IJC
International Journal of Cancer

Use of metformin and outcome of patients with newly diagnosed glioblastoma: Pooled analysis

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Metformin has been linked to improve survival of patients with various cancers. There is little information on survival of glioblastoma patients after use of metformin. We assessed the association between metformin use and survival in a pooled analysis of patient data from 1,731 individuals from the randomized AVAglio, CENTRIC and CORE trials. We performed multivariate Cox analyses for overall survival (OS) and progression-free survival (PFS) comparing patients' use of metformin at baseline and/or during concomitant radiochemotherapy (TMZ/RT). Further exploratory analyses investigated the effect of metformin with a history of diabetes and nonfasting glucose levels in relation to OS or PFS of glioblastoma patients. Metformin alone or in any combination was not significantly associated with OS or PFS (at baseline, hazard ratio [HR] for OS = 0.87; 95% confidence interval [CI] = 0.65–1.16; HR for PFS = 0.84; 95% CI = 0.64–1.10; during TMZ/RT HR for OS = 0.97; 95% CI = 0.68–1.38; HR for PFS = 1.02; 95% CI = 0.74–1.41). We found a statistically nonsignificant association of metformin monotherapy with glioblastoma survival at baseline (HR for OS = 0.68; 95% CI = 0.42–1.10; HR for PFS = 0.57; 95% CI = 0.36–0.91), but not during the TMZ/RT period (HR for OS = 0.90; 95% CI = 0.51–1.56; HR for PFS = 1.05; 95% CI = 0.64–1.73). Diabetes mellitus or increased nonfasting glucose levels were not associated with a difference in OS or PFS in our selected study population. Metformin did not prolong survival of patients with newly diagnosed glioblastoma in our analysis. Additional studies may identify patients with specific tumor characteristics that are associated with potential benefit from treatment with metformin, possibly due to metabolic vulnerabilities.

Introduction

Metformin is the most commonly prescribed agent in the treatment of Type 2 diabetes. Preclinical data and retrospective series suggest improved outcome in cancer patients[1] receiving metformin inducing the initiation of clinical trials and adding metformin to the standard anticancer treatment regimen. The mechanism of action remains elusive, a direct inhibitory effect on tumor cells[2] as well as an indirect inhibition of cancer cells by lowering the levels of circulating glucose and insulin and consequently lesser insulin-like growth-factor signaling have been postulated.[3,4] Direct effects on tumor cells are related to inhibition of complex I of the respiratory chain with a

Key words: glioblastoma, metformin, overall survival, drug repurposing

Abbreviations: CI: confidence interval; HR: hazard ratio; IDH: isocitrate dehydrogenase; MGMT: O^6-methylguanine DNA methyltransferase; MMSE: Mini-Mental State Exam; PFS: progression-free survival; OS: overall survival; RT: radiotherapy; TMZ: temozolomide; WHO: World Health Organization

Additional Supporting Information may be found in the online version of this article.

Financial Disclosures and Conflict of Interests: O. Chinot is a consultant for Roche and received honoraria from Celdex, AbbVie and Servier. M. Weller received research grants from AbbVie, Adastra, MSD, Merck EMD, Novocure, OGD2, Pigur and Roche and honoraria for lectures, advisory board participation or consultation from AbbVie, Basilea, BMS, Celgene, MSD, Merck EMD, Novocure, Orbus, Roche and Tocagen. The other authors do not declare financial disclosures or conflicts of interest related to the article.

Grant sponsor: Wilhelm Sander-Stiftung; **Grant number:** 2009.800.2
DOI: 10.1002/ijc.32337
History: Received 11 Jan 2019; Accepted 18 Mar 2019; Online 13 Apr 2019

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What's new?

Metformin appears to boost survival in some cancers. Here, the authors looked at the diabetes drug's effect on survival in glioblastoma patients. Previous retrospective studies had uncovered a trend toward improved glioblastoma survival in patients treated with metformin. In this study, the authors used pooled data from three prospective randomized clinical trials. They found no association between metformin and survival, nor did they find an association between survival and diabetes diagnosis or increased blood glucose level. However, only a small subset of patients were taking metformin, limiting the study's power.

downstream activation of the adenosine monophosphate-activated kinase and inhibition of the mammalian target of rapamycin.[5]

Numerous studies investigated a potential inhibitory effect of metformin on cancer growth. In glioma models, several studies reported inhibitory effects on proliferation[6–10] and invasion[11] *in vitro* and *in vivo*, and induction of cell death *via* apoptosis[6,9,10] or autophagy.[12] Possible inhibitory mechanisms of metformin have also prompted epidemiological studies as well as prospective clinical trials (reviewed in Ref. 1,13,14).

