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SOMATIC DEFICIENT MISMATCH REPAIR ASSESSED BY IMMUNOHISTOCHEMISTRY AND CLINICAL FEATURES IN BRAZILIAN GLIOBLASTOMA PATIENTS

Background. Glioblastoma (GBM) is the most frequent primary malignant CNS tumor. Deficient mismatch repair (dMMR) is associated with better prognosis and is a biomarker for immunotherapy. Evaluation of MMR by immunohistochemistry (IHC) is accessible, cost effective, sensitive, and specific. **Aim.** Our objective was to investigate MMR proteins in adult GBM patients. **Materials and Methods.** We retrospectively analyzed 68 GBM samples to evaluate the proficiency of MMR genes expression assessed by IHC. Clinicopathologic and molecular features were compared in proficient (pMMR) or dMMR. **Results.** 10 (14.7%) samples showed dMMR, and the most frequent was MSH6 (100%) followed by MSH2, PMS2, and MLH1. We observed heterogeneous expression of dMMR in 5 GBMs. The median overall survival did not differ between pMMR (19.8 months; 0.2—30) and dMMR (16.9 months; 6.4—27.5) ($p = 0.31$). We observed a significantly higher overall survival associated with gross total resection compared to subtotal resection or biopsy (30.7 *vs.* 13.6 months, $p = 0.02$) and MGMT methylated status (29.6 *vs.* 19.8 months, $p = 0.049$). At the analysis time, 10 patients were still alive, all in the pMMR group. **Conclusions.** Our data demonstrated dMMR phenotype assessed by IHC in an expressive portion of GBM patients, however without significant impact on overall survival.

Keywords: brain tumor, glioblastoma, glioma, microsatellite instability, mismatch repair.

Glioblastoma (GBM) comprises 48.6% of all primary malignant brain tumors [1], and standard multimodality treatment includes surgery (aimed at the gross total resection, when feasible), radiotherapy, temozolomide (TMZ)-based chemotherapy, with or without tumor treating

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fields [2—5]. The relapse after frontline therapy is almost universal, and the median overall survival from diagnosis ranges from 14 to 20.9 months [3—5]. Our knowledge of the molecular landscape of GBM has significantly grown in recent years. Yet, the clinical studies have failed to translate such information into the relevant improvements in the treatment options or prognosis [6—9].

A microsatellite instability (MSI) is a condition in which defective or deficient mismatch repair (dMMR) mechanisms in cancer cells result in a hypermutated phenotype, mainly in the microsatellite locations (i.e., repetitive sequences particularly sensitive to DNA mismatch errors) [10]. MSI/dMMR can occur in either hereditary syndromes (Lynch syndrome and constitutional mismatch repair deficiency) or sporadic cancers, and it is characterized by the absence of the expression of one or more proteins namely MutL homologue 1 (MLH1), MutS homologue 2 (MSH2), MutS homologue 6 (MSH6), and postmeiotic segregation increased 2 (PMS2) [11]. Proficient MMR (pMMR) patients have this mechanism preserved. Lynch syndrome is characterized by a monoallelic mutation in the MMR genes resulting in somatic inactivation of a nonmutant allele, which leads to the absence of MMR activity. On the other hand, germline mutation in the constitutional mismatch repair deficiency (CMMRD) is biallelic, expressing dMMR in all tissues [12].

dMMR assessed by immunohistochemistry (IHC) is defined by the European Society for Medical Oncology (ESMO) Translational Research and Precision Medicine Working Group as a loss of the expression of at least one of the MMR-related proteins in tumor cells [13]. MSI/dMMR is a well-established favorable prognostic biomarker in colorectal, gastric, pancreatic, endometrial, and ovarian cancers [14]. dMMR is also considered a selection biomarker in immunotherapy for cancer of the colon, rectum, bile ducts, endometrium, and stomach, among others. In 2017, for the first time, the Food and Drug Administration (FDA) approved immunotherapy as an agnostic treatment based on the presence of dMMR biomarkers regardless of the primary tumor site [15, 16]. The international expert consensus strongly recommends immunotherapy for the treatment of patients with MSI/dMMR solid tumors [13].

