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Original Research Article

Neutrophilia as a biomarker for overall survival in newly diagnosed high-grade glioma patients undergoing chemoradiation

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ABSTRACT

Objective: To study the prognostic value of neutrophil disorders in a retrospective cohort of high-grade glioma patients receiving definitive concurrent temozolomide and radiation.
Materials and methods: Clinical records of consecutive patients treated in our Institution between January 2005 and December 2010 with concurrent temozolomide (75 mg/m^2 daily) and radiation were collected. The prognostic value of pretreatment neutrophilia on survival, defined as a neutrophil count exceeding 7 G/L, was examined.
Results: We identified 164 patients, all treated with concurrent temozolomide-based chemoradiotherapy. Initial surgery was achieved in most (75%), with resection > 90% in 55 patients (34%). Total 151 patients (92%) had glioblastoma, and 13 patients (8%) had WHO grade III glioma. Eighty-two patients (50%) displayed pretreatment neutrophilia. Neutrophilia was not associated with concurrent or adjuvant temodal discontinuation ($p > 0.3$). The 2-year actuarial overall survival was 45%. Steroid consumption, i.e. 60 mg or more of daily prednisolone, increased pretreatment neutrophil count ($p = 0.005$). In univariate analysis, neutrophilia was associated with worse overall survival ($p = 0.019$), as well as age $\geq$ 65 years ($p = 0.009$), surgical resection < 90% ($p = 0.003$) and prednisolone consumption $\geq$ 60 mg/day ($p = 0.016$). In multivariate analysis, neutrophilia ($p = 0.013$), age $\geq$ 65 ($p = 0.001$), and surgical tumor resection < 90% ($p = 0.010$) independently decreased overall survival, while, steroid consumption was not ($p = 0.088$).
Conclusion: In high-grade gliomas treated with concurrent temozolomide and radiation, pretreatment neutrophilia may be a significant prognosis factor for overall survival. In addition with previously available markers, this independent cost-effective biomarker could help identifying patients with worsened prognosis.

Introduction

Glioblastoma is the most common primary brain tumor in adults. Since 2005, standard treatment for glioblastoma patients is maximal safe surgical resection followed by combined radiotherapy and temozolomide chemotherapy [1]. Phase III studies with an addition of targeted antiangiogenic therapies failed to show benefits in overall survival compared to the Stupp protocol [2,3].

Age, performance status (PS), extent of resection, Mini Mental State Examination (MMSE), and O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation status have demonstrated their prognostic impact on patients outcome [4]. The MGMT DNA repair enzyme antagonizes the genotoxic effects of alkylating agents. Its low expression is associated with favorable outcome in patients with glioblastoma undergoing alkylating agent based chemotherapy [5]. Still, prognostic markers to guide individual concomitant and maintenance therapy are mandatory.

In addition to cancer cells, stromal cells, blood vessels and infiltrating inflammatory cells are major components of the tumor microenvironment [6]. As additional markers, prognostic value of

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neutrophil-to-lymphocyte ratio (NLR) > 4 in peripheral blood had previously been described in glioblastoma as an accessible and cost-effective marker of systemic inflammatory responses [7–9]. Still, NLR reflects either neutrophilia, lymphopenia or both. In gliomas, high level of CD4(+) tumour-infiltrating lymphocytes (TILs) combined with low CD8(+) TILs has been associated with poor prognosis in glioblastoma patients [10]. Neutrophils are the most abundant circulating leukocytes, and stand as early immune defense. As leukocytes influence the function and phenotype of CD8+ T cells, neutrophils could represent a potential prognosis biomarker in patients with high-grade gliomas [11]. Recently, tumor-associated neutrophilia has been associated with poor clinical outcome in several human cancers [12–14].

In the current study, prognostic significance of pretreatment systemic neutrophilia was retrospectively examined in a single center cohort of consecutive high-grade (III and IV) glioma adult patients treated with concurrent temozolomide and radiotherapy.

