Routine blood tests do not predict survival in patients with glioblastoma-multivariable analysis of 497 patients

Maas, S.L.N.; Draaisma, K.; Snijders, T.J.; Senders, J.T.; Berendsen, S.; Seute, T.; ... ; Broekman, M.L.D.

Routine Blood Tests Do Not Predict Survival in Patients with Glioblastoma—Multivariable Analysis of 497 Patients

Sybren L.N. Maas[1,2], Kaspar Draaisma[1], Tom J. Snijders[1], Joeky T. Senders[1], Sharon Berendsen[1], Tatjana Seute[1], Raymond M. Schiffelers[3], Wouter W. van Solinge[3], Maarten J. Ten Berg[3], Pierre A. Robe[1], Marike L.D. Broekman[1]

■ BACKGROUND: **Multiple reports have attributed a prognostic value to routine blood tests results for patients with glioblastoma. However, these studies have reported conflicting results and have often had small sample sizes. We sought to validate the prognostic value of the described tests in an independent glioblastoma patient population.**

■ METHODS: **We performed a retrospective single-center multivariable analysis of 497 patients with glioblastoma who had postoperatively undergone radiotherapy and/or chemotherapy to identify the prognostic value for median overall survival of hemoglobin, white blood cell, monocyte, neutrophil, leukocyte, and platelet counts, neutrophil/lymphocyte ratio, C-reactive protein, erythrocyte sedimentation rate, activated partial thromboplastin time, prothrombin time, and lactate dehydrogenase. We also evaluated known prognostic factors for survival such as patient age, intervention type, *IDH1* status, Karnofsky clinical performance status, and postoperative treatment modality.**

■ RESULTS: **In a multivariable model, after correcting for multiple testing bias, biopsy alone (hazard ratio, 0.35; 95% confidence interval, 0.26–0.49; false discovery rate-adjusted $P < 0.001$) and monotherapy after surgery (hazard ratio, 0.46; 95% confidence interval, 0.33–0.66; false discovery rate-adjusted $P < 0.001$) remained significantly associated with worse median overall survival. Patient age and Karnofsky performance status score ≥70 did not significantly influence survival in the multivariable model. No routine blood test included in the multivariable analysis was significantly associated with survival.**

■ CONCLUSIONS: **In the present study, hemoglobin, white blood cell, monocyte, neutrophil, leukocyte, and platelet counts, neutrophil/lymphocyte ratio, C-reactive protein, erythrocyte sedimentation rate, activated partial thromboplastin time, prothrombin time, and lactate dehydrogenase levels did not independently predict for overall survival in patients with glioblastoma.**

INTRODUCTION

In adult patients, glioblastoma is the most common malignant primary brain tumor.[1,2] Although the prognosis for patients with glioblastoma improved with the introduction of a treatment regimen consisting of maximal safe resection, radiotherapy, and concomitant and adjuvant temozolomide, patient survival has remained dismal, with a median overall survival of ~15 months.[3,4] Well-known prognostic factors for glioblastoma include age at diagnosis, clinical performance status, MGMT (O6-methylguanine-DNA methyltransferase) promoter

Key words
- Biomarkers
- Blood tests
- Glioblastoma
- Hemoglobin
- Prognostic markers
- Prognosticators
- Survival

Abbreviations and Acronyms
aPTT: Activated partial thromboplastin time
CI: Confidence interval
CRP: C-reactive protein
ESR: Erythrocyte sedimentation rate
EV: Extracellular vesicle
FDR: False discovery rate
Hb: Hemoglobin
HR: Hazard ratio
IDH: Isocitrate dehydrogenase
KPS: Karnofsky performance scale
LDH: Lactate dehydrogenase
NLR: Neutrophil/lymphocyte ratio
PT: Prothrombin time

From the [1]UMC Utrecht Brain Center, Department of Neurology and Neurosurgery, University Medical Center Utrecht, Utrecht University, Utrecht; and Departments of [2]Pathology and [3]Clinical Chemistry and Hematology, University Medical Center Utrecht, Utrecht, The Netherlands

To whom correspondence should be addressed: Marike L. D. Broekman, M.D., Ph.D., J.D. [E-mail: m.broekman@haaglandenmc.nl]

Sybren L. N. Maas and Kaspar Draaisma contributed equally to the present study.

Citation: World Neurosurg. (2019) 126:e1081-e1091.
https://doi.org/10.1016/j.wneu.2019.03.053

*Journal homepage: **www.journals.elsevier.com/world-neurosurgery***

*Available online: **www.sciencedirect.com***

methylation status, isocitrate dehydrogenase (IDH) status, Mini-Mental State Examination score, extent of resection, and corticosteroid treatment at baseline.[5,6] Virtually all patients with glioblastoma undergoing surgery will undergo a screening blood test before surgery. In general, these tests will be obtained to determine the patient's clinical state and risk of hemorrhagic events. However, the performed tests could simultaneously function as surrogate markers for the physiological state of the patient, including specific changes caused by pathological properties of the tumor. If true, these routine markers could then function as proxy markers for the tumor and could function as prognosticators. This is especially attractive because routine blood tests are standardized, readily available, and less expensive than other potential prognostic markers such as imaging studies or extensive molecular testing of tumor tissue.