In GBM, immunotherapy has been shown to be ineffective in both overall cohort and subgroups stratified by programmed death-ligand 1 programmed cell death protein 1 (PD-L1/PD-1) [17—19]. However, beneficial effects of immunotherapy have been demonstrated in some scenarios, such as biallelic mismatch repair deficiency and pathogenic mutations in a polymerase germline deficiency. Prospectively, one phase 1 trial is in progress in GBM patients and may evaluate pembrolizumab (anti-PD1) in constitutional mismatch repair deficiency and Lynch syndrome (NCT02359565), whose phenotype is dMMR [20, 21]. The beneficial effects of immunotherapy in neoadjuvant surgically resectable recurrent GBM (rGBM), in combination with hypofractionated stereotactic irradiation and in some series in GBM without any identifiable positive biomarker have been reported [20—26]. In contradiction, in rGBM, MSI has been hypothesized as a surrogate of hypermutation induced by temozolomide and seems to be a negative therapeutic biomarker for immunotherapy [27—29].

Few retrospective studies analyzed dMMR in GBMs. The loss of the expression of at least one MMR protein varied from 1.3% to 12.7% in the newly diagnosed GBM (nGBM) and from 13.1% to 33% in rGBM [30—34], but these studies could not confirm that MSI might be associated with a worse prognosis as in primary mismatch repair deficient IDH-mutant astrocytoma and rGBM [27—29]. The limitation in sample size and/or standardization of tests may have contributed to explain the lack of the significant survival differences among dMMR GBM patients previously reported.

dMMR can be tested by IHC, polymerase chain reaction (PCR) or next generation sequencing (NGS). IHC is the preferred method for MSI testing, as recommended by ESMO, with several advantages: it is widely available, rapid, cost effective, and has high sensitivity (92.3%) and specificity (100%) [10, 35]. Such features are particularly relevant in developing and low-income countries, where PCR and NGS costs are seldom covered by public and private health insurance. As IHC is widely used as an initial test, the proper performance of the assay and the correct interpretation of the IHC data are of the main concerns. The absence of nuclear staining in tumor cells was scored "negative or lost or deficient" for expression for that protein in contrast to that stained positively or retained or proficient [36]. Occasionally, IHC is not very clear (around 3.3% in colorectal and endometrial cancer), with weak expression and/or focal expression, which can lead to inconclusive results and misinterpretation of the findings, even with germline mutation [37]. Despite the importance of this screening test, there is no guideline for scoring and interpretation of MMR IHC. When the efficacy of DNA MMR IHC for hypermutated glioma was evaluated, the sensitivity, specificity and overall accuracy were 89%, 99%, and 98%, respectively [38].

Our study was focused on a retrospective analysis of clinicopathological characteristics, expression and pattern of MMR proteins status by IHC, and outcomes in Brazilian GBM patients.

Materials and Methods

The study was approved by the Institutional Review Board and the local Ethics Committee of Hospital BP — a Beneficência Portuguesa de São Paulo (São Paulo, Brazil, number 08127219.1.0000.5483), in accordance with the principles expressed in the Declaration of Helsinki. We retrospectively collected tumor tissue from all adult (18 years old) GBM patients whose cases were consecutively diagnosed in a tertiary neuropathology laboratory (Carmen Lucia Penteado Lancellotti Neuropathology Laboratory, São Paulo, Brazil) between June 2015 and January 2019, according to the 2021 WHO criteria [39]. We reviewed patients' demographic and clinicopathologic data from the medical records, including the age, gender, tumor location, size, resection, histological features, molecular characteristics, and status of disease (newly diagnosed or recurrent). We used the following monoclonal antibodies for immunohistochemical analysis: anti-MLH-1 (clone ES05, Dako, USA), anti-MSH-2 (clone FE11, Dako, USA), anti-MSH-6 (clone SP93, Cell Marque, USA), anti-PMS2 (clone EP51, Dako, USA) with validated protocols and all samples were analyzed with positive and negative (lack of MMR expression) internal (adjacent normal cells) and external controls. Each IHC marker was scored as "proficient" or "deficient". In accordance with the published data on MMR IHC interpretation in glioma patients [38], the tumors that showed uniform positive nuclear staining for the protein were scored as "proficient". In contrast, the absence of nuclear staining of the viable tumor cell nuclei not near areas of necrosis or thermal artifact were scored as "deficient" for expression of that protein. For dMMR tumors, the pattern was classified as "homogeneous or heterogeneous" [38]: "homogeneous" tumors showed the total absence of protein expression in all analyzed tumor areas, whereas "heterogeneous" tumors were not uniform, i.e., showed clusters of "proficient" and "deficient" tumor cells. Malignant viable tumor cells were identified by morphology. IHC for isocitrate dehydrogenase 1 (IDH) (clone H09, Dianova, USA) was performed as the WHO guideline for initial assessment, and all mutant IDH patients were excluded. All IHC were interpreted without the knowledge of mutational studies. Despite the recommendation, IDH sequencing in patients aged < 55 years was

not routinely performed due to lack of insurance coverage for most Brazilian patients. The other immunohistochemical markers performed were GFAP (polyclonal), S100 (polyclonal), ATRX (AX1), Olig2 (olig2), p53 (DO-7 monoclonal), EGFR (31G7), and Ki-67 (MIB1). O-6-methylguanine-DNA-methyltransferase (MGMT) methylation was assessed by a qualitative PCR methylation-specific high-resolution melting (MS-HRM) (Imprint TM DNA modification kit, Sigma).

