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Research article

Focal-to-bilateral tonic-clonic seizures and High-grade CMV-infection are poor survival predictors in Tumor-related Epilepsy Adult-type diffuse gliomas—A single-center study and literature review☆

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A B S T R A C T

Introduction: Previous studies have reported a correlation between a high-grade CMV-infection and an unfavorable prognosis in glioblastoma (GB). Coversely, epilepsy has been associated with a more favorable outcome in GB patients. Despites epilepsy and CMV share similar molecular mechanisms in GB tumoral microenvironment, the correlation between Tumor-Related-Epilepsy (TRE) and CMVinfection remains unexplored. The aim of our study is to examine the correlation between the dregree of CMV infection and seizure types on the survival of TRE Adult-type-diffuse-glioma. To achieve this objective, we conducted a comprehensive literature review to assess our results regarding previous publications

Methods: We conducted a retrospective-observational study on TRE Adult-type-diffuse-gliomas treated at a single center in Mexico from 2010 to 2018. Tumor tissue and cDNA were analyzed by immunochemistry (IHC) for CMV (IE and LA antigens) at the Karolinska Institute in Sweden, and RT-PCR for CMV-gB in Torreon Mexico, respectively. Bivariate analysis (X^{2}-*test*) was performed to evaluate the association between subtypes of Adult-type-diffuse-glioma (IDH-mut grade 4 astrocytoma vs. IDH-wt glioblastoma) and the following variables: type of hemispheric involvement (mesial vs. neocortical involvement), degree of CMV infection (<25%vs. >25% infected-tumoral cells) and seizure types [Focal awareness, focal impaired awareness, and FBTCS]. Kaplan Meier and Cox analyses were performed to determine the risk, $p < 0.05$ was considered statistically significant.

Results: Sixty patients with TRE Adult type diffuse gliomas were included (80% IDH-wt glioblastoma and 20% IDH-mut grade 4astrocytomas). The mean age was 61.5 SD ± 18.4, and 57% were male. Fifty percent of the patients presented with mesial involvement of the hemysphere. Seizure types included focal awareness (15%), focal impaired awareness (43.3%), and FBTCS (41.7%). Ninety percent of cases were treated with Levetiracetam and 33.3% presented Engel-IA postoperative seizure control. More than 90% of samples were positive for CMV-immunohistochemistry (IHC). However, all cDNA analyzed by RT-PCR return negative results. The median of overall survival (OS) was 15 months. High-grade CMV-IE infection (14 vs. 25 months, $p<0.001$), mesial involvement (12 vs. 18 months, $p<0.001$), and FBTCS were associated with worse OS (9 vs.18 months for non-FBTCS). Multivariate analysis demonstrated that high-grade CMV infection (HR = 3.689, $p=0.002$) and FBTCS (HR=7.007, $p<0.001$) were independent unfavorable survival factors.

Conclusions: CMV induces a proinflammatory tumoral microenvironment that contributes to the developmet of epilepsy. Tumor progression could be associated not only with a higher degree of CMV infection but also to epileptogenesis, resulting in a seizure phenotype chracterized by FBTCS and poor survival outcomes. This study represents the first survival analysis in Latin America to include a representative sample of TRE Adult-type diffuse gliomas considering CMV-infection-degree and distinguishing features (such as FBTCS) that might have potential clinical relevance in this group of patients. Further prospective studies are required to validate these results.

Abbreviations

cDNA	complementary DNA
CMV	cytomegalovirus
CMV-IE	Immediate early cytomegalovirus antigen
CMV-LA	Late antigen of cytomegalovirus
COX-2	cyclooxygenase 2
FBTCS	focal to bilateral tonic-clonic seizures
GB	glioblastoma
gB	glycoprotein B of cytomegalovirus
IHC	Immunochemistry
M2	Macrophages type 2
NF-KB	nuclear factor “Kappa-light-chain-enhacer” of activated B-cells
OS	overall survival
PEG2	Prostaglandin E2
PFS	progression-free survival
PRS	Post-recurrence survival
RT-PCR	Real-time polymerase chain reaction
TRE	tumor-related epilepsy

1. Introduction

Glioblastoma (GB) is the most common malignant brain tumor in adults with an incidence of 3.23 cases per 100 thousand inhabitants per-year (CBTRUS, 2021) [1]. Stupp et al. established the current standard of care treatment protocol for GB in 2005: Gross total resection (GTR) followed by Radiotherapy (RT) and Chemotherapy with Temozolomide (TMZ) [2]. An important advancement has been recently obtained with encouraging positive clinical outcomes of Tumor Treating Field (TTF) for GB treatment [3]. Despite multimodal treatment, survival remains extremely poor in GB patients, with a median OS of 14.6 months [4].

Several authors have investigated predictive factors associated with survival in High-grade gliomas (HGG) patients [5,6]. Younger age, IDH-mutant status, methylated-MGMT, a higher KPS score, preoperative tumoral volume, residual tumoral volume (RTV), and extent of resection (EOR) are variables that have been consistently associated with an improved survival [5–7]. Resection guided by intraoperative USG, MRI, and/or 5-ALA are considered as valuable techniques that improve EOR and survival for HGG patients [8,9]. Furthermore, Epilepsy has been described as a favorable independent factor for survival in GB patients at the time of tumoral diagnosis and even during follow-up [10,11].

During the last two decades, several investigators have observed the presence of Cytomegalovirus (CMV) in GB tumors, demonstrating that viral activity is associated with poor prognosis in GB patients. Cobbs et al. (2002) first described the presence of CMV in tumor tissue on all the tumor samples analyzed [12]. Rahbar et al. demonstrated an association between the grade of CMV infection and survival in these patients [13,14]. Subsequently, the Karolinska Institute group from Sweden demonstrated a longer survival in patients with primary, secondary or recurrent GB who were treated with Valganciclovir, an effective antiviral treatment against CMV [15–17].

In Latin America, there is a lack of published studies on this subject. Therefore, our study focuses on evaluating the correlation among survival, seizure types and the degree of CMV infection in tissue samples of patients with tumor-related Epilepsy (TRE) Adult-type-diffuse glioma at a national Mexican reference center.