Materials and methods

Patients and tumors

We examined clinical records of all consecutive patients registered in our institution between January 2005 and December 2010. We excluded patients with pretreatment immune disorder, patients with grade I or II gliomas at diagnosis, and patients treated with hypofractionated chemoradiation. All patients had been referred to a multidisciplinary neuro-oncology tumor board prior treatment initiation. Explorations at diagnosis included both computed tomography (CT) and a brain magnetic-resonance imaging (MRI) with at least T2, FLAIR, T1 and T1 enhanced gadolinium sequences.

Treatment characteristics and follow-up

After prior surgical resection or biopsy, patients received concurrent radiotherapy. Prescribed doses were 60 Gy administered as 2-Gy fractions 5 days per week in patients with glioblastoma, or 59,4 Gy delivered by 1.8 Gy per fraction in patients with grade III gliomas. All patients had daily concomitant oral temozolomide (75 mg per square meter of body-surface area per day for a maximum of 49 days).

Radiotherapy was planned with a dedicated computed tomography (CT) and three-dimensional planning systems; conformal radiotherapy was delivered with linear accelerators with nominal energy of 6 MV or more. Radiotherapy was delivered by a 3D conformal technique.

The concurrent-therapy phase was followed by a 28-day treatment break. During the adjuvant part, patients received temozolomide (150 mg per square meter per day on days 1–5 during the first cycle and 200 mg per square meter per day during subsequent cycles if unacceptable toxic effects did not occur) [1].

Patients were assessed 6 weeks after the completion of treatment with physical examination and imaging studies and then with physical examination every 2–3 months until progression.

Complete blood count analysis

Complete blood count were obtained postoperatively in the week preceding the first chemoradiation for the current analysis. The daily steroid dose considered in the analysis was on the day of the drawing of the blood sample. Patients underwent systematic complete white blood cell counts (WBC) weekly during chemoradiation. Leukocytosis and neutrophilia assessing biological inflammation were defined as blood count over 10 G/L and 7 G/L, respectively. Anemia was defined as hemoglobin count below 13.0 g/dL. Thrombocytosis, lymphopenia, and monocytosis were defined as platelets count over 400 G/L, lymphocytes count below 1.0 G/L, and monocytes count over 1.0 G/L, respectively. We tested these parameters for statistical correlation with OS. The NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count, with 4 being the cutoff for positivity, in accordance with the previously published literature [9].

Statistical analysis

Differences in patient characteristics regarding pretreatment neutrophilia were compared with Fisher test, Student-t test, and by variance analysis. Survival times were defined as the time between the diagnosis and time of death for OS, estimated by the Kaplan Meier method. Patients were censored at the time of last follow-up visit. Survival curves were constructed using the Kaplan-Meier method and statistical comparisons were performed using the log-rank test for univariate analyses. Multivariate analyses were performed using the Cox proportional hazards model with variables with p value <0.1 in univariate analysis. Statistical analyses were performed using R (version 3.3.2).

Results

Patients and tumors

Among 164 patients, median age was 60 years (range: 19–83). Total 13 patients (8%) had grade III glioma, and 151 patients (92%) had glioblastoma.

On initial blood count, before the week of the initial chemoradiation part, median neutrophil count was 7.0 G/L (1.6–16.6). Leukocytosis and neutrophilia were found in 60 patients (37%) and 82 (50%) patients, respectively (Table 1A & Supplementary Fig. S1). Mean neutrophil count were comparable in patients with grade III gliomas or glioblastomas: 7.0 vs. 7.3 G/L, respectively ($p = 0.701$). Patients with daily prednisolone equivalent dose below 60 mg (6.7 G/L) had significantly lower neutrophil counts compared to patients with dose above 60 mg (8.5 G/L; $p = 0.005$) (Supplementary Fig. S2).

