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Original Research

Associations of anticoagulant use with outcome in newly diagnosed glioblastoma

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Abstract ***Background:*** To test the hypothesis that despite bleeding risk, anticoagulants improve the outcome in glioblastoma because of reduced incidence of venous thromboembolic events and modulation of angiogenesis, infiltration and invasion.
Methods: We assessed survival associations of anticoagulant use from baseline up to the start of temozolomide chemoradiotherapy (TMZ/RT) (period I) and from there to the start of maintenance TMZ chemotherapy (period II) by pooling data of three randomised clinical trials in newly diagnosed glioblastoma including 1273 patients. Progression-free survival (PFS) and overall survival (OS) were compared between patients with anticoagulant use versus no use; therapeutic versus prophylactic versus no use; different durations of anticoagulant use versus no use; anticoagulant use versus use of anti-platelet agents versus neither anticoagulant nor anti-platelet agent use. Cox regression models were stratified by trial and adjusted for baseline prognostic factors.

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Results: Anticoagulant use was documented in 75 patients (5.9%) in period I and in 104 patients (10.2%) in period II. Anticoagulant use during period II, but not period I, was associated with inferior OS than no use on multivariate analysis ($p = 0.001$, hazard ratio [HR] = 1.52, 95% confidence interval [CI]: 1.18–1.95). No decrease in OS became apparent when only patients with prophylactic anticoagulant use were considered. No survival association was established for anti-platelet agent use.
Conclusions: Anticoagulant use was not associated with improved OS. Anticoagulants may not exert relevant anti-tumour properties in glioblastoma.

1. Introduction

Venous thromboembolic events (VTEs) are an important complication of glioblastoma [1,2]. Yet, there is no information on their contribution and treatment to morbidity and mortality. Prevention and treatment of VTE in glioblastoma have remained insufficiently studied. There is increased interest in anticoagulants as modifiers of tumour biology which improve outcome. Low-molecular-weight heparin (LMWH) may influence cancer cell adhesion, proliferation, invasion and angiogenesis, in part through coagulation-independent pathways [3–8]. Cohort studies and a Cochrane review have suggested a potential improvement in the outcome in cancer patients treated with anticoagulants [9–12]. Several limitations, however, exist and introduce bias in the interpretation of these results: studies were published more than 15 years ago; patient numbers per study were small; survival was not the primary end-point and cancer types, staging, performance status, clinical status, schedule of chemotherapy, dose and type of anticoagulant and treatment duration varied. Prevention of VTE by LMWH has been evaluated in three studies enrolling patients with World Health Organisation (WHO) 2007 grade III or IV gliomas [13–15], without firm conclusions on modulation of survival. These observations encouraged the evaluation of an association with the outcome of anticoagulant use in the newly diagnosed glioblastoma.

2. Materials and methods

2.1. Patients

To assess associations of anticoagulant use during initial treatment of patients with the newly diagnosed glioblastoma and the outcome, we analysed data from a pooled cohort of patients randomised in three contemporary clinical trials: CENTRIC (cilengitide, temozolomide, and radiation therapy in treating patients with newly diagnosed glioblastoma and methylated gene promoter status) (n = 545) [16], CORE (cilengitide, temozolomide, and radiation therapy in treating patients with newly diagnosed glioblastoma and unmethylated gene promoter status) (n = 265) [17] and avastin in glioblastoma (AVAglio) (n = 463) [18]. All patients from CENTRIC and CORE were included because cilengitide was interpreted to be inactive therapeutically and not to affect the risk of VTE. The control arm only of AVAglio was included to avoid a confounding effect of bevacizumab for progression-free survival (PFS) and incidence of VTE (Fig. 1). Investigations were approved by local institutional review boards. Informed consent was obtained from each patient. For each trial, data sets were received with anonymised individual patient information including the date of randomisation, PFS, OS and the baseline covariates age, gender, WHO performance status, extent of resection, steroid use, Mini-Mental State Examination (MMSE), O^6-methylguanine DNA methyltransferase (*MGMT*) promoter methylation status and details on anticoagulant and anti-platelet agent use.

