PROPOSAL SUMMARY

Host Organization: University Hospital Río Hortega
Project Title: Predicting overall survival in glioblastomas using radiomic features of intraoperative ultrasound. A proposal for the creation of an international database of brain tumor ultrasound images.
Type of study: Ambispective observational
Start date & Duration: 1/10/2021 – 30/03/2022

1. Lay Abstract

Predicting the survival of patients diagnosed with glioblastoma (GBM) is essential to guide surgical strategy and subsequent adjuvant therapies. Intraoperative ultrasound (ioUS) is a low-cost, versatile technique available in most neurosurgical departments. The images from ioUS contain biological information possibly correlated to the tumor's behavior, aggressiveness, and oncological outcomes. Today's advanced image processing techniques require a large amount of data. Therefore, we propose creating an international database aimed to share intraoperative ultrasound images of brain tumors. The acquired data must be processed to extract radiomic or texture characteristics from ioUS images. The rationale is that ultrasound images contain much more information than the human eye can process. Our main objective is to find a relationship between these imaging characteristics and overall survival (OS) in GBM. The predictive models elaborated from this imaging technique will complement those already based on other sources such as magnetic resonance imaging (MRI), genetic and molecular analysis, etc. Predicting survival using an intraoperative imaging technique affordable for most hospitals would greatly benefit the patients' management.

2. Scientific Summary

We plan to carry out a multicentre retrospective study of patients operated with GBM diagnosis between January 2018 and January 2020, in order to set the base for future prospective collection of patients. All cases with an ioUS study will be included. All patients must count with B-mode modality. After an pseudonymization process, the images will be uploaded to a private cloud server. Demographic, clinical, conventional radiological, and molecular variables (IDH, MGMT) will also be collected. OS will be defined as the time elapsed between the histopathological diagnosis and the patient's death. The acquired data must be processed to

obtain a series of radiomic markers to perform the study. A pre-processing stage will be necessary (noise cleaning, despeckling, intensity normalization, filtering) to calculate radiomics measurements (histogram, volumetric, shape, texture, etc.). In the previous stage, a very high number of radiological features per subject will be calculated. Because the number of features is much higher than the data set, to avoid the curse of dimensionality, it will be necessary to reduce their number using feature selection and extraction techniques (standard in pattern recognition and radiomics) that allow choosing those characteristics (or transformations of them) that have greater discriminating power. A predictive model of survival will then be elaborated based on the features selected.

3. Project Details

3.1 Background and Scientific Rationale:

GBM are the most common primary brain neoplasm. The survival of these patients is approximately 15 months despite continuous efforts to find more effective treatments. ioUS is a low-cost, versatile imaging technique available in most neurosurgical departments. These images contain biological information with potential application in surgical practice in addition to being a handy technique for image-guided brain tumor surgery. Advanced modalities, such as contrast-enhanced ultrasound (CEUS), intraoperative ultrasound elastography (IOUS-E), and navigated three-dimensional (3D) ultrasound, currently make it possible to exploit the full potential of this imaging technique to achieve maximal safe tumor resections.

On the other hand, advanced medical image analysis allows the development of predictive survival models using radiomic features. Even though ioUS image quality and resolution could be considered inferior compared to other imaging techniques, such as MRI, there is growing evidence that the ultrasound images contain biological information that can be set as a base to create models that allow classification tasks such as benignity versus malignancy. The ultrasound's capacity as a prognostic marker in response to adjuvant treatments in breast pathology has recently been evaluated. One possible explanation may lie in the fact that echogenicity in the B-mode could be associated with cellularity, which, on the other hand, is related to tumor aggressiveness.

In recent publications, we have explored intraoperative ultrasound's ability to differentiate primary malignant tumors from metastases using artificial intelligence. Besides, we have made the first descriptions about the extraction of brain tumors' ultrasound radiomic characteristics and their correlation with survival. The ultrasound's main limitation is the dependency on the explorer and the variability between the parameters used in image acquisition among different centers. For this reason, image pre-processing is a fundamental step. In this project, we intend to use an ultrasound modality as a reference, the B-mode. Only by gathering a vast set of cases we would be able to test image harmonization techniques to allow the generalization and adoption of our predictive model in other centers. Also, the creation of an international and public database, the **Bra**in **T**umor **i**ntra**o**perative **U**ltra**S**ound **D**ata**B**ase (BraTioUS-DB), will allow the development of additional research lines, since, in addition to B-mode images, other modalities such as elastography, CEUS, vascular studies, etc. will be incorporated prospectively. Furthermore, other histopathological tumor types could also be included.