We performed univariate analyses using the Student's *t*-test for continuous variables, and Chi-squared or Fisher's exact test for evaluating differences in proportions. We used the Kaplan — Meier method to estimate the median overall survival (OS), and the log-rank test to compare patients' survival times. OS was calculated from the time of primary diagnosis to death from any cause or the last available contact date (if available). The survival was expressed as a median with a 95% confidence interval (CI). The differences were considered significant at $p \leq 0.05$. All analyses were performed using SPSS, RRID:SCR_002865, version 25.

Results

We included in the study a total of 68 GBMs patients with available paraffin blocks. Forty-two (61.8%) were male, and 26 (38.2%) were female, with the median age at diagnosis of 58 years (range 21—86 years). In 41 (63.1%) patients, the tumors were < 5 cm and in 62 (91.2%) patients were nGBM. The most frequent location was supratentorial (95.5%). 32 (47%) patients underwent subtotal resection or biopsy, 18 (26.5%) patients were submitted to gross total resection and in 18 (26.5%) patients, the extent of resection was unavailable. By the histological features, 92.6% of the tumors were GBM, 4.4% — gliosarcoma, and 3% — giant cell GBM. 33 (48.5%) tumors were MGMT methylated, 28 (41.2%) tumors were unmethylated, and in 7 (10.3%) patients, MGMT status was not available (missed information or indeterminate result). Median OS in the overall cohort was 17.8 months (95% CI 8.9—26.8 months). At the time of analyses, 14 patients were still alive, all of them were pMMR.

Ten (14.7%) patients showed dMMR phenotype and 58 (85.3%) patients were pMMR. Among the dMMR patients, 5 of 10 showed homogeneous loss of protein expression in all analyzed tumor areas while 5 showed heterogeneous protein expression. The clinicopathological features of the proficient and deficient MMR are presented in Table 1. Median age at diagnosis was 57.5 years in the pMMR group and 59.5 years in the dMMR group. The majority in both groups was nGBM (94.8% pMMR and 80% dMMR). In the dMMR group, the majority was female (60%) whereas in the pMMR group, the majority was male (65%). Tumor location was similar in both groups and the most frequent was supratentorial. Among pMMR patients, the proportion of methylated and unmethylated MGMT was the same (44.8%) whereas in most dMMR cases, MGMT was methylated (70%).

In IHC evaluation, 58 pMMR patients showed a similar patter with the maintained expression of the MMR protein (Figure, *a* and *b*). Among dMMR patients, the most frequent loss of expression was for MSH6 (100%), followed by MSH2 (80%), PMS2 (30%), and MLH1 (0%) (Table 2). Of these, only one was rGBM treated with 2 surgeries, radiotherapy twice, and chemotherapy with temozolomide, irinotecan, and bevacizumab. None showed loss of all MMR expression. In 5 cases, heterogeneity in the loss of repair proteins was observed, with proficient and deficient tumor cells areas (Figure, *c* and *d*). In 5 cases, the loss of expression was homogeneous (Figure, *e* and *f*). Two of these heterogeneous cases were gliosarcoma and both showed PMS2, MSH2 and MSH6 deficient expression only in sarcomatous cells with their preserved expression in glial cells (Figure, *c*).

OS was 19.8 months (9.2—30) in the pMMR group and 16.9 months (6.4—27.5) in the

Table 1. **Clinicopathological features of GBM patients**

Characteristic	Proficient MMR		Deficient MMR	
Age at diagnosis (years)				
Median	57.5 (range 21—86)		59.5 (range 34—78)	
Gender	N = 58	%	N = 10	%
Female	20	35	6	60
Male	38	65	4	40
Tumor location				
Supratentorial	55	95	10	100
Infratentorial	2	3.3	0	0
NA†	1	1.5	0	0
Size (cm)				
5	36	62	5	50
> 5	19	33	5	50
NA†	3	5	0	0
Resection				
Gross total resection	16	27.5	2	20
Subtotal resection or biopsy	29	50	3	30
NA†	13	22.5	5	50
GBM subtypes				
GBM	55	94.8	8	80
Giant cell GBM	2	3.4	0	0
Gliosarcoma	1	1.8	2	20
Status of disease				
Newly GBM	53	91.3	9	90
Recurrent GBM	5	8.7	1	10
MGMT promoter methylation status (PCR)				
Methylated	26	44.8	7	70
Unmethylated	26	44.8	2	20
NA†	6	10.4	1	10