2.2. Statistical analysis

In this prospective retrospective analysis, we hypothesised that anticoagulant use would be associated with longer OS. We thus set out to compare the outcome of patients with and without anticoagulant exposure. Before the analysis, it was decided that the primary hypothesis would correspond to (i) the OS comparison of any anticoagulant use versus no use. Anticoagulant use was evaluated at baseline, corresponding to the time from randomisation into the trial, including the two weeks before, to the date of the first dose of concomitant temozolomide chemoradiotherapy (TMZ/RT) (period I), and during initial treatment, defined as the time interval from the first dose of TMZ/RT until the first dose of maintenance TMZ (period II). A patient was considered taking anticoagulants when at least one dose was taken at any time during the respective periods. Anticoagulant therapy was defined as the use of LMWH, unfractionated heparin, vitamin K antagonists or factor Xa inhibitors. PFS was investigated as an additional time-to-event end-point. Further planned analyses included the comparison of PFS and

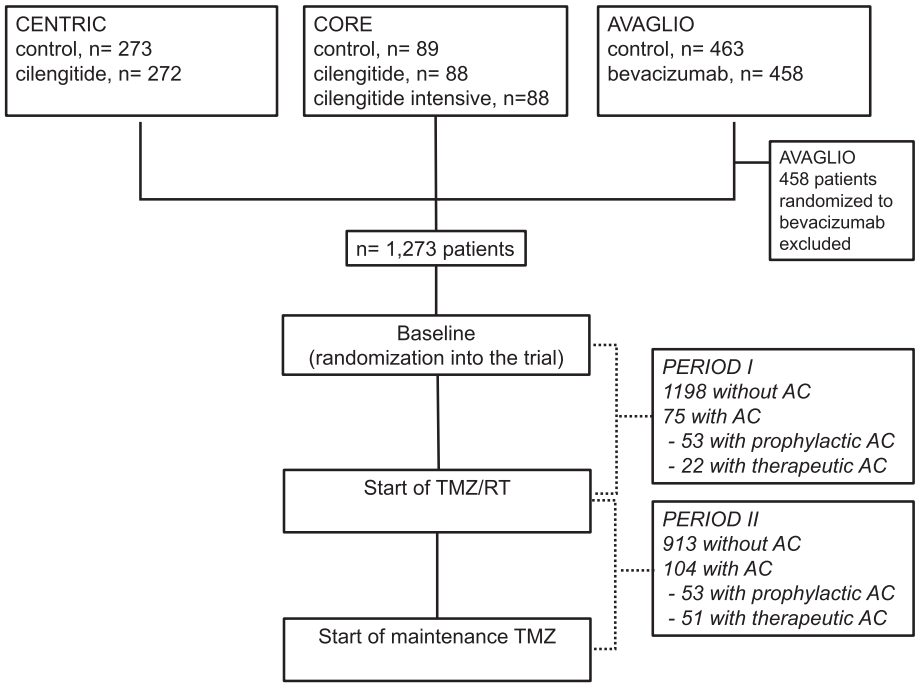

Fig. 1. Consolidated standards of reporting trials (CONSORT) diagram.

OS between (ii) therapeutic versus prophylactic versus no use of anticoagulants, (iii) different durations of anticoagulant use versus no use and (iv) anticoagulant use versus the use of anti-platelet agents versus neither anticoagulant use nor anti-platelet agent use. All analyses were conducted for periods I and II. For PFS analyses in period II, only patients who had not progressed until the first dose of TMZ/RT were included. Four groups were determined to explore the duration of anticoagulant use: 0 days, 1–10 days, 11–30 days and >30 days. If multiple anticoagulants were used, durations of use were added, also for 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. Further exploratory studies were conducted to evaluate whether anticoagulant use beyond period II was associated with the outcome.

Cox proportional hazard models were used to estimate associations of anticoagulant use with the outcome, adjusting for age (continuous), gender (male/female), *MGMT* promotor methylation status (unmethylated/methylated/unknown), WHO performance status (PS = 0 or >0), extent of resection (biopsy only, partial resection or gross total resection), steroid use at baseline (yes/no) and MMSE score (<27 versus ≥27), stratified by trial to account for differences in timing of patient randomisation and imbalances in baseline covariates. Individual log-rank tests were used to assess the prognostic value of these factors. Significance was established at 5% significance level for the primary analysis with a nominal significance level of 2.5% for period I and II. All other analyses were exploratory. SAS version 9.4 (© 2002–2012 by SAS Institute Inc., Cary, NC) was used for baseline covariate description and survival analysis (procedure proportional hazards regression [PHREG]).

3. Results

3.1. Patient characteristics and anticoagulant use

The main characteristics of the 1273 patients examined for period I by trial are summarised in Supplementary Table 1. Baseline demographic variables were similar across trials except for *MGMT* promoter methylation status and extent of resection due to different eligibility criteria to enter the trials. Exclusion criteria related to VTE and anticoagulation and VTE reported in the respective publications are provided in Supplementary Table 2. Median age was 57 years, WHO performance status was 0 in 53.3% of patients and resection was reported as gross total in 47.2% and as partial in 49.1% of patients; 40.9% of patients received steroids at baseline. *MGMT* promoter methylation was detected in 52.2% of the tumors, reflecting the overproportional contribution of CENTRIC that included only patients with *MGMT* promoter methylation. MMSE was 27 or more in 77% of the patients. Compared with the baseline data set, patients who completed TMZ/RT and started TMZ maintenance (period II, n = 1017, 79.9%) were more often aged 49 years or less (27.5% versus 17.2%), male (60.1% versus 52.0%), had more often a WHO

