U.PORTO

FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2021/2022

Nuno Alexandre Mendes Piedade

Sequential Evaluation of Hematology Values as a Prognostic Factor in Glioblastoma Patients

MARÇO, 2022

Nuno Alexandre Mendes Piedade

Sequential Evaluation of Haematology Values as a Prognostic Factor in Glioblastoma Patients

Mestrado Integrado em Medicina

Área: Neurocirurgia

Tipologia: Dissertação

Trabalho efetuado sob a Orientação de:

Professor Doutor Rui Manuel Cardoso Vaz

Coorientação de:

Professor Doutor Paulo José Campos Linhares Vieira

Trabalho organizado de acordo com as normas da revista: Journal of Neuro-Oncology, Springer

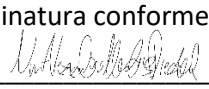
MARÇO, 2022

Eu, Nuno Alexandre Mendes Piedade, abaixo assinado, nº mecanográfico 2016003618, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro ter atuado com absoluta integridade na elaboração deste projeto de opção.

Neste sentido, confirmo que NÃO incorri em plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria de um determinado trabalho intelectual, ou partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores, foram referenciadas, ou redigidas com novas palavras, tendo colocado, neste caso, a citação da fonte bibliográfica.

Faculdade de Medicina da Universidade do Porto, 05/03/2022

Assinatura conforme cartão de identificação:



U.PORTO

FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

UC Dissertação/Projeto (6º Ano) - DECLARAÇÃO DE INTEGRIDADE

NOME

Nuno Alexandre Mendes Piedade

NÚMERO DE ESTUDANTE **E-MAIL**

201603618 namp67432@gmail.com

DESIGNAÇÃO DA ÁREA DO PROJECTO

Neurocirurgia

TÍTULO DISSERTAÇÃO/MONOGRAFIA (riscar o que não interessa)

Sequential Evaluation of Hematology Values as a Prognostic Factor in Glioblastoma Patients

ORIENTADOR

Professor Doutor Rui Manuel Cardoso Vaz

COORIENTADOR (se aplicável)

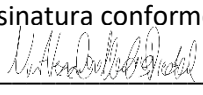
Professor Doutor Paulo José Campos Linhares Vieira

ASSINALE APENAS UMA DAS OPÇÕES:

É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTE TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	**X**
É AUTORIZADA A REPRODUÇÃO PARCIAL DESTE TRABALHO (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	
DE ACORDO COM A LEGISLAÇÃO EM VIGOR, (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) NÃO É PERMITIDA A REPRODUÇÃO DE QUALQUER PARTE DESTE TRABALHO.	

Faculdade de Medicina da Universidade do Porto, 05/03/2022

Assinatura conforme cartão de identificação:



Dedicatória

Um agradecimento ao professor Paulo Linhares pela oportunidade de realizar este trabalho sob a sua orientação e por toda a ajuda prestada. Agradeço também ao professor Manuel Ferreira-Pinto pela dedicação, paciência e tempo que sempre se ofereceu a disponibilizar na realização deste trabalho.

Agradeço aos meus grandes amigos do meu percurso universitário e não-universitário, pela amizade e alegrias partilhadas durante todos estes anos.

Um agradecimento muito especial a toda a minha família e incrível namorada, que sempre me apoiaram incondicionalmente e a quem tudo devo.

Finalmente, deixo um profundo agradecimento àqueles que já cá não estão, mas que me acompanharam e sempre acreditaram no meu sucesso pessoal e académico. Sempre foram uma fonte de coragem (*JP, LC, MM, FP, AC, FM*)!

De facto, o que eramos nós sem as pessoas?

Abstract

Purpose: Inflammatory cells are important promoters of tumour growth considered as prognostic factors. Glioblastoma (GBM) is no different. Hence, we intended to evaluate the inflammatory parameters, NLR, PLR, RDW-CV and RDW-SD in different phases of GBM treatment protocols, in order to understand whether there will be values that allow us to predict patient's prognosis in terms of progression free survival (PFS) and overall survival (OS).

Methods: The population of this study consists of a cohort of 124 patients followed in the Neurosurgery Department of a tertiary care hospital, between January/2014 and December/2018. We performed univariate and multivariate analysis correlating NLR, PLR, RDW-CV, RDW-SV with OS and PFS per treatment time-point.

Results: In our univariate analysis, several hematological parameters were related with OS and PFS. For those same parameters, we observed that pre-chemotherapy NLR value <4 had a better OS compared to values >4 ($p = 0.0007$) and NLR PreS-PosS (difference between pre-surgery and pos-surgery) differ significantly between patients with values < 5 and those with values > 5 ($p = 0.0261$). Additionally, pre-chemotherapy PLR values >9 and NLR values >4 were associated with a lower PFS ($p = 0.0008$; $p = 0.0003$). However, in multivariate analysis we did not show statistically significant values for these same parameters.

Conclusions: The results of the present study demonstrated that higher pre-chemotherapy NLR and PLR values were associated with worse prognosis and NLR PreS-PosS values are associated with better prognosis. However, none of these parameters could be considered independent prognostic factors for GBM.

Keywords: Glioblastoma; Neutrophil-Lymphocyte ratio; Platelet-Lymphocyte ratio; RDW; Overall Survival; Progression Free Survival.

Introduction

Glioblastoma (GBM) is the most aggressive primary brain tumor in adults with dismal prognosis. Current standard treatment is maximal safe surgical resection, plus concomitant radiotherapy and chemotherapy with temozolomide, followed by temozolomide in monotherapy (the Stupp Protocol). However, its efficacy remains poor, with a median survival of 14.6 months[1, 2]. Treatment developments focus on molecular therapies based on the malignancy phenotype, including signal transduction, angiogenesis and, more recently, immunotherapy[3].

It is known there are several biological characteristics that give malignant neoplasms advantages during their development. The existence of hallmarks of cancer has underpinning genomic instability and inflammation[4]. Inflammatory cells are important promoters of tumor growth, creating an environment conducive to angiogenesis and genomic instability[5].

Classic prognostic factors include tumor location, extent of recession, preoperative performance status and patient's age[6, 7]. Recently, genetic and inflammatory parameters were added to the list. In fact, the neutrophil-lymphocyte ratio (NLR) has been pointed as an independent risk factor for some malignancies[8, 9], with a relationship between disease progression and increased neutrophilic and macrophage leukocyte infiltrate[10, 11]. Additionally, it was found that the increase in the neutrophil-lymphocyte ratio (NLR) was a factor of poor prognosis on overall survival (OS) [12]. Platelet-lymphocyte ratio (PLR) was also related to the prognosis of GBM[13-16]. Pre-operative RDW was also linked with prognosis in GBM patients with the patients with higher RDW values having shorter OS[17, 18].

Therefore, the aim of this study is to evaluate the inflammatory parameters, NLR, PLR, RDW-CV and RDW-SD in the different phases of GBM treatment protocols, in order to understand whether there will be values of these same parameters that, throughout the course of the disease, allow us to predict the patient's prognosis in terms of recurrence, progression free survival (PFS) and OS. To our knowledge this is the first study to assess the progression of hematologic markers over the course of the disease.

Methods

Study Design and Settings

Retrospective cohort study with patients with GBM who were submitted to a surgical recession and initiated adjuvant treatment.

Clinical data collection

The study population consists of a cohort of 144 patients followed in the Neurosurgery Department, from January 1, 2014, to December 31, 2018, at Centro Hospitalar Universitário de São João, in Porto. The clinical registries of all patients were reviewed and for each patient, the following data were collected: date of diagnosis, age of diagnosis, date of death, time to recurrence and the type of first and second-line adjuvant treatments. In addition, neutrophil, lymphocyte, platelets, leukocytes, RDW-CV and RDW-SD values were recorded before and after surgery. Additionally, pre-radiochemotherapy and pre-chemotherapy analytical values were also recorded in patients who underwent chemoradiotherapy according the Stupp protocol. In patients under another type of treatment, the analytical values for the moment prior to their treatment were recorded.

The inclusion criteria were: 18 years of age and older; histologically confirmed GBM and tumor resection surgery; patients that have been followed for at least 18 months after tumor resection surgery, because the overall average survival is 15 months[1]. We excluded: all those who do not meet the inclusion criteria; patients that had no treatment performed; patients with no annual data and no tracking data. Regrowth tumors were also excluded.

Of the 144 patients, 14 were excluded because they did not have any treatment. 6 excluded because they did not have hematologic data (either by death or discontinuation of follow-up). There were 124 patients left for the analysis.

OS corresponded to the time between surgery and the date of death or the date of the last follow-up. PFS, corresponds to the time between surgery and the date of tumor progression documented by brain magnetic resonance imaging. In those patients that had no progression we considered that PFS is the time between the first tumor recession and death. To ensure that it is possible to assess OS and PFS in all cases of the study, we set the follow-up date limit to November 30, 2021.

For those patients with missing data in a certain time-point, we still considered their values in other time-points for our analysis. Patients that were loss from follow-up were only considered for the time they were in the study.

Data analysis

In order to probe a possible association between the NLR, PLR, RDW-CV and RDW-SV values in five different timepoints (pre-surgery, post-surgery, pre-radiotherapy and chemotherapy, pre-chemotherapy and progression) with OS and PFS, we first performed a univariate linear regression analysis. Additionally, we calculated the difference between pre-surgery NLR values and the NLR in the other time-points and the difference between pre-chemotherapy NLR and progression NLR in order to evaluate if there is any statistical alteration in these hematologic parameters that could suggest the prognosis. The same was performed for PLR values and we correlated those differences with OS and PFS.

For those who had a statistically significant result we performed a multivariate linear regression analysis correlating each hematologic parameter with OS and PFS, including also relevant population characteristics (gender, age, tumour extension, focality, type of surgery and ECOG Performance Status, second-line adjuvant systemic therapy). We also developed cut-offs for those statistically significant parameters using the median in order to evaluate any relationship in the multivariate analysis. Those same categorical variables were compared with the other clinical and demographic parameters and will be shown at the same table as the continuous hematologic values in the Results section. In the multivariate analysis we didn't consider first-line adjuvant systemic therapy as a cofounder variable because only 3 patients in a group of 124 didn't do Stupp treatment protocol. Finally, we estimated the survival differences between groups using Kaplan-Meier and log rank test.

NRL was calculated as absolute neutrophil count divided by absolute lymphocyte count and PLR was calculated as absolute platelet count divided by absolute lymphocyte count.

We considered as statistically significant a p value < 0.05. The software used for statistical analysis was Stata/SE 17.0.

Results

We included 124 patients. Demographic and clinical characteristics of our cohort are detailed in **Table 1**. The mean age at diagnosis was 60.27 ± 11.18 years. 63.71% were male. The mean OS was 20.252 ± 15.869 and mean PFS was 9.429 ± 10.038.

A supratentorial location were present in 98.39% of patients and 80.65% are unifocal tumors. Sixty-nine patients (55.65%) had only one lobe involved. Gross total resection was achieved in almost half of the patients (46.77%). The first-line adjuvant systemic therapy was chemoradiotherapy according Stupp (77.42% of cases), and TMZ + hypofractonated RT in 20.16%. Regarding to the second-line adjuvant systemic therapy, the most used was Bevacizumab + Lomustine (43.97%). A considerable number of patients did not receive any second-line adjuvant systemic therapy (38.71%).

In the univariate analysis, we were able to relate pre-chemotherapy NLR values with a decrease in OS (P = 0.040). Increases in the progression NLR (P = 0.027) and RDW-CV (P = 0.042) values are also related with a decrease in OS, in the univariate analysis. We also found that a greater difference between pre-surgery NLR and pos-surgery NLR (NLR PreS-PosS) was related with an increase in OS [p = 0.036; coef = 0.265 (0.018; 0.512)] (**Table 2**).

We evaluate these same parameters in a multivariate analysis (**Table 3**). To simplify our analysis, we considered the second-line adjuvant systemic therapy as a dichotomic variable. The type first-line adjuvant systemic therapy was not considered in our multivariate analysis, because 97,58% of patients initiated the stupp protocol. Measured as continues variables, we realize that there was no correlation of pre-chemotherapy NLR (p = 0.914), progression NLR (p = 0.105), progression RDW-CV (p = 0.581) and NLR PreS-PosS with OS. When measured as categorical variables there was also no correlation with OS (p = 0.347; p = 0.175; p = 0.622; p = 0.066, respectively).