Radiotherapy and chemotherapy

All patients underwent a 3D-conformal brain irradiation to a median 60.0 Gy dose (range: 14–60) with concurrent temozolomide (75 mg/m^2 daily during chemoradiation). Median delay from surgery was 1.5 month (range: 0.1–13.3). Most of the patients received the planned treatment doses; adverse events leading to discontinuation of temozolomide involved 11 patients (7%) during chemoradiation phase, and 68 patients (41.5%) during maintenance phase. A total of 5 (3%) and 11 (7%) patients discontinued or stopped concomitant temodal therapy, independent from pretreatment neutrophilia ($p = 0.670$ and $p = 0.371$ respectively). Similar, 15 (11%) patients discontinued concomitant temodal therapy for toxicity, independently from neutrophilia ($p = 0.377$) (Table 1B).

Survival and disease control

145 patients (88%) died during the follow-up (median 28.7 months; range, 0.5–99.0). Estimated 1 and 2-years OS were 75% (95%CI: 68–82) and 45% (95%CI: 37–53), respectively.

Table 1A
Patients characteristics.

Characteristics		Overall population	Neutrophils ≥ 7 G/L (pretreatment)		
			No	Yes	*p*
		n (%) or median [range]			
Patients characteristics					
Number of patients		164 (100%)	82 (50%)	82 (50%)	
Age (years)		60 [19, 83]	59 [19, 83]	60 [23, 81]	0.565
	<65 y	119 (72.6%)	63 (76.8%)	56 (68%)	0.294
	≥65 y	45 (27%)	19 (23%)	26 (31.7%)	
Gender	Male	108 (66%)	59 (72%)	49 (60%)	0.138
	Female	56 (34%)	23 (28%)	33 (40%)	
PS	0	100 (61%)	56 (68%)	44 (54%)	0.078
	≥1	64 (39%)	26 (32%)	38 (46%)	
KPS	≥70	142 (87%)	76 (93%)	66 (80%)	0.039
	<70	22 (13%)	6 (7%)	16 (20%)	
Surgery	Yes	123 (75%)	69 (84%)	54 (66%)	0.012
	No	41 (25%)	13 (16%)	28 (34%)	
	>90%	55 (34%)	30 (37%)	25 (31%)	0.449
WHO Grade	III	13 (8%)	5 (6%)	8 (10%)	0.563
	IV	151 (92%)	77 (94%)	74 (90%)	
Corticosteroid daily dose	(mg)	40 [0, 160]	40 [0, 100]	40 [0, 160]	<0.001
	<60 mg/d	104 (63%)	63 (77%)	41 (50%)	<0.001
	≥60 mg/d	50 (31%)	13 (16%)	37 (45%)	
	NA	10 (6%)	6 (7%)	4 (5%)	
Baseline biology					
Haemoglobin (g/dL)		13.7 [9.6, 17.1]	13.7 [9.6, 16.2]	13.7 [9.8, 17.1]	0.736
Anemia	Yes	50 (30.5%)	23 (28%)	27 (32.9%)	0.611
	No	114 (69.5%)	59 (72%)	55 (67%)	
Platelet (G/L)		250 [121, 953]	248 [140, 463]	258 [121, 953]	0.241
Leukocytes (G/L)		8.9 [3.7, 18.9]	7.2 [3.7, 12.4]	11.2 [7.9, 18.9]	<0.001
Leukocytosis	Yes	60 (37%)	1 (1%)	59 (72%)	<0.001
	No	99 (60%)	78 (95%)	21 (26%)	
	NA	5 (3%)	3 (4%)	2 (2%)	
Neutrophils (G/L)		7.0 [1.6, 16.6]	5.0 [1.6, 6.9]	9.0 [7.0, 16.6]	<0.001
Neutrophilia	Yes	82 (50%)	0 (0%)	82 (100%)	<0.001
	No	82 (50%)	82 (100%)	0 (0%)	
Lymphocytes (G/L)		1.3 [0.2, 3.3]	1.4 [0.5, 2.9]	1.1 [0.2, 3.3]	0.005
Lymphopenia	Yes	40 (24%)	9 (11%)	31 (38%)	<0.001
	No	124 (75.6%)	73 (89%)	51 (62%)	
Monocytes (G/L)		0.5 [0.1, 1.5]	0.5 [0.2, 1.5]	0.5 [0.1, 1.5]	0.050
Monocytosis	Yes	10 (6%)	2 (2%)	8 (10%)	0.010
	No	107 (65%)	49 (60%)	58 (71%)	
	NA	47 (28.7%)	31 (38%)	16 (19%)	
NLR		5.1 [0.7, 83]	3.4 [0.7, 8]	8.9 [2.5, 83]	<0.001
NLR	<4	62 (37.8%)	51 (62%)	11 (13%)	<0.001
	≥4	102 (62%)	31 (38%)	71 (87%)	