performance status of 0 (56.5% versus 40.2%), had used steroids less often at baseline (39.3% versus 47.3%) and had more often an MMSE score of 27 or more (79.8% versus 65.6%) than those who did not (Supplementary Table 3). In period I, only one new VTE (a deep venous thrombosis [DVT]) was documented, which was treated with therapeutic anticoagulation. In period II, new VTEs were documented in 22 of 1017 patients. All new VTEs except one were treated with therapeutic anticoagulation. Among patients with documented isocitrate dehydrogenase mutation (IDH)1^{R132H} status, one of 26 patients (3.8%) with IDH mutation as opposed to 11 of 449 patients (2.5%) without IDH mutation had a VTE documented. Patients with documented VTEs were more often aged ≥50 years (95.5% versus 72.0%) and male (72.7% versus 59.8%), had more often PS = 0 (68.2% versus 56.3%) and MMSE<27 (40.9% versus 19.1%) and had more often used steroids at baseline (81.8% versus 38.4%) than patients without documented VTE (data not shown).

3.2. Anticoagulant use

During period I, 75 patients (5.9%) were on anticoagulants; during period II, 104 patients (10.2%) received anticoagulants. In both periods, LMWH was most commonly used: 5.3% in period I and 8.5% in period II (Table 1, Supplementary Table 4). There were no differences in patient characteristics between patients who received anticoagulants and patients who did not, except that steroid use was more common in patients on anticoagulants in period I (61.3% versus 39.6%, p = 0.001) (Supplementary Table 5) and II (54.8% versus 37.6%, p = 0.005) (Supplementary Table 6). For the overall cohort, median PFS was 7.8 months (95% CI 7.5–8.0), and median OS was 19.9 months (95% CI 18.6–20.9); 826 patients had died. Prognostic factor analysis at baseline confirmed age at diagnosis (p < 0.001), extent of resection (p < 0.001), WHO performance status at diagnosis (p < 0.001), steroid use at baseline (p < 0.001) and *MGMT* promoter methylation status (p = 0.03) as independent prognostic factors for OS (Supplementary Fig. 1).

3.3. Anticoagulant use versus no use (primary analysis)

PFS and OS by anticoagulant use and stratified for trial in this pooled analysis are summarised in Table 2. On multivariate analysis, anticoagulant use was not associated with PFS either in period I (p = 0.25) or II (p = 0.11). No OS difference was observed in period I (HR = 1.13; 95% CI 0.86–1.50, p = 0.37), but in period II, OS was significantly decreased (HR = 1.52; 95% CI 1.18–1.95, p < 0.001) in patients treated with anticoagulants compared with those with no use (Table 3, Fig. 2A–D). No significant difference in OS was

Table 1
Anticoagulant use between the first dose of TMZ/RT and the start of maintenance (period II).

Anticoagulant therapy	CENTRIC (N = 432) N (%)	CORE (N = 215) N (%)	AVAglio (N = 370) N (%)	Total (N = 1017) N (%)
Use of anti-platelets or anticoagulants				
No use	360 (83.3)	172 (80.0)	337 (91.1)	869 (85.4)
Anti-platelet use	19 (4.4)	12 (5.6)	13 (3.5)	44 (4.3)
Anticoagulant use	53 (12.3)	31 (14.4)	20 (5.4)	104 (10.2)
Type of anticoagulant therapy				
No use	379 (87.7)	184 (85.6)	350 (94.6)	913 (89.8)
LMWH	46 (10.6)	23 (10.7)	17 (4.8)	86 (8.5)
Non-fractionated heparin	2 (0.5)	3 (1.4)	0 (0.0)	5 (0.5)
Xa inhibitor	0 (0.0)	1 (0.5)	1 (0.3)	2 (0.2)
LMWH and Vitamin K antagonists	2 (0.5)	2 (0.9)	1 (0.3)	5 (0.5)
LMWH and non-fractionated heparin	2 (0.5)	2 (0.9)	0 (0.0)	4 (0.4)
LMWH and Xa inhibitor	0 (0.0)	0 (0.0)	1 (0.3)	1 (0.1)
LMWH, non-fractionated heparin and Xa inhibitor	1 (0.2)	0 (0.0)	0 (0.0)	1 (0.1)
Type of anticoagulant use				
No use	379 (87.7)	184 (85.6)	350 (94.6)	913 (89.8)
Prophylactic use	28 (6.5)	13 (6.0)	12 (3.2)	53 (5.2)
Therapeutic use	25 (5.8)	18 (8.4)	8 (2.2)	51 (5.0)
Duration of exposure				
0 days	379 (87.7)	184 (85.6)	350 (94.6)	913 (89.8)
1–10 days	16 (3.7)	5 (2.3)	4 (1.1)	25 (2.5)
11–30 days	6 (1.4)	6 (2.8)	2 (0.5)	14 (1.4)
>30 days	31 (7.2)	20 (9.3)	14 (3.8)	65 (6.4)
Exposure in days (exposed patients only)				
Median	35.0	46.0	56.0	43.5
Range	1.0–122.0	1.0–131.0	1.0–106.0	1.0–131.0

LMWH, low-molecular-weight heparin; N, number of patients; RT, radiotherapy; TMZ, temozolomide.

