



# Early platelet variation during concomitant chemo-radiotherapy predicts adjuvant temozolomide-induced thrombocytopenia in newly diagnosed glioblastoma patients

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## Abstract

**Purpose** Temozolomide (TMZ) is known to induce thrombocytopenia but no early predictive test has yet been clearly established. The aim of the study was to retrospectively identify and validate a threshold of early platelet variation predicting TMZ-induced thrombocytopenia during the TMZ phase in patients treated according to the Stupp protocol for glioblastoma.

**Methods** A training set was used to analyze variations in platelet count occurring from the first week (W1) to week 6 (W6) during radiotherapy. Our aim was to identify the most relevant platelet decrease associated with TMZ-induced thrombocytopenia  $\leq 100$  G/L at day 28 during the TMZ phase. The performance of the threshold was confirmed in an independent validation set.

**Results** Overall, 147 patients were included, 85 and 62 in the training and validation sets, respectively. Twenty-seven patients (18%) experienced at least one TMZ-induced thrombocytopenia in the TMZ phase. A platelet decrease at W6  $\geq 35\%$  ( $\Delta W6 \geq 35\%$ ) was identified as the best predictive variation with an AUC of 0.83, a sensitivity of 65%, and a specificity of 96%. In the validation set,  $\Delta W6 \geq 35\%$  platelet variation was identified as an independent marker of TMZ-induced thrombocytopenia during the TMZ phase (OR 15.23 (95% CI 3.5–107.5)) corresponding to sensitivity of 77% (66–87%), specificity of 73% (62–84%), a positive predictive value of 42% (29–54%), and a negative predictive value of 92% (86–99%).

**Conclusion** Platelet decrease at W6  $\geq 35\%$  during the RT-TMZ phase is an early and simple predictive marker of clinically relevant TMZ-induced thrombocytopenia during TMZ maintenance.

**Keywords** Glioblastoma · Temozolomide · Thrombocytopenia · Early platelet variation

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## Introduction

Temozolomide (TMZ), an oral alkylating agent, improves overall survival in patients treated for newly diagnosed glioblastoma multiforme (GBM) [1]. Based on the Stupp protocol, TMZ is delivered concomitantly to radiation therapy (RT) for 6 weeks (RT-TMZ phase) followed by maintenance TMZ monotherapy for at least 6 cycles (TMZ phase). It has been widely reported that TMZ monotherapy is achieved in only one third to one half of patients [2, 3]. Except for tumor progression, TMZ-related toxicities are the main reasons for treatment discontinuation during the TMZ phase and are observed in 5% [4] to 16.5% [5] of patients from phase III clinical trials.

Clinically relevant TMZ-induced thrombocytopenia is defined by a platelet (PLT) count  $\leq 100$  G/L. It may occur during the two TMZ phases in one third of cases in a non-selected population [6]. Grade 1 ( $\leq 100$  G/L) and grade 2 ( $< 75$  and  $> 50$  G/L) [7] TMZ-induced thrombocytopenias induce cycle delay and, in case of prolonged thrombocytopenia, early treatment withdrawal. Grade  $\geq 3$  TMZ-phase thrombocytopenia ( $< 50$  G/L) [7] is observed in 3 to 20% patients [1–5, 8, 9]. Deepness as well as duration of TMZ-induced thrombocytopenia may have significant clinical impacts including treatment discontinuation, PLT transfusion, intracranial hemorrhage [10], and TMZ-related death [9]. Taken together, these findings support that early recognition of high-risk patients with TMZ-induced thrombocytopenia may be of interest to ensure completion of the treatment schedule. Until now, although predictive factors of TMZ-induced myelosuppression have been suggested [6, 11–13], none has been validated [14]. In a study including 52 patients with 19.2% TMZ-induced thrombocytopenia, Preusser et al. reported that the variation of the immature or absolute PLT counts between two consecutive follow-up visits may be a predictor of clinically relevant TMZ-induced thrombocytopenia [15].

