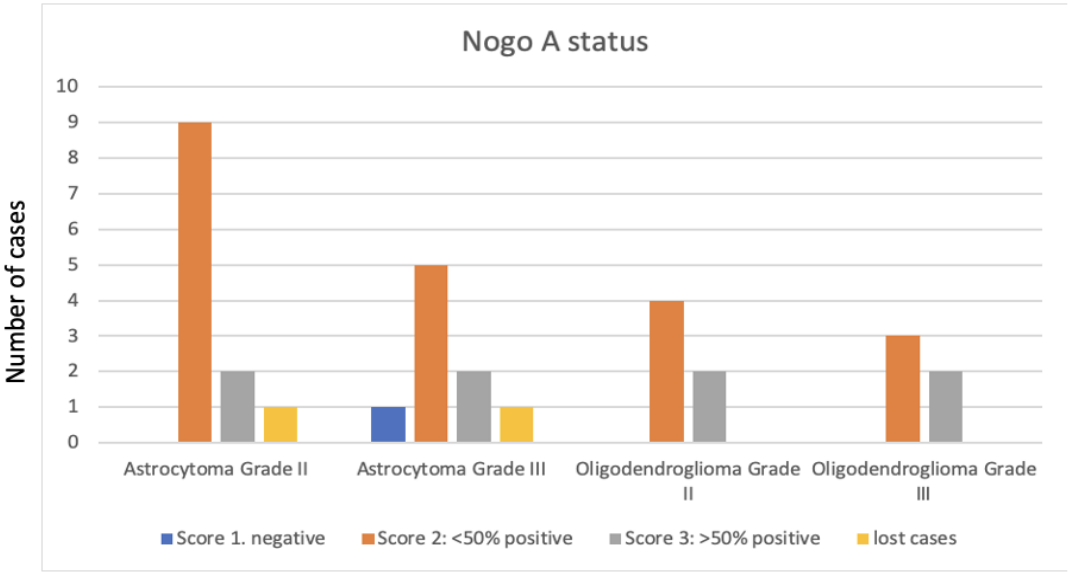

Figure 11: Nogo-A status

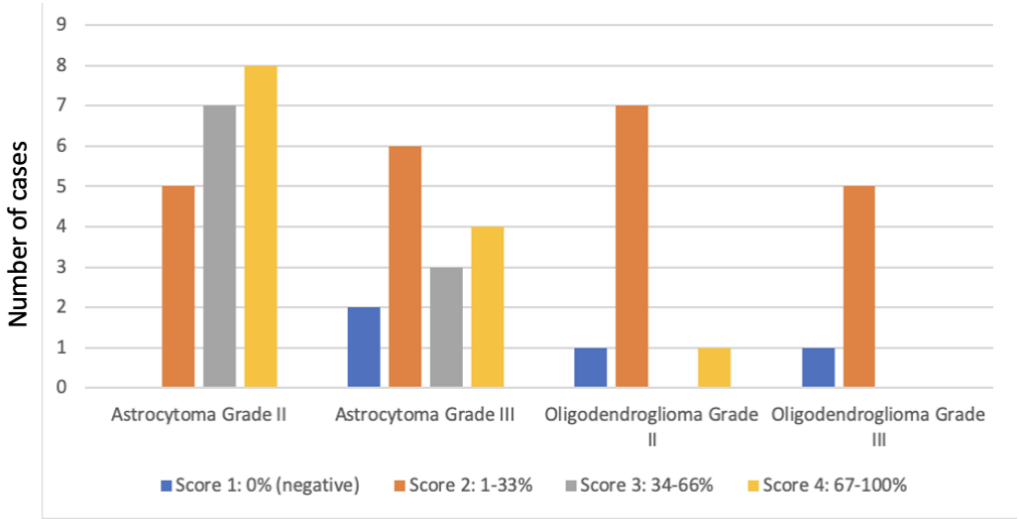

Figure 12: p53 status

Looking at p53 in grade II astrocytomas, all cases were positive with most cases in the two highest possible scores (Score 3 and 4) with 75%. None of the cases were completely negative. (Figure 12) Comparing the results for astrocytoma grade II and III, a shift towards score 1, 2 and 3 can be demonstrated. (Figure 12) With regard to oligodendrogliomas grade II and III, almost entirely Score 1 and 2 could be revealed (8/9 grade II and 6/6 grade III) leading to the assumption that p53 and Olig 2 have a mutual dependency, just as studies have already demonstrated.

Looking at the total amount of mitoses counted in the whole tissue sample, it can be displayed that within grade II tumors (astrocytomas and oligodendrogliomas) only the two lowest scores (Score 1 and 2) are present. Observing grade III, a shift towards the Scores 3 and 4 can be observed with 4/15 (26%) for astrocytomas grade III and 4/6 (66%) for oligodendrogliomas grade III. (Figure 13) Comparing pHH3 and the total amount of mitoses displays that even in grade II astrocytomas or oligodendrogliomas more than 10 mitosis per sample can be found (Score 4) although it is very rare with 8.3% to 16.7% (1/12 for astrocytomas and 1/6 for oligodendrogliomas). Nevertheless, the majority of cases are still categorized within the Score 1 and 2 (83.3%) for grade II astrocytomas and oligodendrogliomas. Examination of grade III illustrates the same shift towards Score 3 and 4 as it can be seen in the counted mitoses (44.4% for astrocytoma and 40.0% for oligodendroglioma). (Figure 14)