Table 2
Unadjusted PFS and OS by anticoagulant use (stratified for trial).

	Variable	Patients (events) (N)	Median survival (95% CI)	Hazard ratio (95% CI)	p-value (Log-rank test)	Survival (%) at 0.25 years (95% CI)	Survival (%) at 0.5 years (95% CI)	Survival (%) at 1 year (95% CI)	Survival (%) at 1.5 years (95% CI)	Survival (%) at 2 years (95% CI)
PFS	**Anticoagulant use at baseline (period I)**									
	No use of anticoagulants	1198 (977)	7.79 (7.62–8.05)	1.00	0.155	79.4 (77.0–81.7)	59.8 (56.8–62.6)	34.1 (31.3–36.9)	23.8 (21.3, 26.4)	14.3 (12.2–16.5)
	Anticoagulant use	75 (61)	4.12 (3.06–9.82)	1.21 (0.93–1.56)		63.0 (50.6–73.1)	45.7 (33.7–56.8)	33.8 (23.0–45.0)	22.5 (13.4, 33.2)	11.0 (4.8–20.2)
	Anticoagulant use between the first TMZ/RT treatment and the first dose of TMZ maintenance (period II)									
	No use of anticoagulants	829 (682)	7.85 (7.52–8.15)	1.00	**0.025**	80.5 (77.6–83.0)	57.7 (54.3–61.1)	37.0 (33.6–40.4)	25.9 (22.9, 29.1)	14.2 (11.6, 17.0)
	Anticoagulant use	82 (72)	7.49 (5.06–10.74)	1.33 (1.04–1.70)		75.6 (64.8–83.5)	53.5 (42.2–63.6)	32.4 (22.5–42.6)	17.1 (9.8, 26.1)	8.9 (3.5, 17.3)
OS	**Anticoagulant use at baseline (period I**									
	No use of anticoagulants	1198 (770)	19.98 (18.46–20.99)	1.00	0.177	97.0 (95.8–97.8)	90.8 (89.0–92.3)	72.7 (70.1–75.2)	54.1 (51.2–56.9)	39.1 (36.2–42.0)
	Anticoagulant use	75 (56)	19.22 (12.06–24.25)	1.21 (0.92–1.58)		93.3 (84.7, 97.2	82.5 (71.8–89.5)	63.1 (50.9–73.0)	53.1 (40.9–63.9)	39.9 (28.5–51.1)
	Anticoagulant use between first TMZ/RT treatment and first dose of TMZ maintenance (period II)									
	No use of anticoagulants	913 (558)	19.94 (18.56–21.03)	1.00	**< 0.0001**	96.0 (94.6, 97.1)	90.5 (88.4–92.2)	70.6 (67.5–73.5)	55.1 (51.7–58.3)	40.4 (37.0–43.7)
	Anticoagulant use	104 (73)	13.67 (10.45–19.09)	1.65 (1.28–2.11)		95.2 (88.8, 98.0)	82.5 (73.7–88.6)	55.2 (45.0–64.2)	42.2 (32.5–51.5)	26.1 (16.9–36.2)

CI, confidence interval; N, number of patients; OS, overall survival; PFS, progression-free survival; TMZ, temozolomide.

observed when patients with anticoagulant use both in period I and in period II were compared with patients who had no anticoagulant use in either period (HR = 1.33; 95% CI 0.87–2.04, p = 0.18). Exploratory analyses beyond period II confirmed the lack of an association of improved outcome with anticoagulant use (Supplementary Table 7).