In this context, the aim of the study was to identify a threshold of PLT variations during the RT-TMZ phase predicting relevant thrombocytopenia during the TMZ phase and to validate it using an independent cohort of patients treated for GBM.

## Materials and methods

### Study population

A retrospective study was conducted from January 2009 to December 2014. Eligible patients had newly diagnosed, histologically proven, and supratentorial GBM; age equal to or greater than 18; received at least 45 Gy in the RT-TMZ phase; a baseline PLT count at diagnosis equal or greater than 100 G/L; and a baseline neutrophil count greater than 1.5 G/L. Patients with active and/or previous history of hematologic

disorder within the 2 years before inclusion were excluded from the study.

TMZ was administered orally at 75 mg/m<sup>2</sup>/day during the RT-TMZ phase from week 1 (W1) to week 6 (W6) concomitantly to RT dosed at 60 Gy. After a 4-week treatment-free interval, the TMZ phase was started at 150 mg/m<sup>2</sup>/day in the first cycle (cycle 1) and 200 mg/m<sup>2</sup>/day (5 days on 28 days per cycle) for at least 6 cycles (cycle 6), as published by Stupp et al. [1]. The doses of TMZ did not exceed the calculated dose for 2 m<sup>2</sup> of body surface area. After 6 cycles of monotherapy, TMZ was continued in non-progressive patients up to 12 cycles. TMZ was not delivered in case of thrombocytopenia  $\leq 100$  G/L, neutropenia  $\leq 1.5$  G/L, and any TMZ-related toxicities of grade  $\geq 3$ . Dose reduction, cycle delay, and/or treatment discontinuation of TMZ schedule were systematically registered during patient follow-up.

### Centralized PLT count measurement

For each patient, a blood count was performed at the beginning of each of the 6 weeks of the RT-TMZ phase (W1 to W6). PLT concentrations were determined by the electric impedance and optical methods using the XN-1000 hematology analyzer (Sysmex Corporation®, Kobe, Japan). In cases of PLT abnormal distribution, a second analysis using an optical method was performed. In cases of a final PLT count lower than 100 G/L or higher than 700 G/L, a blood smear was performed to visually control PLT counts.

### Statistical analysis

The primary endpoint was to identify in a training set the threshold of PLT variation during the RT-TMZ phase that predicts clinically relevant thrombocytopenia during the TMZ phase and to validate its usefulness in a validation cohort.

Patients treated between 2009 and 2012 were enrolled in the training cohort. Explanatory variable was the variation, by patient, of PLT counts during the RT-TMZ phase between absolute PLT count at week 1 (W1) and PLT counts at each week between week 2 ( $\Delta W2$ ) and week 6 ( $\Delta W6$ ). The variable to predict was the occurrence of at least 1 cycle delay, dose reduction, and/or early treatment discontinuation during the TMZ phase due to TMZ-induced thrombocytopenia  $\leq 100$  G/L at day 28 of the cycle, defined as clinically relevant TMZ-induced thrombocytopenia. The attribution of TMZ to the occurrence of thrombocytopenia has been defined as follows: (1) thrombocytopenia occurring within 30 days after TMZ administration, (2) no concomitant treatment having a risk of thrombocytopenia equal or greater than 1/1000 according to the European Medicines Agency's summary of product characteristics, and (3) absence of another cause due to acute pathology after analysis of the performed complementary examinations. The best threshold associated with the occurrence

of at least one clinically relevant TMZ-induced thrombocytopenia was identified using Receiver Operating Curve (ROC). The analysis was performed for each PLT variation from  $\Delta W2$  to  $\Delta W6$ .

Patients treated between 2013 and 2014 were enrolled in the validation cohort. The diagnostic value of the best threshold of PLT variation identified in the training set was tested in the validation cohort to predict the occurrence of clinically relevant TMZ-induced thrombocytopenia. Sensitivity (Se), specificity (Sp), negative predictive value (NPV), and positive predictive value (PPV) were estimated, and the relative risk to experience a clinically relevant TMZ-induced thrombocytopenia was estimated by the odds ratio (OR). Comparisons between the training cohort and the validation cohort for age, gender repartition, recursive partitioning analysis (RPA) stages [16], surgical status, radiotherapy technique (RT), and thrombocytopenia proportion were performed.

