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Survival Associations using Perfusion and Diffusion MRI in Patients with Histologic and Genetic Defined Diffuse Glioma WHO grade II and III.

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Abstract:	ABSTRACT Objective. According to the new WHO 2016 classification for Tumors of the Central Nervous System (CNS), 1p/19q codeletion defines the genetic hallmark that differentiates oligodendrogliomas from diffuse astrocytomas. The aim of our study was to evaluate whether rCBV and ADC histogram analysis can stratify survival in adult patients with genetic defined diffuse glioma grade II and III. Methods. Sixty-seven patients with untreated diffuse gliomas WHO grade II and III and known 1p/19q codeletion status were included retrospectively and analyzed using ADC and rCBV maps based on whole-tumor volume histograms. Overall survival (OS) and progression-free survival (PFS) were analyzed by Kaplan-Meyer and Cox survival analysis adjusted for known survival predictors. Results. Significant longer PFS was associated with homogeneous rCBV distribution - higher rCBVpeak (median, 37 versus 26 months, HR=3.2, P=0.02) in patients with astrocytomas and heterogeneous rCBV distribution - lower rCBVpeak (median, 46 versus 37 months, HR=5.3, P<0.001), higher rCBVmean (median, 44 versus 39 months, HR=7.9, P=0.003) in patients with oligodendrogliomas. ADC parameters (ADCpeak, ADCmean) did not stratify PFS and OS. Conclusion.

	Tumors with heterogeneous perfusion signatures and high average values were associated with longer PFS in patients with oligodendrogliomas. On the contrary, heterogeneous perfusion distribution was associated with poor outcome in patients with diffuse astrocytomas.

Survival Associations using Perfusion and Diffusion MRI in Patients with Histologic and Genetic Defined Diffuse Glioma WHO grade II and III.

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ABSTRACT

Objective.

According to the new WHO 2016 classification for Tumors of the Central Nervous System (CNS), 1p/19q codeletion defines the genetic hallmark that differentiates oligodendrogliomas from diffuse astrocytomas. The aim of our study was to evaluate whether rCBV and ADC histogram analysis can stratify survival in adult patients with genetic defined diffuse glioma grade II and III.

Methods.

Sixty-seven patients with untreated diffuse gliomas WHO grade II and III and known 1p/19q codeletion status were included retrospectively and analyzed using ADC and rCBV maps based on whole-tumor volume histograms. Overall survival (OS) and progression-free survival (PFS) were analyzed by Kaplan-Meyer and Cox survival analysis adjusted for known survival predictors.

Results.

Significant longer PFS was associated with homogeneous rCBV distribution - higher $rCBV_{peak}$ (median, 37 versus 26 months, HR=3.2, P=0.02) in patients with astrocytomas and heterogeneous rCBV distribution - lower $rCBV_{peak}$ (median, 46 versus 37 months, HR=5.3, P<0.001), higher $rCBV_{mean}$ (median, 44 versus 39 months, HR=7.9, P=0.003) in patients with oligodendrogliomas. ADC parameters (ADC_{peak}, ADC_{mean}) did not stratify PFS and OS.

Conclusion.

Tumors with heterogeneous perfusion signatures and high average values were associated with longer PFS in patients with oligodendrogliomas. On the contrary heterogeneous perfusion distribution was associated with poor outcome in patients with diffuse astrocytomas.

Key Words: MR perfusion; MR diffusion; histogram analysis; brain tumors, oligodendroglioma, diffuse astrocytoma.

INTRODUCTION

Diffuse gliomas represent the majority of glial neoplasms, with a range of different biological behavior, treatment strategies and prognoses. The World Health Organization (WHO)

classification of gliomas has been extensively redefined in 2016, reflecting certain molecular aberrations that bring important diagnostic and prognostic information [1, 2]. In the updated guidelines, molecular parameters define the brain tumor diagnosis. Diffusely infiltrating gliomas have now been grouped together not only based upon the behavioral hierarchy and growth patterns but also on the basis of genetic mutations in the IDH1(isocitrate dehydrogenase) and IDH2 genes [2]. The key genotypic feature of an oligodendroglioma, the presence of 1p/19q codeletion, differentiates it from diffuse astrocytoma and is associated with improved radio- and chemotherapeutic sensitivity and survival [3-5].

Histological grade, although suffering from inter- and intraobserver variability as well as tissues sampling error, especially in grade II and III tumors [6-9] is still recognized as an important characteristic for classification of CNS tumors. According to the new 2016 WHO classification, diffuse astrocytoma (WHO grade II) and anaplastic astrocytoma (WHO grade III) are now divided in two sub-categories depending on the mutation of the IDH family of genes (IDH1, IDH2): IDH-mutant and IDH-wildtype. The diagnosis of oligodendroglioma requires mutation of the IDH gene family in addition to 1p/19q codeletion [2].

Functional imaging techniques like diffusion and perfusion MRI are non-invasive methods capable of assessing water movement and blood flow, respectively. Water diffusion reflects cell density and is measured in terms of apparent diffusion coefficient (ADC) [10-12]. Perfusion MRI is associated with tissue vascularization and most importantly neovascularization in tumor, usually by the relative cerebral blood volume (rCBV) [13-15].

These MRI-based parameters have been used with success to assess not only histologic grade in diffuse gliomas, but also for identification of tumors with and without 1p/19q codeletion [16-22]. In a recent study Leu et al. observed that ADC in combination with rCBV, T2 hyperintense volume and contrast enhancement allowed to distinguish between different IDH mutations as well as between IDH mutant with and without 1p/19q codeletion [23]. Furthermore, noninvasive diagnostic alternatives are especially important for repeated monitoring of tumor status as well as for patients with inoperable tumors.

The purpose of our study was to retrospectively evaluate whether ADC and rCBV histogram analyses can stratify progression-free survival (PFS) and overall survival (OS) in patients with diffuse gliomas grade II and III with respect to both oligodendroglial and astrocytic tumors.

MATERIAL AND METHODS

Patients

The study was approved by the institutional and regional medical ethics committees, and all patients signed a consent form. Total 280 consecutive adult patients with a histopathologic diagnosis of diffuse glioma, referred to the regional neurosurgical department between February 2006 and December 2012, were reviewed. Of these, 81 were primary diagnosed as oligodendrogliomas or astrocytomas according to the status of chromosome 1p/19q (1p/19q

codeleted, 1p/19q non-codeleted). From this cohort, 67 patients (33 women, 34 men; mean age, 47 years; range, 18-82 years) met the inclusion criteria as follows: 1) a baseline pre-operative MRI examination from our institution including conventional contrast enhanced T1 weighted images, dynamic susceptibility contrast perfusion-weighted imaging (DSC-PWI) and diffusion-weighted imaging (DWI); 2) minimum 5 years of clinical follow-up; 3) age > 18 years. Patients were excluded for the following reasons: consent not provided (n=4), incomplete imaging **(**n=7), insufficient image quality (n=2) and previously performed biopsy (n=1) Figure 1. Patient data on histopathological and genetic molecular diagnosis, Karnofsky performance status (KPS), comorbidity (diabetes mellitus, cancer, chronic cardiovascular disease and respiratory disease) and administrated treatments are shown in Table 1.

Survival assessments.

The initial follow-up MRI scans were obtained 11-15 weeks after primary surgery and histological diagnosis, and then twice yearly the following five years. Survival data were registered and assessed using patient records.