3.4. Therapeutic versus prophylactic use and duration of anticoagulant use

During period I, 53 patients (4.2%) received prophylactic anticoagulants, and 22 (1.7%) received anticoagulants for therapeutic use (Supplementary Table 3). During period II, 53 patients received prophylactic anticoagulants (5.2%), and 51 received anticoagulants for therapeutic use (5.0%) (Table 1). On multivariate analysis, there was no significant difference of PFS (period I, p = 0.07; period II, p = 0.10). The comparison of OS was significant in period II (p = 0.002) but not in period I (p = 0.31): patients on therapeutic anticoagulation had worse prognosis (HR = 1.75; 95% CI 1.12–2.45, p = 0.001) than those with no use. The difference was not significant for prophylactic use (HR = 1.32; 95% CI 0.92–1.88, p = 0.13) (Tables 4 and 5, upper section, Fig. 2E–F). The durations of anticoagulant use in periods I and II are summarised in Supplementary Table 3 and in Table 1. The comparison by duration of use was not significantly different for PFS (period I, p = 0.20; period II, p = 0.14). OS was significantly different in period II (p < 0.001) but not in period I (p = 0.3). In the former period, patients with more than 30 days of administration had significantly worse prognosis (HR = 2.01; 95% CI 1.51–2.96, p < 0.001) but not when the duration of use was shorter (1–10 days: p = 0.83; 11–30 days, p = 0.72) than those with no use (0 days) (Table 5, middle section). More than 30 days of anticoagulant in period II was associated with decreased OS not only for therapeutic use (HR = 2.15; 95% CI 1.47–3.13, p < 0.0001) but also prophylactic use (HR = 1.87; 95% CI 1.21–2.87, p = 0.005).

3.5. Anticoagulant use versus anti-platelet agent therapy versus use of neither anticoagulant nor anti-platelet use

There was no significant difference in PFS (period I, p = 0.52; period II, p = 0.24). In contrast, similar as before, patients treated with anticoagulants in period II (n = 104, 10.2%) had a significant decrease in OS (HR = 1.51; 95% CI 1.17–1.96, p < 0.001), whereas patients treated with anti-platelet agents (n = 44, 4.3%) (Table 1) did not show such a decrease (HR = 0.98; 95% CI 0.67–1.44, p = 0.94) compared to those with the use of neither anticoagulants nor anti-platelet agents (n = 869, 85.4%) (Table 5, lower section Supplementary Fig. 2).

Table 3
Adjusted hazard ratios for PFS and OS by anticoagulant use on multivariate survival analysis.

	PFS				OS			
	Anticoagulant use at baseline (period I)		Anticoagulant use during concomitant TMZ/RT (period II)		Anticoagulant use at baseline (period I)		Anticoagulant use during concomitant TMZ/RT (period II)	
	Hazard ratio (95% CI)	p value	Hazard ratio (95% CI)	p value	Hazard ratio (95% CI)	p value	Hazard ratio (95% CI)	p value
Anticoagulant use								
No	1	0.25 (df = 1)	1.00	0.11 (df = 1)	1	0.37 (df = 1)	1.00	0.001 (df = 1)
Yes	1.17 (0.90–1.52)		1.23 (0.95–1.58)		1.13 (0.86–1.50)		1.52 (1.18–1.95)	
Age								
Per year	1.02 (1.01–1.02)	<0.001 (df = 1)	1.02 (1.01–1.02)	<0.001 (df = 1)	1.03 (1.02–1.04)	<0.001 (df = 1)	1.03 (1.02–1.03)	<0.001 (df = 1)
Gender								
Male	1.00	0.32 (df = 1)	1.00	0.01 (df = 1)	1.00	0.10 (df = 1)	1.00	0.005 (df = 1)
Female	0.94 (0.83–1.06)		0.83 (0.71–0.96)		0.89 (0.77–1.02)		0.79 (0.67–0.93)	
Extent of surgery								
Biopsy	1.00	<0.001 (df = 2)	1.00	0.004 (df = 2)	1.00	<0.001 (df = 2)	1.00	0.008 (df = 2)
Partial resection	1.25 (0.88–1.78)		1.59 (1.02–2.48)		1.08 (0.73–1.61)		1.12 (0.71–1.77)	
Gross total resection	0.97 (0.69–1.39)		1.27 (0.82–1.99)		0.80 (0.54–1.18)		0.87 (0.55–1.38)	
WHO performance status								
0	1.00	0.01 (df = 1)	1.00	0.06 (df = 1)	1.00	<0.001 (df = 1)	1.00	0.01 (df = 1)
1–2	1.19 (1.04–1.35)		1.16 (0.99–1.35)		1.29 (1.12–1.49)		1.24 (1.05–1.47)	
Steroids use at baseline								
No	1.00	0.008 (df = 1)	1.00	0.09 (df = 1)	1.00	<0.001 (df = 1)	1.00	0.025 (df = 1)
Yes	1.19 (1.05–1.35)		1.14 (0.98–1.33)		1.34 (1.16–1.54)		1.20 (1.02–1.42)	
MGMT promoter								
Unmethylated	1.00	<0.001 (df = 2)	1.00	<0.001 (df = 2)	1.00	<0.001 (df = 2)	1.00	<0.001 (df = 2)
Methylated	0.48 (0.37–0.61)		0.44 (0.34–0.59)		0.37 (0.27–0.49)		0.36 (0.26–0.50)	
Unknown	0.84 (0.66–1.08)		0.87 (0.65–1.16)		0.92 (0.70–1.20)		0.87 (0.64–1.17)	
MMSE								
<27	1.00	0.10 (df = 1)	1.00	0.44 (df = 1)	1.00	0.07 (df = 1)	1.00	0.24 (df = 1)
≥27	0.88 (0.75–1.03)		0.93 (0.77–1.12)		0.86 (0.72–1.01)		0.89 (0.73–1.08)	