3.2 Hypotheses

Intraoperative ultrasound images in B-mode harbour tumor texture features correlated with overall survival in glioblastomas.

3.4 Objectives:

- To determine the relationship between the radiomic features of intraoperative ultrasound B-mode and overall survival in glioblastomas.
- Develop a predictive survival model using the texture features with the highest discriminatory power.
- Validate the model against an external dataset and compare it with currently available predictive models.
- Build a data set that allows exploring various image harmonization techniques that allow the reproducibility of our predictions.
- Establish an international cooperation network (**BraTioUS-DB**) whose objective will be to interchange ultrasound images and clinical data of patients operated on for a brain tumor prospectively from its creation and start-up.

4. Research Plan:

4.1 Study sample:

The cases that meet the inclusion criteria mentioned in the following section will be collected retrospectively to carry out the project's first phase. From January 2018 to January 2020, it is estimated that each center can contribute with a minimum of 20 cases. Therefore, the total sample size for this phase of the study will be approximately 120 patients.

4.2 Selection of patients:

The inclusion criteria will be:

- Adult patients operated between January 2018 and January 2020 with a pathological diagnosis of WHO grade IV astrocytoma (Glioblastoma).
- Intraoperative ultrasound study that includes B-mode images

The exclusion criteria will be:

- Other histopathological diagnoses. Even though the international database will be established in such a way that other tumor types can be included prospectively.
- Artifacts in ultrasound images that make their analysis impossible
- Stereotactic biopsies.

4.3 Data collection (retrospective phase):

Each center will collect the details of the intraoperative acquisition technique of the ultrasound images. These data should include:

- Manufacturer of the ultrasound equipment,
- Type of probe: linear, curve.
- Footprint size
- Frequency range
- Modality. Essential B-mode, other modes optional.
- Acquisition before or after dural opening.
- Co-registered magnetic resonance images when possible (navigated ultrasound).
- Number of explorers who perform the ultrasound study.

Of all the B-mode ultrasound images made by each patient, at least three slices will be selected in which the largest tumor diameter is appreciated, the peritumoral region, and healthy parenchyma, ideally in two planes perpendicular to each other.

After pseudonymizing the images, they will be uploaded from each center to a private cloud server with authentication to ensure data privacy. The computing platform will be Box (www.box.com), a leading company in storing and protecting data worldwide. The contents uploaded to the cloud will be protected with integrated controls, specific permissions and robust user authentication systems. Furthermore, each file will be encrypted with AES 256-bit encryption in various locations (FIPS 140-2 certified). The platform also complies with regulations such as the General Data Protection Regulation (RGPD), the Law of Transfer and Liability of Health Insurance (HIPAA), the Federal Program for Risk Management and Authorizations (FedRAMP). Regarding security, Box Shield (a Box feature) works as a first-level security tool, highlighting, for example, the automatic detection of malware and the prevention of cyberattacks. The details of its characteristics can be consulted at: https://www.box.com/es-419/shield.

Along with the images, the demographic and clinical data of each patient will be recorded:
- Karnofsky Functional Rating Scale (KPS).
- Age
- Sex
- Adjuvant treatment received: chemotherapy/radiotherapy
- Percentage of resection of the enhancing tumor volume and non-enhancing peritumoral region. Based on this percentage, resections will be classified as: 100% complete resection (GTR); Subtotal resection> 90% (SBT) and Partial Resection <90% (PR).
- Immunohistochemical data included in the current WHO classification for pathological diagnoses of glial tumors. IDH, ATRX and MGMT mutation.

