EVALUATION OF PREDICTIVE VALUE OF CT IMAGING BIOMARKERS IN STROKE PATIENTS AFTER ENDOVASCULAR TREATMENT

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ABSTRACT

Background: Ischemic stroke is a high incident disease in the world with a high morbi-mortality, that is why its management is in continuous development. During this development, radiological biomarkers have been studied to improve the early diagnosis to save as much cerebral tissue as is possible. When the diagnosis of stroke is performed it can be treated pharmacological (with rtPA) or surgical (mechanical thrombectomy) depending on time of stroke onset, but the outcome of this treatment is uncertain. Nowadays biomarkers are focus on the diagnosis but not in the prognosis after the treatment and maybe the outcome can be worst if the thrombus is removed or it cannot be clinical improve. This prognosis biomarkers are needed in order to select properly the patients which can be benefited with treatment, to the proper use of medical resources and to provided based on information to the family for a better chose of their familiar treatment.

Objective: The aim of this project is to analyze that biomarkers which can allow us to predict the outcome of a stroke patient treated with mechanical thrombectomy.

Design: A multicentric observational prospective cohort project. Seven third level hospitals (three from Catalonia, two from Madrid and two from Valencia) will participate in this project.

Participants: Patients older than eighteen years old with a stroke treated with mechanical thrombectomy and with all the data collected.

Methods: We will record clinical with a data collection form where all the clinical variables are written. The radiological variables will be obtained from the first CT performed for the diagnosis and will be interpreted by two different neuroradiologist from the investigation team.

Keywords: CT Biomarkers, Stroke, Mechanical thrombectomy, Prognosis.

ABBREVIATIONS

- **NIHSS**: National institute of Health Stroke Scale
- **CT**: Computed Tomography.
- **MRI**: Magnetic Resonance Imaging.
- **MCA**: Middle Cerebral Artery.
- **ASPECTS**: Alberta Stroke Program Early Computed Tomography score.
- **MTT**: Mean Transit Time.
- **CBV**: Cerebral Blood Volume.
- **CBF**: Cerebral Blood Flow.
- **MIP**: Maximum Intensity Projection.
- **DWI**: Diffusion Weighted Imaging.
- **ADC**: Apparent Diffusion Coefficient.
- **PWI**: Perfusion Weighted Imaging.
- **mRS**: modified Rankin Scale.
- **FIV**: Follow-up Infarct Volume.
- **IV**: Intravenous.
- **$cCBF_{max}$**: Maximum Cerebral Flow of Collateral vessels.
- **mTICI**: modified Treatment in Cerebral Ischemia.

INTRODUCTION

Acute cerebral infarct (or stroke) is defined as an abrupt change on cerebral blood flow that changes transitory or permanently the function of a cerebral part. The incidence of this disease is 180-200 cases/100.000/year. Is the most permanently disabling disease in the adult, the second cause of death in men (the fist in women), the second cause of dementia and the first cause of ingress in neurological services (1). The global life time risk of stroke from the age of 25 years onward was approximately 25% among both men and women with geographic variation highest risks in East Asia, Central Europe, and Eastern Europe (2).

There are two main stroke types, the ischemic (around 80% of all strokes) and the hemorrhagic (around 20% of all strokes) (3). Stroke presentation can be transient or permanent which can be form mild to fatal. Classically the definition of transient cerebral infarct was defined by time (the duration of 24 hours from the onset), nowadays is defined as a brief neurological disfunction (less than 1 hour) without evidence of infarct in image techniques (3).

According to the treatment, while the hemorrhagic stroke does not have a well provident interventional acute treatment, the ischemic one has an immediately reperfusion treatment with pharmacologic or endovascular treatment. It is important to discern between these two types of stroke to provide a proper treatment and to improve the outcome of the patient (3,4).

Ischemic stroke is caused by the focal occlusion of one or more arteries. The mechanism of the occlusion determines the subtype of ischemic stroke which can be cardioembolic, small vessel disease, artery-to-artery thromboembolism, occlusive arterial disease or undetermined. These subtypes do not affect the acute treatment but it is important in the secondary prevention (3).

STROKE CODE

The management of a patient with a stroke supposition must be fast in order to minimize the loss of cerebral tissue. The stroke code is a circuit of selection and transport of those patients with a clinic suspicion of a cerebral infarction (3).

At the arrival to the hospital, the neurologist performs the anamnesis asking **time of stroke onset, previous strokes, previous Rankin and perform the NIHSS** (annexed 2), also the **blood pressure and the glycemia** is performed.

The first thing to determine is the presence of stroke and the type, for which computed tomography (CT) or a magnetic resonance (MR) is done(4).

CT IN ACUTE CEREBRAL INFARCTION

Nonenhanced scanning must be performed as soon as possible after the stroke code has been activated. CT is highly sensitive for the depiction of hemorrhagic lesions, and the key role of nonenhanced CT is the detection of hemorrhage or other possible mimics of stroke (eg, neoplasm, arteriovenous malformation) (5) that could be the cause of the neurologic deficit. The second role of nonenhanced CT is the detection of early ischemic signs of established infarction (6).

Hemorrhagic stroke in simple CT is visualized as a spontaneous hyperdense area corresponding with a parenchymal hemorrhagic, secondary to the loss of the cerebral arterial vessel wall solution extravasating blood to the cerebral parenchyma (4).

Ischemic stroke we can see early signs such as (6):

- Subtle hypoattenuation.
- Obscuration and loss of gray matter–white matter differentiation in the basal ganglia.
- Cortical sulcal effacement.
- Loss of the insular ribbon.
- Hyperattenuation of a large vessel (“hyperattenuating MCA sign” or “dot sign”).