Although extensively studied in other cancers, only three previous retrospective studies analyzed survival of patients with glioblastoma with or without treatment with metformin. One retrospective cohort study investigated 276 glioblastoma patients[15] and found a trend toward improved progression-free survival (PFS) in diabetic patients treated with metformin in univariate analyses, but the associations were not observed in the multivariate model. A larger retrospective cohort study included 988 patients[16] and suggested a trend toward a survival benefit in diabetic patients using metformin (hazard ratio [HR] for overall survival [OS] = 0.51, $p = 0.09$). In a large study investigating survival of 1,093 patients with World Health Organization (WHO) Grade III ($n = 231$) or IV glioma ($n = 862$) exposed to metformin, there was a significantly longer OS and PFS of patients with WHO Grade III, but not WHO Grade IV glioma.[17] It was speculated that patients with isocitrate dehydrogenase (IDH)-mutated gliomas may experience more benefit from exposure to metformin.

For a definite insight into the therapeutic potential of metformin, randomized trials are needed. To further explore the likelihood of success of such a trial in patients with newly diagnosed glioblastoma, we used the opportunity to evaluate the effect of antidiabetics in a prospectively collected pooled dataset of three large multicenter randomized clinical trials.

Patients and Methods

Data source and study population

The patient population consisted of randomized patients from the control and experimental arms of the AVAglio (NCT00943826; $n = 921$),[18] the CENTRIC (NCT00689221; $n = 545$)[19] and the CORE (NCT00813943; $n = 265$)[20] trials. All these trials were exploring the addition of a novel antiangiogenic agent added to the standard temozolomide/radiotherapy (TMZ/RT)→TMZ backbone. All trials were aiming for registration and thus the data collected underwent close independent monitoring. Exploratory analyses adjusting for the level of glycemic control were performed with CENTRIC and CORE data only, since those variables were not routinely assessed in the AVAglio trial.

Trials were performed with approval by a local human investigation committee and in accordance with an assurance filed with and approved by the U.S. Department of Health and Human Services, where appropriate. Patient data were protected in accordance with The EU General Data Protection Regulation. Informed consent was given and documented for all study participants.

Exposures

Antidiabetic drug use

Metformin use was defined irrespective of whether it was used as monotherapy or in combination with other antidiabetic agents for the primary analysis. The category was further subclassified into metformin use as monotherapy during radiochemotherapy (20 patients, 1.4%) and metformin use in combination with any other type of antidiabetic agent (combination therapy, 31 patients, 2.2%). Other antidiabetic drugs included insulin, sulfonylureas, thiazolidinediones, alpha-glucosidase inhibitors, dipeptidyl peptidase-4 inhibitors (DPP4) and glinides.

The status of baseline metformin use or other baseline antidiabetic drug use was flagged as "positive" when they were used at any point in the period between the date of randomization minus 2 weeks and the date of the first TMZ treatment dose, and was flagged as "negative" otherwise. The status of the respective metformin use concomitant to the TMZ/RT treatment or other concomitant antidiabetic drug use was flagged as "yes" when they were used in the period between the first TMZ/RT treatment dose and start of the TMZ maintenance phase and "no" otherwise, as described.[21,22] Different durations of baseline or concomitant use were summated, also for patients who used metformin on multiple occasions in the same or overlapping periods to account for the higher dose intensity for these patients. The day of initial surgery for glioblastoma was set as the earliest time point to compute the duration of use.

Statistical analysis

The first time point was fixed at the date of randomization to TMZ plus RT (TMZ/RT) in order to assess the association of baseline use with survival (period I). The second time point

was set at the start of the TMZ maintenance phase (approximately 4 weeks after the end of the 6 weeks of TMZ/RT) in order to assess the association of use with outcome during initial treatment with temozolomide (TMZ/RT period, period II).

For each univariate analysis that was performed, a Kaplan-Meier survival plot was produced and estimates of the median survival time and estimated survival proportion at 1 year (PFS) and 2 years (OS) were computed. A nonparametric stratified log-rank test was implemented to test for differences between the survival distributions.