OS was defined as time from diagnosis to death or last follow-up date when the patient was known to be alive. Censoring was performed after 60 months observation and May 2017 was the date of administrative censoring. PFS was defined as the time from diagnosis to tumor progression, recurrence, death, or the last follow-up date in which the patient showed no disease progression. The definition and date of tumor progression were based on the updated Response assessment in Neuro-Oncology (RANO) criteria [24, 25]. We used 48 months PFS as cut-off to evaluate because several previous studies have reported a median PFS 27-53 months for patients with diffuse glioma [26]. For further analysis, all patients were divided in two groups per OS status: (I) long OS (over 60 months) and (II) short OS (under 60 months) and in two groups per PFS status: (I) long PFS (over 48 months) and (II) short PFS (under 48 months).

M**olecular genetic diagnosis**

1p19q codeletion status analysis was assessed by polymerase chain reaction (PCR) by using at least 4 of 6 microsatellite markers on 1p35-36 and 19q13 in the period 2006-2009 [20]. The multiplex ligation-dependent probe amplification (MLPA) [27] was used after 2009 in our institution and allows detecting chromosomal DNA copy number changes of multiple loci simultaneously. Only patients with a defined 1p/19q codeletion status were included in the final analysis and split in two groups: (I) gliomas with 1p/19q codeletion, defined as oligodendrogliomas and (II) gliomas without 1p/19g codeletion defined as astrocytomas.

IDH 1 and IDH2 mutations were determined for 55% (37/67) of the patients, where IDH-mutant 97.8% (36/37) and IDH-wild type 2.2 % (1/37). Although IDH status was not available in all patients, it has been shown that IDH mutation occurs in all 1p/19q-codeleted tumors and the great majority of grade II/III gliomas fall into the IDH mutant category [2, 28, 29].

MR Imaging

Imaging was performed at 1.5T (Sonata, Symphony, or Avanto; Siemens, Erlangen, Germany) equipped with an 8-channel (Sonata and Symphony imagers) or 12-channel (Avanto imager) phased-array head coil. The MRI protocol included the following sequences: axial T2-weighted fast spin-echo (TR msec/TE msec/section thickness mm, 4000/104/5), coronal fluid-attenuated inversion recovery (TR msec/TE msec/section thickness mm, 9000/108/5), and axial T1-weighted spin-echo (TR msec/TE msec/section thickness mm 500/77/5). Diffusion-weighted images (DWI) were achieved by using an axial echo-planar spin-echo sequence (TR msec/TE msec/section thickness mm 2900/84/5) before the injection of contrast agent. Diffusion was measured in orthogonal directions by use of b-values 0, 500, 1000 sec/mm^2. Perfusion-weighted imaging (PWI) was performed by using a gradient-echo echo-planar imaging technic acquired during contrast agent administration (TR/TE, 1430/46 (12 axial sections) to 1590/52 msec (14 axial sections); bandwidth, 1345 Hz/pixel; voxel size, 1.80 ×1.80 × 5mm3; intersection gap, 1.5 mm. For each section, 50 images were recorded at intervals equal to the TR. After approximately 8 time points, 0.2 mmol/kg of gadobutrol (Gadovist; Bayer Pharma AG, Berlin, Germany) was injected at a rate of 5 mL/s, immediately followed by a 20-mL bolus of saline (Sodiumchloride [9 mg/mL]; B. Braun Melsungen, Melsungen, Germany) injected at a rate of at 5 ml/s. Post-contrast T1-weighted images were acquired after completion of the dynamic susceptibility-weighted contrast-enhanced MR imaging (DSC-MR imaging) [20].

Image processing

The region of interest outlining the entire tumor volume on ADC maps and T2-weighted images was drawn on each slice by two neuroradiologists, blinded to histopathological, genetic/molecular characteristics and clinical outcome (Figure 2). The tumor was defined as regions with hyperintensities on T2-weighted images thought to represent pathologic tissue. Areas of contrast enhancement on contrast-enhanced T1-weighted images were always included. Discrepancies were resolved by consensus reading. Care was taken to avoid areas of cysts and non-tumoral macroscopic vessels evident on both T2-weighted images (tubular structures with flow-void) and on contrast-enhanced T1-weighted images. rCBV maps from DSC MRI were created using established tracer kinetic models, corrected for potential contrast agent leakage from blood-brain-barrier breakdown and normalized to reference tissue [20]. ADC maps from diffusion MRI were created using standard Stejskal-Tanner diffusion approximation [22, 30]. Tumor outlining and processing of ADC and rCBV maps were performed using NordicICE (NordicNeuroLab AS, Bergen, Norway). Whole-tumor normalized histogram distributions of the ADC and rCBV maps were created as described elsewhere [31]. In short, using Matlab 2015 (MathWorks, Natick, Mass), 100 bins histogram were created over an ADC range of 0-300 and an rCBV range of 0-7.5 (ratios; arbitrary units), respectively. To correct for varying tumor sizes, the histograms were normalized by making all areas under the histogram curves equal to one [20]. To reduce the effect of outliers, all ADC and rCBV values below the 5% percentile and over the 95% percentile were excluded.

From this the maximum peak heights of the normalized histogram $rCBV_{peak}$ and ADC_{peak} were statistically used as measures of vascular and cellular tumor heterogeneity, respectively [12, 31]. In addition, mean ADC (ADC_{mean}) and mean rCBV (CBV_{mean}) values of the region of interests were assessed.

Statistical analysis

Associations between MRI parameters (ADC_{peak}, $rCBV_{peak}$, ADC_{mean}, $rCBV_{mean}$) and patient outcome (PFS and OS) were assessed by using Kaplan-Meier survival analysis and Cox regression with time-dependent covariates. Receiver operating characteristic (ROC) analysis was used to determine cutoff for dichotomizing the MRI metrics to clinical outcome and used to estimate values of sensitivity, specificity and area under the curve. A cut-off value for each parameter was determined by maximizing the sum of sensitivity and specificity. Univariate Kaplan-Meier survival analyses were then conducted based on the subgroups obtained from ROC analysis. In addition, Kaplan-Meier and Cox regression analysis were used to determine whether histopathologic (grade II and III) and molecular genetic status was associated with better outcome. For Cox regression, the following time-dependent covariates were included: Karnofsky performance status (KPS), age, comorbidity ("yes" or "no"), character of debut symptoms (epilepsy/focal neurologic deficit/raised intracranial pressure), primary treatment regime including time and extent of surgical resection (subtotal or gross total resection), as well as type and time of adjuvant therapy (radiation therapy dose 1.8Gy×30, chemotherapy with Temozolomide, MSD, Nederland), or radiochemotherapy (dose 1.8Gy×30 with concomitant Temozolomide), time and presence of tumor residuals ("yes" or "no"). For all Cox models, hazard ratios and 95 % confidence intervals were estimated. For all cases, a two-tailed P-value of 0.05 or less was considered statistically significant, before potential correction for multiple comparisons by Holm-Bonferroni analysis. Statistical analysis was performed by using SPSS version 18 software (SPSS, Chicago, III).

RESULTS

Table 2 shows the PFS and OS, as well as the values of $rCBV_{peak}$, $rCBV_{mean}$, ADC_{peak} and ADC_{mean} in the prediction of survival outcome in oligodendrogliomas (oligodendroglioma WHO grade II and anaplastic oligodendroglioma WHO grade III) and diffuse astrocytomas (diffuse astrocytoma WHO grade II and anaplastic astrocytoma WHO grade III). The same parameters calculated for the different subgroups are summarized in Table 3.

