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Evaluating hematologic parameters in newly diagnosed and recurrent glioblastoma: Prognostic utility and clinical trial implications of myelosuppression

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Abstract

Background. Glioblastoma (GBM) patients are treated with radiation therapy, chemotherapy, and corticosteroids, which can cause myelosuppression. To understand the relative prognostic utility of blood-based biomarkers in GBM and its implications for clinical trial design, we examined the incidence, predictors, and prognostic value of lymphopenia, neutrophil-to-lymphocyte ratio (NLR), and platelet count during chemoradiation (CRT) and recurrence.

Methods. This cohort study included 764 newly diagnosed glioblastoma patients treated from 2005 to 2019 with blood counts prior to surgery, within 6 weeks of CRT, and at first recurrence available for automatic extraction from the medical record. Logistic regression was used to evaluate exposures and Kaplan–Meier was used to evaluate outcomes.

Results. Among the cohort, median age was 60.3 years; 87% had Karnofsky performance status ≥ 70, 37.5% had gross total resection, and 90% received temozolomide (TMZ). During CRT, 37.8% (248/656) of patients developed grade 3 or higher lymphopenia. On multivariable analysis (MVA), high NLR during CRT remained an independent predictor for inferior survival (Adjusted Hazard Ratio [AHR] = 1.57, 95% CI = 1.14–2.15) and shorter progression-free survival (AHR = 1.42, 95% CI = 1.05–1.90). Steroid use was associated with lymphopenia (OR = 2.66,1.20–6.00) and high NLR (OR = 3.54,2.08–6.11). Female sex was associated with lymphopenia (OR = 2.33,1.03–5.33). At first recurrence, 28% of patients exhibited grade 3 or higher lymphopenia. High NLR at recurrence was associated with worse subsequent survival on MVA (AHR = 1.69, 95% CI = 1.25–2.27).

Conclusions. High NLR is associated with worse outcomes in newly diagnosed and recurrent glioblastoma. Appropriate eligibility criteria and accounting and reporting of blood-based biomarkers are important in the design and interpretation of newly diagnosed and recurrent glioblastoma trials.

Key Points

- Patients with high neutrophil-to-lymphocyte ratio during chemoradiation and at first recurrence have significantly worse clinical outcomes. Many patients remain lymphopenic at time of first recurrence, and this is an important consideration in trial design.

Importance of the Study

Myelosuppression is a common complication among patients with glioblastoma (GBM) who receive standard therapies and has clinical implications for patient prognosis and clinical trial eligibility. Understanding the extent and effects of myelosuppression in GBM patients can help us evaluate and optimize GBM management and can suggest appropriate cutoffs for blood-based biomarkers cutoff for clinical trial design. Previous literature has studied lymphopenia and neutrophil-to-lymphocyte ratio (NLR) separately; however, their prognostic significance relative to each other is unclear and their impact on immunotherapy trial design remains unexplored. We leveraged a large cohort of GBM patients to explore lymphodepletion, NLR, and thrombocytopenia at different time points over the course of treatments, illuminated their prognostic values, and examine their impact on trial participation.

Glioblastoma (GBM) is the most common malignant primary brain tumor in adults and has a poor prognosis despite maximal standard treatment with surgery, radiation therapy (RT), and temozolomide (TMZ).[1] Lymphopenia and host immunosuppression have been previously demonstrated to be common among GBM patients and are associated with poor outcomes.[2–4] Lymphopenia affects diverse subtypes of cells including B cells, CD4/CD8 T cells, and NK cells, which can be differentially affected by cancer therapy.[5] Contributing factors to myelosuppression include RT, chemotherapy, steroids,[4,6] as well as the possibility of splenic sequestration and T-cell dysfunction.[7]

Lymphopenia has come into greater focus with recent advances and interest in harnessing immunotherapy to treat GBM.[8] In addition to lymphopenia, there has been some evidence of the possible prognostic significance of neutropenia in GBM.[9,10] Lastly, a high ratio between the neutrophil and lymphocyte (NLR) has been shown to be associated with a more pro-inflammatory state and worse outcomes,[11] though its prognostic significance relative to lymphopenia is unclear. Better understanding of these changes to commonly measured blood counts may be important for optimizing management of GBM patients.

In this retrospective cohort study, we studied the effects of lymphopenia, NLR, and thrombocytopenia on patient outcomes and explored parameters that can predict myelosuppression in GBM patients.

