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CLINICAL STUDY

Serum levels of GFAP and EGFR in primary and recurrent high-grade gliomas: correlation to tumor volume, molecular markers, and progression-free survival

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Abstract Our aim was to study the association of two potential serum biomarkers glial fibrillary acidic protein (GFAP) and epidermal growth factor receptor (EGFR) with prognostic markers such as *IDH1* mutation, tumor burden, and survival in patients with high-grade gliomas (HGG). Additionally, our objective was to evaluate the potential of serum EGFR as a surrogate marker for EGFR status in the tumor. Pre-operative serum samples were prospectively collected from patients with primary (n = 17) or recurrent (n = 10) HGG. Serum GFAP and EGFR levels were determined by ELISA and studied for correlation with molecular markers including EGFR amplification, tumor volume in contrast-enhanced T1-weighted MRI, and progression-free survival (PFS). Pre-operative serum GFAP level of ≥0.014 ng/ml was 86 % sensitive and 85 % specific for the diagnosis of glioblastoma. High GFAP was related to the lack of *IDH1* mutation ($P = 0.016$), high Ki67 proliferation index ($P < 0.001$), and poor PFS (HR 5.9, CI 1.2–29.9, $P = 0.032$). Serum GFAP correlated with enhancing tumor volume in primary (r = 0.64 $P = 0.005$), but also in recurrent HGGs (r = 0.76 $P = 0.011$). In contrast, serum EGFR levels did not differ between HGG patients and 13 healthy controls, and were not related to EGFR status in the tumor. We conclude that high serum GFAP associates with *IDH1* mutation-negative HGG, and poor PFS. Correlation with tumor burden in recurrent HGG implicates the potential of serum GFAP for detection of tumor recurrence. Our results suggest that circulating EGFR is not derived from glioma cells and cannot be used as a marker for EGFR status in the tumor.

Keywords High-grade glioma · Serum biomarker · GFAP · EGFR · Tumor volume · Prognosis

Introduction

High-grade gliomas (HGGs, WHO grade III and IV) are aggressive brain tumors, which have a persistently dismal prognosis [1]. The gold standard for diagnosing HGG is histologic evaluation of tumor biopsy. However, inadequate amount of tissue or inherent sampling errors may limit its value [2]. Furthermore, assessing tumor progression with contrast enhanced magnetic resonance imaging (MRI) may be complicated by treatment-related changes such as pseudoprogression or pseudoresponse [3]. A readily achievable blood-based biomarker with diagnostic and prognostic value could bypass these limitations and provide complementary data in clinical decision-making [4].

Glial fibrillary acidic protein (GFAP) is an intermediate filament expressed almost exclusively in astrocytes where it acts as a member of the cytoskeleton [5]. Serum levels of GFAP are known to be elevated after stroke and traumatic

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brain injury [6, 7]. There is increasing evidence that blood levels of GFAP are also elevated in primary HGGs prior to surgical resection implicating that serum GFAP is a diagnostically relevant biomarker [8–10]. However, its prognostic value nor its association to established prognostic markers such as *IDH1* mutation, have not been examined. Also, the previous studies have included only primary HGGs whereas the correlation between serum GFAP level and tumor burden in recurrent HGGs has not been studied.

Overexpression of epidermal growth factor receptor (EGFR), which is often related to amplification of the *EGFR* gene, is a hallmark in primary or de novo glioblastomas [11]. *EGFR* gene amplification is detected in about 40 % of primary glioblastomas and approximately half of these additionally demonstrate EGFRvIII mutation, which results in constitutive signaling activity. This renders EGFR and EGFRvIII attractive targets for therapy [12]. In addition, EGFR amplification carries a diagnostic and prognostic value with an association to glioblastoma and reduced overall survival [13]. EGFR expression and amplification are most commonly determined by immunohistochemistry and chromogenic in situ hybridization (CISH), respectively. Measuring serum levels of EGFR extracellular domain (ECD) has provided additional information on tumor aggressiveness and outcome in various types of malignancies [14–17]. Quaranta et al. found elevated serum EGFR levels also in patients with glioblastomas compared to anaplastic astrocytomas or controls [18]. However, the association of serum EGFR levels to EGFR overexpression and amplification status in tumor tissue or to other molecular markers has not previously been studied.

Our purpose was to study serum levels of GFAP and EGFR in patients with primary and recurrent HGGs, and to examine their association with tumor volumes in T1-weighted post-contrast MRI, prognostic molecular markers, and progression-free survival (PFS). In addition, we wanted to evaluate the potential of serum EGFR as an indicator of tumor EGFR expression by determining the association of serum EGFR levels to tumor EGFR immunohistochemistry and gene amplification.