Patients with a pre-chemotherapy NLR value <4 had a better OS compared to values >4 (mean OS = 25.575 vs 18.401 months). We performed a log-rank test showing that this difference is statistically significant (p = 0.0007). For Progression NLR values, patients with a ratio >6 did not show a worse prognosis compared with values <6 (log rank, p = 0.0606). Progression RDW-CV values <14 seemed to be correlated with worse OS, however the difference was not significant (log rank, p = 0.3109). In other hand, the variable NLR PreS-PosS differ significantly between patients with values < 5 and those with values > 5 (log rank, p = 0.0261) (mean OS 16.817 vs 23.472 months).

On univariate analysis, PFS differ significantly with pre-radiochemotherapy NLR (p = 0.016), pre-chemotherapy NLR (p = 0.021) and pre-chemotherapy PLR (p = 0.043) values. When we compared pre-surgery NLR values with pre-chemoradiotherapy NLR values, we found that a greater difference between them is associated with a higher PFS [p = 0.039; coef = 0.175 (0.009; 0.341)] (**Table 4**).

To simplify multivariate analysis, we considered again the second-line adjuvant systemic therapy as a dichotomic variable and we did not consider the first-line adjuvant systemic therapy in our multivariate analysis for the same reason mentioned before.

On multivariate analysis we did not find any independent prognostic indicator for continuous or categorical variables (**Table 3** and **5**).

Kaplan-Meier analysis (**Fig.1** and **2**) showed that pre-radiochemotherapy NLR values >5 were not significantly different from values <5 in terms of survival (log-rank, $p = 0.5827$). However, greater pre-chemotherapy PLR values (>9) were associated with a lower PFS (log-rank, $p = 0.0008$) (mean PFS 7.448 vs 11.999 months). Pre-chemotherapy NLR > 4 was also associated with shorter PFS (log rank, $p = 0.0003$) (mean PFS 7.381 vs 12.564 months). At last, the variable NLR PreS-PreRTQT (difference between pre-surgery and pre-radiochemotherapy) did not differ significantly between patients with values < -2 and those with values > -2 (log rank p = 0.5846).

Discussion

Significant changes in hematologic values seem to play a significant role in giving accurate information about patient's prognosis in several cancers, including brain tumors.

A prognostic relationship between NLR and GBM has already been described in several studies showing that higher NLR values are associated with worse prognosis[14, 16]. Han et al. related the NLR and the pre-treatment PLR with the prognosis in the GBM, demonstrating greater relevance of the NLR versus the PLR for this purpose[16]. However, it should be noted that some studies did not find a relationship between the NLR and the poor prognosis of GBM [13, 19].

In the univariate analysis we didn't find any relationship between NLR and OS in preoperative patients (p = 0.933). On the other hand, we realized that pre-chemotherapy NLR values were linked with worse OS and PFS (P = 0.040; P = 0.021). However, in the multivariate analysis we found no statistically significant relation with OS (P = 0.914) and PFS (p = 0.169). Higher progression and pre-radiochemotherapy NLR were associated with worse OS (p = 0.027) and better PFS (p = 0.016), respectively. Then we performed a multivariate analysis for these same variables, which showed us no statistically significant relation, considering confounders.

In order to evaluate the effect of NLR in different treatment time-points, we decided to create a variable that corresponds to the difference between the NLR value at the start of the follow-up (pre-surgery NLR value) and the NLR value at other treatment time-point. This simple difference might be relevant to understand if an increase or decrease of certain degree could predict our patient's prognosis. We only developed this variable for NLR, because it is the most extensively studied parameter in literature. In the univariate analysis, a greater difference between pre-surgery NLR and pos-surgery NLR is associated with better prognosis, reflecting an increase in OS (p = 0.036). Meanwhile, a greater PreS-PreRTQT NLR is associated higher PFS (p = 0.039). When we performed a multivariate analysis there was no statistically significant relation with OS or PLR (p = 0.197; p = 0.984).

With these values, and differently from other studies findings[16, 20, 21], we did not find NLR to be an independent prognostic biomarker for patient outcomes.

PLR has been described as a prognostic factor in different cancer types. Wang, et al. demonstrated that PLR is associated with a worse prognosis as an independent prognostic factor[15]. Even though, there are other studies that did not demonstrate this same prognostic relationship[13, 19].

In our study we found that pre-chemotherapy PLR value had a negative effect in PFS in the univariate analysis (P = 0.043) (table 4). However, the multivariate analysis there is no statistically significant relation with PFS (p = 0.656) (table 5).

RDW is another inflammatory parameter associated with cancer progression and prognosis [17, 22-24]. The univariate analysis showed a that progression RDW-CV values are correlated with a worse OS (p = 0.042). In a multivariate analysis, progression RDW-CV values were also related with lower survival, but it was not an independent prognostic factor (coef = -0.729 [-3.351; 1.892], p = 0.581).

The mechanism underlying RDW involvement in tumor prognosis is not totally understood but it is known that some inflammatory markers (for example, C-reactive protein) are increased and linked with higher RDW values[19, 25]. Patrick D Kelly et al. showed in a retrospective cohort that, preoperatively, this analytical parameter was not related to OS in GBM, although there was an increase its value throughout the course of the disease. This suggests some possible systemic inflammatory effect of GBM or treatment[18].

Cut-off values were developed for hematologic parameters that had statistical significance in the univariate analysis. Several studies showed that NLR values higher than 4.0 were associated with worse prognosis[14, 16]. Weng et al. demonstrated significant differences in preoperative NLR value in different gliomas groups, evidencing higher values in the GBM group, relating a lower OS of patients with NLR values >= 4.0, compared to cases of NLR < 4.0 (mean 11.23 vs 18.56, p <0.05).[26] Also Bambury et al. demonstrated that NLR > 4 would have an OS of 7.5 months versus 11.2 months in patients with NLR =<4[20].

In our work, patients with pre-chemotherapy NLR values <4 had a better prognosis compared to values >4, showing a mean OS = 18.401 months and a mean PFS = 12.564 months). For Progression NLR values, patients with a ratio >6 did not show a worse prognosis compared with values <6 (log rank, p = 0.0606). In other hand, a NLR PreS-PosS >5 was associated with more 6.5 months of OS, compared with patients with values <5. This interesting finding might be explained by the fact that inflammation is a critical component of tumour progression[5]. Pre radiochemotherapy NLR values >5 did not show any significant difference in PFS compared with values <5, has we expected based in our univariate analysis. The same happened for our variable NLR PreS-PreRTQT considering the value <-2 or >-2, with a log rank p = 0.5846. At last, we found that pre-chemotherapy PLR values >9 were associated with a lower PFS compared with values <9 (mean PFS 7.448 vs 11.999 months). Still, when performed a multivariate analysis considering these cut-offs and other demographic and clinical characteristics, we could not consider his cut-off as independent prognostic factors for OS and PFS.

Some studies demonstrated that pretreatment RDW was superior to mean cell volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) as a prognostic predictor of clinical outcome in patients newly diagnosed with GBM[17, 27]. Kaisman-Elbaz showed that RDW <14% was a significant favorable prognostic factor for GBM overall survival (particularly for patients under 70 and underwent tumor resection supplemented with oncological treatment)[28]. Also, in several other studies, higher RDW values were linked with gliomas prognosis[29-31]. Even though, we did not find a statistically significant difference in OS comparing progression RDW-CV values <14 and >14, despite the current literature showing otherwise.

Our study has some limitations. We performed a retrospective, single-institution study with a small sample size, but similar to other studies in the area. The hematologic parameters evaluated were presumably collected before any treatment. Moreover, there are more physiological and pathophysiological factors (including diabetes,

inflammatory disease, previous history of recent infection, hypertension, renal and hepatic dysfunction) that may affect NLR, PLR and RDW values that we did not consider as confounders.

In conclusion, the results of the present study demonstrated that higher pre-chemotherapy NLR and PLR values were associated with worse prognosis and NLR PreS-PosS values are associated with better prognosis. However, none of these parameters could be considered independent prognostic factors for GBM.

Therefore, larger prospective studies are needed to verify our findings and clarify the mechanisms underlying the association between NLR, PLR and RDW values and GBM prognosis.

Statements & Declarations

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing Interests

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

Ethics approval

This is a retrospective study. The Ethics committee of Centro Hospitalar Universitário de São João (CHUSJ) / Faculdade de Medicina da Universidade do Porto (FMUP) has confirmed that no ethical approval is required.

References

1. Darefsky, A.S., J.T. King, Jr., and R. Dubrow, *Adult glioblastoma multiforme survival in the temozolomide era: a population-based analysis of Surveillance, Epidemiology, and End Results registries.* Cancer, 2012. **118**(8): p. 2163-72.
2. Miller, C.A., *Pseudoprogression: Patient experience and nursing in uncertainty.* Can J Neurosci Nurs, 2015. **37**(2): p. 35-41.
3. Wirsching, H.G., E. Galanis, and M. Weller, *Glioblastoma.* Handb Clin Neurol, 2016. **134**: p. 381-97.
4. Hanahan, D. and R.A. Weinberg, *Hallmarks of cancer: the next generation.* Cell, 2011. **144**(5): p. 646-74.
5. Coussens, L.M. and Z. Werb, *Inflammation and cancer.* Nature, 2002. **420**(6917): p. 860-7.
6. Lamborn, K.R., S.M. Chang, and M.D. Prados, *Prognostic factors for survival of patients with glioblastoma: recursive partitioning analysis.* Neuro Oncol, 2004. **6**(3): p. 227-35.
7. Lacroix, M., et al., *A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival.* J Neurosurg, 2001. **95**(2): p. 190-8.
8. Donskov, F., *Immunomonitoring and prognostic relevance of neutrophils in clinical trials.* Seminars in Cancer Biology, 2013. **23**(3): p. 200-207.
9. Guthrie, G.J., et al., *The systemic inflammation-based neutrophil-lymphocyte ratio: experience in patients with cancer.* Crit Rev Oncol Hematol, 2013. **88**(1): p. 218-30.
10. Fossati, G., et al., *Neutrophil infiltration into human gliomas.* Acta Neuropathol, 1999. **98**(4): p. 349-54.
11. Atai, N.A., et al., *Osteopontin is up-regulated and associated with neutrophil and macrophage infiltration in glioblastoma.* Immunology, 2011. **132**(1): p. 39-48.
12. Templeton, A.J., et al., *Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis.* J Natl Cancer Inst, 2014. **106**(6): p. dju124.
13. Lopes, M., et al., *Influence of neutrophil-lymphocyte ratio in prognosis of glioblastoma multiforme.* J Neurooncol, 2018. **136**(1): p. 173-180.
14. Zadora, P., et al., *Preoperative neutrophil-lymphocyte count ratio helps predict the grade of glial tumor - a pilot study.* Neurol Neurochir Pol, 2015. **49**(1): p. 41-4.
15. Wang, P.F., et al., *Preoperative inflammation markers and IDH mutation status predict glioblastoma patient survival.* Oncotarget, 2017. **8**(30): p. 50117-50123.
16. Han, S., et al., *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**: p. 617.
17. Liang, R.F., et al., *Significance of Pretreatment Red Blood Cell Distribution Width in Patients with Newly Diagnosed Glioblastoma.* Med Sci Monit, 2017. **23**: p. 3217-3223.
18. Kelly, P.D., et al., *Red blood cell distribution width in glioblastoma.* Clin Neurol Neurosurg, 2021. **213**: p. 107096.
19. Yersal, Ö., et al., *Prognostic significance of pre-treatment neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in patients with glioblastoma.* Mol Clin Oncol, 2018. **9**(4): p. 453-458.
20. Bambury, R.M., et al., *The association of pre-treatment neutrophil to lymphocyte ratio with overall survival in patients with glioblastoma multiforme.* J Neurooncol, 2013. **114**(1): p. 149-54.
21. Alexiou, G.A., E. Vartholomatos, and S. Voulgaris, *Prognostic value of neutrophil-to-lymphocyte ratio in patients with glioblastoma.* J Neurooncol, 2013. **115**(3): p. 521-2.
22. Lee, H., et al., *Elevated red blood cell distribution width as a simple prognostic factor in patients with symptomatic multiple myeloma.* Biomed Res Int, 2014. **2014**: p. 145619.
23. Wan, G.X., et al., *Elevated red cell distribution width contributes to a poor prognosis in patients with esophageal carcinoma.* Clin Chim Acta, 2016. **452**: p. 199-203.