The parametric distribution of the continuous variables was assessed using the Shapiro-Wilk test. The parametric

distributed variables are expressed as the mean  $\pm$  standard deviation (SD). The non-parametric distributed variables are expressed as the median and the interquartile range (IQR). The Student *t* test was used for parametric distributed variables and the Mann-Whitney-Wilcoxon test for the non-parametric distributed variables. In cases of more than two groups, the differences were assessed using the ANOVA or the Kruskal-Wallis test, as appropriate. For the categorical variables, the differences between groups were assessed using the chi-square test or the Fisher exact test. A logistic regression including age, gender, ECOG status, surgery, baseline PLT count, PTV (planning target volume), and the threshold identified in the training set was performed in the validation set to identify variables associated with the occurrence of severe clinically relevant thrombocytopenia during the TMZ phase. The alpha risk threshold was set at 5%, and all of the tests were two-sided. Analyses were performed using R software version 2.13 (R Foundation for Statistical Computing, Vienna, Austria) and ggplot2 packages were used.

**Table 1** Clinical characteristics of patients

		Overall population Number of patients (proportion) n=147	At least one TMZ- induced thrombocytopenia Number of patients (proportion) n=34	P	Training cohort Number of patients (proportion) n=85	Validation cohort Number of patients (proportion) n=62	P
Age at diagnosis	mean [range]	55.1 [18-76]	56.3 [25-71]	0.63	53.7 [18-74]	57 [19-76]	0.12
	<50	44 (30%)	7 (21%)	0.28	30 (35%)	14 (33%)	0.12
	50 - 70	92 (63%)	26 (76%)		51 (60%)	41 (66%)	
	>70	11 (7%)	1 (3%)		4 (5%)	7 (11%)	
Gender	Female	56 (38%)	17 (50%)	0.2	30 (35%)	26 (42%)	0.41
	Male	91 (62%)	17 (50%)		55 (65%)	36 (58%)	
ECOG	0-1	137 (93%)	31 (91%)	0.7	79 (93%)	58 (94%)	0.89
	$\geq 2$	10 (7%)	3 (9%)		6 (7%)	4 (6%)	
BSA		1.9 [1.35-2.76]	1.85 [1.61-2.17]	0.08	1.91 [1.43-2.76]	1.88 [1.35-2.27]	0.46
Creatinine level ( $\mu\text{mol/L}$ )		75.1 [25-174]	76.4 [48-134]	0.71	76.6 [25-136]	73.1 [28-174]	0.001
Surgery	Biopsy	37 (25%)	9 (26%)	0.95	16 (19%)	21 (34%)	0.06
	Partial Resection	34 (23%)	7 (21%)		24 (28%)	10 (16%)	
	GTR	76 (52%)	18 (53%)		45 (53%)	31 (50%)	
RPA class	III	40 (27%)	7 (21%)	0.51	27 (32%)	13 (21%)	0.01
	IV	58 (39%)	17 (50%)		25 (29%)	33 (53%)	
	V-VI	49 (34%)	10 (29%)		33 (39%)	16 (26%)	
Type of radiotherapy	3DCRT	89 (61%)	16 (47%)	0.15	81 (95%)	8 (13%)	<0.001
	IMRT	58 (39%)	18 (53%)		4 (5%)	54 (87%)	
Mean PTV size ( $\text{cm}^3$ )		289 [44-966]	320 [53-849]	0.29	265 [44-751]	323 [46-966]	0.03
Mean RT dose (Gy)		59.3	60	0.06	59.4	59.6	0.7
Thrombocytopenia <sup>a</sup>	grade 1-2	-	18 (53%)	-	10 (12%)	8 (13%)	0.12
	grade 3-4	-	16 (47%)	-	6 (7%)	10 (16%)	

BSA, Body Surface Area; GTR, gross total resection; IMRT, intensity-modulated radiation therapy; RPA, recursive partitioning analysis; RT, radiotherapy; 3DCRT, three dimensional conformal radiation therapy;

<sup>a</sup>according to NCI-CTCAE version 4.03

## Results

### Overall patients' characteristics

A total of 147 patients were included, 85 and 62 patients in the training and the validation set, respectively. As shown in Table 1, the two cohorts were not different except for the RPA stages and for the use of intensity-modulated radiation therapy (IMRT), which were more frequent in the validation set. The median follow-up of the entire population was 46 months (range, 13–85).

