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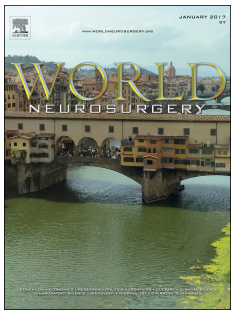

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A survival analysis with identification of prognostic factors, in a series of 110 patients with newly diagnosed glioblastoma, pre and post-introduction of the Stupp regimen: a single-center observational study.

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Abstract

Background: Current treatment protocol in GBM is based on maximal safe resection followed by Stupp protocol. Survival outcomes in cancer can vary in different population groups and outcomes can be conditioned by several factors. In Serbia Temozolomide has been introduced as adjuvant therapy only in 2011. The aims of this study are to confirm the efficacy and safety of Stupp protocol on both OS and PFS and evaluate the influence of the prognostic factors in one of the largest series of patients with GBM, treated over a 2-year period.

Methods: 110 patients complaining of newly diagnosed GBM, underwent surgical removal at the Neurooncology Department of Clinic Center of Serbia in Belgrade, between January 2010 and December 2012. Patients were divided in two groups according to the postoperative treatment. 24 patients, treated before January 2011, received adjuvant standard radiation therapy and BCNU (group A), while 86 patients, operated later that January 2011, received postoperative treatment according to Stupp protocol (group B).

Results: Stupp protocol had significant favorable impact on OS at 1-year follow-up (79.1% in group B versus 62.5% in group A, p= 0.016), while no differences were noted in regards to PFS. Multivariate analysis identified younger age and tumor gross total resection as positive prognostic factors.

Conclusions: The adoption of Stupp protocol has favorable impact, in our series, on OS but not on PFS rate. Furthermore, we noted that wider surgical resection, involving the peritumoral brain zone, as confirmed by the univariate and multivariate represents the most favorable prognostic factor.

Introduction

Glioblastoma Multiforme (GBM) is the most common and aggressive malignant type of primary brain tumor, with an annual incidence of 1 per 100.000 people. Despite recent advances in medical treatment, the prognosis remains extremely poor, with median overall survival (OS) of 14.6-16.7 months from diagnosis and a 2-year survival rate of 26.5%; above all, the vast majority of patients complain of recurrence/regrowth within 1 year from the initial treatment.

Nowadays, standard treatment for newly diagnosed GBM consists of maximum allowed surgical safe resection followed by concomitant chemo-radiotherapy as per Stupp protocol [1-3].

Stupp et *al.* measured a better OS in the group of patients that underwent combined therapy, regardless MGMT (O-6-Methylguanine-DNA Methyltransferase) status; on the contrary, patients presenting methylated variant exhibited an advantage in terms of progression free survival (PFS) as compared to those with the un-methylated gene [1]. In the attempt of defining the impact of demographic and surgical factors on clinical outcomes, it has been underlined that maximal safe resection and eventually performance status and age at surgery resulted positive predictive factors, independently from Stupp protocol [4-6].

Nevertheless, as per health care system bylaws, in Serbia, Temozolomide (TMZ) has been grant as adjuvant chemotherapy only since 2011. Hence, at the Neurosurgery of the Clinical Center of Serbia in Belgrade, before the introduction of the Stupp regimen, GBM patients with good performance status would have received postoperative treatment with radiotherapy followed by Carmustine (BCNU) or Lomustine (CCNU) (from 3 to 6 cycles, depending on hemotoxicity).

The aims of this study are to confirm the efficacy and safety of Stupp protocol on both OS and PFS and evaluate the influence of the prognostic factors in one of the largest series of patients with GBM, treated by mean of modern surgical techniques at a single institution over a short period of time, i.e. 2-year. To authors' knowledge, this is the first survival analysis of GBM patients treated in Serbia, where chronic exposure to environmental carcinogens and poor socio-economic conditions should be claimed as possible factors affecting the outcome of GBM disease.

