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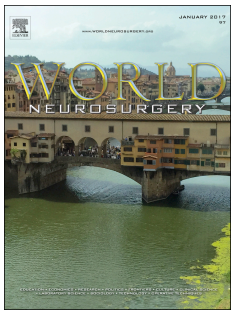

The prognostic role of gender and MGMT methylation status in glioblastoma patients: the female power

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The prognostic role of gender and MGMT methylation status in glioblastoma patients: the female power.

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Abstract

Background: Clinical and molecular factors are essential to define prognosis in glioblastoma (GBM) patients. MGMT methylation status, age, Karnofsky performance status (KPS) and extent of surgical resection are the most relevant prognostic factors. The role of gender in predicting patients prognosis has been investigated showing a slight survival advantage for female patients. **Methods**: We performed a prospective evaluation from a registry called the Project of Emilia Romagna on Neuro-Oncology (PERNO) about prognostic factors in GBM patients who received standard treatment. **Results**: One hundred sixty-nine patients were prospectively evaluated. Among male patients 36 were MGMT methylated (25.7%) and 47 were unmethylated (33.6%); among female patients 32 were methylated (22.9%) and 25 were unmethylated (17.9%). Survival was longer in methylated females than methylated males (p=0.028) but not significantly different between unmethylated female and male patients (P=0.395). In multivariate analysis, gender and MGMT methylation status considered together (methylated female vs methylated male HR=0.459; 95% CI 0.242 – 0.827; P=0.017), age (HR 1.025; 95% CI 1.002 – 1.049; P= 0.032) and KPS (HR 0.965; 95% CI 0.948 – 0.982; P< 0.001) were significantly correlated with survival. **Conclusion**: Survival was consistently longer among MGMT methylated females than males. Gender could be regarded as a further prognostic factor.

Keywords: glioblastoma, gender, MGMT, methylation, PERNO

Introduction

Primary GBMs occur more frequently in male patients with a mean age of diagnosis of 62 years; secondary GBMs have a predilection for female patients with a mean age of 45 years. Glioblastoma prognosis is poor with a median survival of 12-18 months and less than 10% of patients are alive 5 years after diagnosis. Patients surviving over 2 years after diagnosis have a higher survival probability compared to newly diagnosed patients [1, 2].

Extent of surgical resection, performance status and age (> 50 years) are the most important and widely accepted clinical prognostic factors [3-6]. Other factors could impact on prognosis: site of tumors [1]; body surface area (BSA) that is the only factor influencing temozolomide clearance and pharmacokinetics [7]; marital status [8] and care center expertise. About this last topic, in the prospective study of the Project of Emilia-Romagna on Neuro-Oncology (PERNO), overall survival was related to treatment received in a high versus low-volume center [9].

Various prognostic molecular markers have been identified in GBM, including methylation status of the O6-methylguanine-DNA methyltransferase (MGMT) gene promoter, isocitrate dehydrogenase enzyme 1/2 (IDH1/2) mutation, epidermal growth factor receptor (EGFR) overexpression and amplification, glioma-CpGisland methylator phenotype (G-CIMP), tumor protein (TP53) mutation and genetic losses of chromosomes [1].

The role of gender has been investigated as potential prognostic factor.

Male patients have a significant survival advantage than female in the first year after the diagnosis, thereafter the difference is not significant [10].The

results of survival analysis of GBM patients included in the PERNO registry suggest a survival advantage for MGMT methylated females.

Methods

The Project of Emilia-Romagna Region on Neuro-Oncology (PERNO) was created in Italy, in the Emilia-Romagna region in 2008, funded by the Italian Ministry of Health. A prospective study from PERNO registry database about clinical and prognostic features and outcomes of patients with GBM was conducted from January 2009 to December 2010. The main inclusion criteria were: 1) histologically proven GBM according to the WHO classification; 2) age ≥ 18 years; 3) KPS 30-100%, 4) no prior surgery for brain tumor; 5) residence within the Emilia-Romagna region [9,11].

Approval for the project was obtained from the Ethical Committee of each participating center and, before registration, informed written consent of the patient was obtained according to ICH/GCP and national recommendations.

MGMT methylation status was assessed through methylation-specific polymerase chain reaction (MSP).