Note: † NA – not available.

dMMR group (HR 1.44; CI 95%; 0.7—2.95; p = 0.31). As for OS in the dMMR group by IHC patterns, we observed 17.8 months (15.9—19.8) in homogeneous lost and 11 months (0—23.8) in heterogeneous expression (p = 0.56). The extent of the resection at diagnosis and MGMT methylation status were correlated with the improved survival, as expected. In biopsy and subtotal resection (STR) group, OS was 13.6 vs. 30.7 months for gross total resection (GTR) (HR 0.38; CI 95% 0.17—0.88; p = 0.02). In MGMT methylated patients, OS was 29.6 *vs.* 19.8 months for MGMT unmethylated patients (p = 0.049). The additional features (the age at diagnosis ≤50 or >50 years, the status of disease — newly *vs.* recurrent and size) were analyzed but were not statistically significant (Table 3).

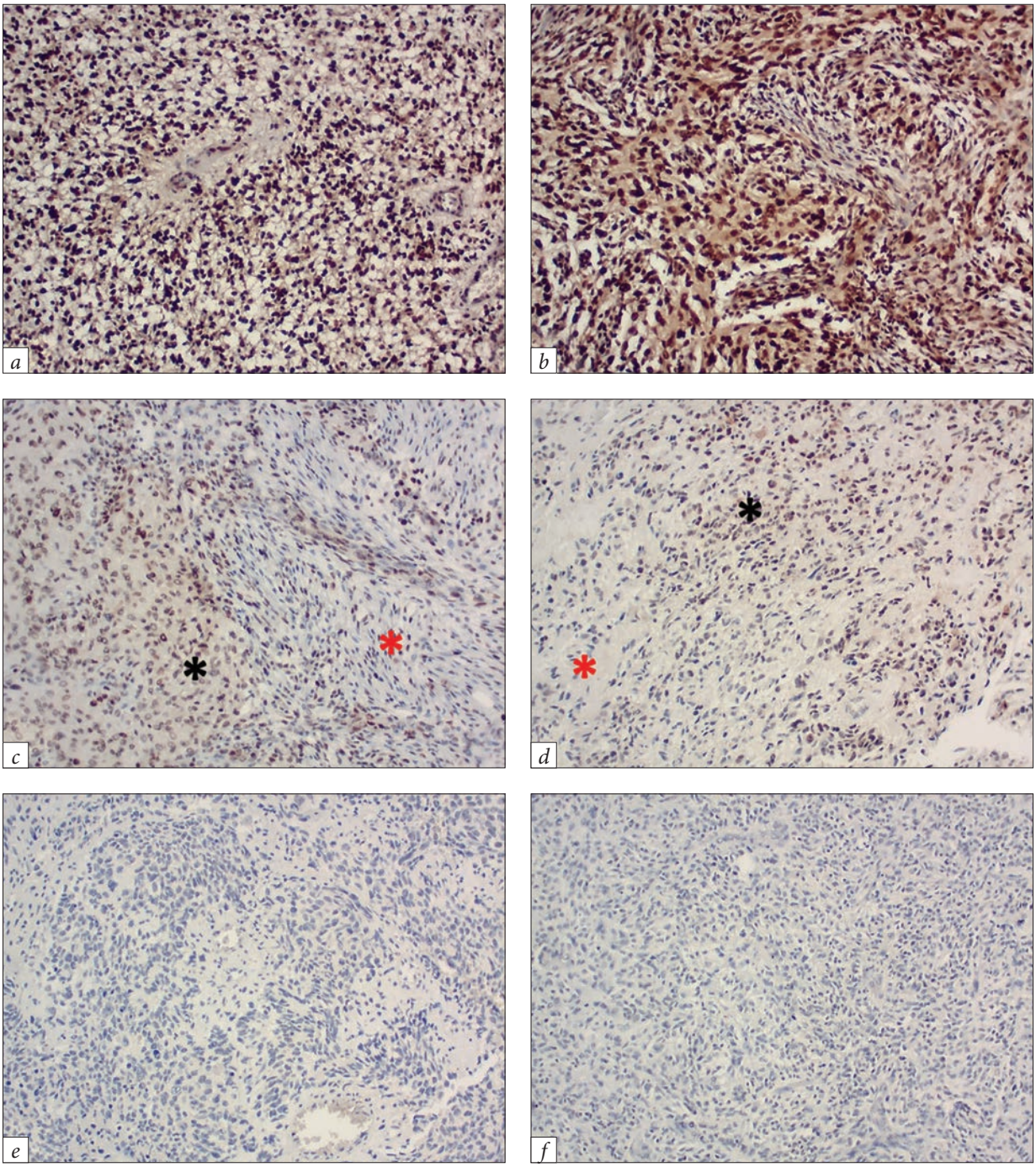

Fig. 1. Representative images of GBM samples. 34-year-old female GBM patient with MLH1 proficient (*a*) (case 8). 78-year-old female gliosarcoma with MLH1 proficient (*b*) and heterogeneous dMMR MSH2 (*c*), with proficient (black asterisk) and deficient (red asterisk) tumor cells areas, respectively (case 1). 48-year-old male GBM patient with heterogeneous loss of MSH6 expression (*d*) and loss of expression of MSH2 (*e*), respectively (case 3). 60-year-old male GBM patient shows homogeneous dMMR MSH2 (*f*) (case 5). All samples were analyzed with positive and negative internal and external controls. Original magnification x 100

Discussion

The identification of any biomarker in GBM with both prognostic and/or therapeutic potentials is important for such an aggressive disease with few treatment options. Although no correlation between hypermutation phenotype with MMR and the response to immunotherapy in GBM patients has been demonstrated, the accurate identification of hypermutation and its genomic signature has potential therapeutic implications and needs more data. MMR genes are important in maintaining genome stability, and there is little data regarding the proficiency of repair genes in GBM both in terms of incidence and impact as a biomarker. The use of an effective, reproducible, and easily accessible method is mandatory in this scenario [40, 41].