Methods

Patient Population

We identified patients, age 18 or older, treated at the Dana-Farber/Brigham and Women's Cancer Center (DFCI/BWH) between 2005 and 2019 who had pathologic confirmation of glioblastoma and had complete blood count data from at least 1 of the 4 prespecified time points that could be extracted from the electronic medical record. Prespecified time points were defined by the following intervals: (1) within 7 days prior to the surgery (preoperative); (2) after surgery but prior to receiving radiation (post-op, pre-RT), (3) during RT or within 42 days of finishing radiation (CRT) and (4) within 4 weeks prior or after the first recurrence. Patient cohorts were aggregated from DFCI/BWH GBM databases, which have been previously published and described,[12,13] and patients with available blood counts through the Mass General Brigham research patient data registry program. Hematologic toxicity was graded per CTCAE version 4.0. A review of the medical records and clinical charts of each patient was performed, and clinical data were obtained retrospectively. If multiple blood count datapoints were available for one of the prespecified time ranges, the lower value was used for analysis. Clinical, demographic, pathologic, and follow-up data were collected from the medical record following approval from the Dana-Farber Harvard Cancer Center IRB with a waiver for informed consent for this retrospective study.

Clinical Variables and Measurements

A review of the medical records for each patient was performed and clinical parameters were collected retrospectively including sex, age, baseline Karnofsky performance status (KPS), histological diagnosis, extent of resection (EOR), RT dose, TMZ use, progression dates, blood counts through hematologic laboratory testing, tumor volume, steroid use during CRT (binary: Yes or no), progression-free survival (PFS), and overall survival. Progression was defined by assessments integrating imaging and clinical notes. complete blood count including white blood cell count including neutrophil, lymphocyte, and platelet

counts were collected at each of the 4 time points. OS was defined as days from initial diagnosis to death and the PFS was defined as days from diagnosis to the day of the first recurrence. For lymphopenia, neutropenia, and thrombocytopenia, we used CTCAE v4.0 criteria as cutoffs: lymphopenia: <500/mm^3 (grade 3), neutropenia: <1000/mm^3 (grade 3), thrombocytopenia: <75 000/mm^3 (grade 1). NLR was calculated by dividing neutrophil count by lymphocyte count, and we defined high NLR as ≥5, as has been previously described. NLR was calculated by dividing neutrophil count by lymphocyte count, and we defined high NLR as ≥5, as has been previously described.[14]

Statistical Analyses

Patient characteristics were assessed with descriptive statistics. The Kaplan–Meier curve equation was used to estimate median OS Cox modeling was used to determine OS and PFS differences between comparison groups. Temporal dynamics of blood counts were analyzed with pairwise Wilcoxon test and multiple hypotheses were controlled using false discovery rate. Univariable and multivariable logistic regressions were conducted for NLR, lymphopenia, and thrombocytopenia to determine clinical variables that are linked to their occurrences. *P*-values < .05 were considered statistically significant. Statistical analysis was conducted using R Statistical Software (Version 4.0.3). To adjust for immortal time bias, we also performed landmark analysis for the results involving PFS and OS from time of RT start.

Results

Patient Characteristics

We identified 764 newly diagnosed adult GBM patients treated at our institution with available blood counts. Baseline patient characteristics are detailed in Table 1. In total, median age of the cohort was 60.3 years old. 40.7% (310/761) of the patients were female and 59.3% (451/761) were male. Among patients with known EOR status, 37.5% (268/715) of patients underwent gross total resection and 62.5% (447/715) received subtotal resection or biopsy (non-gross total resection). Among patients with known radiation dose, the dosage of radiation administered ranged from 3600 to 6300 cGy, with 80.1% (548/684) of the patients receiving ≥5940 cGy. Among patients with known MGMT promoter methylation status, 51.9% (279/538) of patients had methylated MGMT promoter status while 48.1% (259/538) of the patients had an unmethylated MGMT promoter. Among patients with known IDH status, 92.6% (376/406) were IDH wild type and the remaining 7.4% (30/406) were IDH mutant. 90% (564/627) of patients were prescribed TMZ and 87.1% (576/661) of patients had a KPS ≥ 70. Unless terminated for toxicity or disease progression, patients received both concurrent and adjuvant TMZ, patients received both concurrent and adjuvant TMZ. Among 260 patients with known adjuvant TMZ information (260 patients), the median number of adjuvant cycles is 5 with a range of 1–22 cycles. For lymphopenia, neutropenia, and thrombocytopenia, we used criteria for grade 3 toxicity from CTCAE v4.0., for thrombocytopenia, we used criteria defined by previous literature where the cutoff is associated with higher mortality.[15] At pre-op time point, 11.9% (56/469) of patients had grade 3 or higher lymphopenia, none reached grade 3 or higher neutropenia and 0.5% (2/418) had thrombocytopenia. At post-op, pre-CRT time point, 15.4% (97/628) of patients had grade 3 or higher lymphopenia, 0.4% (2/515) had grade 3 or higher neutropenia and 1.3% (7/538) had thrombocytopenia. At post-CRT time point, 37.8% (248/656) of patients had grade 3 or higher lymphopenia, 5.7% (31/544) had grade 3 or higher neutropenia and 9.5% (52/547) had thrombocytopenia. Finally, at first recurrence time point, 27.1% (127/468) of patients had grade 3 or higher lymphopenia, 0.9% (4/453) had grade 3 or higher neutropenia and 4% (18/456) had thrombocytopenia.