Perfusion MRI and PFS/OS.

Histogram analysis of DSC-MR imaging parameters revealed that $rCBV_{peak}$ and $rCBV_{mean}$ were independently associated with PFS in patients with oligodendrogliomas ($p<0.001$; $p=0.003$performed by the Cox regression analysis, where longer PFS (median, 46 versus 37 months) was associated with both higher vascular heterogeneity (lower $rCBV_{peak}$) and higher microvascularity (higher $rCBV_{mean}$). In the group with diffuse astrocytomas, longer PFS (median, 37 versus 26 months) was associated higher $rCBV_{peak}$, reflecting lower vascular

heterogeneity within the tumor. $rCBV_{peak}$ and $rCBV_{mean}$ were also independently associated with PFS in patients with astrocytomas grade III (p=0.009; p=0.002).

Histogram analysis of DSC-MR imaging parameters shows that higher $rCBV_{peak}$ and lower $rCBV_{mean}$ values showed statistically significant longer OS in patients with astrocytomas grade III (median, 54 versus 37 months, p=0.004 and median, 48 versus 23 months, p=0.008, respectively).

Kaplan-Meier curves of representative prognostic parameters of $rCBV_{peak}$ and $rCBV_{mean}$ are depicted in Figure 2. Average rCBV histograms (±1.96 SE) for all oligodendrogliomas and diffuse astrocytomas are shown in Figure 3.

Diffusion MRI and PFS/OS.

There was no significant difference in the ADC parameters (ADC_{peak}, ADC_{mean}) between patients with long and short OS and PFS for neither the oligodendrogliomas, nor astrocytoma groups. A combination of rCBV and ADC parameters did not yield a significant survival association.

Histopathology and PFS/OS.

Median PFS and OS for patients with oligodendroglioma grade II are: 41 and 58 months, anaplastic oligodendroglioma grade III: 43 and 57 months, diffuse astrocytoma grade II: 35 and 56 months and anaplastic astrocytoma grade III: 23 and 34 months, respectively. Based on RANO criteria, 38 patients (56%) showed tumor progression by study completion and 18 patients (30%) were deceased at last follow-up.

Survival outcome for all patients related to diagnosis are shown in Table 4. Patients with oligodendrogliomas have significant longer PFS (median 41 versus 29 months, p=0.01) and OS (median 57 versus 46 months, p=0.002) compared to patients with astrocytomas. Patients with diffuse glioma grade II have significantly longer PFS (median 38 versus 30 months, p=0.05) and OS (median 57 versus 44 months, p=0.006) than patients with diffuse glioma grade III. The corresponding Kaplan-Meier survival curves are shown in Figure 4.

DISCUSSION.

The present study demonstrates that perfusion MRI parameters derived from rCBV maps analyzed by a histogram method are significant predictors of PFS in patients with diffuse gliomas WHO grade II and III and of OS in patients with astrocytomas grade III. These results emphasize the role of MRI-based microvascular blood volume as an independent

prognostic biomarker that may overcome some of the limitations of a histopathological diagnosis, as well as help guide treatment strategy. In particular, the ability of perfusion MRI to identify lesions associated with poor outcome could select for patients in need of a more aggressive therapeutic strategy and shorter intervals between follow-up examinations.

Patients with diffuse astrocytomas WHO grade II/III and a homogenous distribution of rCBV values (high $rCBV_{peak}$) demonstrated longer PFS compared to patients with a heterogeneous distribution (low $rCBV_{peak}$). The opposite finding was observed in patients with oligodendrogliomas, where a high $rCBV_{peak}$ and low $rCBV_{mean}$ were associated with shorter PFS. The rCBV-histogram analysis was also predictive for OS in patients with astrocytoma grade III, where high $rCBV_{peak}$ and low $rCBV_{mean}$ were associated with better outcome. Conversely, the difference in OS was not significant in patients with diffuse astrocytoma grade II and oligodendrogliomas grade II/III. The most obvious reason for this finding is long survival time among patients of both groups, that is in agreement with previous published studies, where observation time was less than 10 years [32-34]. For our analysis we choose 48 months as the cut-off for PFS ('yes/no'), which is in line with median PFS for oligodendrogliomas reported in recently published data [35]. Nevertheless, it may be reasonable to expand the observation time even more, because our material contained a large cohort of patients with grade II gliomas, in which expected median OS could reach 12-15 years and beyond.

Interestingly, we identified a more favorable outcome in the group of patients with oligodendroglial tumors with heterogeneous microvascular anatomy (low $rCBV_{peak}$) and higher vascularity (high $rCBV_{mean}$). These results parallel those of previously published data, suggesting that unlike astrocytic gliomas, high rCBV values do not necessarily indicate aggressive biology associated with poor outcome [36]. The possible biological explanation of a vascular heterogeneous appearance in oligodendrogliomas may be found in the branching network of delicate capillaries typically observed in oligodendrogliomas [29]. Additionally, our data suggest that $rCBV_{peak}$ and $rCBV_{mean}$ in oligodendroglial tumors may reflect differences in [29] vascular biology that in turn impact for radiochemotherapy sensitivity and potentially clinical outcome. It is noteworthy to point out that 1p/19q codeletion has been associated with improved OS and increased benefit of adjuvant PCV (procarbazine-lomustine-vincristine) chemotherapy after radiotherapy [3, 5, 29, 37]. It is worth to note, the similar survival patterns in diffuse astrocytoma grade II and anaplastic oligodendroglioma grade III could indicate that the molecular genetic characteristics may be more important than histological grade.

Several previous reports have focused on the usefulness of both PWI and DWI in unselected glioma patients to differentiate histopathologic grades and, more recently, for stratifying patients into prognostic groups [17, 32, 36, 38-41]. However, distinguishing different histopathologic grades and survival times in patients with diffuse glioma grade II and grade III, especially oligodendroglial tumors, is challenging with considerable overlap between groups. Naturally, previous studies prior to the WHO 2016 classification have focused on OS and PFS of diffuse glioma and therefore paid less attention to molecular genetics. Law et al demonstrated in a large cohort of 189 patients with glioma, where 19% of tumors had oligodendroglial

components, that rCBV can be used to predict median time to progression [33]. Similarly, Spampinato et al included 12 oligodendroglial tumors in a series of 29 evaluated gliomas and found that normalized maximum rCBV (rCBVmax) may predict two-year PFS in patients with gliomas, independent of histopathologic findings. In their study, the correlation between rCBVmax and PFS in oligodendroglial tumors was slightly weaker compared to the group with astrocytic tumors only [40]. Jenkinson et al investigated the relationship between rCBV, genotype and outcome in oligodendroglial tumors and found that rCBV alone was an unreliable indicator for outcome, showing prognostic significance only after stratification for genotype [36]. However, in contrast to our study, all the patients included in their study had been treated with chemotherapy.

In contrast to our findings with perfusion MRI, we did not find a significant association between diffusion MRI and survival. These results are in conflict with previous studies where diffusion-based MRI was used to predict survival time. Cuccarini et al demonstrated in a cohort of 89 patients, that significant longer OS was observed in patients with minimum normalized ADC above cut-off ratio of 1.69. Compared to our study both glioma grade I (pilocytic astrocytoma) and grade IV (glioblastoma) were included in their analysis in addition to glioma grades II/III [34]. Moreover, in more recent study, Neill et al observed median and 90% nADC (histogram metrics of normalized ADC) significantly associated with PFS in patients with recurrent diffuse glioma grade II and III [42]. In our data, oligodendrogliomas with low ADC_{peak} showed trend towards longer OS and PFS, but this difference was not significant and any survival association will need to be confirmed in larger, prospective studies.