Materials and Methods

A total of 110 consecutive patients with histologically confirmed GBM were retrospectively analyzed. The patient cohort consists of adult patients operated, between January 2010 and December 2012, by a surgical team of 4 neurosurgeons at the Department of Neuroncology of Clinical Center of Serbia, in Belgrade.

Patient demographics, clinical preoperative features, extent of surgery, postoperative treatment modalities, date of progression or reoperation, salvage chemotherapy, date of latest follow-up or death were retrieved from electronic database.

Because use of Temozolomide (TMZ) in Serbia started from January 2011, Stupp protocol was adopted as adjuvant scheme for GBM treatment only from that time on.

In the present study, patients were therefore divided into two groups based postoperative treatment protocol received. Group A accounting on 24 patients, were treated before January 2011 and received conformational radiotherapy at the dose of 60 Gy in a daily fractions of 2 Gy 5 days per week, followed by adjuvant 3 to 6-cycles chemotherapy with nitrosoureas (BCNU/CCNU), depending on hemotoxicity [7]; 2 patients of that group didn't complete the radiotherapy protocol due to the rapid progression of the disease.

In Group B we enrolled 86 patients who were administered with Stupp protocol, namely Temozolomide at a dose of 75mg/m^2 every day for 6 weeks and concomitant 60 Gy conformational radiotherapy. After 4 weeks they received 6-cycle adjuvant TMZ at a dose of 150-200mg/m^2, depending on toxicity [6, 8].

A pre and post contrast brain magnetic resonance (MRI), was performed one month after surgery, prior than adjuvant treatments, in order to assess the extent of tumor removal and design the adjuvant treatment protocol.

Entity of tumor removal was defined as in gross total resection (GTR) (removal of all enhancing tumor mass with margins extension when safe), subtotal resection (STR) (< 50% residual enhancing nodular mass) and partial resection (PR) (> 50% residual tumor mass).

MRI complemented by spectroscopy analysis (MRS) was performed per follow-up every 12 weeks after the completion of adjuvant treatment scheme. Effectiveness of postoperative treatment was evaluated according to the RANO criteria [9]; early redo surgery, i.e. within three months after having received chemo-irradiation, was not performed when the MRS showed radionecrosis. Whether early progression was

associated to neurological deterioration, corticosteroids and Temozolomide treatment were continued [10].

We collected several factors, gender, age, MGMT-methylation status, degree of tumor removal and preoperative Eastern Cooperative Oncology Group (ECOG) performance status [5] that were plotted in univariate analysis in between the two groups. Then, the factors with better correlation with OS and PFS were included in multivariate analysis to be claimed as possible prognostic factors.

Patients' population

110 patients (44 women and 66 men) with primary GBM were included in this retrospective single-center study. The median follow-up for the entire cohort was 23.7 months (range 2-72 months). Patient demographic data and clinical features for each group are summarized in Table 1.

Group A (pre-Stupp cohort) accounted for 24 patients, 13 male and 11 female patients, with a median age of 58 years (range 24 – 74 years); Group B (Stupp cohort) includes 55 male and 31 female cases, with a median age of 53 years (range 21 – 74 years).

The preoperative ECOG performance status was stratified in good for 77 (89.5%) patients with an ECOG 0-1 and in poor for 9 (10.5%) patients with an ECOG > 1.

Statistical analysis

Primary endpoint was OS definition while secondary endpoint was PFS definition. Kaplan-Meier Survival curves and Log-rank tests with two-sided were used to determine and compare OS and PFS between treatment groups.

Prognostic factors (age, gender, performance status, MGMT promoter methylation status and extent of resection) were tested to determine the influence on OS and PFS, using Log-rank test and Cox regression test. Differences were considered statistically significant at p-value 0.05. Statistical software used was IBM SPSS Statistics 21.0 (IBM Cooperation, New York, USA).

Results

Tumor features

In the entire cohort, 4 (3.6%) patients had an involvement of the basal ganglia, 2 (1.8%) of the corpus callosum and 2 others (1.8%) of the insula. 19 (17.3%) patients presented multicentric tumor (more than two lobes involved), while 22 (20%) were affected by GBM spreading in two lobes. The GBM involved temporal lobe and occipital lobe in 26 (23.6%) patients, the frontal lobe in 21 (19.1%) cases, while, the parietal lobe, in 12 (10.9%) cases. Finally, there were two rare localizations: one in posterior fossa and the other one at the level of the pineal gland.