KPS: Karnofsky Performance Status; NLR: Neutrophil to Lymphocyte Ratio; PS: Performance Status; WHO: World Health Organization classification.

Table 1B
Treatment characteristics.

Characteristics		Overall population	Neutrophils ≥ 7 G/L (pretreatment)		
			No	Yes	*p*
		n (%) or median [range]			
Treatment characteristics					
Number of patients		164 (100%)	82 (50%)	82 (50%)	
Radiotherapy dose (Gy)		60.0 [14, 60]	60.00 [13.75, 60]	60.00 [34, 60]	0.212
Concomitant TMZ	Yes	164 (100%)	82 (100%)	82 (100%)	NaN
Concomitant BVZ	No	163 (99%)	82 (100%)	81 (99%)	0.495
	Yes	1 (1%)	0 (0%)	1 (1%)	
Concomitant TMZ discontinuation	No	143 (87%)	72 (88%)	71 (87%)	0.542
	Yes	11 (7%)	4 (5%)	7 (8%)	
	NA	10 (6%)	6 (7%)	4 (5%)	
Adjuvant TMZ	No	25 (15%)	11 (13%)	14 (17%)	0.808
	Yes	135 (82%)	69 (84%)	66 (81%)	
	NA	4 (2%)	2 (2%)	2 (2%)	
N adjuvant TMZ cycles		6 [1, 12]	6 [1, 12]	6 [1, 12]	0.481
Adjuvant TMZ discontinuation	No	65 (39.6%)	38 (46%)	27 (32.9%)	0.213
	Yes	68 (41.5%)	30 (36.6%)	38 (46%)	
	NA	31 (18.9%)	14 (17%)	17 (20.7%)	

BVZ: Bevacizumab; Gy: Gray; TMZ: Temozolomide.

Prognostic value of leucocytes disorders

We analyzed the prognostic value of leukocytosis, neutrophilia, and significant prognostic factors as described in previous publications (i.e. PS, Karnofsky Performance Status (KPS), age > 65, gender, resection rate > 90%) [15].

In univariate analysis, the factors significantly associated with poor OS were neutrophilia (p = 0.019), age ≥ 65 (p = 0.009), PS ≥ 1 (p = 0.066), surgical resection < 90% (p = 0.003), prednisolone equivalent daily dose ≥ 60 mg/day (p = 0.016), and absence of maintenance chemotherapy (p = 0.009). KPS > 70 (p = 0.694), anemia (p = 0.789), leukocytosis (p = 0.313), monocytosis (p = 0.384), lymphopenia (p = 0.105), and NLR ≥ 4 (p = 0.090) were not related with OS.

