Original Article

The Applicability of Haarlem Integrated Diagnostic System in Diffuse Glial Tumors and The Role of PDGFRA Amplification as a Marker of Recurrence and Prognosis

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Background: With the help of genetic studies, it is possible to obtain information about diagnosis and prognosis of glial tumors.
Aims: We aimed to categorize the cases according to the new World Health Organization Central Nervous System classification by reconsidering the histologic features of oligodendrogliomas, astrocytomas and oligoastrocytomas. We also evaluated whether these genetic features are prognostic.
Study Design: Diagnostic accuracy study
Methods: The status of molecular changes proposed by current classifications in 60 cases diagnosed with histologically grade II and grade III oligodendrogliomas, astrocytomas and oligoastrocytomas were evaluated. IDH1 (R132H), ATRX, and p53 antibodies were applied immunohistochemically. The 1p / 19q status and PDGFR-α / CEP4 amplification were evaluated by Fluorescence In Situ Hybridization. The SPSS 21.0 statistical package program was used for the selected tests in the statistical analysis of the findings. Sensitivity, specificity, positive predictive level, negative predictive level, and accuracy rate were evaluated in accordance with the specified threshold levels. The significance value was regarded as $p < 0.05$.
Results: The diagnosis changed with genetic alterations. Only 1 case (16%) was left in the same diagnosis as anaplastic oligoastrocytoma. The IDH mutation was 97,5% sensitive in oligodendrogliomas,88.8 in astrocytomas. For predicting oligodendroglial tumor, the sensitivity, spesificity, positive predictive value and negative predictive value of the cut-off value for 1p/19q codeletion was 100%. For predicting astrocytic tumor, the sensitivity, spesificity, positive predictive value and negative predictive value of the cut-off values for ATRX mutation were 72.2%, 100%, 100% and 89.1%, respectively, whereas for p53 mutation 77.7%, 95.1%, 87.5% and 90.6%, respectively. PDGFR-α / CEP4 amplification was not detected in any of the cases. Polisomia was detected in 13 (21%) of the cases. These cases were high grade astrocytomas but there was no statistically significant difference between the integrated diagnostic groups according to CEP4 positivity (p=0,157). There was association between IDH mutation and 1p / 19q loss with longer survival (respectively p=0,147 and p=0,178).
Conclusion: In grade 2 and grade 3 glial tumors, pathological diagnosis is not possible only with histopathological examination. For the diagnosis of oligodendroglial tumor 1p / 19q codeletion, fort he diagnosis of astrocytic tumor ATRX and p53 mutation should be determined. PDGFRA amplification is more common in high grade glial tumors and is not included in the class of glial tumor with intermediate grade. The presence of IDH mutation and 1p / 19q codeletion in glial tumors is associated with good prognosis.
Keywords: Grade II-III glial tumor, WHO 2016 central nervous system classification, genetic changes

Glial tumors are the most prevalent primary brain tumors. Diffuse glial tumors of the central nervous system are classified by World Health Organization (WHO) as astrocytomas (A), oligodendrogliomas (OD) and mixed oligoastrocytomas (OA) according to histological findings (1). A new classification based on genetic evidence was published by WHO in 2016. Molecular and cytogenetic examinations also produced results that further contributed to the diagnosis and prognosis of these tumors in addition to the classification performed mostly by histomorphological methods.
ODs have been found that the combined loss of 1p and 19q is closely associated with classical OD morphology and better prognosis (2).
In glial tumors, point mutations at codon 132 in the IDH1 gene (human cytosolic NADPH- dependent isocitrate dehydrogenase 1) may be observed. The most common mutation is R132H, and the IDH1 protein which is the product of this mutation can be visualized immunohistochemically. Another less common IDH mutation is IDH2, and it is another IDH isoform, which does not yet have commercial primer antibodies for immunohistochemistry (IHC), and can be demonstrated by DNA sequence analysis.

Its most common mutation is R172W (3).
As constitute 10-11% of glial tumors and it is known through the new classification that the genetic alterations that differentiate grade II-III astrocytic tumors from OD are p53 and ATRX mutations (4). p53 mutation rate in all A grades is approximately 30-50% (5). Alpha- thalassemia mental retardation syndrome X gene (ATRX) is located in chromosome Xq21.1, and encodes a protein that is part of the H3.3-ATRX-DAXX chromatin regulatory pathway (6). It is often found together with IDH mutation and they exclude each other with loss of 1p/19q, which are very helpful in diagnosis. However, it is not yet clear whether it has an independent role from other molecular markers in terms of prognosis (7).
High grade astrocytomas (HGA) are characterized by alterations in receptor tyrosine kinase (RTK) signaling, and abnormal platelet-derived growth factor (PDGF) signaling has been demonstrated Although there is no generally accepted predictive value of detecting PDGFR overactivity for glial tumors, this genetic alteration is of great diagnostic and prognostic importance (8).
OA tumors are still very controversial in the 2016 WHO classification. They constitute 0.9% of all brain tumors and 3.3% of primary brain tumors. The diagnosis is OA when oligodendroglial and astrocytic genetic alterations coexist (4).
In the 2016 classification, a group of tumors specified as "NOS (unspecified)" were included. "NOS" is diagnosed when molecular parameters that are the genetic alterations in glioma cannot be examined or if no informative genetic alteration can be detected (4).

Material and Methods

Patients and samples

Between the years 2011–2016, 60 gliomas (19 OD (%31.6), 10 AOD (%16.6), 19 OA (%31.6), 8 AOA (%13.3), 2 A (%3), 2 AA (%3)) were examined.
Archival material from the Department of Pathology was used for histopathological, immunohistochemical, and molecular analyses. Slides of all these cases were reviewed (Figure 1 and figure2). All the cases were classified and graded according to the existing 2016 WHO criteria.
The central ethics committee approved the study (2016/A18). The funding of the study was provided by the scientific research project unit of the hospital.
Immunohistochemistry
IHC was carried out at least on one representative block in all the cases. IHC was performed using primary antibody against the following antigens- IDH1 R132H (Dianova, dilution 1:40), ATRX (Sigma, dilution 1:300) and p53 (Dako, dilution 1:50). Cases showing cytoplasmic positivity for IDH1 in >10% tumor cells were considered positive. Loss of nuclear staining for ATRX in tumor cells (>90%) was considered positive for *ATRX* mutation. Nuclear positivity for p53 in >50% tumor cells was considered positive.

Mutation analysis:

Mutations in exon 4 of IDH1 and IDH2 genes (well-known hotspot regions for oncogenic mutations) (Figure 3) were analyzed by PCR based direct sequencing using representative formalin-fixed paraffin-embedded (FFPE) tumor samples. DNA was extracted from the FFPE tissue by commercially available kit (50) (catalog #: 56404) (QIAGEN, Hilden, Germany) as per manufacturer's instruction. DNA concentrations of samples were assessed spectrophotometrically using a Nanodrop 1000 spectrophotometer (Thermo Scientific, USA). PCR amplifications were performed in a Thermal Cycler (ABI, Applied Biosystems, USA) using HotStarTaq DNA Polymerase kit (catalog #: 203205) (QIAGEN, Hilden, Germany) and appropriate primers;

- IDH1 Forward: 5'CCAAGTCACCAAGGATGCTG'3
- IDH1 Reverse: 5'TCACATTACTGCCAACATGACTT'3
- IDH2 Forward: 5'CCGTCTGGCTGTGTTGTTG'3
- IDH2 Reverse: 5'AGTCTGTCGCCTTGTACTGC'3)