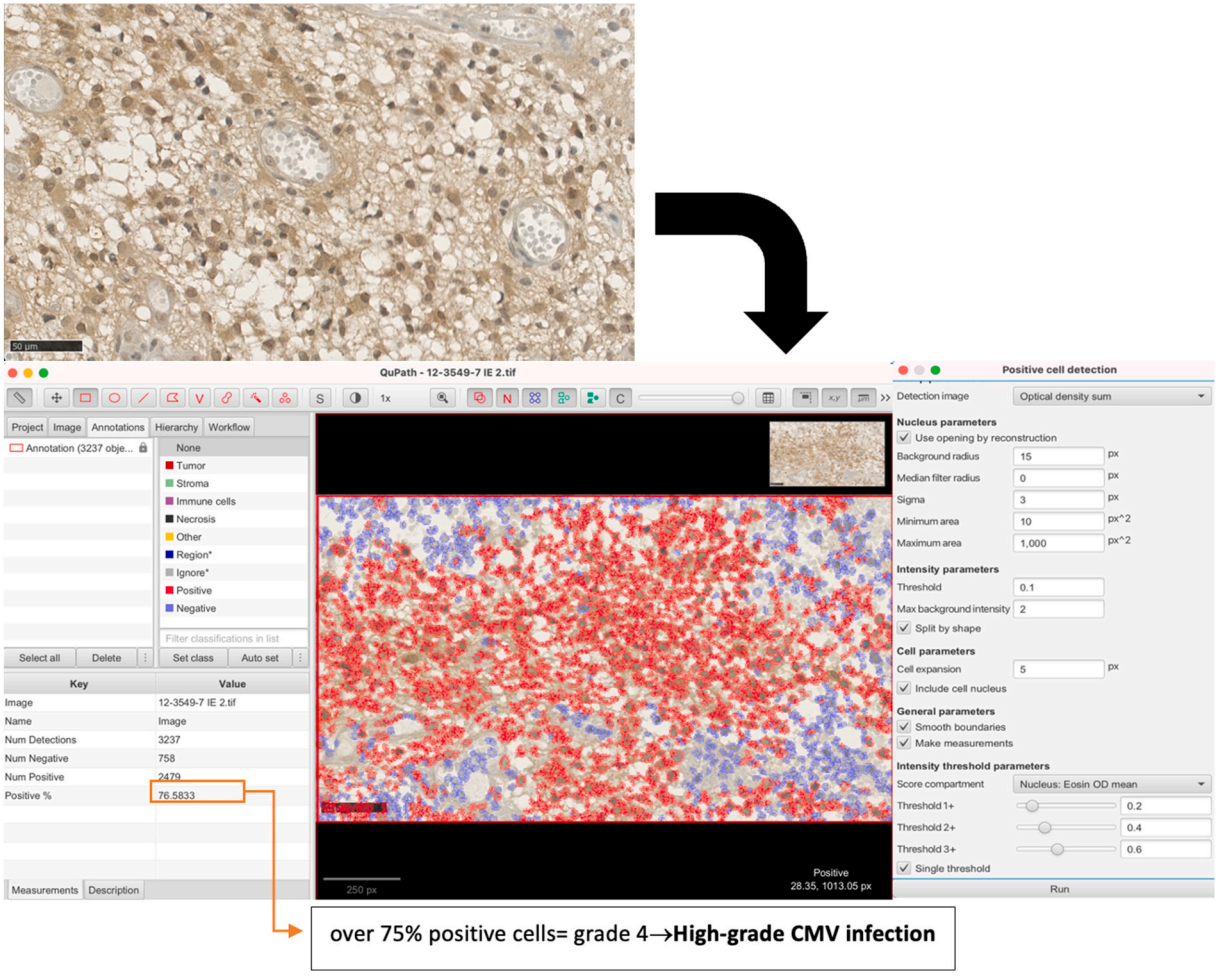

Fig. 1. - Illustrated the counting process of positive cells in the QuPath v0.5.0. program. A scoring system was implemented to categorized CMV infection in low-grade (<25% of positive tumoral cells) and high-grade CMV infection (≥25% of positive tumoral cells).

2. Materials and methods

2.1. Study sample

A retrospective study was conducted in TRE Adult-type diffuse gliomas treated at a single-center in Mexico City (CMN Siglo XXI) from 2010 to 2018, in collaboration with Karolinska Institute in Solna, Sweden and Medical Specialty Hospital from Torreon, Mexico. Inclusion criteria consisted adult patients (≥18 years) diagnosed with Adult-type diffuse glioma according to the up-to-date WHO classification for brain tumors, 2021(IDH-mut astrocytoma WHO grade 4, and IDH-wildtype glioblastomas) and presenting with TRE at the time of tumor diagnosis [18].

2.2. Ethical approval

This study was previously approved by the National Institutional Research Board (R-2021-785-074) and the ethical committee on July 28, 2021. Written informed consent was obtained from all the participants. According to ethical approval, all procedures for this research were conducted on Human subjects following the principles in the Declaration of Helsinki in 1964. Informed consent has been previously obtained for each patient for molecular and/or IHC analysis of tumor tissue and previous surgical procedures, as a routine in our center.

2.2.1. Immunochemistry for cytomegalovirus

A total of 112 formalin-fixed paraffin-embedded (FFPE) blocks of tissue of Adult-type diffuse glioma obtained from the first (n = 60 patients) and second (n = 34) surgery were analyzed for CMV-IE (CMV-immediate early antigen) and CMV-LA (CMV-Late antigen) by immunochemistry (IHC) in the laboratory at the Karolinska Institute in Stockholm, Sweden, following the protocol described by Rahbar et al., [13,14]. Positive cells identification was quantified using the QuPath v0.5.0. program, and a score was assigned by two experienced pathologists categorizing CMV infection as a low-grade (<25% of positive tumoral cells) and high-grade CMV infection (≥25% of positive tumoral cells) base on previous publications (see Fig. 1).

2.2.2. Real-time PCR process for cytomegalovirus

The Real-time PCR process for CMV involved the extraccion of cDNA from 32 frozen tumor tissues using a Qiagen kit. Subsequently, the samples were analyzed with RT-PCR CMV-gB at the Medical Specialties Hospital in Torreon Mexico. The artus® CMV RG PCR kit (Qiagen; Hilden, Germany) was stored at −20 °C,tempered, and the Master Mix was prepared inside the PCR hood as follows, for each reaction: 12.5 μl of CMV RG Master, 2.5 μl of CMV Mg-Sol and 1 μl of CMV RG IC (as internal control) were combined. Sufficient Master Mix was prepared for 40 reactions (32 samples, 4 standards, 1 negative control and 3 extra reactions to compensate for adsorption loss). The reaction set up involved using 0.1 ml microtubes in a Loading Plate at 4 °C withthe assembly performed inside the PCR hood. Each microtube received 15 μl of Master Mix, followed by the addition of 10 μl of each tempered sample. Until this step the samples had remained stored at −20 °C, the tube assembly was carried out in the same wayfor negative control. Additionally, the four standards (for the calibration curve) included in the kit, were preparated in a separate area, with the following concentrations: 1×10^4, 1×10^3, 1×10^2,and 1×10^1 copies/μl. The Rotor-Gene Q 5 plex HRM (Qiagen; Hilden, Germany) was used for PCR; with a template created following the supplier's instructions: a hot-start at 95 °C for 10 min and 45 cycles of 95 °C for 15 s, 65 °C for 30 s and 72 °C for 20 s. The first 10 cycles utilized a Touchdown of 1 °C. Reading was carried out in each cycle at 65 °C in the channels for "FAM/Sybr" (Presence of CMV DNA) and "JOE" (Internal Control).

2.4. Type of hemispheric involvement

Mesial involvement was characterized by tumor spread to a non-six-layer cortex hemispheric regions, typically located in the medial areas of the hemispheres, usually in the temporal and frontal lobes.

Neocortical involvement was defined as tumor spread into a six-layer cortex hemispheric region situated in the dorsolateral or basal areas of the hemispheres.

2.5. Tumor-related epilepsy (TRE)

TRE diagnosis was stablished according to the new classification of ILAE [19]. TRE diagnosis was confirmed in a patient presenting at least one seizure along with the presence of Adult-type diffuse glioma. EEG was performed to validate TRE diagnosis. In our study, seizures were categorized into three groups according to the TRE classification: Focal awareness, focal impaired awareness, and focal to bilateral tonic-clonic seizures (FBTCS)]. For survival analysis, seizure types were categorized into two groups: 1.- non-FBTCS group, including focal awareness and focal impaired awareness seizures and 2.- FBTCS group.

2.6. Residual tumor volume

A control MRI was conducted 24 h after surgery for all cases. Residual tumor volume (RTV) was measured by an expert neuro-radiologist who compared preoperative tumor volume and post-operative tumor volume in T2, Flair and T1-gadolinium MRI

Table 1
Descriptive analysis and demographic data.