In the available data, the prognostic value of specific blood tests on the survival of patients with glioblastoma has been reported. For example, an anemic state at the initial surgery was correlated with reduced overall survival in patients with glioblastoma.[7-10] Others have reported on the effect of the neutrophil/lymphocyte ratio (NLR) on survival. Multiple NLR cutoffs have been studied; however, an NLR of >4.0 has been most prominently associated with reduced survival in the reported data.[11-13] In addition to hemoglobin (Hb) and NLR, the erythrocyte sedimentation rate (ESR), activated partial thromboplastin time (aPTT), prothrombin time (PT), lactate dehydrogenase (LDH), and lymphocyte, neutrophil, and platelet counts have been suggested to be of prognostic value.[10-18]

Although the reports attributing prognostic values to routine blood tests in patients with glioblastoma were descriptive in nature, a pathophysiological relation for some of the markers can be speculated. For example, an anemic state might reflect specific properties of the glioblastoma tumor because anemia can lead to tumor hypoxia. It has been reported that tumor hypoxia can result in therapy resistance and more aggressive tumors.[19,20] The negative predictive value of low serum Hb levels and hypoxia for prognosis has also been described for other malignant tumors, such as head and neck tumors,[21,22] carcinoma of the uterine cervix,[23] and carcinoma of the bladder.[24] Alternatively, an anemic state could have been associated with other chronic diseases negatively affecting the patient's clinical performance. Because performing surgery on anemic patients is not recommended in general, this group of patients could represent a subpopulation for whom postponing surgery to correct the anemic state was not possible.

Neutrophil counts alone or increased NLRs have also been reported to be associated with decreased overall survival in patients with glioblastoma. Neutrophils, long assumed to only play a role in microbial infection and wound healing, are now believed to also influence cancer biology.[25] Neutrophils can facilitate tumor growth by inducing angiogenesis[26] or by accelerating tumor cell migration.[27] The role of neutrophils in the glioblastoma microenvironment is largely unknown; however, neutrophil influx during anti-vascular endothelial growth factor treatment was shown to promote glioma progression in a glioblastoma model.[28,29] It is, therefore, reasonable to assume that increased neutrophil counts could have relevant effects on glioblastoma biology. However, increased neutrophil counts can also be obscured by glucocorticoid (dexamethasone) usage. Glucocorticoid usage can increase circulating neutrophil counts by suppression of spontaneous neutrophil apoptosis.[30] Because dexamethasone usage might be associated with more severe clinical symptoms, its effect on the NLR might be biased to a subpopulation of patients with more advanced disease. Although promising, the interpretation and direct clinical application of the results from most of the studies attributing prognostic values to routine blood tests in patients with glioblastoma has been difficult, because the reported data were obtained from small sample sizes and conflicting results were reported.

The aim of the present study was to validate the reported prognostic value of specific routine blood tests on overall survival in patients with glioblastoma undergoing surgery from a large sample of patients. Thus, we performed a retrospective single-center multivariable analysis on 497 patients undergoing surgery and adjuvant care for primary glioblastoma from 2005 to 2013.

METHODS

Study Design, Patient Population, and Patient Data

For the present retrospective study, data from our hospital's glioblastoma data registry were used. All adult patients with glioblastoma who had undergone surgery from 2005 to 2013 were identified. Only patients who had experienced primary nonrecurrent histologically confirmed glioblastoma receiving adjuvant care and for whom a routine blood test within 5 weeks before and/or after primary surgery was available were included in the present study. Second, because we investigated whether routine blood values can help identify patients with improved or decreased expected survival, we excluded patients who had opted to abstain from any further treatment. Therefore, adjuvant treatment was added as an inclusion criterion.

Our hospital's ethical committee evaluated the study's design and determined that individual patient consent was not required. All data acquisition and management were in accordance with the current privacy and ethics regulations.

Covariates and Outcome

The following pre- and postoperative blood tests were extracted: Hb, white blood cell (WBC), monocyte, neutrophil, lymphocyte, and platelet counts, C-reactive protein (CRP), ESR, aPTT, PT, and LDH.[31] The NLR was calculated by dividing the neutrophil count by the lymphocyte count. If multiple blood samples for an individual patient were present, the findings closest to the date of surgery were selected. To avoid iatrogenic systemic effects from the perioperative phase, the postoperative blood tests obtained within the first 48 hours after surgery were excluded. Furthermore, the patient characteristics, including age at surgery, gender, type of surgery (biopsy or debulking), antineoplastic treatment after surgery (i.e., none or best supportive care, monotherapy with radiotherapy or chemotherapy, chemoradiotherapy), and survival data were extracted from the patients' electronic medical records.

Statistical Analysis

We compared the blood test values obtained before and after the initial surgery and tested for significant differences before and

after surgery using the Wilcoxon signed rank test and McNemar test, as appropriate. Dichotomization was performed using cutoff points determined from reported findings or the values used by our clinical hematology laboratory. For the NLR, a cutoff of 4.0 was used for dichotomization as described in reported studies.[11] Additionally, differences between the preoperative and postoperative NLR (defined as an NLR change >2 points) were analyzed separately.

The overall median survival times were estimated using the Kaplan-Meier method, defining the overall median survival as the interval between date of the first surgery and the date of death. Patients who were lost to follow-up or who were alive at the analysis were censored at the last confirmed in-hospital appointment. The estimated overall median survival was compared for significant differences per stratum of the examined blood tests and demographic parameters using the log-rank method. The correlation with overall survival was tested using the Cox proportional hazards method on univariable analysis for all patient characteristics and blood test values. A multivariable Cox proportional hazards analysis was performed with all variables that showed a significant correlation with survival on univariable analysis and for which sufficient data were available (to ensure a minimum of 10 data points per stratum). In the final multivariable model, the P values were corrected using the Benjamini-Hochberg false discovery rate (FDR) procedure to adjust for multiple testing. A P value <0.05 was chosen to indicate statistical significance on univariable and FDR-adjusted multivariable analysis. All statistical analyses were performed in R statistical software, version 3.3.2 (Foundation for Statistical Computing, Vienna, Austria). The FDR-adjusted P values were calculated using the "p.adjust()" function in the core stats R package. Survival analysis was performed using the survival package, version 2.38.[32]

RESULTS

Study Population

From 2005 to 2013, 503 patients had undergone treatment that included adjuvant care for a histologically confirmed de novo glioblastoma. Of the 503 patients, 6 were excluded from the analysis. For 4 patients we were unable to reconstruct accurate survival data and 2 patients had undergone surgery at a different institution.

