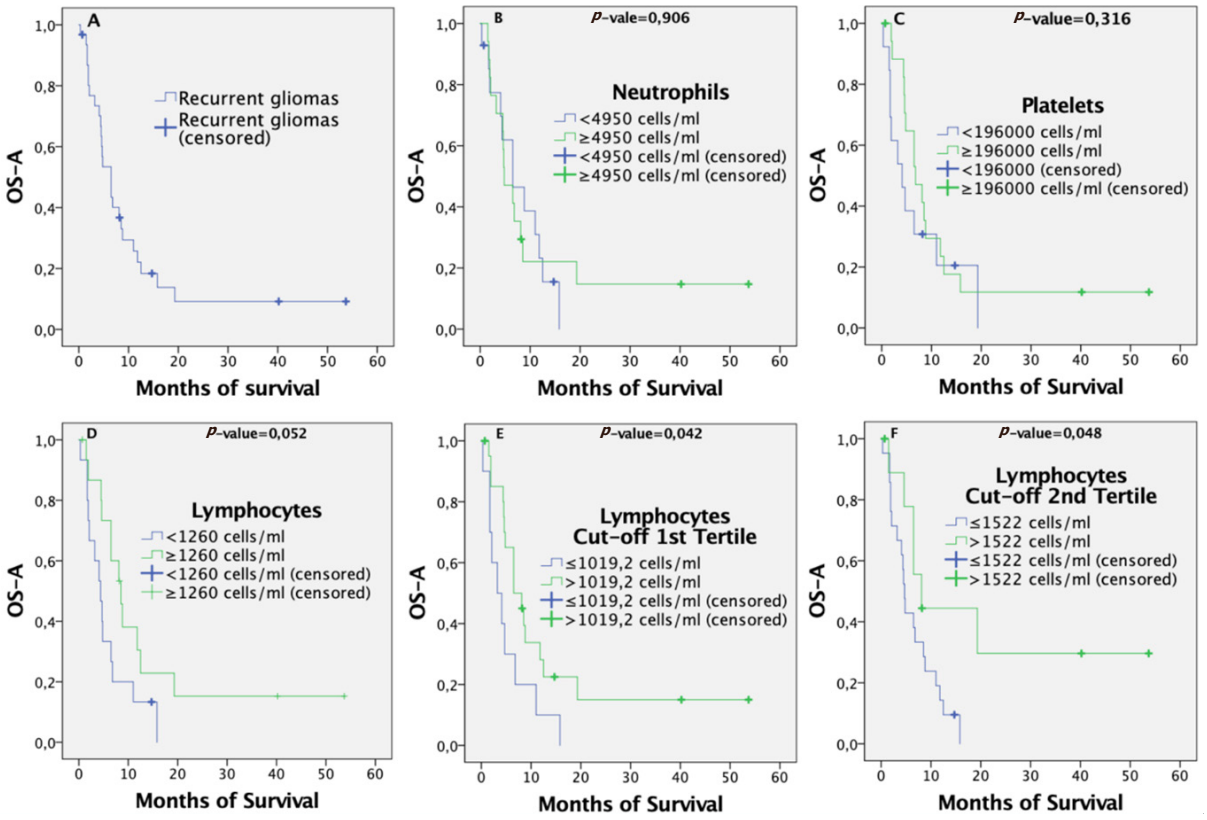

Figure 1. Overall survival from BVZ administration calculated by Kaplan-Meier curves for the total of 31 patients: (A) all patients in the same group; (B) stratified by levels of neutrophils; (C) stratified by levels of platelets; (D-F) stratified by levels of lymphocytes using the median = 1260 cells/mL, the first tertile = 1019 cells/mL, and the second tertile = 1522 cells/mL as the cut-offs, respectively. BVZ: bevacizumab

Table 2. Levels of neutrophils, lymphocytes, platelets (mg/dL), NLR, and PLR blood counted before first cycle of bevacizumab

	Neutrophils (mg/dL)	Lymphocytes (mg/dL)	Platelets (mg/dL)	NLR	PLR
Mean	5.67	1.26	222.03	5.71	207.19
Median	4.95	1.26	196.00	3.97	186.42
SD	2.92	0.50	85.35	6.00	132.98
95%CI	(4.6-6.75)	(1.08-1.45)	(190.73-253.34)	(3.51-7.91)	(158.40-255.97)

SD: standard deviation; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio

Correlation between inflammation variables

Lymphocytes were correlated with platelets ($P = 0.042$), NLR ($P = 0.025$), and PLR ($P < 0.001$) only weakly (correlation coefficient 0.368, -0.401, and -0.589, respectively). As expected, neutrophils were correlated with NLR ($P > 0.001$ and correlation coefficient of 0.676) and platelets with PLR ($P < 0.020$ and correlation coefficient of 0.415). Figure 2C shows that the more NLR increases, the more PLR increases; this association was not statistically significant according to the Spearman test (P-value = 0.053; 95%CI).

