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CD163 Expression as an Independent Prognostic Biomarker in Glioblastoma: A Digital Pathology and Survival Analysis Study

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Abstract

Purpose

Glioblastoma (GBM) remains one of the most lethal malignancies with a median survival of approximately 15 months despite maximal therapy. Tumor-associated macrophages (TAMs) of the M2 phenotype, characterized by CD163 expression, are known to promote immunosuppression, angiogenesis, and invasion. This study aimed to evaluate the prognostic significance of CD163 + macrophage density in GBM patients and to establish a clinically applicable cut-off value for risk stratification.

Methods

A retrospective cohort of 40 patients with histologically confirmed GBM was included. CD163 + cell density (cells/mm²) was quantified on whole-slide immunohistochemical images using QuPath digital pathology software. Correlations between CD163 density and overall survival (OS), progression-free survival (PFS), and recurrence interval (IR) were assessed using Spearman's rank correlation. ROC analysis was performed to determine the optimal cut-off for predicting 12-month mortality. Kaplan–Meier survival analysis with Log-rank test and Cox proportional hazards regression were used to evaluate prognostic impact.

Results

CD163 density showed a significant negative correlation with OS ($\rho = -0.502$, $p = 0.001$). ROC analysis identified an optimal cut-off of 2978.7 cells/mm² (AUC = 0.748). Patients with CD163 ≥ cut-off had significantly shorter median OS compared to those below the cut-off (10.0 vs. 19.0 months; Log-rank $\chi^2 = 7.71$, $p = 0.0055$). In multivariate Cox regression, CD163 remained an independent predictor of OS (HR = 1.75, 95% CI: 1.06–2.89, $p = 0.028$), independent of age.

Conclusion

High CD163 + macrophage density is a strong and independent negative prognostic factor in GBM. The cut-off of 2978.7 cells/mm² effectively stratifies patients into distinct prognostic groups, offering a potential tool for personalized treatment decisions. These findings support the integration of CD163 immunohistochemistry into routine diagnostic workup and highlight TAMs as promising therapeutic targets.

Introduction

Glioblastoma (GBM) is the most common and aggressive primary malignant brain tumor in adults, characterized by rapid progression, diffuse infiltration, and resistance to conventional therapies. Despite maximal safe resection followed by concurrent chemoradiotherapy according to the Stupp protocol, the median overall survival (OS) remains approximately 14.6 months, with a 5-year survival rate of less than

10% [2]. The poor prognosis of GBM is largely attributed to the highly immunosuppressive and heterogeneous tumor microenvironment (TME)[37].

Among the cellular components of the TME, tumor-associated macrophages (TAMs) constitute up to 30–50% of the tumor mass and play a pivotal role in promoting tumor growth, angiogenesis, invasion, and therapy resistance [7]. TAMs exhibit remarkable phenotypic plasticity, ranging from an anti-tumor M1-like phenotype to a pro-tumor M2-like phenotype [7, 8]. The M2-polarized macrophages are characterized by the expression of scavenger receptor CD163 and are associated with immunosuppression through the secretion of IL-10, TGF-β, and other immunomodulatory factors[9, 10].

CD163 has emerged as a reliable immunohistochemical marker for M2-polarized TAMs in various malignancies, including GBM. Several studies have reported an association between high CD163 expression and poor prognosis in GBM[35, 36]. However, the optimal cut-off value for stratifying patients and the independent prognostic value of CD163 when adjusted for other clinical factors remain to be fully established [23]. Additionally, the integration of digital pathology and artificial intelligence-based image analysis has enabled precise and reproducible quantification of CD163 + cells, facilitating the translation of this biomarker into clinical practice.

In this study, we aimed to: (1) evaluate the correlation between CD163 + macrophage density and clinical outcomes (OS, PFS, and recurrence interval) in a cohort of 40 GBM patients; (2) determine the optimal cut-off value of CD163 density for predicting 12-month mortality using ROC analysis; (3) assess the prognostic significance of CD163 through Kaplan–Meier survival analysis and Cox regression; and (4) discuss the potential clinical utility of CD163 as a biomarker for risk stratification and therapeutic targeting.