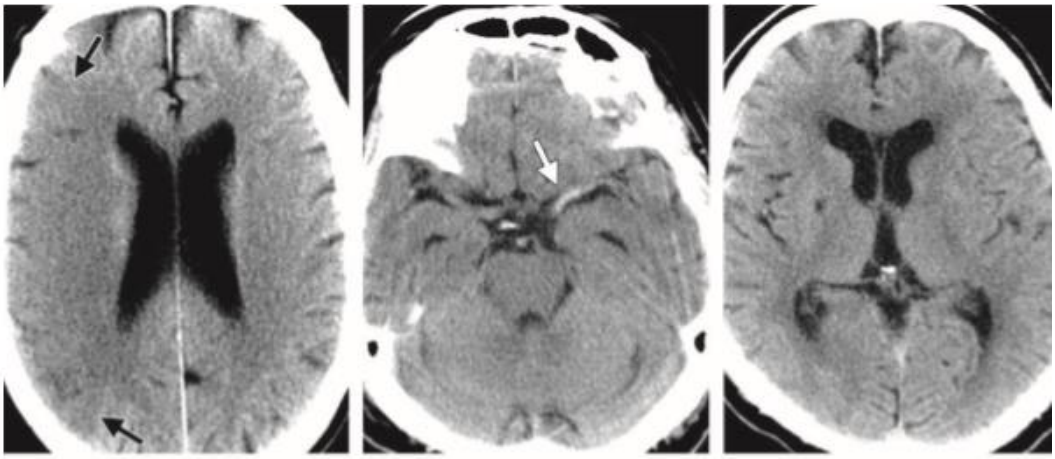

Image 1: Early ischemic CT signs. CT scans show subtle hypoattenuation and sulcal effacement in the right MCA territory (arrows in the left image), a hyperattenuating left MCA (arrow in the middle image), and obscuration and loss of gray matter– white matter differentiation of the left basal ganglia and sulcal effacement in the left MCA territory (right image) (6)**.**

Stroke´s severity is represented by Alberta Stroke Program Early CT Score (ASPECTS). Patient starts with 10 points and It is calculated by the subtraction of every part affected (hypodense) that the scale measure. This scale helps the neuroradiology to reduce the Inter-observer variation as the interpretation of early signs are very observer-dependent.

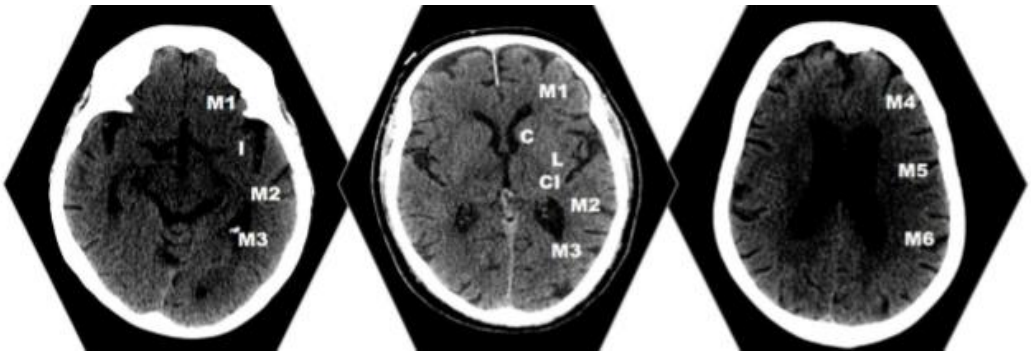

Image 2: We can see the representation of the ASPECTS in a nonenhanced CT image in three different parts of the brain (4)**.**