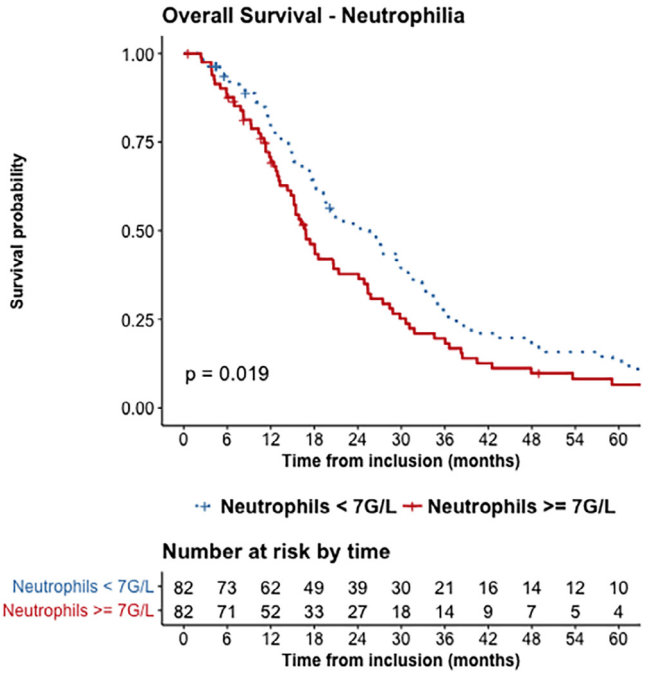

Fig. 1. Estimated overall survival in grade III and IV glioma patients, with or without neutrophilia. *Neutrophilia: neutrophil count ≥ 7 G/L.*

At 2-year follow-up, estimated OS was 51% (95%CI: 41–63%) for patients that had not initial neutrophilia vs. 38% (95%CI: 28–51%) for patients with initial neutrophilia (Fig. 1). In glioblastomas patients, estimated 2-years OS was 48% (95%CI: 38–61%) in absence of neutrophilia vs. 35% (95%CI: 25–48%) if they had (p = 0.014) (Fig. 2).

In multivariate analysis, apart from age ≥ 65, gender, PS, surgical resection quality or corticosteroid requirement, neutrophilia was independently associated with poor OS with hazard ratio (HR) of 1.57 (95%CI: 1.10–2.23, p = 0.013). Age ≥ 65 and surgical resection < 90% were also associated with inferior OS (p = 0.001 and p = 0.010, respectively) (Table 2).

Discussion

The present study associated neutrophilia, as marker of systemic inflammation, with worse survival among high-grade gliomas patients undergoing chemoradiation. A previous study associated elevated neutrophil count with poor OS in patients with glioblastoma, suggesting its relation with more aggressive tumor, and activation of peripheral neutrophils as early sign of tumor progression [16]. Intratumoral neutrophil infiltration also significantly correlated with tumor grade in glioma patients [17]. These results are consistent with the present study, displaying an independent association between neutrophilia and poor prognosis. The lack of prognosis significance from tumor WHO grade (p = 0.062) can be imputed to the small subset of WHO grade III glioma patients. Also, the inclusion of grade III glioma patients undergoing concurrent chemoradiation implies excluding patients treated with adjuvant