Combined information PLR-NLR had predictive value

As association between PLR and NLR was almost significant, we suggest a new patient classification based on both values simultaneously. According to these two variables, patients belong to one of three groups: double positive (both values above their median), double negative (both values below their median), or single positive (the other cases). As the survival of patients with one ratio over the median and the other below it was similar, we classified them into the same group. Median survival was highest for the double-

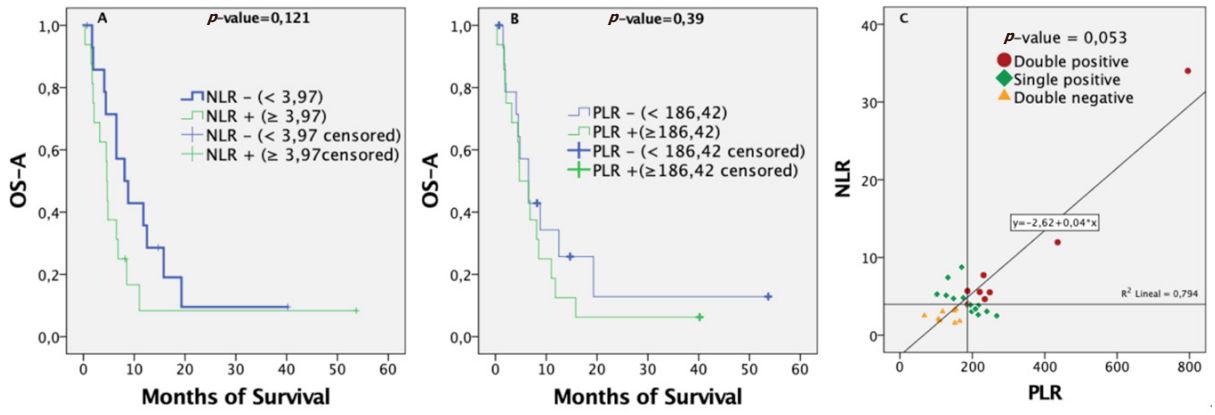

Figure 2. Overall survival from BVZ administration calculated by Kaplan-Meier curves for 31 patients stratified by: (A) NLR positive or negative; (B) PLR positive or negative; (C) Association between NLR and PLR. It seems the more NLR increases, the more PLR increases as well. This association is almost statistically significant following a Spearman test ($P > 0.05$; 95%CI). BVZ: bevacizumab; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio

negative group and lowest for the double-positive group. The survival distribution and its median are displayed in Figure 3A. However, statistical difference between the three groups in terms of mean survival was not found using the Kruskal-Wallis test (*P*-value = 0.485; 95%CI). Figure 3B displays the survival curves after BVZ treatment for the three groups with *P*-value = 0.125. However, comparing the double-negative and single-positive groups, a significant survival difference was observed. The double-positive group had significantly worse prognosis (*P*-value = 0.043, 95%CI) than the non-double-positive group, as shown in Figure 3C.

Similar toxicity for patients classified by combining PLR-NLR information

The median age was 40.5, 52, and 48 years for the double-negative, single-positive, and double-positive groups, respectively. Although other therapies had been provided previously, Table 3 shows that toxicity was similar in the three groups. RT or TMZ did not influence NLR and PLR values. We focused on neutropenia and thrombocytopenia before BVZ administration and the differences were not significant: 25% of the double-negative group, 11.1% of the double-positive group, and 7% of the single-positive group had neutropenia *P*-value of 0.849 stratifying into double positive *vs*. non-double positive). Moreover, 62% of the double-negative group had thrombocytopenia, whereas 22% and 28.6% appeared in the double-positive and single-positive groups, respectively (*P*-value of 0.324 stratifying into two groups). Neither NLR nor PLR was related to hematologic BVZ toxicity: 22% and 21% of double-positive group and single-positive group had neutropenia and not a single case of double-negative had it (*P*-value of 0.555 stratifying into two groups). Fifty percent of double-negative group, 44% of double-positive group, and 28.6% of single-positive group developed thrombocytopenia (*P*-value 0.675 stratifying into two groups).