24. Koma, Y., et al., *Increased red blood cell distribution width associates with cancer stage and prognosis in patients with lung cancer.* PLoS One, 2013. **8**(11): p. e80240.
25. Förhécz, Z., et al., *Red cell distribution width in heart failure: prediction of clinical events and relationship with markers of ineffective erythropoiesis, inflammation, renal function, and nutritional state.* Am Heart J, 2009. **158**(4): p. 659-66.
26. Weng, W., et al., *Preoperative neutrophil-lymphocyte ratio correlated with glioma grading and glioblastoma survival.* Neurol Res, 2018. **40**(11): p. 917-922.
27. Dagistan, Y., E. Dagistan, and V. Citisli, *Evaluation of simple blood counts as inflammation markers for brain tumor patients.* Neurol Neurochir Pol, 2016. **50**(4): p. 231-5.
28. Kaisman-Elbaz, T., et al., *Hemoglobin Levels and Red Blood Cells Distribution Width Highlights Glioblastoma Patients Subgroup With Improved Median Overall Survival.* Front Oncol, 2020. **10**: p. 432.
29. Auezova, R., et al., *Association of preoperative levels of selected blood inflammatory markers with prognosis in gliomas.* Onco Targets Ther, 2016. **9**: p. 6111-6117.
30. Bao, Y., et al., *Preoperative Hematologic Inflammatory Markers as Prognostic Factors in Patients with Glioma.* World Neurosurg, 2018. **119**: p. e710-e716.
31. Perlstein, T.S., et al., *Red blood cell distribution width and mortality risk in a community-based prospective cohort.* Arch Intern Med, 2009. **169**(6): p. 588-94.

Tables and Figures

Table 1 - Demographic and clinical characteristics of the included patients

Characteristics		N	Mean±SD or percentage
Age at diagnosis (years)		124	60.27 ± 11.17
OS (months)		124	20.252 ± 15.869
PFS (months)		124	9.429 ± 10.038
Gender	Male	79	63.71%
	Female	45	36.29%
Location	Supratentorial	122	98.39%
	Infratentorial	2	1.61%
Extension	1 lobe	69	55.65%
	2 or more lobes	55	44.35%
Focality	Unifocal	100	80.65%
	Multifocal	24	19.35%
Surgery resection extent	Total	58	46.77%
	Sub-total	25	20.16%
	Parcial	11	8.87%
	Endoscopic	30	24.19%
First-line adjuvant systemic therapy	Stupp	96	77.42%
	Stupp + hipoRT	25	20.16%
	RT	1	0.81%
	Temozolamide	1	0.81%
	None	1	0.81%
Second-line adjuvant systemic therapy	Stupp	2	1.61%
	Bevacizumab + Lomustine	57	45.97%
	Bevacizumab + Irinotecan	2	1.61%
	Lomustina	6	4.84%
	RT	4	3.23%
	Radiosurgery	2	1.61%
	Temazolamina	1	0.81%
	Temazolamina Continua	2	1.61%
	None	48	38.71%
ECOG Pre-surgery	0	40	32.26%
	1	67	54.03%
	2	15	12.10%
	3	2	1.61%
Pre-surgery NLR		118	8.988± 6.736
Pre-surgery PLR		118	24.693±17.41
Pre-surgery RDW-CV		120	13.037±0.75
Pre-surgery RDW-SD		120	42.023±3.053
Post-surgery NLR		115	17.073±11.471
Post-surgery PLR		115	37.446±25.301
Post-surgery RDW-CV		116	13.356±0.89
Post-surgery RDW-SD		116	43.185±3.361
Pre-radiochemotherapy NLR		123	7.396±8.073
Pre-radiochemotherapy PLR		123	19.147±15.67
Pre-radiochemotherapy RDW-CV		123	14.07±1.171
Pre-radiochemotherapy RDW-SD		123	46.173±4.626
Pre-chemotherapy NLR		101	6.463±6.696
Pre-chemotherapy PLR		101	14.502±15.909
Pre-chemotherapy RDW-CV		101	13.774±1.261
Pre-chemotherapy RDW-SD		101	44.576±5.005
Progression NLR		111	9.088±9.018
Progression PLR		111	18.004±19.931
Progression RDW-CV		111	14.269±1.302
Progression RDW-SD		111	47.475±5.481
NLR PreS-PosS		121	7.226±11.485
NLR PreS-PreRTQT		122	-1.424±10.769
NLR PreS-PreQT		115	-3.177±8.498
NLR PreS-Prog		116	-0.081±10.421
NLR PreQT-Prog		112	-2.704±8.22
PLR PreS-PosS		121	11.129±24.913
PLR PreS-PreRTQT		122	-4.891±22.754
PLR PreS-PreQT		115	-11.836±22.754
PLR PreS-Prog		116	-6.885±25.019
PLR PreQT-Prog		112	4.216±16.847

SD, standard deviation; OS, overall survival; PFS, progression free survival; RT, radiotherapy; ECOG, Eastern Cooperative Oncology Group performance status; PreS-PosS, difference between pre-surgery and pos-surgery; PreS-PreRTQT, difference between pre-surgery and pre-radiochemotherapy; PreS-PreQT, difference between pre-surgery and pre-chemotherapy; PreS-Prog, difference between pre-surgery and progression; PreQT-Prog, difference between pre-chemotherapy and progression.

Table 2 - Univariate Linear Regression of Global Survival based on Analytical Parameters

Univariate OS Analyses				
Time-point	Parameter	Coefficient	Confidence interval	p value
Pre-surgery	NLR	-0.018	[-0.436; 0.400]	0.933
	PLR	0.560	[-0.105; 0.218]	0.490
	RDW-CV	0.721	[-3.061; 4.504]	0.706
	RDW-SD	0.068	[-0.862; 0.997]	0.886
Post-surgery	NLR	0.134	[-0.124; 0.393]	0.305
	PLR	0.062	[-0.055; 0.179]	0.294
	RDW-CV	-0.812	[-4.129; 2.505]	0.629
	RDW-SD	-0.135	[-1.014; 0.743]	0.761
Pre-radio and chemotherapy	NLR	0.167	[-0.186; 0.521]	0.350
	PLR	0. 060	[-0.123; 0.242]	0.518
	RDW-CV	0.653	[-1.762; 3.068]	0.594
	RDW-SD	-0.300	[-0.310; 0.909]	0.333
Pre-chemotherapy	NLR	-0.489	[-0.956; -0.023]	*0.040*
	PLR	-0.175	[-0.373; 0.022]	0.081
	RDW-CV	-1.506	[-4.018; 1.007]	0.237
	RDW-SD	-0.242	[-0.877; 0.394]	0.453
Progression	NLR	-0.351	[-0.661; -0.04]	*0.027*
	PLR	-0.113	[-0.255; 0.029]	0.188
	RDW-CV	-2.243	[-4.4; -0.086]	*0.042*
	RDW-SD	-0.453	[-0.969; 0.062]	0.084
NLR PreS-PosS		0.265	[0.018; 0.512]	*0.036*
NLR PreS-PreRTQT		0.158	[-0.107; 0.423]	0.240
NLR PreS-PreQT		0.032	[-0.316; 0.380]	0.857
NLR PreS-Prog		-0.154	[-0.420; 0.112]	0.253
NLR PreQT-Prog		0.249	[-0.094; 0.593]	0.153
PLR PreS-PosS		0.098	[-0.017; 0.212]	0.093
PLR PreS-PreRTQT		0.025	[-0.101; 0.152]	0.692
PLR PreS-PreQT		-0.009	[-0.138; 0.121]	0.897
PLR PreS-Prog		-0.049	[-0.160; 0.062]	0.385
PLR PreQT-Prog		-0.091	[-0.259; 0.078]	0.289

PreS-PosS, difference between pre-surgery and pos-surgery; PreS-PreRTQT, , difference between pre-surgery and pre-radiochemotherapy; PreS-PreQT, difference between pre-surgery and pre-chemotherapy; PreS-Prog, difference between pre-surgery and progression; PreQT-Prog, difference between pre-chemotherapy and progression.

Table 3 - Multivariate Analysis based on demographic and clinical characteristics

Multivariate OS Analysis			
Parameter	Coefficient	Confidence interval	p-value
Gender	4.091	[-2.553; 10.735]	0.224
Age at diagnosis	0.025	[-0.284; 0.333]	0.875
Extension	0.996	[-6.582; 8.574]	0.794
Focality	-1.891	[-12.05; 8.268]	0.712
Surgery resection extent	0.307	[-2.617; 3.231]	0.835
Second-line adjuvant systemic therapy	11.277	[3.455; 19.099]	*0.005*
ECOG pre-surgery	0.528	[-5.199;6.255]	0.855
NLR pre chemotherapy	-0.03	[-0.576; 0.517]	0.914
NLR Progression	-0.318	[-0.703; 0.067]	0.105
RDWCV progression	-0.729	[-3.351; 1.892]	0.581
NLR PreS-PosS	0.176	[-0.093; 0.446]	0.197
NLR pre chemotherapy cut-off (<4 v >4)	-3.22	[-9.978; 3.539]	0.347
NLR progression cut-off de (<6 v >6)	-4.06	[-9.952; 1.831]	0.175
RDWCV progression cut-off (<14 v >14)	1.478	[-4.441; 7.398]	0.622
NLR PreS-PosS cut-off (<5 v >5)	5.249	[-0.351; 10.848]	0.066

ECOG, Eastern Cooperative Oncology Group performance status; PreS-PosS, difference between pre-surgery and pos-surgery.

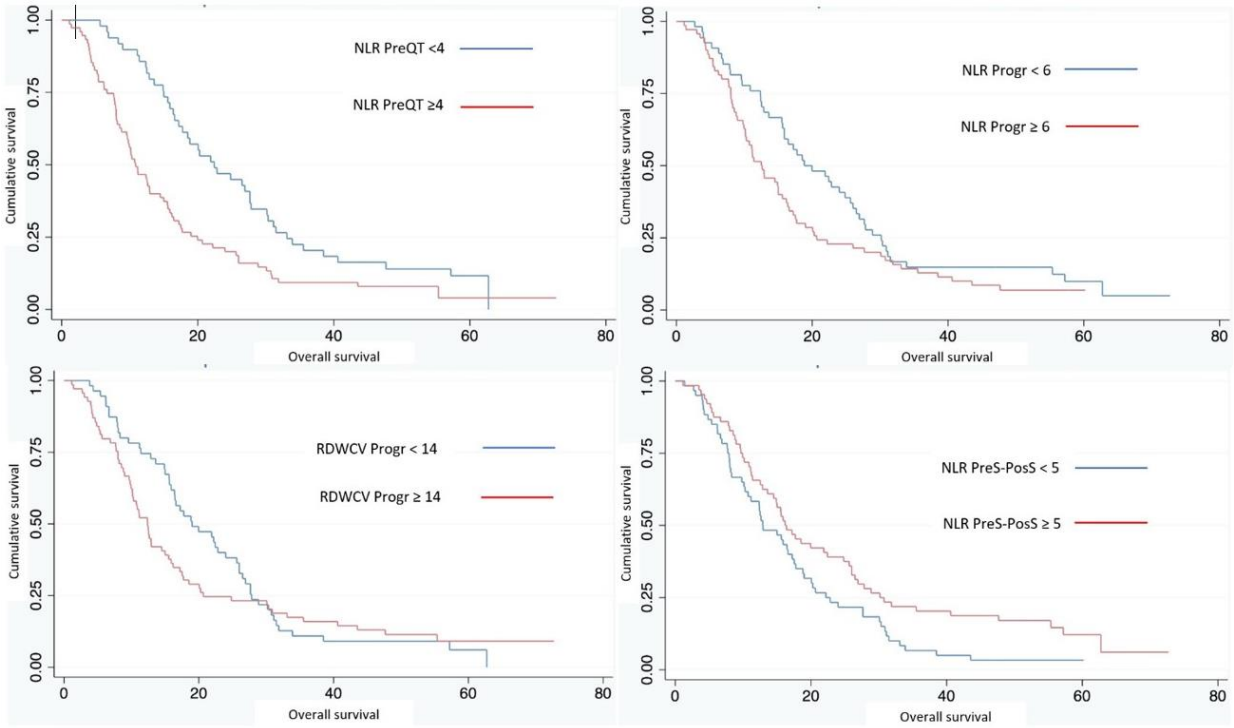


Fig. 1 – Kaplan-Meier plot of overall survival stratified by pre-chemotherapy NLR value (cut-off = 4), progression NLR value (cut-off = 6), progression RDW-CV value (cut-off = 14) and difference between pre-surgery and pos-surgery (cut-off = 5). NLR PreQT, pre-chemotherapy NLR; NLR Progr, progression NLR; RDWCV Progr, progression RDW-CV; NLR PreS-PosS, difference between pre-surgery and pos-surgery.