Table 1. Patient Characteristics

Characteristic	No.	%
Year of Diagnosis		
2005–2009	199	31.3%
2010–2014	212	33.4%
2015–2019	224	35.3%
Age (median)	60.3	
Age (range)	19-94	
Sex		
Female	310	40.7
Male	451	59.3
KPS		
≥70	576	87.1
<70	85	12.9
MGMT promoter status		
Methylated	259	34.0
Unmethylated	279	36.5
Unknown	226	29.5
Surgery		
Biopsy/ Subtotal resection	447	58.6
Gross total resection	268	35.1
Unknown	49	6.4
TMZ		
Yes	564	90.0
No	63	10.0
cGy		
>5940	548	80.1
≤5940	136	19.9
Steroid Use		
Yes	131	46.8
No	149	53.2

Lymphopenia

Lymphopenia is characterized by a significant decrease in the number of lymphocytes, and we defined lymphopenia with CTCAE v4.0 grade 3 lymphocyte count (lymphocyte count < 500 cells/mm^3).[16] We observed temporal changes in lymphocyte measurement related to the treatment course of patients. As expected, patients experienced a statistically significant decrease in lymphocyte count from pre-op to the CRT phase of therapy, with a slight but significant recovery at the first recurrence ($P < .001$, Figure 1A).

We identified several predictors of lymphopenia on logistic regression analysis. Female sex (OR = 2.39, 95% CI = 1.73–3.31, $P < .001$), radiation dose (59.4Gy vs. < 59.4Gy, OR = 1.74, 95%CI = 1.11–2.79, $P = .018$), and steroid use during CRT (OR = 2.04, 95% CI = 1.21–3.47, $P = .008$) were significantly associated with higher risk for lymphopenia on UVA. We did not observe significant differences in lymphopenia amongst IDH mutant tumors (75% vs. 64% in IDH-wild-type tumors, $P = .3$) nor amongst MGMT methylated tumors (59% vs. 67% in MGMT unmethylated tumors, $P = .08$). Finally, on MVA, female sex, and steroid use remained significantly associated with increased risk for lymphopenia (OR = 2.33 and 2.66, 95% CI = 1.033–5.33 and 1.20–6.07, $P = .04$ and .02, respectively) (Supplementary Table 1).

We further observed clinical variables, including lymphocyte count, associated with overall survival (OS) and progression-free survival (PFS). On univariable analysis (UVA), lower KPS, low radiation dose, subtotal resection or biopsy, older age, and MGMT promoter unmethylated status were associated with inferior survival ($P < .001$). Lymphopenia during CRT was associated with inferior survival ($P = .02$, Supplementary Figure 1A) but not PFS (p = 0.15, Supplementary Figure 1B). Lymphopenia during CRT remains associated with OS (AHR = 1.44, 95% CI = 1.12–1.86, $P = .005$) after adjustment for age, KPS, RT dose, EOR, and MGMT promoter methylation. No association was found between OS and lymphopenia at other time points (preoperative, pre-RT). Lymphopenia during CRT was also associated with shorter PFS after adjustment of clinical covariables (AHR = 1.28, 95% CI = 1.001–1.624, $P = .049$). No association was found between PFS and lymphopenia at other time points.

Neutrophil-to-Lymphocyte Ratio

A significant decrease in NLR was observed from preoperative to pre-RT to CRT time points (Figure 1B).

Patients were dichotomized as high versus low by median NLR (4.9). Among patients with high NLR, 63% exhibited grade 3 or 4 lymphopenia during CRT and had a longer median survival than those who do not have high-grade lymphopenia (Supplementary Table 2). For clinical predictors of higher NLR during CRT, we found that EOR (subtotal resection or biopsy, OR = 1.56, 95% CI = 1.09–2.23, $P = .016$) and steroid use (OR = 3.54, 95%CI = 2.08–6.11, $P < .001$) were significantly associated on UVA. On logistic regression MVA, steroid use was the only significant independent predictor for increased NLR value (OR = 3.58, 95% CI = 1.92–6.78, $P < .001$) (Supplementary Table 3).