Information about repair gene proficiency by IHC and the clinical impact in GBM patients like our population is scarce (Table 4). Tepeoglu et al. [32] evaluated 71 GBM patients assessed by IHC. They detected a loss of at least one of the MMR proteins in 7 (9.9%) patients, and the OS was lower in the dMMR group (30.65 months *vs.* 10.71 months, $p = 0.059$). Çakir et al. [33] observed 8.5% dMMR in 47 nGBM and 33.3% in 27 rGBM evaluated by IHC but did not find significant impact on survival. Almuhaisen et al. [42] found 6% dMMR in 96 HGG in adolescents and young adults. The somatic dMMR is usually associated with the acquired alteration along the molecular evolution of diffuse gliomas and not as an early mutation in the oncogenesis of GBM [43], justifying a higher frequency in rGBM. In our study, we demonstrated 10/68 (14.7%) GBMs with somatic dMMR assessed by IHC across the entire cohort, but with homogeneous loss only in 5/10 (50%), similar to the previous reports on GBM (~7%). We observed a clearly heterogeneous pattern of loss in 5/10 cases, which may signify MMR mutation but needs further mutational investigation in our series. This pattern is important in glioma patients and was described by McCord et al. [38] who evaluated 100 gliomas. 8 (8%) of them showed a loss of at least one MMR protein by IHC (all of them were hypermutated by NGS), and in 4/8 tumors the pattern of MMR loss was heterogeneous. In nGBM, we observed 9/62 (14.5%) dMMR, a slightly superior frequency when compared with previous reports, and 1/6 (16%) in rGBM (case 2) with a homogeneous loss.

Viana-Pereira et al. [44] evaluated 73 adults with high grade glioma (HGG) and found 5 (6.8%) MSI patients by PCR. Among these, only

Table 2. **Evaluation of MMR protein expression in MMR-deficient GBM samples**

Case	Gender	GBM type	MLH1	PMS2	MSH2	MSH6	Pattern of MMR IHC loss	OS (months)
1	F	Gliosarcoma	proficient	deficient	deficient	deficient	heterogeneous	30.7
2	F	GBM	proficient	proficient	deficient	deficient	homogeneous	29.6
3	M	GBM	proficient	proficient	deficient	deficient	heterogeneous	11.0
4	M	GBM	proficient	proficient	deficient	deficient	heterogeneous	5.1
5	M	GBM	proficient	proficient	deficient	deficient	homogeneous	37.1
6	F	GBM	proficient	proficient	proficient	deficient	homogeneous	16.9
7	M	Gliosarcoma	proficient	deficient	deficient	deficient	heterogeneous	31.7
8	F	GBM	proficient	proficient	deficient	deficient	homogeneous	17.8
9	F	GBM	proficient	proficient	deficient	deficient	homogeneous	8.7
10	F	GBM	proficient	deficient	proficient	deficient	heterogeneous	0.2