Data of methylation status of MGMT promoter were available only in 62 (56.4%) patients, of which 57 (66.3%) belong to group B: 28 (45.2%) had methylated MGMT promoter and 34 (54.8%) presented un-methylated MGMT promoter.

Extent of tumor removal

At primary surgery overall GTR, STR and PR were achieved respectively in 77 (70%), 19 (17.3%) and 14 (12.7%) patients; in Group A GTR was observed in 17 (71%) patients, STR in 4 (17%) and partial in 3 (12%) patients, whereas in Group B gross total tumor resection was achieved in 60 (70%) cases, subtotal resection in 15 (17%) and partial in 11 (13%) patients.

Recurrence and Regrowth

In our series, 102 (93%) patients showed tumor recurrence and 34 (33%) underwent re-operation, 28 were for the Stupp group (32.5%) and 6 patients in pre-Stupp group (25%). In patients with progression or relapse, who didn't undergo second surgery, salvage chemotherapy and/or symptomatic therapy was considered. Of these cases, 39 patients of Group B received 6-cycles BCNU treatment, according to second-line management scheme, after Temozolomide-based chemotherapy [11], whether those in Group A received only symptomatic drugs, after adjuvant chemo-radiotherapy.

Follow-up and survival outcomes

At the end of the follow up 97 patients (88.2%) died: 22 patients (91.7%, 22/24) belonged to group A and 75 (87%, 75/86) to group B. The median OS in the entire cohort was 17 months (14.43-19.57 months), i.e. 19 and 13 months respectively in

group B and in group A. 1-year and 2-years OS rates were 79.1% and 34.9% in those patients who were administered of the Stupp protocol while it was found of 62.5% and 12.5% in those cases that received RT and adjuvant BCNU or CCNU. This difference indeed resulted statistically significant (Log-rank test, p= 0.016).

On the other side, the median PFS was 11 months in Group B and 9 months in Group A, resulting not significant (Log-rank, p= 0.143) (Figure 1 and Figure 2).

Different factors were found to positively affect the OS, when performing univariate analysis. Hence, younger patients (<50 years) survived longer with a median OS of 20 months, than older patients 13 months (> 60 years) and 16 months (50-60 years), being this a significant positive correlation (Log-rank, p= 0.002).

As well, those patients presenting with a preoperative good ECOG performance status (0-1) showed a median OS of 19 months, significantly better than those diagnosed with a poor ECOG (2-3-4), who had a median OS of 13 months (Log-rank, p= 0.001).

Similar results were noted in terms of PFS: indeed younger age and good performance status correlated with better PFS (younger age: Log-rank, p= 0.001; ECOG 0-1: Log-rank, p= 0.003).

Finally, we observed that GTR led a better OS, (median survival of 20 months) as compared to STR (median survival, 13 months) and PR (median survival, 11 months), again being this a significant positive correlation (Log-rank, p= 0.036). Similar results were noted in terms of PFS: indeed gross total removal correlated with better PFS (Log-rank, p= 0.022) (please see Table 2).

The multivariate analysis revealed that there was no statistically significant correlation between Stupp protocol and both OS (p=0.115 at Cox Hazard multivariate analysis) and PFS (p=0.574 at Cox Hazard multivariate analysis,). On the other side, revealed that GTR was an independent favorable prognostic factor for both OS (p=0.046) and PFS (p=0.036). Furthermore, similar result was retrieved for younger age in terms of OS (p=0.009) and PFS (p=0.021) (please see table 3).