Lymphocyte level was the best independent predictor

Lymphocyte level before BVZ administration was the best independent predictor of overall survival, as displayed in Table 4 (HR = 0.34; 95%CI: 0.145-0.81; *P* = 0.015). The area under the ROC curve was 0.823 and the optimal cut-off value was 1645 cell/mL (0.80 sensitivity and 0.85 specificity). Interestingly, the number of BVZ cycles was not related to lymphopenia, and differences in the distribution of age, sex, KPS, extent of resection, alcohol/tobacco use, and histological diagnoses were not significant.

Lymphocyte level and outcome depending on tumor localization

Figure 4 shows seven different localizations assessed using MRI: frontal (four patients), temporal (four patients), parietal (three patients), cerebellar (one patient), occipital (four patients), overlapping (thirteen

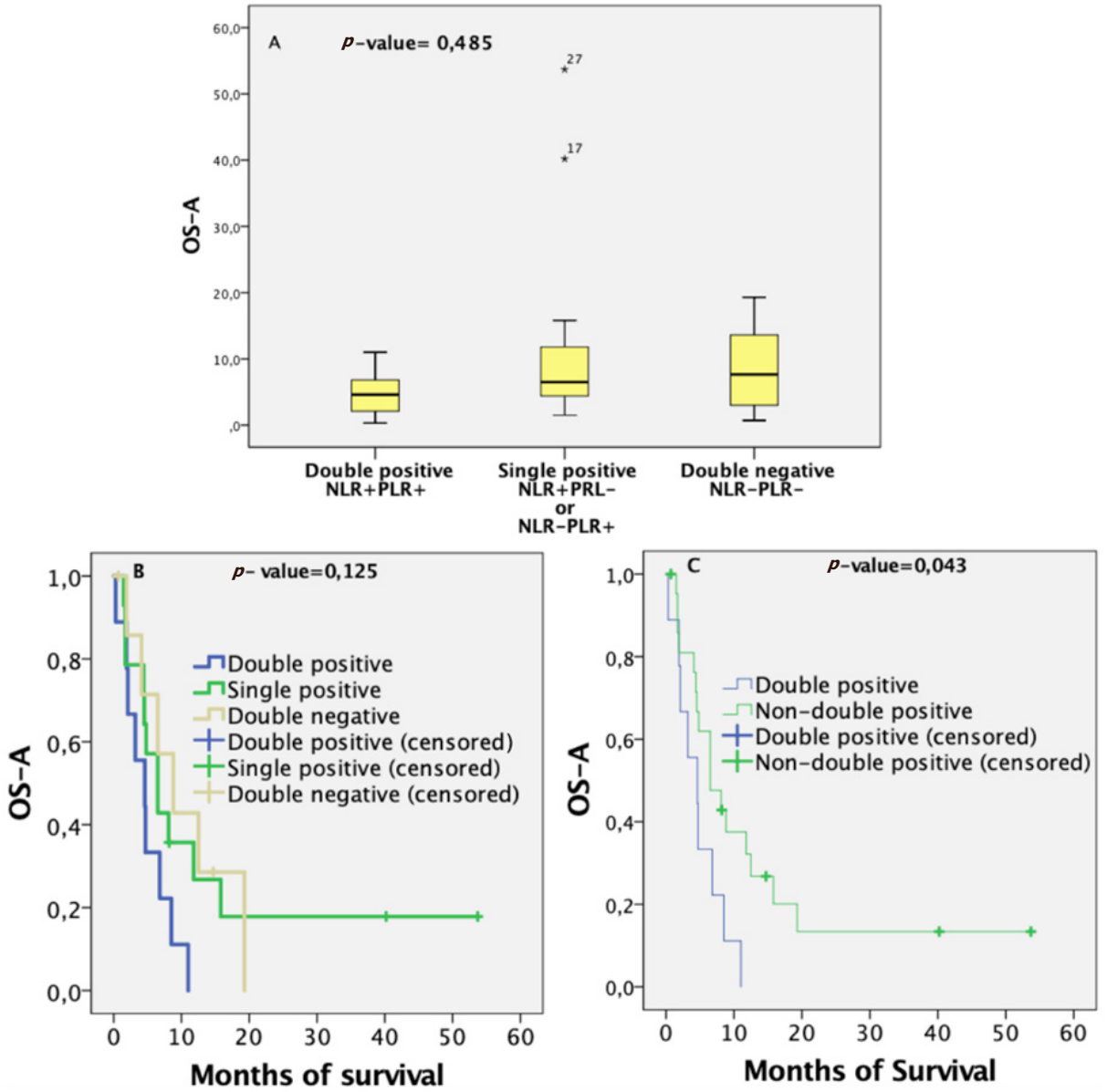

Figure 3. Predictive survival ability combining PLR-NLR information: (A) diagram boxes comparing median survival after BVZ administration among the three groups; (B) overall survival from BVZ administration calculated by Kaplan-Meier curves for 31 patients stratified into three groups, namely double positive, double negative, and single positive; (C) overall survival from BVZ administration calculated by Kaplan-Meier curves for 31 patients stratified into a double-positive group and otherwise. BVZ: bevacizumab; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio

patients), and diencephalon (two patients). Figure 4A exhibits the distribution of pretreatment lymphocyte levels depending on tumor localization. Patients with frontal or temporal tumors tended to show lower lymphocyte values. Figure 4B shows the OS after BVZ treatment for the same groups of patients. The three patients with the best outcome were found in the overlapping group. The parietal group had a high lymphocyte level but also a high OS with a small standard deviation in both cases. No statistical differences were found between patients with tumors in the left and right hemispheres, in terms of either lymphocyte levels or survival.

Limitations

The main limitations of our study are its retrospective design and the small and diverse sample.

Table 3. Clinical characteristics of patients according to PLR-NLR groups