There is important difference between the tumor outlining in our study and previous reports [17, 30, 43]. In most reported studies, apparent tumor necrosis was excluded from the region-of-interest. In contrast, the histogram-based approach which we used in our analysis assesses the entire tumor volume. Removing areas of macroscopic necrosis but not regions of micronecrosis beyond visual inspection (i.e. below image spatial resolution), is subjective and does not reflect the real tumor heterogeneity. These differences may possible explain contradictory results with previous published studies, where diffusion-based MRI was used to predict survival time.

There are some limitations in our study that must be considered. First, manual region-of-interest identification is complicated because the diffuse glioma has infiltrating-appearing margin with indistinct borders beyond radiologic visualization. Second, the PCR with microsatellites had been used to assess 1p19q codeletion during the period from 2006 to 2009, which is considered less sensitive than MLPA analysis that has been used in our institution since 2009. Finally, IDH1 and IDH2 mutations were determined in only 55% of patients; however, the *IDH* mutation rate in 1p/19q codeleted tumors is close to 100 % [29, 44, 45].

In conclusion, our results indicate that perfusion MRI provides sensitive prognostic markers for OS and PFS in patients with diffuse gliomas and might serve as an independent factor to predict prognosis. Thus, imaging-based biomarkers of vascularity may therefore constitute as

a non-invasive supplement to histopathologic and molecular genetic markers and provide important information to guide treatment.

Figure 1. Flowchart demonstrates study cohort with exclusion criteria.

Figure 2. Histograms generation for patients with diffuse glioma.

a) Total volume was segmented on axial ADC maps in a 65 years old male with oligodendroglioma. b) The anatomic T2-weighted MR image and rCBV overlay for the same patient. c) Whole-volume rCBV histogram is given with average rCBV histograms (±1, 96 SE) of all diffuse gliomas with different survival time.

Figure 3. Kaplan-Meier analysis of progression-free survival in patients with oligodendroglioma

a) $rCBV_{peak}$, b) $rCBV_{mean}$, and in patients with diffuse astrocytoma c) $rCBV_{peak}$. Overall survival in patients with anaplastic astrocytoma d) $rCBV_{peak}$, e) $rCBV_{mean}$. For all graphs, the y-axis represents the percentage surviving.

Figure 4. Kaplan-Meier survival curves showing the progression-free survival (a,c) and overall survival (b,d) in patients with oligodendrogliomas and diffuse astrocytomas (a,b) and in patients based on diffuse glioma pathology grade (c,d).

References.

1. Aldape K, Burger PC, Perry A. Clinicopathologic aspects of 1p/19q loss and the diagnosis of oligodendroglioma. *Archives of pathology & laboratory medicine*. 2007;131:242-51.
2. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta neuropathologica*. 2016;131:803-20.
3. van den Bent MJ, Brandes AA, Taphoorn MJ, et al. Adjuvant procarbazine, lomustine, and vincristine chemotherapy in newly diagnosed anaplastic oligodendroglioma: long-term follow-up of EORTC brain tumor group study 26951. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2013;31:344-50.
4. Lecavalier-Barsoum M, Quon H, Abdulkarim B. Adjuvant treatment of anaplastic oligodendrogliomas and oligoastrocytomas. *The Cochrane database of systematic reviews*. 2014:Cd007104.
5. Cairncross G, Wang M, Shaw E, et al. Phase III trial of chemoradiotherapy for anaplastic oligodendroglioma: long-term results of RTOG 9402. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2013;31:337-43.

6. Giannini C, Scheithauer BW, Weaver AL, et al. Oligodendrogliomas: reproducibility and prognostic value of histologic diagnosis and grading. *Journal of neuropathology and experimental neurology*. 2001;60:248-62.
7. Giannini C, Burger PC, Berkey BA, et al. Anaplastic oligodendroglial tumors: refining the correlation among histopathology, 1p 19q deletion and clinical outcome in Intergroup Radiation Therapy Oncology Group Trial 9402. *Brain pathology (Zurich, Switzerland)*. 2008;18:360-9.
8. van den Bent MJ. Interobserver variation of the histopathological diagnosis in clinical trials on glioma: a clinician's perspective. *Acta neuropathologica*. 2010;120:297-304.
9. Hattori N, Hirose Y, Sasaki H, et al. World Health Organization grade II-III astrocytomas consist of genetically distinct tumor lineages. *Cancer science*. 2016;107:1159-64.
10. Gupta RK, Cloughesy TF, Sinha U, et al. Relationships between choline magnetic resonance spectroscopy, apparent diffusion coefficient and quantitative histopathology in human glioma. *Journal of neuro-oncology*. 2000;50:215-26.
11. Sugahara T, Korogi Y, Kochi M, et al. Usefulness of diffusion-weighted MRI with echo-planar technique in the evaluation of cellularity in gliomas. *Journal of magnetic resonance imaging : JMRI*. 1999;9:53-60.
12. Jenkinson MD, du Plessis DG, Smith TS, et al. Cellularity and apparent diffusion coefficient in oligodendroglial tumours characterized by genotype. *Journal of neuro-oncology*. 2010;96:385-92.
13. Edelman RR, Mattle HP, Atkinson DJ, et al. Cerebral blood flow: assessment with dynamic contrast-enhanced T2*-weighted MR imaging at 1.5 T. *Radiology*. 1990;176:211-20.
14. Aronen HJ, Gazit IE, Louis DN, et al. Cerebral blood volume maps of gliomas: comparison with tumor grade and histologic findings. *Radiology*. 1994;191:41-51.
15. Knopp EA, Cha S, Johnson G, et al. Glial neoplasms: dynamic contrast-enhanced T2*-weighted MR imaging. *Radiology*. 1999;211:791-8.
16. Chawla S, Krejza J, Vossough A, et al. Differentiation between oligodendroglioma genotypes using dynamic susceptibility contrast perfusion-weighted imaging and proton MR spectroscopy. *AJNR American journal of neuroradiology*. 2013;34:1542-9.
17. Kapoor GS, Gocke TA, Chawla S, et al. Magnetic resonance perfusion-weighted imaging defines angiogenic subtypes of oligodendroglioma according to 1p19q and EGFR status. *Journal of neuro-oncology*. 2009;92:373-86.
18. Lev MH, Ozsunar Y, Henson JW, et al. Glial tumor grading and outcome prediction using dynamic spin-echo MR susceptibility mapping compared with conventional contrast-enhanced MR: confounding effect of elevated rCBV of oligodendrogliomas [corrected]. *AJNR American journal of neuroradiology*. 2004;25:214-21.
19. Fellah S, Caudal D, De Paula AM, et al. Multimodal MR imaging (diffusion, perfusion, and spectroscopy): is it possible to distinguish oligodendroglial tumor grade and 1p/19q codeletion in the pretherapeutic diagnosis? *AJNR American journal of neuroradiology*. 2013;34:1326-33.
20. Emblem KE, Scheie D, Due-Tonnessen P, et al. Histogram analysis of MR imaging-derived cerebral blood volume maps: combined glioma grading and identification of low-grade oligodendroglial subtypes. *AJNR American journal of neuroradiology*. 2008;29:1664-70.
21. Kang Y, Choi SH, Kim YJ, et al. Gliomas: Histogram analysis of apparent diffusion coefficient maps with standard- or high-b-value diffusion-weighted MR imaging--correlation with tumor grade. *Radiology*. 2011;261:882-90.
22. Jenkinson MD, Smith TS, Brodbelt AR, et al. Apparent diffusion coefficients in oligodendroglial tumors characterized by genotype. *Journal of magnetic resonance imaging : JMRI*. 2007;26:1405-12.
23. Leu K, Ott GA, Lai A, et al. Perfusion and diffusion MRI signatures in histologic and genetic subtypes of WHO grade II-III diffuse gliomas. *Journal of neuro-oncology*. 2017.
24. Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2010;28:1963-72.