Materials and Methods

Study Design and Patient Population

This retrospective cohort study included 40 patients with histologically confirmed glioblastoma (WHO grade IV) who underwent surgical resection at the Neurosurgery Service of QSUNT between January and December 2024. Patients with incomplete clinical data or unavailable tumor tissue for immunohistochemical analysis were excluded. From an initial pool of 56 GBM cases identified in the Pathology Service records, 16 patients were excluded due to insufficient follow-up data or incomplete clinical records, leaving 40 patients for final analysis. The study was approved by the Institutional Ethics Committee and was conducted in accordance with the Declaration of Helsinki.

Clinical Data Collection

Demographic and clinical data, including age, sex, date of diagnosis, date of surgery, date of recurrence (if applicable), date of progression, date of last follow-up, and date of death, were extracted from

electronic medical records. Overall survival (OS) was defined as the time from surgery to death or last follow-up. Progression-free survival (PFS) was defined as the time from surgery to first radiological or clinical progression. Recurrence interval (IR) was defined as the time from surgery to first radiological confirmation of recurrence.

Immunohistochemistry and Digital Quantification

Formalin-fixed, paraffin-embedded (FFPE) tumor tissue sections (3–4 μm) were stained with anti-CD163 monoclonal antibody (clone MRQ-26, Roche Diagnostics, Mannheim, Germany) using standard immunohistochemical protocols. Immunohistochemical staining for CD163 was performed using the fully automated Bench Mark ULTRA PLUS slide staining system (Roche Diagnostics). The system automatically performs baking, deparaffinization, cell conditioning, and staining. Following staining, sections were counterstained with hematoxylin, dehydrated, and coverslipped.

Following immunohistochemical staining, all slides were digitized using the VENTANA DP 200 whole-slide brightfield scanner (Roche Diagnostics, Basel, Switzerland) at 20× magnification. The scanner utilizes dynamic focus optical technology and built-in calibrations to produce high-resolution images. Images were acquired in BIF format and subsequently exported as TIFF files for quantitative analysis of CD163 + cells, using QuPath software (version 0.4.3). For each case, areas of necrosis, hemorrhage, and technical artifacts were excluded from analysis. The mean number of CD163-positive cells per mm^2 was calculated from at least 5 randomly selected high-power fields (HPFs) within the viable tumor area. Standard deviation (SD) was also recorded to assess intratumoral heterogeneity.

Statistical Analysis

All statistical analyses were performed using AI statistical analysis with subscription. Operate AI was used as an assistive tool to generate code for the Cox proportional hazards regression model. All AI-generated codes and suggestions were independently verified by the authors, and the final interpretation of all statistical results remains the sole responsibility of the authors. Spearman correlation, ROC analysis, Kaplan–Meier survival analysis, and Log-rank testing were performed using standard statistical packages.

The Shapiro–Wilk test was used to assess normality of continuous variables. Given the non-normal distribution of the data ($p < 0.05$ for all variables), correlations between CD163 density and clinical parameters (OS, PFS, IR) were evaluated using Spearman's rank correlation coefficient (ρ).

Receiver operating characteristic (ROC) curve analysis was performed to determine the optimal CD163 cut-off for predicting 12-month mortality. The cut-off was selected by maximizing the Youden index (sensitivity + specificity − 1). Area under the curve (AUC) with 95% confidence interval (CI) was calculated.

Kaplan–Meier survival curves were constructed to compare OS between the high-CD163 and low-CD163 groups, with differences assessed using the Log-rank (Mantel-Cox) test. Cox proportional hazards regression was used to estimate hazard ratios (HR) with 95% CI for univariate and multivariate analyses. In the multivariate model, CD163 (log10-transformed to improve normality) and age (categorized as < 65 vs. ≥65 years) were included as covariates.

