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**Title and sponsors:** Multicentre validation of Hemodynamic MTS biomarkers from preoperative and postradioteraphy MRI in Patients with Glioblastoma: predictors of overall survival. Juan M García-Gómez, Fernando Aparici, Carlos Botella, Jose Muñoz, Elies Fuster-Garcia, Javier Juan-Albarracín, Raquel Faubel, Sabina Asensio. 1 Biomedical Data Science Lab. Universitat Politecnica de Valencia. 2 Hospital Universitari i Politecnic La Fe Valencia.

**Background**
Despite an aggressive therapeutic approach, the prognosis for most patients with glioblastoma (GBM) remains poor (Stupp 2005). The relationship between non-invasive MRI biomarkers at preoperative, postradioteraphy and follow-up stages, and the survival time in GBM patients will be useful to plan an optimal strategy for the management of the disease (Wangaryattawanich, 2013).

The Hemodynamic Multiparametric Tissue Signature (HTS) provides an automated unsupervised method to describe the heterogeneity of the enhancing tumor and edema areas in terms of the angiogenic process located at these regions. HTS considers 4 habitats within the GBM: 1) the HAT habitat, which refers to the high angiogenic enhancing tumor part of the GBM, 2) the LAT habitat, which refers to the less angiogenic enhancing tumor area of the GBM, 2) the IPE habitat, which refers to the potentially tumour infiltrated peripheral edema, and 4) the VPE habitat, which refers to the vasogenic peripheral edema of the GBM (Juan-Albarracín, 2016). Our preliminary Cox survival regression study based on HTS biomarkers yielded statistically significant proportional hazard ratios (HR) and $R^2$ coefficients (p-value < 0.05 with False Discovery Rate correction $\alpha < 0.05$) for different combinations of perfusion biomarkers and HTS habitats. Relative Cerebral Blood Volume (rCBV) and relative Cerebral Blood Flow (rCBF) in combination with HAT and LAT habitats yielded HR ranging from 1.613 to 3.003.

**Objectives**

The purpose of this study is to analyse the significance of HTS biomarkers obtained from pre-treatment, postradioteraphy and follow-up MR Images (MRI) to predict the survival of patients with glioblastoma.
There are several specific objectives:
- To identify four habitats within the GBM using MRI and HTS
- To analyse the relation between the HTS habitats obtained from the first preoperative MRI and the overall survival of the patient
- To analyse the relation between HTS habitats obtained from the first preoperative MRI and the progression-free survival of the patient
- To analyse the relation between the HTS habitats obtained from the post radiotherapy MRI and the overall survival of the patient
- To analyse the relation between HTS habitats obtained from the post radiotherapy MRI and the progression-free survival of the patient
- To discover other interesting relations between the HTS habitats obtained from preoperative, postradioterapy and flow-up images and the clinical conditions of the patients

Hypothesis

Our conceptual hypothesis is that there is a significant correlation between the perfusion biomarkers located at several HTS habitats and the patient’s overall survival.

METHODOLOGY

Design: Multicenter observational retrospective cohort study with data collected from hospital information systems. The cohort will be built with patients diagnosed with GBM in the hospitals involved in the study with a MRI pre-treatment since 1st of January of 2012 to 1st January of 2017.

Selection criteria

Inclusion criteria:
- Patients diagnosed with Glioblastoma grade IV WHO with histopathological confirmation
- Age > 18 years at diagnosis
- Patients with access to the preoperative, and postradiotherapy MRI studies using 1.5T or 3T scanners, including
  o Pre and post gadolinium T1-weighted MRI
  o T2-weighted MRI
  o FLAIR MRI
  o Dynamic Susceptibility Contrast (DSC) T2*-weighted perfusion
  o Dynamic Contrast Enhancement (DCE) T1-weighted perfusion (optional)
  o Diffusion Weighted Imaging (DWI) (optional)
- WHO performance score between 0 and 2
- Patients with Karnofsky Performance Score (KPS) of ≥ 70%

Exclusion criteria:
- Patient with congestive heart failure within 6 months prior to study entry (New York Heart Association >= Grade 3)
- Uncontrolled or significant cardiovascular disease, including:
  o Myocardial infarction and transient ischemic attack or stroke within 6 months prior to enrollment
  o Uncontrolled angina within 6 months
  o Diagnosed or suspected congenital long QT syndrome
  o Any history of clinically significant ventricular arrhythmias (such as ventricular tachycardia, ventricular fibrillation, or Torsades de pointes);
    o Clinically significant abnormality on electrocardiogram (ECG)
- Pulmonary disease including or greater than grade 2 dyspnea or laryngeal edema, grade 3 pulmonary edema or pulmonary hypertension according to CTCAE 4.03

Variables

Mandatory variables collected will be:

- Socio-demographic data
  o Age at diagnosis (integer)
  o Gender (male/female/other)
  o Ethnicity (self-reported ethnicity group: Asian, Black, White, Other/Unknown)
  o Date of exitus (dd/mm/yy)
- Clinical data
  o Date of preoperative MRI study (dd/mm/yy)
  o Glioblastoma histopathological confirmation after surgery (yes/no)
  o Date of histopathological confirmation (dd/mm/yy)
  o Karnofsky Performance Status Scale at diagnosis [0..100]
  o ECOG/WHO/Zubrod score at diagnosis [0: Asymptomatic, 1: Symptomatic but completely ambulatory, 2: Symptomatic, <50% in bed during the day, 3: Symptomatic, >50% in bed during the day, 4: Bedbound, 5: Death]
  o Laterality (left-handed, right-handed, unknown)
  o Date of recurrence (dd/mm/yy or no)
  o Criteria for diagnosing recurrence (text)
- Surgery
  o Resection (Total, sub-total or biopsy)
  o Volume of resected tumor (cms)
  o Percentage of Volume of remaining tumor after surgical resection (%)
- Chemotherapy
  o Radiotherapy-Chemotherapy Standard treatment (Complete, Incomplete, No, Only RT, Only CT)
  o Radiotherapy (Gy)
  o Adjuvant Temozolomide (yes/no)
  o Adjuvant Temozolomide cycles (integer)
  o Bevacizumab (yes/no)
  o Other second-line treatments (text)
  o Administration of steroids at any time (yes/no)
- Image findings
  o Main tumor location (frontal lobe, parietal lobe, occipital lobe, temporal lobe, thalamus, cerebellum, brain stem)
  o Subventricular GBM (yes/no)
  o Involving cortex (yes/no)
  o Localization of the tumor, side (left/right)
- Observations
  o Observations: discontinuation, adherence, compliance, modifications of the treatment, etc (text)
- Previous Diabetes / hypertension / dyslipidemia (yes/no)
- Preoperative, Postradioteraphy and last follow-up MR Images:
  o Morphological MRI (preferred sequences and protocol):
    ▪ Pre-gadolinium Spoiled Gradient Echo T1-weighted exam
    ▪ Post-gadolinium Spoiled Gradient Echo T1-weighted exam
    ▪ Fast Spin Echo T2-weighted exam
    ▪ FLAIR exam
  o T2*-weighted Dynamic Susceptibility Contrast Perfusion MRI (preferred sequence and protocol)
    ▪ Gradient Echo-Echo Planar Image (GE-EPI)
    ▪ TE/TR = 30ms / 1-1.2 seconds
    ▪ Flip angle = 72°
    ▪ Reps = 120 (# of images per slice, collected over time)
    ▪ Slice thickness = 4-5mm
    ▪ FOV = 22-24cm, Matrix = 96
    ▪ After approximately 1 minute of baseline collection, inject a bolus of Gd-chelated contrast agent, typically 0.1mmol/kg at 3-5cc/sec.
  o T1-weighted Dynamic Contrast Enhanced Perfusion MRI (optional)
  o Diffusion Weighted MRI (b={0,1000} s/mm^2, intermediate b as optional)

Optional variables for glioblastoma profiling and patient stratification will be:

- Molecular profile
  o EGFR mutation/amplification/overexpression (yes/no, yes/no, yes/no)
  o PTEN loss/mutation (yes/no, yes/no)
  o CDKN2A loss (yes/no)
  o NES overexpression (yes/no)
  o Notch & Shh pathway activation (yes/no)
  o NF1 loss/mutation (yes/no, yes/no)
  o TP53 loss/mutation
  o PTEN loss/mutation
  o MET overexpression (yes/no)
  o CHI3L1 overexpression (yes/no)
  o CD44 overexpression (yes/no)
  o MERTK overexpression (yes/no)
  o TNF family & NFkB pathways activation (yes/no, yes/no)
  o NEFL mutation/amplification/overexpression (yes/no, yes/no, yes/no)
  o GABRA1 mutation/amplification/overexpression (yes/no, yes/no, yes/no)
  o SYT1 mutation/amplification/overexpression (yes/no, yes/no, yes/no)
  o SLC12A5 mutation/amplification/overexpression (yes/no, yes/no, yes/no)
Outcomes

Primary outcomes: The main outcomes will be survival time (in days) estimated since the date of the preoperative MRI to the exitus date. Exitus date will be collected from clinical records and should be confirmed by the main investigator at each center.

Secondary outcomes: Progression-free survival (in days), estimated since the date of the preoperative MRI to the date of recurrence.

Exploratory outcomes: Correlation between MTS habitats in longitudinal studies.

Data source: Data will be collected from Hospital Information System (HIS), and Picture Archiving and Communication System (PACS) of each center involved in the study.

Statistics

Cox regression, Kaplan–Meier estimator, and multiple linear regression analysis will be used to assess survival significance of each biomarker at each HTS habitat. The predictive value will be compared with models based on clinical and volumetric image variables: Age, KPS and VASARI features. Moreover, the HTS-based models will be compared to models based on hemodynamic biomarkers (CBF, CBV, $K_{trans}$ and $v_e$) and diffusion biomarkers (ADC) extracted from automatic segmentations of the edema and the enhancing tumor. Finally, Sørensen–Dice coefficient will be used to measure the correlation between MTS habitats in longitudinal studies.
Ethical issues
The study will be developed according to the Declaration of Helsinki about ethical principles for clinical research in humans. By the other hand, the study will be following Good Clinical Practices Guidelines and The International Conference on Harmonisation (ICH) and regulatory requirements of the institutions involved.

According to the design of the study – an observational, retrospective study based on secondary data- and taking into account that the patients are dead by the time of the data collection, informed consent exemption will be justified. Nevertheless, all personal data collected will be properly anonymized and identified by a code. Only the main researcher in each center involved could identify that code with clinical records. Treatment, communication and data cession of all participants will be managing according to Ley Orgánica 15/99 de Protección de Datos de Carácter Personal.

Study protocol will be approved by ethical committees’ authorities including ethical committees of the centers involved in the study like the Universitat Politècnica de València. Results of the study will be published according to STROBE declaration in a relevant scientific journal.

Bibliography