### TMZ-induced thrombocytopenia in the entire population

Overall, 34 patients (23%) experienced clinically relevant TMZ-induced thrombocytopenia corresponding to 16 (18.8%) and 18 (29%) patients in the training and validation set, respectively, as shown in Table 2. For each cohort, this side effect was observed respectively in 8 (9.4%) and 12 (19%) patients during the RT-TMZ phase, in 14 (16.4%) and 13 (20.9%) patients during the TMZ phase, and in 6 (7.1%) and 7 (9.7%) patients in both sequences.

Among the 27 patients who experienced clinically relevant TMZ-induced thrombocytopenia in the TMZ phase, 23 patients (85%) were within the first 6 cycles, 16 patients (57%) had a mean TMZ dose reduction of 25% for the remaining cycles, and three patients (11%) underwent hospitalization for PLT transfusion without bleeding. The main reason for TMZ withdrawal was tumor progression in 73% of cases. Regarding TMZ-related toxicities, a grade 3

thrombocytopenia (< 50 G/L) of more than 60 days was the only reason for early treatment discontinuation and concerned six patients (4%). There were no significant differences in age (mean 56.3 vs. 54.8 years,  $P = 0.56$ ), body surface area (mean 1.85 vs. 1.9 m<sup>2</sup>,  $P = 0.08$ ), serum creatinine level (mean 76.4 vs. 75.1 μmol/L,  $P = 0.71$ ), proportion of women (50 vs. 36%,  $P = 0.2$ ), proportion of patients receiving corticosteroid treatment (74 vs. 58%,  $P = 0.12$ ), or corticosteroid doses (mean 30 vs. 35 mg of prednisone equivalent,  $P = 0.56$ ) between patients with or without clinically relevant TMZ-induced thrombocytopenia. Anti-epileptic drug (AED) exposure was not associated with the occurrence of TMZ-induced thrombocytopenia with 15% of patients exposed to levetiracetam ( $n = 87$ ), 33% of patients exposed to gabapentin ( $n = 7$ ), and 18% of patients who received no AED ( $n = 53$ ,  $P = 0.5$ ).

### Determination of the threshold of PLT variation

A significant difference in median absolute PLT counts was observed during the last 2 weeks of the RT-TMZ phase between patients with or without clinically relevant TMZ-induced thrombocytopenia in the TMZ phase (respectively, 169 ± 40 G/L vs. 241 ± 20 G/L at W5,  $P = 0.001$  and 130 ± 40 G/L vs. 229 ± 20 G/L at W6,  $P < 0.001$ , Fig. 1a). Furthermore, the patients who experienced clinically relevant TMZ-induced thrombocytopenia during the TMZ phase had a significantly deeper decrease in PLT compared to patients without this side effect (respectively, -24 ± 15% vs. -2 ± 5% at W5,  $P = 0.01$  and -44 ± 16% vs. -9 ± 5% at W6,  $P < 0.001$  (Fig. 1b)). As a consequence, the highest AUCs were observed for ΔW5 and ΔW6 at 0.82 and 0.83, respectively. In