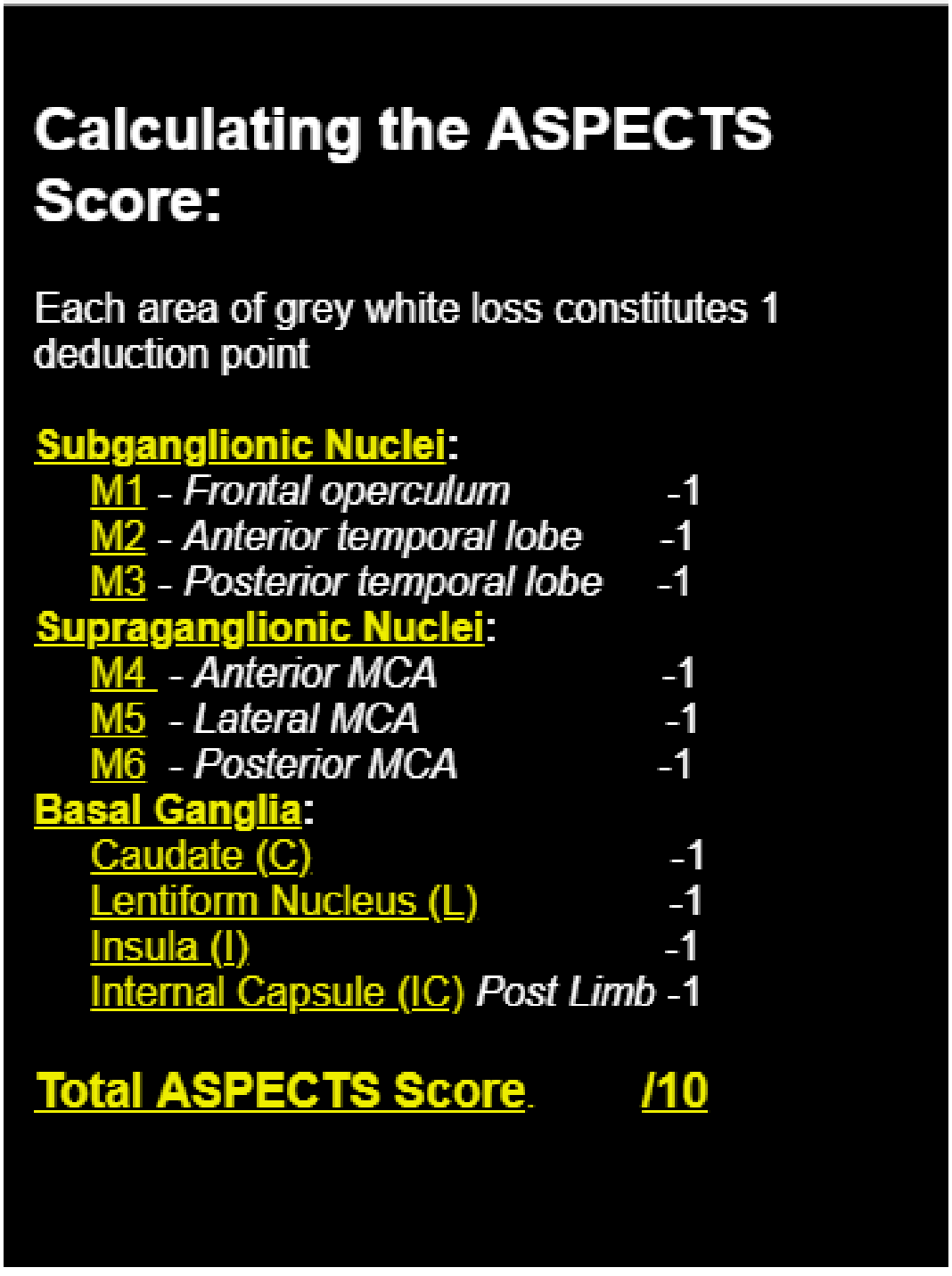

Image 3: We can see the score of every part of the brain that is measured in the ASPECTS (7).

After the diagnosis of ischemic stroke, a perfusion-CT is performed so we can determine the ischemic penumbra and the infarct core. The ischemic penumbra is defined as proportion of the ischemic territory that is still potentially salvageable, if an appropriate treatment is given (8). The infarct core is defined the irreversible injured cerebral tissue.

The perfusion-CT gives us the mean transit time (MTT), the cerebral blood volume (CBV) and the cerebral blood flow (CBF). With the comparison of these volumes we can obtain the ischemic penumbra and the infarction core (6).

Table 1 (4):

	MTT	BCF	BCV	SIMPLE CT
Ischemic penumbra	↑	↓	NORMAL	NO CHANGES
Infarction core	↑	↓↓	↓↓	HYPODENSITY