A p-value < 0.05 was considered statistically significant. All tests were two-sided.

Results

Patient Characteristics

A total of 40 patients (25 males, 62.5%; 15 females, 37.5%) with a mean age of 61.86 ± 9.13 years were included. Thirty-four patients (85.0%) presented with primary GBM, while 6 (15.0%) had recurrent disease. At the end of follow-up, 38 patients (95.0%) were deceased, while 2 patients (5.0%) remained alive and were censored at their last documented follow-up. The median overall survival for the entire cohort was 17.5 months.

Characteristics of the study population as shown in Table 1.

Table 1
Characteristics of the study population (n = 40). a Abbreviations in table: GBM, glioblastoma; SD, standard deviation.

Characteristic	Value
Age (years), mean ± SD	61.86 ± 9.13
Sex, n (%)	
Male	25 (62.5%)
Female	15 (37.5%)
Disease status, n (%)	
Primary GBM	34 (85.0%)
Recurrent GBM	6 (15.0%)
Survival status, n (%)	
Deceased	38 (95.0%)
Alive (censored)	2 (5.0%)

Descriptive Statistics

The mean CD163 density was 2180.0 cells/mm² (SD = 1558.2), with a median of 2081.0 cells/mm² (IQR: 1197.5–3217.8). Mean OS was 17.05 ± 8.79 months (median: 17.5, IQR: 11.5–23.0), mean PFS was 11.88 ± 7.37 months (median: 11.0, IQR: 5.0–18.2), and mean IR was 11.74 ± 6.05 months (median: 11.0, IQR: 7.0–16.0). As shown in Table 2.

Table 2

Descriptive statistics for survival parameters and CD163 and Spearman correlations between CD163 and clinical outcomes (n = 40). Abbreviations in table: OS, overall survival; PFS, progression-free survival; IR, recurrence interval; SD, standard deviation; IQR, interquartile range; ρ, Spearman's rank correlation coefficient. Statistically significant at $p < 0.05$.

Variable	Mean	SD	Median	IQR	Range	Spearman ρ	p-value
OS (months)	17.05	8.79	17.5	11.5–23.0	0–37	**−0.502**	0.001
PFS (months)	11.88	7.37	11.0	5.0–18.2	0–30	**−0.438**	0.005
IR (months)	11.74	6.05	11.0	7.0–16.0	2–24	**−0.168**	0.403
CD163 (cells/mm²)	2180.0	1558.2	2081.0	1197.5–3217.8	75–5357		

Correlation Between CD163 Density and Clinical Outcomes

CD163 density showed a significant negative correlation with OS (ρ = −0.502, p = 0.001). A negative trend was also observed for PFS (ρ = −0.438, p = 0.005), indicating a statistically significant correlation. No significant correlation was found between CD163 density and recurrence interval (IR) (ρ = −0.168, p = 0.403). As shown in Table 2.

ROC Analysis and Optimal Cut-off Determination

ROC analysis for predicting 12-month mortality yielded an AUC of 0.748 (95% CI: 0.58–0.91). The optimal cut-off value of CD163 density was determined to be 2978.7 cells/mm², with a sensitivity of 60.0% and specificity of 83.3%. Based on this cut-off, patients were stratified into two groups: low CD163 (< 2978.7 cells/mm²; n = 29) and high CD163 (≥ 2978.7 cells/mm²; n = 11).

Kaplan–Meier Survival Analysis

Kaplan–Meier analysis revealed a significant difference in OS between the two groups (Log-rank χ^2 = 7.71, p = 0.0055). Patients with low CD163 had a median OS of 19.0 months, compared to 10.0 months in the high-CD163 group—a clinically meaningful difference of 9 months. Survival probabilities at 6, 12, and 24 months were consistently higher in the low-CD163 group (89.7%, 72.4%, and 48.3%, respectively) compared to the high-CD163 group (63.6%, 45.5%, and 18.2%, respectively).