Table 4 - Univariate Linear Regression of PFS based on Analytical Parameters

Univariate PFS Analyses				
Time-point	Parameter	Coefficient	Confidence interval	P value
Pre-surgery	NLR	-0.670	[-0.347; 0.212]	0.636
	PLR	0.005	[-0.103; 0.114]	0.922
	RDW-CV	-0.087	[-2.558; 2.385]	0.945
	RDW-SD	-0.084	[-0.691; 0.523]	0.784
Post-surgery	NLR	0.003	[-0.164; 0.171]	0.967
	PLR	0.020	[-0.056; 0.096]	0.530
	RDW-CV	-0.250	[-2.391; 1.890]	0.817
	RDW-SD	0.053	[-0.514; 0.620]	0.853
Pre-radio and chemotherapy	NLR	0.269	[0.050; 0.488]	***0.016***
	PLR	0.019	[-0.096; 0.135]	0.744
	RDW-CV	-0.442	[-1.893; 1.009]	0.548
	RDW--SD	-0.115	[-0.482; 0.252]	0.537
Pre-chemotherapy	NLR	-0.342	[-0.632; -0.052]	*0.021*
	PLR	-0.127	[-0.249; -0.004]	*0.043*
	RDWCV	0.658	[-0.917; 2.233]	0.409
	RDW-SD	0.240	[-0.155; 0.636]	0.231
Progression	NLR	-0.079	[-0.220; 0.063]	0.272
	PLR	-0.024	[-0.088; 0.04]	0.456
	RDW-CV	-0.539	[-1.518; 0.440]	0.278
	RDW-SD	-0.070	[-0.304; 0.163]	0.553
NLR PreS-PosS		0.068	[-0.091; 0.228]	0.396
NLR PreS-PreRTQT		0.175	[0.009; 0.341]	*0.039*
NLR PreS-PreQT		-0.061	[-0.272; 0.149]	0.564
NLR PreS-Prog		-0.044	[-0.164; 0.076]	0.471
NLR PreQT-Prog		0.009	[-0.147; 0.166]	0.906
PLR PreS-PosS		0.035	[-0.038; 0.109]	0.344
PLR PreS-PreRTQT		0.001	[-0.079; 0.081]	0.974
PLR PreS-PreQT		-0.029	[-0.108; 0.049]	0.461
PLR PreS-Prog		-0.015	[-0.065; 0.035]	0.550
PLR PreQT-Prog		0.011	[-0.066; 0.087]	0.784

PreS-PosS, difference between pre-surgery and pos-surgery; PreS-PreRTQT, difference between pre-surgery and pre-radiochemotherapy; PreS-PreQT, difference between pre-surgery and pre-chemotherapy; PreS-Prog, difference between pre-surgery and progression; PreQT-Prog, difference between pre-chemotherapy and progression.

Table 5 - Multivariate Analysis based on demografic and clinical characteristics

Multivariate PFS Analysis			
Parameter	Coefficient	Confidence interval	p-value
Gender	1.785	[-2.547; 6.117]	0.415
Age at diagnosis	-0.042	[-0.246; 0.163]	0.687
Extension	-2.075	[-6.857; 2.708]	0.391
Focality	-0.348	[-6.905; 6.209]	0.916
Surgery resection extent	-0.377	[2.251; 1.497]	0.690
Second-line adjuvant systemic therapy	-4.12	[-8.961; 0.721]	0.094
ECOG Pre-surgery	2.655	[-0.716; 6.026]]	0.121
NLR Pre-chemoradiotherapy	0.085	[-0.370; 0.540]	0.710
NLR pre-chemotherapy	-0.61	[-1.484; 0.264]	0.169
PLR pre-chemotherapy	0.081	[-0.280; 0.443]	0.656
NLR PreS-PreRTQT	-0.003	[-0.305; 0.299]	0.984
NLR Pre-chemoradiotherapy cut off (<5 v >5)	2.052	[-2.098; 6.203]	0.329
PLR_pre-chemotherapy cutoff (<9 v >9)	-2.449	[-8.254; 3.355]	0.405
NLR pre-chemotherapy cutoff (<4 v >4)	-3.885	[-9.946; 2.176]	0.207
NLR PreS-PreRTQT cutoff (<-2 v >-2)	0.600	[-3.332; 4.533]	0.763

ECOG, Eastern Cooperative Oncology Group performance status; PreS-PreRTQT, difference between pre-surgery and pre-radiochemotherapy

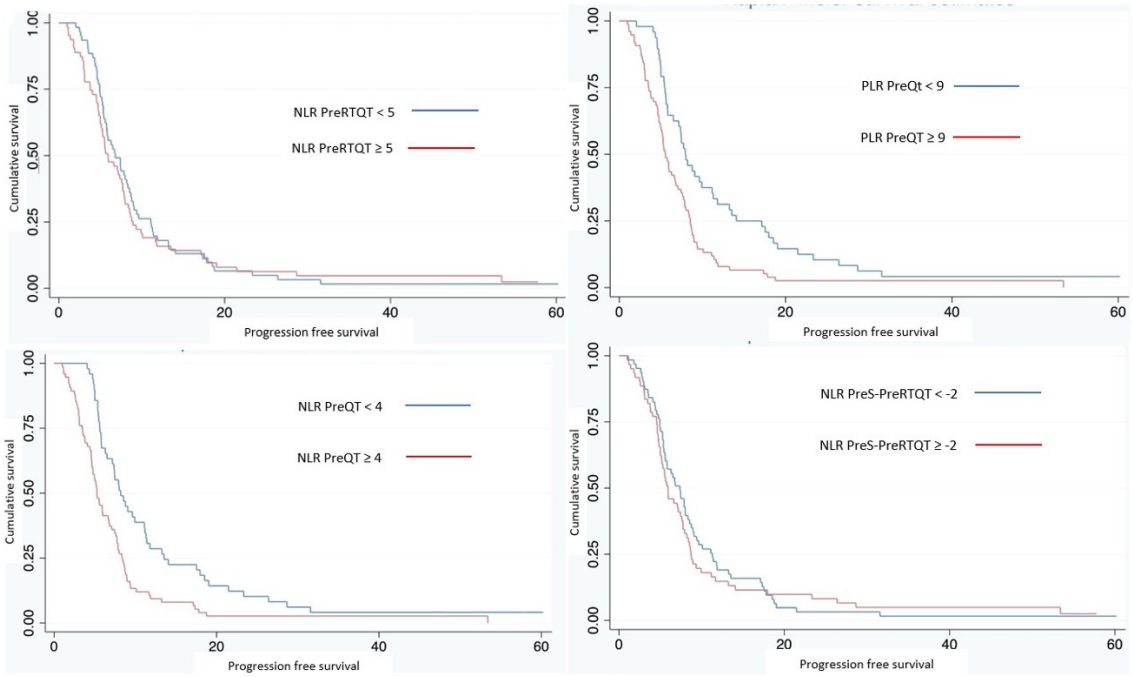


Fig. 2 - Kaplan-Meier plot of progression free survival stratified by pre-radiochemotherapy NLR value (cut-off = 5), pre-chemotherapy PLR value (cut-off = 9), pre-chemotherapy NLR value (cut-off = 4) and difference between pre-surgery and pre-radiochemotherapy (cut-off = -2). NLR PreRTQT, pre-radiochemotherapy NLR; PLR PreQT, pre-chemotherapy PLR; NLR PreQT, pre-chemotherapy NLR; NLR PreS-PreRTQT, difference between pre-surgery and pre-radiochemotherapy.

Reporting Guidelines

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	(a) Page 6 - "The population of this study consists of a cohort of 124 patients followed in the Neurosurgery Department of a tertiary care hospital, between January/2014 and December/2018."
			(b) Page 6 - "in our univariate analysis…"; "the results of the present study…"
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 7 -"Glioblastoma (GBM) is the most aggressive primary brain tumor in…"
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 7 - "Therefore, the aim of this study is to evaluate the inflammatory parameters, NLR, PLR, RDW-CV and RDW-SD in the different phases…allow us to predict the patient's prognosis in terms of recurrence, progression free survival (PFS) and OS."
Methods			
Study design	4	Present key elements of study design early in the paper	Page 7 - "Retrospective cohort study with patients with GBM who were submitted to a surgical recession and initiated adjuvant treatment."
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 7 - "The study population consists of a cohort of 144 patients followed in the Neurosurgery Department, from January 1, 2014, to December 31, 2018, at…"

Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	Page 8 – (a) "The inclusion criteria were: 18 years of age and..."; "To ensure that it is possible to assess OS and PFS in all cases of the study, we set the follow-up date limit to November 30, 2021." (b) not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 9 - "OS corresponded to the time between surgery and the date of death or the date of the last follow-up. PFS, corresponds to …"
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 8 - "The clinical registries of all patients were reviewed and for each patient, the following data were collected…"; "OS corresponded to the time between…"
Bias	9	Describe any efforts to address potential sources of bias	Page 8 - "For those who had a statistically significant result we performed a multivariate linear regression analysis correlating each hematologic parameter with OS and PFS, including also relevant population characteristics…"
Study size	10	Explain how the study size was arrived at	The study size calculation was not performed since the scarce availability of suitable patients for this study.
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 8 - "In our study we decided to initially make a univariate analysis for NLR, PLR, RDW-CV, RDW-SV correlating..."

Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	(a) Page 8 - "In our study we decided to initially…"; "For those who had a statistically significant result…" (b) Page 8 - "For those who had a statistically significant result we performed a multivariate analysis correlating each hematologic parameter with OS and PFS considering ..."; "We also developed cut-offs for those statistically significant parameters..."; "Those same categorical variables were compared with the other clinical and demographic parameters and ..."
		(*b*) Describe any methods used to examine subgroups and interactions	(c) Page 8 - "For those patients with missing data in a certain time-point, we still considered their values in other time-points for our analysis."
		(*c*) Explain how missing data were addressed	(d) Page 8 - "Patients that were loss from follow-up were only considered for the time they were in the study." (e) not applicable
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	(a) Information included in Table 1 (Page 15); (b) and (c) not appliable.
		(b) Give reasons for non-participation at each stage	

			(c) Consider use of a flow diagram	
Descriptive data		14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	(a) Table 1 (Page 15) Page 8 - "We included 124 patients. Demographic and clinical characteristics of our cohort are detailed in Table 1 and 2. The mean age at diagnosis was…"
			(b) Indicate number of participants with missing data for each variable of interest	(b) Information included in Table 1 (Page 15) and in the paragraph: "Of the 144 patients, 14 were…" (Page 8)
			(c) Summarise follow-up time (eg, average and total amount)	(c) Page 8 - "patients that have been followed for at least 18 months after tumour resection surgery, because the overall average survival is 15 months"; "To ensure that it is possible to assess OS and PFS in all cases of the study, we set the follow-up date limit to November 30, 2021."
Outcome data		15*	Report numbers of outcome events or summary measures over time	Page 9 - "In the univariate analysis, we were able to relate pre-chemotherapy NLR values with…" "On univariate analysis, PFS differ…"
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	(a) Page 9 - "We evaluate these same parameters in a multivariate analysis (table 3). To simplify our analysis, we considered the second-line adjuvant systemic therapy as a dichotomic variable. The type first-line adjuvant systemic therapy was not considered in our multivariate analysis, because 97,58% of patients initiated the stupp protocol. (…)" (b) and (c) not applicable	
		(*b*) Report category boundaries when continuous variables were categorized		
		(*c*) If relevant, consider translating estimates of relative risk into		

		absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Page 9 - "Patients with a pre-chemotherapy NLR value <4 had a better OS compared to…"
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 12 - "…the results of the present study demonstrated that higher pre-chemotherapy NLR and PLR values were associated with worse prognosis and NLR PreS-PosS values are associated with better prognosis. However, none of these parameters could be considered independent prognostic factors for GBM."
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 11 - "Our study has some limitations. We performed a retrospective, single-institution study with …"
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 11 - "Still, when performed a multivariate analysis considering these cut-offs and other demographic and clinical characteristics, we could not consider these cut-off as independent prognostic factors for OS and PFS." "Some studies demonstrated that pretreatment RDW was superior..."
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 12 - "In conclusion, the results of the present study…"
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 12 - Included in "Statements & Declarations"

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Submission guidelines according to Journal Of Neuro-Oncology, Springer

Types of papers

The Journal welcomes original reports dealing with both Laboratory Investigations and Clinical Studies related to tumors of the nervous system. Although we do not accept Case Reports, Letters to the Editor may be considered. Occasionally, the journal will also publish Review articles on selected topics. Original Laboratory Investigations and Clinical Studies of unusually high impact and timely findings in the field may be submitted as a "Priority Report" for expedited review and publication. Please contact the Editorial Office if you feel your paper warrants that treatment.