4.4 Data collection (prospective phase):

Once the international collaborative network has been consolidated and the online platforms have been enabled, glioblastomas and other tumor strains will begin to be collected prospectively.
The inclusion criteria will be all patients undergoing surgical intervention with a pathological diagnosis of a primary brain tumor with an intraoperative ultrasound study. Ultrasound modalities will not be limited only to B-Mode, but also, depending on each center's availability, to other modalities such as strain-type elastography, shear-wave elastography, and post-contrast ultrasound (CEUS), Doppler and Microvascular modalities (i.e., eFlow, fineFlow, among others) and navigated ultrasound.
The clinical and demographic data will be the same as those detailed for the retrospective phase of the project.
The cases stored prospectively will be made available to the participating centers. Creating an extensive database of ultrasound of brain tumors will allow the development of new projects and lines of research in the future. For its use, each center may prepare proposals for the use of

the database that must be approved by the network members and, if required, also by the Ethics Committees of each participating institution.

4.5 Pre-processing of images and estimation of radiomic markers

The acquired data must be processed to obtain a series of radiomic markers to perform the study. A pre-processing or harmonization stage will be necessary (cropping, noise cleaning, speckling removal, histogram/intensity normalization) to calculate radiomics measurements.

4.6 Segmentation

The segmentation process will be performed semi-automatically by an expert neurosurgeon and by a senior neuroradiologist. Segmentation of the area corresponding to the tumor will be performed, avoiding regions cystic/necrotic. The peritumoral area with evidently altered echogenicity will also be segmented. The Dice similarity coefficient will be used to assess the variability between the segmentations made by both examiners.

4.7 Radiomic Feature Extraction

In a previous step, the images will be pre-processed by resampling at 1 x 1 mm size. Intensity discretization will also carry out using 256 gray levels and a relative rescaling of intensities using the three-sigma method (± 3 standard deviations [SD]). The next step will be to extract the segmented regions' radiomic features using open-source software Pyradiomics / LifEx.

4.8 Data analysis

Because the number of features is much higher than the data set, it will be necessary to reduce their number using feature selection and extraction techniques that allow choosing those characteristics that have greater discriminating power.

A predictive model of survival will then be elaborated based on the selected features. The performance evaluation strategy of the predictive survival model will be adjusted to the sample size. The options to be tested will be five and ten-fold cross-validation, the random split of the sample into a training cohort and testing cohort (unseen data), and an external validation cohort formed by one of the collaborating centers.

5. Team and Scientific Potential:

The research group that we have assembled has already collaborated in the past and has carried out various tasks focused on integrating non-invasive diagnostic tools for neurological pathologies and creating new imaging methods applicable to neurosurgical patients.

The principal investigator (S.C.) is currently collaborating with an international network dedicated to the study and advanced analysis of images in glioblastomas, ReSPOND (Radiomics Signatures for PrecisiON Diagnostics) consortium on glioblastoma (GBM).
At the national level, S.C. collaborates with the recently established National Brain Metastasis Network (RENACER), a project promoted by the National Center for Oncological Research.
Our center has also collaborated for several years with the National Registry of Central Nervous System Tumors (RETSINE) of the Spanish Research Group in Neuro-Oncology.
Since 2019, the IP is the Coordinator of the Central Nervous System Tumors Committee of the Río Hortega University Hospital.
Our team has collaborated with the Image Processing Laboratory (IPL) of the Higher Technical School of Telecommunications Engineering (HTST) of the University of Valladolid (www.lpi.tel.uva.es), which has been studying diagnostic image processing for years, performing multiple projects and having high-level expertise in this field.

The project will have the collaboration of the following international centers:

1. Dr. Giussepe Maria Della Pepa (Fondazione Policlinico A. Gemelli, IRCSS, Rome, Italy)
2. Dr. Aliasgar V Moiyadi (Tata Memorial Center, Mumbai, India)
3. Dr. Massimiliano del Bene (Fondazione Istituto Neurologico Carlo Besta, Milano, Italy)
4. Dr. Benjamin Saß (Philipps University of Marburg, Marburg, Germany)
5. Dr. Ilyess Zemmoura (CHRU de Tours and Inserm U1253, Tours, France).