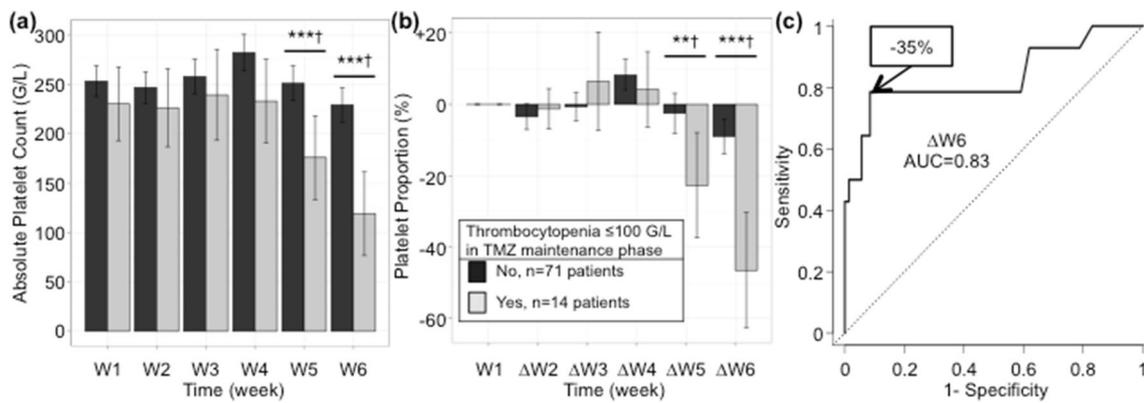
**Table 2** Reasons for TMZ schedule changes in the entire cohort

	RT-TMZ phase		TMZ phase	
	Number of events	Number of patients (proportion)	Number of events	Number of patients (proportion)
Overall TMZ schedule changes <sup>a</sup>	25	25 (17%)	61	57 (40%)
Hematologic toxicities				
Grade 1-4 thrombocytopenia	19	19 (13%)	30	27 (18%)
Grade 2-4 neutropenia	0	0	5	4 (2.7%)
Non-hematologic toxicities				
Grade 3 asthenia	1	1 (0.7%)	8	8 (5%)
Grade 3 nausea	0	0	4	3 (2%)
Infection <sup>b</sup>	1	1 (0.7%)	5	5 (3.4%)
Neurological Symptoms	1	1 (0.7%)	3	3 (2%)
Acute Renal Failure	1	1 (0.7%)	1	1 (0.7%)
Vein Thrombosis	1	1 (0.7%)	0	0
Other causes	1	1 (0.7%)	6	6 (4.1%)

RT-TMZ, radiotherapy with concurrent temozolomide; TMZ, temozolomide

<sup>a</sup> defined as cycle delay, with or without dose reduction in the TMZ-schedule

<sup>b</sup> apart from febrile neutropenia which is included in neutropenia



**Fig. 1** Identification of the best predictor threshold in the training cohort ( $n = 85$ ). **a** Histograms represent the absolute platelet count means by week in the RT-TMZ phase. Dark histograms represent patients with no TMZ-induced thrombocytopenia  $\leq 100$  G/L at day 28 in the maintenance phase, and light gray histograms represent patients with TMZ-induced thrombocytopenia  $\leq 100$  G/L at day 28 in the maintenance phase. Lines represent 95% confidence intervals (95% CI). **b** Histograms represent the mean variation in percentage (%) of platelet count between week 1

(baseline) and each week during the RT-TMZ phase. **c** An ROC curve represents the variations of platelet count between baseline and week 6 ( $\Delta W6$ ) associated with TMZ-induced thrombocytopenia occurrence during TMZ phase. A decrease of platelet count of 35% at week 6 was identified as the threshold that maximizes sensibility and sensitivity. AUC, area under the curve;  $**P = 0.01$ ;  $***P = 0.001$ ; the dagger symbol indicates Student's  $t$  test