1 patient presented dMMR by IHC. Rodríguez-Hernández et al. [30] described 43% dMMR assessed by IHC in 76 HGG patients. The loss of any MMR protein expression was not correlated with the PCR-MSI status. The longer overall survival in dMMR HGG patients observed in the study cohort (dMMR, 13.8 months; pMMR, 10.1 months; p = 0.045) was not confirmed in an independent validation cohort in the same trial; however, the treatment approach was different between the cohorts. Indraccolo et al. [31] analyzed 57 pairs of GBM samples (newly and recurrent GBM) and observed 25.9% loss of reactivity in rGBM and 3.6% in nGBM assessed by IHC. In this trial, alteration of MMR genes by IHC was not confirmed by PCR and the whole exome sequencing (WES) in any of analyzed cases, and they could not demonstrate any impact on the OS. In studies [30, 31, 44], the available tests for the detection of MMR (IHC) and MSI (PCR and WES) were compared, suggesting that there is no trustworthy relationship between the methods in this population. Kim et al. [45] studied 740 primary brain tumors and observed ~2% dMMR, and the loss of nuclear MMR protein was 100% correlated with the MMR gene mutation in their cases, suggesting that IHC is sufficient to identify MMRD in brain tumors, similarly to McCord et al. [38], who previously showed high sensitivity, specificity, and overall accuracy of MMR IHC for hypermutated gliomas. Pan-cancer genome analysis projects utilizing data from The Cancer Genome Atlas (TCGA) identified 0.25% to 1.3% MSI rate in GBM samples evaluated by different sequencing methods [46—51], showing a lower MSI frequency than other methods, perhaps due to the quality/quantity of the information it provides. In the absence of conclusive data, currently all tests are recommended by international guidelines regardless of the primary tumor, including neuro-oncological tumors [10, 13]. Since the FDA approved immunotherapy in dMMR patients [10, 16, 52], the MMR status in all tumors has become relevant, mainly in GBM patients, whose therapeutic options are limited. The correct interpretation of MMR IHC is a concern because we observed a heterogeneous protein expression, which had been previously described not only in gliomas [38] but also in colorectal and endometrial cancer [37], and possible association with germline mutation or clinical impact have not been well elucidated. We observed a loss of MMR protein expression in areas of necrosis and thermal injury (not shown). In this scenario, a guideline to standard IHC interpretation is imperative.

Table 3. **Univariate survival analysis according to clinical features**

	OS (months)	CI 95%	*p*
pMMR	19.8	9.2—30	0.314
dMMR	16.9	6.4—27.5	
Homogeneous dMMR	17.8	15.9—19.8	0.562
Heterogeneous dMMR	11	0—23.8	
nGBM	17.8	12.5—23.2	0.760
rGBM	10.1	0—37.8	
≤50 years	16.1	10—22.7	0.496
>50 years	26.9	22.1—31.8	
≤ 5 cm	13.6	3.2—24.1	0.680
> 5 cm	16.9	6—27.9	
Biopsy + STR	13.6	5.34—22	0.020*
GTR	30.7	21—40.4	
MGMT methylated	29.6	13.4—45.8	0.049*
MGMT unmethylated	19.8	10.7—28.9	

Note: * The difference is significant ($p < 0.05$).

The efforts to select patients by predictive and/or therapeutic biomarkers, who can benefit from immune-checkpoint inhibitors in a known immunologically cold tumor are highly desirable. Although not established as a definitive test to evaluate the MMR status in GBMs, IHC is an accessible and low-cost test and has a high correlation with microsatellite instability in most tumors. Our choice to assess MMR status by IHC was because the PCR and NGS costs are usually not covered by insurance as there is no valida-

Table 4. **Summary of published retrospective series that evaluated MMR expression by IHC**