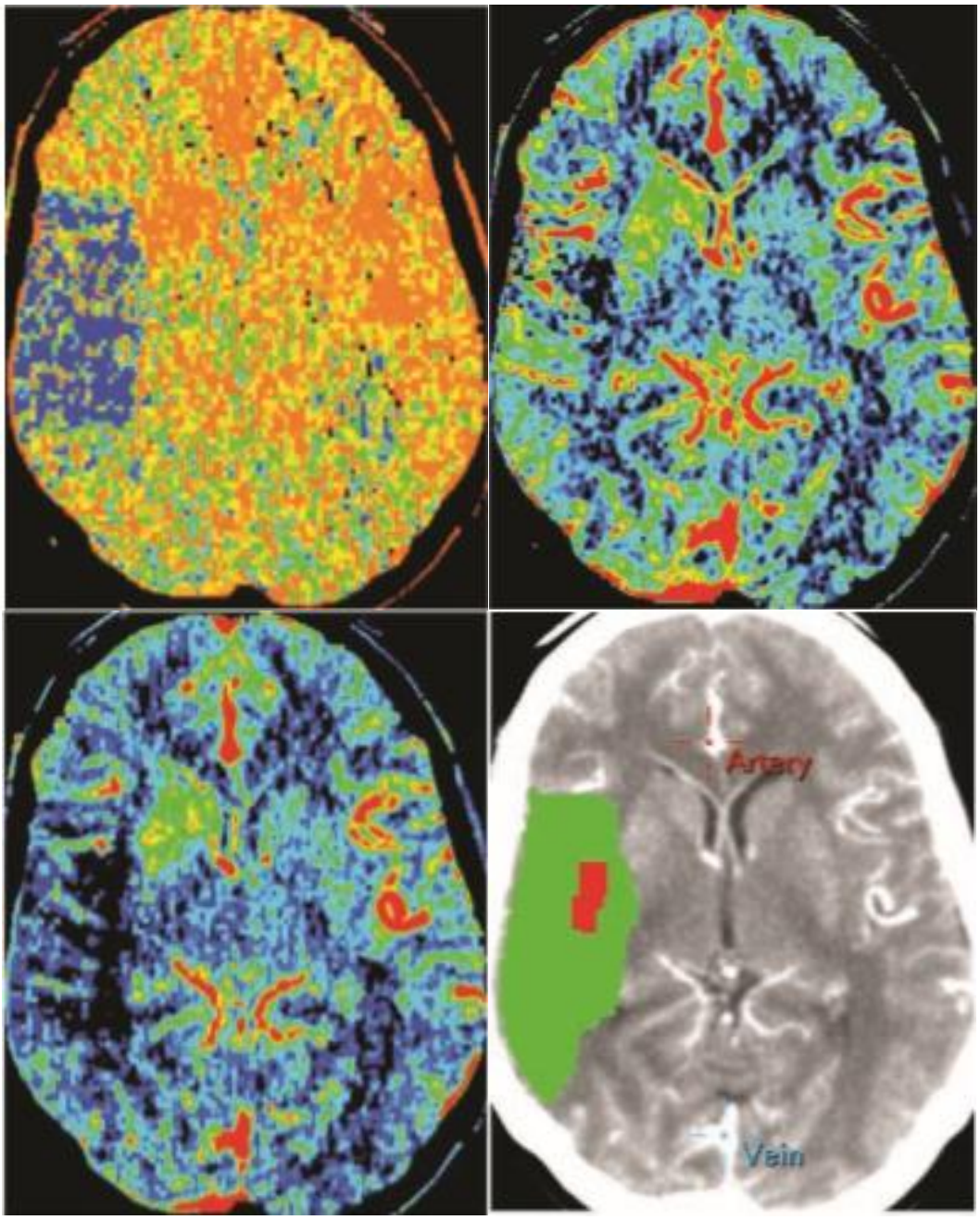

Image 4: We can see a Perfusion CT map of MTT (upper left image), CBV (upper right), CBF (down left) and a summary map (down right) showing an altered MTT and CBF in the right frontotemporal area, suggestive of ischemia, and a reduced subcortical area with decreased CBV, suggestive of an infarcted core (6)**.**

At the same time an Angio-TC of supra-aortic trunks and intracranial vessels for the selection of endovascular treatment patients. It helps us to determine the

occlusion point, the presence or absence of carotid/vertebral dissection and the collateral vascularity (4,7).

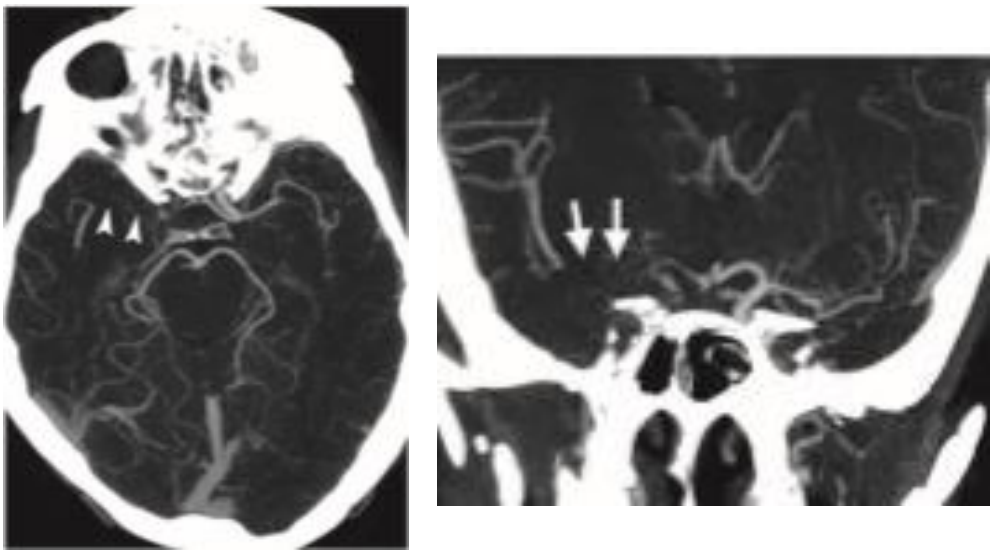

Image 5: We can see Axial (left) and coronal (right) MIP reformatted Angio-CT images show obstruction of the right MCA (arrows) (6)**.**

The collateral vascularity is more associate with the infarct growth and penumbra salvage than the time elapsed (9). So for that it is important to treat those persons with a good collaterals.