CI, confidence interval; df, degree of freedom; MGMT, O6-methylguanine DNA methyltransferase; MMSE, Mini-Mental State Examination; OS, overall survival; PFS, progression-free survival; PS, performance status; RT, radiotherapy, TMZ, temozolomide; WHO, World Health Organisation.

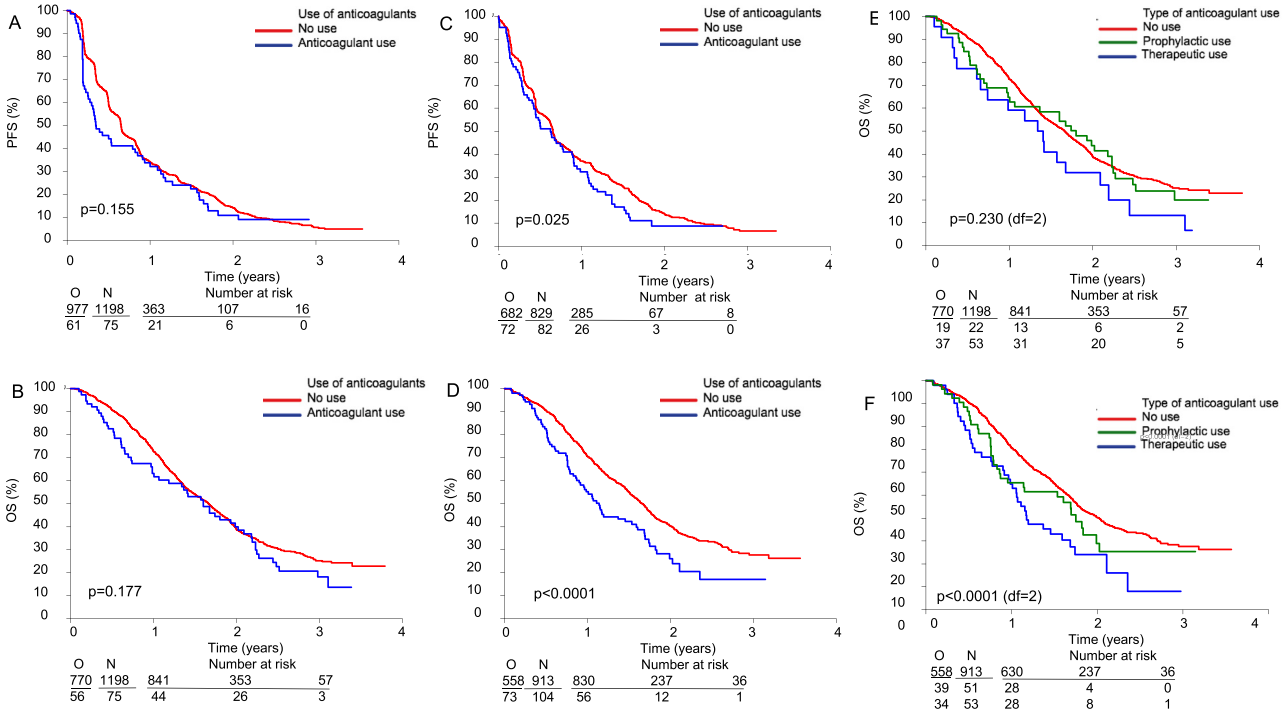

Fig. 2. Prognostic significance of anticoagulant use in the newly diagnosed glioblastoma. A–D: PFS (A, B) or OS (C, D) by use versus no use at baseline (period I) (A, C) or from the first TMZ/RT dose to the start of TMZ maintenance (period II) (B, D). E, D: OS by no use versus prophylactic versus therapeutic use in periods I (E) and II (F) (univariate p values provided).

4. Discussion

This analysis was conducted to support the hypothesis that anticoagulants might improve the outcome in patients with the newly diagnosed glioblastoma. This hypothesis was based on immature clinical data [13–15] and biological properties of heparin [3–8] but never tested because the only prospective trial [15] was terminated early for the lack of drug supply. We used the patient cohorts of three contemporary clinical trials [16–18] to support a disease-modifying role of anticoagulants. Given the type of clinical trial data available, we examined specifically two periods, the time from randomisation to the start of concomitant TMZ/RT (period I) and from then to the first dose of maintenance TMZ (period II). Data sets did not allow for more in-depth analyses of the total dose of anticoagulants or days with versus without anticoagulants. Data analysis beyond the start of maintenance TMZ was hampered by the fact that patients increasingly dropped out for any per-protocol allowed withdrawal including progression, toxicity and refusal, resulting in increasingly limited sample size over time.