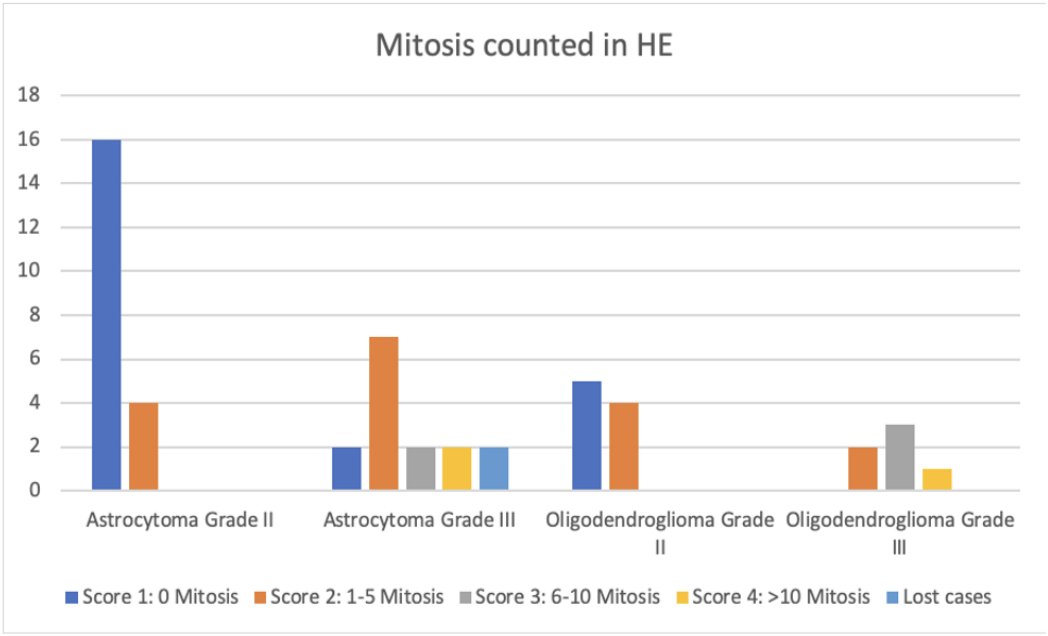

Figure 13: Mitosis counted in HE

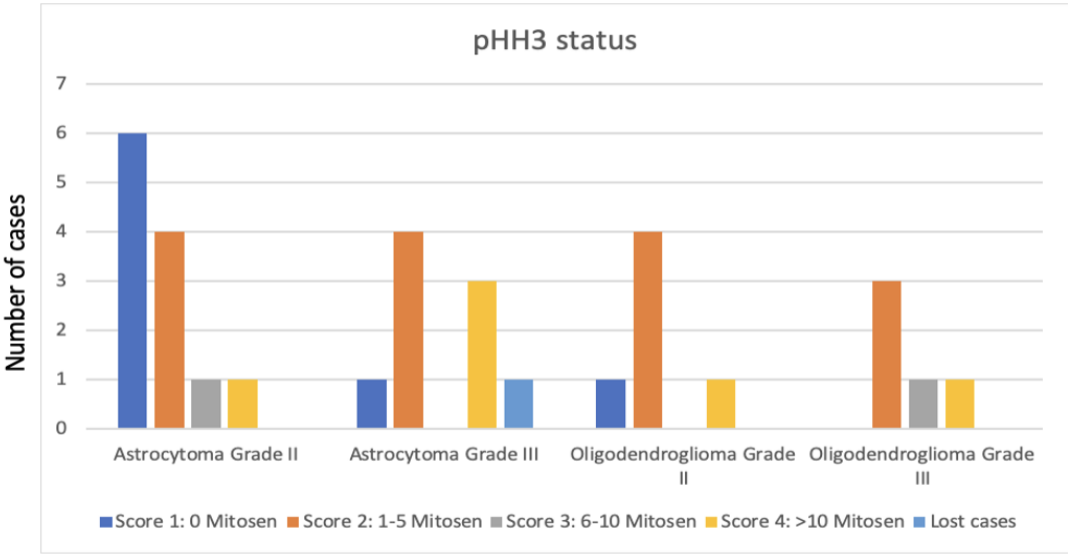

Figure 14: pHH3 status

Comparing additional markers, such as EZH2 and Ki67/MIb1 with the results of pHH3 and the counted mitoses, demonstrate interesting results. For EZH2 the same shift as in pHH3 form Score 1 and 2 in grade II tumors towards Score 3 and 4 in grade III tumors can be displayed. In astrocytomas and oligodendrogliomas grade II 12/12 and 6/6 cases were categorized in Score 1 and 2. Within the next higher grade III for astrocytomas 6/9 (66.7%) are still ranked in Score 1 and 2 but 2/9 (22.2%) were pushed into Score 3 and 4. Similar results can be seen for oligodendrogliomas grade III. 3/5 (60.0%) remain in Score 1 and 2 but 2/5 (40.0%)

were pushed towards Score 3 and 4. (Figure 16) Observing Ki67/MIB1 during the study showed grade II astrocytomas and oligodendrogliomas harbored in the Scores 1 and 2 but the swing to redeploy towards Score 3 and 4 cannot be seen as intense as with EZH2. In astrocytomas from previously 20/20 Score 1 and 2 grade II tumors compared to grade III 1/15 (6.7%) were rescored in Score 3 and 0/15 in Score 4. The remaining 93.3% were still ranked in Score 1 and 2. The same picture can be seen for Oligodendrogliomas. 9/9 grade II tumors were in Score 2 and zero in Score 1. For grade III cases 1/6 (16.7%) shifted towards Score 3 and the remaining 5/6 (83.3%) stayed in Score 2. (Figure 15)

