European Journal of Cancer 107 (2019) 15–27

Available online at www.sciencedirect.com

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journal homepage: www.ejcancer.com

Original Research

Contrast enhancement is a prognostic factor in *IDH1/2* mutant, but not in wild-type WHO grade II/III glioma as confirmed by machine learning

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Received 14 July 2018; received in revised form 29 October 2018; accepted 30 October 2018
Available online 7 December 2018

KEYWORDS
IDH mutation;
Glioma;
Contrast enhancement;
Co-del 1p/19q;
Machine learning

Abstract ***Background:*** Mutation of the isocitrate dehydrogenase (*IDH*) gene and co-deletion on chromosome 1p/19q is becoming increasingly relevant for the evaluation of clinical outcome in glioma. Among the imaging parameters, contrast enhancement (CE) in WHO II/III glioma has been reported to indicate poor outcome in the past. We aimed at reassessing the prognostic value of CE in these tumours within the framework of molecular markers using a machine learning approach (random survival forests [RSF]) as well as conventional Cox regression modelling.

Methods: 301 patients with WHO grade II (n = 181) or grade III glioma (n = 120) were stratified according to their molecular profile. Pre-operative magnetic resonance imaging (MRI) was reviewed and volumetric analyses of CE and T_2 volumes were performed followed by conventional univariate and multivariate Cox analyses. Furthermore, the dataset was split into

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https://doi.org/10.1016/j.ejca.2018.10.019

discovery and validation datasets, and RSFs were trained on the discovery dataset to predict the individual risk of each patient. Concordance indices for Cox and RSF models were determined and the variable importance of explanatory variables was assessed using the minimal-depth concept.

Results: In *IDH* mut tumours only, both conventional Cox regression modelling and RSF analyses showed that CE on initial MRI is a prognostic factor for survival with dependence on volume ($p < 0.05$). In contrast, presence of CE on initial MRI was not associated with outcome in *IDH* wt tumours.

Conclusions: In patients with diffuse *IDH* wt gliomas WHO grade II/III, CE is not associated with survival, whereas in tumours with an *IDH* mutation, presence of CE on initial MRI is linked to inferior survival.

1. Introduction

Disease course in diffuse glioma depends on demographic, histological, molecular and radiological features. Currently, a three-way classification based on isocitrate dehydrogenase (*IDH*) mutation and co-del 1p/19q has been implemented into the updated WHO classification with consequences for risk stratification and treatment planning [1–3]. Furthermore, malignancy of a glioma is often assumed in case of contrast enhancement (CE) on initial magnetic resonance imaging (MRI) [4–6]. So far, *IDH* mutation has been described to be associated with radiological features, such as tumour size, location and CE in malignant gliomas [7–10]. However, in this context, WHO II tumours had always been analysed separately from WHO III gliomas. Recent studies indicate that molecular markers such as *IDH* mutation and co-del 1p/19q are clinically more relevant than grading [11–14]. This suggests that diffuse gliomas WHO II and III should be analysed with respect to their molecular properties.

The prognostic value of pre-therapeutic parameters is often determined by modelling end-points like overall survival (OS) and progression-free survival (PFS) with Cox proportional hazard models. Although well established, these models rely on assumptions, and appropriate variable selection in multivariate analysis is controversial [15]. Modern machine learning techniques offer alternatives for statistical modelling in survival settings; random survival forests (RSFs) are particularly promising in that they can deal with correlated variables, do not rely on restrictive assumptions and provide an intuitive measure of the importance of individual variables [16]. RSFs are based on a combination of multiple decision trees and as a consequence can identify and capture interactions between variables.

The present study uses both conventional Cox regression and RSF analysis to investigate the prognostic role of CE in patients with gliomas WHO grade II/III in light of *IDH* mutation status and co-deletion 1p/19q.

2. Materials and methods

2.1. Patient cohort

Data of all newly diagnosed patients with a histological diagnosis of a WHO grade II or grade III glioma undergoing either biopsy or surgery between 2005 and 2015 in the Department of Neurosurgery of the LMU Munich were retrospectively analysed. We included all cases since 2010, as in those cases *IDH* mutation testing was regularly performed as part of the clinical routine, as well as all cases since 2005, where information on co-deletion 1p/19q was already available and *IDH* mutation testing could be performed retrospectively. Furthermore, data on clinical and imaging follow-up had to be available; patients who previously received a 'wait-and-see' strategy in case of a non-enhancing tumour and who were referred to our centre in case of a new CE were excluded from the study. The study was approved by the ethic committee of the Ludwig-Maximilians University, Munich. Stereotactic biopsies were performed using high-resolution contrast-enhanced T1-weighted MRI. In case of CE, this area was used for biopsy. Medical records were reviewed for demographic and clinical data, such as age and Karnofsky Performance Score (KPS) at diagnosis, imaging data and treatment parameters. Decisions on choice of treatment modality, such as the surgical procedure and adjuvant therapy, were made by an interdisciplinary tumour board. Informed consent was obtained from all patients. Date of death or last follow-up and date of progression were obtained on the basis of regular follow-up investigations scheduled every 6 months in WHO grade II and every 3 months in WHO grade III patients. Date of last follow-up was 30th September 2017. OS was calculated from the date of first diagnosis until death or date of last follow-up. PFS was calculated from date of first diagnosis until radiological or clinical progression according to MacDonald criteria, as many patients were included before publication of RANO criteria [17,18].

2.2. Magnetic resonance imaging

MRI was performed on 3.0 T scanners (Philips Intera 3T, Andover, MA, and Signa HDxt, GE Healthcare, Milwaukee, WI). The standardised protocol comprised an axial diffusion-weighted, an axial T_2-weighted, an axial fluid-attenuated inversion recovery (FLAIR) sequence, and T_1-weighted gradient-echo sequences before and after the administration of a gadolinium-based contrast agent. Evaluation of CE was performed on T_1-weighted gradient-echo sequences by experienced investigators (B.S., M.L., B.E.W.) taking into account any CE, which was not related to vascular structures, regardless of its size and appearance. A volumetric analysis of the CE volume and T_2-based volume was performed using the Brainlab 2.0 neuronavigational software as described previously [19]. A more detailed description can be found in the supplementary material section.

2.3. Histology and grading

All tumours were formalin-fixed and paraffin-embedded according to standard procedures. And 2-μm sections were cut and stained with haematoxylin and eosin (H&E) before histopathological grading. Grading was independently assessed by two experienced neuropathologists (U.S. and A.G.); all incongruent cases were discussed until a consensus concerning the diagnosis was reached. Analyses were done blinded for molecular and clinical parameters.

2.4. Sequencing and co-del 1p/19q analysis

IDH1 and *IDH2* mutations were examined by pyrosequencing, while co-del 1p/19q was detected using microsatellite markers as described previously [20]. Detailed description can be found in the supplement.

2.5. Statistical analysis and machine learning

Conventional statistical analyses were performed using the SPPS 22.0 software. Demographic data were analysed via chi-square test in case of categorical variables and via Wilcoxon test in case of continuously scaled as variables. Univariate and multivariate Cox regression modelling was performed for survival analyses.