Clinical Studies

Scientific reports of the results of original clinical research. Manuscript body text is limited to 3000 words. Abstract text is limited to 250 words and should be in a structured format (see specifics below). There may be a maximum of 5 tables and figures (total), and up to 50 references.

Structured format of Abstracts

The following abstract format applies to all submissions except for invited editorials and letters to the editor:

Introduction: Provide a background or rationale for the study. Also state the objective of the study.

Methods: Describe the study design, procedures, and materials for the study.

Results: Detail the pertinent data and the significant findings or observations made.

Conclusions: Provide an interpretation of the study findings, summarize their implications, and provide possible next steps for future investigation.

Graphical Abstracts

A graphical abstract is a single, concise, pictorial and visual summary of the main findings of the article. This could be a figure that is specially designed to capture the content of the article for readers at a single glance or a streaming video.

Technical Requirements for Streaming

Ø 4 Resolution: 16:9 or 4:3

Ø 4 Maximum file size: 25 GB

Ø 4 Minimum video duration: 1 sec

Ø 4 Supported file formats: avi, wmv, mp4, mov, m2p, mp2, mpg, mpeg, flv, mxf, mts, m4v, 3gp

Manuscript Submission

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

Permissions

Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

Online Submission

Please follow the hyperlink "Submit manuscript" and upload all of your manuscript files following the instructions given on the screen.

Source Files

Please ensure you provide all relevant editable source files at every submission and revision. Failing to submit a complete set of editable source files will result in your article not being considered for review. For your manuscript text please always submit in common word processing formats such as .docx or LaTeX.

ORCID ID

This publication requires that the corresponding author provides his/her ORCiD ID before proceeding with submission.

For more information about this journal's ORCiD policy, please visit the ORCID FAQ

Title Page

Title Page

Please make sure your title page contains the following information.

Title

The title should be concise and informative.

Author information

- The name(s) of the author(s)
- The affiliation(s) of the author(s), i.e. institution, (department), city, (state), country
- A clear indication and an active e-mail address of the corresponding author
- If available, the 16-digit ORCID of the author(s)

If address information is provided with the affiliation(s) it will also be published.

For authors that are (temporarily) unaffiliated we will only capture their city and country of residence, not their e-mail address unless specifically requested.

Abstract

Please provide a structured abstract of 150 to 250 words which should be divided into the following sections:

- Purpose (stating the main purposes and research question)
- Methods
- Results
- Conclusion

For life science journals only (when applicable)

- Trial registration number and date of registration for prospectively registered trials
- Trial registration number and date of registration followed by "retrospectively registered", for retrospectively registered trials

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Text

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.

- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX. We recommend using Springer Nature's LaTeX template.

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

References

Citation

Reference citations in the text should be identified by numbers in square brackets. Some examples:

1. Negotiation research spans many disciplines [3].

2. This result was later contradicted by Becker and Seligman [5].

3. This effect has been widely studied [1-3, 7].

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text.

The entries in the list should be numbered consecutively.

If available, please always include DOIs as full DOI links in your reference list (e.g. “https://doi.org/abc”).

- Journal article

  Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955-8

  Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted:

  Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

- Article by DOI

  Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. https://doi.org/10.1007/s001090000086

- Book

  South J, Blass B (2001) The future of modern genomics. Blackwell, London

- Book chapter

  Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

- Online document

  Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

- Dissertation

  Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations, see

ISSN.org LTWA

If you are unsure, please use the full journal title.

Authors preparing their manuscript in LaTeX can use the bibliography style file sn-basic.bst which is included in the Springer Nature Article Template.

Statements & Declarations

The following statements must be included in your submitted manuscript under the heading 'Statements and Declarations'. This should be placed after the References section. Please note that submissions that do not include required statements will be returned as incomplete.

Funding

Please describe any sources of funding that have supported the work. The statement should include details of any grants received (please give the name of the funding agency and grant number).

Example statements:

"This work was supported by [...] (Grant numbers [...] and [...]). Author A.B. has received research support from Company A."

"The authors declare that no funds, grants, or other support were received during the preparation of this manuscript."

Competing Interests

Authors are required to disclose financial or non-financial interests that are directly or indirectly related to the work submitted for publication. Interests within the last 3 years of beginning the work (conducting the research and preparing the work for submission) should be reported. Interests outside the 3-year time frame must be disclosed if they could reasonably be perceived as influencing the submitted work.

Example statements:

"Financial interests: Author A and B declare they have no financial interests. Author C has received speaker and consultant honoraria from Company M. Dr. C has received speaker honorarium and research funding from Company M and Company N. Author D has received travel support from Company O. Non-financial interests: Author D has served on advisory boards for Company M and Company N."

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

Please refer to the "Competing Interests" section below for more information on how to complete these sections.

Author Contributions

Authors are encouraged to include a statement that specifies the contribution of every author to the research and preparation of the manuscript.

Example statement:

"All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [full name],

[full name] and [full name]. The first draft of the manuscript was written by [full name] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript."

Please refer to the "Authorship Principles " section below for more information on how to complete this section.

Data Availability

This journal encourages authors to provide an optional statement of data availability in their article. Data Availability Statements should include information on where data supporting the results reported in the article can be found, including, where applicable, hyperlinks to publicly archived datasets analysed or generated during the study. Data availability statements can also indicate whether data are available on request from the authors and where no data are available, if appropriate.

Example statements:

"The datasets generated during and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT LINK TO DATASETS]"

"The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request."

Please refer to the "Research Data Policy and Data Availability" section below for more information on how to complete this section.

In addition to the above, manuscripts that report the results of studies involving humans and/or animals should include the following declarations:

Ethics approval

Authors of research involving human or animal subjects should include a statement that confirms that the study was approved (or granted exemption) by the appropriate institutional and/or national research ethics committee (including the name of the ethics committee and reference number, if available). For research involving animals, their data or biological material, authors should supply detailed information on the

ethical treatment of their animals in their submission. If a study was granted exemption or did not require ethics approval, this should also be detailed in the manuscript.

"This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University B (Date.../No....)."

"This is an observational study. The XYZ Research Ethics Committee has confirmed that no ethical approval is required."

For detailed information on relevant ethical standards and criteria, please refer to the sections on "Research involving human participants, their data or biological material", "Research involving animals, their data or biological material".

Consent to participate

For all research involving human subjects, freely-given, informed consent to participate in the study must be obtained from participants (or their parent or legal guardian in the case of children under 16) and a statement to this effect should appear in the manuscript.

Example statement:

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

"Written informed consent was obtained from the parents."

Please refer to the section on "Informed Consent" for additional help with completing this information.

Consent to publish

Individuals may consent to participate in a study, but object to having their data published in a journal article. If your manuscript contains any individual person's data in any form (including any individual details, images or videos), consent for publication must be obtained from that person, or in the case of children, their parent or legal guardian. This is in

particular applicable to case studies. A statement confirming that consent to publish has been received from all participants should appear in the manuscript.

Example statement:

"The authors affirm that human research participants provided informed consent for publication of the images in Figure(s) 1a, 1b and 1c."

Please refer to the section on "Informed Consent" for additional help with completing this information.

Tables

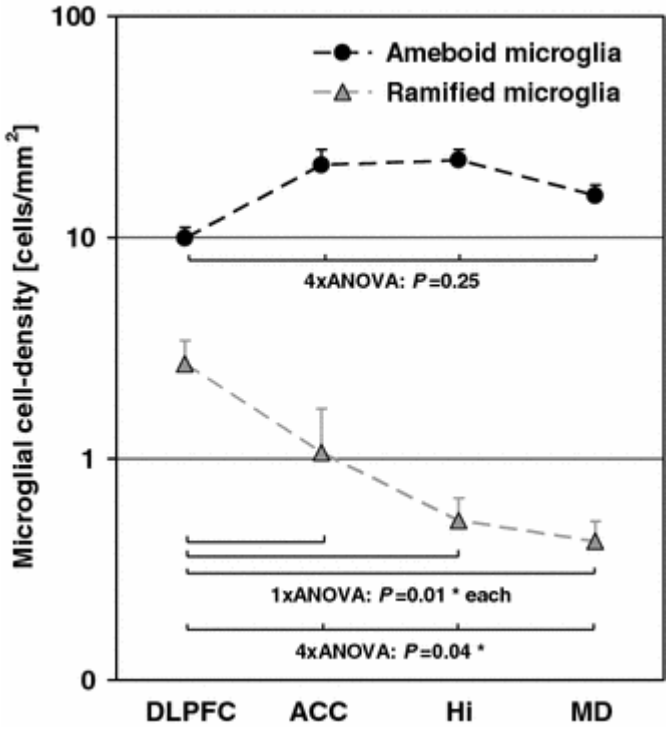
- All tables are to be numbered using Arabic numerals.
- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.
- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Artwork and Illustrations Guidelines

Electronic Figure Submission

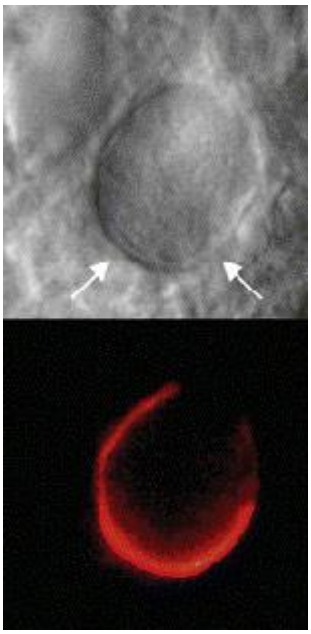
- Supply all figures electronically.
- Indicate what graphics program was used to create the artwork.
- For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.
- Vector graphics containing fonts must have the fonts embedded in the files.
- Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

Line Art



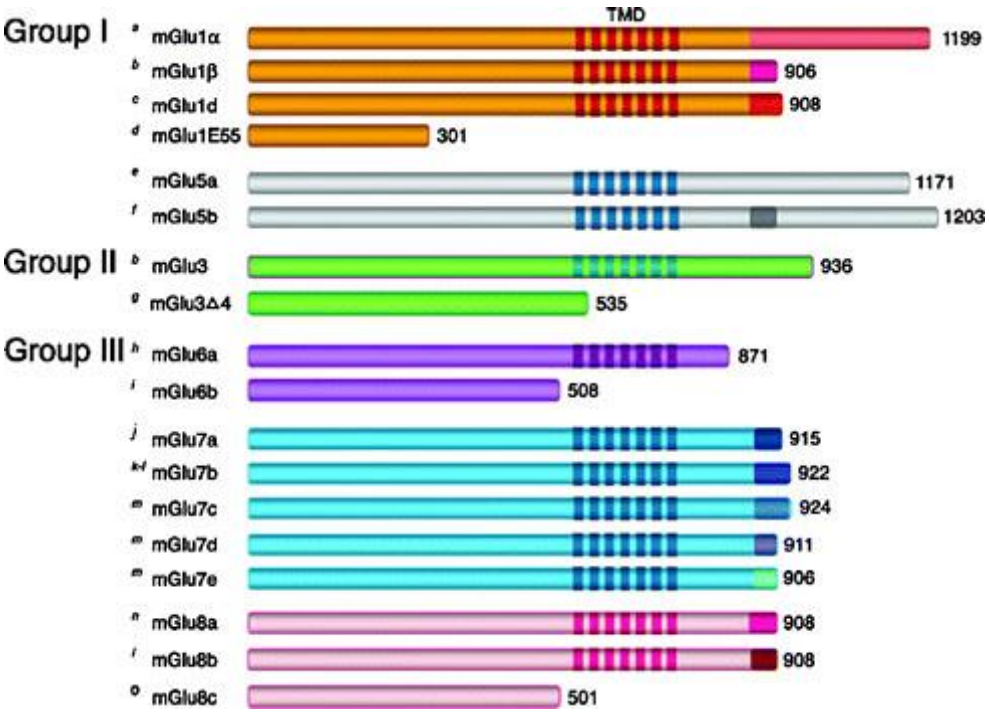
- Definition: Black and white graphic with no shading.
- Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
- All lines should be at least 0.1 mm (0.3 pt) wide.
- Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
- Vector graphics containing fonts must have the fonts embedded in the files.