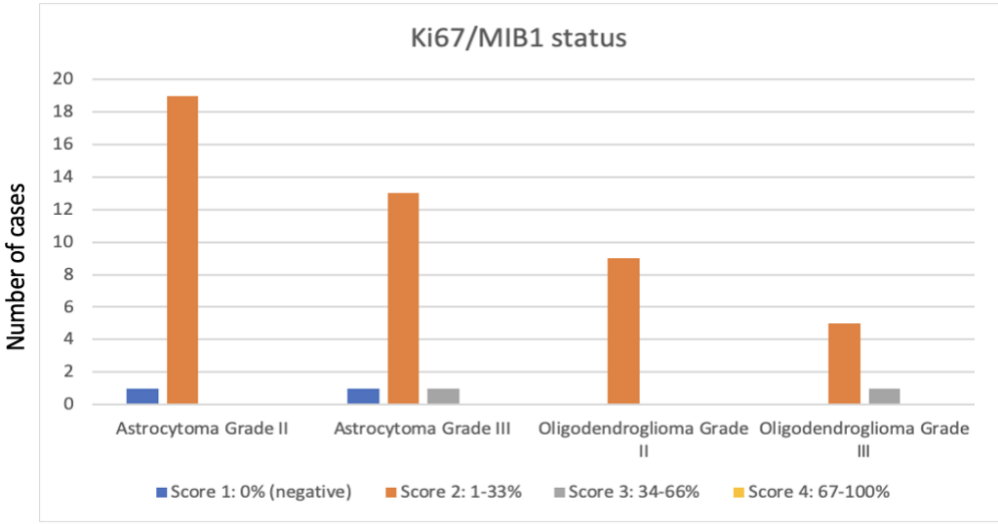

Figure 15: Ki67/MIB1 status

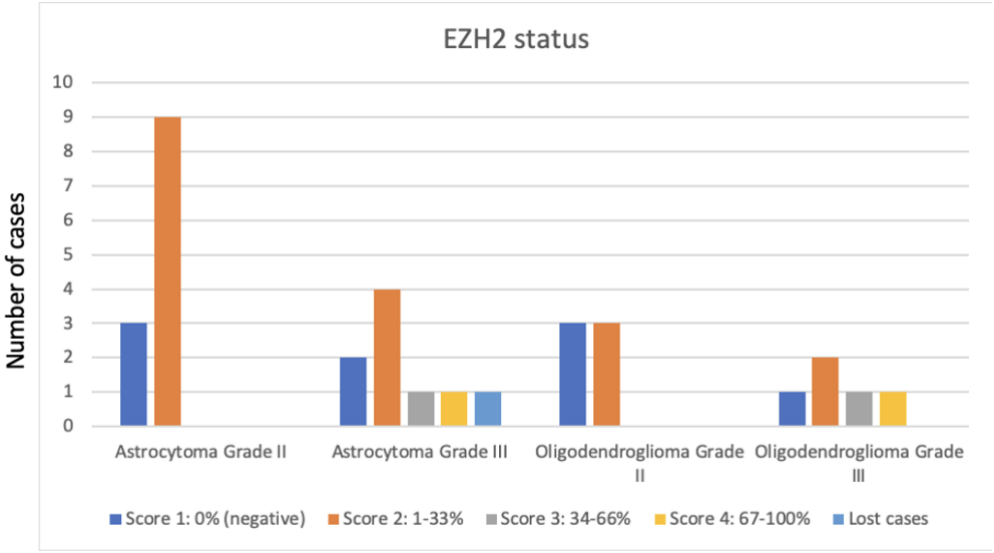

Figure 16: EZH2 status

Looking at Cyclin D1 and WT1 as potential markers for proliferative and mitotic activity, a shift in astrocytomas grade II to grade III can be seen just like in pHH3 or Ki67/MIB1 but for oligodendrogliomas grade II and grade III this phenomenon could not be displayed during the study. (Figure 17 and 18)