PCR reactions were run as total volume of 50 μl reaction mixtures consisting of nuclease free water, 5 μl 10x PCR Buffer, 2 μl 25mM MgCl2, 1.5 μl 10 mM dNTP mix (ABI, Applied Biosystems, USA), 6 μl of each primer (4pmol/μl), 0.25 μl of Hot Start Taq DNA polymerase and 200 ng of each tumor DNA. After an initial denaturation at 95°C for 15 minutes, 42 cycles were performed of 30 seconds denaturation at 95°C, 30 seconds annealing at 58°C and 45 seconds extension at 72°C, followed by a final extension of 10 minutes at 72°C. The intensity of PCR products were checked by running 5 μl of each PCR reaction with 2 μl of loading dye on a 2% agarose gel. Reagent contamination control was achieved by examining lane for "No DNA" blank tube. Then, all succeeded PCR products were purified using QIAquick PCR Purification Kit (catalog #: 28106) (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The purified amplicons were submitted to direct sequencing in both directions (forward and reverse) using reagents from the Big Dye Terminator v3.1 Cycle Sequencing kit (ABI, Applied Biosystems, USA) in accordance with the manufacturer's protocol. After ethanol precipitation, subsequent products were run on the ABI-3730 (48 capillary) automatic sequencer (Applied Biosystems, USA). Bidirectional sequence traces were analyzed with SeqScape® Software v3.0 (Applied Biosystems, USA) and manually reviewed.

FISH 1p/19q and PDGFRA/CEP4

FISH analysis was performed on 5-micron thick FFPE tissue samples.
Deparaffinization, pre-hybridization and hybridization steps have been done according to the datasheet. One hundred tumors cells were analyzed on fluorescent microscope (Olympus BX61; Olympus Optical, Japan). The cells were captured on a computer system with a digital camera (XLMM, Dage-MTI, IN, USA) and compatible software (Duet®, Bioview Ltd., Israel). Dual-color paired probes for 1p and 1q (1p36 Spectrum Orange and 1q25 Spectrum Green, Vysis LSI probes, Abbott Molecular, Des Plaines, IL) were hybridized simultaneously on one slide and similarly for 19q and 19p (19q13 Spectrum Orange and 19p13 Spectrum Green, Vysis LSI probes, Abbott Molecular, Des Plaines, IL) were used on a separate slide. Using a fluorescence microscope with an oil immersion 100x objective, each hybridization was analyzed by locating an area with a high proportional density of neoplastic nuclei and evaluating scoring a minimum of 100 non- overlapping interphase

nuclei for the numbers of green and orange signals in each nucleus. With the probe pairs configured as they are, we calculated the proportion of orange (target chromosome arm) to green (comparison/control chromosome arm) signals. Based on laboratory experience a proportion <0.80 represents a deletion. The ratio of SpectrumOrange to SpectrumGreen signals (total orange/total green) was calculated (Figure 4).
PDGFR-α Break Apart was studied in chromosome 4q12 loci with separate bi-color probes (green and orange) (Cytotest, RO, USA). Orange signal was obtained when 3' of the target probes were hybridized in the 4q12 loci, whereas green signal was obtained when 5' of the target probes were hybridized. CEP4 4p11-q11 aqua probe was added as control probe. Aqua signal was obtained by centromeric region hybridization. In normal (negative) cells, two aqua and two fusion yellow (orange + green) signals were observed.
In an abnormal cell with an increase in the number of copies in the 4q12 loci, more than two fusion yellow and aqua signals were observed. If all 3 signals increase at the same time and the number of cells with >2 and <6 signals is more than 10%, polysomia is detected. Polysomia detected cases were separated into two groups: 3-4 signals were evaluated as low-level polysomia, and 5-6 signals were evaluated as high-level polysomia (Figure 5).
PDGFR-α/CEP4 centromere ratio was assessed.
Amplification: If the PDGFR-α/CEP4 signal ratio was ≥2, it was evaluated as amplification (9).

Statistical analysis

In our study, SPSS version 21.0 (Statistical Package for the Social Sciences, Chicago, IL, USA) was used for statistical analysis of data.
Survival using the Kaplan–Meier method was calculated based on date of initial surgery and pathological confirmation and the date of death. The results were evaluated in 95% confidence interval.
Sensitivity, specificity, positive predictive level (PPV), negative predictive level (NPV) and accuracy rate were evaluated in accordance with the specified threshold levels. The significance value was regarded as $p < 0.05$.
We performed post hoc power analysis by G*Power 3.1.9.2. While Type II error (β) probability was observed less than 0.01 (power >0,99) for combined diagnostic algorithm and single ATRX, P53 mutations and 1p19q deletions but less than 0.40 (power<0.60) for IDH mutation and CEP4 amplification.

Results

The demographic and clinical characteristics of the 75 patients are shown in Table 1.
Histopathologically, 19 cases were diagnosed with OD (%31.6), 10 with AOD (%16.6), 19 with OA (%31.6), 8 with AOA (%13.3), 2 with A (%3), and 2 with AA (%3). We reclassified all of the cases according to the genetic alterations detected. Overall, there was a diagnosis change in 28 cases (46.6%) (Figure 6). 1 case was left as AOA since it had both 1p/19q loss and ATRX mutation.
Immunohistochemical analyses using IDH1 (R132H) antibody showed positive results in 52 cases (86.6%) and negative results in 8 cases (13.3%).
Guanine to Adenine point mutation in the IDH1 R132H region was detected by sequencing in 37 out of 52 cases immunohistochemically expressing IDH1, but no mutation was detected in 4 cases by sequencing although IDH1 expression was immunohistochemically confirmed in repeated experiments.
In 8 cases in which IDH1 expression were not detected via immunohistochemical analysis, point mutation in IDH1 R132H region was detected by sequencing in 3 cases, and guanine to adenine and thymine to adenine change in IDH2 R172K and R172W regions were detected by sequencing in 2 cases. In the remaining 3 cases, no IDH mutation was found by immunohistochemical methods or Sanger sequence analysis.
ATRX mutation and 1p/19q loss are mutually exclusive genetic alterations ($p < 0.001$) (Table 2).
In the evaluation of CEP4 polysomia, polysomia was detected in 13 out of 50 cases with positive signal (26%). 7 (53.8%) of these cases had high-level polysomia, while 6 (46.1%) had low-level polysomia. CEP4 amplification showed sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), respectively, 42.8%, 79.4%, 46.1%, 77.1% for astrocytoma (Table 3).
For predicting glial tumor, the sensitivity, specifity, PPV, NPV and the accuracy of the cut-off value for ATRX mutation, p53 mutation and 1p/19q codeletion were indicated in Table 3. ATRX mutation showed a sensitivity of 72.2%, a specificity of 100%, a PPV of 100%, and a NPV of 89.1% for astrocytoma (p=0,002). p53 mutation showed a sensitivity of 77.7%, a specificty of 95.1%, a PPV of 87.5%, and a NPV of 90.6% (p=0,005). For predicting oligodendroglial tumor, the sensitivity, spesificity, PPV and NPV of the cut-off value for 1p/19q codeletion was 100% (p<0,001).
The cases with the best survival rates were diagnosed with OD, and the cases with the worst survival were diagnosed with A, AA, and AOD (Figure 7). There was no statistically significant difference (p= 0.901).
The survival time of cases with IDH mutation with 1p/19q loss were longer (p= 0 ,174 and p= 0,178 respectively) (Figure 8 and Figure 9).
A survival rate of 76.2% was observed in cases with negative PGFR-α amplification. A survival rate of 74.2% was observed in cases with negative CEP4 polysomia, and 75% in cases with positive CEP4 polysomia (p = 0.966).