Of the 497 included patients (Table 1), 297 were men (59.8%). The median age was 62.2 years (range, 21.0–88.1). Of the 497 patients, 359 had undergone tumor resection (72.2%) and 138 had undergone biopsy (27.8%). After surgery, 335 patients underwent chemoradiotherapy (67.4%) and 162 received monotherapy with either radiotherapy or chemotherapy (32.6%). The Karnofsky performance scale (KPS) was available for 99.0% of the patients. For 59.5%, the *IDH1* mutation status using R132H immunohistochemical staining was available. Laboratory data before surgery were available for 493 patients (99.2%) and were obtained with a median of 2.9 days (range, 0–32.9) before surgery. Laboratory results were available for 340 patients ≥48 hours after surgery (median, 18.2 days; range, 2–35). For 336 patients, data were available for both pre- and postoperative blood tests. The median survival was 15.1 months for patients receiving radiotherapy plus concomitant and adjuvant temozolomide. However, the median survival patients undergoing monotherapy was a median of 7.9 months.

Table 1. Baseline Characteristics of Study Population ($n = 497$)

Characteristic	Patients (*n*; %)
Age (years)	
Median	62.2
Range	21–88.1
Age group	
<50 years	88 (17.7)
50–70 years	298 (60.0)
>70 years	111 (22.3)
Gender	
Male	297 (59.8)
Female	200 (40.2)
Extent of surgery	
Biopsy	138 (27.8)
Debulking	359 (72.2)
Treatment after surgery	
Monotherapy (radio- or chemotherapy)	162 (32.6)
Chemoradiotherapy	335 (67.4)
Karnofsky performance scale score	
<70	92 (18.5)
≥70	400 (80.5)
Missing	5 (1.0)
IDH1 status (R132H staining)	
IDH1 wild-type	276 (55.5)
IDH1 mutated	20 (4.0)
Missing	201 (40.4)
Laboratory data	
Before surgery	493 (99.2)
Interval (days)	
Median	2.9
Range	0–32.9
Missing	4 (0.8)
After surgery	340 (68.4)
Interval (days)	
Median	18.2
Range	2–35
Missing	157 (31.6)
Before and after surgery	336 (67.6)
Missing	161 (32.4)

Table 2. Blood-Based Biomarkers Before versus After Surgery

Variable	Preoperatively	Postoperatively	Test	*P* Value
Hemoglobin (mmol/L)				
Median	9.2	8.4		
Range	5.7–12.7	4.5–10.6	Wilcoxon signed rank test	<0.001
<8.6 (male); <7.4 (female)	43 (8.7)	128 (25.8)		
>8.6 (male), >7.4 (female)	438 (88.1)	202 (40.6)	McNemar test	< 0.001
NT	16	167		
NLR				
Median	6.8	5		
Range	0.1–46.9	0.2–80.8	Wilcoxon signed rank test	<0.001
<4	143 (28.8)	130 (26.2)		
>4	336 (67.6)	200 (40.2)	McNemar test	<0.001
NT	18	167		
Neutrophil count				
Median	9.5	7.5		
Range	1.3–27.3	1.2–29.9	Wilcoxon signed rank test	<0.001
$<8.3 \times 10^9$/L	198 (39.8)	195 (39.2)		
$>8.3 \times 10^9$/L	281 (56.5)	138 (27.8)	McNemar test	<0.001
NT	18	164		
Lymphocyte count				
Median	1.4	1.5		
Range	0.1–63.7	0.3–79	Wilcoxon signed rank test	0.113
$<4 \times 10^9$/L	472 (95.0)	328 (66.0)		
$>4 \times 10^9$/L	9 (1.8)	4 (0.8)	McNemar test	<0.001
NT	16	165		
Monocyte count				
Median	0.7	0.7		
Range	0–2.6	0.2–2.1	Wilcoxon signed rank test	0.688
$<0.8 \times 10^9$/L	314 (63.2)	232 (46.7)		
$>0.8 \times 10^9$/L	167 (33.6)	100 (20.1)	McNemar test	0.001
NT	16	165		
WBC count				
Median	11.9	9.9		
Range	1–71.5	3.3–256	Wilcoxon signed rank test	<0.001
$<4 \times 10^9$/L	2 (0.4)	1 (0.2)		
$>4 \times 10^9$/L	478 (96.2)	330 (66.4)	McNemar test	<0.001
$<10 \times 10^9$/L	243 (48.9)	233 (46.9)		
$>10 \times 10^9$/L	237 (47.7)	98 (19.7)	McNemar test	0.890
NT	17	166		
CRP				
Median	2	19.5		
Range	0.5–214	0.5–410	Wilcoxon signed rank test	<0.001