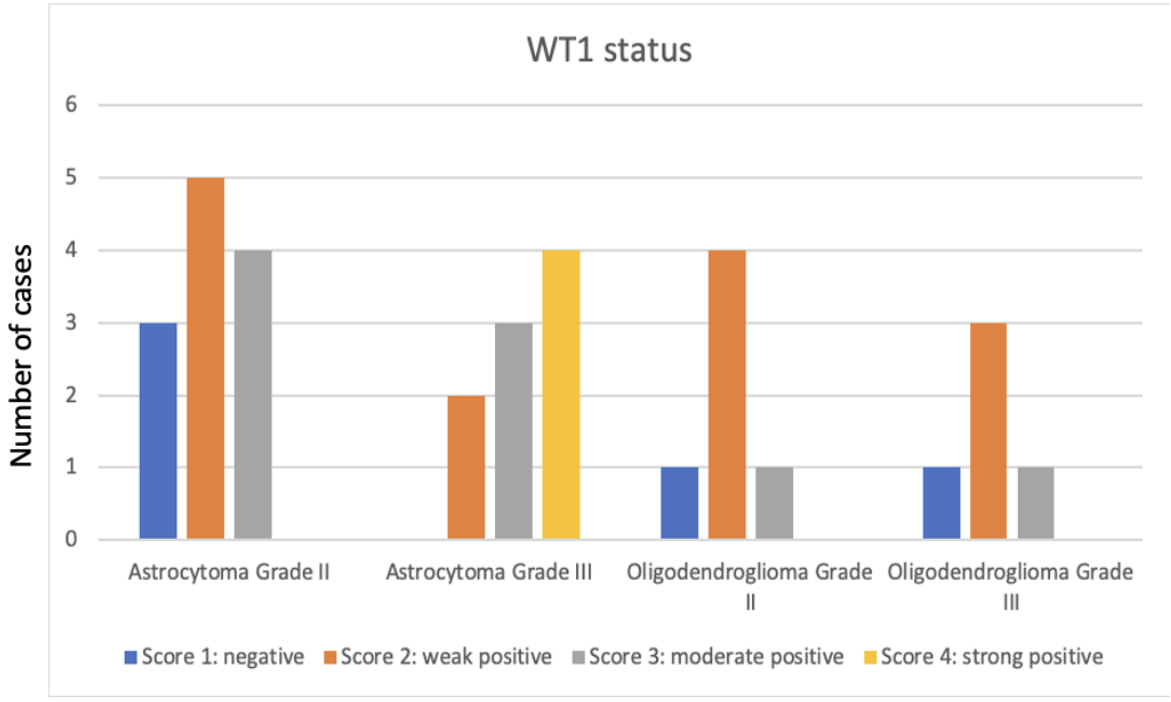

Figure 17: WT1 status

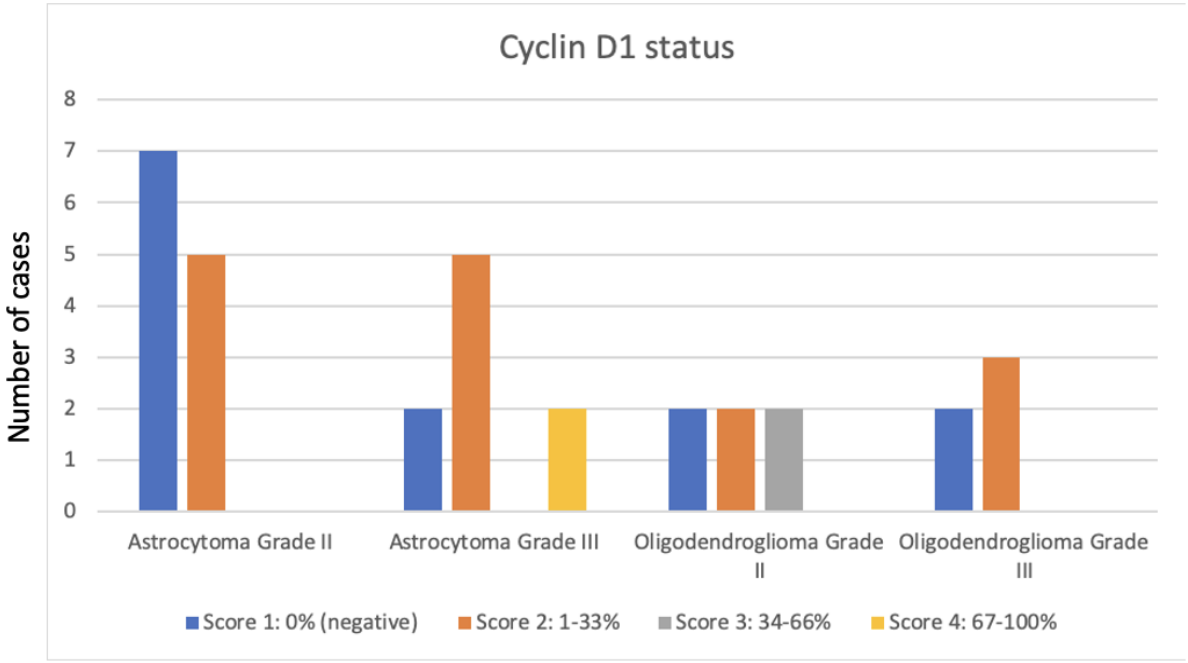

Figure 18: Cyclin D1 status

4 Discussion

From the first classification of brain tumors by Rudolf Virchow in 1863 to our most recent WHO 2016 Classification, more than 150 years have passed. (Virchow 1863) Within that time period, our understanding and scientific progress has increased exponentially, not just with brain tumors but with tumors and medicine in general. (Louis 2012; Banan and Hartmann 2017; Johnson et al. 2017) With rapidly enhancing technologies and research findings, such as molecular data and genetic alterations in gliomas, this trend will continue and the gap between major evolutions will shrink more and more.

The first part of this research project was trying to find out to what extent diagnoses might change while reclassifying cases, originally diagnosed with the former WHO 2007 Classification, with the now most recent WHO 2016 Classification or if even a change in diagnosis can be displayed. The results showed that nearly one third (32.0%) of all diagnoses made with the previous 2007 Classification changed while using the most recent WHO 2016 standards of classifying low grade gliomas. Furthermore, we could display the major cause of change for the diagnosis, which is the lack of 1p19q codeletion. More precisely, 40.0% of the cases changed from previous oligodendrogliomas to now astrocytomas (lack of 1p19q codeletion) whilst only 4.3% of all cases showed reverse changes of entity from previously astrocytomas to now oligodendrogliomas (present 1p19q codeletion). An additional closer look revealed that there are three different groups of change. (1) only the diagnosis changed (24.0%), (2) only the WHO grade changed (6.0%) and (3) both the diagnosis and the WHO grade changed (8.0%). Inclusion of 1p19q codeletions into the diagnostic procedure is one of the main aspects in the WHO 2016 Classification and has an immense impact of the tumors' entity as displayed above. (Theeler et al. 2012) Correct diagnosis has not only drastic and immediate significances for the pathologist but also - and more severe significances for the patient. (Louis et al. 2014; Louis et al. 2016) A change of the tumors' entity can lead

to major shifts in the patient management meaning that the patients' life expectancy, treatment, progress of the disease and many more might change radically.(Giannini et al. 2001; Van Den Bent 2010; Louis et al. 2016)