Materials and methods

Study subjects and sample collection

Pre-operative blood samples were prospectively obtained from 27 patients with radiologically suspected primary (n = 17) or recurrent (n = 10) HGG who were scheduled for tumor resection between 2011 and 2013 (mean age 53 years; women 63 %). Mean interval between blood sampling and surgical resection was 18 days. Additionally, post-operative blood samples were collected 2–5 days after surgical resection from 20 of these patients. Patient characteristics are presented in Table 1. The study was approved by the local ethics committee, and all patients gave written informed consent before participation. Serum samples of 13 healthy subjects (mean age 54 years; women 69 %) without a history of cancer or neurological symptoms were used as controls. Pre-operative and control blood samples were collected in Vacuette Z Serum Clot Activator (Greiner Bio-One, Kremsmünster, Austria) and post-operative blood samples in Venosafe plastic tubes (Terumo Europe N.V.). After adequate coagulation, samples were centrifuged at 2500×*g* for 10 min within 2 h, and supernatants were stored at −70 °C.

Serum GFAP and EGFR measurements

Serum GFAP and EGFR levels were determined using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits from BioVendor (Brno, Czech Republic) and OncogeneScience (Cambridge, USA), respectively. Both assays were performed according to the instructions by the manufacturer. Serum samples, quality controls and standards were diluted 1:3 for GFAP ELISA and 1:50 for EGFR ELISA. All standards and test specimens were run in duplicates with the volume of 100 µl pipetted into each ELISA well. A biotin-labelled anti-GFAP-antibody and an alkaline phosphatase-labelled anti-EGFR-antibody specifically recognizing the ECD of EGFR were employed as detector antibodies. The absorbance was measured by reading the plate at 450 nm for GFAP and at 650 nm for EGFR. Reported concentration values are the mean absorbances of the duplicates. The limit of detection defined as the mean absorbance of the blanks (calibrator diluent) + 3 SD ($A_{blank} + 3 \times SD_{blank}$) was measured and calculated as 0.014 ng/ml for GFAP. All values below this detection limit were defined as 0 ng/ml, which also was applied in case the other absorbance measurement of a duplicate was below the detection limit. Serum EGFR ELISA assays for HGG patients and control subjects were performed at separate times and, therefore, 24 samples from HGG patients were re-analyzed for serum EGFR to test the reliability of the measurement.

Immunohistochemistry and molecular markers

Formalin-fixed paraffin-embedded tumor tissues obtained during surgical resection were sectioned at 3 µm and used for analyses. GFAP, EGFR, and Ki67 were studied by immunohistochemistry using primary antibodies (clones EP671Y, 5B7, and 30-9, respectively; Ventana Medical Systems, Strasbourg, France). *IDH1* mutation was studied by immunohistochemistry with an IDH1 R132H antibody

Table 1 Clinical data, imaging abnormalities, GFAP and EGFR serum levels, and molecular markers in tumor tissue of the high-grade glioma patients studied