Machine learning analysis was performed with R (version 3.4.4), using the framework mlr [21,22]. A short description of the RSF algorithm principle can be found in the supplementary material section. To ensure unbiased evaluation of predictive performance, the data were split into discovery and validation datasets with a 2:1 ratio. Two RSFs with 5000 trees each were trained on the discovery dataset with the target variables PFS and OS, respectively, using the package randomForestSRC [23]. Age, KPS, presence of co-del 1p/9q, surgery versus biopsy, adjuvant treatment, WHO grade, presence of CE and presence of *IDH* mutation were used as explanatory variables. To minimise the risk of overfitting, default settings of the RSF algorithm were used (mtry = 3, nodesize = 3). The trained RSF models were used to predict an individual risk of each patient in the discovery and validation datasets; predictions in the discovery dataset were made with out-of-bag data. As a measure of predictive performance, the concordance indices in each dataset were calculated. The validation dataset was split into high- and low-risk groups using the median of the predicted risk, and a Kaplan–Meier analysis was performed.

To investigate whether the RSF model for OS captured the interaction between CE and *IDH* mutation, the 48-month survival was predicted for all patients in the discovery dataset and compared in notched boxplots with respect to CE and *IDH* mutation. In notched boxplots, non-overlapping notches suggest a significant difference in medians. In case that the RSF model correctly captured the interaction between CE and *IDH*, it was expected that notches overlap in patients with *IDH* wt, and not in patients with *IDH* mutation.

Table 1
Patient characteristics.

Parameter	All patients	*IDH* wt	*IDH* mut
Total number	301	93	208
%		30.9	69.1
WHO grading			
Grade II	181	34	147
%	60.1	36.5	70.7
Grade III	120	59	61
%	39.9	63.5	29.3
Age < 45 years	173	31	142
%	57.5	33.3	68.2
KPS < 80	10	7	3
%	0.3	7.5	1.4
Resection	64	5	59
%	21.3	5.4	28.4
Biopsy	237	88	149
%	78.7	94.6	71.6
Radiochemotherapy	30	23	7
%	10	24.7	3.3
EBRT	50	28	22
%	16.6	30.1	40.6
Chemotherapy	124	25	99
%	41.2	26.9	47.6
Wait-and-see	97	17	80
%	32.2	18.2	38.5
Contrast enhancement (CE)	121	45	76
%	40.2	58.4	36.5
CE: WHO II	48	8	40
%	39.7	17.8	52.6
CE: WHO III	73	37	36
%	60.3	82.2	47.4

EBRT = external beam radiation therapy; *IDH* = isocitrate dehydrogenase; KPS = Karnofsky performance scale; WHO = World Health Organisation.
Percentages are in-column values.

The importance of explanatory variables was determined from the trained RSF models using the minimal-depth concept [16]. The minimal depth is the forest-averaged distance to the tree trunk at which an explanatory variable is used for data splitting; variables with a strong predictive performance have a low minimal depth.

3. Results

A total number of 301 patients with either WHO grade II (n = 181) or WHO grade III (n = 120) were included. Of them, 208 (69.1%) patients had an *IDH* mutation, that is, 147 of 181 (81.2%) of WHO grade II and 61 of 120 (50.8%) of WHO grade III gliomas. The prevalence of *IDH2* mutation was 6.7% in our study population. Open tumour resection was performed in 64 patients, while 237 patients received stereotactic biopsy. The large number of biopsies reflects the high number of patients with eloquently located tumours referred to our centre. A difference in surgical procedure based on *IDH* mutation status can be ruled out, as both biopsy and resection were performed in order to obtain molecular characteristics.

Treatment differed significantly between WHO groups ($p < 0.0001$), reflecting the fact that patients with a grade II tumour were less likely to receive therapy than patients with a grade III tumour, whereas presence of CE did not influence choice of treatment modality within the WHO groups ($p = 0.19$ in the WHO II/ $p = 0.7$ in the WHO III group, see also Table 1 for detailed treatment description).

3.1. Molecular groups and outcome

Median follow-up time in the entire study population was 64.1 months for the survivors. During the follow-up period, 215 patients were alive, 86 patients died and 134 had experienced tumour progression. Median OS in the entire group was not reached. Mean estimated survival was 147.7 months (95% confidence interval [CI], 133.8–161.6), and median PFS was 38.1 months (95% CI, 31.2–44.9).

Stratification according to the three molecular subgroups revealed the following distribution of survival times: *IDH* wt patients had the worst outcome while IDH mut/co-del 1p/19q did best ($p < 0.001$, see Table 2, Fig. 1). Further univariate and multivariate analyses were performed within the *IDH* wt versus *IDH* mut group, the latter consisting both of 1p/19q co-deleted and non-co-

Table 2
PFS and OS times according to molecular pattern and CE.

Parameters	PFS (median, 95% CI) (months)	HR (95% CI fo	*p*-value	Events/ patients	OS (median, 95% CI) (months)	HR (95% CI for HR)	*p*-value	Events/ patients
IDH wt	15.1 (13.5–16.8)	0.41 (0.33–0.51)	<0.001	134/301	25.7 (20.0–31.4)	0.18 (0.11–0.24)	<0.001	86/301
IDH mut/no co-del 1p/19q	53.8 (30.0–77.3)				113.2[a] (102.9–123.4)			
IDH mut/co-del 1p/19q	68.8 (47.5–74.2)				198.1[a] (181.2–215.1)			
CE								
Entire group								
CE yes	32.3 (19.9–44.8)	0.59 (0.42–0.84)	0.002	134/301	92.0 (60.9–123.1)	0.46 (0.33–0.76)	0.001	86/301
CE no	71.8 (50.6–93.0)				164.8[a] (147.3–182.2)			
WHO II								
CE yes	50.2 (16.4–84.0)	0.73 (0.44–1.21)	0.22	71/181	167.8 (150.7–185.0)	0.77 (0.30–2.08)	0.62	23/181
CE no	77.8 (52.1–103.5)				181.1 (161.5–200.7)			
WHO III								
CE yes	23.0 (15.4–30.6)	0.74 (0.44–1.25)	0.26	63/120	56.5 (27.2–85.4)	0.72 (0.42–1.22	0.30	63/120
CE no	32.8 (0.5–70.5)				59.3 (52.7–101.5)			
***IDH* wt**								
CE yes	14.8 (6.6–22.9)	0.76 (0.45–1.29)	0.31	56[b]/93	24.5 (16.2–32.8)	0.77 (0.46–1.27)	0.30	60/93
CE no	15.9 (13.7–18.1)				27.4 (18.6–36.2)			
***IDH* mut**								
CE yes	47.3 (22.7–72.0)	0.60 (0.38–0.93)	0.02	78/208	140.9 (120.0–199.4)	0.31 (0.14–0.69)	0.002	26/208
CE no	89.3 (67.2–111.3)				196.1 (178.0–213.4)			
***IDH* mut/no co-del 1p/19q**								
CE yes	36.8 (21.1–52.4)	0.48 (0.26–0.90)	0.02	41/88	99.3[a] (79.2–119.5)	0.39 (0.15–0.98)	0.04	18/88
CE no	86.4 (42.8–93.9)				112.4[a] (102.7–122.2)			
***IDH* mut/co-del 1p/19q**								
CE yes	87.9 (31.8–144.0)	0.62 (0.33–1.20)	0.15	37/120	151.1[a] (126.3–175.9)	0.08 (0.01–0.69)	0.003	8/120
CE no	106.0[a] (88.0–124.1)				214.8[a] (199.6–230.1)			

PFS = progression-free survival; OS = overall survival; CI = confidence interval; HR = hazard ratio; *IDH1/2* = isocitrate dehydrogenase $^1/_2$; mut = mutation; co-del 1p/19q = co-deletion on chromosomes 1p/19q; WHO = World Health Organisation; CE = contrast enhancement.