Treatment

GBM patients younger than 70 years received the standard postsurgical treatment [12]. For patients older than 70 years the decision about schedule and time of radiotherapy and the choice to administer chemotherapy were

taken by oncologists and radiotherapists, based on patient's performance status, clinical conditions and comorbidities.

Temozolomide (TMZ) concomitant with radiotherapy was administered at the dose of 75 mg/m^2 for 7 days per week until the end of radiotherapy. Adjuvant TMZ was administered at the dose of 150-200 mg/m^2 on days 1-5 every 28 days for up to 12 cycles or until radiological and/or clinical progression or unacceptable toxicities [9,11].

Statistical analysis

Data are reported as median, range and frequency. Survival data (median survival times with 95% confidence interval) were calculated by Kaplan-Meier procedure and were analyzed by log-rank test. Multivariate analysis was done through Backward Stepwise Cox proportional hazard model. The hazard ratios (HRs) were computed with 95% confidence intervals (95% CI). Two-tailed P values less than 0.05 were considered significant. The SPSS (Version 13.0 for Windows; SPSS Inc., Chicago, IL, USA) was used as statistical software.

Results

One hundred sixty-nine GBM patients were treated with TMZ concomitant with and adjuvant to radiotherapy. Median age was 60 years (range 29 – 82 years). Ninety-nine patients were male (58.6%) and 70 were female (41.4%). According to MGMT methylation status assessment, 68 patients were MGMT

methylated (48.6%), 72 were unmethylated (51.4%). Only four patients received hypofractionated treatment: one was methylated female, one was unmethylated female, one was methylated male and one was with unknown methylation status female. Patients‘ characteristics are described in **Table 1**.

Survival

Survival was similar between male patients (15.6 months 95% CI 12.9 – 18.4) and female patients (16.7 months 95% CI 13.5 – 19.9) (P= 0.519).

Overall survival (OS) in MGMT methylated patients was 20.0 months (95% CI 14.2 – 25.9) versus 14.1 months (95% CI 11.0 – 17.2) in unmethylated patients (P= 0.001).

Combining gender and methylation status, at univariate analysis, we observed a significant survival improvement for methylated females compared to methylated males: the female methylated group did not reach the median survival, however overall survival at 12 months (OS12) was significantly higher with respect to male methylated group (78.1% vs 66.7% P= 0.028). No significant difference was found between unmethylated females and unmethylated males (P=0.158) **[Figure 1]**.

Multivariate analysis **[Table 2]** showed that gender and MGMT methylation status simultaneously considered (P< 0.000), age (P= 0.032) and performance status (P= 0.000) were the most important prognostic factors. Radical resection rate was not significantly different between female methylated group and other groups (62.5% vs 51.4%, P= 0,574).

Discussion

Age, performance status and extent of surgical resection are well known prognostic factors for GBM.

In a randomized EORTC-NCIC phase III trial [13] in newly diagnosed GBM, survival was not significantly different between female and male patients who received radiotherapy alone (survival difference 1,4 months) or standard treatment (survival difference 2,2 months). Based on these results, EORTC nomogram [14] included MGMT promoter methylation status, age at diagnosis, KPS, extent of surgery and mini-mental state evaluation (MMSE) as important prognostic factors but it did not include gender. More recently, a combined analysis of data from two independent clinical trials (NRG Oncology/Radiation Therapy Oncology Group 0525 and 0825) was used to develop a validated nomogram for estimation of survival in 1354 newly diagnosed GBM patients. It showed that gender was independently associated with survival, being male gender associated with shorter survival [14]. Particularly, a slight survival benefit for female versus male patients was confirmed also in the group of MGMT methylated patients, giving more convincing evidence of this association.

In a retrospective study of 105 newly diagnosed GBM patients we also evaluated the interaction between gender and GBM molecular features [15]. We found that female patients with MGMT methylation presented an improved survival compared to other groups (median OS 28.6 months, P= 0.008).

It was hypothesized that pharmacodynamic, pharmacokinetic and molecular factors can create some differences among male and female patients, but a clear explanation of this is still unknown.

Females have a different body fat distribution, which may influence the absorption of the drug and a lower glomerular filtration rate with a consequent decreased elimination.