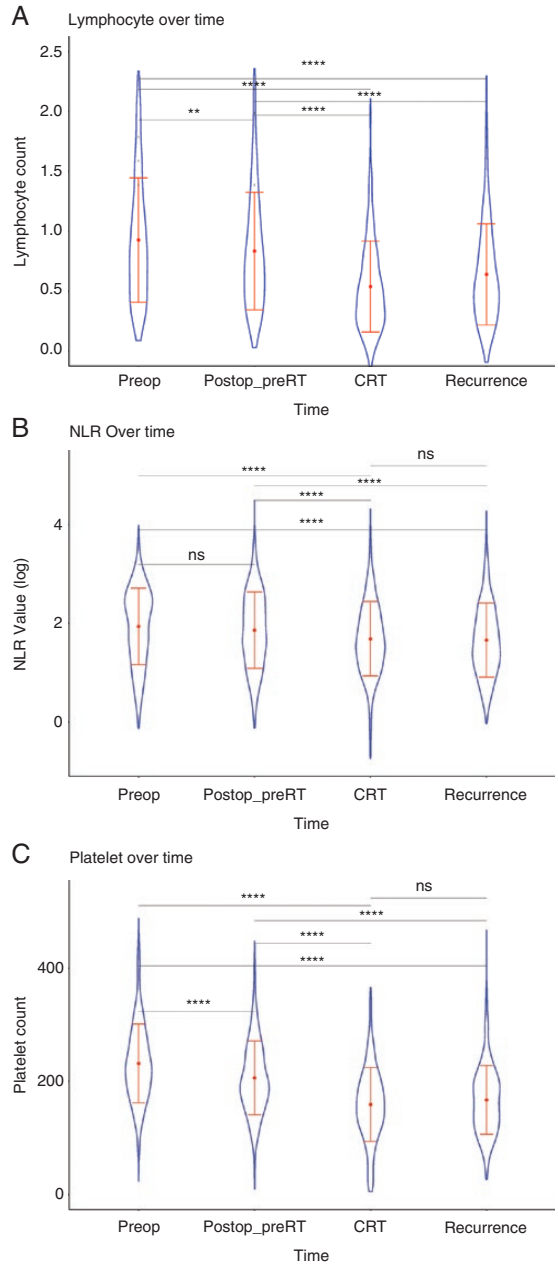

Figure 1. Absolute lymphocyte count (A), neutrophil-to-lymphocyte ratio (B), and platelet count (C) at 4-time points: (1) preoperative, (2) prior to radiation therapy start, (3) during chemoradiation phase of therapy (within 6 weeks of completing radiation therapy) and (4) at time of first recurrence. Median and interquartile range is shown. Significant differences between time points are annotated accordingly.

For survival analysis UVA, high NLR at each of the 4 time points were associated with worse OS (Figure 2A) and high NLR during CRT was also associated with worse PFS ($P < .001$, Figure 2B). On MVA, high NLR *during CRT* remained significantly associated with worse OS (AHR = 1.62, 95% CI = 1.23–2.14, $P < .001$) and PFS (AHR = 1.41, 95% CI = 1.09–1.83, $P = .01$). Furthermore, for patients who