Age	n (%)
<65 years	21 (35)
≥65 years	39 (65)
Gender	
Male	34 (56.7)
Female	26 (43.3)
Localization	
Frontal	26 (43.3)
Temporal	21 (35)
Parietal	22 (36.7)
Occipital	11 (18.3)
Central core/Thalamic	1 (1.7)
Right-Hemisphery	39 (65)
Left-Hemisphery	21 (35)
Tumor Extension	
2-3 Lobes	42 (70)
1 Lobe	17 (28.3)
Central core involvement	1 (1.7)
Type of hemispheric involvement	
with Mesial involvement	30 (50)
with Neocortical involvement	30 (50)
Tumor size	
<30 cm^2	30 (50)
≥30 cm^2	30 (50)
Extent of resection	
GTR	39 (65)
STR	18 (30)
Biopsy	3 (5)
Second surgery (yes)	34 (56.7)
Preoperative KPS	
KPS<60	11 (18.3)
KPS ≥60	49 (81.7)
Steroidal treatment (yes)	60 (100)
Start time of adjuvant therapy (RT/TMZ) from surgery	
<6 weeks	53 (88.3)
≥6 weeks	7 (11.7)
RT interrupted	0 (0)
RT dose (Gy)	
<60 Gy	14 (23.3)
≥60 Gy	46 (76.7)
No. TMZ cycles	
<6	25 (41.7)
≥6	35 (58.3)
Bevacizumab (yes)	5 (8.3)
Stereotactic Radiosurgery (SRS) (yes)	0 (0)
RTOG-RPA	
III	11 (18.3)
IV	29 (48.3)
V	9 (15)
VI	11 (18.3)
Adult-type Diffuse Gliomas	
IDH- wt glioblastoma	48 (80)
IDH- mut astrocytoma WHO grade 4	12 (20)
MGMT	
MGMT-Methylated	37 (61.7)
MGMT-Unmethylated	23 (38.3)
Grade of CMV infection.	n = 60)
First surgery	
IE-CMV Low-grade of Infection	12 (20)
IE-CMV High-grade of Infection	48 (80)
LA-CMV Low-grade of Infection	18 (30)
LA-CMV High-grade of Infection	42 (70)
Grade of CMV infection.	(n = 34)
Second surgery	
IE-CMV Low-grade of Infection	7 (20.6)
IE-CMV High-grade of Infection	27 (79.4)
LA-CMV Low-grade of Infection	9 (26.5)
LA-CMV High-grade of Infection	25 (73.5)
TRE seizures type	

(*continued on next page*)

Table 1 (*continued*)

Age	n (%)
Focal awareness	9 (15)
Focal impaired awareness	26 (43.3)
Focal to bilateral tonic-clonic seizures	25 (41.7)
Epilepsy Treatment	
Levetiracetam	57 (95)
Fenitoina	2 (3.3)
Valproato	4 (6.7)
Lamotrigina*	15 (25)
CBMZ*	1 (1.7)
Intraoperative Seizures	4 (6.6)
Engel	
IA	20 (33.3)
IB	22 (36.7)
IC	1 (1.7)
IIA	1 (1.7)
IIB	11 (18.3)
IIIA	3 (5)
IVA	2 (3.3)

Low-grade of CMV Infection (<15% infected tumor cells), High-grade of Infection (≥15% infected tumor cells). *As an adjuvant treatment with Levetiracetam. CBMZ = carbamacepine.

sequences. FreeSurfer software (https://surfer.nmr.mgh.harvard.edu/) was utilized for this purpose. EOR was defined as follows: GTR was considered in cases with a complete tumor resection notified by the neurosurgeon and confirmed by postoperative MRI showing a RTV<1.5 cm^3. Subtotal resection (STR) was indentified in cases with evidence of a RTV >1.5 cm^3 in postoperative MRI when the neurosurgeon reported ~90% of tumoral resection.Biopsy was documented when a stereotactic procedure was utilized to obtain tissue

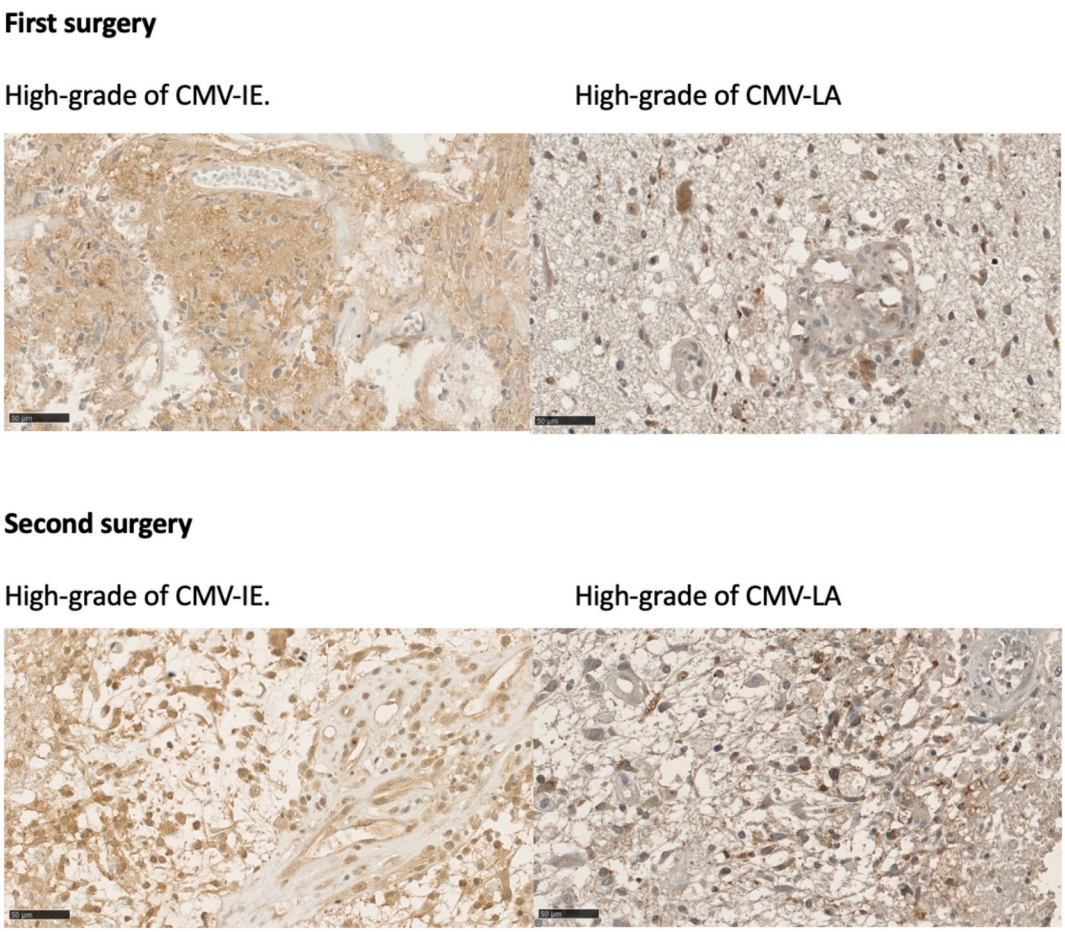

Fig. 2. - High-grade CMV infection in tumor tissue by immunochemistry for CMV-LA and CMV-IE in first and second surgery.

for diagnosis purposes.

2.7. Survival

Progression-free survival (PFS) was established from the time of the first surgery until recurrence, as defined by RANO criteria [20]. Overall survival (OS) was calculated from the time of the first surgery until death. Post-recurrence survival (PRS) was determined from the time of recurrence until death.

2.8. Pre-operative and outcome scores

Preoperative status was evaluated using Karnofsky's performance status (KPS). Outcome assessment was performed using the

Table 2
Bivariate analysis: TRE seizure types.