Some evidences [16,17] showed that sexual dimorphism might modulate the biology of GBM. In mice models, male exhibited higher proliferation and enhanced tumorigenesis compared to female counterparts [16]. Further, the tumors showed a more extensive necrosis in male patients than in female, due to the association of tumor cell death with MYC increased activity in female and with TP53 activity in male patients [17].

In 2016, a report by Schiffgens et al. analyzed sex-specific differences in relation to survival in GBM patients through the evaluation of a novel biomarker that promotes tumor progression: the Wnt receptor Frizzled-7 (FZD7). In this study, methylated MGMT promoter was found to be significantly associated with longer survival in female patients. Interestingly, combining FZD7 and MGMT methylation, sex-specific differences were deleted [18]. In addition, the role of gender has to be reminded when considering toxicity. The incidence of grade 3-4 hematological toxicity in GBM patients treated with temozolomide is in the range of 7% [12].

In a trial of 445 patients treated with TMZ, a higher incidence (11%) of significant myelotoxicity was reported in female patients. Older female

patients who received higher doses had a major chance to develop both neutropenia and thrombocytopenia [7].

To demonstrate the modulation of gender on the risk of severe myelotoxicity, Armstrong et al. conducted a retrospective review of all GBM patients treated with temozolomide from 1997 to 2004 at M.D. Anderson Cancer Center. They found that grade 3-4 myelotoxicity was significantly different according to the gender, being 16% in female and 7% in male patients, respectively (P= 0.015). Patients carrying the G allele of MGMT (rs2308327) presented a 240% increase in risk of myelotoxicity (95% CI, 0.99 – 5.84) [19].

Other myelotoxic effects of TMZ are represented by aplastic anemia and myelodysplastic syndromes (MDS) and gender might have a role in predicting these conditions too. Villano et al. [20] reported all TMZ associated hematologic events reported in the FDA Med-Watch database between 1997 and 2008 and showed that aplastic anemia was observed in 39 out of 112 cases. Of these 39 patients, 11 died above all for infection. Moreover, these severe and often fatal hematologic toxicities seemed to occur two or three times more frequently in female than in male patients [21].

According to EORTC-NCIC phase III trial and NRG Oncology/Radiation Therapy Oncology Group 0525 and 0825 independent trials, our results from the prospective PERNO registry suggest that females have a survival advantage, though the difference is not significant (16.7 vs 15.6 months, P= 0.519).

MGMT methylation status confirms its role of important prognostic factor (median survival 20.0 months for methylated group vs 14.1 months for unmethylated group, P= 0.001).

When considering MGMT methylation status with gender, we observed a survival benefit in methylated female patients (met vs unmet p< 0.001) but, interestingly, not in male patients (met vs unmet P= 0.395).

At multivariate analysis, simultaneous evaluation of patients' gender and MGMT methylation status has a significant prognostic role: MGMT methylation is associated with longer survival in female patients when compared to unmethylated females (HR 4.108, 95% CI 2.105 – 8.016, P= 0.000), to methylated males (HR 0.459, 95% CI 0.242 – 0.827, P= 0.017), to unmethylated males (HR 0.327 95% CI 0.176 – 0.607 P= 0.000).

Conclusion

The results of this PERNO registry analysis is of particular interest to drive oncologists in their clinical choices to select GBM patients to treat with standard treatment. MGMT methylated promoter has already been shown to be associated with a better prognosis but also gender should be considered as a potential prognostic factor and a predictive factor for myelotoxicity.

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Disclosure

The authors have declared no conflicts of interest. All authors have read and approved the manuscript and have participated to a sufficient extent to be named as authors.