With the second part of this project, we targeted to assess possible meaningful immunohistological markers with regard to the WHO 2016 Classification. This should help the observer to diagnose the patient more precisely with the correct tumor entity, all in the light of required tests like 1p19q or IDH1 for an integrated diagnosis still need to be implemented. In order to further differentiate oligodendrogliomas from astrocytomas, lineage markers such as Olig 1 and Olig 2 are potentially useful. (Ligon et al. 2004) These markers are not necessarily included in the WHO 2016 Classification but can help the pathologist on finding the right diagnosis. Both lineage genes Olig 1 and Olig 2 encode basic helix-loop-helix (bHLH) transcription factors that are expressed in the CNS and regulate main steps of early oligodendrocyte development. (Ligon et al. 2004; Ligon et al. 2006) Studies in rodent CNS have shown that Olig 1 can promote the formation of an chondroitin sulfate proteoglycan-positive glial progenitor and is furthermore exclusively seen in oligodendrocytes and their progenitor cells. (Lu et al. 2001) Research on human CNS biopsies have exposed that Olig 1 and Olig 2 genes are higher and more uniformly expressed in oligodendrogliomas, however it can also be found in astrocytomas. (Ligon et al. 2006) Human Olig 2 alone is known to be specifically expressed in oligodendrogliomas and oligodendrocytes and it was not discovered in non-oligodendroglial tumors. (Yannick et al. 2001) In summary, exclusive Olig expression cannot objectively indicate, whether or not the tumor is an oligodendroglioma or astrocytoma, but Olig 2 can be used as a marker for diffuse gliomas in general. (Ligon et al. 2004; Ligon et al. 2006) Even though Olig 2 indicates an oligodendroglioma, further immunohistochemistry should be carried out, such as the astrocytic glial cell markers GFAP and S100, in order to differentiate between oligodendrogliomas and astrocytomas. (Lu et al. 2001) Just as in the above-mentioned studies, for Olig 2 our study revealed a highly staining intensity

for both, grade II and III oligodendrogliomas (Score 3) and not a single case was negative. Nonetheless, for astrocytomas grade II and III few Score 3 cases were also investigated. Therefore Olig 2 alone is not capable of identifying solely oligodendrogliomas and further markers are necessary.

The widely known p53 suppressor protein that plays a role in various cellular processes like apoptosis, response to DNA damage, cell cycle arrest, angiogenesis and differentiation, can also be mutated in diffuse low grade gliomas. (Sanson et al. 2004; Louis 2006; Ludwig and Kornblum 2017) The p53 protein is encoded by the TP53 gene on chromosome 17p and a disruption of p53 increases genetic instability. (Sanson et al. 2004; Louis 2006) The role of p53 in gliomas varies. Inactivating TP53 mutations are found in about 50 to over 80% of astrocytomas depending on different studies but only in 13% of oligodendrogliomas. (Louis 2006; Mittal et al. 2011) Accordingly, p53 inactivation is seen as the early stage of astrocytoma formation. This was also demonstrated in different mutation studies and mouse modeling. (Louis 2006) Furthermore, TP53 mutation and 1p19q codeletion largely appears to be mutually, leading to the conclusion that p53 inactivation contributes to astrocytoma tumorigenesis while 1p19q and a negative TP53 status mostly leads towards oligodendroglioma formation. (Louis 2006; Mittal et al. 2011) Alterations in the p53 pathway found in low grade gliomas are thought to stimulate progression to high grade tumors. (Ludwig and Kornblum 2017) In these high grade gliomas, the p53 pathway may be deregulated by other alterations like amplification of MDM2 or MDM4 and 9p deletion that leads to a loss of the ARF product of the CDKN2A gene. (Louis 2006) Not only astrocytomas but also around 70 to 80% of glioblastomas are known to have mutations in the p53 pathway and secondary glioblastomas often have direct mutations in the TP53 gene. (Ludwig and Kornblum 2017) Looking at astrocytomas grade II in our study, 75.0% of all cases can be harbored in the two highest scores (Score 3 and 4). Comparing astrocytomas grade II and III a shift from Score 3 and 4 towards lower scores with less staining intensity (Score 1 and 2) could be revealed. For oligodendrogliomas grade II and III

entirely Score 1 and 2 were found. As a result, p53 and Olig 2 have a mutual dependency, interact inversely proportional and can help identifying oligodendrogliomas in association with the 1p19q status.

In order to categorize the proliferative activity of tumors we then investigated the immunohistochemical markers pHH3, Ki67/Mib1, EZH2, WT1 and Cyclin D1 as well as the total amount of mitoses counted in HE. To accurately diagnose diffuse astrocytomas with their WHO grade (II or III) the tumor's proliferative potential should be investigated. Differentiating between different WHO grades is vital for the patient's prognosis and treatment. (Colman et al. 2006) Unfortunately, methods for displaying the proliferative potential, like the number of mitoses per 10 high-power fields (mitotic index) in Hematoxylin-Eosin (HE) staining have interobserver discordance and therefore lack objectivity. Studies displayed that Phospho-Histone H3 (pHH3) staining can be seen as a reliable and simple method for detecting mitoses and consequently result in a true mitotic index because the pHH3 mitotic index compared with the standard mitotic index count in HE, was significantly associated with each other. (Colman et al. 2006) Phospho-Histone H3 (pHH3) can therefore be labeled as an accurate immunomarker for cells within the mitotic phase and help the pathologist to more precisely grade diffuse gliomas into grade II and III (Colman et al. 2006; Zhu et al. 2016). When looking at the total amount of mitoses counted in HE we could display that for grade II tumors only Score 1 and 2 could be assessed. Looking at grade III cases, a shift towards the higher scores with more mitoses (Score 3 and 4) was observed. The comparison between the individual counted mitoses in HE samples and the immunohistochemical marker pHH3 displayed the same shifts towards higher scores for grade III tumors but due to the more objective way of staining, pHH3 might be the better diagnostic tool than counting mitoses, which can lead to inter observer discordance.