Discussion

In our study, molecular examinations were performed in accordance with the criteria of 2016 WHO CNST classification recommended for grade II and grade III ODs, As and OAs, and new integrative diagnoses were established. Diagnosis changed completely in 28 cases out of 60 cases evaluated in this study (46.6%).
The aim of this study was to investigate the contributions of 1p/19q loss typically observed in oligodendroglial tumors, and ATRX mutation and p53 expression observed in astrocytic tumors and IDH mutation, which is known to have prognostic value in glial tumors and to re-diagnose cases according to the new classification. In addition, PDGFR- α/CEP4 amplification, which is more frequently seen in HGA, was also included in the study to assess its implications in pathology applications.
In older classifications, OA diagnosis was very common, whereas in the new classification OA diagnosis covers a very small area. There are still cases diagnosed with OA (1).

Similar to our study, in a study on 43 OA cases, Felix Sahm et al. assessed 1p/19q, IDH, p53 and ATRX status of cases. Based on the results of the study, loss of 1p/19q, ATRX mutation and p53 nuclear expression were observed simultaneously in one case. This case was described as a molecular hybrid since it exhibited genetic alterations specific to ODs and As (10).
In another study, ATRX expression and loss of 1p/19q were simultaneously observed in only 3 of 1041 glial tumors (11).
IDH1 mutation is the first genetic event during gliomagenesis in ODs and As. Following IDH mutation, different genetic alterations occur depending on tumor specific differentiation and behavior pattern (12). IDH mutation has been an important area of diagnosis and prognosis, and contributes greatly to routine pathological studies. Capability to examine mutation assessments by IHC has enabled these assessments to be rapidly included in routine pathology practices. Parsons et al. investigated IDH mutation in 22 cases of glioblastoma and called attention to this topic (13). A more recent study of 1010 glial tumors from 6 centers examined the role of IDH mutation in glial tumorigenesis in general (14).
Today, it is known that 90% of mutations in IDH gene are IDH1(R132H) mutations (13). IDH1 mutation rates are reported as 42.9-100% in OD, 46-100% in AOD, 74-90% in A, and 27.3-73% in AA (15).
In our study, IDH1 (R132H) expression was detected in 23 (92%) OD cases, 12 (80%) AOD cases, 1 (100%) OD NOS case, 12 (85.7%) A cases, 3 (75%) AA cases and 1 (100%) AOA case. The incidence of IDH1 (R132H) expression that we detected in all intermediate-grade glial tumors included in the study is consistent with the high values reported in the literature (15).
There are commercially available primary antibodies for IDH1 (R132H) mutation assessment, and no commercial antibodies are available for other commonly identified IDH1 regions such as IDH1 R132G, R132S, and R132C and for IDH2 assessment. Consistent with the literature, there are cases in our study in which immunohistochemical IDH1 expression was not detected, whereas mutation in IDH1 (R132) was detected in the same region following Sanger sequence analysis (12). These results suggest that the specificity of the antibody used may not be high. Therefore, if there is a strong suspicion of false-negative IDH1 cases based on immunohistochemical assays, IDH mutation should be properly assessed using other methods.
IDH2 mutation is less frequent than IDH1 mutation, and they are mutually exclusive (3). In our study, IDH1 and IDH2 mutations were not observed simultaneously when DNA sequence analysis was performed.
Assessing 1p/19q loss in oligodendroglial neoplasias is an important aid in establishing a diagnosis (2). Cairncross et al. (1998) were the first ones to report that the combined loss of chromosome 1p/19q was predictive for chemotherapy response, and an effective prognostic factor for longer survival in anaplastic OD patients (16).
1p and 19q loss rates we observed in oligodendroglial tumors are consistent with those reported in the literature (17). Our results confirm the hypothesis that there are no oligodendroglial tumors without 1p/19q loss (4).
In our study we found a statistically negative correlation between ATRX mutation and 1p/19q loss ($p < 0.001$).
PDGFRA-α/CEP4 amplification and increase in gene copy numbers were assessed by FISH method in all cases, and no amplification was detected. CEP4 polysomia was detected mostly in astrocytic high grade tumors, consistent with the literature (18). In HGAs, *PDGFRA* amplification is common and can manifest as high-level or low-level amplifications. *PDGFRA* amplification increases with grade and is associated with a less favorable prognosis (19). Although biologically, this is probably more accurately described as a "high-level polysomy" rather than true gene amplification, the definition nonetheless correlates strongly with clinical outcome, including therapeutic response to tyrosine kinase inhibitors (20).
When survival rate was assessed according to the diagnoses of the cases, best survival rate was obtained in OD cases. Several studies have demonstrated that survival is better in ODs than in low-grade As. In a study conducted by Ohgaki et al. on 987 cases, median survival time was 11.6 years in OD, and 5.6 years in low-grade As. These values are similar for grade III tumors: a study conducted by the European Cancer Treatment and Research Organization - Brain Tumor Group, which included 368 patients, demonstrated that median survival time was 34.7 months in AOD (21).
IDH mutant tumors have better prognosis regardless of grade (12, 22). In our study, survivalrate was higher in cases with IDH mutation, but no statistically difference was there ($p= 0.147$).
The presence of 1p/19q loss has been associated with long overall survival in patients regardless of treatment (21). In our study, survival rate was 64.3% in cases without 1p/19q loss, and 81.6% in cases with 1p/19q loss ($p = 0.178$).
In summary, molecular glioma research has significantly advanced the understanding of glioma pathogenesis and identified a number of diagnostic, prognostic and/or predictive molecular markers that currently are on their way into clinical application. The presence or absence of *IDH* mutations, ATRX mutation, p53 mutation and 1p/19q codeletion can be used to define gliomas with characteristic distributions of clinical behavior, acquired genetic alterations and associated germline variants. This framework could be further refined through the incorporation of alterations in *PDGFRA* or other alterations that might be useful to consider in the diagnosis of glioma.

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References

1. Louis DN, Ohgaki H, Wiestler OD et al. The 2007 WHO Classification of Tumours of the Central Nervous System. Acta Neuropathol. (Berl.) 2007;114(2):97–109.
2. Aldape K, Burger PC, Perry A. Clinicopathologic aspects of 1p/19q loss and the diagnosis of oligodendroglioma. Arch Pathol Lab Med. 2007 Feb;131(2):242-51.
3. Cohen AL, Holmen SL, Colman H. IDH1 and IDH2 mutations in gliomas. Curr Neurol Neurosci Rep. 2013 May;13(5):345.
4. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. 2016 Jun;131(6):803-20.
5. Macaulay RJ. Impending Impact of Molecular Pathology on Classifying Adult Diffuse Gliomas. Cancer Control.