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Table 1

Table 1: Patients' characteristics

Patients' characteristics	**N (tot. 169) and %**
Age	
< 70 years	*141(83%)*
≥ 70 years	*28 (17%)*
Gender	
Male	*99 (58.6%)*
Female	*70 (41.4%)*
KPS (Karnofsky performance status)	
< 70%	*10 (5.9%)*
≥70%	*159 (94.1%)*
MGMT methylation status	
Methylated	*68 (40.3%)*
Unmethylated	*62 (36.7%)*
Age and MGMT methylation status	
Methylated males	*36 (25.7%) median age 60 years*
Unmethylated males	*47 (33.6%) median age 60 years*
Methylated females	*32 (22.9%) median age 58 years*
Unmethylated females	*25 (17.9%) median age 63 years*
Surgery	
Biopsy	*23 (13.6%)*
Partial resection	*65 (38.5%)*
Complete resection	*81 (47.9%)*

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Table 2

Table2: Results from multivariate analysis

	MULTIVARIATE ANALYSIS		
	HR	95%CI	P
Group			<0.001
- methylated female vs methylated male	0.459	0.242 - 0.827	0.017
- methylated male vs unmethylated female	0.530	0.301 - 0.934	0.028
- methylated female vs unmethylated male	0.327	0.176 - 0.607	<0.001
- methylated female vs unmethylated female	0.243	0.125 - 0.475	<0.001
Age	1.025	1.002 - 1.049	0.032
KPS	0.965	0.948 - 0.982	<0.001
MGMT methylation			- 1
Gender			- 1
			1

1 Not included in the forward stepwise selection model

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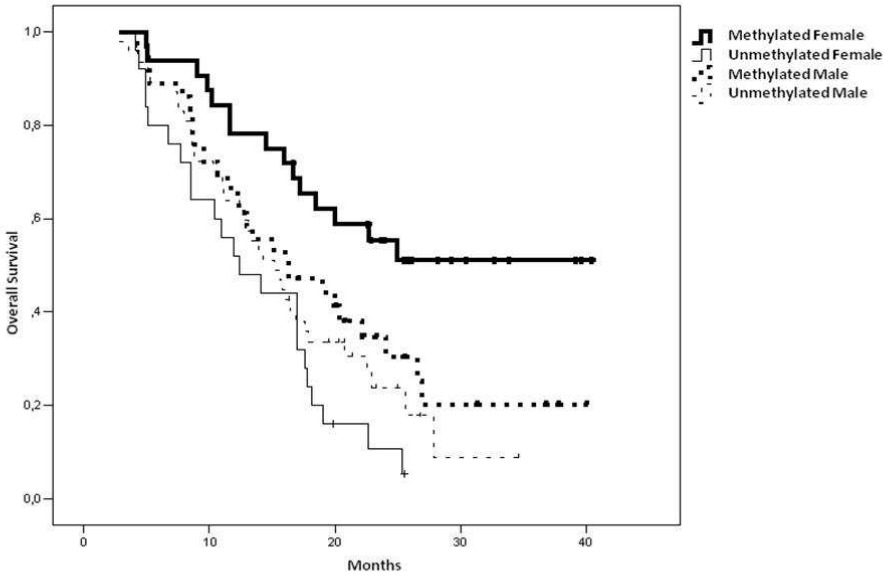
Figures

Figure 1

Fig. 1: Survival status when considering gender and MGMT methylation status



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Highlights

- MGMT methylation is a relevant prognostic factor for glioblastoma patients.
- From literature a prognostic advantage for female patients has been reported.
- In this analysis we report a survival benefit for methylated female GBM patients.

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Abbreviations

BSA: body surface area

EGFR: epidermal growth factor receptor

FZD7: Wnt receptor Frizzled-7

GBM: glioblastoma

G-CIMP: glioma-CpGisland methylator phenotype

HR: Hazard ratio

ICH/GCP: International Conference of Harmonisation/Good Clinica Practice

IDH: isocitrate dehydrogenase enzyme

KPS: Karnofsky performance status

MDS. Myelodysplastic syndromes

MGMT: O6-methylguanine-DNA methyltransferase

MMSE: mini-mental state evaluation

MSP: methylation-specific polymerase chain reaction

OS: overall survival

PERNO: Project of Emilia-Romagna on Neuro-Oncology

TMZ: Temozolomide

TP53: tumor protein 53

WHO: World Health Organization

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Disclosure form

All named authors have read the manuscript and have agreed to submit the paper in its present form.

The manuscript has not been and will not be submitted simultaneously to another journal, in whole or in part.

The paper reports previously unpublished work.

All those named as authors have made a sufficient contribution to the work.

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The authors declare they have observed appropriate ethical guidelines and legislation in conducting the study described in the paper.

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