Variable	Focal awareness n (%)	Focal impaired awareness n (%)	FBTCS n (%)	p
Age				
<65 years	3 (14.3)	17 (81)	1 (4.8)	<**0.001***
≥65 years	6 (15.4)	9 (23.1)	24 (61.5)	
Gender				
Male	5 (14.7)	16 (47.1)	13 (38.2)	0.788
Female	4 (15.4)	10 (38.5)	12 (46.2)	
Localization				
Right-Hemisphery	6 (15.4)	17 (43.6)	16 (41)	0.988
Left-Hemisphery	3 (14.3)	9 (42.9)	9 (42.9)	
Type of hemispheric involvement				
with Neocortical involvement	5 (16.7)	25 (83.3)	0 (0)	<**0.001***
with mesial involvement	4 (13.3)	1 (3.3)	25 (83.3)	
Tumor size				
<30 cm^2	7 (23.3)	13 (43.3)	10 (33.3)	0.151
≥30 cm^2	2 (6.7)	13 (43.3)	15 (50)	
EOR				
GTR	3 (7.7)	17 (43.6)	19 (48.7)	0.071
STR or Biopsy	6 (28.6)	9 (42.9)	6 (28.6)	
Second surgery (yes)	8 (23.5)	15 (44.1)	11 (32.4)	0.066
Preoperative KPS				
KPS<60	1 (9.1)	2 (18.2)	8 (72.7)	0.067
KPS ≥60	8 (16.3)	24 (49)	17 (34.7)	
Start time of adjuvant therapy (RT/TMZ) from surgery				
<6 weeks	8 (15.1)	22 (41.5)	23 (43.3)	0.713
≥6 weeks	1 (14.3)	4 (57.1)	2 (28.6)	
RT dose (Gy)				
<60 Gy	2 (14.3)	2 (14.3)	10 (71.4)	**0.024***
≥60 Gy	7 (15.2)	24 (52.2)	15 (32.6)	
No. TMZ cycles				
<6	4 (16)	6 (24)	15 (60)	**0.028***
≥6	5 (14.3)	20 (57.1)	10 (28.6)	
RTOG-RPA III	1 (9.1)	8 (72.7)	2 (18.2)	0.092
RTOG-RPA IV-VI	8 (16.3)	18 (36.7)	23 (46.9)	
Adult-type Diffuse Gliomas				
IDH- wt glioblastoma	6 (12.5)	19 (39.6)	23 (47.9)	0.133
IDH- mut astrocytoma WHO grade 4	3 (25)	7 (58.3)	2 (16.7)	
MGMT				
MGMT-Methylated	4 (10.8)	18 (48.6)	15 (40.5)	0.409
MGMT-Unmethylated	5 (21.7)	8 (34.8)	10 (43.5)	
Grade of CMV infection.				
First surgery				
IE-CMV Low-grade of Infection	1 (8.3)	9 (75)	2 (16.7)	**0.046***
IE-CMV High-grade of Infection	8 (16.7)	17 (35.4)	23 (47.9)	
Epilepsy Treatment				
Levetiracetam	8 (14)	25 (43.9)	24 (42.1)	0.659
Fenitoina	0 (0)	1 (50)	1 (50)	0.833
Valproato	0 (0)	1 (25)	3 (75)	0.347
Lamotrigina*	4 (26.7)	2 (13.3)	9 (60)	**0.023***
CBMZ*	1 (100)	0 (0)	0 (0)	0.056
Intraoperative Seizures	2 (50)	0 (0)	2 (50)	0.066
Engel IA	0 (0)	16 (80)	4 (20)	<**0.001***
Engel IB-IV	9 (22.5)	10 (25)	21 (52.5)	

EOR = Extent of resection. FBTCS= Focal to bilateral tonic-clonic seizures. Low-grade CMV Infection (<15% infected tumor cells), High-grade Infection (≥15% infected tumor cells).

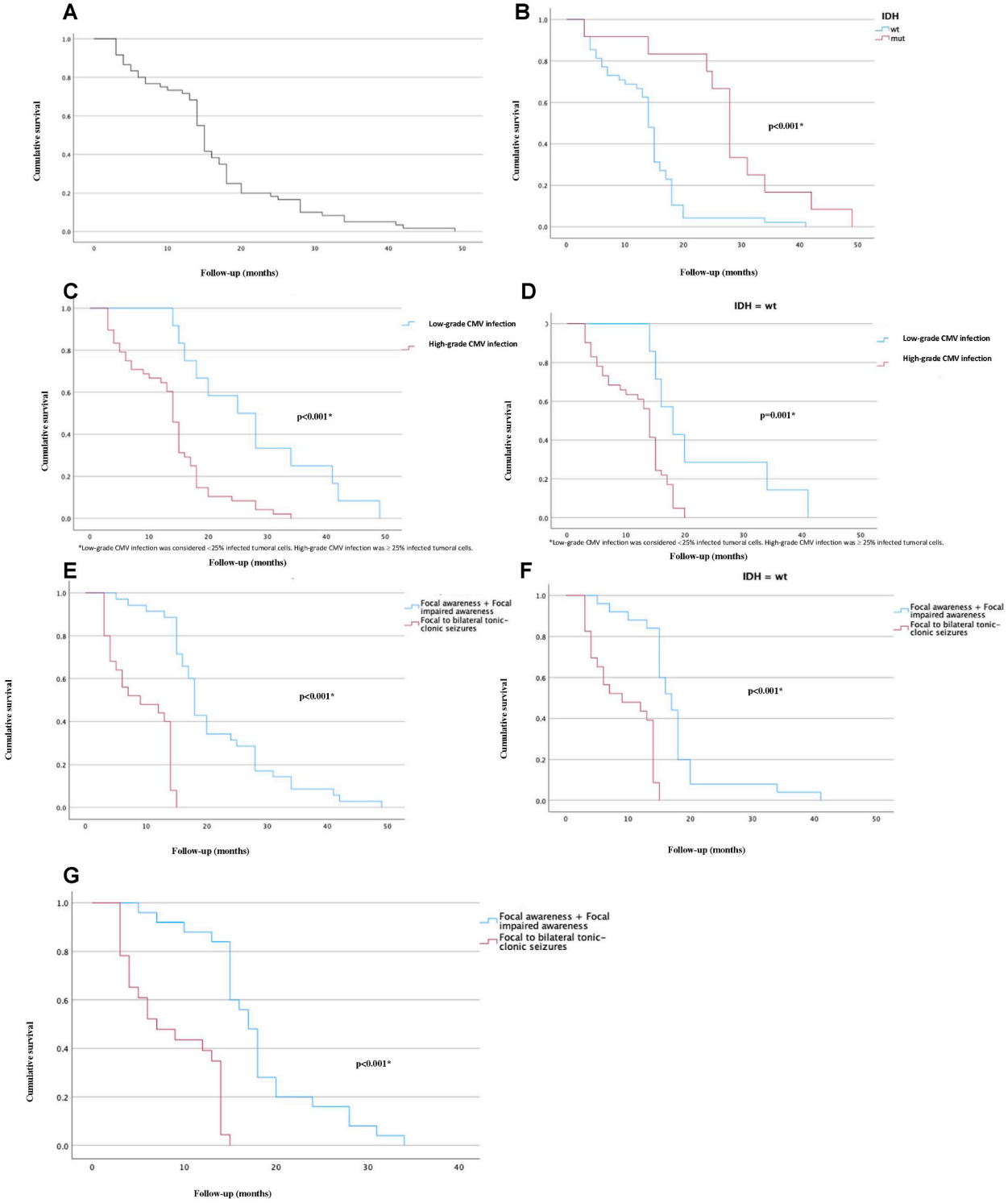

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Fig. 3. Kaplan Meier analysis. A.- The median OS was 15 SD±0.477 in all TRE Adult-type diffuse glioma patients (n=60). **B.-** IDH-wt glioblastoma patients (n=48) presented poorer OS compared with IDH-mut grade 4 astrocytomas (median OS 14 vs. 18 months, p<0.001). **C.-** Patients with a high-grade CMV-IE infection demostrated inferior OS compared with low-grade CMV infection (median OS 14 vs. 25 months, p<0.001) in the complete cohort of TRE Adult-type diffuse glioma cases. **D.-** Among IDH-wt glioblastoma patients (n=48), cases with a high-grade CMV-IE infection showed poorer OS compared with low-grade CMV infection (14 vs. 18 months, p=0.001). **E.-** Patients diagnosed with FBTCS presented a poor OS compared with non-FBTCS (median of OS 9 vs. 18 months, p<0.001) in all Adult-type diffuse glioma cases. **F.-** IDH-wt glioblastoma patients (n=48) with FBTCS presented poorer OS compared with non-FBTCS cases (9 vs. 17 months, p<0.001). **G.-** Patients with FBTCS and high-grade CMV-IE infection demostrated inferior OS compared with non-FBTCS patients (7 vs. 17 months, p<0.001).

Radiation Therapy Oncology Group recursive partitioning analysis (RTOG-RPA) model for all patients. Seizure control duringTRE follow-up was evaluated using Engel scale.

2.9. Statistical analysis

For statistical analysis, we included all 94 samples stained for CMV- IHC, obtained from both the first (n = 60) and second surgery (n = 34). A bivariate analysis (X^2-*test*) was performed to evaluate the difference in thr degree of CMV infection (<25% tumoral positive cells vs. ≥ 25%), and seizure types with clinical variables. Kaplan Meier and Cox analysis were performed to establish the risk in TRE Adult-type diffuse glioma (p<0.05 was considered statistically significant). IBM SPSS Statistics version 29.0 was used for the analysis.