Kaplan–Meier survival analysis revealed a significant difference in overall survival between the two CD163 groups (Log-rank χ^2 = 7.71, p = 0.0055) (Table 3, Fig. 1). Patients with CD163 below the cut-off

had a median OS of 19.0 months, compared to 10.0 months in the high-CD163 group.

Table 3
Kaplan–Meier survival outcomes by CD163 group. a Abbreviations in table: OS, overall survival; CD163, CD163-positive cell density. Log-rank (Mantel-Cox) test; $\chi^2 = 7.71$. Statistically significant at $p < 0.05$.

Parameter	CD163 < 2978.7 (n = 29)	CD163 ≥ 2978.7 (n = 11)	Difference
Median OS (months)	19.0	10.0	9.0 months
6-month survival	89.7%	63.6%	26.1%
12-month survival	72.4%	45.5%	26.9%
24-month survival	48.3%	18.2%	30.1%
Log-rank test	$\chi^2 = 7.71$	p = 0.0055	Highly significant

Cox Regression Analysis

In univariate Cox regression, CD163 (log10-transformed) was significantly associated with OS (HR = 1.82, 95% CI: 1.15–2.88, p = 0.011). In multivariate analysis adjusting for age (≥ 65 years), CD163 remained an independent predictor of OS (HR = 1.75, 95% CI: 1.06–2.89, p = 0.028). Age was not a significant independent predictor in this model (HR = 1.45, 95% CI: 0.78–2.68, p = 0.240).

Discussion

This study demonstrates that high CD163 + macrophage density is a strong and independent negative prognostic factor in glioblastoma. Using digital pathology-based quantification, we identified an optimal cut-off of 2978.7 cells/mm² that effectively stratifies patients into distinct prognostic groups, with a 9-month difference in median OS between low- and high-CD163 groups (19.0 vs. 10.0 months, p = 0.0055).

Correlation with Clinical Outcomes

The negative correlation between CD163 density and OS ($\rho = -0.502$, p = 0.001) is consistent with multiple previous studies. In a large meta-analysis, high CD163 expression was associated with significantly worse OS in GBM [20]. Similarly, a recent study reported that patients with high CD163 + TAM density had a median OS of 12.0 months compared to 42.0 months in those with low density [26]. Our findings extend these observations by providing a specific, reproducible cut-off value derived from ROC analysis, which may facilitate clinical implementation.

The correlation between CD163 density and PFS ($\rho = -0.438$, p = 0.005) in our cohort suggests that M2-polarized macrophages also contribute to earlier disease progression. However, the lack of a significant correlation with recurrence interval (IR) may be attributed to the relatively small sample size (n = 40) and the heterogeneity of post-recurrence treatments. It is also possible that CD163 predominantly influences

overall survival through mechanisms beyond early progression, such as resistance to chemoradiotherapy or the development of cachexia and systemic immunosuppression [8, 10, 12].

Prognostic Independence

The independent prognostic value of CD163 in multivariate analysis, even after adjusting for age, underscores its potential as a clinically useful biomarker. In our model, CD163 had a higher HR (1.75) than age (1.45), suggesting that the macrophage infiltrate may be a more powerful predictor of outcome than chronological age in this cohort. This finding aligns with the emerging concept that the tumor immune microenvironment is a key determinant of GBM prognosis.

Biological Mechanisms

From a biological perspective, the mechanisms underlying the prognostic impact of CD163 + TAMs are well-established (Fig. 2).