Clinical data and MRI							Pre- and post-operative serum levels				Molecular markers tumor tissue							
Pt no.	Age/sex	Stage	Gr	Dg	PFS (mo)	V_{Gad} (cm^3)	Pre GFAP (ng/ml)	Post GFAP (ng/ml)	Pre EGFR (ng/ml)	Post EGFR (ng/ml)	IDH1 mut	EGFR ampl	EGFR IHC C	EGFR IHC H	EGFR IHC loc	Ki67	1p19q del	MGMTmet
1	49/F	Primary	3	AA	–(29.4)[a]	0.57	0	NA	47.8	NA	Yes	No	1	3	B	16	0	Yes
2	62/F	Primary	3	AA	–(14.4)[a]	1.71	0	0.069	54.7	38.4	No	No	3	3	B	12	0	Yes
3	44/F	Primary	3	AA	–(21.0)[a]	0	0	0.333	35.9	45.3	Yes	No	1	3	B	3	0	Yes
4	49/M	Primary	3	AA	–(13.0)[a]	0	0.023	0	54.4	63.9	No	No	3	3	B	14	0	Yes
5	57/F	Primary	3	AA	10.4	0.16	0	0	49.3	51.2	No	Yes	3	3	B	13	0	No
6	18/M	Primary	3	AA	10.9	0	0.034	0.531	50.5	47.6	No	No	2	3	B	35	0	Yes
7	49/F	Primary	3	AA	–(10.7)[a]	0.81	0	0	50.0	50.6	Yes	No	2	3	M	5	0	Yes
8	54/F	Primary	3	AO	13.6	0.03	0	NA	74.1	NA	Yes	No	2	3	B	10	1	Yes
9	60/M	Primary	3	AOA	–(21.6)[a]	0	0	0	45.3	44.3	Yes	No	3	3	B	33	0	Yes
10	71/M	Primary	4	GBMO	0	1.91	0.160	1.005	45.8	40.7	No	No	3	3	B	76	0	Yes
11	37/M	Primary	4	GBMO	–(21.0)[a]	4.32	0	0.387	61.5	47.1	No	No	3	3	B	30	0	Yes
12	35/F	Primary	4	GBM	0	1.27	0.025	NA	54.9	NA	No	No	1	1	C	59	0	–[c]
13	63/F	Primary	4	GBM	24.0	12.2	0.037	0.076	56.2	54.0	No	No	1	3	C	24	0	Yes
14	62/F	Primary	4	GBM	0	11.5	0.067	0.137	49.7	42.6	No	No	2	3	B	53	0	Yes
15	67/F	Primary	4	GBM	0	14.8	0.387	2.491	52.0	53.2	No	Yes	3	3	B	65	0	Yes
16	70/M	Primary	4	GBM	0	21.5	0.115	NA	32.8	NA	No	No	1	3	M	88	0	No
17	76/F	Primary	4	GS	0	34.7	0.085	0	49.4	54.0	No	No	1	2	M	90	0	Yes
18	28/M	Recurrent	3	AA	81.2	0	0	0.046	52.0	47.6	Yes	No	1	3	B	11	0	Yes
19	51/M	Recurrent	3	AO	60.9	0	0	1.566	56.8	51.3	No	No	3	3	B	16	1	Yes
20	42/F	Recurrent	3	AOA	24.3	0	0	0	48.6	57.3	Yes	No	2	3	B	8	0	Yes
21	46/M	Recurrent	3	AOA	39.0	28.2	0.096	NA	38.8	NA	Yes	No	2	3	C	38	1	Yes
22	42/F	Recurrent	4	GBM	24.0	0.04	0.041	NA	43.7	NA	No	Yes	3	3	M	42	0	Yes
23	68/F	Recurrent	4	GBM	23.1	27.9	0.099	0.385	62.9	58.1	No	Yes	3	3	B	17	0	Yes
24	64/F	Recurrent	4	GBM	34.2	12.8	0.037	0.222	79.4	71.2	No	Yes	3	3	B	31	0	Yes
25	42/F	Recurrent	4	GBM	5.5	28.0	0.027	1.609	42.8	48.0	No	Yes	0	2	B	55	1	No
26	61/F	Recurrent	4	sGBM	16.1	2.59	0	NA	50.2	NA	No	Yes	1	2	C	19	0	No
27	57/M	Recurrent	4	GBM	11.0	3.03[b]	0.020	0	55.8	56.4	No	Yes	1	3	B	37	0	No

Pt no patient number, *F* female, *M* male, *Gr* grade, *Dg* diagnosis, *AA* anaplastic astrocytoma, *AO* anaplastic oligodendroglioma, *AOA* anaplastic oligoastrocytoma, *GBMO* glioblastoma with oligodendroglioma component, *GBM* glioblastoma, *sGBM* secondary GBM, *GS* gliosarcoma, *PFS* progression-free survival, V_{Gad} tumor volume with gadolinium enhancement in MRI-T1-Gad, *NA* not analyzed (no post-operative blood samples), *IDH1 mut IDH1* mutation, *EGFR ampl* EGFR amplification, *EGFR IHC* EGFR immunohistochemistry, *C* most common intensity (0–3), *H* highest intensity (0–3), *loc* location of staining, *C* cytoplasmic, *M* membranous, *B* both, *1p19q del* 1p19q deletion, *MGMT met MGMT* promoter methylation

[a] No tumor progression during follow up (follow up mo), [b] tumor volume defined from contrast enhanced CT (no MRI due to cardiac pacemaker), [c] failed for technical reasons

(clone H09; Dianova, Hamburg, Germany), which detects the most common R132H mutation of the *IDH1* gene [19]. Immunostainings were performed with BenchMark XT Autostainer and antibody detection with *ultra*VIEW Universal DAB Detection Kit (Ventana Medical Systems, Tucson, Arizona). *EGFR* amplification was studied by silver in situ hybridization [20] and 1p/19q codeletion by fluorescent in situ hybridization using Vysis 1p36/1q25 and 19q13/19p13 FISH probe kit (Abbot Laboratories, Abbott Park, IL). *MGMT* gene promoter methylation was analyzed by pyrosequencing [21]. EGFR immunohistochemistry was reported using a scoring system described previously by Ålgars et al. [20]. Three scoring parameters were used: the highest staining intensity (minimum 10 % of tumor area), the most common staining intensity, and the localization of staining (membranous, cytoplasmic, or both). Staining intensities were classified as: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong).