Cox models were used for multivariate survival analyses. Each model was adjusted for the prognostic factors such as age (continuous), gender (male or female), O^6-methylguanine DNA methyltransferase (MGMT) promotor methylation status (unmethylated, methylated or unknown), WHO performance status (PS) (status = 0 or status >0), extent of initial resection (biopsy only, partial resection or complete resection), steroid use at baseline (yes or no) and Mini-Mental State Exam (MMSE) score (score <27 or score ≥27), and in addition stratified for trial. In the AVAglio trial, bevacizumab significantly improved PFS compared to the placebo group; therefore, treatment arm was introduced as an additional stratification factor for this trial in the analysis of PFS. Significance was established at the 5% significance level. In the descriptive analyses, frequencies with a difference of 10% or more were considered clinically relevant. Differences in steroid use were formally assessed with a chi square test.

We used SAS version 9.4 (SAS Institute Inc., Cary, NC) for description of baseline covariates and survival analyses (PROC PHREG).

Results

Patient cohort

A total of 1,731 patients pooled from CENTRIC (545 patients), CORE (265 patients) and AVAglio (921 patients) with newly diagnosed glioblastoma were assessed for survival according to use of metformin. The distribution of baseline demographic variables was similar over the three trials and four strata, except for MGMT promotor methylation status and extent of surgery. The two cilengitide trials were designed for patients with a methylated (CENTRIC) or unmethylated (CORE) tumor. In AVAglio, MGMT promotor methylation data were missing in 24% of patients. These observations were therefore labeled as "unknown" as they would otherwise be lost during complete case survival analysis. Patients who finished the initial 6 weeks of TMZ/RT and continued with the TMZ maintenance period were younger than those who did not and more often male. They had a better WHO performance score, had more often an MMSE score ≥27 and used less often steroids at baseline (39.4% *vs.* 47.5%, $p < 0.01$) (Table 1 and Appendix Table S1).

In contrast, patients who used antidiabetic drugs at baseline were older than those who did not, more often male and had more often a WHO PS > 0. Patients who used antidiabetic drugs other than metformin at baseline also used steroids more often at baseline compared to patients using metformin or no antidiabetics (other antidiabetic drugs: 52.0% *vs.* metformin: 41.9% *vs.* no use: 40.5%, $p = 0.27$). Similar results were obtained for patients using antidiabetic drugs in the TMZ/RT period (Appendix Table S2).

A total of 124/1,731 patients (7.2%) used any antidiabetic treatment at randomization. At the time of starting maintenance TMZ (approximately 4 weeks after the end of TMZ/RT), 6.3% (89/1,413) were receiving some antidiabetic treatment. Seventy-four patients (4.3%) received metformin at baseline (Period 1), and 51 patients (3.6%) during Period 2. The distribution of baseline antidiabetic drug use during the TMZ/RT period in terms of prevalence, most common types and duration of exposure was similar over the three trials and no differences were noted between the standard and bevacizumab arm of the AVAglio trial (Appendix Table S3).

Association between metformin and survival

Baseline metformin use and metformin use during the TMZ/RT period was not statistically associated with a difference in OS or PFS compared to no use of metformin at baseline (HR for OS = 0.87; 95% confidence interval [CI] = 0.65–1.16; HR for PFS = 0.84; 95% CI = 0.64–1.10) or during the TMZ/RT period (HR for OS = 0.97; 95% CI = 0.68–1.38; HR for PFS = 1.02; 95% CI = 0.0.74–1.41; Table 2, Appendix Tables S4–S9, Fig. 1). Similar results were obtained for OS and PFS when metformin use was compared to no use of any antidiabetics (Table 2, Appendix Fig. S1, Appendix Tables S8 and S9). Although there was also no overall significant difference in OS or PFS when comparing metformin use as monotherapy or metformin use as combination therapy or use of other antidiabetic drugs to no use of antidiabetic drugs both at baseline (p for OS = 0.364, p for PFS = 0.072) and during TMZ/RT (p for OS = 0.256, p for PFS = 0.556) (Table 2, Appendix Fig. S2, Appendix Tables S8 and S9), there was a borderline statistically significant HR for the baseline metformin monotherapy comparison for OS (HR = 0.68; 95% CI = 0.42–1.10) and a significant HR for PFS (HR = 0.57; 95% CI = 0.36–0.91). During the TMZ/RT period, the HR was 0.90 for OS (95% CI = 0.51–1.56) and 1.05 for PFS (95% CI = 0.64–1.73). For antidiabetic drugs, other than metformin there was a trend for worse survival, especially when used during RT/TMZ (Table 2).