2015 Apr;22(2):200-5.
6. Haberler C, Wöhrer A. Clinical Neuropathology practice news 2-2014: ATRX, a new candidate biomarker in gliomas. Clin Neuropathol. 2014 Mar-Apr;33(2):108-11.
7. Eckel-Passow JE, Lachance DH, Molinaro AM, Walsh KM, Decker PA, Sicotte H et al. Glioma Groups Based on 1p/19q, IDH, and TERT Promoter Mutations in Tumors. N Engl J Med. 2015 Jun 25;372(26):2499-508.
8. Koschmann C, Zamler D, MacKay A, Robinson D, Wu YM, Doherty R et al. Characterizing and targeting PDGFRA alterations in pediatric high-grade glioma. Oncotarget. 2016 Oct 4;7(40):65696-65706.
9. Zneimer, S. Cytogenetic Abnormalities: Chromosomal, FISH, and Microarray-Based Clinical Reporting and Interpretation of Result. John Wiley & Sons, 2014.
10. Sahm F, Reuss D, Koelsche C, Capper D, Schittenhelm J, Heim S et al. Farewell to oligoastrocytoma: in situ molecular genetics favor classification as either oligodendroglioma or astrocytoma. Acta Neuropathol. 2014 Oct;128(4):551-9.
11. Huse JT, Diamond EL, Wang L, Rosenblum MK. Mixed glioma with molecular features of composite oligodendroglioma and astrocytoma: a true "oligoastrocytoma"? Acta Neuropathol. 2015 Jan;129(1):151-3.
12. Appin CL, Brat DJ. Molecular genetics of gliomas. Cancer J. 2014 Jan-Feb;20(1):66-72.
13. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P et al. An integrated genomic analysis of human glioblastoma multiforme. Science. 2008 Sep 26;321(5897):1807-12.
14. Hartmann C, Meyer J, Balss J, Capper D, Mueller W, Christians A et al. Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas. Acta Neuropathol. 2009 Oct;118(4):469-74.
15. Balss J, Meyer J, Mueller W, Korshunov A, Hartmann C, von Deimling A. Analysis of the IDH1 codon 132 mutation in brain tumors. Acta Neuropathol. 2008 Dec;116(6):597-602.
16. Cairncross JG, Ueki K, Zlatescu MC, Lisle DK, Finkelstein DM, Hammond RR et al. Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. J Natl Cancer Inst. 1998 Oct 7;90(19):1473-9.
17. Riemenschneider MJ, Jeuken JW, Wesseling P, Reifenberger G. Molecular diagnostics of gliomas: state of the art. Acta Neuropathol. 2010 Nov;120(5):567-84.
18. Puputti M, Tynninen O, Sihto H, Blom T, Mäenpää H, Isola J et al. Amplification of KIT, PDGFRA, VEGFR2, and EGFR in gliomas. Mol Cancer Res. 2006 Dec;4(12):927-34.
19. Phillips JJ, Aranda D, Ellison DW, Judkins AR, Croul SE, Brat DJ, Ligon KL,Horbinski C, Venneti S, Zadeh G, Santi M, Zhou S, Appin CL, Sioletic S, Sullivan LM, Martinez-Lage M, Robinson AE, Yong WH, Cloughesy T, Lai A, Phillips HS, Marshall R, Mueller S, Haas-Kogan DA, Molinaro AM, Perry A. PDGFRA amplification is common in pediatric and adult high-grade astrocytomas and identifies a poor prognostic group in IDH1 mutant glioblastoma. Brain Pathol. 2013 Sep;23(5):565-73.
20. Hirsch FR, Herbst RS, Olsen C, Chansky K, Crowley J, Kelly K, Franklin WA,Bunn PA Jr, Varella-Garcia M, Gandara DR. Increased EGFR gene copy number detected by fluorescent in situ hybridization predicts outcome in non-small-cell lung cancer patients treated with cetuximab and chemotherapy. J Clin Oncol. 2008 Jul 10;26(20):3351-7.
21. Li YX, Shi Z, Aibaidula A, Chen H, Tang Q, Li KK et al. Not all 1p/19q non-codeleted oligodendroglial tumors are astrocytic. Oncotarget. 2016 Oct 4;7(40):64615-64630.
22. Franceschi E, Mura A, De Biase D, Tallini G, Pession A, Foschini MP, Danieli D, Pizzolitto S, Zunarelli E, Lanza G, Bartolini D, Silini EM, Visani M, Di Oto E, Tosoni A, Minichillo S, Lamberti G, Lanese A, Paccapelo A, Bartolini S, Brandes AA. The role of clinical and molecular factors in low-grade gliomas: what is their impact on survival? Future Oncol. 2018 Jul;14(16):1559-1567.

Table 1. Clinical and molecular characteristics of the cases. Abbreviations: mo month, OS overall survival, FISH fluorescent in situ hybridization, IHC immunohistochemistry, OD Oligodendroglioma, AOD Anaplastic oligodendroglioma, OA Oligastrocytoma, AOA Anaplastic oligoastrocytoma, A Astrocytoma, AA Anaplastic astrocytoma

Clinicopathological and Mutational Features		Histopathological Diagnosis		
		OD-AOD	A-AA	OA-AOA
	No	n (%)	n (%)	n (%)
Number of Patients	60	29 (48.3)	4 (6.6)	27 (60)
Age (years)	Mean (range)	41.07 (24-66)	33.5 (30-42)	39.03 (12-69)
Gender	Male	13 (44.8)	3 (75)	20 (74)
	Female	16 (55.7)	1 (25)	7 (25.9)
Mean OS (mo)		23.4 (6-66)	10.5 (6-12)	32.6 (1-156)
1p, 19q FISH	Co-deletion	25 (96.1)	0 (0)	14 (51.8)
	No co-deletion	1 (3.8)	4 (100)	13 (48.1)
ATRX IHC	Mutant (loss)	1 (3.4)	4 (100)	9 (33.3)
	Wildtype (retained)	28 (96.5)	0 (0)	18 (66.6)
p53 IHC	Expression	1 (3.4)	4 (100)	15 (55.5)
	No expression	28 (96.5)	0 (0)	12 (44.4)
IDH-1 IHC	Expression	25 (86.2)	3 (75)	24 (88.8)
	No expression	4 (13.7)	1 (25)	3 (11.1)
IDH sequencing	Mutant	23 (95.8)	3 (100)	16 (69.5)
	Wildtype	1 (4.1)	0 (0)	7 (30.4)
CEP4 FISH	Polysomy	4 (17.3)	3 (75)	5 (25)
	No Polysomy	19 (82.6)	1 (25)	15 (75)
PDGFRA FISH	Amplification	0 (0)	0 (0)	0 (0)

Table 2. Correlation of the genetic changes in the study (*p <0,05).						
	ATRX mut	p53	IDH1mut	IDH2mut	IDH1exp	CEP4
1p/19q deletion	-0,699**	-0,659**	-0,139	0,255	0,430	-0,234
ATRX mut		0,528**	0,447**	-,205	,100	,269
p53			-,143	-,229	-,189	,234
IDH1mut				-0,459	,485*	,333
IDH2mut					-,414	-,293
IDH1exp						,015

Table 3: The sensitivity, specificity, positive predictive value, negative predictive value and the accuracy of the cut-off values of ATRX mutation, p53 mutation and CEP4 polysomy for astrocytoma and 1p/19q codeletion for oligodendroglioma				
Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
ATRX mutation	72.2 (46.4-89.2)	100 (89.3-100)	100 (71.6-100)	89.1 (75.6-95.9)
p53 mutation	77.7 (51.9-92.6)	95.1 (82.1-99.1)	87.5 (60.4-97.8)	90.6 (76.9-96.9)
1p/19q codeletion	100 (89-100)	100 (78.1-100)	100 (89-100)	100 (78.1-100)
CEP4 polysomy	42.8 (18.8-70.3)	79.4 (61.5-90.6)	46.1 (20.4-73.8)	77.1 (59.4-88.9)
PPV: positive predictive value; NPV: negative predictive value				