Continues

Table 2. Continued

Variable	Preoperatively	Postoperatively	Test	*P* Value
<10 mg/L	200 (40.2)	56 (11.3)		
>10 mg/L	16 (3.2)	90 (18.1)	McNemar test	<0.001
NT	281	351		
ESR				
Median	5	32		
Range	1—41	2—121	Wilcoxon signed rank test	0.039
<11 mm/hour (male); <24 mm/hour (female)	133 (26.8)	6 (1.2)		
>11 mm/hour (male), >24 mm/hour (female)	20 (4.0)	15 (3.0)	McNemar test	0.011
NT	344	476		
Platelet count				
Median	257.8	242.5		
Range	72.4—884.1	61—744.3	Wilcoxon signed rank test	<0.001
$<450 \times 10^3/mm^3$	465 (93.6)	320 (64.4)		
$>450 \times 10^3/mm^3$	16 (3.2)	9 (1.8)	McNemar test	<0.001
NT	16	168		
PT				
Median	13.1	13.1		
Range	11.6—15.6	11.8—15.8	Wilcoxon signed rank test	0.971
<12.2 seconds (decreased)	18 (3.6)	1 (0.2)		
>12.2 seconds	394 (79.3)	39 (7.9)	McNemar test	<0.001
<15.2 seconds	410 (82.5)	39 (7.9)		
>15.2 seconds (prolonged)	2 (0.4)	1 (0.2)	McNemar test	<0.001
NT	85	457		
aPTT				
Median	30	31.5		
Range	22—48	22—46	Wilcoxon signed rank test	0.008
<27 seconds (decreased)	79 (15.9)	7 (1.4)		
>27 seconds	337 (67.8)	39 (7.9)	McNemar test	<0.001
<37 seconds	390 (78.5)	39 (7.9)		
>37 seconds (prolonged)	26 (5.2)	7 (1.4)	McNemar test	0.137
NT	81	451		
LDH				
Median	214	257.5		
Range	116—819)	93—894	Wilcoxon signed rank test	<0.001
<250 U/L	101 (20.3)	91 (18.3)		
>250 U/L	46 (9.3)	109 (21.9)	McNemar test	<0.001
NT	350	297		

NT, Not tested; NLR, neutrophil/lymphocyte ratio; WBC, white blood cell; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PT, prothrombin time; aPTT, activated partial thromboplastin time; LDH, lactate dehydrogenase.

Comparison of Pre- and Postoperative Laboratory Blood Test Values

For all analyzed blood values, we obtained more data before surgery compared with postoperatively (Table 2). To simplify our analysis, we hypothesized that if the obtained values did not significantly differ preoperatively compared with postoperatively, we could focus the analysis on the preoperative data only. However, a comparative analysis of the pre- and postoperative laboratory blood values indicated significant changes in 9 of 12 blood values. The median Hb level, NLR, neutrophil count, WBC count, and platelet count all significantly decreased after surgery. In contrast, the CRP, ESR, aPTT, and LDH significantly increased after surgery. The lymphocyte count, monocyte count, and PT did not change preoperatively compared with postoperatively. Therefore, because most of the values did change significantly, we decided to include both the pre- and postoperative values in the subsequent analysis.

Correlation Between Routine Blood Tests and Overall Survival

As expected, on univariable analysis (Table 3), the established prognostic variables of age at diagnosis, extent of surgery, type of treatment after surgery, KPS, and *IDH1* mutation status were all significantly associated with survival. Patients aged <50 years had a median survival of 17.4 months compared with 12.4 months for patients aged 50–70 years (hazard ratio [HR], 1.66; 95% confidence interval [CI], 1.26–2.20; $P < 0.001$) and 8.4 months for patients aged >70 years (HR, 2.82; 95% CI, 2.03–3.92; $P < 0.001$). Patients who had only undergone a biopsy had a median survival of 8.3 months compared with 14.2 months for the patients who had undergone resection (HR, 0.42; 95% CI, 0.34–0.52; $P < 0.001$). A combination of radiotherapy and chemotherapy was associated with a longer median survival of 15.1 months (HR, 0.34; 95% CI, 0.27–0.42; $P < 0.001$) compared with 7.9 months for postoperative monotherapy. The patients with a KPS score of ≥70 had median survival of 13.1 months compared with 9.1 months for those with a KPS score <70 (HR, 0.68; 95% CI, 0.53–0.87; $P = 0.003$). Positive IDH1 R132H staining was associated with increased survival from 13.6 months survival for wild-type to 54.7 months with positive IDH1 staining (HR, 0.32; 95% CI, 0.17–0.6; $P < 0.001$). No significant change was found in survival for patients undergoing surgery from 2005 to 2007, 2008 to 2010, and 2011 to 2013.

On univariable analysis, some of the measured blood values were also significantly associated with patient survival. Neutrophil counts $>8.3 \times 10^9$/L both preoperatively and postoperatively showed reduced patient survival. Preoperatively, this correlated with a reduction in median survival from 14.3 to 11.7 months (HR, 1.35; 95% CI, 1.1–1.66; $P = 0.005$). In contrast, postoperatively, a reduction from 15.1 to 12.0 months was observed (HR, 1.45; 95% CI, 1.13–1.87; $P = 0.004$). For the preoperative and postoperative NLR values >4, significant reductions in overall survival were observed. As a post hoc assessment of the influence of the NLR on patient survival, we calculated the effect of an increase or reduction of the NLR from preoperatively to postoperatively of ≥2 points. No significant changes in survival were observed for alterations in the NLR of ≥2 points in either direction. A WBC count $>10 \times 10^9$/L preoperatively yielded reduced patient survival. For preoperatively elevated WBC counts, survival was 12 months compared with 14.1 months (HR, 1.26; 95% CI, 1.02–1.56; $P = 0.032$). In contrast, postoperatively elevated WBC counts decreased survival from 14.3 to 12.4 months (HR, 1.31; 95% CI, 1.02–1.68; $P = 0.033$). An aPTT <27 seconds preoperatively was associated with reduced survival compared with a normal aPTT (range, 27–37 seconds) and an increased aPTT time (>37 seconds). A preoperative LDH >250 U/L was associated with a reduction in survival (11.7 vs. 13.8 months; HR, 1.46; 95% CI, 1.01–2.11; $P = 0.043$).