[a] Mean values (median not reached).

[b] In 4 of 60 patients out of the IDH wt group, no date of progression was documented before death.

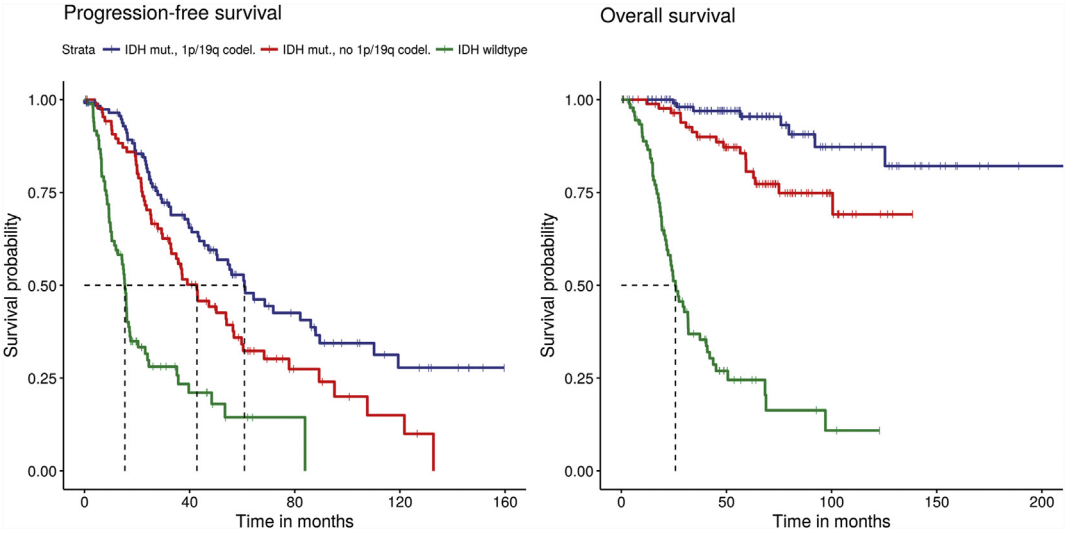

Fig. 1. Progression-free survival (left, $p < 0.001$) and overall survival (right $p < 0.001$) according to the *IDH* mutation and 1p/19q co-deletion status. IDH = isocitrate dehydrogenase.

deleted patients. A separate analysis according to co-deletion 1p/19q in the *IDH* mut group could not be performed due to the low number of events.

3.2. CE, volumetric parameters and molecular subgroups

CE was present in 40.2% of all patients. WHO III tumours were more likely to present with CE (60.8%) than WHO II tumours (26.5%, $p < 0.001$). In WHO grade II and III tumours pooled together, presence of CE was associated with shorter PFS/OS while when WHO II and WHO III were analysed separately, the presence of CE enhancement was associated neither with PFS nor with OS (see Table 2, Fig. 2).

CE occurred more often in *IDH* wt tumours (45/93; 48.4%) compared to *IDH* mut gliomas (76/208; 36.5%); however, this difference was not statistically significant ($p = 0.057$). Mean CE volume was 1.51 ml; no statistical difference in volume was observed between *IDH* mut (1.76 ml) and *IDH* wt tumours (1.40 ml; $p = 0.38$).

Stratification of outcome revealed CE not to be associated with prognosis in *IDH* wt patients, while it was significantly associated with outcome in the two *IDH* mut groups (see Table 2, Figs. 3 and 4).

Assessment of T_2 volume revealed that *IDH* mut tumours were larger (mean volume, 76.8 ml [standard deviation, 86.7 ml]) than *IDH* wt tumours (53.3 ml [standard deviation, 54.1]; $p = 0.005$). While there was no correlation of T_2 tumour volume with outcome in the *IDH* wt group ($p = 0.41$ for PFS/$p = 0.22$ for OS), smaller-sized *IDH*-mutated tumours had longer PFS

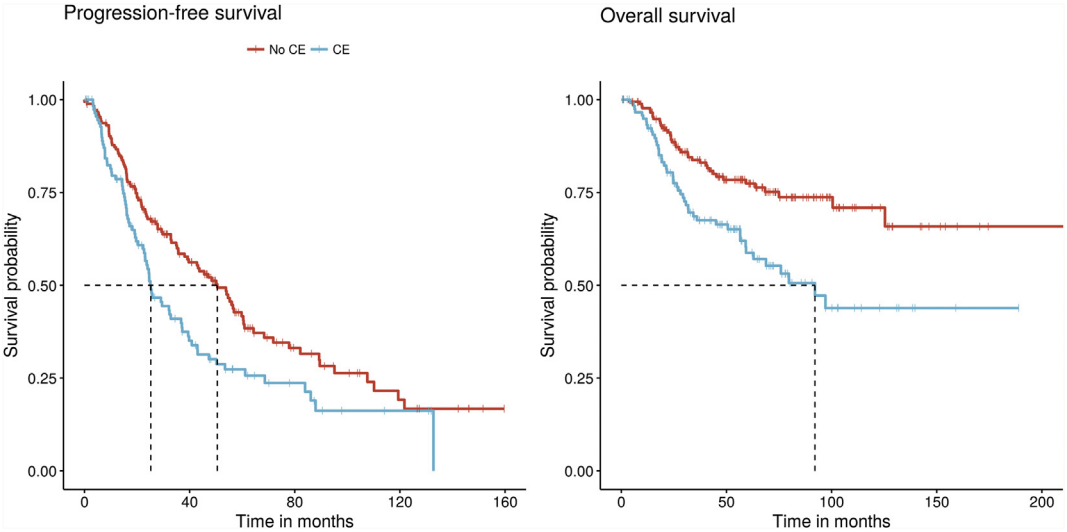

Fig. 2. Apparent influence of contrast enhancement on progression-free survival (left, $p = 0.002$) and overall survival (right, $p = 0.001$) when WHO grade II and WHO III are pooled together. WHO = World Health Organisation.