	Double Positive (*n* = 9)	Single Positive (*n* = 14)	Double Negative (*n* = 8)	*P*-value (3 groups)	*P*-value (2 groups)
Pre-BVZ toxicity Anemia	(Yes/No) 3/6	(Yes/No) 5/9	(Yes/No) 3/5	0.984	0.873
Neutropenia	1/8 (11.1%/88.9%)	1/13 (7%/93%)	2/6 (25%/75%)	0.477	0.849
Lymphopenia	5/4	11/3	7/1	0.285	0.129
Leukopenia	1/8	2/12	2/6	0.716	0.627
Thrombocytopenia	2/7 (22%/78%)	4/10 (28.6%/71.4%)	5/3 (62.5%/37.5)	0.171	0.324
Post-BVZ toxicity Anemia	(Yes/No) 4/5	(Yes/No) 7/7	(Yes/No) 1/7	0.203	0.675
Neutropenia	2/7 (22%/78%)	3/11 (21%/78.5%)	0/8 (0%/100%)	0.354	0.555
Lymphopenia	7/2	10/4	5/3	0.786	0.593
Leukopenia	1/8	1/13	0/8	0.642	0.499
Thrombocytopenia	4/5 (44%/56%)	4/10 (28.6%/71.4%)	4/4 (50%/50%)	0.560	0.675

NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; BVZ: bevacizumab; 3 groups: double positive, single positive, or double negative; 2 groups: double positive *vs.* non-double positive

Table 4. Uni- and multivariable analysis (Cox regression) for the most representative variables associated with OS after BVZ treatment

Variables	OS-A (univariable)		OS-A (multivariable)	
	HR, (95%CI)	*P*-value	HR, (95%CI)	*P*-value
Age as continuous variable	1.02 (0.96-1.05)	0.306		
Gender	1.25 (0.52-2.98)	0.619		
Neutrophil Level (cells/mL)	1.03 (0.89-1.19)	0.704		
Median: < 4950 *vs*. ≥ 4950	0.95 (0.43-2.11)	0.906		
Platelet Level (cells/mL)	1.00 (0.99-1.00)	0.145		
Median: < 196,000 *vs*. ≥ 196,000	0.33 (0.68-3.23)	0.316		
Lymphocyte Level (cells/mL)	0.34 (0.145-0.81)	0.015*		
Median: < 1260 *vs*. ≥ 1260	2.15 (0.97-4.80)	0.052		
First tertile: < 1019 *vs*. ≥ 1019	2.25 (1.00-5.04)	0.042*		
Second tertile: < 1522 *vs*. ≥ 1522	2.62 (0.96-7.10)	0.048*		
NLR	1.04 (0.99-1.10)	0.105		
Median: (< 3.97 *vs*. ≥ 3.97)	0.54 (0.24-1.20)	0.121		
PLR	1.00 (1.00-1.00)	0.249		
Median: (< 186.42 *vs*. ≥ 186.42)	0.71 (0.32-1.56)	0.390		
PLR-NLR (Double positive *vs*. non-double positive)	2.35 (0.99-5.56)	0.043*		
Neutropenia	0.93 (0.32-2.72)	0.890		
Lymphopenia	2.23 (0.82-6.10)	0.118		
Leukopenia	0.77 (20.29-2.06)	0.604		
Thrombocytopenia	1.20 (0.52-2.73)	0.682		
Anemia	2.22 (0.92-5.33)	0.076		
Lymphocyte Level (cells/mL)			0.42 (0.16-1.10)	0.078*
PLR-NLR (Double positive *vs*. non-double positive)			1.50 (0.55-4.04)	0.436