25. Eisele SC, Wen PY, Lee EQ. Assessment of Brain Tumor Response: RANO and Its Offspring. *Current treatment options in oncology*. 2016;17:35.
26. Ahluwalia MS, Xie H, Dahiya S, et al. Efficacy and patient-reported outcomes with dose-intense temozolomide in patients with newly diagnosed pure and mixed anaplastic oligodendroglioma: a phase II multicenter study. *Journal of neuro-oncology*. 2015;122:111-9.
27. Horbinski C. Practical molecular diagnostics in neuropathology: making a tough job a little easier. *Seminars in diagnostic pathology*. 2010;27:105-13.
28. Leeper HE, Caron AA, Decker PA, et al. IDH mutation, 1p19q codeletion and ATRX loss in WHO grade II gliomas. *Oncotarget*. 2015;6:30295-305.
29. Wesseling P, van den Bent M, Perry A. Oligodendroglioma: pathology, molecular mechanisms and markers. *Acta neuropathologica*. 2015;129:809-27.
30. Pope WB, Kim HJ, Huo J, et al. Recurrent glioblastoma multiforme: ADC histogram analysis predicts response to bevacizumab treatment. *Radiology*. 2009;252:182-9.
31. Emblem KE, Nedregaard B, Nome T, et al. Glioma grading by using histogram analysis of blood volume heterogeneity from MR-derived cerebral blood volume maps. *Radiology*. 2008;247:808-17.
32. Bisdas S, Kirkpatrick M, Giglio P, et al. Cerebral blood volume measurements by perfusion-weighted MR imaging in gliomas: ready for prime time in predicting short-term outcome and recurrent disease? *AJNR American journal of neuroradiology*. 2009;30:681-8.
33. Law M, Young RJ, Babb JS, et al. Gliomas: predicting time to progression or survival with cerebral blood volume measurements at dynamic susceptibility-weighted contrast-enhanced perfusion MR imaging. *Radiology*. 2008;247:490-8.
34. Cuccarini V, Erbetta A, Farinotti M, et al. Advanced MRI may complement histological diagnosis of lower grade gliomas and help in predicting survival. *Journal of neuro-oncology*. 2016;126:279-88.
35. Jaeckle KA. Oligodendroglial tumors. *Seminars in oncology*. 2014;41:468-77.
36. Jenkinson MD, Smith TS, Joyce KA, et al. Cerebral blood volume, genotype and chemosensitivity in oligodendroglial tumours. *Neuroradiology*. 2006;48:703-13.
37. Wick W, Hartmann C, Engel C, et al. NOA-04 randomized phase III trial of sequential radiochemotherapy of anaplastic glioma with procarbazine, lomustine, and vincristine or temozolomide. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2009;27:5874-80.
38. Deike K, Wiestler B, Graf M, et al. Prognostic value of combined visualization of MR diffusion and perfusion maps in glioblastoma. *Journal of neuro-oncology*. 2016;126:463-72.
39. Mangla R, Ginat DT, Kamalian S, et al. Correlation between progression free survival and dynamic susceptibility contrast MRI perfusion in WHO grade III glioma subtypes. *Journal of neuro-oncology*. 2014;116:325-31.
40. Spampinato MV, Schiarelli C, Cianfoni A, et al. Correlation between cerebral blood volume measurements by perfusion-weighted magnetic resonance imaging and two-year progression-free survival in gliomas. *The neuroradiology journal*. 2013;26:385-95.
41. Zonari P, Baraldi P, Crisi G. Multimodal MRI in the characterization of glial neoplasms: the combined role of single-voxel MR spectroscopy, diffusion imaging and echo-planar perfusion imaging. *Neuroradiology*. 2007;49:795-803.
42. Neill E, Luks T, Dayal M, et al. Quantitative multi-modal MR imaging as a non-invasive prognostic tool for patients with recurrent low-grade glioma. *Journal of neuro-oncology*. 2017;132:171-9.
43. Whitmore RG, Krejza J, Kapoor GS, et al. Prediction of oligodendroglial tumor subtype and grade using perfusion weighted magnetic resonance imaging. *Journal of neurosurgery*. 2007;107:600-9.
44. Reuss DE, Sahm F, Schrimpf D, et al. ATRX and IDH1-R132H immunohistochemistry with subsequent copy number analysis and IDH sequencing as a basis for an "integrated" diagnostic

approach for adult astrocytoma, oligodendroglioma and glioblastoma. *Acta neuropathologica*. 2015;129:133-46.
45. Wang XW, Ciccarino P, Rossetto M, et al. IDH mutations: genotype-phenotype correlation and prognostic impact. *BioMed research international*. 2014;2014:540236.