3. Results

3.1. Descriptive results

This study included a total of 60 patients diagnosed with TRE Adult-type diffuse glioma. Among them, 20% (n = 12) were IDH-mutant astrocytomas grade 4, and 80% (n = 48) were IDH-wt glioblastomas. All patients experienced recurrence and disease progression, but only 56.7% (n = 34) had a second surgery. The mean age was 61.5 years SD±18.4 (range 18–85), and 57% were male. Demographic results are presented in Table 1.

More than 90% of the tumor samples were positive for CMV by IHC at the first surgery (n=60, CMV-IE=96% and CMV-LA=90%) and at the second surgery (n=34, CMV-IE=100% and CMV-LA=94%) (Fig. 2). RT-PCR analysis for CMV yielded negative results in all cDNA samples analyzed. Interestingly, 85% of cases with a high-grade of CMV-IE infection were IDH-wt glioblastomas (p=0.036).

Regarding hemispheric involvement, 50% of cases presented mesial involvement, and among them, 69.2% of patients were older than 65 years (p < 0.001).

Concerning TRE, focal awareness (15%), focal impaired awareness (43.3%), and FBTCS (41.7%) were reported. Among cases with FBTCS, 61.5% of were older than 65 years (p<0.001), and 83.3% (n=25) of cases with mesial involvement presented FBTCS (p < 0.001).

During follow-up, 33.3% of the patients presented Engel IA. In our series, 52.5% of FBTCS cases presented Engel IB-IV (p<0.001). Additionally,75% of cases with neocortical involvement reported Engel IA, compared to 25% of cases with mesial involvement (p=0.006).

Stimulation-related intraoperative seizures (IOS) occurred in 4 cases with mesial involvement.

Levetiracetam was the most common antiseizure medication (ASM) used as monotherapy(95%, n=57). Four patients initially treated with Valproic acid (VA) had to switch to Levetiracetam due to elevated serum hepatic tests. Double therapy (Levetiracetam and Lamotrigine) was administered to 25% of patients, with 60% (n=9) of them presented FBTCS (p=0.023). Gross total resection (GTR) was obtained in 65% of cases.

In the bivariate analysis, 48% of FBTCS cases presented a high grade of CMV infection (p=0.046, likelihood ratio 0.043) (see Table 2). However, there was no significant difference between the degree of CMV infection and hemispheric involvement.

The median PFS was 10±7.6 months (range 3-34), median OS was 15 SD±10.2 (range 3–49), and median PRS was 3 SD±6 months (range 0–30) (**see** Fig. 3 **A-B**).

3.2. Cytomegalovirus infection degree in tumor tissue and survival

Patients with a low-grade CMV-IE infection at first surgery (<25%) had longer PFS and PRS and better OS than patients who presented with a high-grade CMV-IE infection in their tumors (≥25%); the median PFS was 18 vs. 9 months (p = 0.002). Median OS was 25 vs. 14 months (p<0.001) and median PRS was 5 vs. 2 months (p=0.015) (**see** Fig. 3-C**).**

IDH-wt glioblastoma cases with low-grade CMV-IE infection showed a significantly longer PFS and better OS in comparison with high-grade CMV-IE infection (median of PFS was 16 vs. 8 months, p=0.003, median OS was 18 vs. 14 months, p=0.001) (**see** Fig. 3-D**).**

3.3. Tumor-related epilepsy and survival

Patients with FBTCS presented poor PFS (median of 6 months vs. 15 months for non-FBTCS, p<0.001), the worst OS (median of 9 vs. 18 months, p<0.001), and inferior PRS (median of 0 months vs. 5 months, p<0.001) compared to the other seizure types in Kaplan-Meier analysis (**see** Fig. 3-E**).**

Regarding ASM, 95% of our patients were treated with Levetiracetam, and the mean of OS was superior in these patients compared with patients non-treated with Levetiracetam (16.3 vs. 15.3 months, p=0.730). Patients treated with VA (n=4) or double-therapy (Levetiracetam + Lamotrigine) exhibited poor OS (VA median OS was 3 vs. 15 months, p=0.027. Double-therapy median OS 7 vs. 15 months, p=0.004).

IDH-wt glioblastoma patients with FBTCS presented poor PFS (median of 6 vs. 14 months, p<0.001) and inferior OS (9 vs. 17 months, p<0.001) compared with non-FBTCS cases (**see** Fig. 3-F**).**

Patients with high-grade CMV-IE and FBTCS presented poor PFS (median 6 vs. 14 months for non-FBTCS, p<0.001) and worse OS (median 7 vs. 17 months, p<0.001) compared to other seizure types (**see** Fig. 3-G**).**

3.4. Cox analysis

Cox analysis identified a high-grade CMV-IE infection (HR=2.927, 95% CI 1.433–5.978, p=0.003), FBTCS (HR=3.296, 95% CI 1.632–6.654, p<0.001) and IDH-wt glioblastoma (HR=2.802, 95% CI 1.128–6.957, p=0.026) as independen risk factors for tumoral progression (PFS) see Table 3.

We observed similar results in the Cox analysis for OS [a high-grade CMV-IE infection (HR=3.689, 95% CI 1.617–8.415, p=0.002), FBTCS (HR=7.007, 95% CI 2.980–16.471, p<0.001) and IDH-wt glioblastoma (HR=4.443, 95% CI 1.464–13.487, p=0.008) were indentified as independent risk factors] (see Table 4).

Results showed that preoperative KPS was the unique independent favorable survival factor. Every 10-point increase in preoperative KPS corresponded to an accumulative 3.2% reduction in the risk of recurrence (PFS, HR=0.968, 95% CI 0.947–0.989, p=0.003) and 2.1% reduction in the risk of death (OS, HR=0.979, 95% IC 0.962–0.997, p=0.023).

4. Discussion

Glioblastoma is the most common malignant brain tumor in adults. The median OS reported is 14.6 months [4] and continues to be disappointing despite the current gold standard therapy introduced by Stupp et al. (2005) that includes GTR followed by concomitant and adjuvant RT and TMZ [2].

Factors such as young age, a higher KPS, supramaximal resection (SMR) or GTR, IDH-mut and MGMT-methylated status are associated with a better survival in patients with GB [5–7]. Even though over 400 trials have been conducted since the instauration of the Stupp protocol for patients with GB in 2005 [27], no new therapy -except for tumor-treating-fields has demonstrated any improvement in survival of these patients [3]. It is therefore a great challenge to find new strategies and treatments to improve outcome in GB patients, and this matter is hence of high priority. During the past two decades, the presence and activity of CMV in tumoral tissue has been related to poor prognosis in GB patients [12–14,16]. Epilepsy has been associated with a better outcome at the time of GB diagnosis or during follow-up [10,11]. In Latin America, no previous publications have studied the relationship among these factors in relation to GB patients survival. The present study evaluates the presence and correlation of the degree of CMV infection in TRE Adult-type diffuse glioma tissue specimens, and seizure types with survival in Mexican patients.

4.1. Cytomegalovirus infection in tumor tissue affects survival time

Cobbs et al. reported for the first time in 2002 a very high prevalence (100%) of this virus in tumor tissue samples from GB patients

Table 3
Survival COX-analysis for progression-free survival (PFS). HR < 1- better PFS, HR > 1 worse PFS.

Variable	HR	95% CI (Lower)	95% CI (Upper)	p
Age	1.000	0.978	1.022	0.967
Preoperative KPS	0.968	0.947	0.989	**0.003***
EOR				
GTR	1			
STR/Biopsy	1.299	0.662	2.550	0.447
CMV-IE first-tumor surgery				
CMV-IE low-grade infection	1			
CMV-IE high-grade infection	2.927	1.433	5.978	**0.003***
TRE seizures type				
No-FBTCS	1			
FBTCS	3.296	1.632	6.654	< **0.001***
Adult-type Diffuse Gliomas				
IDH- mut astrocytoma WHO grade 4	1			
IDH- wt glioblastoma	2.802	1.128	6.957	**0.026***
MGMT status				
MGMT-methylated	1			
MGMT-unmethylated	1.177	0.643	2.153	0.598

$p < 0.05$ were statically significant.
EOR = Extent of resection. FBTCS= Focal to bilateral tonic-clonic seizures. Low-grade CMV Infection (<15% infected tumor cells), High-grade Infection (≥15% infected tumor cells).