M2-polarized macrophages promote tumor progression through: (1) secretion of immunosuppressive cytokines (IL-10, TGF-β) that inhibit cytotoxic T-cell activity; (2) production of angiogenic factors (VEGF) that stimulate neovascularization; (3) release of matrix metalloproteinases that facilitate invasion; and (4) induction of resistance to temozolomide and radiotherapy. CD163 itself may also play a direct role in tumor cell proliferation through its interaction with haptoglobin-hemoglobin complexes, which have been shown to activate signaling pathways in GBM cells. [3, 4]

Clinical Implications

The identification of a clear cut-off value (2978.7 cells/mm²) has important clinical implications. Patients with CD163 levels above this threshold could be considered for more aggressive surveillance, earlier second-line therapies, or enrollment in clinical trials evaluating immunomodulatory agents. Furthermore, CD163 represents a potential therapeutic target. Strategies aimed at reprogramming M2 macrophages toward an M1-like phenotype—using agents such as CSF-1R inhibitors, CD47 blockers, or TLR agonists—are currently under investigation and may be particularly beneficial for patients with high CD163 expression.

Digital Pathology and Artificial Intelligence

The integration of artificial intelligence with digital pathology is transforming modern oncology. Digital pathology enables complete digitization of histopathological slides, creating the foundation for advanced analyses. AI algorithms, particularly deep learning networks, are significantly increasing the accuracy and efficiency of image analysis, enabling automated tumor detection and classification, identification of prognostic molecular biomarkers, and prediction of treatment response and patient outcomes. In GBM, these technologies are addressing the high heterogeneity of the tumor and the weak correlation between histological phenotypes and prognosis.[27] Recent studies have used convolutional neural networks (CNNs) to accurately map the spatial distribution of transcriptional subtypes of GBM

and identify tumor regions associated with worse outcomes, achieving high performance (AUC > 0.80) in classifying molecular subtypes [28].

Limitations

Several limitations should be acknowledged. First, the retrospective design and relatively small sample size (n = 40) limit the generalizability of our findings and precluded more complex multivariate models. Second, we did not have data on MGMT promoter methylation status, which is a known prognostic and predictive factor in GBM. Third, the cut-off value was derived from a single-center cohort and requires external validation in independent, larger datasets. Fourth, the use of a single marker (CD163) may not fully capture the complexity of TAM polarization; additional markers such as CD206, CD204, or the CD163/CD8 ratio could provide complementary information.

Future Directions

Future studies should aim to validate our findings in prospective, multi-center cohorts with larger sample sizes. The integration of CD163 quantification into routine pathological reporting, potentially through AI-assisted digital pathology platforms, could facilitate widespread adoption. Additionally, exploring the relationship between CD163 density and response to specific therapies, particularly immunotherapies and anti-angiogenic agents—may help identify patient subgroups most likely to benefit from these treatments.

Conclusion

In conclusion, this study confirms that high CD163 + macrophage density is a strong, independent, and negative prognostic factor in glioblastoma. The cut-off value of 2978.7 cells/mm² effectively stratifies patients into groups with significantly different outcomes (median OS: 19.0 vs. 10.0 months, p = 0.0055). CD163 remained an independent predictor of OS in multivariate analysis after adjusting for age (HR = 1.75, p = 0.028). These findings support the routine assessment of CD163 in GBM biopsies as a prognostic biomarker and highlight the potential of targeting tumor-associated macrophages as a therapeutic strategy in this devastating disease.

Declarations

Competing Interests:

The authors have no relevant financial or non-financial interests to disclose.

Ethics Approval:

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the University of Medicine, Tirana.

Consent to Participate:

Informed consent was obtained from all individual participants included in the study.

Consent to Publish:

The authors affirm that human research participants provided informed consent for publication of the data included in this study.

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Author Contribution

E.N. and M.I. conceived and designed the study. E.N. performed material preparation, data collection, and statistical analysis. E.N. wrote the main manuscript text. M.I. provided supervision, contributed to the interpretation of histopathological materials, and critically revised the manuscript for important intellectual content. All authors reviewed and approved the final manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. Aggregated data supporting the findings of this study are presented within the manuscript. Individual patient-level data are not publicly available due to privacy and ethical restrictions in accordance with the institutional ethics committee and the General Data Protection Regulation (GDPR) requirements.