Enhancing and necrotic tumor volumes in MRI

Tumor areas with contrast enhancement and necrosis were defined from pre-operative MRI acquired on clinical basis. Mean interval between MRI and blood sampling was 18 days. Tumor volume with gadolinium enhancement was delineated in T1-weighted post-contrast images (MRI-T1-Gad) using iPlan RT Treatment Planning Software (Brainlab, Munich, Germany) by thresholding the enhancing tumor volume on visual basis and then manually subtracting the hyperintense volume on pre-contrast T1-weighted images. Necrotic tumor volume was manually outlined in pre-operative MRI-T1-Gad with guidance from standard T2-weighted and FLAIR images. Enhancing residual tumor volumes were correspondingly delineated in post-operative MRI-T1-Gad performed 1–4 days after operation. Extent of tumor resection (EOR%) was calculated from tumor volumes with gadolinium enhancement in pre- and post-operative MRI-T1-Gad in those patients with available post-operative serum samples.

Statistical analysis

Data are presented as mean ± SD. Comparisons of serum protein levels between groups were made using Kruskal–Wallis test and Mann–Whitney *U* test with Bonferroni correction, or one-way ANOVA. Spearman's correlation was used to compare serum protein levels and tumor volumes, GFAP IHC, and Ki67 proliferation index. Comparisons of serum protein levels according to molecular marker status was performed using Mann–Whitney *U* test or Independent-samples *T* test. EGFR IHC was compared to serum EGFR using Kruskal–Wallis test and to EGFR amplification using Crosstabs with Pearson χ^2. Receiver operating characteristic (ROC) curve analysis was used to evaluate the ability of serum GFAP and EGFR values to discriminate glioblastoma from anaplastic glioma and control subjects. A GFAP cut-off value was determined according to ROC curve analysis and the cutoff value was then applied in Kaplan–Meier curve with log-rank test. Univariate Cox regression model was used to analyze survival data for PFS. PFS was defined as the time from first surgical resection (also for recurrent HGGs) to first tumor progression in follow-up MRI, deterioration in clinical symptoms, or end of follow-up. Agreement between repeated EGFR ELISA measurements was assessed with intraclass correlation coefficient ICC(3,1) and Coefficient of Repeatability (CR). Difference between pre- and post-operative serum protein levels was evaluated using Wilcoxon signed-rank or paired-samples *T* test. Two-tailed *P* values <0.05 were regarded as significant. Statistical analyses were conducted using SPSS 21 for Mac (SPSS, Inc., Chicago, IL, USA).

Results

Pre-operative serum GFAP

12 patients (86 %) with glioblastoma and three patients (23 %) with anaplastic glioma had pre-operatively detectable serum GFAP levels ($\geq$0.014 ng/ml). All control subjects but one showed zero serum GFAP (detection limit 0.014 ng/ml). Serum GFAP was significantly higher in glioblastoma patients (0.079 ± 0.100) compared to anaplastic glioma patients (0.012 ± 0.028; $P = 0.003$) or controls ($P = 0.001$) (Fig. 1a). No difference was observed between anaplastic glioma patients and control subjects ($P = 1.000$). Serum GFAP levels for primary and recurrent HGGs were 0.055 ± 0.098 and 0.032 ± 0.038, respectively ($P = 0.979$).

Pre-operative serum GFAP values significantly correlated to enhancing tumor volume and necrotic tumor volume in MRI-T1-Gad both in primary ($r = 0.64$; $P = 0.005$ and $r = 0.73$; $P = 0.001$, respectively) and in recurrent HGGs ($r = 0.76$; $P = 0.011$ and $r = 0.64$; $P = 0.047$, respectively) (Fig. 2). Additionally, enhancing tumor volume correlated to necrotic tumor volume ($r = 0.73$; $P < 0.001$).

Patients with HGGs carrying *IDH1* gene mutation showed significantly lower serum GFAP levels (0.012 ± 0.033 ng/ml) compared to patients with *IDH1* mutation-negative HGGs (0.061 ± 0.091 ng/ml; $P = 0.016$). In relation to 1p19q co-deletion or MGMT promoter methylation status, however, no variation in serum GFAP level was observed ($P = 0.775$ and $P = 0.864$, respectively). In contrast, serum GFAP values correlated to Ki67 proliferation index ($r = 0.78$;