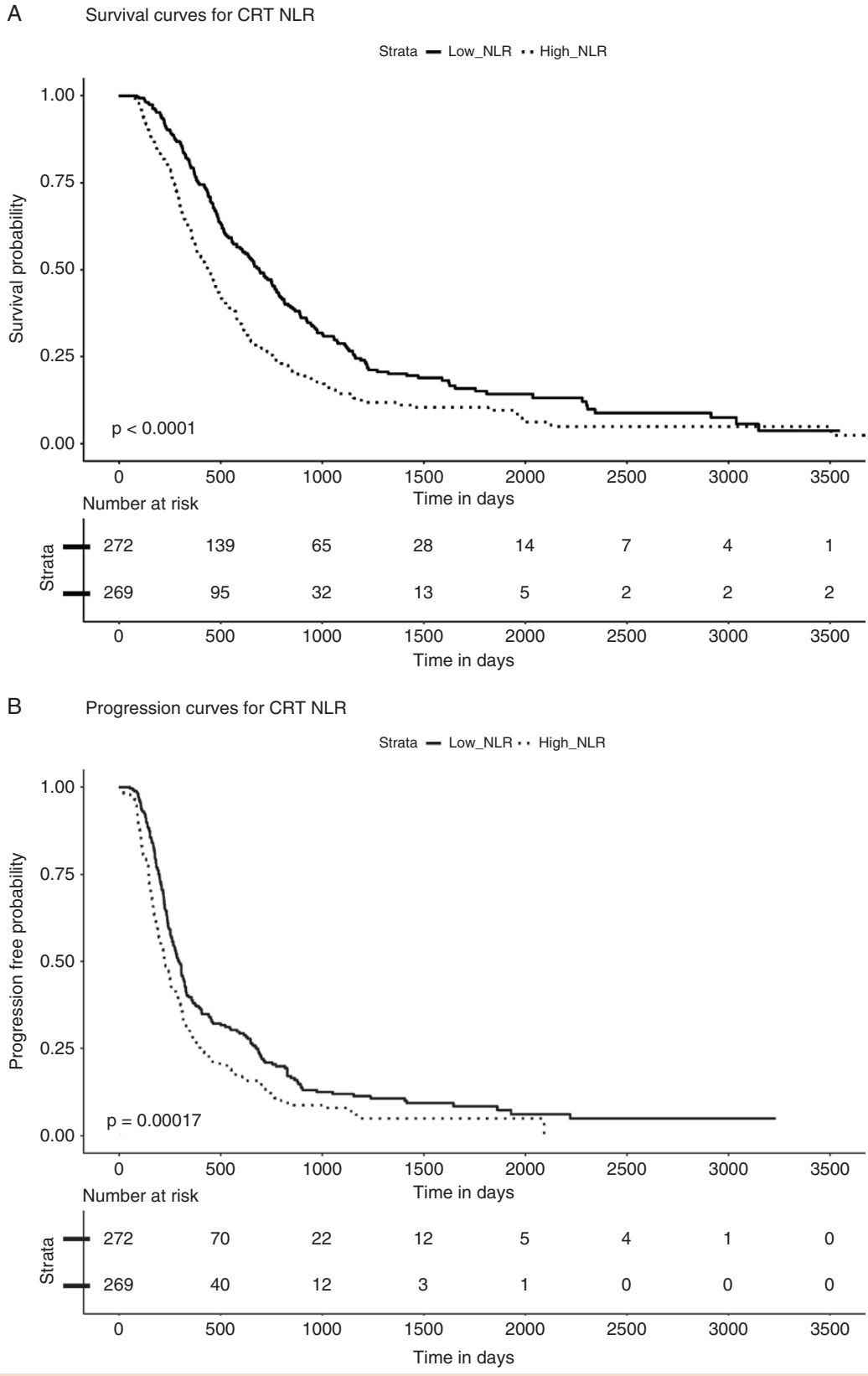

Figure 2. Kaplan–Meier survival curves for overall survival (A) and progression-free survival (B) for patients stratified by high versus low neutrophil-to-lymphocyte ratio during chemoradiation.

did not receive steroids, the association between higher NLR during CRT and worse OS remained significant after adjusting for clinical variables including KPS status, EOR, age, MGMT status and sex (AHR = 1.88, 95% CI = 1.06–3.37, $P = .03$); similarly, high NLR was not significantly associated with PFS (AHR = 1.31, 95% CI = 0.77–2.22, $P = .31$).

Thrombocytopenia and Neutropenia

As with other hematologic parameters, there was a significant decreasing trend in platelet count across the first 3 time points followed by a recovery in platelet count at first recurrence (Figure 1C). For clinical predictors, none was significantly associated with a higher risk for thrombocytopenia. We found that thrombocytopenia during chemoradiation is not significantly associated with either OS or PFS on UVA. Thrombocytopenia also did not have a significant association with PFS at any time point. We found that neutropenia during chemoradiation is not significantly associated with either OS ($P = .884$) or PFS ($P = .938$) in UVA nor MVA ($P > 0.05$).

Relative Prognostic Impact of Hematologic Parameters

Since multiple hematologic parameters were associated with clinical outcomes, we performed an MVA including all hematologic parameters to better characterize relative prognostic utility. Therefore, the MVA included lymphopenia, NLR, and thrombocytopenia during CRT along with relevant clinical covariates (Table 2). We tested the variance inflation factor between blood counts and found no values over 1.5, and significant multicollinearity was not detected.[17] High NLR during CRT remained associated with inferior OS (AHR = 1.58, 95% CI = 1.15–2.17, $P = .005$) and PFS (AHR = 1.49, 95%CI = 1.11–2.00, $P = .008$). Lymphopenia during CRT were not associated with neither OS nor PFS ($P = .77$, $P = .45$, respectively) in this analysis. Thrombocytopenia during CRT was associated with increase in PFS (AHR = 0.57, 95%CI = 0.35–0.91, $P = .02$) but not significantly associated with OS in this analysis. Given concerns for immortal time bias,[18] landmark analysis was performed without a meaningful change of results. This study was initiated prior to the 2021 World Health Organization classification of CNS tumors.[19] We repeated analyses for patients with known IDH wildtype status and observed that the results were similar in this analysis (Supplementary Table 4).