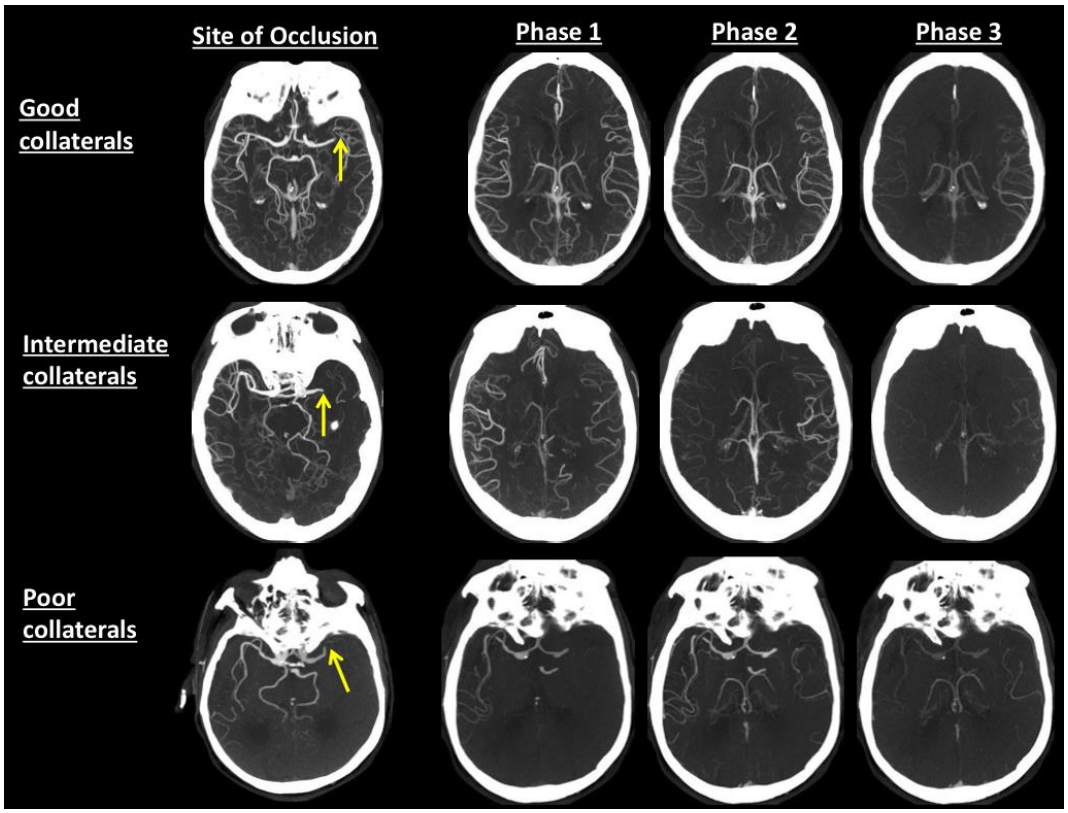

Image 6: We can see a descriptive image of the collateral vascularity(10)**.**

MRI IN ACUTE CEREBRAL INFARCTION

In the ischemic stroke the multimodal MRI provide information that also allow to distinguish between ischemic penumbra and infarction core. Different forms of MRI imaging are performed to determinate ischemic penumbra and infarction core.

DWI measures the net movement of water in tissue due to random (Brownian) molecular motion of water and shows hyperintense ischemic tissue changes within minutes to a few hours after arterial occlusion due to a reduction of the apparent diffusion coefficient (ADC). The ADC reduction occurs primarily in the intracellular space associated with disruption in membrane ionic homeostasis and cytotoxic edema. Decreases in the ADC and increased signal on DWI studies in many instances represent irreversible ischemia. To differentiate acute from subacute or older lesions (T2 shine-through), DWI is used in conjunction with T2-weighted images and ADC maps (11).

PWI allows the measurement of capillary perfusion. The method most commonly used in clinical practice and research is the dynamic susceptibility contrast-enhanced technique, in which paramagnetic contrast agent is injected as an IV bolus and the signal change is tracked by susceptibility-weighted, T2-weighted magnetic resonance (MR) sequences. Relative cerebrovascular hemodynamic measures reflecting cerebral blood volume, mean transit time, time to peak, and cerebral blood flow (CBF) can be derived from the MR signal intensity-over-time curve in a semiquantitative fashion. Parameter maps display the area of critically reduced perfusion (11).

Nowadays the use of multimodal MRI in stroke is very reduced and multimodal CT is the predominant image acquisition in the stroke disease, even there is no difference in the prediction of the functional outcome of endovascular treatment (12).

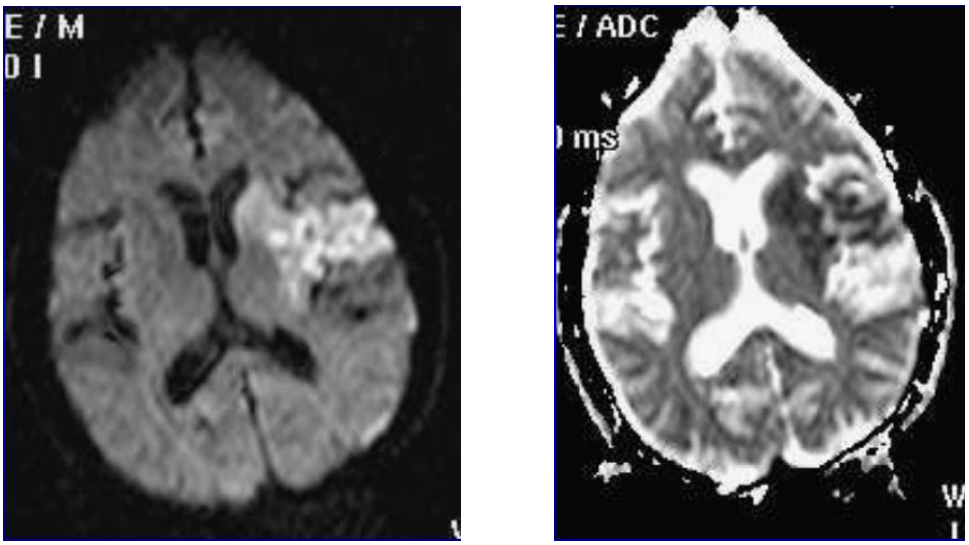

Image 7: We can see an hyperintense section in the temporal lobule in the DRM (left image) and the same section with but hypodense in the ADC (right image) corresponding to a cytotoxic edema which means a stroke (images courtesy of Hospital Josep Trueta).

MANAGEMENT AND TREATMENT OF STROKE

Nowadays, the management of a stroke is determined by time elapsed since time of stroke onset.

Radiologic diagnosis:

- <4,5 hours: Multimodal CT (Nonenhanced-CT + Angio-CT + Perfusion-CT).
- 4,5-8 hours: MR multimodal or Multimodal CT (Nonenhanced-CT + Angio-CT + Perfusion-CT).
- Wake up stroke/ vertebrobasilar <4 hours: MR multimodal or multimodal CT (Nonenhanced-CT is performed + Angio-CT + Perfusion-CT).

Treatment:

- <4,5 hours: rtPA (1st option) and endovascular treatment as 2nd option (1st if rtPA contraindicated).
- 4,5 – 8 hours: rtPA (if low risk of bleeding or absence of large vessel thrombose) or endovascular treatment (if high risk of bleeding or presence of large vessel thrombose) (4).

The latest articles plead about changing the selection of treatment depending on the viable tissue instead on the time (13,14). With these articles the treatment of stroke could be done by endovascular treatment regardless the time of stroke onset.

After the treatment or the decision of no treatment, all patients are hospitalized in the stroke unit.

PROGNOSTIC

The prognostic of the patient is uncertain. In the clinical practice there are not biomarkers that allow us to predict the functional outcome.