Association between metformin and survival in the subgroup of patients with diabetes

Next, we analyzed the subgroup of patients with diabetes. Of all randomized patients, 150 (8.7%) had a history of diabetes and its distribution was similar over the three trials and four strata (Appendix Table S10). Baseline patient and clinical characteristics according to a history of diabetes are shown in Appendix Table S11. There was no statistically significant difference in OS or PFS when comparing patients with or without a history of diabetes after adjustment for important prognostic factors (Appendix Tables S12 and S13). Among the subgroup of

Table 1. Baseline patient and clinical characteristics

	Pooled patient population Total (*N* = 1,731) *N* (%)	Started TMZ maintenance period	
		No (*N* = 318) *N* (%)	Yes (*N* = 1,413) *N* (%)
Age (years)			
Median	57.0	60.0	56.0
Range	18.0–84.0	24.0–81.0	18.0–84.0
Age category			
≥50	1,291 (74.6)	269 (84.6)	1,022 (72.3)
‹50	440 (25.4)	49 (15.4)	391 (27.7)
Gender			
Male	1,026 (59.3)	161 (50.6)	865 (61.2)
Female	705 (40.7)	157 (49.4)	548 (38.8)
Extent of surgery			
Partial resection	835 (48.2)	165 (51.9)	670 (47.4)
Complete resection	789 (45.6)	128 (40.3)	661 (46.8)
Biopsy	104 (6.0)	25 (7.9)	79 (5.6)
Missing	3 (0.2)	0 (0.0)	3 (0.2)
WHO performance status (PS)			
PS = 0	905 (52.3)	123 (38.7)	782 (55.3)
PS › 0	823 (47.5)	192 (60.4)	631 (44.7)
Missing	3 (0.2)	3 (0.9)	0 (0.0)
Steroid use at baseline			
No	1,019 (58.9)	166 (52.2)	853 (60.4)
Yes	708 (40.9)	151 (47.5)	557 (39.4)
Missing	4 (0.2)	1 (0.3)	3 (0.2)
MGMT			
Methylated	781 (45.1)	151 (47.5)	630 (44.6)
Unmethylated	727 (42.0)	123 (38.7)	604 (42.7)
Unknown	223 (12.9)	44 (13.8)	179 (12.7)
MMSE			
≥27	1,325 (76.5)	207 (65.1)	1,118 (79.1)
‹27	384 (22.2)	102 (32.1)	282 (20.0)
Missing	22 (1.3)	9 (2.8)	13 (0.9)

patients with diabetes, the use of metformin was not associated with OS or PFS, neither at baseline nor during the TMZ/RT period in none of the comparisons (Appendix Table S14).

Association between metformin and survival according to glucose levels

Of all randomized patients in CENTRIC and CORE, 92.3% had a nonfasting glucose level ≤200 mg/dL (11.1 mmol/L) at baseline. Patients with blood glucose values >11.1 mmol/L at baseline or during TMZ/RT had more often used steroids at baseline (63.2% *vs.* 38.2% and 53.6% *vs.* 35.8%, Appendix Table S11 and data not shown) and more often used metformin in combination with other antidiabetic drugs compared to metformin as monotherapy or no use of antidiabetic drugs (Appendix Table S15). There was no statistically significant difference in OS or PFS between having a nonfasting blood glucose level >11.1 mmol/L compared to ≤11.1 mmol/L after adjustment for important prognostic factors at baseline (Appendix Table S16, results for PFS not shown) or during TMZ/RT (Appendix Table S17, results for PFS not shown). The HR for OS in patients with nonfasting blood glucose level >11.1 mmol/L during TMZ/RT was 1.61 (95% CI = 1.00–2.58). After additional adjustment for nonfasting blood glucose levels, the use of metformin was not associated with OS or PFS, neither at baseline nor during the TMZ/RT period in none of the comparisons (Appendix Table S18).