Subgroup Analysis: Patients Receiving Standard Chemoradiation

In a subgroup analysis, we restricted the cohort to only those who received standard-of-care therapy with ≥ 59.4Gy radiation dose and concurrent and adjuvant TMZ ($n = 418$). High NLR during CRT remained an independent predictor for OS (AHR = 1.66, 95% CI = 1.15–2.39, $P = .007$) but not PFS ($P = .07$). Other significant variables associated with worse OS include lower KPS (AHR = 4.14, 95% CI = 1.85–9.28, $P < .001$), non-GTR (AHR = 1.46, 95% CI = 1.07–1.99 $P = .02$), older age (AHR = 1.03, 95% CI = 1.01–1.04, $P < .001$), unmethylated MGMT promoter status (AHR = 2.61, 95% CI = 1.90–3.59, $P < .001$), male sex (AHR = 1.48, 95% CI = 1.06–2.07, $P = .02$).

Subgroup Analysis: Patients Receiving Standard Chemoradiation With Available Steroid and Tumor Volume Data

Amongst patients who received standard 6 weeks chemoradiation with available information on steroid use and tumor volumes ($n = 77$), the association between higher NLR during CRT and worse OS persisted after adjusting for steroid and tumor volume (AHR = 4.69, 95% CI = 2.08–10.58, $P < .001$); however, high NLR was not significantly associated with PFS (AHR = 2.10, 95% CI = 0.99–4.50, $P = .053$). Furthermore, for patients who did not receive steroids, the association between higher NLR during CRT and worse OS remained significant after adjusting for clinical variables including KPS status, EOR, age, MGMT status, and sex (AHR = 1.88, 95% CI = 1.06–3.37, $P = .03$); similarly, high NLR was not significantly associated with PFS in this case (AHR = 1.31, 95% CI = 0.77–2.22, $P = .31$).

Table 2. Multivariable Survival Analyses Incorporating Hematological Parameters

Multivariable	AHR (OS)	95% CI (OS)	*P*-values (OS)	AHR (PFS)	95% CI (PFS)	*P*-values (PFS)
KPS > 70	0.71	0.42–1.21	0.21	0.98	0.56–1.72	0.95
GTR	1.39	1.06–1.83	0.02	1.24	0.96–1.61	0.10
Age	1.04	1.03–1.05	<0.001	1.02	1.01–1.04	<0.001
MGMT status	0.41	0.31–0.54	<0.001	0.40	0.30–0.52	<0.001
Male sex	1.48	1.10–1.98	0.01	0.98	0.74–1.30	0.89
NLR during CRT	1.58	1.15–2.17	0.005	1.49	1.11–2.00	0.008
Lymphopenia during CRT	0.95	0.68–1.33	0.77	1.13	0.82–1.57	0.45
Thrombocytopenia during CRT	0.79	0.47–1.32	0.36	0.57	0.35–0.91	0.02

PFS, Progresson-free survival.

First Recurrence

Finally, we evaluated whether high NLR and lymphopenia at time of first recurrence were predictors of survival, specifically for time from first recurrence to death ($OS_{recurrence}$, $n = 609$). After adjustments of clinical covariates, high NLR value at time of first recurrence was associated with inferior survival after recurrence (AHR = 1.69, 95% CI = 1.25–2.27, $P < .001$). Lymphopenia at time of first recurrence was not associated with $OS_{recurrence}$ (AHR = 1.23, 95% CI = 0.91–1.69, $P = .17$). With respect to predictors of high NLR at this timepoint, steroid use during CRT (AHR = 1.86, 95% CI = 1.03–3.40, $P = .042$) and age (AHR = 1.02, 95% CI = 1.00–1.05, $P = .049$) were significantly associated with high NLR at first recurrence.

Impact of Myelosuppression on Clinical Trial Eligibility at Recurrence

To better understand how hematologic parameters may affect clinical trial eligibility at first recurrence, we assessed the potential impact on patient enrollment for a hypothetical recurrent GBM trial based on differing inclusion criteria (Table 3). A substantial number of patients would be ineligible for trials due to lymphopenia. For example, if a prospective recurrent GBM trial required absolute lymphocyte count > 0.5 K/uL or > 0.75 K/uL, 28% and 56% of patients in our cohort would be ineligible, respectively (Table 3). Other hematologic parameters did not have such an impactful effect on patient eligibility for trials.