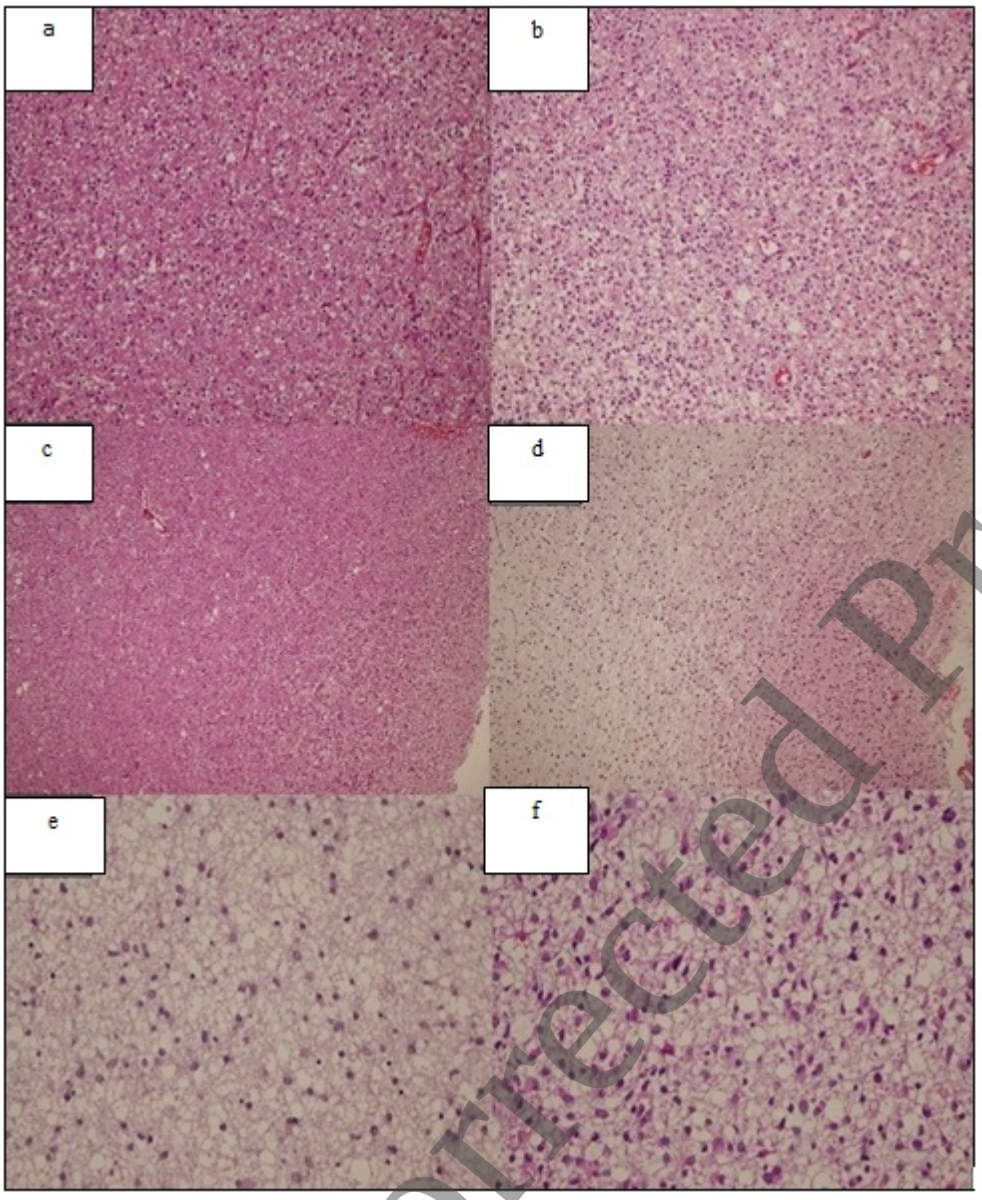

Figure 1. (a-f). Microscopic photographs of specimens from histopathological diagnoses (H / E, original magnifications: a, b: x200 c, d: x100, e, f: x400). Oligodendrogliom (a). Anaplastic oligodendrogliomas (b). Oligoastrocytoma (c). Anaplastic oligoastrocytoma (d). Astrocytoma (e). Anaplastic astrocytoma (f).

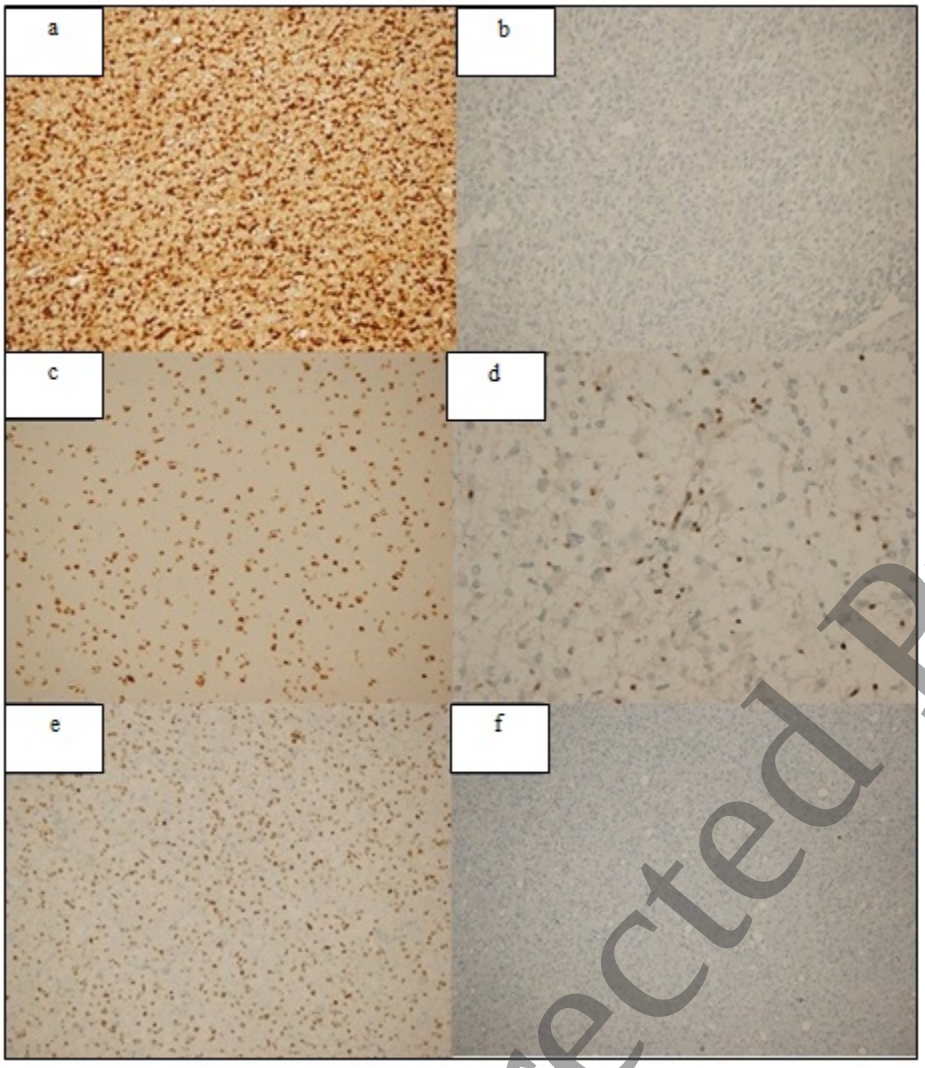

Figure 2.(a-f). Microscopic photographs of immunohistochemical changes (original magnifications: a, b, c, e: x200, d:x 400, f: x100). Cytoplasmic and nuclear positivity with IDH1 (R132H) antibody (a). Negative with IDH1 (R132H) antibody (b). Nuclear positivity (non-mutant) with ATRX antibody (c). Nuclear negativity (mutant) with ATRX antibody, positivity in vessel walls and reactive glial cells (d). Nuclear positivity with P53 antibody (90%, score 3+) (e). Nuclear negativity with P53 antibody (0%, score 0) (f).