Table 4
Survival COX-analysis for Overall survival (OS). HR < 1- better survival, HR > 1 worse survival.

Variable	HR	95% CI (Lower)	95% CI (Upper)	p
Age	1.011	0.989	1.032	0.338
Preoperative KPS	0.979	0.962	0.997	**0.023***
EOR				
GTR	1			
STR/Biopsy	1.869	00.928	3.764	0.080
CMV-IE first-tumor surgery				
CMV-IE low-grade infection	1			
CMV-IE high-grade infection	3.689	1.617	8.415	**0.002***
TRE seizures type				
No FBTCS	1			
Focal to bilateral tonic-clonic seizures	7.007	2.980	16.471	<**0.001***
Adult-type Diffuse Gliomas				
IDH- mut astrocytoma WHO grade 4	1			
IDH- wt glioblastoma	4.443	1.464	13.487	**0.008***
MGMT status				
MGMT-methylated	1			
MGMT-unmethylated	1.282	0.679	2.424	0.444

$p < 0.05$ were statically significant.
EOR = Extent of resection. FBTCS= Focal to bilateral tonic-clonic seizures. Low-grade CMV Infection (<15% infected tumor cells), High-grade Infection (≥15% infected tumor cells).

analyzed in their series [12]. Posteriorly, other authors tried to replicate this study in different centers around the world with heterogeneous results [28]. Rahbar et al. presented additional evidence of a correlation between the grade of CMV infection and survival: a low-grade of CMV-IE infection was associated with an OS longer than 18 months (OR 6.604, 95% CI = 1.359–32.094, p = 0.019) [13]. Later, this group also demonstrated that a low grade of CMV infection in GB tissue was associated with a higher OS compared to a high grade of CMV infection; 33 vs.13 months, HR:2.2, 95% CI = 1.0515–4.3796, p = 0.036 [14]. GB patients with positive CMV serology seemed to have shorted OS compared to patients with negative CMV serology [29].

Due to the heterogeneous results concerning to detection of CMV, Farias et al. published a meta-analysis in 2019 reporting the presence of CMV in 62% of the glioblastoma tumors analyzed [30]. Cai et al. (2020) reported a meta-analysis about the impact of CMV infection in GB patients related to prognosis [31] and suggested that the presence of CMV has no relationship with a better outcome. Among seven papers reporting CMV tumoral presence, three of them showed that CMV-degree infection was indeed a significant risk factor for GB survival (p < 0.05) [31]. It seems that the most important factor in relation to glioblastoma outcome is the degree of CMV infection, and that the merely presence of the virus has no pronostic value.

Considering the diversity in techniques for detecting CMV, the Karolinska Institute carried out a systematic review [32]. It has been shown that the diagnostic methods used for CMV detection are highly variable among different centers and that the presence of CMV proteins in tumoral tissue specimens with optimized techniques appears to be the most reliable method for the detection of this virus in tumor tissue specimens [32,33], detecting CMV in 84.2% of 1653 tumor specimens examined. Previously published negative results for CMV detection in tumoral tissue turned out from the implementation of an inadequate technique (PCR, sensitivity of 29%) and antibodies (CCH2+DDG9, sensitivity of 7.4%), and the fact that the authors did not use the Optimized IHC technique [32]. IHC is superior to the PCR technique because it does not only demonstrates CMV in tumoral tissue with a higher sensitivity (84% vs. 29%) [32], but also because identifies the degree of CMV infection [13,14]. Another advantage is that CMV proteins (e.g. IE, LA, and pp65) detected by IHC are more stable than DNA and RNA detected by PCR in tumoral tissue. Additionally, for PCR fresh frozen tissue is required for an adequate extraction of genetical material, and many times the process of tissue fixation can destroy the DNA [32].

This virus is often reactivated in brain tumor patients undergoing radiation therapy against the brain [34,35], almost half of the patients suffer a reactivation of the virus after RT and often develop CMV encephalitis-like symptoms and rapid recurrence. These observations support a frequent presence of CMV in glioblastoma and a potentially poor outcome in patients with active CMV infections in these tumors. Data supporting this hypothesis is the demonstration of a highly improved OS time in GB patients receiving anti-viral medications as add-on-to-standard therapy. This favorable outcome is identified in patients with primary, recurrent, and secondary GB when survival time is compared to contemporary control patients with the same baseline therapy [15–17,36]. Similar unexpected prolonged survival time has been observed in GB patients undergoing dendritic cell pp65 mRNA vaccination against CMV or adoptive therapy with CMV-specific T cells [37–39]. These observations claim for randomized clinical trials to prove or refute this promising positive treatment effect with anti-CMV therapy in patients with GB. Currently, the VIGAS 2 trial evaluates valganciclovir in 220 GB patients and is recruiting patients in Sweden and Norway (NCT04116411).

In our study, more than 90% of the tumor tissue samples were positive for CMV by IHC, but all cDNA samples tested by RT-PCR were negative. RT-PCR has a sensitivity of 29% compared to 50–100% of IHC for CMV detection in GB tumoral tissue specimens [32]. For this reason, it is not surprising that RT-PCR failed to demonstrate the presence of CMV in tumoral cDNA samples in our study. A systematic review reported that the *Optimized immunohistochemical technique* identified the virus in 1391 (84.2%) of 1653 samples demonstrating that is the *gold standard technique* for CMV detection in tumoral tissue [32], identifying that PCR and sequencing techniques frequently fail to detect CMV in tumor specimens. For that reason, our study was designed to use the optimized ICH technique for every patient with a complete slide instead of performing a TMA (tissue microarrays) technique. We must emphasize that

our goal was not focused on detecting positivity; our objective was centered on detecting any association between the degree of CMV infection and survival. Previous studies with an inadequate technique and antibodies were not able to detect the CMV. Although we obtained negative results with the PCR technique, we only analyzed 35 samples exclusively for the gB protein. CMV produces nearly 750 proteins, and only one negative protein detection (gB protein) with the least sensitive technique to detect CMV in tumoral tissue (PCR) cannot be considered as an overall negative result.

Previous reports emphasize the importance regarding survival and the degree of CMV infection in tumoral tissue [13,14,31]. Our results demonstrated that GB patients with a low-grade of CMV-IE infection at first surgery were associated with a longer PFS (18 vs. 8 months, $p = 0.002$), PRS (5 vs. 2 months, $p = 0.015$), and better OS time (25 vs. 14 months, $p < 0.001$) than patients who presented with a high-grade CMV- infection (≥25%). In the Cox analysis, we found that high-grade CMV-IE infection was a risk-independent factor in both PFS (HR = 2.927, 95% CI 1.433–5.978, $p = 0.003$) and OS (HR = 3.689, 95% CI 1.617–8.415, $p = 0.002$) in Adult-type diffuse glioma. These observations are in line with the previous studies performed by Rabhar et al. who demonstrated that the grade of CMV infection in GB tumor tissue specimens assessed with IHC detection of CMV IE proteins with optimized staining protocols, has a prognostic value [13,14]. Treatment-designed studies data have demonstrated an improved survival in patients receiving anti-CMV therapy [15–17].