The catalytic subunit of the Polycomb repressive complex 2 (PRC2), Enhancer of zeste homolog 2 (EZH2), is a further prognostic and predictive marker in gliomas.

Polycomb group of genes can function as transcription regulators through modification of chromatin. (Purkait et al. 2015) EZH2 plays an important role in the regulation of cell proliferation and the cell cycle by affecting tri-methylation at lysin residual of histone H3 (H3K27me3). (Lin et al. 2015; Purkait et al. 2015) Regarding immunohistological analyses, only a few studies are present for EZH2 in gliomas. These studies reveled higher EZH2 expression in the nuclei of high grade tumors, more precisely in grade III astrocytic tumors. (Ahmed et al. 2016) In general, around 25-30% of grade II astrocytomas showed positivity for EZH2 and around 70% for grade III astrocytomas. Similar correlation was found for oligodendrogliomas grade II and grade III. (Purkait et al. 2015) For EZH2 our study revealed a shift from Score 1 and 2 in grade II tumors towards Score 3 and 4 in grade III tumors, analogues to pHH3.

Another helpful immunomarker in order to assess the mitotic activity of a diffuse glioma is Ki67/MIB1. As mentioned above, it can be difficult for the observer to evaluate mitotic figures with HE stained histological slides. (Skjulsvik et al. 2014) The nuclear protein Ki67 is specific to indicate the proliferative phase of a tumor cell. Hence, monoclonal Ki67 antibodies can be seen as a reliable indicator to more precisely show proliferative activity in tumor cells and then help grading gliomas. (Huang et al. 2009; Paulus 2009; Zeng et al. 2015) As Skjulsvik et al. revealed, Ki67/MIB1 correlated significantly with increasing grade in diffuse gliomas but even though it might help differentiate among high and low grade gliomas, it is not possible to accurately categorize diffuse gliomas as grade II or III. (Skjulsvik et al. 2014) As a consequence, Ki67 alone shouldn’t be used to classify the histological grade but can lead to the right WHO grade in combination with other immunohistochemical markers. Not only can Ki67 be a useful marker in order to grade tumors, it also functions as a prognostic and predictive marker. Regarding the prognostic and predictive validity of Ki67, literature showed that high Ki67 expression was strongly associated with shorter progression-free survival compared to moderate and low expression. (Zeng et al. 2015) Our project Ki67/Mib1 indicated

a less drastic shift from Score 1 and 2 towards Score 3 and 4 with only 6.7% of grade III cases being in Score 3 and 0% in Score 4. That leads to the causal inference that Ki67/Mib1 might be a more sensitive marker for the proliferative activity, while being compared to EZH2.

The zinc finger pleiotropic transcription factor WT1 (Wilms' Tumor) can be used as a prognostic and predictive marker in gliomas as well. First it was defined as a tumor suppressor gene but from today's knowledge level it can also act as an oncogene in several contexts. (Rauscher et al. 2014; Kijima et al. 2016) Furthermore, it promotes growth and differentiation, as well as migration and invasion of cells. (Kijima et al. 2016) WT1 plays a role in the resistance against apoptotic therapies by modifying the antiapoptotic protein Bcl-xL and by silencing WT1, the tumors sensitivity to cisplatin chemotherapy increases. (Rauscher et al. 2014) Immunohistological analysis by Rauscher et al. showed that WT1 is expressed in astrocytic and oligodendroglial brain tumors and the expression increases with the WHO grade. Additionally, the study showed that WT1 in astrocytic tumors is associated with older age and the absence of IDH1 mutation. (Rauscher et al. 2014)

The cyclin protein family and their catalytic partners, the cyclin dependent kinases (CDKs), play a great role in regulating the cell cycle. In interaction with CDKs, cyclins guide cells from one phase of the cell cycle to the next one. (Chakrabarty et al. 1996; Hui et al. 2013) Cyclins can be grouped into the mitotic cyclins (cyclin B1) and the G1/S cyclins (cyclin D1, cyclin E1) with the function of regulating the corresponding phase. (Chakrabarty et al. 1996; Hui et al. 2013) More precisely, cyclin D1 and E1 have a role in the proliferation, invasion, differentiation, apoptosis and angiogenesis of cells and tumor cells and can therefore be seen as key oncogenes. Up to date, the true mechanism of cyclin D1 and E1 and how the work is not fully elucidated. (Hui et al. 2013) A study displayed that cyclin D1 can be used as an immunohistological marker to identify the proliferating potential of tumor cells in diffuse gliomas. Strong correlations were found between cyclin D1 and Ki67/MIB1

in grade II astrocytomas but for grade III astrocytomas this correlation could not be affirmed. (Chakrabarty et al. 1996) In comparison to Ki67/MIB1, the general cyclin D1 expression in all grades of gliomas was higher which leads to the suggestion that cyclin D1 cells represent a group in which proliferation and therefore Ki67/MIB1 expression can still take place. In general, cyclin D1 overexpression can be found in astrocytic tumors with an increasing expression in high grade malignant tumors. (Chakrabarty et al. 1996) Looking at our results for Cyclin D1 and WT1, in astrocytomas grade II and III Cyclin D1 and WT1 have equal sifts as in pHH3 and Ki67/Mib1. For oligodendrogliomas grade II and III this phenomenon could not be displayed. These markers consequently tend to show a certain diagnostic value but there are more precise and sensitive ones.