Discussion

Temozolomide is a second-generation alkylating cell cycle non-specific agent and has been available in Serbia since 2011 for treatment of glioblastoma. Cytotoxicity of this agent is borne through DNA methylation, which result later in the failure of mismatch

repair mechanisms. There is a conspicuous number of factors that can influence the cellular response to TMZ, and among them, O6-Methyguanine-DNA Methyltransferase is one of the most important. As compared to traditional alkylating agents, such as nitrosoureas, the effects of TMZ response in malignant glioma have been remarkable: partial and complete response rates to TMZ have been reported approximately up to 30%, whereas barely reached 10% for traditional chemotherapeutic agents [12], confirmed by significantly higher OS and PFS in the group of patients that received TMZ [13].

The introduction in the current clinical practice of Stupp regimen, consisting in combination of radiotherapy with TMZ, further ameliorated median survival rates, by taking advantage of synergism demonstrated between TMZ and RT [6]. As demonstrated in the randomized EORTC-NCIC trial [1, 6] and further studies [14-16] alone, an OS was found superior in group of patients receiving Stupp protocol as compared to those treated with radiotherapy alone, being respectively 14.6 months and 12.1.

The results of the present study confirm these data, revealing that better results in terms of overall survival were achieved in patients treated with Stupp protocol, as compared to those, who have radiotherapy with BCNU/CCNU (19 vs 13 months) ($p= 0.016$) (see Figure 1).

Nevertheless, there are several factors, which could define OS and, therefore, should be taken into account for ruling out the prognosis of GBM. It has been demonstrated that glioblastoma has an infiltrative pattern of spread, which consists in necrotic core, a rim of proliferative cells and a margin of invasive cells. Analyzing several biopsy samples obtained in GBM margins, it was noted that non-tumor cells mixed with GBM infiltrating cells are a common finding within 2 cm from the edge of the surgical cavity. Indeed, local recurrence occurs almost in all GBM patients and, despite PFS detected by Stupp et *al*. was evidently influenced by Temozolomide concomitant to radiotherapy ($p< 0.0001$), the rate of relapse was still high, being 88.8% at 2-years in CCRT group and 98.2% at 2-years in RT alone group [1].

On the contrary, some authors [17] observed that the addition of TMZ does not change the pattern of progression of GBM after radiotherapy.

In our series, the rate of relapse seems to be the same in two groups (79.7% in Group A vs 88.5% months in Group B) ($p = 0.143$) and the statistical analysis underlines there was not statistically significant correlation between STUPP protocol and PFS.

Furthermore, this treatment protocol doesn't figure out as an independent prognostic factor, at multivariate analysis, on OS and PFS. Therefore, it is reasonable to consider the efficacy of TMZ related to the others factors, such as the entity of surgical resection, tumor genetic profiling and patients' inner features. Recently [18-20], the surgical radicality has been considered one of the most important prognostic factor. Indeed, GTR compared to either STR or PR, correlates with a decreased mortality and disease progression rates. Furthermore, it has been found a correlation between extent of resection and 6-months PFS. Indeed, the extensive tumor resection of the T1-contrast enhancing zone and a partial resection of the peritumoral region, positively correlates with better OS and PFS [21]. The current series supports that hypothesis. Indeed, the GTR, achieved in 69% of GBM patients, has significant correlation on both OS and PFS either at the univariate and multivariate analysis. These results, once again, highlight the importance of GTR as an independent favorable prognostic factor: indeed, as already reported in other series in the pertinent literature [18, 22], we found that the surgical outcome mostly defines the patient survival, independently from tumor genetic profiling, patient clinical status and features and adjuvant treatments adopted.

However, it is important keeping in mind the related risk of “more extensive surgery”, and we would like to underline that our surgical outcomes were the result of the long experience of a single neurosurgical team and the availability of modern surgical instrumentations, such as CUSA and intraoperative neuromonitoring.

On the other side, to rule out differences in terms of OS and PFS, it is worth considering that the effect of Temozolomide could be conditioned by molecular heterogeneity and presence of stem cell-like cells [23-25], frequently observed in GBM tumors. These cells are responsible of tumor relapse by changing treatment susceptibility. Moreover, a chronic exposure to environmental carcinogens could be claimed as another possible factor affecting the outcome and prognosis of GBM.