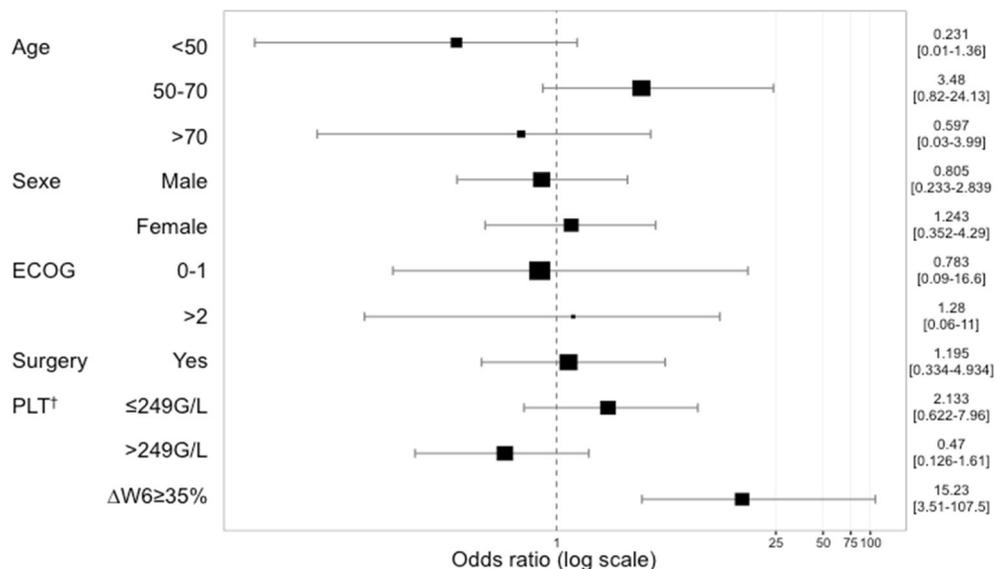
ROC curve analysis, the PLT decrease  $\Delta W6 \geq 35\%$  was identified as the best predictor of clinically relevant TMZ-induced thrombocytopenia during the TMZ phase (Fig. 1c). This threshold was associated with a Se at 65% (55–75%), a Sp at 96% (91–99%), a PPV at 79% (70–87%), and an NPV at 96% (86–97%).

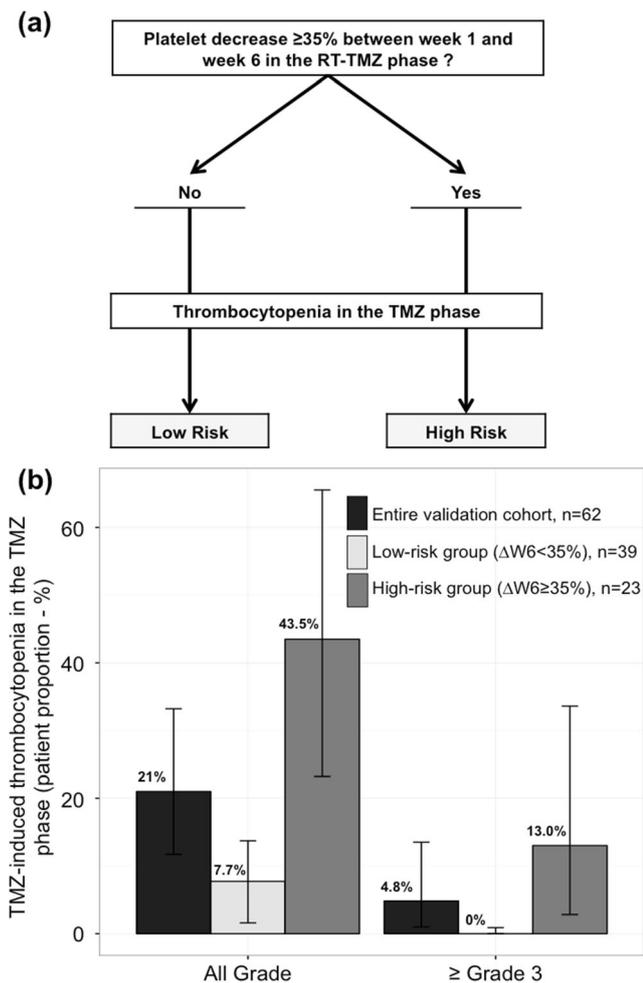
**Validation of the  $\Delta W6 \geq 35\%$  threshold**

$\Delta W6 \geq 35\%$  was observed in 23 patients of the validation cohort (37% (25–49%)). Among the 13 patients with clinically relevant TMZ-induced thrombocytopenia during TMZ phase, the presence of the  $\Delta W6 \geq 35\%$  threshold during the RT-TMZ phase ( $n = 10$ ) was associated with a Se at 77% (66–87%), a Sp at 73% (62–84%), a PPV of 42% (29–54%), and an