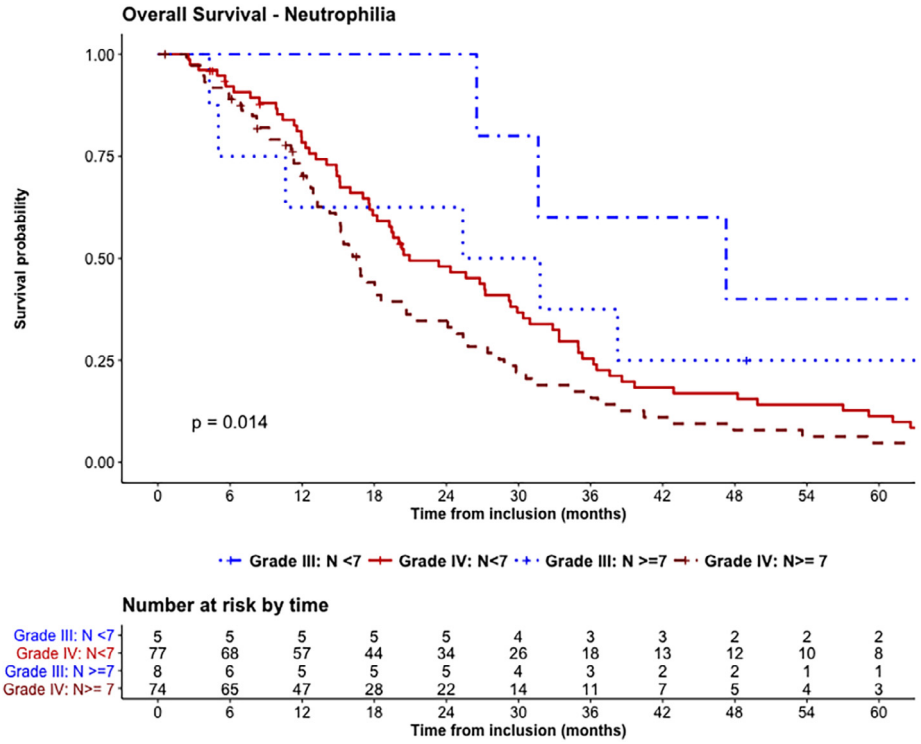

Fig. 2. Estimated overall survival in patients with or without neutrophilia, stratifying on WHO tumor grade. *N: neutrophil count, < or ≥ 7 G/L.*

Table 2
Results of univariate (log-rank) and multivariate (Cox) analyses (significant factors in bold) for overall survival.

	UNIVARIATE	MULTIVARIATE			
		HR	Lower 95%	Upper 95%	p
Neutrophilia (vs. absence)	0.019	1.57	1.10	2.23	**0.013**
Age ≥ 65y (vs. <65y)	0.009	1.92	1.29	2.87	**0.001**
Female gender (vs. Male gender)	0.102	–	–	–	0.053
WHO Grade IV (vs. Grade III)	0.062	–	–	–	0.160
PS = 0 (vs. PS ≥ 1)	0.066	–	–	–	0.498
Resection > 90% (vs. <90% or biopsy)	0.003	0.60	0.41	0.88	**0.010**
Prednisolone ≥ 60 mg/d (vs. <60 mg/d)	0.016	–	–	–	0.088

Neutrophilia: neutrophil count ≥ 7 G/L; PS: Performance status; WHO: World Health Organization.

PCV and presenting more favourable tumor phenotypes (IDH1 or TERT promoter mutations, 1p19q codeletion) [18].

Neutrophils both can exert antitumoral (N1 phenotype) or protumoral (N2 phenotype) activity, depending on the tumor micro environment [19]. There is also an complex interplay between tumor associated neutrophils and tumor infiltrating lymphocytes [19,20]. NLR is the most studied prognosis biomarker derived from peripheral blood cell count analysis in glioblastoma patients [7–9]. It owns potential double information, both immunosuppressive state through lymphopenia, and inflammatory condition through neutrophilia. We studied the most commonly published NLR cutoff at 4 [7–9]. Despite their immunosuppressive state, partially related with long-term steroid use, patients with glioblastoma often have an increase in circulating neutrophils [17]. As corticosteroids consumption increase neutrophil and decrease lymphocyte counts, it may heavily influence NLR. A recent change or tapering schedule in steroid dosage administration was previously related with significant white blood cells variations [21]. In the context of brain tumors, CD4 lymphopenia was associated with worse prognosis [10]. In glioblastoma patients, both neutrophils in peripheral blood and in the tumor microenvironment have been associated with a major role in glioblastoma-induced T-cell suppression [22]. In our population, NLR was not able to predict patient survival ($p = 0.090$), neither lymphopenia ($p = 0.105$). Moreover, neutrophilia was independently associated with patients OS, even after adjusting on corticosteroid daily dose ($p = 0.013$) in the multivariate analysis.