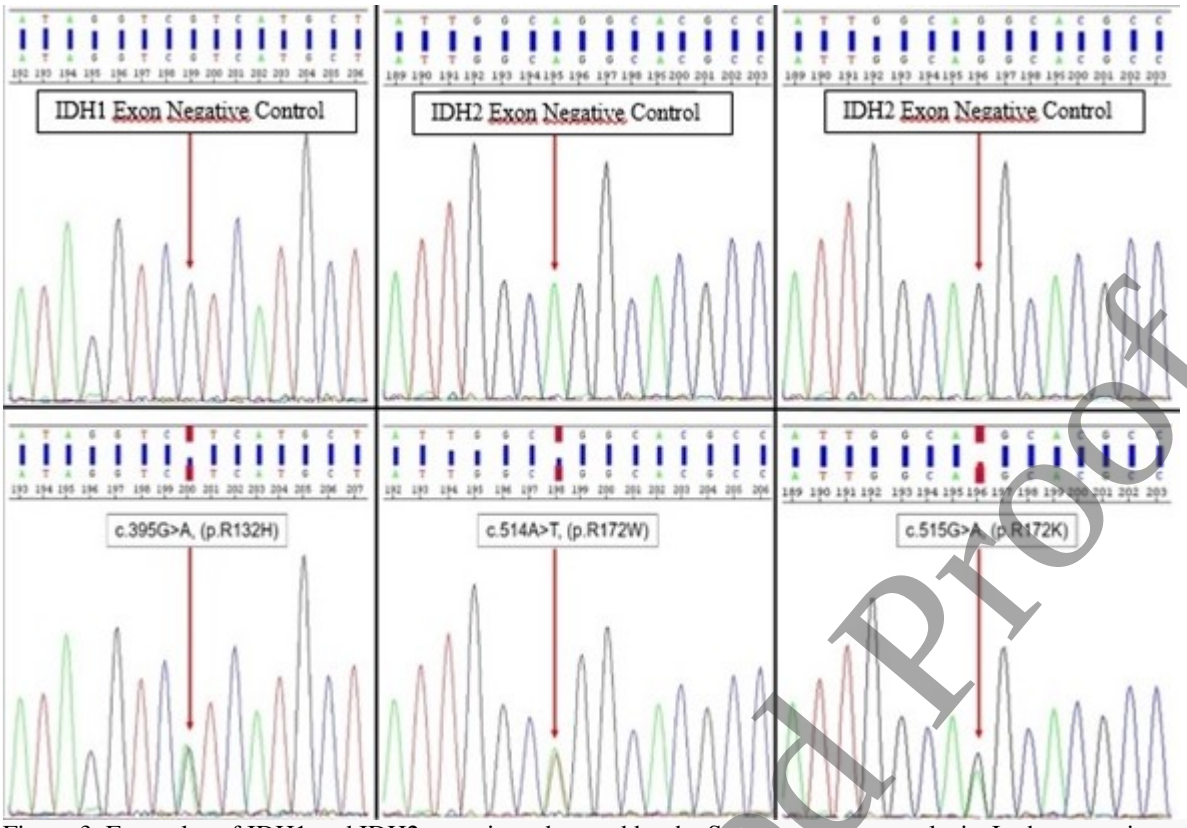

Figure 3. Examples of IDH1 and IDH2 mutations detected by the Sanger sequence analysis. In the upper image, IDH1 and IDH2 gene exogenous negative control for exon 4 and in the bottom image sample forward sequence electrophoresis of the mutated patients.

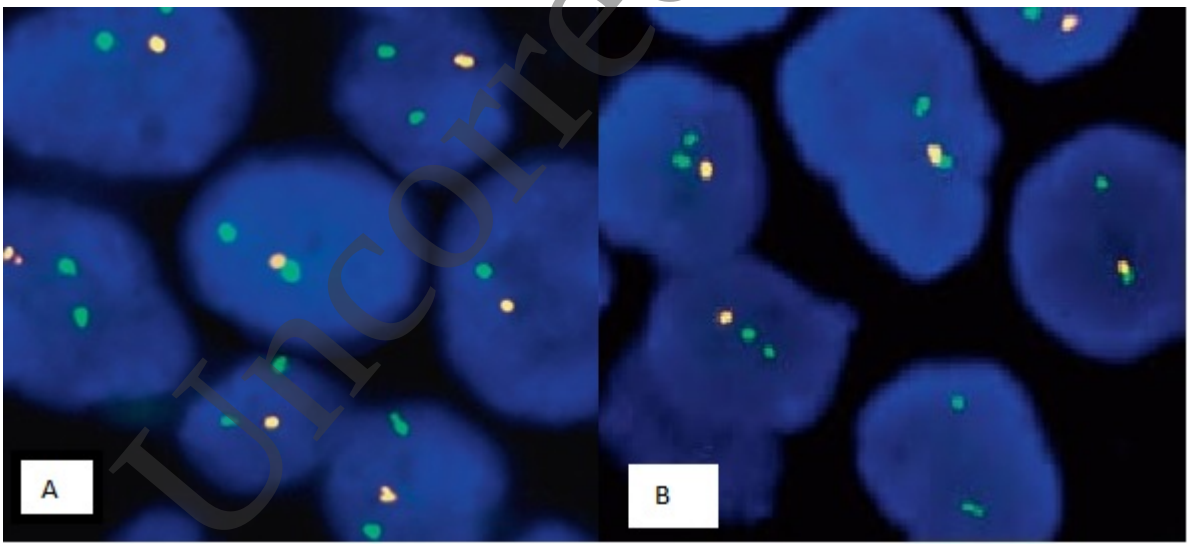

Figure 4. (a,b). Microscopic photographs of 1p / 19q FISH studies. OD case showing 1p loss (red signal: 1p36, green signal 1q25) (a). Loss of 19q at the same time (red signal: 19q13, green signal 19p13) (b).