Discussion

In this pooled analysis of three randomized prospective multicenter clinical trials, the use of metformin was not associated with significant differences in OS or PFS in patients with newly diagnosed glioblastoma. Similar results were obtained in the

Table 2. Overview of adjusted estimates for the primary and secondary analyses

Comparison	Period	Group	OS			PFS		
			HR	95% CI	*p*-value	HR	95% CI	*p*-value
Metformin use *vs.* no metformin use	Baseline	Metformin (in any combination)	0.87	(0.65–1.16)	0.327	0.84	(0.64–1.10)	0.204
	TMZ/RT	Metformin (in any combination)	0.97	(0.68–1.38)	0.854	1.02	(0.74–1.41)	0.912
Metformin use and other antidiabetic drug use *vs.* no antidiabetic drug use	Baseline	Metformin (in any combination)	0.87	(0.65–1.16)	0.343	0.84	(0.64–1.11)	0.219
		Other antidiabetics	1.14	(0.82–1.58)	0.434	1.19	(0.87–1.63)	0.284
	TMZ/RT	Metformin (in any combination)	0.98	(0.69–1.39)	0.912	1.03	(0.74–1.42)	0.869
		Other antidiabetics	1.46	(1.00–2.13)	0.05	1.32	(0.90–1.92)	0.151
Metformin as monotherapy, metformin in combination and other antidiabetic drug use *vs.* no antidiabetic drug use	Baseline	Metformin (monotherapy)	0.68	(0.42–1.10)	0.115	0.57	(0.36–0.91)	0.019
		Combination therapy	1.02	(0.72–1.46)	0.901	1.09	(0.79–1.51)	0.612
		Other antidiabetics	1.14	(0.82–1.58)	0.433	1.19	(0.87–1.63)	0.284
	TMZ/RT	Metformin (monotherapy)	0.90	(0.51–1.56)	0.695	1.05	(0.64–1.73)	0.851
		Combination therapy	1.04	(0.67–1.64)	0.849	1.01	(0.67–1.54)	0.951
		Other antidiabetics	1.46	(1.00–2.13)	0.05	1.32	(0.90–1.92)	0.151