Figure 1

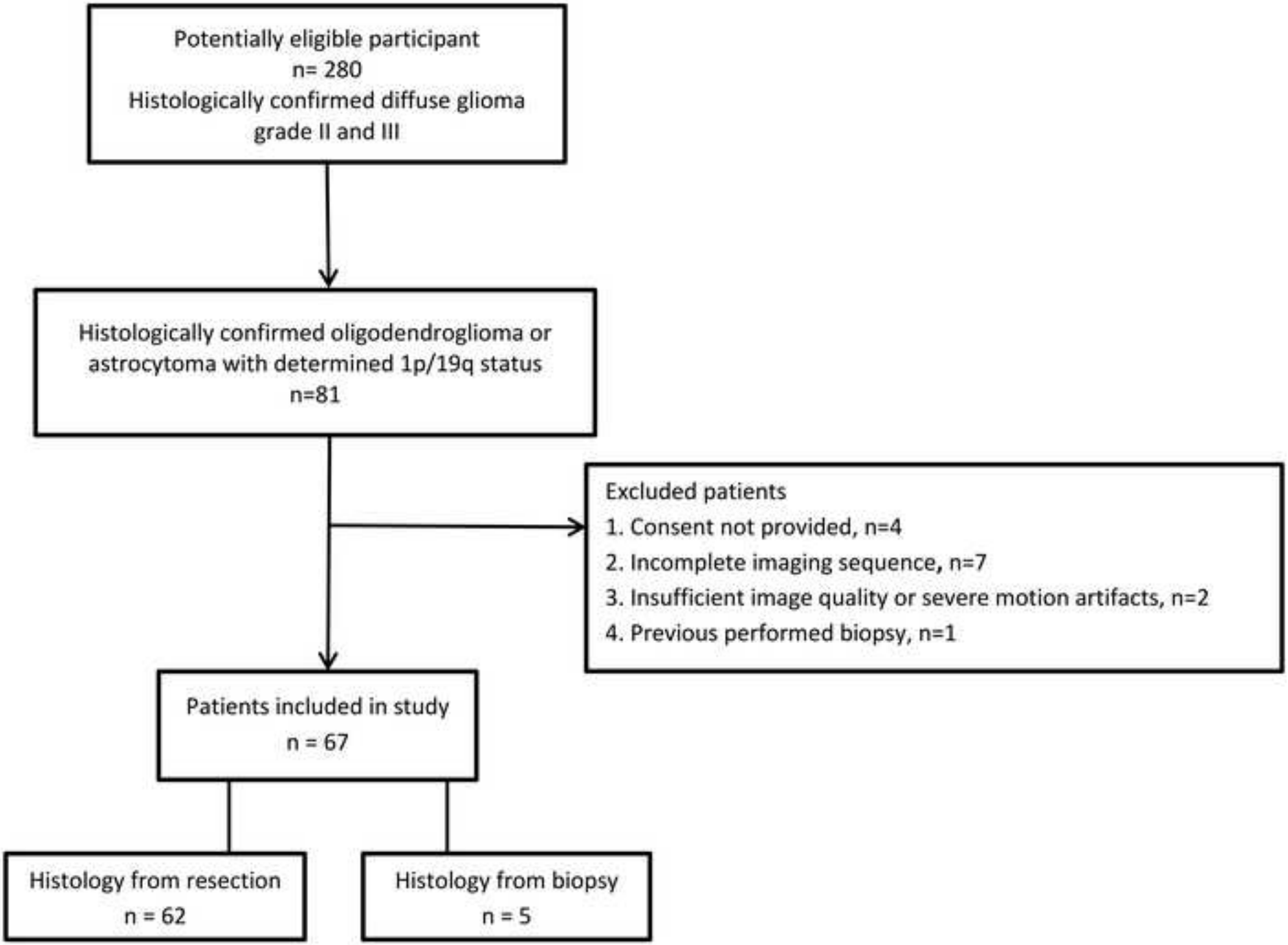

Figure 3

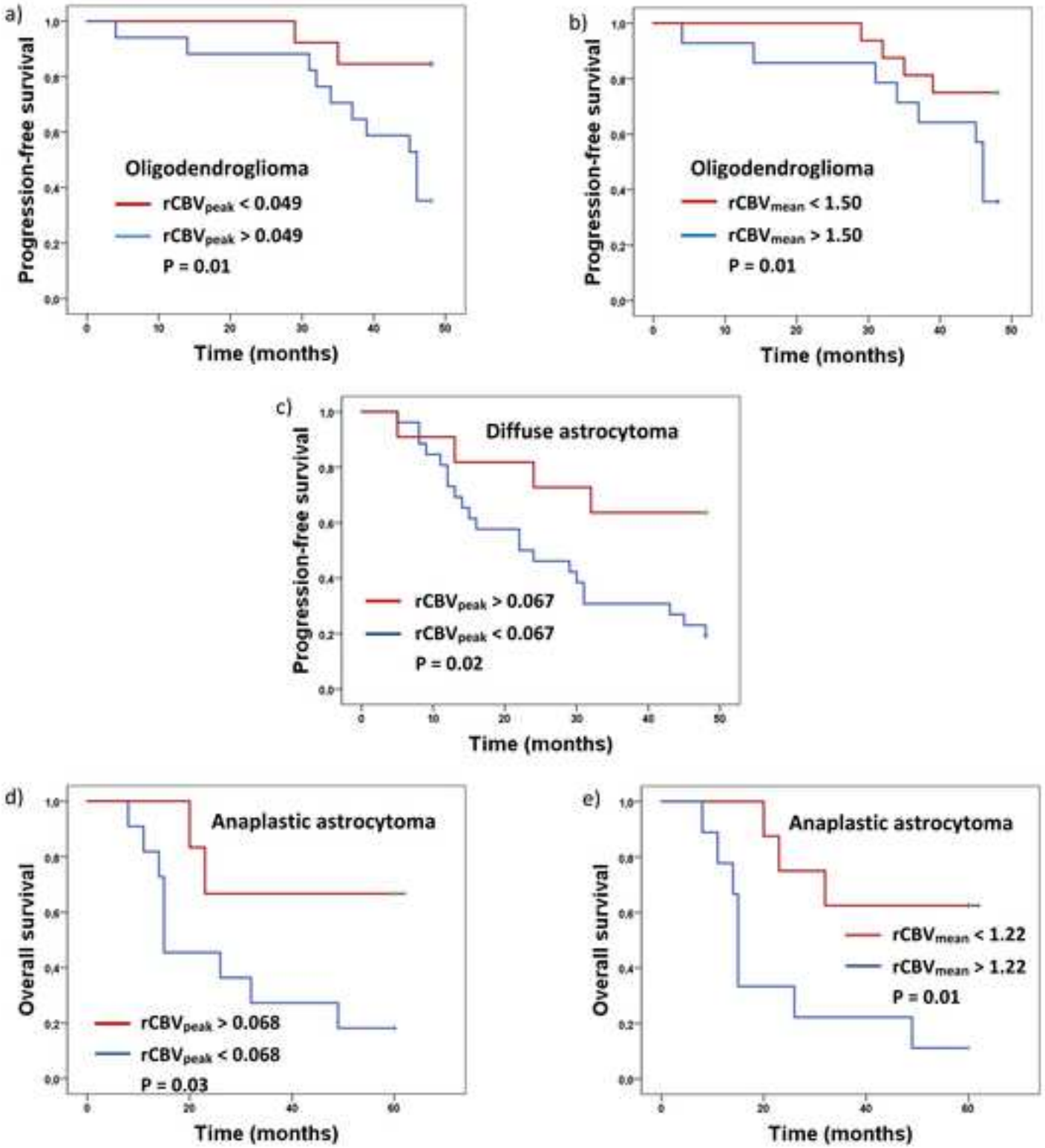

Figure 2

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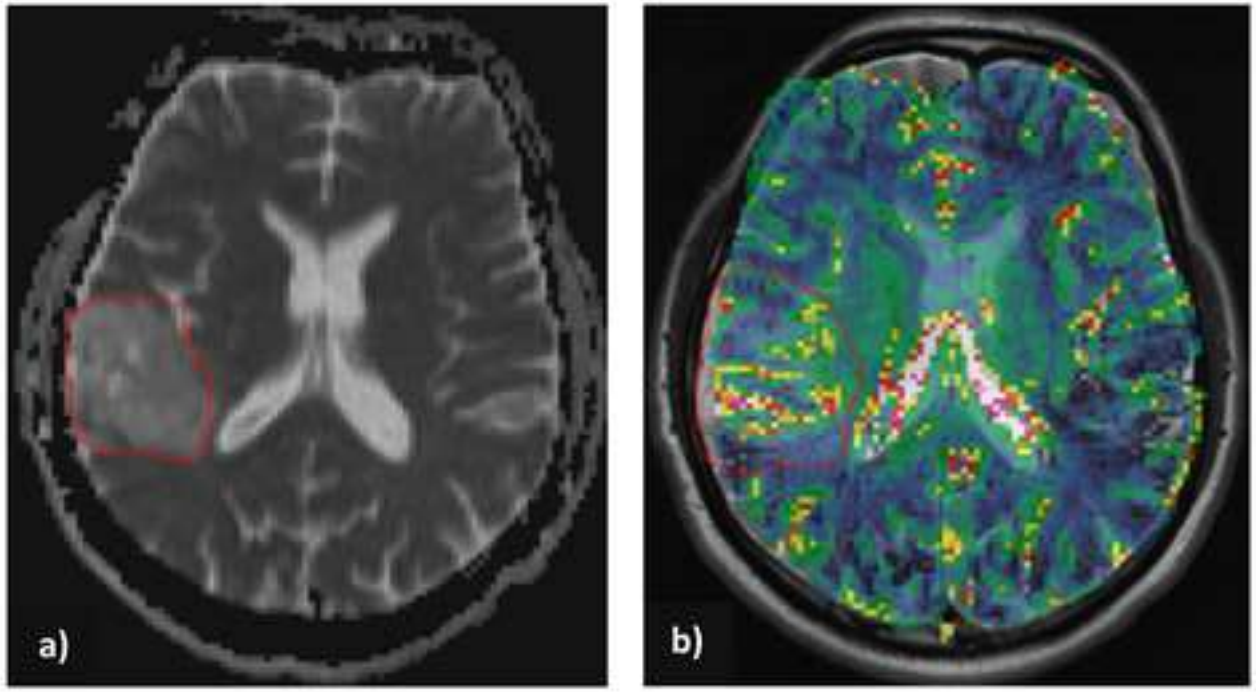