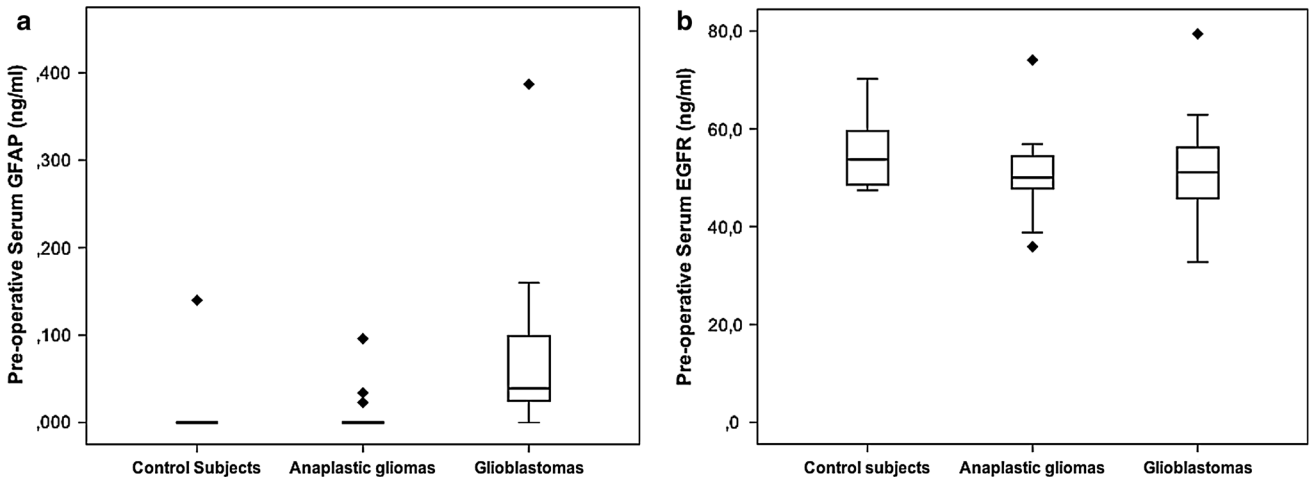

Fig. 1 *Box plot* of serum GFAP (**a**) and serum EGFR (**b**) values in healthy control subjects (n = 13) and patients with primary or recurrent anaplastic glioma (n = 13) or glioblastoma (n = 14) before surgery. GFAP values were significantly elevated in glioblastoma patients when compared with anaplastic glioma ($P = 0.003$) or control subjects ($P = 0.001$)

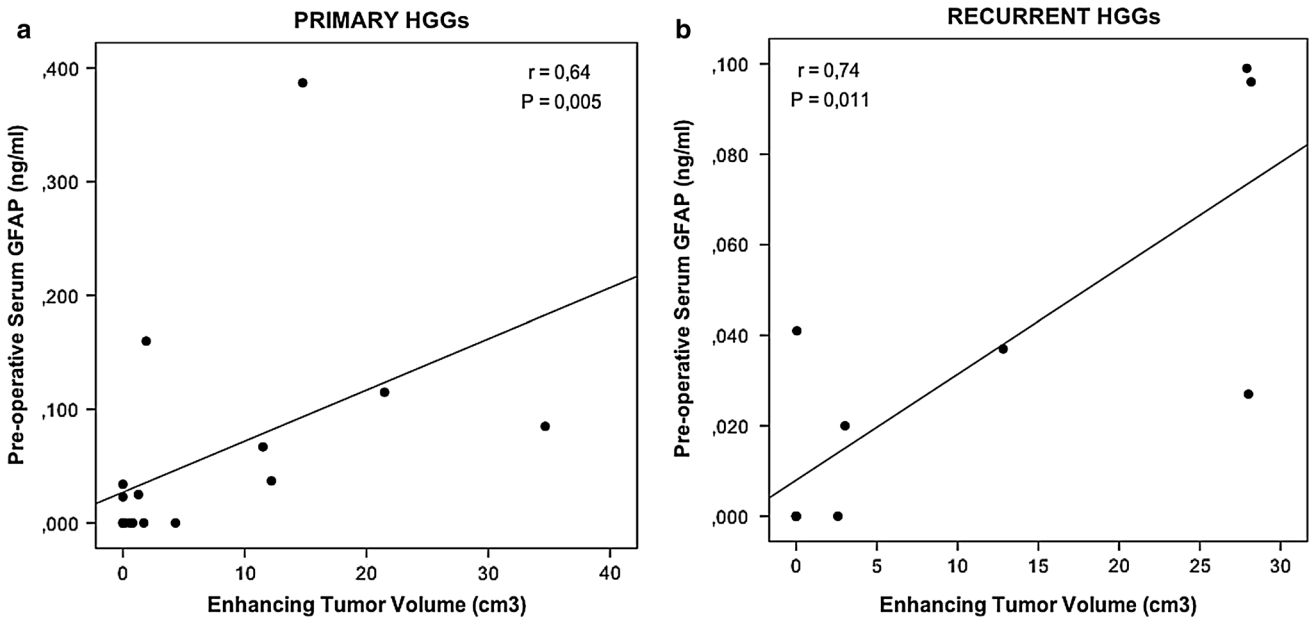

Fig. 2 Serum GFAP values correlate with enhancing tumor volume in MRI-T1-Gad in patients with primary (**a**) and recurrent (**b**) HGG before surgery

$P < 0.001$). All HGGs expressed GFAP detected by immunohistochemistry (GFAP positive cells 87 ± 20 %), the extent of which, however, did not correlate to serum GFAP levels ($P = 0.761$).