Our working group has previously published several articles about applying ioUS and advanced analysis of MRI. Our original works constitute the first descriptions in the literature about the semi-quantitative analysis of strain-type elastography in brain tumors and peritumoral regions, predicting tumor consistency in meningiomas combining radiomic features of MRI and ioUS elastography. Similarly, we are the first research group to describe the use of artificial intelligence techniques (Machine and Deep Learning) and radiomic analysis of intraoperative ultrasound images of brain tumors.

6. Ethics:

The data will originate from measurements, comparisons and validations. They will be used to meet project objectives and in peer-reviewed conferences and publications. The types of data generated in the investigation will be of type:

- Observational: data captured in real-time (neuroimaging)
- Simulation: data generated from test models (mathematical)
- Compiled: data mining, compiled databases, etc.
- Reference: clustered data sets (radiomic neuroimaging measurements).

Most of the data will be in ASCII (American Standard Code for Information Interchange) data files, for example, Comma Separated Variables (CSV) format, which can be imported into rich text files for word processing or into spreadsheets. If specialized software is used, information on free readers will be provided. Neuroimaging data will be collected in Digital Imaging and Communication On Medicine (DICOM) format.

All data associated with scientific publications will be openly available by default unless there is a specific reason for not publishing the data. There will be no restrictions on the use of published data, but users should acknowledge the study group and the data source in any resulting publication. The research team members will impose a strict policy on all employees, co-workers, subcontractors, etc., who have access to the data. This policy will include but is not limited to, not allowing copies on local devices during data processing.

The transfer of data about human beings to the repository will only be considered when: informed consents, ethical approval and, where applicable, approval of local data protection authorities cover the purpose for which the data is intended to be used. Since this study involves patients diagnosed with glioblastomas, the cases have perished at the time of data collection. Therefore, the exemption of informed consent will be requested for the retrospective phase of the study (see Annex). On the contrary, for the prospective collection of cases, the informed consent model will be used (see Annex). All data to be transferred to the repository will be pseudonymized. The data owner/data provider will be responsible for the pseudonymization process and ensure that identifiable variables are not transferred to the repository.

7. Communication Plan for Dissemination:

This project's results will be disseminated through its publication in scientific journals and international conferences focused on neuroimaging, ultrasound, neurosurgery, and neuro-oncology.

8. Outputs: Impact, potential beneficiaries and added value:

The research work, aligned with one of the items of the Horizon 2020 program (Health, Demographic Change and Wellbeing) in addition to two of the United Nations Sustainable Development Goals (Health and well-being, 3; Industry, Innovation and Infrastructure, 9), has a great potential for impact, both at a scientific-technical level and a socio-economic level.

Concerning the scientific-technical, it is considered that the development of models for pre-processing, advanced analysis of ultrasound images that apply to commercial scanners and that, in turn, derive in descriptors that generate a large amount of useful information about the macro and microstructural properties of brain tumors, will lead to an increase in the use of advanced ultrasound techniques in clinical practice and will generate an increase in scientific research projects focused on these imaging technique. Furthermore, such models' implementation will substantially improve radiological care (diagnosis, early detection and treatment).

About the socio-economic aspect, it is considered that the advance in the analysis and interpretation of ultrasound images, together with its future application in clinical settings, will not only have a significant influence on the quality of life of patients, as well as the possible economic implications that this entails, but it will also increase the efficiency of community health systems thanks to the reduction of the time required for acquisitions and their subsequent processing.

Besides, predicting survival of GBM patients opens the door for the creation of alliances with other workgroups and specialists (radiologists, pathologists, biologists, engineers, oncologists, radiation oncologists, neurosurgeons) to use the information provided by our model for the application of new oncological therapies, both pharmacological and radiotherapy. The ultimate goal will always be to seek more efficient therapeutic options and improve patients' quality of life suffering from this pathology.

9. Funding

For the first phase of the project, the collection of images retrospectively, each participating center will assume the responsibility for collecting and uploading the data.
Other financing sources will be sought to maintain cloud server services and external agents specialized in image processing.