4.2. *CMV induces a pro-inflammatory tumoral microenvironment and promotes epileptogenesis*

CMV is a well-known oncovirus that fulfills all the hallmarks of cancer [40]. CMV and epilepsy share the same inflammatory pathway. Normally CMV infects the macrophages precursors ($CD34^+$ cells) and produces a latent infection. In the tumor microenvironment (TME) CMV can induce the transformation of macrophages type 1 in type 2 (M2) by cmvIL-10, or in tumor-associated macrophages (TMA). Additionally, CMV can induce a lytic infection in epithelial cells by upregulating a diversity of cytokines that turn on an immune system evasion and subsequent tumoral progression. US28 of CMV can induce the transcription of NF-KB, upregulating the COX-2, PGE2, VEGF, and IL-6 expression in the TME [40,41]. Regarding epileptogenesis, glioma immune cells interactions

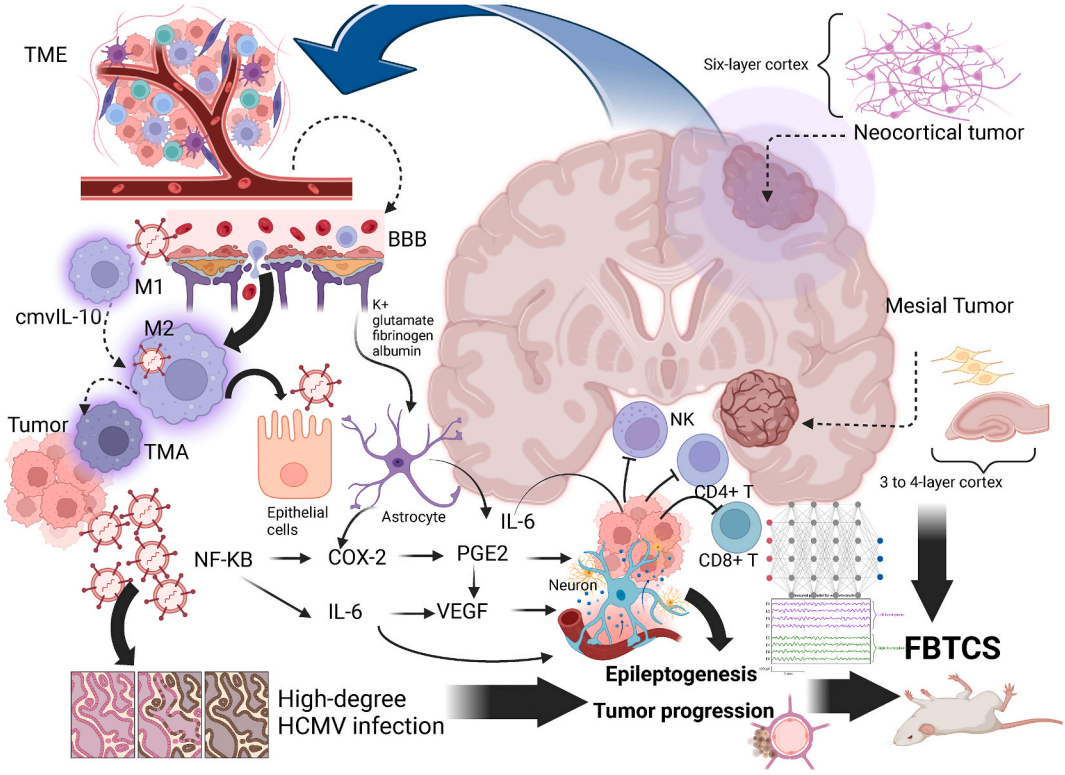

Fig. 4. - Schematic representation of TME inflammatory pathway for CMV-infection, epileptogenesis, and tumoral progression. TRE adult-type diffuse gliomas can develop neocortical or mesial hemispheric involvement. Within the tumor microenvironment, a brain-blood-barrier (BBB) disruption allows the migration of macrophages, immune cells, and other local extracellular components (e.g. K+, glutamate, fibrinogen) which compromise astrocyte cells and produce cytokines (IL-6) and proinflammatory markers (COX-2, PGE2). These molecular factors induce immune system inhibition and inflammation of neurons surrounding the tumor with a subsequent local "neuronal-inflammation" and an increase in excitability resulting in epileptogenesis (the presence of an abnormal neuronal network that generates seizures) related to tumoral progression. A High grade CMV infection can be associated with an active proinflammatory process in TME, progression of tumoral growth, and the development of epileptogenesis with FBTCS as a clinical manifestation. Tumors with mesial involvement have an increased frequency of FBTCS presentation. Created with BioRender.com.

Table 5
Epilepsy on GB survival: Literature review.

Author	Place	n	IDH-wt n (%)	Epilepsy -onset symptom n (%)	Epilepsy during follow up n (%)	ASM	Seizure at time of GB diagnosis (preoperative seizures) Median OS (95% CI)			Postoperative seizures Median OS (95% CI)			Observations	Type of study
							Epilepsy	No-Epilepsy	p	Epilepsy	No-Epilepsy	p		
Shin et al. (2017) [21]	Minnesota USA	122	NS	31 (25.4)	58 (48)	Levetiracetam (86%)	2.14 years	1.07 years	Seizure HR 0.52, 95% CI 0.27–0.95 p = 0.033*	1.66 years	0.87 years	HR 0.72, 95% CI0.43–1.21, p = 0.22	67% had more than 1 seizure 41% Generalized seizures 72% partial seizures	Retrospective study
Dobran et al. (2018) [22]	Catania, Italy	139	NS	50 (35.9)	–	–	10 ± 2.3 months	7 ± 1.6 months	0.07	Age (<65 vs.>65 years) 12 ± 2.2 months EOR (GTR vs subtotal/ Biopsy) 14 ± 2.9 months	Age (<65 vs.>65 years) 8 ± 2.4 months EOR (GTR vs subtotal/ Biopsy) 7 ± 1.6 months	p = 0.0001*, OR = 4.36, 95% IC 2.06–9.26 p = 0.017*, OR = 2.34, 95% IC 1.16–4.75	OS for the type of seizures (**p = 0.043***): -Focal without impairment of consciousness (n = 10) 14 ± 2.3 months -Focal with impairment of consciousness (n = 13) 8 ± 1.5 months -**FBTCS (n = 27) 6.5 ± 1.1 months** Independent favorable OS factors in COX-analysis (p < 0.05): Age, frontal localization, GTR, TMZ, and RT.	Retrospective study
Toledo et al. [23],	Barcelona, Spain	134	113 (84)	37 (27.6)	68 (51)	Prophylactic ASM. Levetiracetam 42.5% (n = 57)	85.5% at 1-year follow-up 12.9% at 2-year follow-up 6.2% at 5-year follow-up	37.7% at 1-year follow-up 48.7% at 2-year follow-up 25.6% at 5-year follow-up	<0.001*	29.5% at 1-year follow-up 10.4% at 2-year follow-up 2.2% at 5-year follow-up	71% at 1-year follow-up 33.8% at 2-year follow-up 19.9% at 5-year follow-up	<0.001*	26% become drug resistant. Aged and IDH had significant impacts on OS (p < 0.001) Prophylactic AED doesn't improve OS (p < 0.001) Type of seizures Focal without impairment of consciousness (32.3%) Focal with impairment of consciousness (24.3%) Focal evolving to bilateral convulsive seizure (43.2%). Seizures associated with GB or death (17.9%, n = 24)	Retrospective study

(*continued on next page*)

Table 5 (*continued*)

Author	Place	n	IDH-wt n (%)	Epilepsy -onset symptom n (%)	Epilepsy during follow up n (%)	ASM	Seizure at time of GB diagnosis (preoperative seizures) Median OS (95% CI)			Postoperative seizures Median OS (95% CI)			Observations	Type of study
							Epilepsy	No-Epilepsy	p	Epilepsy	No-Epilepsy	p		
Toledo et al. [24],	Barcelona, Spain	56	NS	15 (26.6)	25 (44.6)	Levetiracetam (n = 24,42.9%)	–			aged ≤60 years (n = 24) 100% survival at 20 months follow-up	aged ≤60 years (n = 24) 58.3% survival at 20 months follow-up	0.032*	Medical refractory epilepsy (n = 10, 17.9%) Status epilepticus (n = 9, 16.1%) Type of seizures Focal without impairment of consciousness (n = 5, 20.7%) Focal with impairment of consciousness (n = 6,24.1%) Focal evolving to bilateral convulsive seizure (n = 14. 55.2%). Seizures as the presenting symptom of glioblastoma predicted longer survival in adults younger than 60 years. The IDH1 R132H mutation and p53 overexpression (>40%) were associated with seizures at presentation	Prospective study
Ozbek et al. [25],	Samsun Turkey	76	NS	11(14)	–	–	13 months	9 months	0.026*				Univariate analysis (p < 0.05): Aged, KPS, RT dose. Multivariate analysis (p < 0.05): Aged (RR 2.094, CI95% 1.224–3.563, p = 0.007), presence of seizure prior GB dx (RR 0.460, CI95% 0.217–0.975, p = 0.043), RT Dose (RR 0.486, CI95% 0.269–0.878, p = 0.017)	Retrospective study
Berendse et al. [10],	Utrecht, Netherlands	647	NS	212 (32.9)	–	Levetiracetam 92 (43.4%) No ASM prophylactic	–	–	–	13.2 months (95% CI: 11.4–14.9)	8.4 months (95% CI: 7.4–9.5)	<0.0001*	Multivariate analysis: Aged (HR 0.75, p < 0.01), KPS (HR 0.61, p < 0.01), EOR (HR 0.71,	Retrospective study