**P*-values significant. OS: overall survival; BVZ: bevacizumab; OS-A: overall survival from bevacizumab administration; HR: hazard ratio; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio

DISCUSSION

Low-grade gliomas may undergo aggressive transformation, becoming high-grade gliomas, and they also tend to recur after a few months[37]. It is still unclear what the best therapeutic option for those recurrent glioma patients who have already received the standard radio- and chemotherapy actually is. Scientists are trying to find: (1) new treatments to prolong OS and quality of life; and (2) biomarkers that predict the response to these treatments. The FDA approved BVZ as a single agent for treatment of recurrent GBM with poor or refractory response to other therapies[17]. The European Medicines Agency rejected this indication, although some studies have questioned the decision[18]. No new agents have been approved

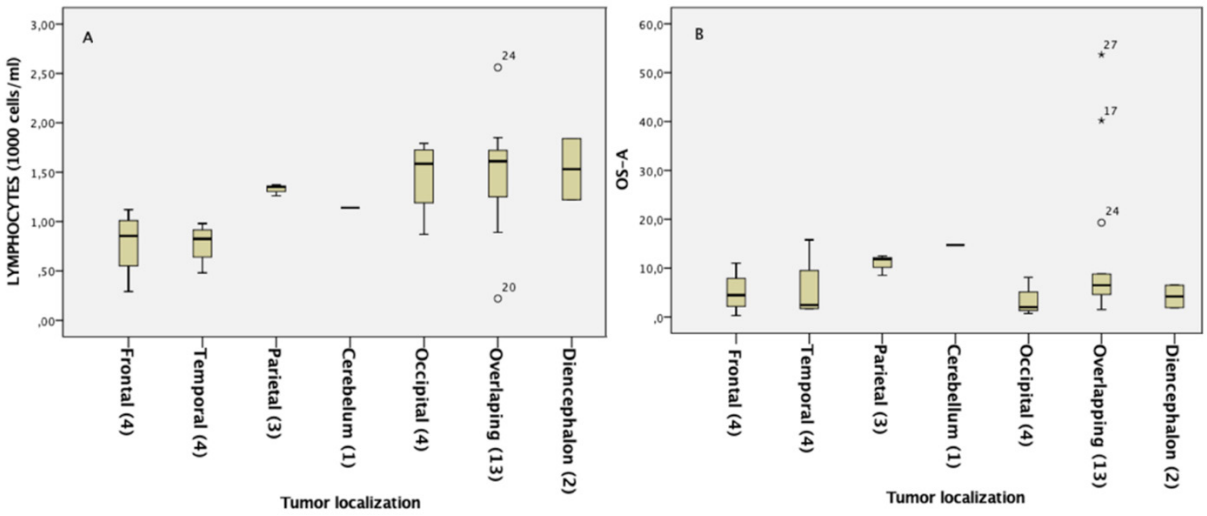

Figure 4. Lymphocyte levels and outcomes with patients classified by tumor localization: (A) lymphocyte level diagram box depending on tumor localization; (B) diagram box of overall survival from BVZ administration depending on tumor localization. BVZ: bevacizumab

for second-line therapy of GBM in Europe since the approval of TMZ in 1999. It has been reported that BVZ enhances the effect of RT and TMZ, allowing chemotherapy and oxygen to better perfuse within the tumor[14]. BVZ can attenuate tumor-associated brain edema and thus improve patient symptoms due to a reduction in steroid use[10]. Nevertheless, it seems that only some patients benefit; thus, it would be useful to find biomarkers that determine those who might respond beforehand[18].

Inflammation and cancer are linked: high levels of neutrophils and platelets are related to poor prognosis for several cancers while high lymphocytic infiltration is associated with improved survival and superior response to systemic therapy[25,28]. However, there are few studies focused on prognostic inflammatory biomarkers for recurrent gliomas[30-35] and even fewer focused on BVZ response after recurrence.