ROC analysis for the differentiation of glioblastoma from anaplastic glioma or control patients produced a serum GFAP cut-off value of 0.014 ng/ml with a sensitivity of 86 % and a specificity of 85 % (AUC 0.86; $P < 0.001$; CI 0.72–0.99). Univariate Cox regression analysis revealed serum GFAP value of ≥0.014 ng/ml to be a significant predictor of PFS in primary HGGs (Hazard ratio 5.9; $P = 0.032$; CI 1.2–29.9). The Kaplan–Meier curve (Fig. 3) illustrates that serum GFAP ≥ 0.014 ng/ml was related to poor PFS in patients with primary HGG compared to HGG patients with serum GFAP < 0.014 ng/ml ($P = 0.008$; Log rank).

Pre-operative serum EGFR

No statistical difference was observed in pre-operative serum EGFR values between glioblastoma patients (52.6 ± 11.0 ng/ml), anaplastic glioma patients (50.6 ± 9.2 ng/ml), and control subjects (55.8 ± 7.8 ng/ml $P = 0.391$) (Fig. 1b). Accordingly, ROC analysis of serum EGFR values

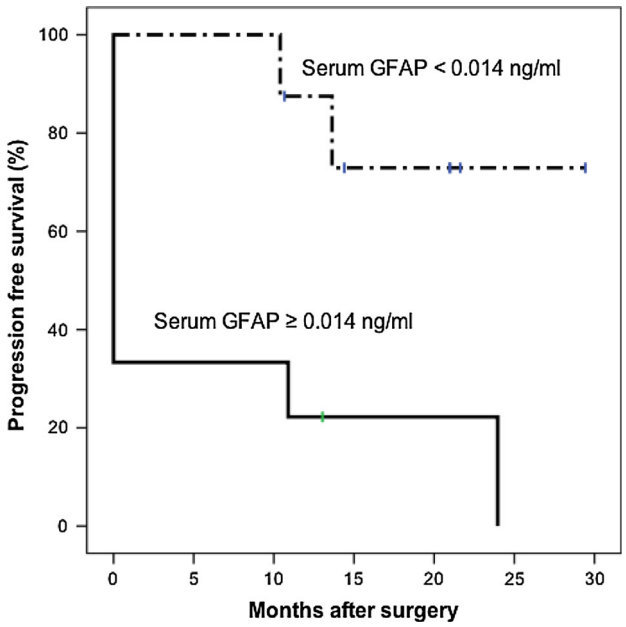

Fig. 3 Kaplan–Meier progression-free survival curves of primary HGG patients (n = 17) divided according to serum GFAP threshold level of 0.014 ng/ml. Censored data are indicated by *vertical lines*

resulted in poor discrimination between glioblastoma and anaplastic glioma patients (AUC 0.57; $P = 0.528$; CI 0.35–0.79). Serum EGFR values did not correlate to enhancing or necrotic tumor volume in MRI-T1-Gad ($P = 0.985$ and $P = 0.261$, respectively), nor to Ki67 proliferation index ($P = 0.235$). Furthermore, the status of *IDH1* mutation, 1p19q co-deletion, or *MGMT* promoter methylation were not associated to different levels of serum EGFR ($P = 0.392$, $P = 0.762$, and $P = 0.197$, respectively).

Serum EGFR concentration was not related to tumor *EGFR* gene amplification or immunohistochemistry. Similar serum EGFR values were observed in HGG patients with EGFR amplification (54.5 ± 12.0 ng/ml) and without EGFR amplification (50.5 ± 9.2 ng/ml; $P = 0.351$). All HGGs studied showed positive staining in EGFR immunohistochemistry. Highest staining intensity was three in most specimens, whereas the most common staining intensity varied from 0 to 3. Location of EGFR IHC staining most commonly was both cytoplasmic and membranous. However, the intensity or the location of the staining was not associated to serum EGFR concentration ($P = 0.418$ and $P = 0.206$, respectively) nor to *EGFR* amplification ($P = 0.091$ and $P = 0.943$, respectively). Elevated (>71.3 ng/ml) or diminished serum EGFR values (<40.2 ng/ml; healthy controls serum EGFR mean ± 2SD) were not associated to PFS in patients with primary HGG (HR 1.5; $P = 0.707$; CI 0.2–12.2 and HR 0.9; $P = 0.958$; CI 0.1–7.6, respectively). For repeated EGFR ELISA measurements, ICC(3,1) was 0.764 (CI 0.532–0.890) and CR 14.5 ng/ml indicating moderate agreement between measurements.