Discussion

In this study, we leveraged a large single-institution cohort of newly diagnosed GBM patients to examine the impact of hematologic parameters including lymphopenia, NLR, and thrombocytopenia. While we evaluated hematologic parameters at several time points, nadir values during chemoradiation demonstrated the greatest prognostic utility. We found that the presence of lymphopenia and high NLR during CRT can predict poor patient outcomes whereas the presence of thrombocytopenia might predict better PFS. We also observed that among these candidate blood-based biomarkers, high NLR during CRT appeared to be most pronounced in its association with worse OS and PFS after adjustment of clinical covariates.

Neutrophil-to-Lymphocyte Ratio

Several prognostic factors in GBM are consistently associated with clinical outcomes, but there remains significant variability in patient outcomes after adjustment of factors such as age, performance status, EOR, and MGMT promoter methylation status.[20] NLR has been posited as a marker of systemic inflammation and its prognostic utility has been demonstrated across several cancers.[21] Several studies have demonstrated that high NLR (≥4 or ≥5) may be associated with decreased overall survival in GBM.[14,22,23] In contrast to prior studies, our study was conducted in a substantially larger cohort of patients across multiple time points and with adjustment of relevant clinical covariables.

As another important distinction, prior studies have not demonstrated the prognostic value of NLR relative to lymphopenia.[24] Our results indicate that high NLR was consistently significant after adjustment of other variables including lymphopenia. This suggests NLR during CRT provides augmented prognostic information relative to the information gleaned from lymphocyte count alone. Supporting this, 37% of patients with high NLR during CRT did not exhibit CTCAE grade 3 or 4 lymphopenia (Supplementary Table 3). High NLR patients without grade 3 or 4 lymphopenia had a numerically worse median survival than high NLR patients with grade 3 lymphopenia. If neutrophil counts rather than lymphopenia is driving the association between NLR and OS, this may suggest that chronic inflammation (and relevant associated cytokines,

Table 3. Patients Eligible for a Trial at Different Cutpoints for Hematologic Parameters at Time of First Recurrence

	Recurrent Patients Eligible Per Hematologic Criteria (%, ***n***)
Lymphocyte > 0.25	94% (442/468)
Lymphocyte > 0.5	72% (336/468)
Lymphocyte > 0.75	44% (205/468)
Lymphocyte > 1	25% (118/468)
Neutrophil > 0.5	99.8% (452/453)
Neutrophil > 1	99% (449/453)
Neutrophil > 1.5	98% (444/453)
Neutrophil > 2	87% (393/453)
PLT > 50	99% (450/456)
PLT > 75	96% (438/456)
PLT > 100	87% (396/456)
PLT > 150	58% (265/456)

etc.) may underlie poor survival, rather than T-cell loss and dysfunction. Prior work has also suggested that high NLR may correspond to lower infiltration of antitumor immune cells in the tumor microenvironment.[16,17] While further study is required, it is notable that the median NLR for GBM patients (4.9) in our cohort is higher than what has been reported in other cancers,[21,25] suggestive that NLR may be a more important biomarker in GBM relative to other cancer settings. Of note, we observed an association of high NLR with both PFS and OS, which has not been previously reported.[24] Our results raise the possibility that NLR may serve as an easy-to-obtain, predictive marker of biological behavior of the tumor or responsiveness to standard chemoradiation therapy given its association with tumor progression and OS. One may speculate that this is consistent with the hypothesis that high NLR is representative of a pro-inflammatory state that may hasten glioblastoma progression. Thus, our results rigorously add to a growing body of evidence to support prognostic utility of NLR, though prospective validation is warranted.

With increased interest in use of external datasets for the design and analysis of GBM trials,[26] understanding covariates that explain variability in outcomes has been of renewed interest. Obtained from inexpensive, routinely obtained bloodwork, NLR could represent a valuable prognostic biomarker. For clinical trials in the newly diagnosed setting, our findings emphasize the need for reporting of hematologic parameters such as NLR given their possible impact in interpreting results. If validated, there could also be consideration of stratifying patients for NLR or adjusting for this in multivariable analyses of clinical outcomes. We also demonstrate that high NLR at time of first recurrence is associated with inferior overall survival. While there have been several studies evaluating hematologic parameters in the newly diagnosed setting,[2–4,24] this finding may be particularly valuable as there is a scarcity of data on the prognostic significance of hematologic parameters in recurrent GBM patients.[27]