Some blood biomarkers have been propose to be potential prognosticators of the stroke outcome such as plasma levels of high-sensitivity C-reactive protein, complement 3, matrix metalloproteinase 9, hepatocyte growth factor, LpPLA2, NT-proBNP, cystatinC0, rheumatoid factor, homocysteine and antiphosphatidylserine antibodies in blood collected within 24 hours of stroke onset (15). The main problem is that these biomarkers in clinical practice remains unclear as they do not have a specific value because the stroke is a dynamic disease and also changes with a reperfusion treatment.

In clinical practice there are not biomarkers for the complications of stroke such as malignant brain edema, a complication with high mortality and few treatment (16) or in the post endovascular treatment such as early re-occlusion in the next 48 hours (17). Futile revascularization, defined as a poor clinical outcome despite successful revascularization, has been seen in latest articles that can be predicted by Alberta Stroke Program Early CT Score (ASPECTS) applied to Angio-CT source images with a value ≤5 as the cut-off to predict futile recanalization (18). Even this the outcome is unclear in half of the patients treated.

The need of suitable and reliable biomarkers to predict the outcome of a stroke is need in clinic not only for a proper use of the resources, it is also needed to report patient's family the outcome and not making false expectations.

BIOMARKERS

The term "biomarker", a portmanteau of "biological marker", refers to a broad subcategory of medical signs (that is, objective indications of medical state observed from outside the patient) which can be measured accurately and reproducibly (19).

Biomarkers have multiple applications such as: **prediction** at patient at risk of disease, **detection** of disease (diagnosis), **staging** the extension of the disease, **grading** disease aggressiveness and prognosis and **assessment of response to treatment**. The radiological biomarkers are useful for all these applications (20).

Two main prerequisites are needed for the effective use of imaging biomarkers:

- Standardization: Concerns the acquisition and post-processing parameters of the image. It is an essential step to ensure reproducibility across different centers and machines (20)
- Validation: The sensitivity, specificity, precision, and reproducibility of new biomarkers have to be rigorously tested (21). The changes in biomarker values should be correlated to the biological effect and clinical endpoints (qualification)(20).

OBJECTIVE AND HYPOTHESIS

Hypothesis: Diverse biomarkers can be obtained by CT such as grade of arterial occlusion, composition of thrombus, collateral circulation, infarct volume and penumbra extension. They can be used to select patient tributary to mechanical thrombectomy and predict their treatment response and their functional evolution in ischemic stroke.

Objective: Analyze radiological biomarkers utility in selection of patients to be treated with mechanical thrombectomy in acute cerebral infarct.

Secondary objectives:

- Analyze the predictable capacity of the response to the treatment
- Validation of prognostic estimation of the clinic evolution.

SUBJECTS AND METHODS

PROJECT DESIGN

Multicentric (Hospital Universitari de Bellvitge, Hospital Universitari Vall d´Hebron, Hospital Clinic de Barcelona and Hospital Can Ruti) prospective cohort studio during two and a half years analyzing the radiological images taken during the stroke code and the clinical outcome with mRS at 90 days. It is a prospective longitudinal observational project.

PROJECT POPULATION

People over 18 years who has suffered a stroke whose has been treated with mechanical thrombectomy. Those patients will be selected from two third level hospitals (Hospital Universitari de Bellvitge, Hospital Universitari Vall d´Hebron, Hospital Clinic de Barcelona and Hospital Can Ruti) participating in this project.

INCLUSION AND EXCLUSION CRITERIA

These criteria are the same as the nowadays used in the clinical practice (22) adding the center inclusion criteria:

1) <u>Clinical inclusion criteria</u>

- Sudden focal neurological deficit attributable to a cerebral stroke.
- Clearly defined time of onset, allowing initiation of IV treatment within 3 hours and IA treatment within 6 hours of symptoms onset.
- Clearly defined time of onset, allowing initiation of IV treatment within 3 hours and IA treatment within 6 hours of symptoms onset.
- Age between 18 and 80 years.
- Availability of an interventional neuroradiologist.

2) <u>Clinical and laboratory exclusion criteria</u>

- Severe stroke as assessed clinically and/or adequate imaging techniques.
- Rapidly improving minor neurological deficit.

- Clinical presentation suggestive of a subarachnoid hemorrhage (even if CT scan is negative).
- Seizure at onset of stroke.
- Coma at onset.
- Prior stroke within the last 3 months.
- Any history of prior stroke and concomitant diabetes mellitus.
- Major surgery or significant trauma in past 3 month.
- Recent or present acute or dangerous bleeding.
- Known hemorrhagic diathesis.
- Patients in treatment with oral anticoagulants.
- Administration of heparin within the previous 48 hours and a PTT exceeding the normal higher limit for the laboratory.
- Previous history of or suspected intracranial hemorrhage.
- Previous history of central nervous system damage (neoplasm, aneurysm, intracranial surgery).
- Documented ulcerative gastrointestinal disease in the last 3 months, esophageal varices.
- Arterial aneurism, vascular malformations.
- Neoplasm with increased bleeding risk.
- Bacterial endocarditis, pericarditis.
- Acute pancreatitis.
- Severe liver disease, including hepatic failure, cirrhosis, portal hypertension (esophageal varices) and active hepatitis.
- Severe hypertension: 185 mmHg or PAD >110 mm Hg uncontrolled or requiring continuous IV therapy.
- Baseline blood glucose 400 mg per deciliter (22mmol/L).
- Known contrast sensitivity.
- Women of childbearing potential or known to be breastfeeding.
- Prognosis very poor regardless of therapy (<6 months of life expectancy).
- Disability preceding stroke (e.g., modified Rankin scale >1).
- Unlikely to be available for follow-up.
- Refuses consent.