Post-operative serum GFAP and EGFR levels

Post-operative serum GFAP levels were elevated from pre-operative levels in 65 % of the HGG patients with available post-operative serum samples (n = 20). The average increase in post-operative serum GFAP levels was 0.39 ± 0.63 ng/ml, which was statistically significant ($P = 0.003$; Fig. 4a). This increase in serum GFAP or the separate post-operative values, however, showed no correlation to either enhancing residual tumor volume in post-surgical MRI or to the EOR% ($P = 0.583$; $P = 0.719$; $P = 0.372$; $P = 0.508$, respectively). Post-operative serum EGFR levels did not differ from those observed pre-

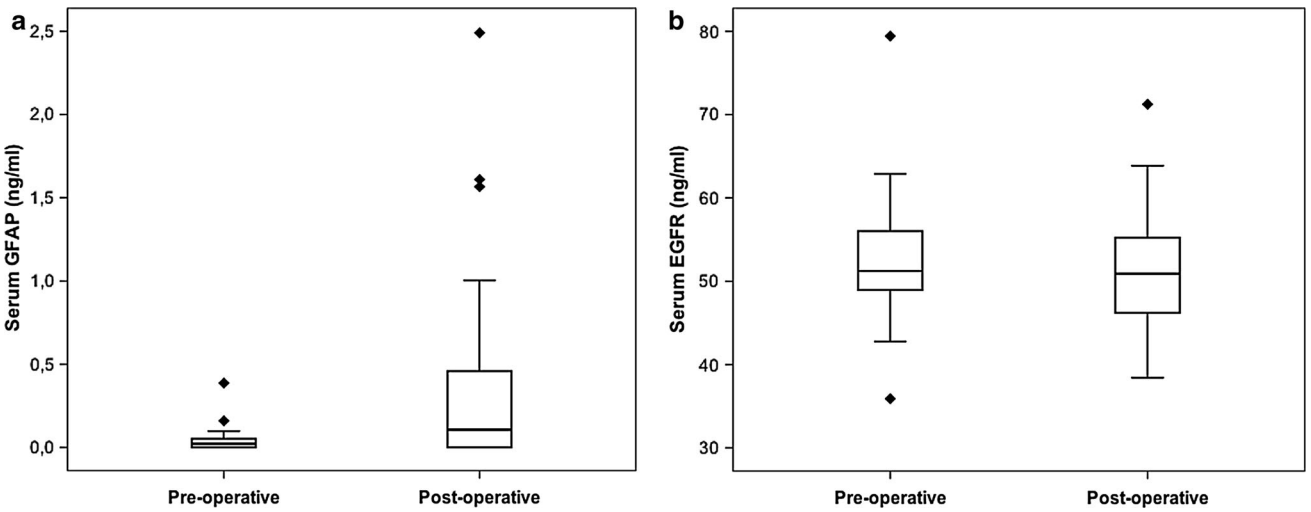

Fig. 4 *Box plot* of pre- and post-operative serum GFAP (**a**) and serum EGFR (**b**) values in 20 HGG patients with blood samples 2–5 days after surgery. Significant increase was observed in GFAP values after surgical resection of the tumor ($P = 0.003$)

operatively ($P = 0.354$; Fig. 4b), nor did they correlate to enhancing residual tumor volume or EOR%. The range of EOR% in those patients with available post-operative serum samples was 63–100 % (mean 89 ± 13 %).

Post-operative serum samples were obtained on day 2 (n = 5), day 3 (n = 11), day 4 (n = 3), or day 5 (n = 1). No significant difference in mean serum GFAP or EGFR values was observed between these post-operative days ($P = 0.280$ and $P = 0.260$, respectively).

Discussion

In this study we found that serum GFAP is a potential biomarker for diagnosis of recurrence since GFAP levels significantly correlated with tumor burden in recurrent HGGs. Serum GFAP may thus be helpful in the follow-up of patients with HGG who often present controversial findings on MRI after oncologic therapy. Additionally, serum GFAP may confer prognostic value since it seems to be associated with *IDH1* mutation-negative tumors and short PFS. In contrast, serum EGFR provides little value in patients with HGG since it showed no difference compared to healthy control subjects and no association with tumor burden, *EGFR* amplification or protein expression.

Serum GFAP provides diagnostic and prognostic value in primary and recurrent HGGs

We found that serum GFAP was detectable in most glioblastoma patients but only 3 out of 13 anaplastic glioma patients. Accordingly, serum GFAP level above 0.014 ng/ml provided a sensitivity of 86 % and specificity of 85 % for the diagnosis of GBM. Our results correspond to previous studies confirming the diagnostic value of serum GFAP in differentiating GBM from gliomas of lower grade [8, 9, 22]. High serum GFAP value in one control subject remains unclarified as there was no history of brain trauma, brain MRI was normal, and after 10 months of follow-up this subject remained neurologically symptom-free.