NPV of 92% (86–99%). In multivariate analysis, the  $\Delta W6$  threshold was the only predictor of clinically relevant TMZ-induced thrombocytopenia during the TMZ phase: OR 15.23 (3.51–107.5),  $P < 0.001$  (Fig. 2). In the validation cohort and as shown in Fig. 3a, the  $\Delta W6 \geq 35\%$  threshold may identify at the end of the RT-TMZ phase a low-risk group and a high-risk group of patients who will experience TMZ-induced thrombocytopenia during the TMZ phase. Indeed, if overall 13 patients (21%) in the validation cohort ( $n = 62$ ) had a grade 1 TMZ-induced thrombocytopenia  $\leq 100$  G/L, when considering the low-risk group ( $n = 39$ ), only three patients (7.7%) experienced a grade 1 TMZ-induced thrombocytopenia  $\leq 100$  G/L compared to 10 patients (43.5%) in the high-risk group ( $n = 23$ ; Fig. 3b). In the low-risk group, the three patients who experienced TMZ-induced thrombocytopenia

**Fig. 2** Forest plot showing the odds ratios and confidence intervals from multivariate analyses of the TMZ-induced thrombocytopenia  $\leq 100$  G/L in the TMZ phase in the validation cohort ( $n = 62$ ). Black squares represent the odds ratio, and the size is proportional to the number of patients. The lines represent the 95% confidence interval. The dagger symbol indicates baseline platelet (PLT) count (median in the whole population); the double dagger indicates median planning target volume (PTV)





**Fig. 3** Identification of three groups at risk for TMZ-induced thrombocytopenia in the TMZ phase. **a** Algorithm to estimate the risk of TMZ-induced thrombocytopenia  $\leq 100$  G/L at day 28 in the TMZ phase for patients suffering from newly diagnosed GBM and who planned to receive the Stupp protocol. **b** Histograms represent proportions of patients who experienced all grade and  $\geq$  grade 3 TMZ-induced thrombocytopenia in the TMZ phase in the entire validation cohort (black), according to platelet decrease at week 6 lower than 35% (low-risk group, light gray) and equal to or higher than 35% (high-risk group, dark gray). Lines represent 95% confidence intervals

continued the TMZ schedule with subsequent dose reduction until tumor progression and no greater than or equal to grade 3 thrombocytopenia was observed.

## Discussion

The present study shows that a decrease  $\geq 35\%$  of PLT between the first and the last week of the RT-TMZ phase ( $\Delta W6 \geq 35\%$ ) was a predictor of clinically relevant TMZ-induced thrombocytopenia during the TMZ phase. Using training and validation sets, our results indicate that a  $\Delta W6 \geq 35\%$  was significantly associated with the risk of this side effect during the TMZ phase with a sensitivity of 77%, a

specificity of 73%, a PPV of 42%, and an NPV of 92% and was also identified as independent marker in multivariate analysis in the validation cohort. To our knowledge, these findings have not been previously reported to date and are very likely useful to further evaluate interventional preventive and curative procedures in these patients. In our population, the presence of a  $\Delta W6 \geq 35\%$  was observed in approximately one third of cases and may represent an early parameter to modify the follow-up in this subgroup. In our training set, the threshold of  $\Delta W6 \geq 35\%$  platelet variation during RT-TMZ identified 10/13 (77%) of patients who experienced cycle delay or early treatment withdrawal during TMZ phase with a risk at 4.7% of false negative.

Previously, the utility of PLT count variations has been investigated in a series of 52 patients treated for GBM [15]. In contrast to our work, the authors focused on PLT changes from the last to the current follow-up visit for the prediction of severe TMZ-induced thrombocytopenia at the next follow-up visit. The results showed a moderate diagnostic potential of this PLT time course analysis with an AUC less than 0.70 and a highest observed PPV of 57%. Given those findings, our study included a larger sample size of patients with also a specific design with training and validation sets. These results support that the  $\Delta W6 \geq 35\%$  appears to be a relevant marker of TMZ-induced thrombocytopenia. In particular, the high VPV of  $\Delta W6 \geq 35\%$  PLT variation during RT-TMZ allows for reliable identification of the patients that will not experience a TMZ-induced thrombocytopenia during the TMZ phase.