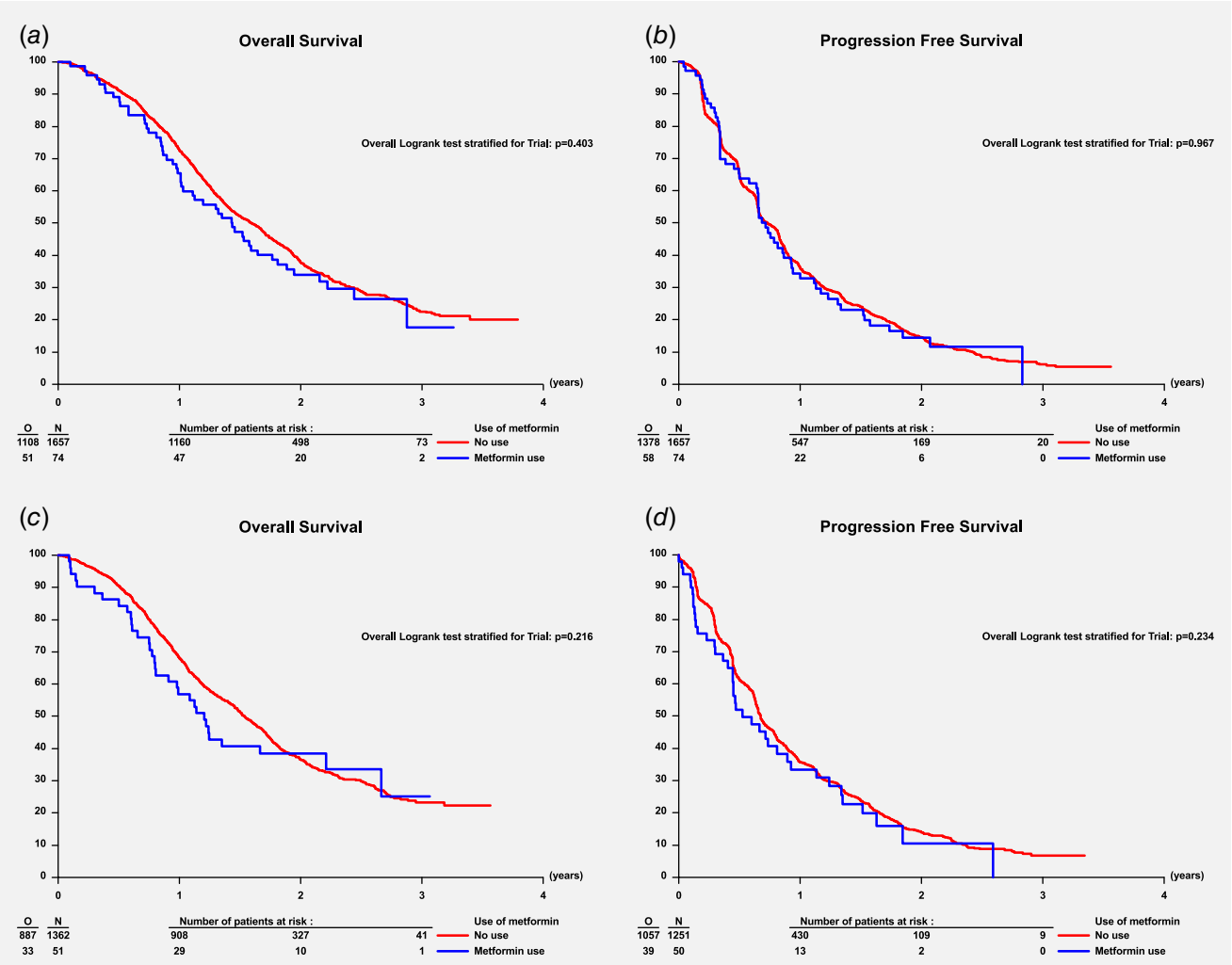

Figure 1. Kaplan-Meier survival plots for use of metformin in any combination. Kaplan-Meier survival plots for OS (*a*) and PFS (*b*) at baseline according to the use of metformin in any combination. Kaplan-Meier survival plots for OS (*c*) and PFS (*d*) according to the use of metformin in any combination during TMZ/RT.

subset of patients with a history of diabetes or after adjustment for nonfasting glucose levels. We found a borderline statistically significant association of metformin monotherapy with glioblastoma survival at baseline, but not during RT/TMZ. Neither a diagnosis of diabetes nor the presence of increased nonfasting glucose levels was significantly associated with OS or PFS of glioblastoma patients in our cohort, but long-term measures of glycemic control were not available in our study.

A decreased OS in glioblastoma patients with diabetes as previously described[16,23] could not be demonstrated in our study. Similarly, persistently increased blood glucose levels were reported to be associated with reduced survival in patients with glioblastoma,[24] high-grade glioma[23,25] or low-grade glioma.[26] Our analyses do not confirm these observations. Increased nonfasting blood glucose levels were not associated with worse survival although there was an indication for worse OS (HR = 1.61; 95% CI = 1.00–2.58) when detected during TMZ/RT. The present analysis differed from previous investigations because our study population consisted exclusively of patients with newly diagnosed glioblastoma and they were all treated with TMZ/RT standard treatment. Chambless *et al.* investigated patients with either WHO Grade III or IV glioma, which underwent different treatment regimens in a retrospective study. The small patient population consisted of 15 patients (9%) with Type 2 diabetes mellitus among the 171 patients with high-grade glioma. Furthermore, they adjusted only for a few key factors not including gender, steroid use at baseline, MGMT status and MMSE. We hypothesize that patients with lower grade gliomas[15,23,25] are more likely to experience survival-detrimental effects of diabetes or hyperglycemia due to their higher life expectancy as compared to patients with primary glioblastoma. Also, patients included in clinical trials are likely to be returning to clinic more frequently, undergoing more blood tests ultimately leading to a better glycemic control, whereas patients with an uncontrolled diabetes may be excluded upfront from the trial. The fact that in our study over 90% of patients had documented normal blood glucose levels (≤11.1 mmol/L) underscores this observation.