3) Center inclusion criteria:

- The center must have a CT stroke protocol.
- The center must have endovascular treatment with professional experience enough out of the learning curve.
- The center must perform the mechanical thrombectomy with the same endovascular retriever system.

SAMPLE SIZE

In a bilateral contrast with an α risk of 5%, a statistical power of 80% supposing a moderate relative risk and with a 10% of patient tracing lost. 215 patients in each arm will be needed (exposed and no exposed) making a total of 430 patients.

The computations were carried out with Prof. Marc Saez' software based on the library 'pwr of the free statistical environment R (version 3.5.1).

SAMPLE COLLECTION

Every patient who has been treated by mechanical thrombectomy in a context of a stroke and with the accomplish of the inclusion criteria will be invite to participate in this project. One of the investigators will explain the project objective and the consent form will be sign.

PROJECT VARIABLES

The variables analyzed in the main objective are the following:

1) DEPENDENT VARIABLE:
    - *Modified Ranking Scale (mRS)*: defined between good outcome (mRS between 0-2) and poor outcome (mRS between 3-6) (annexed 1).

2) INDEPENDENT VARIABLE:
    - *Length of arterial occlusion*: A clot >8mm is associated with a poor result in rtPA treatment measured in NECT (23). In the mechanical thrombectomy maybe the length have not relevant impact on recanalization, neurological outcome, or intraprocedural complications (24). It may be an indication of direct mechanical thrombectomy treatment rather than rtPA treatment.
    - *Composition of thrombus*: Measuring clot density in Hounsfield units (HU) on admission NECT, PECT and Angio-CT. With these images can differentiate in dense or soft thrombus (25) in a range from over 18.4 to 40.35 Hounsfield Units is an independent predictor of poor clinical outcome (26).
    - *Collateral circulation*: With a Angio-CT we can evaluate the collaterally by maximum cerebral flow of collateral vessels ($cCBF_{max}$). It will be measured in mL/100 g/min. A $cCBF_{max}$ > 64 in mL/100 g/min is a predictor of good outcome.
    - *Follow-up infarct volume*: in NCCT we can measure the infarct volume in mL. Large FIV are associated with worst outcome, and especially FIV >131mL (27).
    - *Ischemic penumbra extension*: Defined as the mismatch between MTT, BCV and BCF in the perfusion CT measured in mL(4).

As it is an observational design is possible that exist confusion in the relation of interest. I have considered the following ones:

- Sociodemographic variables:
    - Age: measured in years.
    - Biological sex: collected as male or female.
    - Provenance: as there are differences on incidence.
    - Socioeconomic factors proxied by education and occupation.
- Process variables:
    - Intervention time: measured in minutes.
    - Numbers of tries to remove the thrombus: measured in ordinal numbers.
    - Prior antiplatelet use: defined as yes/no.

- mTICI scale: is a 0-3 grade defined as: grade 0→ no perfusion; grade 1→ antegrade reperfusion past the initial occlusion, but limited distal branch filling with little or slow distal reperfusion. grade 2is divided in grade 2a→ antegrade reperfusion of less than half of the occluded target artery previously ischemic territory grade 2b→ antegrade reperfusion of more than half of the previously occluded target artery ischemic territory grade 3→ complete antegrade reperfusion of the previously occluded target artery ischemic territory, with absence of visualized occlusion in all distal branches (28).

- Clinical variables:
  - Blood pressure in the emergency room: Measuring the systolic and diastolic pressure in mmHg.
  - Baseline National Institutes of Health Stroke Scale (NIHSS) score (annex 2): Is a scale with a value between 0-42 points.

- Medical history variables:
  - Smoking: Defined as current smoker/ex-smoker/ no smoker.
  - Hypertension: Defined as yes/no.
  - Diabetes mellitus: Defined as yes/no.
  - Hyperlipidemia: Defined as yes/no.
  - History of stroke or transient ischemic attack: Defined as yes/no.
  - Atrial fibrillation: Defined as yes/no.

DATA COLLECTION

Data will be obtained from the diverse centers after the mechanical thrombectomy. The images will be obtained with a CT protocol in acute ischemic stroke (admission): Nonenhanced-CT, Angio-CT, Perfusion-CT (CBF, CBV and MTT).

Schedule 3: Imaging protocol (6)*:*

Parameter	Imaging Technique		
	Nonenhanced CT	Perfusion CT	CT Angiography
Mode	Axial scanning	Cine imaging	Helical scanning
Gantry angle (°)	Orbital view	Orbital view	0
Section thickness (mm)	5	5*	0.625
Pitch	. . .	. . .	0.531:1
Kilovolt peak (kVp)	120	80	120
Milliamperage (mA)	250	150	Automatic (200–400) with noise index of 12
Rotation time (sec)	0.8	1	0.4
Contrast material			
Volume (mL)	. . .	50	60
Injection rate (mL/sec)	. . .	4	3.5
Saline flush (mL)	. . .	. . .	30†
Delay (sec)	. . .	5	Bolus tracking on ascending aorta
Attenuation values (bolus tracking) (HU)	. . .	. . .	70
Acquisition time (sec)	. . .	45	~10

Image 7: parameters of the CT in each different technique (6)

The intervention must be performed by an interventional neuroradiologist and in all the centers must perform the intervention with the same procedures and the same material.

The FIV will be obtain in the follow-up of the patient during the hospital stance at seven days with a CT.

A data form has been made to gather all the clinical, procedure and image information needed to this project (annex 4).

The images will be analyzed by the neuroradiologists from the investigator team. After 90 days from the mechanical thrombectomy the mRS will be obtain during the follow up.

STATISTICAL ANALYSIS

The methods summarized are the following:

1. All the patients with the inclusion and exclusion criteria meet will enter and those excluded will be specified the reason.