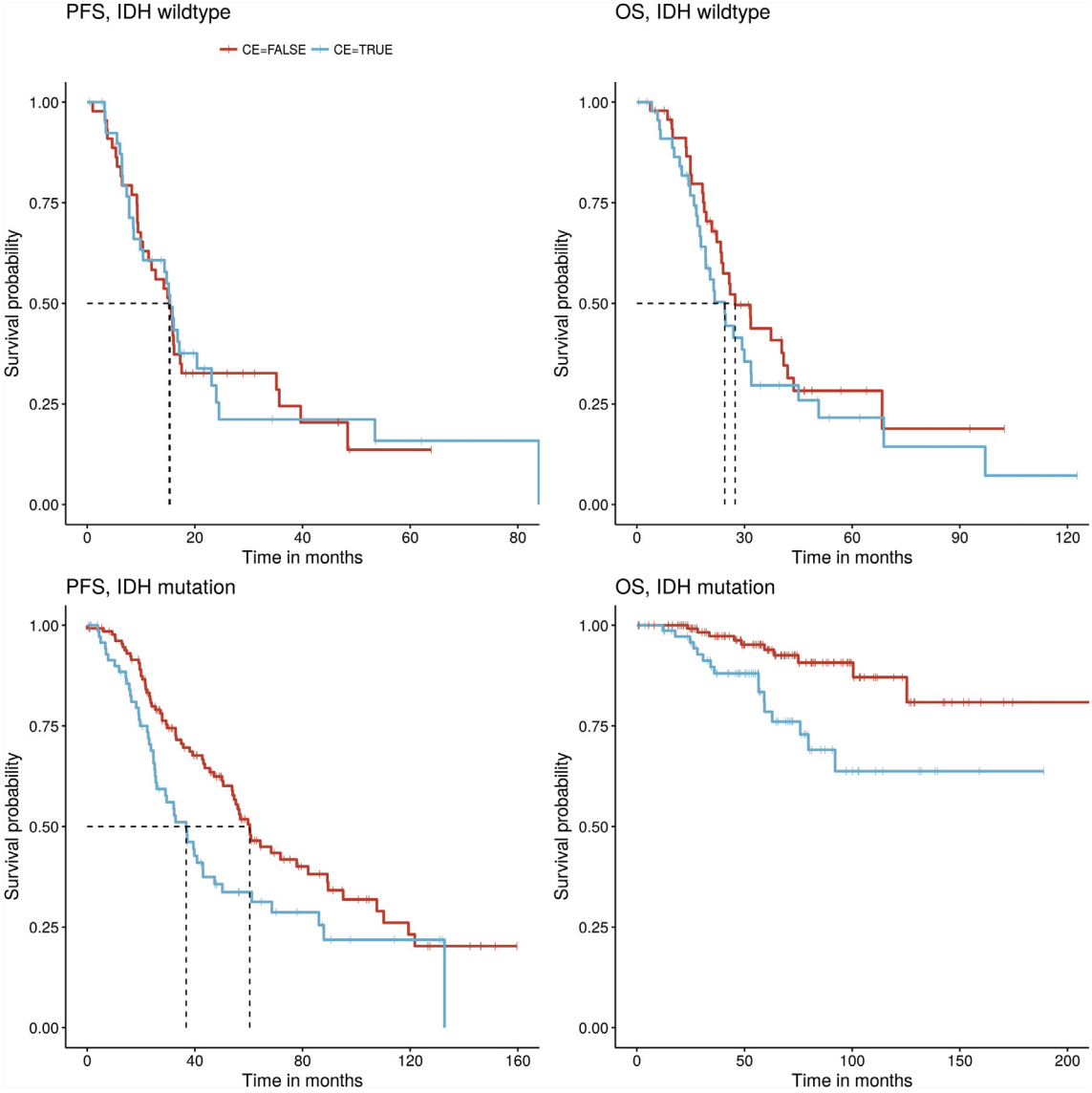

Fig. 3. While contrast enhancement (CE) was not associated with progression-free survival (PFS; upper left, $p = 0.31$) or overall survival (OS; upper right, $p = 0.30$) in the *IDH* wt group, presence of CE on initial magnetic resonance imaging was a negative prognostic factor for PFS (lower left, $p = 0.02$) and OS (lower right, $p = 0.002$) in the *IDH* mut group. IDH = isocitrate dehydrogenase.

($p < 0.001$) but not OS ($p = 0.15$) times; this result, however, should be regarded cautiously due to the relatively low number of events for OS compared to the number of events for PFS.

3.3. Molecular markers within cohorts with/without CE

Within the cohorts of tumours either with or without CE, the stratification according to the molecular subclassification remained associated with prognosis: patients with *IDH* mut/co-del 1p/19q glioma had the best outcome in either cohort followed by *IDH* mut/no co-del 1p/19q and the *IDH* wt tumours ($p < 0.0001$). For Kaplan-Meier-curves, see Fig. 5.

3.4. Statistical analysis and machine learning

3.4.1. Conventional Cox regression analyses

Univariate analysis revealed that lower age, lower KPS values at initial diagnosis, WHO grade II, resection, no CE at initial MRI, presence of *IDH* mutation and prolonged interval until initiation of adjuvant therapy are associated with prolonged OS times (Table 3).

As WHO grade was highly intercorrelated with mode of adjuvant treatment, we calculated alternate Cox-regression models containing either variable (see Table 4 for model with WHO grade and Suppl. Table 1 for model with adjuvant treatment). Furthermore, separate Cox-regression models including T_2 and CE volumes as well as 'presence of CE' and 'CE volume' were