Halftone Art



- Definition: Photographs, drawings, or paintings with fine shading, etc.
- If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.
- Halftones should have a minimum resolution of 300 dpi.

Combination Art



- Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.
- Combination artwork should have a minimum resolution of 600 dpi.

Color Art

- Color art is free of charge for online publication.
- If black and white will be shown in the print version, make sure that the main information will still be visible. Many colors are not distinguishable from one another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent.
- If the figures will be printed in black and white, do not refer to color in the captions.
- Color illustrations should be submitted as RGB (8 bits per channel).

Figure Lettering

- To add lettering, it is best to use Helvetica or Arial (sans serif fonts).
- Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).
- Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.
- Avoid effects such as shading, outline letters, etc.
- Do not include titles or captions within your illustrations.

Figure Numbering

- All figures are to be numbered using Arabic numerals.
- Figures should always be cited in text in consecutive numerical order.
- Figure parts should be denoted by lowercase letters (a, b, c, etc.).
- If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures,"A1, A2, A3, etc." Figures in online appendices [Supplementary Information (SI)] should, however, be numbered separately.

Figure Captions

- Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.
- Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.
- No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.
- Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.
- Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

- Figures should be submitted separately from the text, if possible.
- When preparing your figures, size figures to fit in the column width.
- For large-sized journals the figures should be 84 mm (for double-column text areas), or 174 mm (for single-column text areas) wide and not higher than 234 mm.
- For small-sized journals, the figures should be 119 mm wide and not higher than 195 mm.

Permissions

If you include figures that have already been published elsewhere, you must obtain permission from the copyright owner(s) for both the print and online format. Please be aware that some publishers do not grant electronic rights for free and that Springer will not be able to refund any costs that may have occurred to receive these permissions. In such cases, material from other sources should be used.

Accessibility

In order to give people of all abilities and disabilities access to the content of your figures, please make sure that

- All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware)

- Patterns are used instead of or in addition to colors for conveying information (colorblind users would then be able to distinguish the visual elements)
- Any figure lettering has a contrast ratio of at least 4.5:1

Supplementary Information (SI)

Springer accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature can add dimension to the author's article, as certain information cannot be printed or is more convenient in electronic form.

Before submitting research datasets as Supplementary Information, authors should read the journal's Research data policy. We encourage research data to be archived in data repositories wherever possible.

Submission

- Supply all supplementary material in standard file formats.
- Please include in each file the following information: article title, journal name, author names; affiliation and e-mail address of the corresponding author.
- To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.
- High resolution (streamable quality) videos can be submitted up to a maximum of 25GB; low resolution videos should not be larger than 5GB.

Audio, Video, and Animations

- Aspect ratio: 16:9 or 4:3
- Maximum file size: 25 GB for high resolution files; 5 GB for low resolution files
- Minimum video duration: 1 sec
- Supported file formats: avi, wmv, mp4, mov, m2p, mp2, mpg, mpeg, flv, mxf, mts, m4v, 3gp

Text and Presentations

- Submit your material in PDF format; .doc or .ppt files are not suitable for long-term viability.
- A collection of figures may also be combined in a PDF file.

Spreadsheets

- Spreadsheets should be submitted as .csv or .xlsx files (MS Excel).

Specialized Formats

- Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

Collecting Multiple Files

- It is possible to collect multiple files in a .zip or .gz file.

Numbering

- If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables.
- Refer to the supplementary files as “Online Resource”, e.g., "... as shown in the animation (Online Resource 3)", “... additional data are given in Online Resource 4”.
- Name the files consecutively, e.g. “ESM_3.mpg”, “ESM_4.pdf”.

Captions

- For each supplementary material, please supply a concise caption describing the content of the file.

Processing of supplementary files

- Supplementary Information (SI) will be published as received from the author without any conversion, editing, or reformatting.

Accessibility

In order to give people of all abilities and disabilities access to the content of your supplementary files, please make sure that

- The manuscript contains a descriptive caption for each supplementary material
- Video files do not contain anything that flashes more than three times per second (so that users prone to seizures caused by such effects are not put at risk)

Back to top

English Language Editing

For editors and reviewers to accurately assess the work presented in your manuscript you need to ensure the English language is of sufficient quality to be understood. If you need help with writing in English you should consider:

- Getting a fast, free online grammar check.
- Asking a colleague who is proficient in English to review your manuscript for clarity.
- Visiting the English language tutorial which covers the common mistakes when writing in English.
- Using a professional language editing service where editors will improve the English to ensure that your meaning is clear and identify problems that require your review. Two such services are provided by our affiliates Nature Research Editing Service and American Journal Experts. Springer authors are entitled to a 10% discount on their first submission to either of these services, simply follow the links below.

Free online grammar check

English language tutorial

Nature Research Editing Service

American Journal Experts

Please note that the use of a language editing service is not a requirement for publication in this journal and does not imply or guarantee that the article will be selected for peer review or accepted.

If your manuscript is accepted it will be checked by our copyeditors for spelling and formal style before publication.

Ethical Responsibilities of Authors

This journal is committed to upholding the integrity of the scientific record. As a member of the Committee on Publication Ethics (COPE) the journal will follow the COPE guidelines on how to deal with potential acts of misconduct.

Authors should refrain from misrepresenting research results which could damage the trust in the journal, the professionalism of scientific authorship, and ultimately the entire scientific endeavour. Maintaining integrity of the research and its presentation is helped by following the rules of good scientific practice, which include*:

- The manuscript should not be submitted to more than one journal for simultaneous consideration.
- The submitted work should be original and should not have been published elsewhere in any form or language (partially or in full), unless the new work concerns an expansion of previous work. (Please provide transparency on the re-use of material to avoid the concerns about text-recycling ('self-plagiarism').
- A single study should not be split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (i.e. 'salami-slicing/publishing').
- Concurrent or secondary publication is sometimes justifiable, provided certain conditions are met. Examples include: translations or a manuscript that is intended for a different group of readers.
- Results should be presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation (including image based manipulation). Authors should adhere to discipline-specific rules for acquiring, selecting and processing data.
- No data, text, or theories by others are presented as if they were the author's own ('plagiarism'). Proper acknowledgements to other works must be given (this includes material that is closely copied (near verbatim), summarized and/or paraphrased), quotation marks (to indicate words taken from another source) are used for verbatim copying of material, and permissions secured for material that is copyrighted.

Important note: the journal may use software to screen for plagiarism.

- Authors should make sure they have permissions for the use of software, questionnaires/(web) surveys and scales in their studies (if appropriate).
- Research articles and non-research articles (e.g. Opinion, Review, and Commentary articles) must cite appropriate and relevant literature in support of the claims made. Excessive and inappropriate self-citation or coordinated efforts among several authors to collectively self-cite is strongly discouraged.
- Authors should avoid untrue statements about an entity (who can be an individual person or a company) or descriptions of their behavior or actions that could potentially be seen as personal attacks or allegations about that person.
- Research that may be misapplied to pose a threat to public health or national security should be clearly identified in the manuscript (e.g. dual use of research). Examples include creation of harmful consequences of biological agents or toxins, disruption of immunity of vaccines, unusual hazards in the use of chemicals, weaponization of research/technology (amongst others).
- Authors are strongly advised to ensure the author group, the Corresponding Author, and the order of authors are all correct at submission. Adding and/or deleting authors during the revision stages is generally not permitted, but in some cases may be warranted. Reasons for changes in authorship should be explained in detail. Please note that changes to authorship cannot be made after acceptance of a manuscript.

*All of the above are guidelines and authors need to make sure to respect third parties rights such as copyright and/or moral rights.

Upon request authors should be prepared to send relevant documentation or data in order to verify the validity of the results presented. This could be in the form of raw data, samples, records, etc. Sensitive information in the form of confidential or proprietary data is excluded.

If there is suspicion of misbehavior or alleged fraud the Journal and/or Publisher will carry out an investigation following COPE guidelines. If, after

investigation, there are valid concerns, the author(s) concerned will be contacted under their given e-mail address and given an opportunity to address the issue. Depending on the situation, this may result in the Journal's and/or Publisher's implementation of the following measures, including, but not limited to:

- If the manuscript is still under consideration, it may be rejected and returned to the author.
- If the article has already been published online, depending on the nature and severity of the infraction:

  - an erratum/correction may be placed with the article

  - an expression of concern may be placed with the article

  - or in severe cases retraction of the article may occur.

The reason will be given in the published erratum/correction, expression of concern or retraction note. Please note that retraction means that the article is **maintained on the platform**, watermarked "retracted" and the explanation for the retraction is provided in a note linked to the watermarked article.

- The author's institution may be informed
- A notice of suspected transgression of ethical standards in the peer review system may be included as part of the author's and article's bibliographic record.

Fundamental errors

Authors have an obligation to correct mistakes once they discover a significant error or inaccuracy in their published article. The author(s) is/are requested to contact the journal and explain in what sense the error is impacting the article. A decision on how to correct the literature will depend on the nature of the error. This may be a correction or retraction. The retraction note should provide transparency which parts of the article are impacted by the error.

Suggesting / excluding reviewers

Authors are welcome to suggest suitable reviewers and/or request the exclusion of certain individuals when they submit their manuscripts. When

suggesting reviewers, authors should make sure they are totally independent and not connected to the work in any way. It is strongly recommended to suggest a mix of reviewers from different countries and different institutions. When suggesting reviewers, the Corresponding Author must provide an institutional email address for each suggested reviewer, or, if this is not possible to include other means of verifying the identity such as a link to a personal homepage, a link to the publication record or a researcher or author ID in the submission letter. Please note that the Journal may not use the suggestions, but suggestions are appreciated and may help facilitate the peer review process.

Authorship principles

These guidelines describe authorship principles and good authorship practices to which prospective authors should adhere to.

Authorship clarified

The Journal and Publisher assume all authors agreed with the content and that all gave explicit consent to submit and that they obtained consent from the responsible authorities at the institute/organization where the work has been carried out, **before** the work is submitted.

The Publisher does not prescribe the kinds of contributions that warrant authorship. It is recommended that authors adhere to the guidelines for authorship that are applicable in their specific research field. In absence of specific guidelines it is recommended to adhere to the following guidelines*:

All authors whose names appear on the submission

1) made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work;

2) drafted the work or revised it critically for important intellectual content;

3) approved the version to be published; and

4) agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

* Based on/adapted from:

ICMJE, Defining the Role of Authors and Contributors,

Transparency in authors' contributions and responsibilities to promote integrity in scientific publication, McNutt at all, PNAS February 27, 2018

Disclosures and declarations

All authors are requested to include information regarding sources of funding, financial or non-financial interests, study-specific approval by the appropriate ethics committee for research involving humans and/or animals, informed consent if the research involved human participants, and a statement on welfare of animals if the research involved animals (as appropriate).

The decision whether such information should be included is not only dependent on the scope of the journal, but also the scope of the article. Work submitted for publication may have implications for public health or general welfare and in those cases it is the responsibility of all authors to include the appropriate disclosures and declarations.

Data transparency

All authors are requested to make sure that all data and materials as well as software application or custom code support their published claims and comply with field standards. Please note that journals may have individual policies on (sharing) research data in concordance with disciplinary norms and expectations.