10. Recent publications

1. Intraoperative Ultrasonographic Elastography: A Semi-Quantitative Analysis of Brain Tumor Elasticity Patterns and Peritumoral Region. **Cepeda S**, Barrena C, Arrese I, Fernandez-Pérez G, Sarabia R. World Neurosurg. 2020 Mar;135:e258-e270.doi:10.1016/j.wneu.2019.11.133.
2. Meningioma Consistency Can Be Defined by Combining the Radiomic Features of Magnetic Resonance Imaging and Ultrasound Elastography. A Pilot Study Using Machine Learning Classifiers. **Cepeda S**, Arrese I, García-García S, Velasco-Casares M, Escudero-Caro T, Zamora T,Sarabia R.World Neurosurg. 2021 Feb;146:e1147-e1159. doi:10.1016/j.wneu.2020.11.113.
3. Comparison of Intraoperative Ultrasound B-Mode and Strain Elastography for the Differentiation of Glioblastomas From Solitary Brain Metastases. An Automated Deep Learning Approach for Image Analysis. **Cepeda S**, García-García S, Arrese I, Fernández-Pérez G, Velasco-Casares M, Fajardo-Puentes M, Zamora T, Sarabia R. Front. Oncol. 2021 Feb 2;10:590756. doi: 10.3389/fonc.2020.590756
4. Is there a relationship between the elasticity of brain tumors, changes in diffusion tensor imaging and histological findings? A pilot study using intraoperative ultrasound elastography. **Cepeda S**, García-García S, Velasco-Casares M, Fernández-Pérez g, Zamora T, Arrese I, Sarabia R. Brain Sciences. 2021; 11(2):271. doi:10.3390/brainsci11020271
5. Relationship between the overall survival in glioblastomas and radiomic features of intraoperative ultrasound: A feasibility study. **Cepeda S,** García-García S, Arrese I, Velasco-Casares M, Sarabia R. J Ultrasound. 2021 Feb 16. doi: 10.1007/s40477-021-00569-9. Online ahead of print.
6. Acute changes in diffusion tensor-derived metrics and its correlation with the motor outcome in gliomas adjacent to the corticospinal tract. **Cepeda S**, García-García S, Arrese I, Velasco-Casares M, Sarabia R. Surg Neurol Int. February 2021;12:51. doi:10.25259/SNI_862_2020
7. Letter to the Editor. Intraoperative ultrasound elastography applied in meningioma surgery. **Cepeda S**, Sarabia R. Neurosurg Focus. 2021 May;50(5):E23. doi: 10.3171/2021.1.FOCUS2115.
8. Predicting short-term survival after total resection in glioblastomas by machine learning-based radiomic analysis of preoperative MRI. **Cepeda S**, Perez-Nuñez A, Garcia-Garcia S, Garcia-Perez D, Arrese I, Jimenez-Roldan L, Garcia-Galindo M, Gonzalez P, Velasco-Casares M, Zamora T, Sarabia R. 23 June 2021, PREPRINT available at Research Square [https://doi.org/10.21203/rs.3.rs-640526/v3]
9. Advantages and limitations of intraoperative ultrasound strain elastography applied in brain tumor surgery: a single-center experience. **Cepeda S**, García-García S, Arrese I, Velasco-Casares M, Sarabia

R., 25 June 2021, PREPRINT available at Research Square [https://doi.org/10.21203/rs.3.rs-654010/v1]

10. Hernando-Pérez E, Pérez-Riesgo E, **Cepeda S**, Arrese I, Sarabia R, Villalobos C, Núñez L (2021) Differential Ca^{2+} responses and Store operated Ca^{2+} Entry in Primary Cells from Human Brain Tumors. *Biochim Biophys Acta Mol Cell Res. 2021 Jul;1868(8):119060. doi: 10.1016/j.bbamcr.2021.119060.Epub 2021 May 14*.
11. Evaluation of the reporting quality of clinical practice guidelines on gliomas using the RIGHT checklist. Yang Y, Ma Y, Lu J, Du S, Zhang J, Meng H, Chen Z, Zhang Q, Zhang X, Shi W, Girolamo F**, Cepeda S**, Kang J.. Ann Transl Med 2021;9(12):1002. doi: 10.21037/atm-21-2604