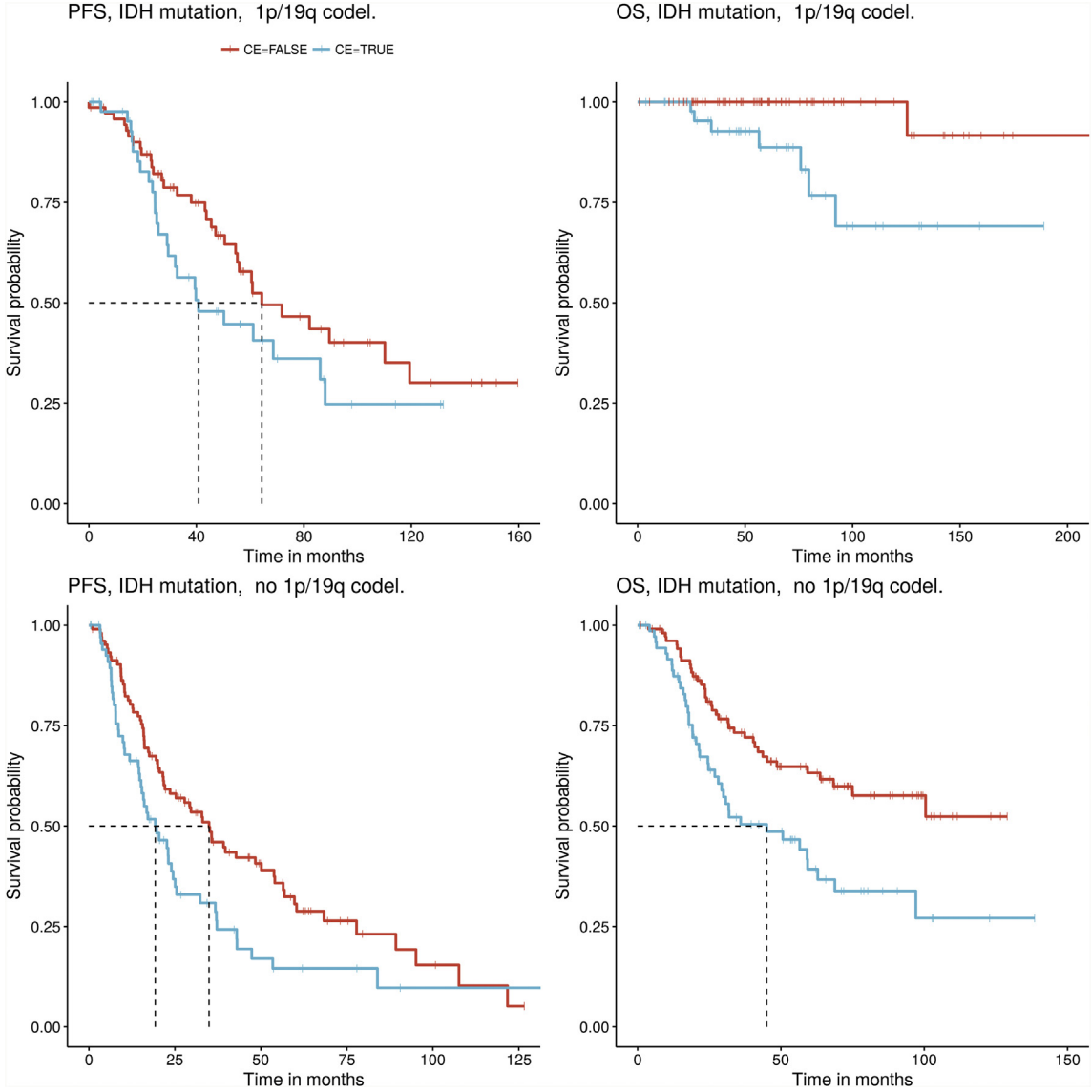

Fig. 4. ontrast enhancement remains a prognostic factor for progression-free survival (PFS) and overall survival (OS) in the *IDH* mut group, independent of the 1p/19q co-del status: PFS (upper left, $p = 0.15$) and OS (upper right, $p = 0.003$) in the co-del; PFS (lower left, $p = 0.02$) and OS (lower right, $p = 0.04$) in the non-co-del group. IDH = isocitrate dehydrogenase.

performed (Tables 5 and 6). Regardless of the model calculated, presence of CE remained an independent prognostic factor associated with poor outcome in the *IDH* mut, but not *IDH* wt group.

4. RSF analysis

The trained RSF model for OS achieved concordance indices of c = 0.859 on the discovery dataset and c = 0.849 on the validation dataset, whereas the RSF for PFS achieved c = 0.704 on the discovery data and c = 0.714 on the validation data. The individual risk of the end-points OS and PFS was predicted on the validation set and used to stratify the patients into high- and low-risk groups. The resulting Kaplan–Meier analysis (Fig. 6) demonstrates that the machine learning–predicted risks are strongly associated with OS and PFS, respectively.

The predicted 48-month survival (Fig. 7) for patients with *IDH* wt is much lower than for patients with *IDH* mutation, in accord with Figs. 1 and 3. In addition, the trained model suggests that the presence of CE plays a relevant role in patients with *IDH* mutation, whereas CE does not appear to have an influence in the predictions for patients with *IDH* wt. This is in good agreement with the results in Fig. 3.

Fig. 8 displays the minimal depth of each explanatory variable as a measure of its prediction of outcome. With respect to OS, the presence or absence of *IDH* mutation closely followed by age is of highest importance. These variables have also the highest importance for the prediction of tumour progression.

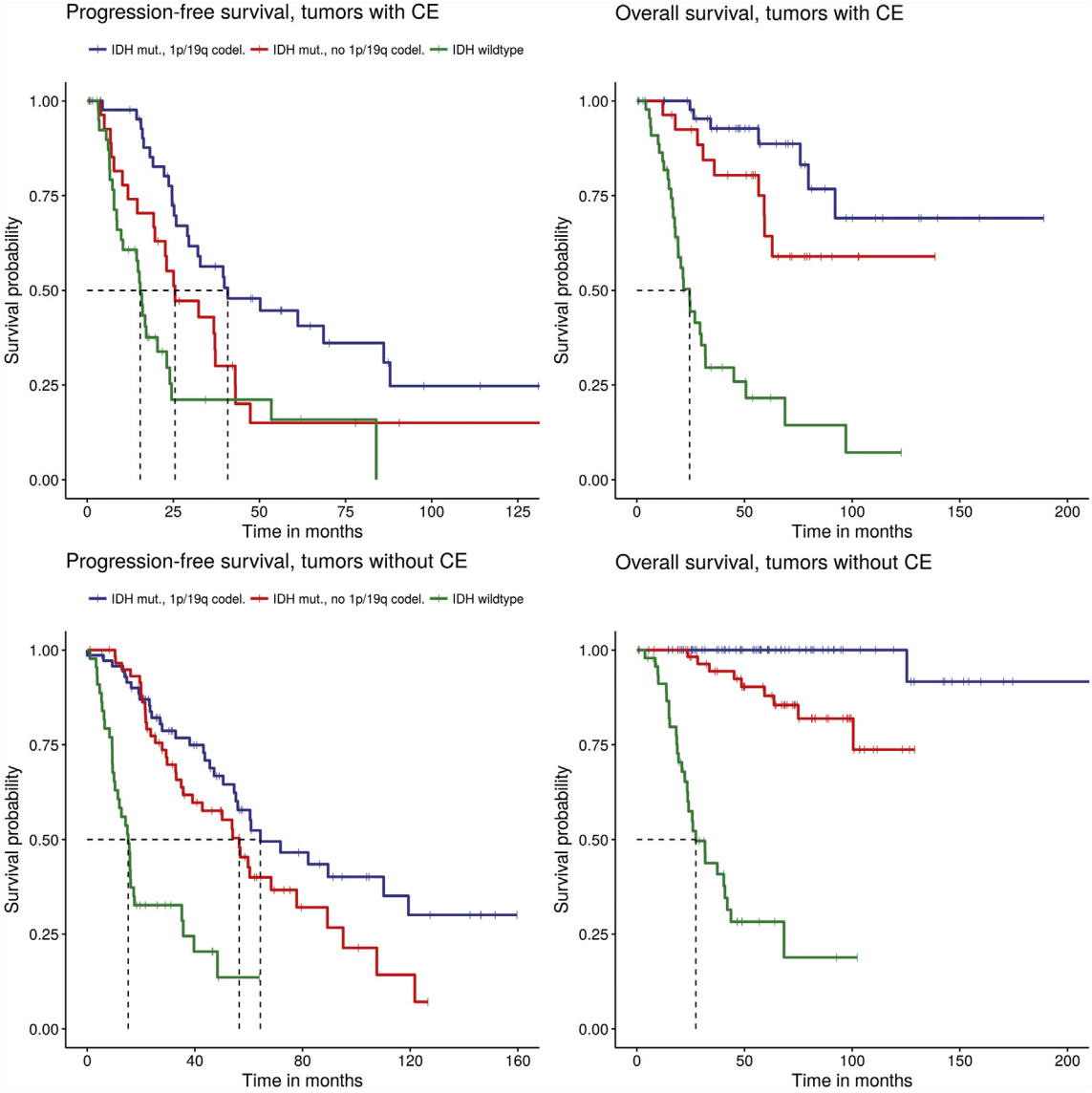

Fig. 5. Stratification of tumours according to presence of contrast enhancement (CE; upper row, $p < 0.001$ both) and absence of CE (lower row, $p < 0.001$ both) and *IDH* mut/1p/19q co-del status. IDH = isocitrate dehydrogenase.