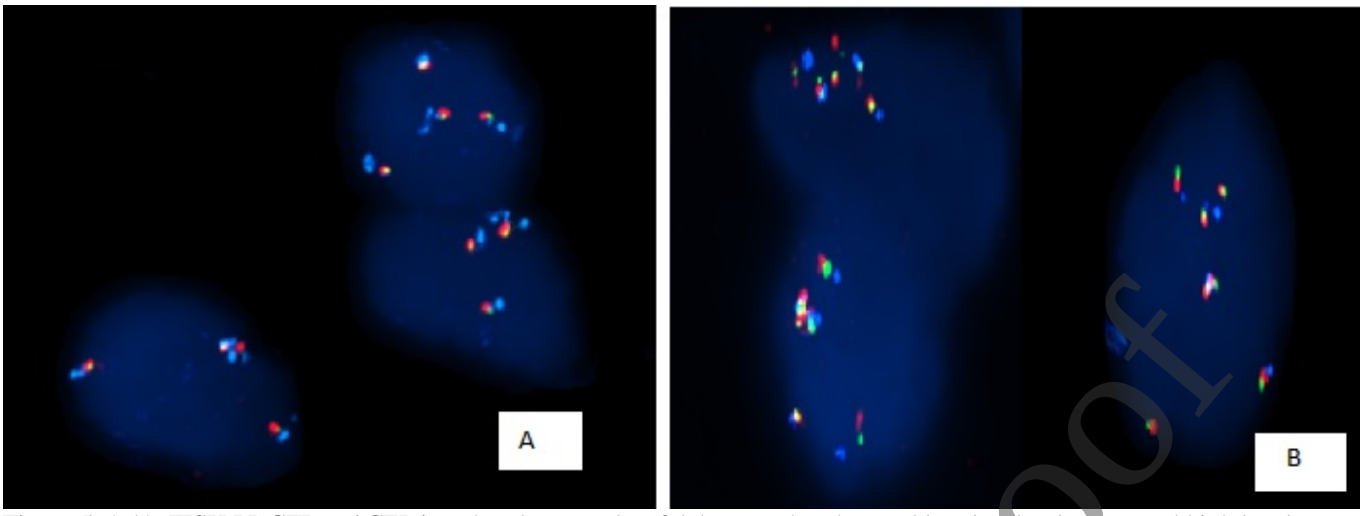

Figure 5. (a,b). FISH PDGFR-α / CEP4 study; photographs of AA cases that detected low level polysomy and high level polysomy. low-level polysomy (a). high-level polysomy (b).

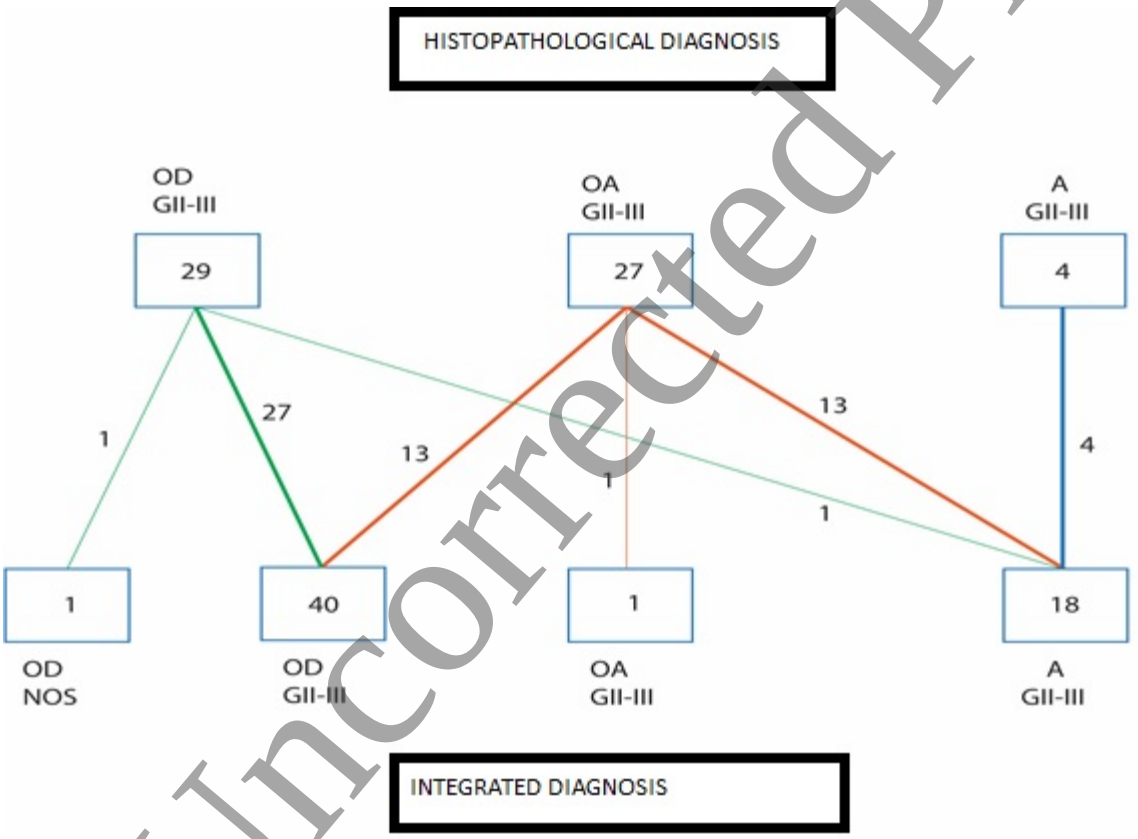

Figure 6. Numerical responses of histopathological diagnosis (first diagnosis) to integrated diagnosis (new diagnosis) (G: Grade)