The frequencies of all grades of TMZ-induced thrombocytopenia in our population are consistent with those previously reported in Caucasian patients 23% (34/147) versus 21 to 27.3%, respectively, [8, 9, 17]. However, we observed a higher proportion of TMZ-induced thrombocytopenia during the RT-TMZ phase in the validation cohort (21 vs. 16.5%). A higher proportion of RPA class IV (53 vs. 29%) and a higher mean tumor volume (323 vs. 265 cm<sup>3</sup>) in the validation set could explain these different proportions. The difference in RPA stage could be partly explained, in part, by the higher rate of biopsy in the validation cohort (34 vs. 19%) as no difference was seen for Karnofsky performance status between the two cohorts. However, the proportion of gross tumor resection was similar as well as in the subgroup of patients with  $\Delta W6 \geq 35\%$ . Lastly, regarding the impact of tumor volume radiation, which is associated with hematological toxicities [18], no significant difference in the mean PTV size was observed between the cohort of patients experiencing thrombocytopenia and the entire population (320 vs. 289 cm<sup>3</sup>). These findings demonstrate that the  $\Delta W6 \geq 35\%$  threshold has been determined and validated in two similar cohorts.

Until now, the association between clinically relevant TMZ-induced thrombocytopenia and PLT decrease due to individual constitutional predisposition remains debated. The threshold of 100 G/L of PLT was chosen in agreement with the summary of the European public assessment report for TMZ, which is currently used in our institution. This threshold during the RT-TMZ phase may not be adapted to all patients regarding pharmacogenomics predisposition. Indeed, it has been shown that a low expression of the *MGMT* gene in circulating monocytes in the peripheral blood was associated with an increased risk of TMZ-induced hematological toxicities [19]. The relationship between several antisense polymorphisms located in the *MGMT* gene and TMZ-induced myelotoxicity was explored but none have yet been validated in larger and prospective cohorts [11–13]. Given the retrospective design of our study, we were not able to perform these molecular analyses in the population experiencing rapid PLT decrease. A rapid decrease of PLT count during 6 weeks of the RT-TMZ phase, even if the threshold of 100 G/L is not reached, could be a good marker to detect pharmacogenomics predisposition. The analyses of constitutional *MGMT* promoter methylation and the exploration of antisense polymorphisms of the *MGMT* gene and *MGMT* protein expression in myeloid progenitors and megakaryoblasts from the bone marrow from patients with  $\Delta W6 \geq 35\%$  could be of major interest.

There were several limitations in our study mainly due to its retrospective design. However, among the 172 consecutive patients treated during the study period, only a small portion ( $n = 25$ ) was excluded from the final analysis, including 24 patients who did not receive TMZ in the TMZ phase and one patient who had no centralized PLT count during the RT-TMZ phase. Moreover, it is important to underline that we used a robust method, including a first step for PLT cutoff identification and a second step for its validation. Moreover, considering that the TMZ schedule changes due to TMZ-induced thrombocytopenias which were not standardized, we did not analyze their impact on patient outcome. Lastly, methylation of the *MGMT* promoter and assessment of mutation of *IDH1/IDH2* were not routinely performed. A prospective study focusing on PLT monitoring and including all of these factors is ongoing in our center (GLIOPLAK trial, [clinicaltrials.gov](https://clinicaltrials.gov) identifier NCT02617745).

In conclusion, our study based on discovery and validation cohorts showed that a PLT decrease  $\Delta W6 \geq 35\%$  in the RT-TMZ phase might identify patients with a significant risk of clinically relevant TMZ-induced thrombocytopenia during the TMZ phase. Further prospective interventional studies based on this marker are also warranted.

### Compliance with ethical standards

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

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