Study	Population	Method	Results	OS
Viana-Pereira et al. (2011) [44]	144 HGG (71 pediatric and 73 adult patients)	PCR in all cases IHC only MSI by PCR	IHC was performed only in MSI patients by PCR 14/71 pediatric patient (19.7%) were MSI by PCR (MSI Low was defined with 1 or 2 markers by PCR); 76.9% of them dMMR 5/73 adults (6.8%) MSI by PCR, only 1 dMMR (MLH1 negative).	OS not available
Rodriguez-Hernandez et al. (2013) [30]	96 newly diagnosed astrocytoma II—IV (19 astro grade III and 57 GBM)	IHC and PCR	PCR was performed only in dMMR by IHC 41/96 (43%) dMMR by IHC: 37 (42%) were MSI low (<30% instability markers) 4 (5%) were MSI high (≥30% instability markers), only 1 of them dMMR dMMR protein IHC expression was not associated with PCR-MSI status	HGG OS in study cohort: dMMR, 13.8 months; pMMR, 10.1 months (p = = 0.045) This difference in OS was not confirmed in an independent validation cohort.
Indraccolo et al. (2018) [31]	57 pairs of GBM samples (diagnosis and relapse)	IHC, PCR, and WES	IHC was performed in all samples; PCR in 40 samples, and WES in 9 samples 2/56 (3.6%) dMMR by IHC in nGBM patients 14/54 (25.9%) dMMR by IHC in rGBM patients Among 12 dMMR analyzed, no MSI by PCR was detected. 3 MSH-6 negative (IHC) samples showed no functional mutations in MMR genes (WES)	No significant difference in OS
Tepeoglu et al. (2019) [32]	71 GBM	IHC	7 (9.9%) dMMR	OS: dMMR, 10.71 ± 5.2 months; pMMR, 30.65 ± ± 5.1 months (p = 0.059)
Çakir et al. (2020) [33]	47 nGBM 27 rGBM	IHC	4 (8.5%) nGBM 9 (33.3%) rGBM	OS: dMMR, 7 months; pMMR, 10 months (p = = 0.953)
Almuhaisen et al. (2020) [42]	96 HGG (15—39 years)	IHC	6 (6%) dMMR	OS not available

Table 4. (ending)

Study	Population	Method	Results	OS
Kim et al. (2021) [38]	740 primary brain tumors	IHC MSI PCR NGS Sanger	13 (2%) dMMR = 9 GBM, 2 astrocytoma grade 4, 1 diffuse midline glioma H3K27A, 1 pleomorphic xanthoastrocytoma	A trend for lower PFS in patients with MMRD-HGG than that in patients without MMRD- HGG ($p = 0.69$, $p = 0.64$)
McCord et al. (2020) [38]	100 gliomas	NGS for hypermutation analysis IHC	8/100 (8%) dMMR (all of them were hypermutators)	OS not available
Yamada et al. (2022)	68 GBM	IHC	14.5% dMMR all cohort (7.3% homogeneous loss of MMR expression) 15.3% nGBM 9% rGBM	OS: dMMR, 16.9 months (9.6—24.2); pMMR 24.2 months (14.9—33.4) ($p = = 0.120$)

tion by the national health regulatory agency about the use of NGS in solid tumors. In addition, sequencing is known to bring more information and seems to be more reliable in detecting dMMR/MSI, but its access is prohibitive for a large portion of patients. This discussion is certainly relevant and indicates the need to standardize the definition and the appropriate method for evaluating the proficiency of repair genes in GBM patients (Table 4). Furthermore, none of these tests have already been proved as a biomarker for immune checkpoint inhibitor treatment in GBM patients.

The most frequent loss of expression in our series was observed for MSH6, followed by MSH2, PMS2, and MLH1 similar to the published series [30, 31]. A specific molecular signature associated with loss of MSH6 expression in rGBM can be induced by TMZ [28, 41] and supposed to be a negative biomarker for response to TMZ and immunotherapy [28].

The patient with a dMMR rGBM (case 2) showed loss of MSH2 and MSH6 and survived for 30 months, but (unfortunately) no sequencing could be performed to confirm the presence of mutations. IHC is not able to differentiate germline from somatic mutations, but it may be indicative, especially in CMMRD patients, where all cells lose MMR protein expression. In the case of suspected family inheritance, the germ test is mandatory to confirm the mutation [12]. In our series, despite not having a germinative test, none of the cases presented global loss of expression. In nGBMs, the dMMR incidence is low and prognostic data are scarce [28, 43]. Our incidence (14.7%) was slightly superior when compared with the previous reports (3.3%—8%), but if we consider only the patients who presented a homogeneous loss, it is similar to the data published.

Both phenotypic and genotypic intratumoral heterogeneity of GBM is well established [43, 53, 54]. The information about the heterogenous expression of repair genes in GBM patients is interesting but scarce as if this finding has any prognostic or therapeutic implication deserves further studies [38, 55].