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Figures

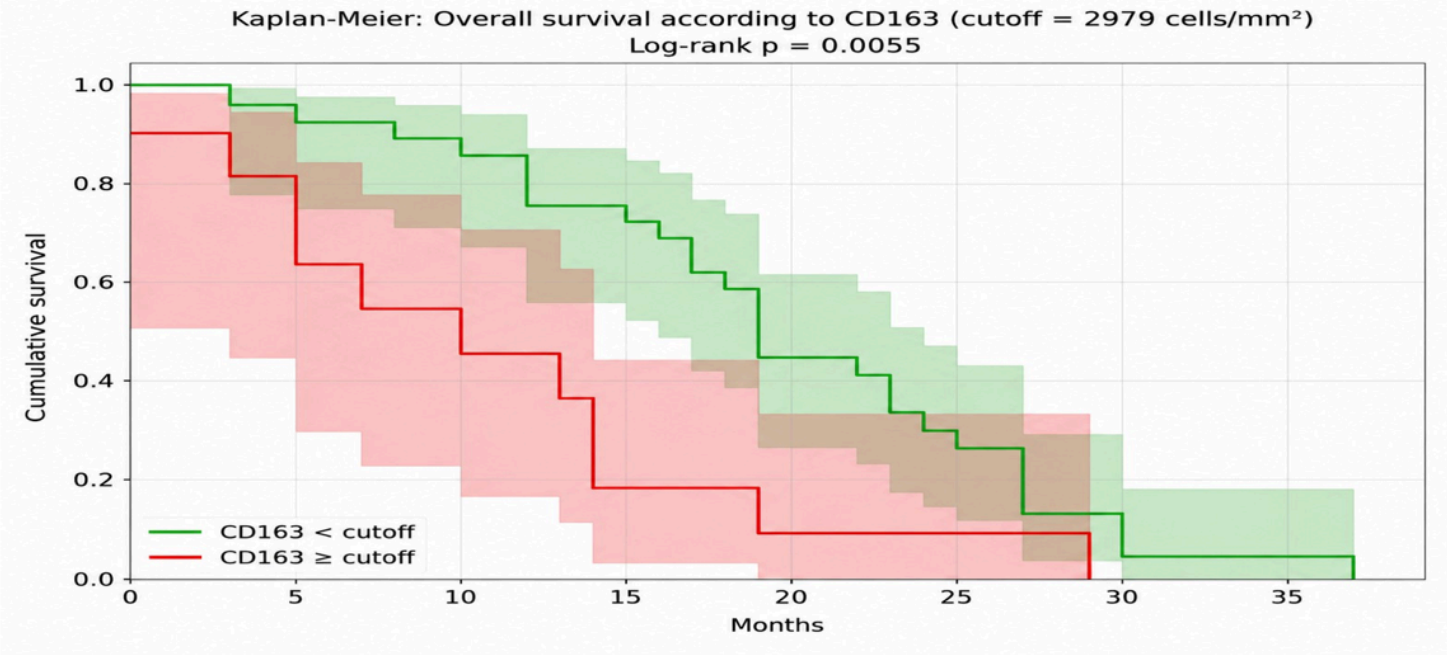


Figure 1

Kaplan–Meier survival curves for overall survival according to CD163 expression level. Patients with CD163 density ≥ 2978.7 cells/mm² (high CD163, n=11) had significantly shorter survival compared to those with CD163 < 2978.7 cells/mm² (low CD163, n=29) (Log-rank $\chi^2 = 7.71$, $p = 0.0055$).