Table 3
Univariate analysis of PFS/OS.

Parameter	PFS			OS		
	p-value	HR	95% CI	*p*-value	HR	95% CI
Age[a]	0.03	1.01	1.00–1.03	<0.001	1.04	1.03–1.06
KPS[a]	0.03	0.97	0.94–1.00	0.002	0.95	0.92–0.98
WHO grade II versus WHO grade III	<0.001	0.47	0.34–0.67	<0.001	0.15	0.10–0.28
IDH-mut versus wild type	<0.001	0.22	0.15–0.32	<0.001	0.08	0.05–0.12
Co-del 1p/19q yes versus no	<0.001	0.38	0.26–0.56	<0.001	0.12	0.06–0.25
Resection versus. biopsy	0.56	0.89	0.59–1.33	0.04	0.54	0.30–0.98
CE no versus yes	0.002	0.59	0.42–0.84	0.001	0.46	0.33–0.76
Adjuvant treatment[b]	<0.001	0.68	0.58–0.82	<0.001	0.43	0.35–0.53
CE volume[a]	<0.001	1.05	1.02–1.08	0.059	1.02	1.00–1.06
T2 volume[a]	0.04	1.00	1.00–1.01	0.81	1.00	0.99–1.01

CE, contrast enhancement; CI, confidence interval; EBRT, external beam radiation therapy; HR, hazard ratio; *IDH*, isocitrate dehydrogenase; KPS, Karnofsky performance scale; PFS, progression-free survival; OS, overall survival; WHO, World Health Organisation.

[a] Continuously scaled.

[b] Wait-and-see' versus 'chemotherapy' versus 'radiotherapy' versus 'radiochemotherapy'.

Table 4
Multivariate analysis for PFS/OS for the entire group, as well as according to *IDH* mutation status.

Parameters	Entire group					
	PFS			OS		
	p-value	HR	95% CI	*p*-value	HR	95% CI
CE no versus yes	0.20	0.80	0.57–1.13	0.50	0.84	0.51–1.39
Age[a]	0.64	1.00	0.99–1.02	0.02	1.02	1.00–1.04
KPS[a]	0.74	0.99	0.97–1.02	0.76	1.00	0.96–1.03
WHO grading II versus III	0.02	0.66	0.36–0.78	<0.0001	0.28	0.16–0.50
Biopsy versus resection	0.53	0.78	0.68–1.49	0.46	0.78	0.39–1.53
Co-del 1p/19q yes versus no	0.02	0.64	0.44–0.93	0.002	0.27	0.12–0.62
IDH-mut versus wild type	<0.0001	0.37	0.25–0.56	<0.0001	0.16	0.09–0.30
Parameter	***IDH* mut**					
	PFS			**OS**		
	***p*-value**	**HR**	**95% CI**	***p*-value**	**HR**	**95% CI**
CE no versus yes	0.03	0.56	0.36–0.95	0.01	0.38	0.16–0.86
Age[a]	0.05	0.98	0.96–0.99	0.78	0.99	0.96–1.03
KPS[a]	0.15	0.97	0.93–1.01	0.68	0.99	0.92–1.05
WHO grading II versus III	0.85	1.05	0.63–1.61	0.01	0.32	0.13–0.75
Biopsy versus resection	0.38	0.80	0.49–1.31	0.98	1.01	0.44–2.33
Co-del 1p/19q yes versus no	0.03	0.58	0.36–0.93	0.02	0.35	0.14–0.85
Parameters	***IDH* wt**					
	PFS			**OS**		
	***p*-value**	**HR**	**95% CI**	***p*-value**	**HR**	**95% CI**
CE no versus yes	0.53	0.81	0.43–1.52	0.22	0.70	0.39–1.23
Age[a]	0.02	1.03	1.01–1.05	0.14	1.02	1.00–1.04
KPS[a]	0.75	1.01	0.96–1.05	0.93	1.00	0.96–1.04
WHO grading II versus III	0.02	0.45	0.23–1.04	<0.001	0.25	0.15–0.46
Biopsy versus resection	0.98	1.02	0.23–4.41	0.83	0.88	0.26–2.94
Co-del 1p/19q yes versus no	na	na	na	na	na	na

CE, contrast enhancement; CI, confidence interval; HR, hazard ratio; co-del 1p/19q, co-deletion on chromosomes 1p and 19q; IDH, isocitrate dehydrogenase; KPS, Karnofsky performance scale; PFS, progression-free survival; OS, overall survival; WHO, World Health Organisation.
[a] Continuously scaled.

5. Discussion

In clinical practice, CE has often been assumed to be an indicator of dismal prognosis, merely due to its association with malignant progression [24–27]. However, whereas age and tumour size have been shown to be prognostic in prospective trials, few or inconclusive data exist for CE [28,29]. Recent findings suggest that molecular markers might characterise different tumour populations, leading to refined grading systems in the current WHO classification [1,2]. In our study, we re-evaluate CE as a prognostic factor within the framework of these molecular markers.

Using conventional Cox modelling and a machine learning analysis, we reveal that patients with tumours harbouring an *IDH* mutation have longer PFS and OS times when lacking CE on initial MRI. Recent reports analysing the association between CE and *IDH* mutation in 'malignant glioma' lumping grade III and grade IV together suggested that CE is a prognostic factor for outcome in *IDH* mutated, but not in *IDH* wt tumours [7,8,30]. The findings of our study not only confirm these data for WHO III but also show that this interrelation is true for WHO grade II tumours as well. On the contrary, CE was not associated with prognosis in *IDH* wt patients. Thus, absence of CE is not a favourable sign in *IDH* wt WHO II glioma and should not result in under-treatment.

Moreover, the association between CE and outcome in the *IDH* mut group was observed both in 1p/19q co-deleted and non-co-deleted tumours. Fig. 5 shows that the influence of molecular status on outcome seems to be the same both within the group 'CE yes' and 'CE no', with patients harbouring *IDH* mut, 1p/19q co-deleted tumours having the best outcome, followed by patients diagnosed with *IDH* mut, non-co-deleted tumours. This finding supports the hypothesis that *IDH* mutation is an early event in tumourigenesis, which occurs much earlier than co-del 1p/19q or CE and that the latter is a secondary event, which can further discriminate tumours with different prognosis [31,32]. In addition, volumetric tumour size in T_2-weighted MRI was associated with PFS in *IDH* mut, but not *IDH* wt gliomas, indicating that *IDH* wt status might be more powerful than tumour size. This is in line with recently published findings concerning the value of non-enhancing tumour volume in *IDH* mut glioma [33].