To further study the effects of the factors identified to significantly influence patient survival on univariable analysis, the significant factors identified in the univariable analysis were assessed on multivariable analysis. The factors were only included for which sufficient data were available to ensure a minimum of 10 data points per stratum. Therefore, *IDH1* mutation status, preoperative aPTT, and preoperative LDH levels could not be included in the multivariable analysis (Table 3). On multivariable analysis, after correcting for multiple testing bias, biopsy alone (HR, 0.35; 95% CI, 0.26–0.49; FDR-adjusted $P < 0.001$), and monotherapy after surgery (HR, 0.46; 95% CI, 0.33–0.66; FDR-adjusted $P < 0.001$) remained significantly associated with worse median overall survival. Patient age and KPS score ≥70 did not significantly influence survival in the multivariable model. No routine blood test included in the multivariable analysis was significantly associated with survival.

DISCUSSION

The results of the present study have indicated that the routine blood values for patients with glioblastoma receiving postoperative chemotherapy and/or radiotherapy had no significant predictive value. In our multivariable model, after correcting for multiple testing bias, the extent of surgery and adjuvant treatment modality were significantly associated with patient survival.

Our results are in line with those from some reports on the prognostic value of routine blood tests. Two studies analyzed the influence of the lymphocyte count on the prognosis of patients with glioblastoma and found no significant correlation.[11,15] For some blood tests, however, our results were in contradiction with the reported data. The prognostic value of the Hb levels has been reported by 5 studies.[7-10,33] Four of these reported a negative prognostic value for low Hb levels[7-10]; however, 1 study found no statistically significant difference.[33] For several other blood values, significant prognostic values were reported that we could not confirm. Three studies found a significant correlation between an NLR >4.0 and a poor prognosis.[11-13] One study reported a significant prognostic value for LDH in a multivariable model.[10] CRP was determined to be a significant prognostic factor in 1 study[16] but was not significant in another.[14] Preoperative thrombocytosis was concluded to predict for poor overall survival.[17] In contrast, a decrease in the platelet count during glioblastoma treatment with concurrent radiotherapy and temozolomide has been correlated with an increase in survival.[15] On multivariable analysis, only prolonged aPTT and decreased PT appeared to significantly shorten survival.[17] Increased serum albumin levels also correlated with increased survival in patients with glioblastoma.[18]

Table 3. Prognostic Effect of All Factors on Overall Median Survival

			Univariable			Multivariable			
Variable	**Patients (*n*)**	**OS (%)**	**HR**	**95% CI**	***P* Value**	**HR**	**95% CI**	***P* Value**	**FDR-Adjusted *P* Value**
Overall	497	12.4							
Age (years)									
<50	88	17.4							
50–70	298	12.4	1.66	1.26–2.2	< 0.001	1.48	1.03–2.11	0.033	0.119
>70	111	8.4	2.82	2.03–3.92	< 0.001	1.5	0.94–2.38	0.089	0.163
Gender									
Male	297	12.9							
Female	200	11.8	1.05	0.85–1.28	0.666				
Extent of surgery									
Biopsy	138	8.3							
Debulking	359	14.2	0.42	0.34–0.52	< 0.001	0.35	0.26–0.49	< 0.001	< 0.001
Surgery year									
2005–2007	114	12.3							
2008–2010	167	11.7	1.16	0.9–1.48	0.254				
2011–2013	216	13.1	0.92	0.71–1.19	0.526				
Treatment after surgery									
Monotherapy (RT or ChT)	162	7.9							
ChT-RT	335	15.1	0.34	0.27–0.42	< 0.001	0.46	0.33–0.66	< 0.001	< 0.001
KPS score									
<70	92	9.1							
≥70	400	13.1	0.68	0.53–0.87	0.003	0.91	0.62–1.33	0.616	0.616
IDH1 R132H staining									
Negative	276	13.6							
Positive	20	54.7	0.35	0.18–0.65	0.001				
Hb, preoperative									
<8.6 (male), <7.4 (female)	43	12							
>8.4 (male), >7.4 (female)	438	12.4	0.8	0.57–1.13	0.201				
Hb, postoperative									
<8.6 (male), <7.4 (female)	128	13.6							
>8.4 (male), >7.4 (female)	202	13.6	1	0.78–1.28	0.984				
NLR, preoperative									
<4	143	14.8							
>4	336	11.8	1.27	1.01–1.58	0.037	1.11	0.75–1.65	0.607	0.616
NLR, postoperative									
<4	130	15.1							
>4	200	12.5	1.34	1.04–1.73	0.026	0.9	0.64–1.25	0.516	0.616

Univariable and multivariable analyses were performed to identify significant correlations between clinical and blood-based variables on glioblastoma patient survival.
OS, overall survival; HR, hazard ratio; CI, confidence interval; FDR, false discovery rate; RT, radiotherapy; ChT, chemotherapy; RT-ChT, chemoradiotherapy; KPS, Karnofsky performance scale; IDH1, isocitrate dehydrogenase 1; Hb, hemoglobin; NLR, neutrophil/lymphocyte ratio; WBC, white blood cell; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PT, prothrombin time; aPTT, activated partial thromboplastin time; LDH, lactate dehydrogenase.