To our knowledge, no one has analyzed the pretreatment levels of inflammatory indices with survival in recurrent gliomas treated with BVZ. We analyzed the correlation between survival and inflammatory components measured before BVZ administration in 31 recurrent gliomas: neutrophils, lymphocytes, platelet counts, the ratios of NLR and PLR, and the combination PLR-NLR.

Our results suggest that pre-BVZ levels of neutrophils and platelets do not seem to act as biomarkers. However, the group of patients with high levels of lymphocytes lived longer than the others. This result is robust since several cut-off values showed significant differences between groups. We recommend using a threshold for lymphocytes between 1019 cells/mL (first tertile) and 1522 cells/mL (third tertile). Then, we do not know if lymphocyte infiltration before BVZ treatment could improve overall survival for recurrent glioma patients.

Since lymphocytes were the most powerful biomarker, we analyzed if it was related to the location of the tumor. Those patients with frontal or temporal tumors tended to have lower lymphocyte values. The three patients with the best outcome were found in the overlapping group. The parietal group had a high lymphocyte level but also a high OS with small standard deviation in both cases. Unfortunately, the low number of individuals per group did not allow us to determine differences between groups. However, these preliminary results could inspire other groups and motivate a deeper study.

The relation between survival and pretreatment NLR or PLR was not statistically significant and survival curves where similar (P-value = 0.121 and P-value = 0.39, respectively). The Spearman correlation p-value

between NLR and PLR was 0.053. We would expect to have a more significant correlation value with a larger number of patients. It is noteworthy that the survival curve for NLR-PLR positive patients was below the survival curve of NLR-PLR negative patients. This result fits very well with previous results that a NLR > 4 prior to second surgery was a poor prognostic factor in GBM, and later progression was associated with longer survival in patients but not in longer survival after second surgery[32].

The combined inflammatory information PLR-NLR may affect the evolution of high-grade gliomas at recurrence treated with bevacizumab. In fact, the double-positive category is associated with the worst prognosis (*P*-value = 0.043). This merger had not been carried out in the studies that were previously reviewed. We hypothesize that patients with double-positive PLR-NLR have more angiogenesis and the BVZ effect is smaller, having worse prognosis. Nevertheless, a deeper study would be necessary.

Regarding toxicity, we found fewer patients suffering lymphopenia and leukopenia with treatment at recurrence than at diagnosis. However, more patients had anemia, neutropenia, or thrombocytopenia at recurrence. Some of them also received other therapies after progression such as second surgery or TMZ. Although 55.17% of patients received Stupp treatment after progression (4.5 average cycles), later neutropenia was not affected. A complication when studying recurrence is the possible modification of our results due to previous administration of therapies or development of infections. In principle, the toxicity the patients experience in prior therapy could influence posterior NLR and PLR levels. It is believed that patients with more toxicity respond better to therapy. In our study, toxicity was similar in all groups: neither NLR nor PLR was related to hematological BVZ toxicity.

This study classified recurrent glioma patients who receive more benefit from the treatment with BVZ considering new immune markers: both lymphocyte levels and the combination of PLR-NLR could be employed as non-invasive hematological prognostic markers for recurrent high-grade gliomas treated with BVZ. However, lymphocyte level before BVZ administration was the best independent predictor of overall survival from bevacizumab administration (OS-A). A close relationship appeared between inflammation and angiogenesis, although this is not very well understood yet.

DECLARATIONS

Acknowledgments

We are most grateful to the Hospital Universitario de Gran Canaria Doctor Negrin Facility of Las Palmas de Gran Canaria and especially to the Radio Oncology Service for their technical support.

Authors' contributions

Wrote the paper, edited figures, developed statistical analysis and discussed results: Martínez-González A
Collected data and designed the protocol: Cabrera R
Designed the study and protocol: Lloret M
Designed the study and protocol, discussed results and revised the manuscript: Lara PC
All authors approved the final version of the manuscript.

Availability of data and materials

Not applicable.

Financial support and sponsorship

This work has been supported by Ministerio de Economía y Competitividad/FEDER, Spain [grant number MTM2015-71200-R] and James S. Mc. Donnell Foundation 21st Century Science Initiative in Mathematical and Complex Systems Approaches for Brain Cancer [Special Initiative Collaborative, Planning Grant 220020420 and Collaborative award 220020450].

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

This study was carried out in accordance to the protocol "PATRORA" approved by the Agencia Española de Medicamentos y Productos Sanitarios (ref: PLJ-BEV-2016-01) and by the Hospital Universitario de Gran Canaria Doctor Negrin committee (ref: 170007).

Consent for publication

Not applicable.

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