Preclinical study showed that T cells from patients with glioblastoma displayed minimal proliferation, IFN-γ production, and increasing numbers of degranulated neutrophils within the peripheral circulation [23]. As neutrophils promotes inflammation through infections and tissue damage conditions, it could promote fast-growing tumor phenotype associated with necrosis and treatment resistance. Recent clinical and experimental data suggested that corticosteroids decreases the effectiveness of radiotherapy- and chemotherapy-induced genotoxic stress, and reduces OS in glioblastoma [24]. Beyond the established adverse effect profile of protracted corticosteroid use, this analysis substantiates the request for prudent and restricted use of corticosteroids in glioblastoma patients, and anti-Vascular Endothelial Growth Factor (VEGF)-antibody increased tumour cell death, without improving OS when combined with radiotherapy [24]. Bevacizumab treatment reduced steroid use, yet no survival difference was seen [2,3]. These findings emphasizes the importance of identifying alternative agents such as VEGF antagonists for managing oedema in glioblastoma patients [24]. In the present study, higher steroid daily doses correlated with higher pretreatment neutrophil count ($p = 0.005$). Steroïds decreased OS in univariate analysis ($p = 0.016$) but was not independently associated with OS ($p = 0.088$). Taken together, these findings and the present study suggests that neutrophilia may independently predict OS, through definition of more aggressive disease and high amount of necrosis, independently from glucocorticoid intakes.

The dichotomy of neutrophils activity is partially described: they both can exert antitumoral or protumoral activity (N1 or N2 phenotype respectively) [25]. Glioblastoma patients displays decreased function of natural killer cells and T cells, and high peripheral release of both TGF-β [16,26]. Past studies established that tumor associated neutrophils are polarized from an protumor N2 to an antitumor N1 phenotype in the absence of TGF-β, and radiation therapy may induces the N1 polarization [27]. Targeting granulocytes, TGF-β or S100A4 may be effective approaches to inhibit the glioma malignant phenotype and diminish antiangiogenic therapy resistance. Clinical trials are currently planned, ongoing or completed, e.g. SAPPHIRE trial (NCT00761280) for the targeted inhibition of TGF-β2. Still, therapeutic targeting neutrophils, key keepers against host infection will be challenging.

The strength of this study is the robust association between neutrophilia and poor prognosis in both anaplastic gliomas and glioblastoma in a homogenous cohort of high-grade glioma patients treated with concurrent temozolomide + RT. Our findings are consistent with previously published and biological recent discoveries. We highlighted prognosis value from neutrophil count before chemoradiation, independently from patients steroids requirements. Finally, we described independence from pretreatment neutrophilia and temozolomide discontinuation. Main limitations included its retrospective design, blood cell count evaluation before chemoradiation thus after surgery in most patients, and the absence of any notion of active smoking. Epigenetic silencing of the MGMT (O6-methylguanine–DNA methyltransferase) DNA-repair gene by promoter methylation compromises DNA repair and has been associated with longer survival in patients with glioblastoma who receive alkylating agents. Thus, the absence of MGMT status hampers seriously the validity of the multivariate analysis on survival. In the context of glioblastoma, corticosteroids may compromise patients' survival [24]. In the present study, we revealed that neutrophilia was a prognosis biomarker, even after adjustment for the level of corticosteroid consumption. However, these data being retrospective, this result does not allow us to affirm the absence of correlation between survival, neutrophilia and corticosteroid consumption. Also, we were not able to assess neutrophils functional status in this population.

Conclusion

Pretreatment hematologic profile with initial neutrophilia may be a clinically relevant prognosis biomarker in high-grade gliomas patients treated with concomitant temozolomide and radiation. In addition with previously established prognosis factors, this independent biomarker may help identifying patients in future prognostic risk scores. Neutrophil count should be prospectively

monitored in further clinical trials investigating targeted therapies or immunotherapies.

Conflict of interest statement

None declared.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ctro.2018.04.002.

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