As widely accepted in the literature [1, 5, 26-28], younger age and good preoperative performance status present favorable impact. In the current study, we also found that younger age (< 50y) and preoperative Performance Status (ECOG= 0-1) exhibited a correlation with longer survival, as showed by the univariate analysis. On the other side, the multivariate analysis revealed that only younger age is an independent prognostic factor for OS and PFS (see table 3). In our study, the MGMT promoter

methylation has not an impact on patient survival, nevertheless, the rate of MGMT methylation status was available in only 62 patients (56.4%) among the whole cohort.

These data are not congruent with pertinent literature where MGMT methylation has been proposed as a positive predictive factor. Stupp et *al*. and other studies [1, 6, 29-31], identified the methylation of MGMT as a possible genetic factor influencing the overall survival and, eventually, it was defined as one of the strongest predictor for ruling out the outcome of concomitant RT/TMZ therapy regimen. However, its role is less clear when evaluating the effectiveness of other treatment modalities, including RT plus nitrosoureas adjuvant chemotherapy or RT alone or TMZ alone [32, 33].

Furthermore, recent studies [34-36] remarked the high level of false-positive and false-negative methylated gene, because of mosaic methylation patterns with variable grade of methylation. Besides, tumor heterogeneity may be the reason for variation in treatment response, representing further contribution to the definition of resistance mechanisms [23, 34-37].

Study Limitations

We would like to underline that results retrieved in the present study are influenced by several limitations, mostly being related to socio-economic conditions and standard of Health care system in Serbia.

For instance, it is well renown that the IDH mutation could affect GBM patients' survival rates and that, according to such item, these lesions could be further classified. In this scenario, we had the chance to enroll a very limited sample of patients in which MGMT methylation essay had been performed. In this small subgroup of patients such laboratory investigation has been run in a facility in Switzerland by mean of methylation specific PCR analysis.

Finally, it stands clear that the two groups could figure out as not homogeneous for comparison, as the group B is quite larger than group A; since January 2011, per Serbian Health care system disposition, all patients complaining of GBM have been admitted to Stupp protocol, so inevitably the gap in terms of number of patients in the two groups have been enlarging. A statistically significant level between the two groups did not emerge, but our analysis aimed to present the different impact of several predictive factors in terms of Overall Survival (OS) and Progression Free Survival (PFS) in a large series of patients, treated at a single institution, receiving two different management schemes, over a short period of time.

Nonetheless, considering such heterogeneity we are conscious that the overall survival analysis could have result biased.

Conclusions

Significant survival benefits have been achieved with the introduction of Stupp protocol in the management of GBM patients at the Clinic Center for neurosurgery of Belgrade. However, the adoption of Stupp protocol has favorable impact, in our series, on OS but not on PFS rate. We noted that a safe and wide surgical resection, involving the PBZ, permits a longer PFS in both groups analyzed, as also confirmed by the univariate and multivariate analysis. Indeed, we would like to underline, once again, that GTR represents the most favorable prognostic factor for both OS and PFS. However, future refinement of surgical techniques, identification of genetic and epigenetic phenomena in order to better understand GBM pathogenic mechanisms could eventually further improve prognosis of this disease.

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Table 1: Demographic data and peculiar features of GBM patients divided in the two groups according to postoperative treatment protocol.

	Group A RT+BCNU/CCNU (n=24)	**Group B** Stupp protocol (n=86)
Gender		
Man	13 (54%)	55 (64%)
Woman	11 (46%)	31 (36%)
Age (years)		
<50	5 (21%)	27 (31%)
51-60	9 (37%)	32 (38%)
>61	10 (42%)	27 (31%)
Performance Status		
ECOG 0-1	10 (42%)	77 (90%)
ECOG 2-3-4	14 (58%)	9 (10%)
Location		
Temporal	19 (22,1%)	4 (16,7%)
Two lobes	21 (24,4%)	1 (4,2%)
Frontal	13 (15,1%)	8 (33,4%)
Multicentric	14 (16,3%)	5 (20,8%)
Parietal	9 (10,5%)	3 (12,5%)
Rare	10 (11,6%)	3 (12,5%)
Rate of resection		
GTR	17 (71%)	60 (70%)
STR	4 (17%)	15 (17%)
PR	3 (12%)	11 (13%)
MGMT promoter status		
Methylated	2 (8%)	26 (30%)
Unmethylated	3 (12%)	31 (36%)
Unknown	19 (80%)	29 (34%)
Progression		
Yes	24 (100%)	79 (92%)
No	0 (0%)	7 (8%)
Alive/dead		
Alive	2 (8%)	11 (13%)
Dead	22 (92%)	75 (87%)