5 Summary

In the past decades, a major revolution in the diagnosis of low grade gliomas occurred. The current literature shows that there are already many immunohistological markers that trend to have a diagnostic value even though they are not in routine use yet. Furthermore, with rapid research ongoing, a huge possibility for further immunohistological test was build. From my point of view, the next big step in the field of gliomas will be that the diagnosis can purely be made by molecular and genetic tests and therefore create fully objective defined groups. These groups can then form the foundation of further research, just as we can now see it in the WHO 2016 Classifications' more narrowly defined groups and consequently improve the diagnostic algorithm, therapy, patient care and outcome within that particular group. I additionally think that in the near future not only the now present molecular biomarkers and genetic determinants will be important, rather newer, more precise and specific ones will be developed for the different entities. Also, the way of analyzing molecular data might change to a more precise way, meaning immunohistochemistry or FISH might not be the gold standard in the future because by then, better and more reliable test are created.

6 References

Ahmed S, Rashed H, Hegazy A, Mohamed AM, Elmesallamy W (2016): Prognostic value of ALDH1, EZH2 and Ki-67 in astrocytic gliomas. Turk Patoloji Derg 32, 70–81

Bailey PCH (1926): A classification of the tumours of the glioma group on a histogenetic basis with a correlated study of prognosis. Can Med Assoc J 1925

Banan R, Hartmann C (2017): The new WHO 2016 classification of brain tumors—what neurosurgeons need to know. Acta Neurochir (Wien) 159, 403–418

Brat DJ, Perry A (2010): Astrocytic and Oligodendroglial Gliomas. 63–101

Bromberg JEC, van den Bent MJ (2009): Oligodendrogliomas: Molecular Biology and Treatment. Oncologist 14, 155–163

Capper D, Reifenberger G (2015): Klassifikation von Gliomen. Nervenarzt 86, 672–683

Chakrabarty A, Bridges LR, Gray S (1996): Cyclin D1 in astrocytic tumours: An immunohistochemical study. Neuropathol Appl Neurobiol 22, 311–316

Cohen AL, Holmen SL, Colman H (2013): IDH1 and IDH2 mutations in gliomas. Curr Neurol Neurosci Rep 13, 1–7

Colman H, Giannini C, Huang L, Gonzalez J, Hess K, Bruner J, Fuller G, Langford L, Pelloski C, Aaron J, et al. (2006): Assessment and prognostic significance of mitotic index using the mitosis marker phospho-histone H3 in low and intermediate-grade infiltrating astrocytomas. Am J Surg Pathol 30, 657–664

Daumas-Duport C, Scheithauer B, O'Fallon J, Kelly P (1988): Grading of astrocytomas: A simple and reproducible method. Cancer 62, 2152–2165

Giannini C, Scheithauer BW, Weaver AL, Burger PC, Kros JM, Mork S, Graeber MB, Bauserman S, Buckner JC, Burton J, et al. (2001): Oligodendrogliomas: Reproducibility and prognostic value of histologic diagnosis and grading. J Neuropathol Exp Neurol 60, 248–262

Goodenberger ML, Jenkins RB (2012): Genetics of adult glioma. Cancer Genet 205, 613–621

Huang L, Jiang T, Yuan F, Li GL, Cui Y, Liu EZ, Wang ZC (2009): Correlation of

chromosomes 1p and 19q status and expressions of O 6-methylguanine DNA methyltransferase (MGMT), p53 and Ki-67 in diffuse gliomas of World Health Organization (WHO) grades II and III: A clinicopathological study. Neuropathol Appl Neurobiol 35, 367–379

Hui W, Yuntao L, Lun L, WenSheng L, ChaoFeng L, HaiYong H, Yueyang B (2013): MicroRNA-195 Inhibits the Proliferation of Human Glioma Cells by Directly Targeting Cyclin D1 and Cyclin E1. PLoS One 8

Huse JT, Phillips HS, Brennan CW (2011): Molecular subclassification of diffuse gliomas: Seeing order in the chaos. Glia 59, 1190–1199

Huse JT, Diamond EL, Wang L, Rosenblum MK (2015): Mixed glioma with molecular features of composite oligodendroglioma and astrocytoma: A true “Oligoastrocytoma”? Acta Neuropathol 129, 151–153

Johnson DR, Guerin JB, Giannini C, Morris JM, Eckel LJ, Kaufmann TJ (2017): 2016 Updates to the WHO Brain Tumor Classification System: What the Radiologist Needs to Know. Radiographics 37, 2164–2180

Kernohan JW, Mabon RF (1949): A simplified classification of the gliomas. Proc Staff Meet Mayo Clin 24, 71–75

Kijima N, Hashimoto N, Chiba Y, Fujimoto Y, Sugiyama H, Yoshimine T (2016): Functional Roles of Wilms’ Tumor 1 (WT1) in Malignant Brain Tumors. Wilm Tumor 1, 261–272

Kim YH, Nobusawa S, Mittelbronn M, Paulus W, Brokinkel B, Keyvani K, Sure U, Wrede K, Nakazato Y, Tanaka Y, et al. (2010): Molecular classification of low-grade diffuse gliomas. Am J Pathol 177, 2708–2714

Komori T (2017): The 2016 WHO Classification of Tumours of the Central Nervous System: The Major Points of Revision. Neurol Med Chir (Tokyo) 57, 301–311

Labussiere M, Sanson M, Idbaih A, Delattre J-Y (2010a): IDH1 gene mutations: a new paradigm in glioma prognosis and therapy? Oncologist 15, 196–9

Labussiere M, Wang XW, Idbaih A, Ducray F, Sanson M (2010b): Prognostic markers in gliomas. Futur Oncol 6, 733–739

Ligon KL, Alberta JA, Kho AT, Weiss J, Kwaan MR, Nutt CL, Louis DN, Stiles CD, Rowitch DH (2004): The oligodendroglial lineage marker OLIG2 is universally expressed in diffuse gliomas. J Neuropathol Exp Neurol 63, 499–509