Table 5
Multivariate analysis for PFS/OS for the entire group, as well as according to *IDH* mutation status (CE volume).

Parameters	Entire group					
	PFS			OS		
	p-value	HR	95% CI	*p*-value	HR	95% CI
CE volume[a]	0.001	1.05	1.02–1.05	0.22	1.03	0.99–1.07
Age[a]	0.57	1.00	0.99–1.02	0.02	1.02	1.00–1.05
KPS[a]	0.48	0.99	0.96–1.02	0.92	1.00	0.97–1.04
WHO grading II versus III	0.16	0.76	0.52–1.11	<0.0001	0.27	0.16–0.49
Biopsy versus resection	0.33	0.79	0.49–1.31	0.46	0.78	0.55–1.53
IDH-mut versus wild type	<0.001	0.29	0.18–0.45	<0.001	0.16	0.09–0.30
Co-del 1p/19q yes versus no	0.01	0.55	0.35–0.85	0.001	0.26	0.11–0.59
Parameter	***IDH* mut**					
	PFS			**OS**		
	***p*-value**	**HR**	**95% CI**	***p*-value**	**HR**	**95% CI**
CE volume[a]	<0.001	1.07	1.03–1.10	0.029	1.05	1.01–1.10
Age[a]	0.04	0.98	0.96–1.00	0.88	0.99	0.96–1.04
KPS[a]	0.16	0.97	0.94–1.01	0.99	1.00	0.94–1.07
WHO grading II versus III	0.91	1.03	0.61–1.72	0.004	0.29	0.12–0.68
Biopsy versus resection	0.50	0.84	0.51–1.39	0.96	1.02	0.34–2.48
Co-del 1p/19q yes versus no	0.04	0.61	0.38–0.97	0.04	0.39	0.16–0.95
Parameters	***IDH* wt**					
	PFS			**OS**		
	***p*-value**	**HR**	**95% CI**	***p*-value**	**HR**	**95% CI**
CE volume[a]	0.55	1.02	0.97–1.07	0.08	0.96	0.91–1.01
Age[a]	0.02	1.02	1.00–1.04	0.14	1.01	0.99–1.04
KPS[a]	0.52	1.02	0.97–1.07	0.72	0.99	0.95–1.04
WHO grading II versus III	0.03	0.48	0.27–0.95	0.001	0.24	0.12–0.45
Biopsy versus resection	0.90	1.10	0.25–4.76	0.97	0.99	0.29–3.13
Co-del 1p/19q yes versus no	na	na	na	na	na	na

CE, contrast enhancement; CI, confidence interval; HR, hazard ratio; co-del 1p/19q, co-deletion on chromosomes 1p and 19q; IDH, isocitrate dehydrogenase; KPS, Karnofsky performance scale; PFS, progression-free survival; OS, overall survival; WHO, World Health Organisation.
[a] Continuously scaled.

Our machine learning analysis confirmed the results of conventional Cox regression models. The trained RSF achieved very good prognostic performance, on both the discovery and validation datasets. The distinct separation of groups in the Kaplan-Meier analysis confirms the excellent performance on the validation dataset and indicates that the RSF model works well on previously unseen data. Moreover, the model detects an influence of CE on 48-month survival only in the subgroup with *IDH* mutation (Fig. 7). These findings match the results in Figs. 1 and 3 and indicate that the machine learning model automatically captures the interaction between CE and *IDH* with regard to OS.

Furthermore, the RSF analysis allows for additional insight into the importance of individual variables for OS and PFS prognosis. For both end-points, *IDH* mutation status and age have the lowest minimal depths of all prognostic factors, which indicates high variable importance.

Generally, we have demonstrated that a machine learning approach based on RSF provides a relatively simple and intuitive alternative to the more established Cox regression models, with the additional benefit of deeper insights into the relation between predictors and outcome. In addition, an excellent predictive performance not only on the discovery dataset but also on previously unseen validation data was demonstrated. The risk of potential overfitting was mitigated through rigorous separation of discovery and validation datasets.

Our study has two main limitations: its retrospective, single-centre design and the different treatment regimens in WHO II and grade III tumours. As our hypothesis concerning the effect of grading and CE in diffuse lower grade astrocytoma was based on the data generated at our centre, it requires a (preferably prospective) validation in a comparably large cohort.

Concerning the second limitation, up to now, patients with WHO III glioma were more likely to receive an aggressive treatment. While the proportion of patients receiving an adjuvant therapy following surgical procedure was higher in the WHO III group, presence of CE did not influence treatment decisions within the WHO groups. Furthermore, in contrast to previous reports, our study population included a large proportion of patients undergoing stereotactic biopsy. However, while adjuvant treatment was correlated with OS in the univariate analysis, neither mode of surgery (biopsy or

Table 6
Multivariate analysis for PFS/OS for the entire group, as well as according to *IDH* mutation status (T_2 volume).

Parameters	Entire group					
	PFS			OS		
	p-value	HR	95% CI	*p*-value	HR	95% CI
T_2 volume[a]	0.005	1.00	0.99–1.01	0.75	1.00	0.99–1.01
Age[a]	0.88	1.00	0.99–1.02	0.03	1.02	1.00–1.05
KPS[a]	0.44	0.99	0.96–1.02	0.98	1.00	0.96–1.04
WHO grading II versus III	0.19	0.76	0.51–1.14	<0.0001	0.27	0.16–0.47
Biopsy versus resection	0.11	0.68	0.42–1.09	0.32	0.70	0.36–1.40
IDH-mut versus wild type	<0.001	0.27	0.17–0.42	<0.0001	0.16	0.09–0.31
Co-del 1p/19q yes versus no	0.01	0.55	0.35–0.87	0.002	0.26	0.11–0.62
Parameter	***IDH* mut**					
	PFS			**OS**		
	***p*-value**	**HR**	**95% CI**	***p*-value**	**HR**	**95% CI**
T_2 volume[a]	<0.0001	1.01	1.00–1.02	0.10	1.00	0.99–1.01
Age[a]	0.009	0.97	0.95–0.99	0.65	0.99	0.95–1.03
KPS[a]	0.44	0.99	0.95–1.02	0.61	0.98	0.92–1.05
WHO grading II versus III	0.98	0.99	0.59–1.68	0.003	0.27	0.12–0.64
Biopsy versus resection	0.11	0.66	0.39–1.10	0.51	0.75	0.32–1.75
Co-del 1p/19q yes versus no	0.02	0.55	0.34–0.89	0.02	0.33	0.13–0.86
Parameters	***IDH* wt**					
	PFS			**OS**		
	***p*-value**	**HR**	**95% CI**	***p*-value**	**HR**	**95% CI**
T_2 volume[a]	0.52	0.99	0.99–1.00	0.84	1.00	0.99–1.01
Age[a]	0.02	1.02	1.00–1.05	0.29	0.98	0.99–1.03
KPS[a]	0.56	1.01	0.96–1.06	0.95	1.00	0.96–1.05
WHO grading II versus III	0.02	0.47	0.25–0.89	0.001	0.25	0.12–0.51
Biopsy versus resection	0.92	1.01	0.25–4.54	0.97	0.98	0.29–3.22
Co-del 1p/19q yes versus no	na	na	na	na	na	na

CI, confidence interval; HR, hazard ratio; co-del 1p/19q, co-deletion on chromosomes 1p and 19q; IDH, isocitrate dehydrogenase; KPS, Karnofsky performance scale; PFS, progression-free survival; OS, overall survival; WHO, World Health Organisation.
[a] Continuously scaled.

resection) nor mode of adjuvant treatment influenced outcome in the multivariate or RSF analysis. Thus, these two factors do not constitute a limitation per se, but should be kept in mind when comparing our results with those of previous studies.