***ECOG**: Eastern Cooperative Oncology Group Scale, **MGMT**: O6-alkylguanine DNA alkyltransferase, **GTR**: Gross Total Resection, **PR**: Partial Resection, **STR**: Subtotal Resection.*

Table 2: Table showing the influence of different factors on the Overall Survival and Progression-Free Survival rates as per univariate survival analysis on the entire GBM patients cohort. (P-value < 0.05 at Log-rank test).

	N° patients	Median (months)	95% CI (median)	p-value
OS				
Gender				
Male	66	16	14.02-17.98	0.299
Female	44	21	18.22-23.78	
Age (years)				
< 50	32	20	3.37-36.63	**0.002**
> 50	78	16	13.12-18.89	
Performance Status				
ECOG 0-1	87	19	15.96-22.05	**0.001**
ECOG 2-3-4	23	13	9.51-16.46	
Extent of surgery				
GTR	77	20	16.56-23.44	
STR	19	13	10.89-15.11	**0.036**
PR	14	11	4.89-17.11	
MGMT promoter status				
Methylated	29	21	14.67-27.33	0.429
Unmethylated	33	15	12.77-17.23	
Postoperative protocol				
Stupp protocol	86	19	15.97-22.03	**0.016**
RT+BCNU/CCNU	24	13	10.95-15.05	
PFS				
Gender				
Male	66	9	8.36-9.64	0.318
Female	44	12	8.88-15.11	
Age (years)				
< 50	32	14	8.80-19.20	**0.002**
> 50	78	9	7.68-10.32	
Performance Status				
ECOG 0-1	87	10	8.12-11.88	**0.003**
ECOG 2-3-4	23	7	3.48-10.52	
Extent of surgery				
GTR	77	11	9.11-12.90	**0.022**
STR	19	9	6.19-11.81	
PR	14	6	2.33-9.67	
MGMT promoter status				
Methylated	29	14	6.32-21.68	0.532
Unmethylated	33	9	7.84-10.06	
Postoperative protocol				
Stupp protocol	86	11	9.23-12.78	0.143
RT+BCNU/CCNU	24	9	5.45-12.55	

***CI**: confidence interval, **BCNU**: Carmustine, **CCNU**: Lomustine, **ECOG**: Eastern Cooperative Oncology Group Scale, **MGMT**: O6-alkylguanine DNA alkyltransferase, **OS**: Overall Survival,*

***GTR**: Gross Total Resection, **PFS**: Progression-Free Survival, PR: Partial Resection, **STR**: Subtotal Resection, **RT**: Radiotherapy.*

Table 3: Table showing results of Cox Hazard multivariate analysis on both OS and PFS. Only independent prognostic factors for which statistical significance univariate analysis was found, were used for the multivariate test (P-value < 0.05).

OS				
	p-value	HR	95% CI	
Preoperative ECOG performance status	0.165	0.68	0.39	1.18
Entity of resection	**0.046**	0.64	0.41	0.99
Age at surgery	**0.009**	0.51	0.31	0.84
Stupp Protocol	0.115	0.64	0.37	1.14
PFS				
	p-value	HR	95% CI	
Preoperative ECOG performance status	0.117	0.64	0.36	1.12
Entity of resection	**0.032**	0.61	0.39	0.96
Age at surgery	**0.021**	0.56	0.34	0.92
Stupp Protocol	0.574	0.85	0.49	1.49