Survival of patients with glioblastoma after use of metformin has been investigated in only few prior studies. A study on 276 patients with primary glioblastoma did not demonstrate an increased PFS after adjustment for important confounding factors.[15] Welch *et al.* reported that the use of metformin monotherapy was among the most important predictors for survival in their retrospective analysis on 988 patients.[16] That study differs from ours, as it was not derived from a clinical trial population, pooled primary and secondary GBM and included various oncologic treatment regimens. Patients also had a median glucose level of 198.5 mg/dL (including fasting and nonfasting glucose levels), which is markedly higher than in our study (105 mg/dL, Q1: 92.1 to Q3: 128); their study was not adjusted for MGMT status.[16] In another retrospective study in 1,093 patients with high-grade glioma from southeast Germany, only patients with WHO Grade III glioma had a significantly longer survival with metformin treatment (HR for OS = 0.30; 95% CI = 0.11–0.81), whereas patients with glioblastoma did not (HR = 0.83; 95% CI = 0.57–1.20).[17]

Our results are in contrast to several experimental preclinical studies suggesting inhibitory effects of metformin on glioma cells.[6–12,27–29] One major drawback of those studies may however be that metformin doses used in cell culture are frequently by far higher than the concentrations measured in the brain of diabetic patients. *In vitro* studies often used metformin doses in the millimolar (10^{-3}, mM) range,[6,7,9–12,28,29] whereas metformin doses in the brain of diabetic patients have been measured in the micromolar (10^{-6}, μM) range.[30] Whether doses of metformin may be increased in patients with cancer as compared to patients with diabetes has not yet been investigated. Also, metformin doses in the μM range showed inhibitory effects on glioblastoma cells in extended treatments (7–15 days).[10] Potentially, a subset of glioma patients with metabolic vulnerabilities may be more susceptible to metformin,[31] for example, patients with gliomas classified as a proneural subtype or gliomas harboring mutations in the IDH genes,[32] which could also explain the positive associations in Grade III patients in one study.[17] We were unable to stratify according to IDH-mutational status, because studies were performed before this marker was known or incorporated into routine practice. Nevertheless, the pooled trials were focusing on primary glioblastoma, thus less than 5% of the tumors are expected to harbor an IDH mutation.

Interestingly, even with low sample sizes, there was an indication for better survival in patients with metformin monotherapy at baseline, but this was not present when investigating during TMZ/RT. Besides possible glioma-inhibitory effects of metformin monotherapy, it has to be considered that diabetic patients with less severe and long-lasting diabetes may have been more likely to be treated with metformin monotherapy, which might explain those associations. On the other hand, steroid use due to increased intracranial pressure and clinical worsening may have led to diabetic decompensations and subsequent use of metformin. Thereby, any possible effects of metformin may have been negated by negative effects of steroids. Furthermore, one may speculate that missing survival-detrimental effects of diabetes may be related to beneficial effects of metformin in a significant number of diabetic patients leading to overall null associations.

Our study has several limitations: the analyses were unplanned and retrospective, and the subgroups of patients treated with metformin were small representing less than 7% of patients. Therefore, a lack of power may have led to false-negative results. Furthermore, we were not able to perform dose–response analyses, that is, investigating increasing doses and durations of metformin in relation to glioblastoma survival although there was a significant range of duration of metformin use between patients within the respective trials. However, we investigated metformin use at two different

periods of time (at baseline and during TMZ/RT) and metformin is mostly used at stable drug doses of 1–2.5 g/day. We were not able to analyze long-term glycemic control using HbA1c values, repeated measures of short-term glycemic control using fasting glucose levels, levels of circulating insulin or injected insulin, or preexisting hyperglycemia *vs.* perioperative steroid induced hyperglycemia. Nonfasting glucose levels may well be confounded by glucose intake and may serve only as an inaccurate approximation for the investigation of glycemic control. Although documentation of use of co-medications in patients within clinical trials is supposedly good, there may still be the possibility that co-medications were not completely documented. Possibly, patients with gliomas that already developed under metformin are resistant to the drug during the later course of disease. Therefore, metformin use before the diagnosis of glioma may have confounded our analysis on metformin and outcome of patients with primary glioblastoma. Finally, although we adjusted our analysis for multiple confounding factors, there may have been residual confounding, for example, due to older age and worse PS in the group of patients on antidiabetic medication at baseline.

In summary, we did not observe an association between the use of metformin and survival in patients with newly diagnosed glioblastoma. We conclude that, at least at commonly used therapeutic doses, metformin has no evident impact on outcome of patients with GBM, but those results are limited by the retrospective nature of our study and limited sample size.

Additional prospective studies may identify patients with specific tumor characteristics that are associated with potential benefit from metformin.

Acknowledgements

Els Genbrugge's fellowship at EORTC (Brussels, Belgium) was supported by a grant from the EORTC Brain Tumor Group. This study was partly supported by the Wilhelm Sander-Stiftung, Munich and Ingolstadt, Germany (to P.H.).

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