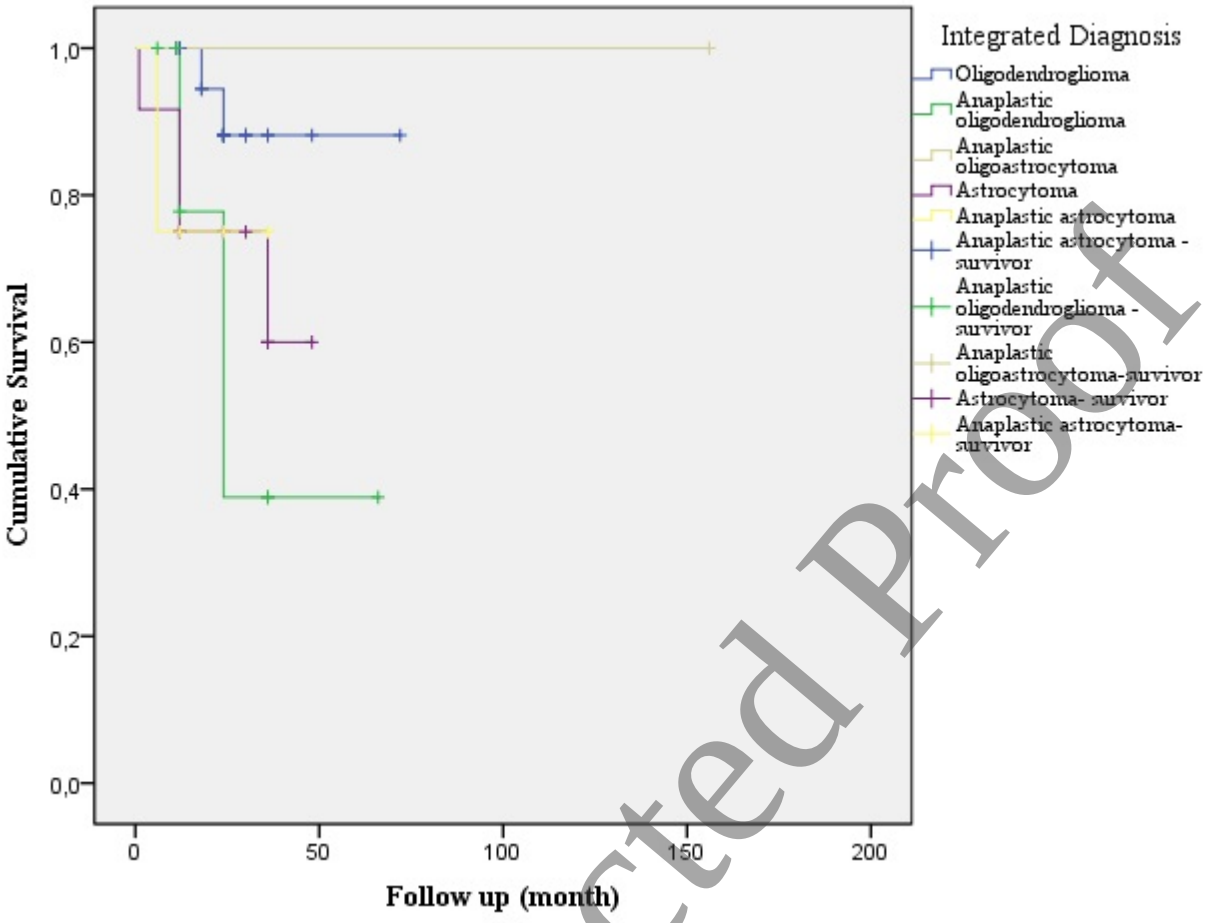

Figure 7. Survival times according to integrated diagnosis

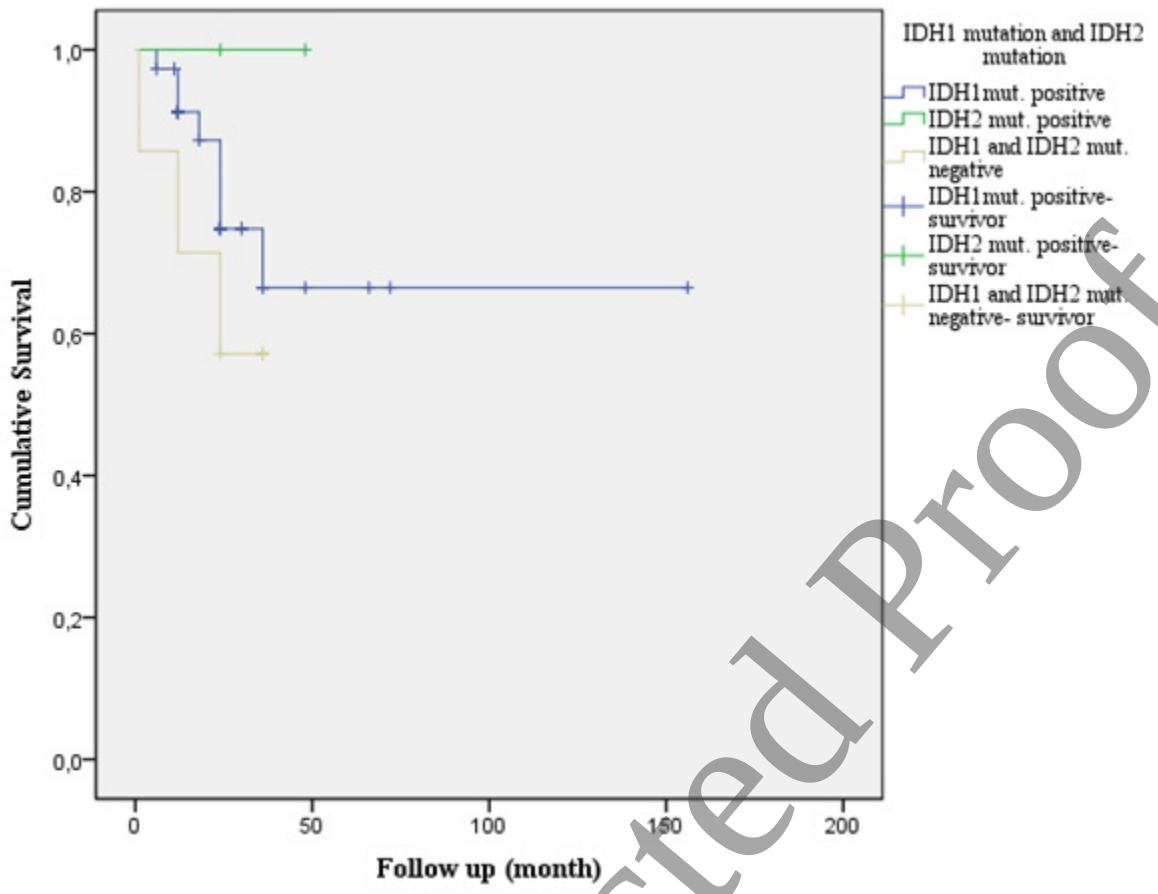

Figure 8. Survival in cases which IDH1 and IDH2 mutations are detected and mutations are not detected

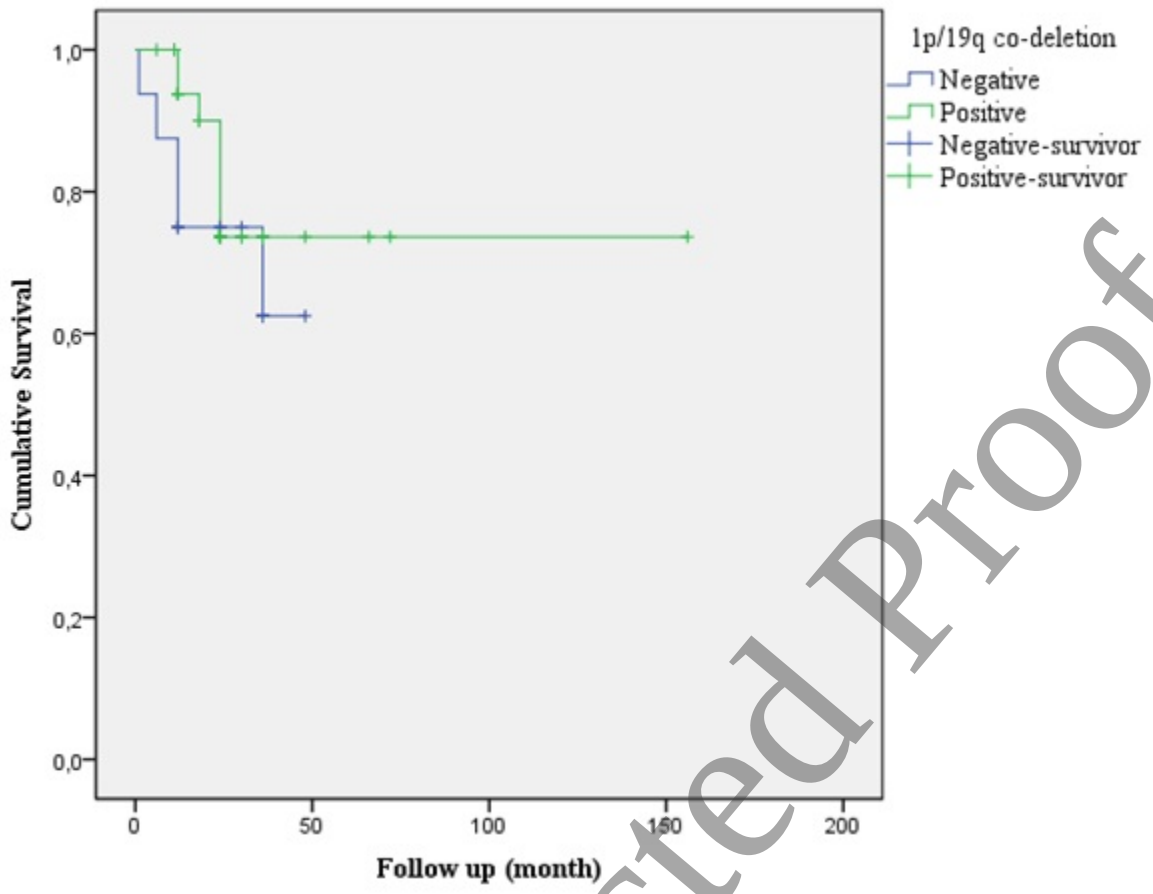

Figure 9. Survival with and without 1p / 19q loss