The correlation of serum GFAP to tumor burden in recurrent HGGs is of interest and suggests a possible value of serum GFAP as a biomarker for tumor recurrence. Previously the association between serum GFAP and tumor volume has been evaluated only in primary HGGs [8, 10]. Clearly, difficulties in determining true recurrence in the era of pseudoprogression and pseudoregression resulting from novel oncologic therapies underlines the potential importance of our finding. However, a longitudinal follow-up with a larger patient population is warranted to study in more detail the ability of serum GFAP to detect recurrent HGGs at the earliest possible stage.

Serum GFAP was related to both enhancing and necrotic tumor volume in MRI-T1-Gad. Tumor necrosis might therefore partially explain the elevated serum GFAP levels associated with bulky tumors. In a previous study, Jung et al. found a correlation between serum GFAP levels and the histologic measure of necrotic GFAP positive tumor cells in patients with GBM, further emphasizing that the size of the tumor and the amount of GFAP positive cells, necrosis and the disruption of BBB, may all be factors involved in the elevation of serum GFAP levels in patients with HGG [8].

To our knowledge, this study demonstrates for the first time that serum GFAP level is related to *IDH1* mutation status in HGGs. *IDH1* mutation in gliomas is regarded as the most powerful prognostic marker for a favourable outcome compared to their *IDH1* mutation-negative counterparts [23]. Relation of high serum GFAP to *IDH1* mutation-negative HGGs, and also correlation to high Ki67 proliferation index implicate the highly aggressive characteristics of HGGs associated with high serum GFAP. Furthermore, using 0.014 ng/ml as a cutoff value we found high serum GFAP to be a significant prognostic marker for a poor PFS in patients with primary HGG. On the contrary, a previous study reported a non-significant trend for a more favourable overall survival in GBM patients with higher serum GFAP levels [22]. The authors speculated that this is due to decreasing GFAP expression with increasing malignancy grade. However, we found no such difference in tumor GFAP expression between anaplastic gliomas and glioblastomas ($P = 0.435$).

The increase in serum GFAP levels after surgical tumor resection is consistent to a previous study where plasma GFAP values were elevated 24–48 h after surgery in 83 % of patients including both low-grade and high-grade gliomas [9]. Furthermore, post-operative serum GFAP values in our cohort showed no correlation to either enhancing residual tumor volume or to EOR%. These results indicate that post-operative blood levels of GFAP represent brain injury induced by the surgery rather than being a measure of residual tumor burden.

Serum EGFR is not related to EGFR status in the tumor tissue

In contrast to a previous study [18], we were unable to detect any difference in serum EGFR levels between patients with GBM, anaplastic glioma, and healthy controls. We used the same ELISA assay as Quaranta et al., but included also anaplastic oligodendrogliomas, oligoastrocytomas, and GBMs with oligodendroglioma component. However, this is not likely to explain the discrepancy since HGGs with or without oligodendroglioma component

presented with similar pre-operative serum EGFR values ($P = 1.0$).

Circulating EGFR concentrations are altered in various cancers. Compared to controls, lower serum EGFR levels have been detected in patients with ovarian and breast cancer, whereas elevated concentrations have been associated with cervical and gastric carcinomas, and pleural mesotheliomas [14–17, 24]. We found no such alteration in serum EGFR concentrations in HGG patients compared to healthy controls. Furthermore, EGFR gene amplification or protein overexpression in tumor tissue was not related to circulating EGFR levels. These results suggest that tumor cells are not likely to be the major source of circulating EGFR in patients with HGG, which is supported by the fact, that serum EGFR levels did not correlate to tumor burden in MRI. According to our findings, BBB disruption does not affect the release of ECD of EGFR into circulation. Our results indicate that serum EGFR has no diagnostic or prognostic value in patients with HGG and we further believe that serum EGFR is not applicable as a predictive marker for efficacy or treatments targeting EGFR.

Our study confronts limitations. First, ELISA tests for GFAP or EGFR have not yet been standardized. Second, the number of patients was limited and, therefore, our preliminary results require confirmation in larger cohorts.

Conclusion

This is the first study to show the potential of serum GFAP as a biomarker for tumor recurrence in patients with HGG since it correlated with recurrent tumor burden. Serum GFAP may thus offer a valuable tool in the follow-up of HGG patients. However, larger prospective trial to confirm this relationship is warranted. Furthermore, our results suggest a prognostic value for serum GFAP since it associated with *IDH1* mutation status and PFS. In contrast, we conclude that circulating EGFR is not related to tumor EGFR expression and thus provides little value in planning and follow-up of EGFR-targeted therapies.

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Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethical standards All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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