Role of the Corresponding Author

One author is assigned as Corresponding Author and acts on behalf of all co-authors and ensures that questions related to the accuracy or integrity of any part of the work are appropriately addressed.

The Corresponding Author is responsible for the following requirements:

- ensuring that all listed authors have approved the manuscript before submission, including the names and order of authors;
- managing all communication between the Journal and all co-authors, before and after publication;*
- providing transparency on re-use of material and mention any unpublished material (for example manuscripts in press) included in the manuscript in a cover letter to the Editor;
- making sure disclosures, declarations and transparency on data statements from all authors are included in the manuscript as appropriate (see above).

* The requirement of managing all communication between the journal and all co-authors during submission and proofing may be delegated to a Contact or Submitting Author. In this case please make sure the Corresponding Author is clearly indicated in the manuscript.

Author contributions

In absence of specific instructions and in research fields where it is possible to describe discrete efforts, the Publisher recommends authors to include contribution statements in the work that specifies the contribution of every author in order to promote transparency. These contributions should be listed at the separate title page.

Examples of such statement(s) are shown below:

• Free text:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [full name], [full name] and [full name]. The first draft of the manuscript was written by [full name] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Example: CRediT taxonomy:

• Conceptualization: [full name], …; Methodology: [full name], …; Formal analysis and investigation: [full name], …; Writing - original draft preparation: [full name, …]; Writing - review and editing: [full name], …;

Funding acquisition: [full name], …; Resources: [full name], …; Supervision: [full name],….

For **review articles** where discrete statements are less applicable a statement should be included who had the idea for the article, who performed the literature search and data analysis, and who drafted and/or critically revised the work.

For articles that are based primarily on the **student's dissertation or thesis**, it is recommended that the student is usually listed as principal author:

A Graduate Student's Guide to Determining Authorship Credit and Authorship Order, APA Science Student Council 2006

Affiliation

The primary affiliation for each author should be the institution where the majority of their work was done. If an author has subsequently moved, the current address may additionally be stated. Addresses will not be updated or changed after publication of the article.

Changes to authorship

Authors are strongly advised to ensure the correct author group, the Corresponding Author, and the order of authors at submission. Changes of authorship by adding or deleting authors, and/or changes in Corresponding Author, and/or changes in the sequence of authors are **not** accepted **after acceptance** of a manuscript.

- **Please note that author names will be published exactly as they appear on the accepted submission!**

Please make sure that the names of all authors are present and correctly spelled, and that addresses and affiliations are current.

Adding and/or deleting authors at revision stage are generally not permitted, but in some cases it may be warranted. Reasons for these changes in authorship should be explained. Approval of the change during revision is at the discretion of the Editor-in-Chief. Please note that

journals may have individual policies on adding and/or deleting authors during revision stage.

Author identification

Authors are recommended to use their ORCID ID when submitting an article for consideration or acquire an ORCID ID via the submission process.

Deceased or incapacitated authors

For cases in which a co-author dies or is incapacitated during the writing, submission, or peer-review process, and the co-authors feel it is appropriate to include the author, co-authors should obtain approval from a (legal) representative which could be a direct relative.

Authorship issues or disputes

In the case of an authorship dispute during peer review or after acceptance and publication, the Journal will not be in a position to investigate or adjudicate. Authors will be asked to resolve the dispute themselves. If they are unable the Journal reserves the right to withdraw a manuscript from the editorial process or in case of a published paper raise the issue with the authors' institution(s) and abide by its guidelines.

Confidentiality

Authors should treat all communication with the Journal as confidential which includes correspondence with direct representatives from the Journal such as Editors-in-Chief and/or Handling Editors and reviewers' reports unless explicit consent has been received to share informatio

Compliance with Ethical Standards

To ensure objectivity and transparency in research and to ensure that accepted principles of ethical and professional conduct have been followed, authors should include information regarding sources of funding, potential conflicts of interest (financial or non-financial), informed consent if the research involved human participants, and a statement on welfare of animals if the research involved animals.

Authors should include the following statements (if applicable) in a separate section entitled “Compliance with Ethical Standards” when submitting a paper:

- Disclosure of potential conflicts of interest
- Research involving Human Participants and/or Animals
- Informed consent

Please note that standards could vary slightly per journal dependent on their peer review policies (i.e. single or double blind peer review) as well as per journal subject discipline. Before submitting your article check the instructions following this section carefully.

The corresponding author should be prepared to collect documentation of compliance with ethical standards and send if requested during peer review or after publication.

The Editors reserve the right to reject manuscripts that do not comply with the above-mentioned guidelines. The author will be held responsible for false statements or failure to fulfill the above-mentioned guidelines.

Competing Interests

Authors are requested to disclose interests that are directly or indirectly related to the work submitted for publication. Interests within the last 3 years of beginning the work (conducting the research and preparing the work for submission) should be reported. Interests outside the 3-year time frame must be disclosed if they could reasonably be perceived as influencing the submitted work. Disclosure of interests provides a complete and transparent process and helps readers form their own judgments of potential bias. This is not meant to imply that a financial relationship with an organization that sponsored the research or compensation received for consultancy work is inappropriate.

Editorial Board Members and Editors are required to declare any competing interests and may be excluded from the peer review process if a competing interest exists. In addition, they should exclude themselves from handling manuscripts in cases where there is a competing interest. This may include – but is not limited to – having previously published with

one or more of the authors, and sharing the same institution as one or more of the authors. Where an Editor or Editorial Board Member is on the author list they must declare this in the competing interests section on the submitted manuscript. If they are an author or have any other competing interest regarding a specific manuscript, another Editor or member of the Editorial Board will be assigned to assume responsibility for overseeing peer review. These submissions are subject to the exact same review process as any other manuscript. Editorial Board Members are welcome to submit papers to the journal. These submissions are not given any priority over other manuscripts, and Editorial Board Member status has no bearing on editorial consideration.

Interests that should be considered and disclosed but are not limited to the following:

Funding: Research grants from funding agencies (please give the research funder and the grant number) and/or research support (including salaries, equipment, supplies, reimbursement for attending symposia, and other expenses) by organizations that may gain or lose financially through publication of this manuscript.

Employment: Recent (while engaged in the research project), present or anticipated employment by any organization that may gain or lose financially through publication of this manuscript. This includes multiple affiliations (if applicable).

Financial interests: Stocks or shares in companies (including holdings of spouse and/or children) that may gain or lose financially through publication of this manuscript; consultation fees or other forms of remuneration from organizations that may gain or lose financially; patents or patent applications whose value may be affected by publication of this manuscript.

It is difficult to specify a threshold at which a financial interest becomes significant, any such figure is necessarily arbitrary, so one possible practical guideline is the following: "Any undeclared financial interest that could embarrass the author were it to become publicly known after the work was published."

Non-financial interests: In addition, authors are requested to disclose interests that go beyond financial interests that could impart bias on the work submitted for publication such as professional interests, personal relationships or personal beliefs (amongst others). Examples include, but are not limited to: position on editorial board, advisory board or board of directors or other type of management relationships; writing and/or consulting for educational purposes; expert witness; mentoring relations; and so forth.

Primary research articles require a disclosure statement. Review articles present an expert synthesis of evidence and may be treated as an authoritative work on a subject. Review articles therefore require a disclosure statement.Other article types such as editorials, book reviews, comments (amongst others) may, dependent on their content, require a disclosure statement. If you are unclear whether your article type requires a disclosure statement, please contact the Editor-in-Chief.

Please note that, in addition to the above requirements, funding information (given that funding is a potential competing interest (as mentioned above)) needs to be disclosed upon submission of the manuscript in the peer review system. This information will automatically be added to the Record of CrossMark, however it is **not added** to the manuscript itself. Under 'summary of requirements' (see below) funding information should be included in the '**Declarations**' section.

Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Funding' and/or 'Competing interests'. Other declarations include Ethics approval, Consent, Data, Material and/or Code availability and Authors' contribution statements.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

When all authors have the same (or no) conflicts and/or funding it is sufficient to use one blanket statement.

Examples of statements to be used when funding has been received:

- Partial financial support was received from [...]
- The research leading to these results received funding from [...] under Grant Agreement No[...].
- This study was funded by [...]
- This work was supported by [...] (Grant numbers [...] and [...]

Examples of statements to be used when there is no funding:

- The authors did not receive support from any organization for the submitted work.
- No funding was received to assist with the preparation of this manuscript.
- No funding was received for conducting this study.
- No funds, grants, or other support was received.

Examples of statements to be used when there are interests to declare:

- **Financial interests:** Author A has received research support from Company A. Author B has received a speaker honorarium from Company Wand owns stock in Company X. Author C is consultant to company Y.

  Non-financial interests: Author C is an unpaid member of committee Z.

- **Financial interests:** The authors declare they have no financial interests.

  Non-financial interests: Author A is on the board of directors of Y and receives no compensation as member of the board of directors.

- **Financial interests:** Author A received a speaking fee from Y for Z. Author B receives a salary from association X. X where s/he is the Executive Director.

  Non-financial interests: none.

- **Financial interests:** Author A and B declare they have no financial interests. Author C has received speaker and consultant honoraria from Company M and Company N. Dr. C has received speaker

honorarium and research funding from Company M and Company O. Author D has received travel support from Company O.

Non-financial interests: Author D has served on advisory boards for Company M, Company N and Company O.

Examples of statements to be used when authors have nothing to declare:

- The authors have no relevant financial or non-financial interests to disclose.
- The authors have no competing interests to declare that are relevant to the content of this article.
- All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.
- The authors have no financial or proprietary interests in any material discussed in this article.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

Research involving human participants, their data or biological material

Ethics approval

When reporting a study that involved human participants, their data or biological material, authors should include a statement that confirms that the study was approved (or granted exemption) by the appropriate institutional and/or national research ethics committee (including the name of the ethics committee) and certify that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. If doubt exists whether the research was conducted in accordance with the 1964 Helsinki Declaration or comparable standards, the authors must explain the reasons for their approach, and demonstrate that an independent ethics committee or institutional review board explicitly approved the doubtful aspects of the study. If a study was

granted exemption from requiring ethics approval, this should also be detailed in the manuscript (including the reasons for the exemption).

Retrospective ethics approval

If a study has not been granted ethics committee approval prior to commencing, retrospective ethics approval usually cannot be obtained and it may not be possible to consider the manuscript for peer review. The decision on whether to proceed to peer review in such cases is at the Editor's discretion.

Ethics approval for retrospective studies

Although retrospective studies are conducted on already available data or biological material (for which formal consent may not be needed or is difficult to obtain) ethics approval may be required dependent on the law and the national ethical guidelines of a country. Authors should check with their institution to make sure they are complying with the specific requirements of their country.

Ethics approval for case studies

Case reports require ethics approval. Most institutions will have specific policies on this subject. Authors should check with their institution to make sure they are complying with the specific requirements of their institution and seek ethics approval where needed. Authors should be aware to secure informed consent from the individual (or parent or guardian if the participant is a minor or incapable) See also section on **Informed Consent**.

Cell lines

If human cells are used, authors must declare in the manuscript: what cell lines were used by describing the source of the cell line, including when and from where it was obtained, whether the cell line has recently been authenticated and by what method. If cells were bought from a life science company the following need to be given in the manuscript: name of company (that provided the cells), cell type, number of cell line, and batch of cells.

It is recommended that authors check the NCBI database for misidentification and contamination of human cell lines. This step will alert authors to possible problems with the cell line and may save considerable time and effort.

Further information is available from the International Cell Line Authentication Committee (ICLAC).

Authors should include a statement that confirms that an institutional or independent ethics committee (including the name of the ethics committee) approved the study and that informed consent was obtained from the donor or next of kin.

Research Resource Identifiers (RRID)

Research Resource Identifiers (RRID) are persistent unique identifiers (effectively similar to a DOI) for research resources. This journal encourages authors to adopt RRIDs when reporting key biological resources (antibodies, cell lines, model organisms and tools) in their manuscripts.

Examples:

Organism: *Filip1*$^{tm1a(KOMP)Wtsi}$ **RRID:MMRRC_055641-UCD**

Cell Line: RST307 cell line **RRID:CVCL_C321**

Antibody: Luciferase antibody DSHB Cat# LUC-3, **RRID:AB_2722109**

Plasmid: mRuby3 plasmid **RRID:Addgene_104005**

Software: ImageJ Version 1.2.4 **RRID:SCR_003070**

RRIDs are provided by the Resource Identification Portal. Many commonly used research resources already have designated RRIDs. The portal also provides authors links so that they can quickly register a new resource and obtain an RRID.