***CI**: confidence interval, **ECOG**: Eastern Cooperative Oncology Group Scale, **HR**: hazard ratio, **OS**: Overall Survival, **PFS**: Progression-Free Survival.*

Figure 1: Influence of postoperative protocol on Overall Survival

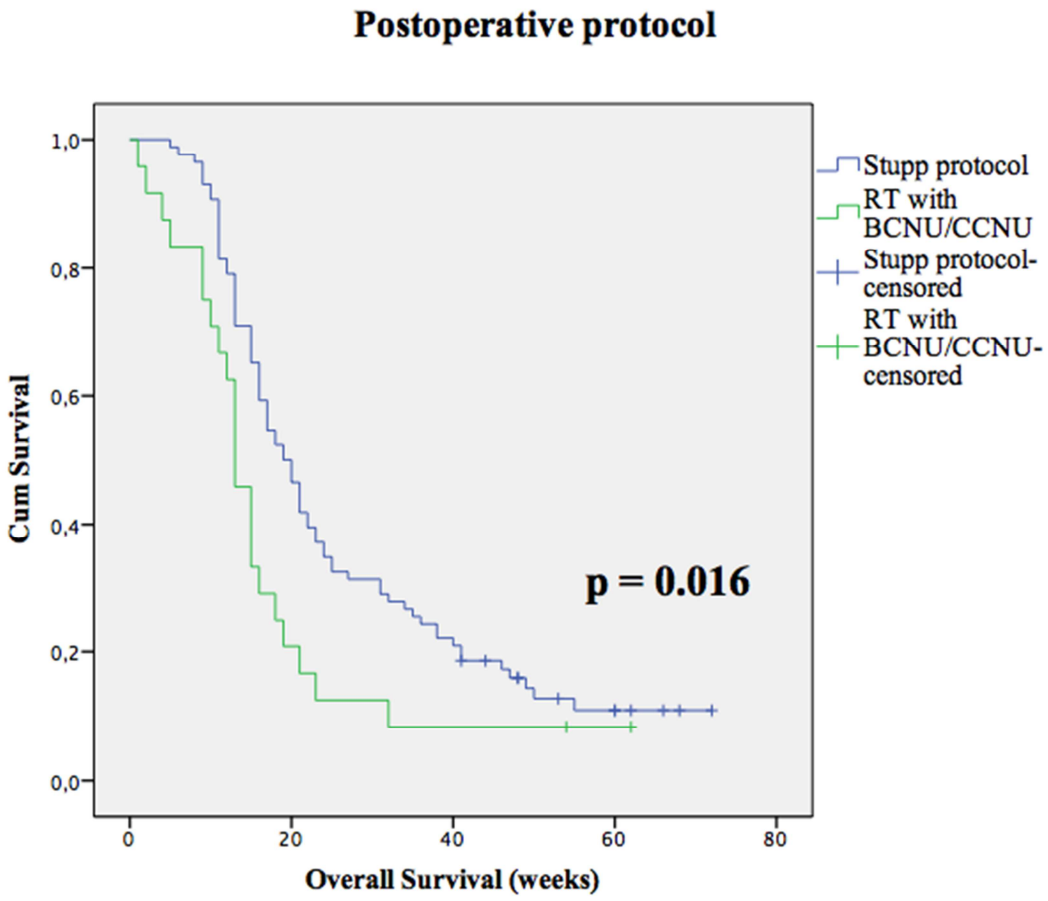

Figure 1: Kaplan-Meier survival analysis of the influence of Stupp protocol and RT with BCNU/CCNU protocol on Overall Survival in the entire cohort. P-value was calculated using Log-rank test. ***BCNU***: Carmustine, ***CCNU***: Lomustine, ***RT***: radiotherapy.