Regarding survival, in general cancers, the dMMR/MSI somatic phenotype is associated with a better prognosis and is also used as a the-

rapeutic biomarker for immunotherapy [43, 52]. However, in our series, we observed a shorter survival in patients with this phenotypic presentation, 19.8 *vs.* 16.9 months, without statistical significance. Survival difference associated with MMR was reported in the previously published GBM series with dubious results, both with numerically longer and shorter survivals. Unlike colon, endometrial, and ovarian cancers, the loss of expression of repair gene proteins in CNS tumors does not necessarily result in the hypermutated phenotype [45]. The impact of this difference in gliomas is not well established in the literature yet.

The limitations of our study include the relatively small number of patients, the retrospective nature, the information on MMR based solely on IHC analysis, the lack of germline evaluation, and the absence of complete clinical information since the data source was a laboratory of pathology. These limitations are mainly justified by the shortage of a unified record of medical information and lack of access to complementary tests in our country.

Our study adds information on the IHC detection of dMMR in GBMs, especially in the initial presentation as seen in most of our samples (91.2%). Furthermore, we observed a heterogeneous loss of expression of MMR proteins in 50% (5/10) of our patients. Further studies may clarify whether this finding has any therapeutic or prognostic impact.

In conclusion, the role of repair gene deficiency in GBMs is not yet clearly elucidated. Despite being rare in the population studied, the identification of a potential predictive biomarker for therapy response is highly desirable for such an aggressive disease, particularly with using a widely available test like IHC.

Declarations

Ethics approval and consent to participate

The study was approved by the Institutional Review Board and the local Ethics Committee of Hospital BP — a Beneficência Portuguesa de São Paulo (São Paulo, Brazil, number 08127219.1.0000.5483), in accordance with the principles expressed in the Declaration of Helsinki. As data collection was anonymized without patient contact or intervention, informed consent was dismissed by the ethics committee.

Our manuscript does not contain any individual person's data in any form.

All authors have approved the contents of this paper and have agreed to the submission policies for publication.

Availability of data and materials

Not applicable.

Competing interests

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Authors' contributions

CAFY: study design, data analysis, manuscript writing and submission; SMFM: study design, data analysis, supervision, manuscript writing and revision; LLFA: data analysis; CLPL: study design, data analysis, manuscript revision and supervision.

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ІМУНОГІСТОХІМІЧНЕ ДОСЛІДЖЕННЯ ДЕФЕКТІВ СИСТЕМИ РЕПАРАЦІЇ ТА ЗВ'ЯЗКУ ЇХ ІЗ КЛІНІЧНИМ ПЕРЕБІГОМ ГЛІОБЛАСТОМИ У ХВОРИХ БРАЗИЛІЇ

Вступ. Гліобластома (ГБМ) є найчастішою діагностованою первинною злоякісною пухлиною центральної нервової системи. Дефект системи репарації неспарених основ (MMR) пов'язаний з кращим прогнозом і є біомаркером, що свідчить на користь проведення імунотерапії. Оцінка системи MMP методом імуногістохімії є доступною, економічно ефективною, чутливою та специфічною. **Метою** було дослідити експресію білків MMR у дорослих хворих на ГБМ. **Матеріали та методи.** Імуногістохімічне дослідження експресії маркерів MMP проведено в пухлинній тканині 68 хворих на ГБМ ретроспективної групи. Клініко-патологічні та молекулярні особливості порівнювали з наявністю чи відсутністю маркерів MMR. **Результати.** Дефектність за маркерами MMR виявлено в 10 (14,7%) зразках тканини ГБМ. Найчастіше дефектність виявлялась за MSH6 (100%) і рідше для MSH2, PMS2 і MLH1. У 5 зразках ГБМ показано гетерогенність експресії білків MMR. Не виявлено різниці показників загальної виживаності хворих залежно від статусу MMR: 19,8 місяців (0,2—30) в разі наявності маркерів в порівнянні з 16,9 місяцями (6,4—27,5) в разі їх відсутності ($p = 0,31$). Визначено, що вищі показники загальної виживаності асоціюються з тотальною резекцією (30,7 місяців у порівнянні з 13.6 місяцями, $p = 0,02$) і статусом метилування MGMT (29,6 місяців у порівнянні з 19,8 місяцями, $p = 0,049$). **Висновки.** Отже, проведений нами аналіз результатів гістохімічного дослідження маркерів системи MMR у тканині ГБМ не виявив зв'язку з показниками загальної виживаності хворих.

Ключові слова: пухлини головного мозку, гліобластома, гліома, мікросателітна нестабільність, репарація неспарених основ.