Clinical Trial Registration

The World Health Organization (WHO) definition of a clinical trial is "any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes". The WHO defines health interventions as "A health intervention is an act performed for, with or on behalf of a person or population whose purpose is to assess, improve, maintain, promote or modify health, functioning or health conditions" and a health-related outcome is generally defined as a change in the health of a person or population as a result of an intervention.

To ensure the integrity of the reporting of patient-centered trials, authors must register prospective clinical trials (phase II to IV trials) in suitable publicly available repositories. For example www.clinicaltrials.gov or any of the primary registries that participate in the WHO International Clinical Trials Registry Platform.

The trial registration number (TRN) and date of registration should be included as the last line of the manuscript abstract.

For clinical trials that have not been registered prospectively, authors are encouraged to register retrospectively to ensure the complete publication of all results. The trial registration number (TRN), date of registration and the words 'retrospectively registered' should be included as the last line of the manuscript abstract.

Standards of reporting

Springer Nature advocates complete and transparent reporting of biomedical and biological research and research with biological applications. Authors are recommended to adhere to the minimum reporting guidelines hosted by the EQUATOR Network when preparing their manuscript.

Exact requirements may vary depending on the journal; please refer to the journal's Instructions for Authors.

Checklists are available for a number of study designs, including:

Randomised trials (CONSORT) and Study protocols (SPIRIT)

Observational studies (STROBE)

Systematic reviews and meta-analyses (PRISMA) and protocols (Prisma-P)

Diagnostic/prognostic studies (STARD) and (TRIPOD)

Case reports (CARE)

Clinical practice guidelines (AGREE) and (RIGHT)

Qualitative research (SRQR) and (COREQ)

Animal pre-clinical studies (ARRIVE)

Quality improvement studies (SQUIRE)

Economic evaluations (CHEERS)

Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Ethics approval'.

Examples of statements to be used when ethics approval has been obtained:

• All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of the Medical University of A (No. ...).

• This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University B (Date.../No. ...).

• Approval was obtained from the ethics committee of University C. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

• The questionnaire and methodology for this study was approved by the Human Research Ethics committee of the University of D (Ethics approval number: ...).

Examples of statements to be used for a retrospective study:

• Ethical approval was waived by the local Ethics Committee of University A in view of the retrospective nature of the study and all the procedures being performed were part of the routine care.

• This research study was conducted retrospectively from data obtained for clinical purposes. We consulted extensively with the IRB of XYZ who determined that our study did not need ethical approval. An IRB official waiver of ethical approval was granted from the IRB of XYZ.

• This retrospective chart review study involving human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Human Investigation Committee (IRB) of University B approved this study.

Examples of statements to be used when no ethical approval is required/exemption granted:

• This is an observational study. The XYZ Research Ethics Committee has confirmed that no ethical approval is required.

• The data reproduced from Article X utilized human tissue that was procured via our Biobank AB, which provides de-identified samples. This study was reviewed and deemed exempt by our XYZ Institutional Review Board. The BioBank protocols are in accordance with the ethical standards of our institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves

the right to reject submissions that do not meet the guidelines described in this section.

Informed consent

All individuals have individual rights that are not to be infringed. Individual participants in studies have, for example, the right to decide what happens to the (identifiable) personal data gathered, to what they have said during a study or an interview, as well as to any photograph that was taken. This is especially true concerning images of vulnerable people (e.g. minors, patients, refugees, etc) or the use of images in sensitive contexts. In many instances authors will need to secure written consent before including images.

Identifying details (names, dates of birth, identity numbers, biometrical characteristics (such as facial features, fingerprint, writing style, voice pattern, DNA or other distinguishing characteristic) and other information) of the participants that were studied should not be published in written descriptions, photographs, and genetic profiles unless the information is essential for scholarly purposes and the participant (or parent/guardian if the participant is a minor or incapable or legal representative) gave written informed consent for publication. Complete anonymity is difficult to achieve in some cases. Detailed descriptions of individual participants, whether of their whole bodies or of body sections, may lead to disclosure of their identity. Under certain circumstances consent is not required as long as information is anonymized and the submission does not include images that may identify the person.

Informed consent for publication should be obtained if there is any doubt. For example, masking the eye region in photographs of participants is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic profiles, authors should provide assurance that alterations do not distort meaning.

Exceptions where it is not necessary to obtain consent:

• Images such as x rays, laparoscopic images, ultrasound images, brain scans, pathology slides unless there is a concern about identifying information in which case, authors should ensure that consent is obtained.

• Reuse of images: If images are being reused from prior publications, the Publisher will assume that the prior publication obtained the relevant information regarding consent. Authors should provide the appropriate attribution for republished images.

Consent and already available data and/or biologic material

Regardless of whether material is collected from living or dead patients, they (family or guardian if the deceased has not made a pre-mortem decision) must have given prior written consent. The aspect of confidentiality as well as any wishes from the deceased should be respected.

Data protection, confidentiality and privacy

When biological material is donated for or data is generated as part of a research project authors should ensure, as part of the informed consent procedure, that the participants are made aware what kind of (personal) data will be processed, how it will be used and for what purpose. In case of data acquired via a biobank/biorepository, it is possible they apply a broad consent which allows research participants to consent to a broad range of uses of their data and samples which is regarded by research ethics committees as specific enough to be considered "informed". However, authors should always check the specific biobank/biorepository policies or any other type of data provider policies (in case of non-bio research) to be sure that this is the case.

Consent to Participate

For all research involving human subjects, freely-given, informed consent to participate in the study must be obtained from participants (or their parent or legal guardian in the case of children under 16) and a statement to this effect should appear in the manuscript. In the case of articles describing human transplantation studies, authors must include a statement declaring that no organs/tissues were obtained from prisoners and must also name the institution(s)/clinic(s)/department(s) via which organs/tissues were obtained. For manuscripts reporting studies involving vulnerable groups where there is the potential for coercion or where consent may not have been fully informed, extra care will be taken by the

editor and may be referred to the Springer Nature Research Integrity Group.

Consent to Publish

Individuals may consent to participate in a study, but object to having their data published in a journal article. Authors should make sure to also seek consent from individuals to publish their data prior to submitting their paper to a journal. This is in particular applicable to case studies. A consent to publish form can be found

here. (Download docx, 36 kB)

Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Consent to participate' and/or 'Consent to publish'. Other declarations include Funding, Competing interests, Ethics approval, Consent, Data and/or Code availability and Authors' contribution statements.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

Sample statements for **"Consent to participate"**:

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

Informed consent was obtained from legal guardians.

Written informed consent was obtained from the parents.

Verbal informed consent was obtained prior to the interview.

Sample statements for **"Consent to publish"**:

The authors affirm that human research participants provided informed consent for publication of the images in Figure(s) 1a, 1b and 1c.

The participant has consented to the submission of the case report to the journal.

Patients signed informed consent regarding publishing their data and photographs.

Sample statements if identifying information about participants is available in the article:

Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

Images will be removed from publication if authors have not obtained informed consent or the paper may be removed and replaced with a notice explaining the reason for removal.

Back to top

Research Data Policy and Data Availability Statements

This journal operates a type 2 research data policy (life sciences). A submission to the journal implies that materials described in the manuscript, including all relevant raw data, will be freely available to any researcher wishing to use them for non-commercial purposes, without breaching participant confidentiality.

The journal strongly encourages that all datasets on which the conclusions of the paper rely should be available to readers. We encourage authors to ensure that their datasets are either deposited in publicly available repositories (where available and appropriate) or presented in the main manuscript or additional supporting files whenever possible. Please see Springer Nature's information on recommended repositories.

List of Repositories

Research Data Policy

General repositories - for all types of research data - such as figshare and Dryad may be used where appropriate.

Datasets that are assigned digital object identifiers (DOIs) by a data repository may be cited in the reference list. Data citations should include the minimum information recommended by DataCite: authors, title, publisher (repository name), identifier.

DataCite

Where a widely established research community expectation for data archiving in public repositories exists, submission to a community-endorsed, public repository is mandatory. Persistent identifiers (such as DOIs and accession numbers) for relevant datasets must be provided in the paper.

If the journal that you're submitting to uses double-blind peer review and you are providing reviewers with access to your data (for example via a repository link, supplementary information or data on request), it is strongly suggested that the authorship in the data is also blinded. There are data repositories that can assist with this and/or will create a link to mask the authorship of your data.

For the following types of data set, submission to a community-endorsed, public repository is mandatory:

Mandatory deposition	Suitable repositories
Protein sequences	Uniprot
DNA and RNA sequences	Genbank DNA DataBank of Japan (DDBJ) EMBL Nucleotide Sequence Database (ENA)
DNA and RNA sequencing data	NCBI Trace Archive NCBI Sequence Read Archive (SRA)
Genetic polymorphisms	dbSNP dbVar European Variation Archive (EVA)
Linked genotype and phenotype data	dbGAP The European Genome-phenome Archive (EGA)
Macromolecular structure	Worldwide Protein Data Bank (wwPDB) Biological Magnetic Resonance Data Bank (BMRB) Electron Microscopy Data Bank (EMDB)
Microarray data (must be MIAME compliant)	Gene Expression Omnibus (GEO) ArrayExpress
Crystallographic data for small molecules	Cambridge Structural Database

Data availability

The journal encourages authors to provide a statement of Data availability in their article. Data availability statements should include information on where data supporting the results reported in the article can be found, including, where applicable, hyperlinks to publicly archived datasets analysed or generated during the study. Data availability statements can also indicate whether data are available on request from the authors and where no data are available, if appropriate.

Data Availability statements can take one of the following forms (or a combination of more than one if required for multiple datasets):

- 1. The datasets generated during and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS]
- 2. The datasets generated during and/or analysed during the current study are not publicly available due [REASON WHY DATA

ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.

- 3. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.
- 4. Data sharing not applicable to this article as no datasets were generated or analysed during the current study.
- 5. All data generated or analysed during this study are included in this published article [and its supplementary information files].

More examples of template data availability statements, which include examples of openly available and restricted access datasets, are available:

Data availability statements

Authors who need help understanding our data sharing policies, help finding a suitable data repository, or help organising and sharing research data can access our Author Support portal for additional guidance.

After Acceptance

Upon acceptance, your article will be exported to Production to undergo typesetting. Once typesetting is complete, you will receive a link asking you to confirm your affiliation, choose the publishing model for your article as well as arrange rights and payment of any associated publication cost.

Once you have completed this, your article will be processed and you will receive the proofs.

Article publishing agreement

Depending on the ownership of the journal and its policies, you will either grant the Publisher an exclusive licence to publish the article or will be asked to transfer copyright of the article to the Publisher.

Offprints

Offprints can be ordered by the corresponding author.

Color illustrations

Publication of color illustrations is free of charge.

Proof reading

The purpose of the proof is to check for typesetting or conversion errors and the completeness and accuracy of the text, tables and figures. Substantial changes in content, e.g., new results, corrected values, title and authorship, are not allowed without the approval of the Editor.

After online publication, further changes can only be made in the form of an Erratum, which will be hyperlinked to the article.

Online First

The article will be published online after receipt of the corrected proofs. This is the official first publication citable with the DOI. After release of the printed version, the paper can also be cited by issue and page numbers.

Open Choice

Open Choice allows you to publish open access in more than 1850 Springer Nature journals, making your research more visible and accessible immediately on publication.

Article processing charges (APCs) vary by journal – view the full list

Benefits:

- Increased researcher engagement: Open Choice enables access by anyone with an internet connection, immediately on publication.
- Higher visibility and impact: In Springer hybrid journals, OA articles are accessed 4 times more often on average, and cited 1.7 more times on average*.
- Easy compliance with funder and institutional mandates: Many funders require open access publishing, and some take compliance into account when assessing future grant applications.

It is easy to find funding to support open access – please see our funding and support pages for more information.

*) Within the first three years of publication. Springer Nature hybrid journal OA impact analysis, 2018.

Open Choice

Funding and Support pages

Open access publishing

To find out more about publishing your work Open Access in *Journal of Neuro-Oncology*, including information on fees, funding and licenses, visit our Open access publishing page.