Ligon KL, Fancy SPJ, Franklin RJM, Rowitch DH (2006): Olig gene function in CNS development and disease. Glia 54, 1–10

Lin L, Zheng Y, Tu Y, Wang Z, Liu H, Lu X, Xu L, Yuan J (2015): MicroRNA-144 suppresses tumorigenesis and tumor progression of astrocytoma by targeting EZH2. Hum Pathol 46, 971–980

Louis DN (2006): Molecular Pathology of Malignant Gliomas. Annu Rev Pathol Mech Dis 1, 97–117

Louis DN (2012): The next step in brain tumor classification: „let us now praise famous men“. Or molecules? Acta Neuropathol 124, 761–762

Louis DN, Perry A, Burger P, Ellison DW, Reifenberger G, von Deimling A, Aldape K, Brat D, Collins VP, Eberhart C, et al. (2014): International Society Of Neuropathology--Haarlem consensus guidelines for nervous system tumor classification and grading. Brain Pathol 24, 429–435

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

Lu QR, Park JK, Noll E, Chan JA, Alberta J, Yuk D, Alzamora MG, Louis DN, Stiles CD, Rowitch DH, Black PM (2001): Oligodendrocyte lineage genes (OLIG) as molecular markers for human glial brain tumors. Proc Natl Acad Sci 98, 10851–10856

Ludwig K, Kornblum HI (2017): Molecular markers in glioma. J Neurooncol 134, 505–512

Mittal S, Szlaczky MC, R.Barger G (2011): Low-grade gliomas in adults. 1–18

Oberheim Bush NA, Chang S (2016): Treatment Strategies for Low-Grade Glioma in Adults. J Oncol Pract 12, 1235–1241

Paulus W (2009): GFAP, Ki67 and IDH1: perhaps the golden triad of glioma immunohistochemistry. Acta Neuropathol 118, 603–604

Purkait S, Sharma V, Jha P, Sharma MC, Suri V, Suri A, Sharma BS, Sarkar C (2015): EZH2 expression in gliomas: Correlation with CDKN2A gene deletion/ p16 loss and MIB-1 proliferation index. Neuropathology 35, 421–431

Rauscher J, Beschorner R, Gierke M, Bisdas S, Braun C, Ebner FH, Schittenhelm

J (2014): WT1 expression increases with malignancy and indicates unfavourable outcome in astrocytoma. J Clin Pathol 67, 556–561

Sanson M, Thillet J, Hoang-Xuan K (2004): Molecular changes in gliomas. Curr Opin Oncol 16, 607–613

Scheithauer BW (2009): Development of the WHO Classification of Tumors of the Central Nervous System. Brain Pathol 19, 551–564

Senetta R, Verdun di Cantogno L, Chiusa L, Castellano I, Gugliotta P, Sapino A, Cassoni P (2013): A „weighted“ fluorescence in situ hybridization strengthens the favorable prognostic value of 1p/19q codeletion in pure and mixed oligodendroglial tumors. J Neuropathol Exp Neurol 72, 432–41

Siegal T (2015): Clinical impact of molecular biomarkers in gliomas. J Clin Neurosci 22, 437–444

Skjulsvik AJ, Mørk JN, Torp MO, Torp SH (2014): Ki-67/MIB-1 immunostaining in a cohort of human gliomas. Int J Clin Exp Pathol 7, 8905–8910

Theeler BJ, Yung WKA, Fuller GN, De Groot JF (2012): Moving toward molecular classification of diffuse gliomas in adults. Neurology 79, 1917–1926

Van Den Bent M, Smits M, Kros JM, Chang SM (2018): Diffuse Infiltrating Oligodendroglioma and Astrocytoma. 35

Van Den Bent MJ (2010): Interobserver variation of the histopathological diagnosis in clinical trials on glioma: A clinician’s perspective. Acta Neuropathol 120, 297–304

Van Den Bent MJ, Weller M, Wen PY, Kros JM, Aldape K, Chang S (2017): A clinical perspective on the 2016 WHO brain tumor classification and routine molecular diagnostics. Neuro Oncol 19, 614–624

Virchow R (1863): Die Krankhaften Geschwülste.

Wesseling P, Kros JM, Jeuken JWM (2011): The pathological diagnosis of diffuse gliomas: Towards a smart synthesis of microscopic and molecular information in a multidisciplinary context. Diagnostic Histopathol 17, 486–494

Wilcox P, Li CCY, 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

Yan H, Parsons W, Jin G (2009): IDH1 and IDH2 Mutations in Gliomas. N Engl J Med 360, 765–773

Yannick M, Sanson M, Mokhtari K, Leuraud P, Kujas M, Delattre JY, Poirier J, Zalc B, Hoang-Xuan K (2001): OLIG2 as a specific marker of oligodendroglial tumour cells. Lancet 358, 298–300

Zeng A, Hu Q, Liu Y, Wang Z, Cui X, Li R, Yan W, You Y (2015): IDH1/2 mutation status combined with Ki-67 labeling index defines distinct prognostic groups in glioma. Oncotarget 6

Zhu P, Zhang C-B, Yang P, Chen J, Liu Y-Q, Hu H-M, Huang H, Bao Z-S, Zhang W, Kong W-J, Jiang T (2016): Phosphohistone H3 (pHH3) is a prognostic and epithelial to mesenchymal transition marker in diffuse gliomas. Oncotarget 7

Zülch KJ, Avtsyn AP, Barnar RO, Brucher JM, Earle KM, Fankhauser R, Ishida Y, Kunicki A, Olvera Rabiela JE, Rubinstein LJ, Sobin LH (1979): Histological typing of tumours of the central nervous system. Office of Publications, World Health Organization, Geneva Geneva

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