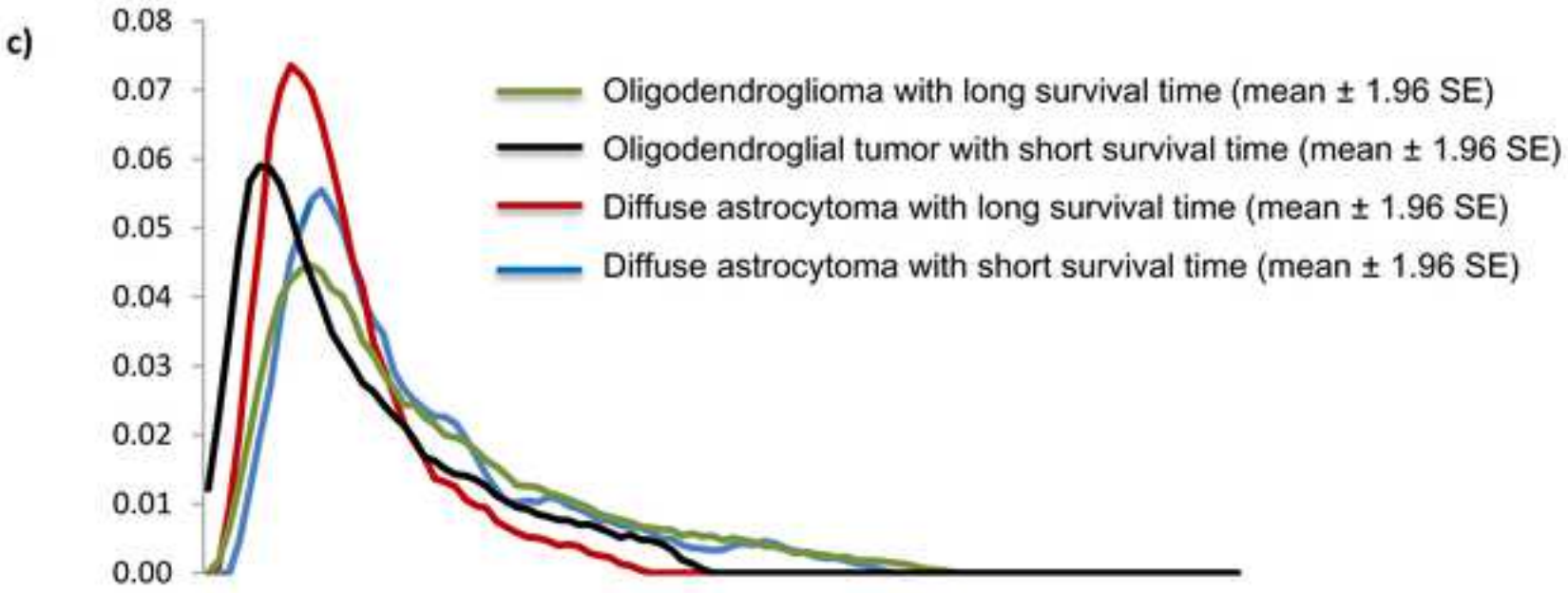

Figure 4

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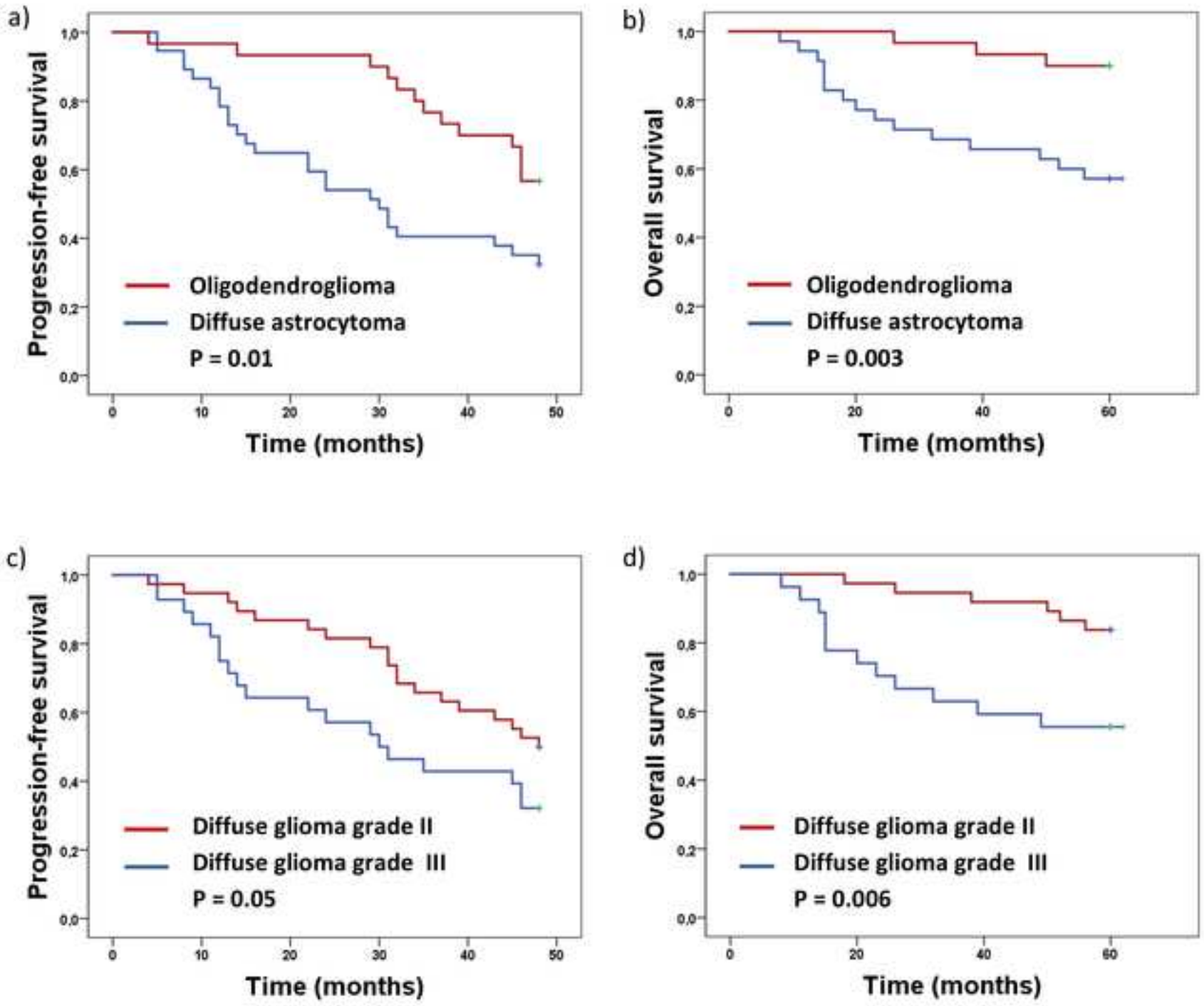

Table

Table 1. Patient diagnosis, KPS, previous surgery, comorbidity, clinical manifestation and treatment.