Anticoagulant use at study registration or during concomitant chemoradiotherapy (TMZ/RT, defined here as period II) was not associated with a significant increase in PFS or OS (Table 2). In contrast, therapeutic anticoagulant use during period II was associated with inferior OS (Fig. 2F). Longer exposure to anticoagulants was associated with more loss of survival days (Table 5), suggesting dose dependence; this was even true for patients on prophylactic use only. In an effort to

Table 4
Estimates of median OS and OS at 2 years by the type of anticoagulant use.

	Type of anticoagulant	Patients (N)	Observed events (O)	Non-parametric		
				Median (months) (95% CI)*	% at 2 years (95% CI)	P-value (log-rank test)*
Baseline (period I)	No use	1198	770	19.98 (18.46–20.99)	39.1 (36.2–42.0)	0.230
	Prophylactic use	53	37	21.59 (11.79–26.78)	43.7 (29.6–56.9)	
	Therapeutic use	22	19	16.48 (7.29–25.07)	31.8 (14.2–51.1)	
First TMZ/RT dose to first TMZ maintenance dose (period II)	No use	913	558	19.94 (18.56–21.03)	40.4 (37.0–43.7)	<0.0001
	Prophylactic use	53	34	18.43 (9.46–21.85)	29.0 (15.8–43.5)	
	Therapeutic use	51	39	12.71 (9.13–16.46)	24.0 (12.4–37.6)	

* The p-value refers to the median (months). CI, confidence interval; N, number of patients; O, number of observed events; RT, radiotherapy; TMZ, temozolomide.

Table 5
Adjusted hazard ratios for PFS and OS by the type of anticoagulant use, duration of anticoagulant use and anticoagulant use versus anti-platelet agent therapy on multivariate survival analysis.

	PFS				OS			
	Anticoagulant use at baseline (period I)		Anticoagulant use during concomitant TMZ/RT (period II)		Anticoagulant use at baseline (period I)		Anticoagulant use during concomitant TMZ/RT (period II)	
	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Type of anticoagulant use		0.07 (df = 2)		0.10 (df = 2)		0.31 (df = 2)		0.002 (df = 2)
No use	1.00		1.00		1.00		1.00	
Prophylactic use	1.02 (0.73–1.43)	0.91	1.05 (0.74–1.49)	0.77	1.02 (0.73–1.43)	0.90	1.32 (0.92–1.88)	0.13
Therapeutic use	1.74 (0.90–2.27)	0.02	1.46 (1.03–2.05)	0.03	1.43 (0.90–2.27)	0.13	1.75 (1.25–2.45)	0.001
Duration of anticoagulant use		0.20 (df = 3)		0.14 (df = 3)		0.33 (df = 3)		<0.001 (df = 3)
No use (0 days)	1.00		1.00		1.00		1.00	
1–10 days	0.86 (0.46–1.62)	0.65	1.89 (0.54–1.45)	0.64	0.76 (0.38–1.53)	0.44	1.94 (0.54–1.64)	0.83
11–30 days	1.14 (0.81–1.59)	0.45	0.47 (0.82–2.63)	0.19	1.16 (0.83–1.63)	0.39	0.87 (0.41–1.85)	0.72
>30 days	1.72 (1.00–2.94)	0.05	1.36 (0.99–1.87)	0.06	1.55 (0.86–2.77)	0.14	2.01 (1.51–2.69)	<0.001
Anticoagulant use versus anti-platelet agent therapy		0.52 (df = 2)		0.24 (df = 2)		0.32 (df = 2)		0.006 (df = 2)
No use	1.00		1.00		1.00		1.00	
Anti-platelet use	1.01 (0.72–1.42)	0.94	0.11 (0.78–1.58)	0.56	0.79 (0.54–1.15)	0.22	0.98 (0.67–1.44)	0.94
Anticoagulant use	1.17 (0.90–1.52)	0.25	1.24 (0.96–1.59)	0.10	1.12 (0.85–1.48)	0.41	1.51 (1.17–1.96)	0.001

CI, confidence interval; df, degree of freedom; OS, overall survival; PFS, progression-free survival; PS, performance status; RT, radiotherapy; TMZ, temozolomide.

control for comorbidities, we noted that no such effect was seen for patients treated with anti-platelet agents (Supplementary Fig. 2).