6. Conclusion

In patients with diffuse *IDH* wt gliomas WHO grade II and III, CE is not associated with survival. In patients with an *IDH* mutation, presence of CE on initial MRI is

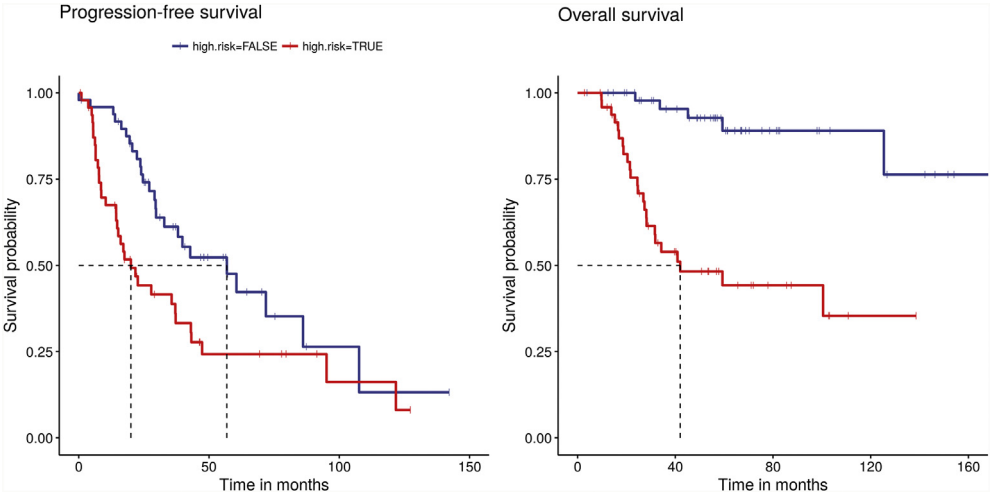

Fig. 6. Kaplan-Maier analysis of random survival forest analysis: the predicted risk of progression-free survival (left, $p < 0.001$) and overall survival (right, $p < 0.001$) is a highly significant prognostic factor.

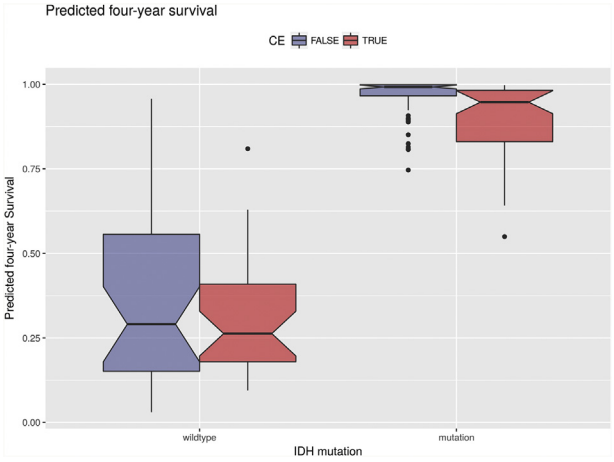

Fig. 7. Four-year overall survival as predicted by random survival forests, stratified by *IDH* mutation status and contrast enhancement (CE). The predictions for *IDH* wt tumours do not depend on CE, whereas the predicted 4-year survival in tumours with *IDH* mutation is shorter when CE is present. Notches in the boxplot indicate a 95% confidence interval, and non-overlapping notches indicate significant difference in medians. IDH = isocitrate dehydrogenase.

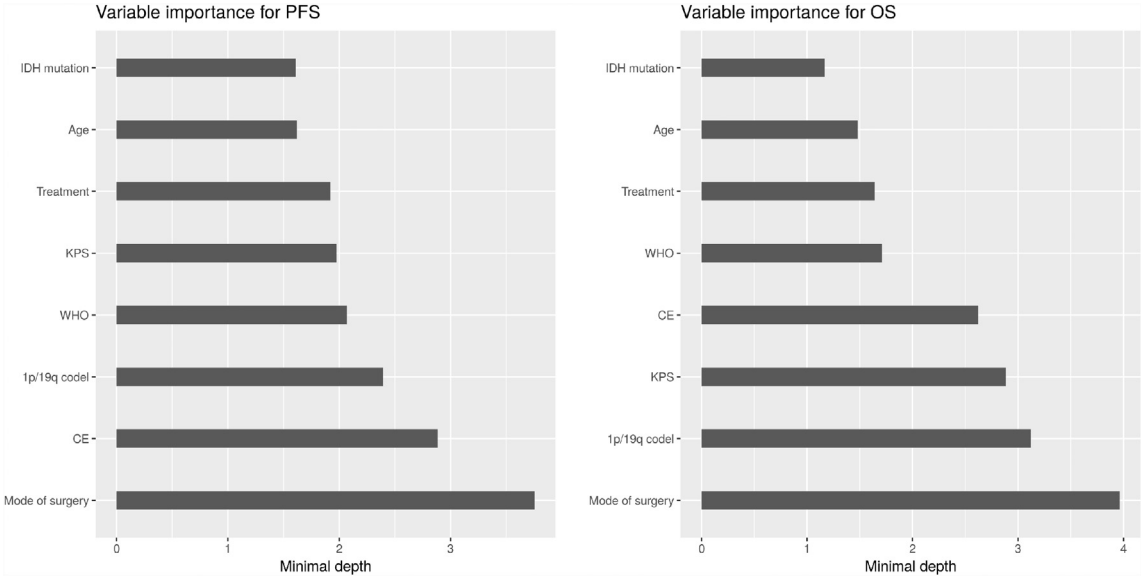

Fig. 8. Variable importance prediction for progression-free survival (left) and overall survival (right) in the entire group.

linked to inferior survival. Hence, the value of CE as a prognostic marker has to be redefined with respect to the molecular signature of the tumour. Further multicentre trials are necessary to validate our results.

Funding

This study was supported by a generous grant of the Remark-Moolenaars family.

Conflict of interest statement

Bogdana Suchorska, Ulrich Schüller, Annamaria Biczok, Markus Lenski, Nathalie Lisa Albert, Armin Giese, Friedrich-Wilhelm Kreth, Birgit Ertl-Wagner and Michael Ingrisch declare not to have any potential conflicts of interest. Joerg-Christian Tonn is a consultant/advisory board member for Merck Serono, Roche, and Celldex and has received speakers bureau honoraria from Brainlab, Merck Serono, Roche and Siemens.

Acknowledgements

We thank Janina Mielke, Anna Krieger and Michael Ruiter for expert technical help.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2018.10.019.

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