Lymphopenia

Lymphodepletion, common in GBM,[2,4] has been of increasing focus with recent interest in immunotherapy. Both treatment-naïve and treatment-induced lymphopenia have been associated with poor prognosis in GBM.[3] Several mechanisms have been posited to contribute to lymphopenia including the size of radiation field,[28] temozolomide chemotherapy,[29] lymphotoxic effects of steroids,[30] splenic sequestration, and T-cell dysfunction.[7] Unsurprisingly, steroid use was associated with lymphopenia in our study, as has been noted in other studies. Moving forward, minimizing the use of steroids and limited RT fields, or use of proton RT,[31] can reduce risk of lymphopenia, which may be particularly relevant as immunotherapies continue to be investigated for GBM.[32] Of note, in agreement with prior findings by Le Rhun et al.,[24] females were more likely to develop lymphopenia relative to males. These findings support recent work demonstrating broad-based sex differences in GBM,[33] as well as sex-based differences in hematologic adverse events in oncology patients.[34]

Thrombocytopenia

Thrombocytopenia has long been known to occur in GBM patients treated with chemoradiation.[35] Our results suggested a possible association of thrombocytopenia with improved PFS, which could correlate with findings from a prior study that suggested that increase in platelet count is associated with worse outcomes for GBM patients.[36] Further study is needed to better explore the prognostic significance of thrombocytopenia in GBM patients.

Recurrent GBM Trial Eligibility

In addition to prognostic utility, persistent myelosuppression after chemoradiation presents challenges in clinical trial design in the recurrent GBM setting; this is particularly relevant as there are no effective second-line therapies and clinical trials are an important consideration for patients.[37] With the rise of immunotherapy trials, the selection of hematologic parameters required for eligibility criteria of clinical trials can be challenging, and there is a need to balance concerns that lymphopenia may compromise novel immune-based therapies with the need to open and expand clinical trial eligibility for patients who are otherwise appropriate trial candidates.[38,39] Of note, it is not known if baseline lymphocyte counts predict outcomes to immunotherapy in GBM; some data suggests that lymphopenia is associated with inferior antitumor response from immunotherapy in other cancers,[40,41] while other work suggests that baseline lymphopenia should not be used to exclude patients from immunotherapy clinical trials.[42]

To examine potential impact of hematologic parameters on trial eligibility in recurrent GBM, we evaluated impact of different thresholds for eligibility with these parameters. As expected, while various cutoff values for neutrophil or platelet count had minimal effect on excluding patients from a prospective trial, the baseline lymphocyte count had a meaningful impact on the trial eligibility of recurrent GBM patients (Table 3). At first recurrence, 28% of patients (127/468) had a lymphocyte count < 500 cells/uL and 55% (258/468) had lymphocyte count below 750 cells/uL; thus, a significant number of patients would not be eligible for a recurrent GBM clinical trial if eligibility criteria required a baseline absolute lymphocyte count above these levels. Better understanding of the relationship between baseline lymphocyte count and immunotherapy efficacy may help guide lymphopenia-related eligibility criteria. A more liberal minimum baseline lymphocyte count such as > 500 cells/uL or 750 cells/uL may be appropriate for recurrent GBM immunotherapy trials to not overly restrict accessibility and generalizability of such trials.

This is a retrospective study with inherent limitations. Patients were excluded if blood counts could not be automatically extracted, and it is unclear if there were any unintended biases that arose from this selection strategy.

As certain variables were not available for all patients, subgroup analyses for patients with steroid and tumor volume data were completed to evaluate the impact of these relevant parameters on the analysis. Steroid use is an important consideration as it can identify patients

with worsening clinical status but is also associated with lymphopenia and high NLR. For example, while a subgroup analysis of patients that did not require steroids showed that NLR remained associated with prognosis, steroid use was also associated with high NLR (Supplementary Table 3). These findings highlight that there remains a complex interplay between steroid use and hematologic parameters.

Conclusion

High NLR during chemoradiation is an independent predictor of PFS and OS in newly diagnosed GBM. The prognostic utility of high NLR during CRT appears to be superior to lymphopenia alone in both the newly diagnosed and recurrent GBM setting. Many patients continue to exhibit myelosuppression at recurrence, and this should be accounted for in the design of immunotherapy trials in recurrent GBM.

Supplementary material

Supplementary material is available online at *Neuro-Oncology Advances* online.

Keywords:

blood-based biomarkers | clinical trial design | Glioblastoma | lymphopenia | neutrophil-to-lymphocyte ratio (NLR)

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Conflict of interest statement

LN reports: Consulting for Ono, Brave Bio, Genmab. Advisory board for Ono, Kite/Gilead. Royalty from Wolters Kluwer (UpToDate). Clinical trial support from Merck, Astra Zeneca, Kazia, Ono, Bristol Myers Squibb. All other authors do not report relevant disclosures. RR reports research support from Project Data Sphere.

Authorship statement

All authors contributed to the data collection. Study conception, design and data analysis were performed by Davy Deng and Rifaquat Rahman. The first draft of the manuscript was written by Davy Deng and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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