Continues

Table 3. Continued

Variable	Patients (*n*)	OS (%)	Univariable			Multivariable			FDR-Adjusted *P* Value
			HR	95% CI	*P* Value	HR	95% CI	*P* Value	
NLR increase									
Difference <2 points	252	13.5							
Difference >2 points	65	12.4	1.17	0.86–1.6	0.322				
NLR decrease									
Difference <2 points	186	13.8							
Difference >2 points	131	12.3	1.05	0.82–1.36	0.691				
Neutrophil count, preoperative									
$<8.3 \times 10^9$/L	198	14.3							
$>8.3 \times 10^9$/L	281	11.7	1.35	1.1–1.66	0.004	1.59	0.88–2.87	0.123	0.194
Neutrophil count, postoperative									
$<8.3 \times 10^9$/L	195	15.1							
$>8.3 \times 10^9$/L	138	12	1.45	1.13–1.87	0.004	1.73	1.01–2.96	0.047	0.130
Lymphocyte count, preoperative									
$<4 \times 10^9$/L	472	12.4							
$>4 \times 10^9$/L	9	23.2	0.73	0.35–1.55	0.412				
Lymphocyte count, postoperative									
$<4 \times 10^9$/L	328	13.4							
$>4 \times 10^9$/L	4	18.2	0.96	0.24–3.87	0.955				
Monocyte count, preoperative									
$<0.8 \times 10^9$/L	314	12							
$>0.8 \times 10^9$/L	167	13.4	0.94	0.76–1.15	0.540				
Monocyte count, postoperative									
$<0.8 \times 10^9$/L	232	13.6							
$>0.8 \times 10^9$/L	100	12.6	1.18	0.91–1.55	0.214				
WBC count, preoperative									
$<10 \times 10^9$/L	176	14.1							
$>10 \times 10^9$/L	304	12	1.26	1.02–1.56	0.032	0.77	0.45–1.34	0.359	0.494
WBC count, postoperative									
$<10 \times 10^9$/L	171	14.3							
$>10 \times 10^9$/L	160	12.4	1.31	1.02–1.68	0.033	0.64	0.38–1.06	0.085	0.163
CRP, preoperative									
<10 mg/L	200	12.3							
>10 mg/L	16	9.6	1.54	0.9–2.62	0.113				
CRP, postoperative									
<10 mg/L	56	13							
>10 mg/L	90	12.4	0.85	0.57–1.25	0.402				

Continues

Table 3. Continued

Variable	Patients (*n*)	OS (%)	Univariable			Multivariable			FDR-Adjusted *P* Value
			HR	95% CI	*P* Value	HR	95% CI	*P* Value	
ESR, preoperative									
<11 mm/hour (male), <24 mm/hour (female)	133	12.5							
>11 mm/hour (male), >24 mm/hour (female)	20	10.1	1.32	0.81–2.16	0.260				
ESR, postoperative									
>11 mm/hour (male), >24 mm/hour (female)	6	11.7							
>11 mm/hour (male), >24 mm/hour (female)	15	11.4	1.07	0.34–3.38	0.908				
Platelet count, preoperative									
$<450 \times 10^3/mm^3$	465	12.4							
$>450 \times 10^3/mm^3$	16	12.7	0.87	0.49–1.56	0.649				
Platelet count, postoperative									
$<450 \times 10^3/mm^3$	320	13.5							
$>450 \times 10^3/mm^3$	9	20.6	0.74	0.35–1.58	0.441				
PT, preoperative									
<12.2 seconds	18	11.2							
12.2–15.2 seconds	392	12.4	0.98	0.58–1.64	0.926				
>15.2 seconds	2	20.2	0.95	0.22–4.16	0.946				
aPTT, preoperative									
<27 seconds	79	9.9							
27–37 seconds	311	13	0.71	0.54–0.93	0.014				
>37 seconds	26	14.5	0.51	0.3–0.86	0.011				
aPTT, postoperative									
<27 seconds	7	6.8							
27–37 seconds	32	10.6	0.9	0.34–2.37	0.835				
>37 seconds	7	10.8	1.22	0.37–4.08	0.744				
LDH, preoperative									
< 250 U/L	101	13.8							
> 250 U/L	46	11.7	1.46	1.01–2.11	0.043				
LDH, postoperative									
< 250 U/L	91	15.1							
> 250 U/L	109	13.5	1.08	0.78–1.5	0.624				

Univariable and multivariable analyses were performed to identify significant correlations between clinical and blood-based variables on glioblastoma patient survival.

OS, overall survival; HR, hazard ratio; CI, confidence interval; FDR, false discovery rate; RT, radiotherapy; ChT, chemotherapy; RT-ChT, chemoradiotherapy; KPS, Karnofsky performance scale; IDH1, isocitrate dehydrogenase 1; Hb, hemoglobin; NLR, neutrophil/lymphocyte ratio; WBC, white blood cell; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PT, prothrombin time; aPTT, activated partial thromboplastin time; LDH, lactate dehydrogenase.

The reason we were unable to confirm some of the reported significant values remains to be elucidated. Some studies were underpowered (<100 patients), which could have introduced bias owing to outliers in the data. Another reason could have been because some of the studies reported data only on specific blood values that showed significant correlations with patient survival.

This could have introduced publication bias because studies showing insignificant results were not reported.

In our study, patient age was not significantly associated with patient survival. This is in contrast to the results reported by others.[5,6] Most probably, the findings could be explained by the greater percentages of monotherapy and biopsy-alone status in the group of patients aged >70 years. The percentage of patients receiving chemoradiotherapy was 88%, 73%, and 37% for those aged <50, 50–70, and >70 years, respectively. The corresponding percentages of biopsies as the surgical intervention were 23%, 27%, and 35%. Dichotomization of the data set into patient groups aged <70 and >70 years did not result in a significant association for age group status in the multivariable model (data not shown).