(*continued on next page*)

Table 5 (*continued*)

Author	Place	n	IDH-wt n (%)	Epilepsy -onset symptom n (%)	Epilepsy during follow up n (%)	ASM	Seizure at time of GB diagnosis (preoperative seizures) Median OS (95% CI)			Postoperative seizures Median OS (95% CI)			Observations	Type of study
							Epilepsy	No-Epilepsy	p	Epilepsy	No-Epilepsy	p		
													p p < 0.005), RT and TMZ (HR0.08, p p < 0.005), **Epilepsy (HR: 0.75 (95% CI: 0.61–0.92), p < 0.01).**	
Flanigan et al. [26],	California, USA	443	NS	63 (14.22) (seizure-only n = 45)	124 (28)	–	15.1 months (13.0–18.1) "Seizure-only" vs. Seizures + another symptoms = 26.8 months (18.2–34.2)	13.1 months (11.1–14.7) 12.9 months (11.1–14.2)	Seizure (HR 0.81, 95% CI 0.65–1.01, p = 0.067) Seizure HR 0.48 (0.34–0.67), p < 0.001*				Temporal lobe localization = seizure 43% vs. 32% no seizure (p = 0.033) Tumoral size seizure 4.1 ± 1.5 vs. 4.9 ± 1.4 no-seizure p < 0.001* Univariate analysis in preoperative-seizures (n = 63). vs. symptoms post-seizure (26.8 (18.2–34.2) vs. 10.1 (6.7–16.6) months, HR = 4.7 (2.31–9.31) p < 0.001* Multivariate analysis: **seizures only HR 0.54, CI 95% 0.37–0.75, p < 0.001**	Retrospective study

ASM = antiseizure medication, FBTCS= Focal to bilateral tonic-clonic seizures, NS = no specified.

contribute to a local hyperexcitability state resulting on an abnormal epileptic network. Epileptogenesis is an active process. Blood-brain barrier (BBB) disruption is required to initiate tumoral-related epileptogenesis, with the exposition of astrocytes induced by cytokines (IL-6) and other proinflammatory markers (COX-2, PGE2) that promote the immune system inhibition and neuro-inflammation associated to abnormal neuronal excitability [42,43] (see Fig. 4).

4.3. Tumor-related epilepsy affects GB survival

TRE is a well-recognized condition in low-grade gliomas. The prevalence of epilepsy in GB patients has been previously reported to fluctuate between 25 and 50% [23,44]. Epilepsy in patients with GB plays an important role with impact in their quality of life and also appears to affect the survival of these patients. Drug-resistance has been documented in 20% of GB series [23,44]. We designed our study to include Adult-type diffuse glioma patients with TRE at the time of tumoral diagnosis, and FBTCS was reported on 41.7%. Previous publications have focused on identifying seizures at GB diagnosis and during follow-up, identifying their impact on survival [10,21–24,26]. Some authors have reported epilepsy as an independent favorable factor in survival [10,21,26] (see Table 5). In the present study, patients with FBTCS presented the shortest PFS (median of 6 months vs. 15 months for non-FBTCS, $p < 0.001$), the worst OS (median of 9 vs. 18 months, $p < 0.001$), and the poorest PRS (median of 0 months vs. 5 months, $p < 0.001$) compared with the other seizure types in the Kaplan-Meier analysis. We also observed that FBTCS represent a significant independent risk factors for both PFS (HR = 3.296, 95% CI 1.632–6.654, $p < 0.001$) and OS (HR = 7.007, 95% CI 2.980–16.471, $p < 0.001$) in the Cox analysis.

Regarding to ASM, 95% of our patients were treated with Levetiracetam and the mean of OS was superior in these patients compared with patients non-treated with Levetiracetam (16.3 vs. 15.3 months, $p = 0.730$). Although VA has been previously associated with a better OS [45], in our series all VA cases (n = 4) presented with a poor outcome. This result could be related with the fact that in our study most of the cases were routinely treated with Levetiracetam. The four VA patients mentioned presented altered hepatic tests and had to be turned to Levetiracetam. Double-therapy was also associated with a poor OS probably because of drug-resistance.

Other authors have implied that the molecular mechanisms related to epileptogenesis may promote tumor progression [44]. CMV (as an oncovirus), epileptogenesis, and IDH-type behavior share similar molecular mechanisms for tumoral growth and disease progression, [40,44]. In our study, patients with a high-grade CMV infection and FBTCS showed a worse OS compared with other seizure types (median 7 vs. 17 months for non-FBTCS, <0.001). Additionally, IDH-wt glioblastoma patients with FBTCS presented poor survival compared with non-FBTCS cases (median OS 9 vs. 17 months, $p < 0.001$). 83.3% of cases with mesial involvement presented FBTCS (<0.001). It seems that patients with a high grade of CMV infection develop an active proinflammatory microenvironment, which promotes epileptogenesis and subsequent clinical presentation of the FBTCS type associated with this process. Further studies are required to confirm this hypothesis.

4.4. Limitations

In general, our study has limitations because it is a retrospective study that included only patients with TRE, hence our series does not represent a GB general population sample. 81% of the cases (n = 49) presented a preoperative KPS ≥60 points, probably associated with an early diagnosis due to a seizure-related presentation. Nevertheless, there was no statistical difference ($p > 0.05$) among IDH-wt glioblastoma, seizure type, and degree of CMV infection (results were not shown) with preoperative KPS. Only 22.4% (n = 11) of the patients with a KPS higher than 60 points presented a postoperative RTOGPA of 3 and an adequate seizure control (Engel IA) was reported in 38%. Given the low number of IDH-mut astrocytomas grade 4 cases (n = 12) we did not include them for a separate survival analysis, as performed for IDH-wt glioblastoma (n = 48), and only IDH-wt GB were analyzed as a part of the Adult-type diffuse glioma sample. Further research is required to analyze IDH-mut astrocytomas grade 4 in a larger cohort. MGMT-unmethylated status was not included to assess prognosis in our sample. Additionally, in relation to ASM effect on survival, results should not be generalized due to small samples of VA patients.

5. Conclusions

CMV induces a proinflammatory tumoral microenvironment (TME) that inhibits the immune system and promotes tumoral progression. Tumoral progression was associated not only with a higher degree of CMV infection but also with epileptogenesis which determines a clinical phenotype of FBTCS, and poor survival. This study represents the first survival analysis in Latin America including seizure type and CMV-infection-degree in TRE Adult-type diffuse gliomas. The relevance and accuracy of these results are based on an adequate implementation of the optimized immunohistochemical technique to identify CMV infection degree in tumoral tissue. A high degree of CMV infection seems associated with an active pro-inflammatory TME increasing the epileptogenesis processes associated with identifiable clinical features (FBTCS). These may have clinical relevance and could be outcome predictors for this group of patients. Further prospective studies are required to validate these results.

Ethics statement

This study was reviewed and approved by the National Institutional Research Board with the approval number (R-2021-785-074) and the ethical committee.

All participants/patients provided informed consent to participate in the study.

All participants/patients provided informed consent for the publication of their anonymized case details and images.

According to ethical approval, all procedures for this research were conducted on Human subjects following the principles in the Declaration of Helsinki in 1964. Previous informed consent was obtained for each patient for molecular and/or IHC analysis in tumoral tissue and previous surgery as a routine in our center.

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This research did not receive any specific funding.

Data availability statement

Has data associated with your study been deposited into a publicly available repository?
The database of the current study is available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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