Figure 2: Influence of postoperative protocol on Progression-Free Survival

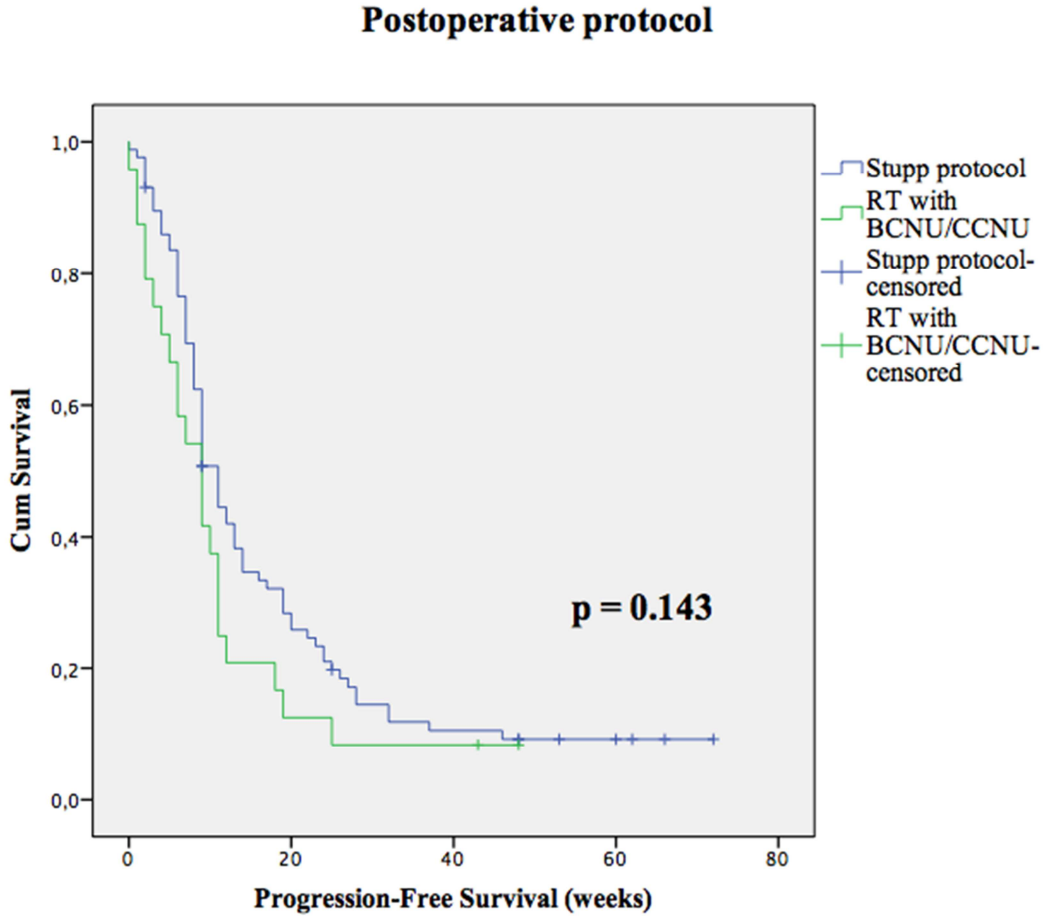

Figure 2: Kaplan-Meier survival analysis of influence of Stupp protocol and RT with BCNU/CCNU protocol on Progression-Free Survival in the entire cohort. P-value was calculated using Log-rank test. ***BCNU***: Carmustine, ***CCNU***: Lomustine, ***RT***: radiotherapy.

Highlights

- GBM is the most aggressive malignant type of primary brain tumor
- TMZ improves OS but not PFS rate
- The younger age and the good preoperative PS are independent prognostic factors
- GTR represents an independent prognostic factor only for PFS

ABBREVIATIONS:

GBM: Glioblastoma Multiforme; TMZ: Temozolomide; RT: Radiotherapy; OS: Overall survival; PFS: Progression Free Survival; BCNU: Carmustine; CCNU: Lomustine; MGMT: O-6-Methylguanine-DNA Methyltransferase; MRI: Magnetic Resonance Imaging; CT: Computed Tomography; MRS: Magnetic Resonance Spectroscopy; GTR: Gross Total Resection; STR: Subtotal Resection; PR: Partial Resection; ECOG: Eastern Cooperative Oncology Group; PS: Performance Status; PBZ: Peritumoral Brain Zone.