The present study also had some limitations. First, owing to its retrospective and single-center design, we were dependent on the physician's choices and reports. For example, not every blood test evaluated in the present study was a part of the routine preoperative evaluation and was thus only obtained for clinical indications. In line with this, Hb was a part of the routine evaluation for 96.8% of the patients before surgery. In contrast, information on the preoperative LDH levels (not a part of the routine evaluation) was only available for 29.6% of the patients. Also, we did not have any data on the MGMT (O6-methylguanine-DNA methyltransferase) promoter methylation status or Mini-Mental State Examination, which are known prognostic markers for glioblastoma.[5,6] Additionally, because the studied population preceded the 2016 WHO classification (in which the assessment of *IDH* mutation status is required[2]), information on *IDH1* status was only available for 55.1% of the patients. Finally, as discussed, glucocorticoid usage can influence the neutrophil counts and its associated NLR. However, we could not correct for glucocorticoid usage, because data on its usage were not available. Future research on the potential prognostic values of routine blood values should ideally be performed prospectively. To further strengthen the value of such a study, the participating patients should all undergo the same tests to avoid selection bias regarding the applied tests. Thus, the true (biological) value of the specific routine blood values could be tested.

As an alternative to routine blood tests, other tests might have more potential. These tests include tests of circulating tumor cells,[34] extracellular vesicles (EVs),[35] nucleic acids,[36] proteins, and oncometabolites (as reviewed for glioblastoma by Kros et al.[37]). However, these potential valuable markers also have technical challenges. For example, EVs—nano-size plasma membrane-contained vesicles that contain RNA and proteins from its donor cell[35,38]—in serum from patients with glioblastoma can contain tumor-derived proteins, micro-RNAs and mRNA, including epidermal growth factor receptor variant III mRNA.[39] Because the lipid bilayer of EVs protects their contents from degradation in the blood, they are interesting candidates for clinical monitoring of patients with glioblastoma. However, before these vesicles can be used routinely in the clinic, several challenges should be addressed. For example, epidermal growth factor receptor variant III is only present in ~30% of patients with glioblastoma, suggesting that other tumor-specific RNAs are needed to detect and monitor glioblastomas and their response to therapy. In addition, the experimental protocols used to investigate EV-derived RNA can greatly influence the detection sensitivity and specificity of a mutation.[3,40,41] Given these, and other challenges, these alternatives to routine blood tests have clinical potential but have yet to be validated for routine use in patients with glioblastoma.

CONCLUSION

In the present study, no prognostic value was found for the results from routine blood tests in patients with glioblastoma treated with chemotherapy and/or radiation therapy. Although the wide availability, low cost, and common use of these routine tests make them attractive as clinical prognosticators, we could not confirm their clinical relevance.

REFERENCES

1. Ostrom QT, Gittleman H, Farah P, et al. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2006-2010. *Neurooncol*. 2013;15(suppl 2): ii1-ii56.
2. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol*. 2016;131:803-820.
3. Brennan CW, Verhaak RGW, McKenna A, et al. The somatic genomic landscape of glioblastoma. *Cell*. 2013;155:462-477.
4. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial . 2009;10:459-466.
5. Mineo J-F, Bordron A, Baroncini M, et al. Prognosis factors of survival time in patients with glioblastoma multiforme: a multivariate analysis of 340 patients. *Acta Neurochir (Wien)*. 2007;149: 245-252 [discussion: 252-253].
6. Gorlia T, van den Bent MJ, Hegi ME, et al. Nomograms for predicting survival of patients with newly diagnosed glioblastoma: prognostic factor analysis of EORTC and NCIC trial 26981-22981/CE.3. *Lancet Oncol*. 2008;9:29-38.
7. Céfaro GA, Genovesi D, Vinciguerra A, et al. Prognostic impact of hemoglobin level and other factors in patients with high-grade gliomas treated with postoperative radiochemotherapy and sequential chemotherapy based on temozolomide: a 10-year experience at a single institution. *Strahlenther Onkol*. 2011;187:778-783.
8. Lally BE, Colasanto JM, Fischer JJ, Knisely JPS. Is there an optimal hemoglobin level for patients with glioblastoma multiforme? *Cancer J*. 2004;10: 391-396.
9. Odrazka K, Petera J, Kohlova T, et al. Prognostic impact of hemoglobin level prior to radiotherapy on survival in patients with glioblastoma. *Strahlenther Onkol*. 2003;179:615-619.
10. Lutterbach J, Sauerbrei W, Guttenberger R. Multivariate analysis of prognostic factors in patients with glioblastoma. *Strahlenther Onkol*. 2003; 179:8-15.
11. Bambury RM, Teo MY, Power DG, et al. The association of pre-treatment neutrophil to lymphocyte ratio with overall survival in patients with glioblastoma multiforme. *J Neurooncol*. 2013;114: 149-154.
12. McNamara MG, Lwin Z, Jiang H, et al. Factors impacting survival following second surgery in patients with glioblastoma in the temozolomide treatment era, incorporating neutrophil/lymphocyte ratio and time to first progression. *J Neurooncol*. 2014;117:147-152.
13. Han S, Liu Y, Li Q, Li Z, Hou H, Wu A. Pre-treatment neutrophil-to-lymphocyte ratio is associated with neutrophil and T-cell infiltration and predicts clinical outcome in patients with glioblastoma. *BMC Cancer*. 2015;15:617.

14. Reynés G, Vila V, Martín M, et al. Circulating markers of angiogenesis, inflammation, and coagulation in patients with glioblastoma. *J Neurooncol*. 2010;102:35-41.