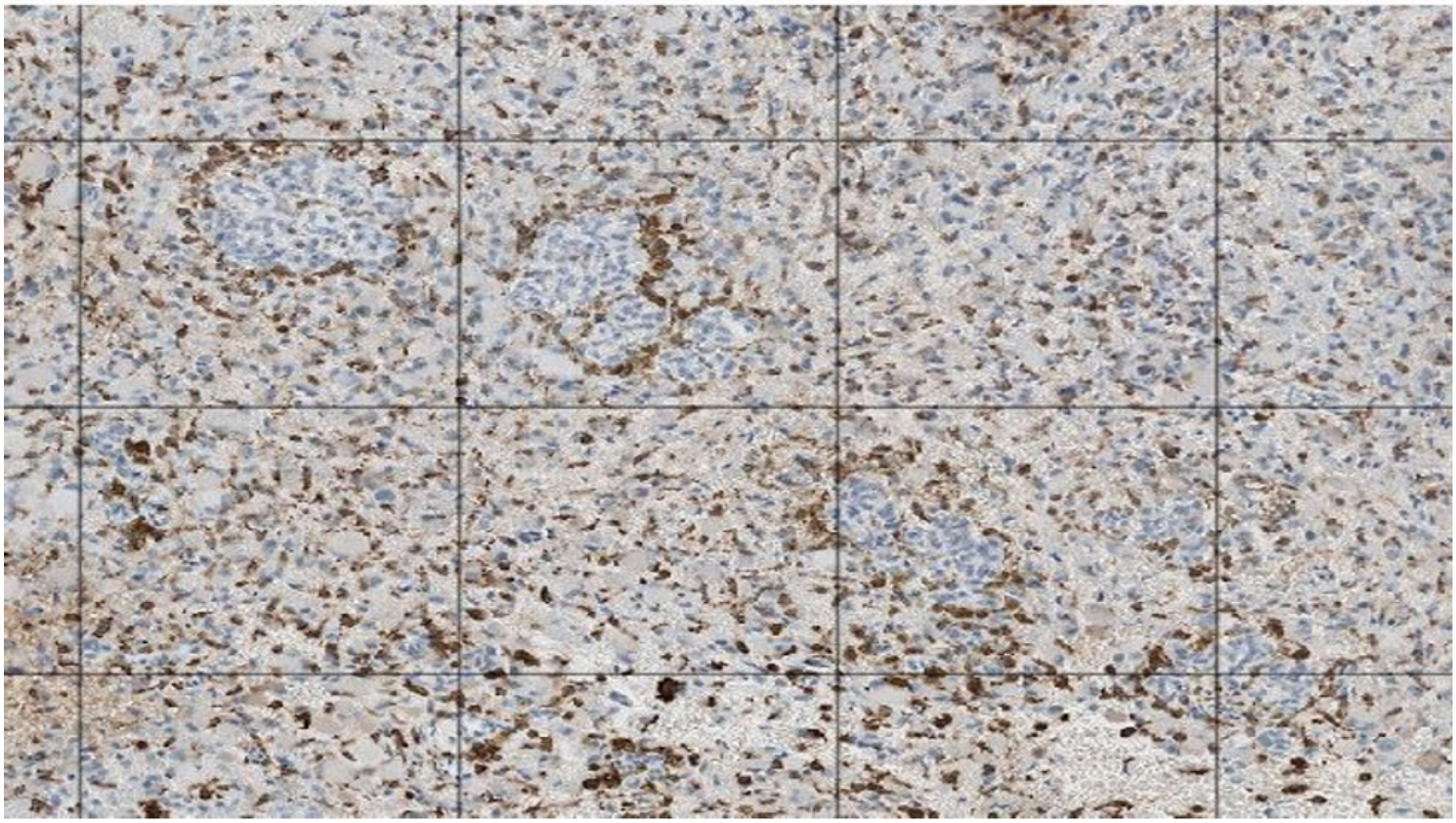


Figure 2

Immunohistochemical staining for CD163 in a glioblastoma specimen from a patient with short overall survival (OS = 6 months). This extensive infiltration of M2-polarized macrophages, coupled with intense aberrant angiogenesis, is indicative of a profoundly immunosuppressive and aggressive tumor microenvironment, correlating with rapid disease progression and unfavorable prognosis. Original magnification: ×20; scale bar: 100 μm. Counterstain: hematoxylin (blue nuclei).