The lack of a PFS association suggests that anticoagulation did not interfere with the efficacy of first-line therapy. Because all documented new VTEs except one were treated with therapeutic anticoagulation and because all patients on therapeutic anticoagulation likely had prior VTE, although not always documented, we cannot distinguish whether VTE or therapeutic anticoagulation defines the patient population at risk. Corticosteroid use has been linked to increased risk of VTE [19] and to inferior survival [20]. Steroid use was associated with anticoagulant use (Supplementary Tables 4–6), but anticoagulant use remained associated with inferior outcome on multivariable analysis (Table 3). Although no data on the cause of death are available, lethal cerebral hemorrhages potentially related to therapeutic anticoagulation would probably have been captured. Thus, anticoagulants per se may not compromise survival, but VTEs, once they occur, should be adequately treated. Finally, therapeutic anticoagulation may merely identify a group of patients with poor disease control.

The risk of VTEs in patients with gliomas has recently been linked to the absence of IDH mutations [21], but no such relationship was demonstrated in this cohort; however, overall, only 26 patients with IDH-mutant glioblastoma may have been too low to confirm this association.

Although prophylactic rather than therapeutic use of anticoagulants was not confirmed to be associated with inferior OS, the trend was still negative. Admittedly, one might argue that patients kept on prophylactic anticoagulants were considered high risk by their physicians and represent a worse prognosis population, but our synopsis of clinical patient characteristics does not support this (Supplementary Tables 5 and 6).

Our study suffers from several limitations. Owing to its retrospective nature, imbalances in important unmeasured prognostic factors or determinants of anticoagulant use could not be corrected for in the adjusted analyses. In addition, subgroups of patients treated with anticoagulants were small. Finally, exposure time to anticoagulants captured by this analysis may have been too short to allow identifying a positive disease-modifying effect of anticoagulants, although our exploratory analyses did not support this view (Supplementary Table 7). Moreover, we assume that potentially beneficial effects of anticoagulation in the tumor microenvironment might be particularly relevant during radiotherapy which has been associated with increased invasiveness and inflammatory signalling supporting tumor growth [22–24], and there was no indication for improved PFS, although periods I and II together comprise a significant part of the PFS time of many patients. For the patients treated with

anticoagulants beyond period II (2.7–9.1%), exploratory analyses did not show improved PFS or OS either (Supplementary Table 7). Yet, we cannot rule that patients who do not require anticoagulants for VTE might still derive benefit from therapeutic anticoagulation for tumor control.

Nevertheless, no better data sets to address this question are available and this analysis lends no support for randomised clinical trials of primary prophylaxis with anticoagulants in newly diagnosed glioblastoma that aim at prolonging survival, rather, prevention of VTE-associated morbidity and mortality should remain the focus.

Conflict of interest statement

E.L.R. has received research grants from Mundipharma and Amgen and honoraria for lectures from Mundipharma and Novartis. E.G. declares no conflict of interest. R.S. declares fees to the institution from AbbVie, Celgene, Novartis, Merck KGaA (Darmstadt) and MSD, all outside the submitted work, and travel assistance from Novocure; and spouse is a full-time employee of Celgene. O.L.C. has received a research grant from Roche and honoraria for lectures or board participation or consulting from Abbvie, BMS, Celldex, Immatics, Ipsen and Roche. L.B.N. declares scientific advisory board participation for Merck and BMS. T.C. declares compensation by providing consultative services to VBL, INSYS, Roche, Tocagen, Human Longevity, Sunovion, Boston Biomedical, Alexion, Wellcome trust, Novogen, Novocure, GW Pharma, AbbVie, Cortice and BMS. D.R. has received research grants from Acerta Pharma, Agenus, Celldex Therapeutics, EMD Serono, Incyte, Inovio, Midatech and Tragara and has received honoraria for lectures or advisory board participation or consulting from Abbvie, Agenus, Amgen, BMS, Cavion, Celldex, EMD Serono, Genentech/Roche, Inovio, Juno Pharmaceuticals, Merck, Midatech, Momenta Pharmaceuticals, Novartis, Novocure, Oncorus, Oxigene, Regeneron and Stemline Therapeutics. W.W. has received research support from Apogenix GmbH, Merck, Sharp & Dohme (MSD), Pfizer and Roche and honoraria for lectures from MSD. T.G. declares no conflict of interest. M.W. has received research grants from Abbvie, Acceleron, Actelion, Bayer, Merck, Sharp & Dohme (MSD), Merck (EMD), Novocure, OGD2, Piqur and Roche and honoraria for lectures or advisory board participation or consulting from Abbvie, BMS, Celldex, Merck, Sharp & Dohme (MSD), Merck (EMD), Novocure, Pfizer, Roche, Teva and Tocagen.

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Appendix A. Supplementary data

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