15. Williams M, Liu ZW, Woolf D, et al. Change in platelet levels during radiotherapy with concurrent and adjuvant temozolomide for the treatment of glioblastoma: a novel prognostic factor for survival. *J Cancer Res Clin Oncol*. 2012;138:1683-1688.

16. Strojnik T, Smigoc T, Lah TT. Prognostic value of erythrocyte sedimentation rate and C-reactive protein in the blood of patients with glioma. *Anticancer Res*. 2014;34:339-347.

17. Brockmann MA, Giese A, Mueller K, et al. Preoperative thrombocytosis predicts poor survival in patients with glioblastoma. *Neuro Oncol*. 2007;9:335-342.

18. Han S. The prognostic role of preoperative serum albumin levels in glioblastoma patients. *BMC Cancer*. 2015;15:108.

19. Wilson WR, Hay MP. Targeting hypoxia in cancer therapy. *Nat Rev Cancer*. 2011;11:393-410.

20. Vaupel P. The role of hypoxia-induced factors in tumor progression. *Oncologist*. 2004;9(suppl 5):10-17.

21. Rudat V, Stadler P, Becker A, et al. Predictive value of the tumor oxygenation by means of pO_2 histography in patients with advanced head and neck cancer. *Strahlenther Onkol*. 2001;177:462-468.

22. Brizel DM, Dodge RK, Clough RW, Dewhirst MW. Oxygenation of head and neck cancer: changes during radiotherapy and impact on treatment outcome. *Radiother Oncol*. 1999;53:113-117.

23. Pedersen D, Søgaard H, Overgaard J, Bentzen SM. Prognostic value of pretreatment factors in patients with locally advanced carcinoma of the uterine cervix treated by radiotherapy alone. *Acta Oncol*. 1995;34:787-795.

24. Quilty PM, Duncan W. The influence of hemoglobin level on the regression and long term local control of transitional cell carcinoma of the bladder following photon irradiation. *Int J Radiat Oncol Biol Phys*. 1986;12:1735-1742.

25. Coffelt SB, Wellenstein MD, de Visser KE. Neutrophils in cancer: neutral no more. *Nat Rev Cancer*. 2016;16:431-446.

26. Yang L, DeBusk LM, Fukuda K, et al. Expansion of myeloid immune suppressor Gr+CD11b+ cells in tumor-bearing host directly promotes tumor angiogenesis. *Cancer Cell*. 2004;6:409-421.

27. Bald T, Quast T, Landsberg J, et al. Ultraviolet-radiation-induced inflammation promotes angiotropism and metastasis in melanoma. *Nature*. 2014;507:109-113.

28. Broekman ML, Maas SLN, Abels ER, Mempel TR, Krichevsky AM, Breakefield XO. Multidimensional communication in the microenvirons of glioblastoma. *Nat Rev Neurol*. 2018;14:482-495.

29. Liang J, Piao Y, Holmes L, et al. Neutrophils promote the malignant glioma phenotype through S100A4. *Clin Cancer Res*. 2014;20:187-198.

30. Liles WC, Dale DC, Klebanoff SJ. Glucocorticoids inhibit apoptosis of human neutrophils. *Blood*. 1995;86:3181-3188.

31. Berg ten MJ, Huisman A, van den Bemt PMLA, Schobben AFAM, Egberts ACG, van Solinge WW. Linking laboratory and medication data: new opportunities for pharmacoepidemiological research. *Clin Chem Lab Med*. 2007;45:13-19.

32. Therneau TM, Grambsch PM. *Modeling Survival Data: Extending the Cox Model*. New York, NY: Springer Science & Business Media; 2000.

33. Fiorentino A, Fusco V. Elderly patients affected by glioblastoma treated with radiotherapy: the role of serum hemoglobin level. *Int J Neurosci*. 2012;123:133-137.

34. Alix-Panabières C, Pantel K. Challenges in circulating tumour cell research. *Nat Rev Cancer*. 2014;14:623-631.

35. Maas SLN, Breakefield XO, Weaver AM. Extracellular vesicles: unique intercellular delivery vehicles. *Trends Cell Biol*. 2017;27:172-188.

36. Aravanis AM, Lee M, Klausner RD. Next-generation sequencing of circulating tumor DNA for early cancer detection. *Cell*. 2017;168:571-574.

37. Kros JM, Mustafa DM, Dekker LJM, Sillevis Smitt PAE, Luider TM, Zheng P-P. Circulating glioma biomarkers. *Neuro Oncol*. 2015;17:343-360.

38. Abels ER, Breakefield XO. Introduction to extracellular vesicles: biogenesis, RNA cargo selection, content, release, and uptake. *Cell Mol Neurobiol*. 2016;36:301-312.

39. Skog J, Würdinger T, van Rijn S, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol*. 2008;10:1470-1476.

40. Mateescu B, Kowal EJK, van Balkom BWM, et al. Obstacles and opportunities in the functional analysis of extracellular vesicle RNA—an ISEV position paper. *J Extracell Vesicles*. 2017;6:1286095.

41. Théry C, Witwer KW, Aikawa E, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles*. 2018;7:1535750.

Conflict of interest statement: The present study was financially supported, in part, by the Hersenstichting Nederland (Foundation Concerned with Diseases of the Brain), the Schumacher Kramer Stichting, and the T & P Bohnenn Foundation.

Received 10 January 2019; accepted 6 March 2019

Citation: World Neurosurg. (2019) 126:e1081-e1091. https://doi.org/10.1016/j.wneu.2019.03.053

Journal homepage: ***www.journals.elsevier.com/world-neurosurgery***

Available online: ***www.sciencedirect.com***