Diagnosis	No. of patients	Grade[a]	KPS[b]	Comorbidity[c]	Primary symptoms[d]	Resection data[f]	Adjuvant therapy[g]
Oligodendroglioma IDH mutant and 1p/19 codeletion	30	20/10	24/6	24/6	18/8/3	11/19	5/2/10
Diffuse astrocytoma	37	17/20	28/9	23/14	10/14/1	25/12	2/12/17

[a] No. of patients with WHO grade II/ no. of patients with WHO grade III.
[b] No. of patients with Karnofsky performance scale ≥ 70/ no. of patients with Karnofsky performance scale < 70.
[c] No. of patients without chronical diseases/ no. of patients with chronical diseases.
[d] No. of patient with seizures/ no. of patients with symptoms of increased intracranial pressure / no. of patients with focal neurologic deficit.
[f] No. of subtotal resections/ no. of gross total resections.
[g] No. of patients who underwent chemotherapy/radiation therapy/radio-chemotherapy.

Table 2. Diagnostic effectiveness, survival outcomes of $rCBV_{peak}$, $rCBV_{mean}$, ADC_{peak}, ADC_{mean} in patients with diffuse glioma WHO grade II and III.

	Progression-free survival				Overall survival			
	Long PFS Mean ± SD	**Short PFS Mean ± SD**	**P - value**	**Cox model HR (CI 95%)**	**Long OS Mean ± SD**	**Short OS Mean ± SD**	**P - value**	**Cox model HR (CI 95%)**
Oligodendroglioma, IDH-mutant and 1p/19q codeletion								
$rCBV_{peak}$	0.045± 0.009	0.059± 0.009	<**0.001**	**5.3 (1.1; 8)**	0.051± 0.011	0.052± 0.007	0.81	-
$rCBV_{mean}$	1.70± 0.33	1.36± 0.22	**0.003**	**7.9 (1.3; 6.5)**	1.55± 0.34	1.54± 0.26	0.92	-
ADC_{peak}	0.58± 0.027	0.060± 0.016	0.79	-	0.060± 0.024	0.048± 0.003	0.36	-
ADC_{mean}	1181.8± 132	1111.7± 100	0.11	-	1154± 127	1128± 82	0.66	-
Diffuse astrocytoma								
$rCBV_{peak}$	0.073± 0.026	0.055± 0.011	**0.02**	**3.2 (1.1; 9.5)**	0.063± 0.022	0.056± 0.011	0.41	-
$rCBV_{mean}$	1.38± 0.74	1.52± 0.42	0.54	-	1.49± 0.59	1.50± 0.46	0.83	-
ADC_{peak}	0.055± 0.029	0.058± 0.021	0.22	-	0.056± 0.025	0.057± 0.020	0.58	-
ADC_{mean}	1311.5± 250	1215.1± 214.	0.26	-	1256± 199	1219± 255	0.41	-

HR: hazard ratio; SD: standard deviation; CI: confidence interval; rCBV: relative cerebral blood volume; ADC: apparent diffusion coefficient.

Table 3. Diagnostic Effectiveness, Survival outcomes of $rCBV_{peak}$, $rCBV_{mean}$, ADC_{peak}, ADC_{mean} in patients with oligodendroglioma and astrocytoma (WHO grade II and III).

	Progression-free survival				Overall survival			
	Long PFS Mean ± SD	**Short PFS Mean ± SD**	**P - value**	**Cox model HR (CI 95%)**	**Long OS Mean ± SD**	**Short OS Mean ± SD**	**P - value**	**Cox model HR (CI 95%)**
Oligodendroglioma grade II								
$rCBV_{peak}$	0.045± 0.01	0.060± 0.08	**0.04**	**7.2 (1.2; 7.2)**	0.051± 0.012	0.056	0.51	-
$rCBV_{mean}$	1.27± 0.39	1.39± 0.23	0.051	-	1,59± 0.38	1.40± 1.14	0.51	-
ADC_{peak}	0.065± 0.031	0.060± 0.016	0.63	-	0.064± 0.026	0.045± 0.002	0.26	-
ADC_{mean}	1152± 106	1099± 93	0.24	-	1123± 105	1174± 28	0.44	-
Oligodendroglioma grade III								
$rCBV_{peak}$	0.045± 0.006	0.058± 0.01	0.06	-	0.051± 0.01	0.044	0,60	-
$rCBV_{mean}$	1.65± 0.19	1.29± 0.21	**0.03**	**5.3 (1.0; 5.4)**	1.47± 0.25	1.82	0.20	-
ADC_{peak}	0.046± 0.011	0.062± 0.021	0.25	-	0.052± 0.018	0.052	0.80	-
ADC_{mean}	1235± 167	1140± 124	0.47	-	1215± 149	1036	0.40	-
Astrocytoma grade II								
$rCBV_{peak}$	0.076± 0.028	0.052± 0.012	**0.05**	**1.2 (0.5; 6.2)**	0.062± 0.025	0.059± 0.014	0.77	-
$rCBV_{mean}$	1.38± 0.87	1.67± 0.54	0.39	-	1.58± 0.68	1.61± 0.85	0.95	-
ADC_{peak}	0.048± 0.019	0.056± 0.022	0.44	-	0.055± 0.020	0.048± 0.025	0.64	-
ADC_{mean}	1358± 234	1260± 288	0.42	-	1258± 195	1382± 441	0.62	-
Astrocytoma grade III								
$rCBV_{peak}$	0.079± 0.007	0.056± 0.011	**0.009**	**3,2 (1.1; 6.4)**	0.072± 0.011	0.055± 0.01	**0.004**	**3.2 (1.2; 5.2)**
$rCBV_{mean}$	1.15± 0.051	1.43± 0.27	**0.002**	**3.1 (1.2;7.8)**	1.181± 0.053	1.458± 0.273	**0.008**	**6.8 (1.8; 7.0)**
ADC_{peak}	0.081± 0.045	0.058± 0.020	0.49	-	0.061± 0.039	0.060± 0.018	0.95	
ADC_{mean}	1094± 186	1185± 139	0.47	-	1198± 183	1160± 135	0.66	

HR: hazard ratio; SD: standard deviation; CI: confidence interval; rCBV: relative cerebral blood volume; ADC: apparent diffusion coefficient.

Table 4. Survival related to histopathologic and genetic subtypes in patients with diffuse glioma grade II and III.

	Progression-free survival		Overall survival	
	P-value	Cox model HR (CI 95%)	P-value	Cox model HR (CI 95%)
Oligodendroglioma vs diffuse astrocytoma	0.01	0.4 (0.2; 0.8)	0.003	0.2 (0.05; 0.6)
Diffuse glioma grade III vs grade II	0.05	2.0 (1.2; 9.8)	0.006	3.6 (1.3; 9.6)

HR: hazard ratio; SD: standard deviation; CI: confidence interval; rCBV: relative cerebral blood volume; ADC: apparent diffusion coefficient.