2. In the statistical analysis the following hypothesis will be contrast:

1) The mRS will not be related with length of arterial occlusion
2) The mRS will not be related with composition of thrombus
3) The mRS will not be related with collateral circulation
4) The mRS will not be related with infarct volume
5) The mRS will not be related with ischemic penumbra extension

3. Descriptive analyses: On the one hand, categorical variables would be expressed in percentages; on the other hand, continuous variables would be represented in mean +/- standard deviation, when we assume a normal distribution. However, if there is not a normal distribution, median and interquartile range (IQR) will be the summary measures. Always stratifying by the groups of mRS (0-2/3-6)

4. Bivariate inference: to test hypothesis number 2 chi square test will be applied or the Fisher' exact test (if the expected frequencies were lower than 5). The rest of the hypothesis will be tested by means of the t-Student (continuous variables) and the U-Mann-Whitney (discrete variables).

5. Multivariate analyses: In order to assess the relationship between mRS and the independent variables, logistic regressions will be estimated, adjusting for the covariables in order to control for confounding.

The statistical analysis is performed with the SPSS v21, considering statistically significant with a p value ≤0,05.:

IMAGING ANALYSIS

All images will be analyzed by two neuroradiologists, blinded to the patients' clinical story, admission and follow-up images.

ETHICAL ASPECTS

This project is conducted according to the Declaration of Helsinki of Ethical Principles for Medical Research Involving Human Subjects signed by the World Health Association the October 2013 and by the Law 14/2007 of July 3rd of investigational project with invasive procedure.

The management of personal data required in this project, its communication, the personal data cession of all the patients and the confidentiality is in compliance with Law 15/1999 of December 13th on the Protection of Personal Data, the European Parliament and Council regulation 2016/679 of April 27th and with the organic law 3/2018 of December the 5 th of the Data Protection.

Patient names and surnames, data and clinical history information will be codified in order to maintain the anonymity when introducing and processing this information into a database, according to the mentioned Law and guaranty of digital right.

The data access will be only available for the research team, the Ethical and Clinical Investigation Committee, the pertinent health authorities and those responsible for data analysis. The access to this information for a third person is not allow and the investigator, the hospital and the collaborator must sign a statement attesting to having read and approved the final protocol, and agree with the national and international ethical aspects with the investigation.

This project will be presented in the Ethical and Clinical Investigation Committee by single verdict and final project will be presented to each of the participating centers for they approval.

The aim of this project is to analyze the previous clinic and image biomarkers post treatment. The patient will be explained the aim of this project, ask permission for the use of its image and informing about the data privacy. For all of this a subject information sheet and consentient form (annex 3).

PROJECT LIMITATIONS

- In the emergencies room of the hospital not always a neuroradiology will be present. Some avoidable mistakes could be made (5).
- The Inter-observer variation cannot be avoided.
- The time of observation cannot be long enough to see the change.
- As it is a multicentric project coordination difficulties and data dump in the data base can be a problem.

EXECUTION PLAN AND SCHEDULE

STAGE 0: PROJECT PREPARATION

Activity 1. Operative protocol elaboration:

During the first 2 months the first meeting of the research team will be done in order to elaborate the main objective and the work plan.

A bibliographic research must be performed to define and write a protocol for Ethical and Clinical Investigation Committee.

The inclusion and exclusion criteria should be those used in the moment of the project perform. They can change according to the DAWN and DEFUSE3 projects. Even other centers can be invited if the inclusion criteria are accomplished.

Activity 2. Committee explanation and ethical approval:

During the following 2 months the principal investigator will request all the permissions to the Ethical committee to the project approbation and the final protocol with the corrections of the Ethical committee will be done if it is needed.

STAGE 1: DATA COLLECTION

Activity 4. Data collection:

During the following sixteen months the data will be collected from the diverse hospitals and this data will be turn over into a data base by the investigator team. In the follow up of the patients the mRS at 90 days will be performed.

After the sixteen months, three months more are needed to fulfill the data base and to do the phone calls of the latest patients to obtain the mRS at 90 days.

Activity 5. Periodic meetings:

Periodic meetings each six months will be performed to analyses the data collection and to solve problems.

STAGE 2: ANALYSIS AND FINAL EVALUATION

Activity 6. Statistical analysis:

A qualified statistical from the research team analyses the data during the following two months obtained preliminary results.

Activity 7. Data analysis:

The research team interpret the data obtained and perform an article with the final conclusion during the following two months.

In this stage also, a review of the images for the purpose of identifying another biomarker can be performed.

Activity 8. Results publication:

The results will be sent to the different specialized medical journals and try to exposed it in neurological congress.

STAGE		FIRST YEAR				SECOND YEAR				THIRD YEAR	
		1-3 MONTH	4-6 MONTH	7-9 MONTH	10-12 MONTH	1-3 MONTH	4-6 MONTH	7-9 MONTH	10-12 MONTH	1-3 MONTH	4-6 MONTH
STAGE 0	PROTOCOL DESIGNE										
	COMMITTEE EXPLANATION										
	ETHICAL APPROVAL										
	FINAL PROJECT										
STAGE 1	DATA COLLECTION										
	PERIODIC MEETINGS										
STAGE 2	STATISTICAL ANALISE										
	DATA ANALISE										
	RESOULTS PUBLICATION										

BUDGET

SERVICE	COST	QUANTITY	SUBTOTAL
PERSONAL EXPENSES			
PROYECT MANAGER	4750.00 €/Month	27 Months	128,250.00€
EXECUTION EXPENSES			
COALIFY STATISTICAL	40.00 €/Hour	30 Hours	1,200.00 €
TRAVELS AND DIETS	200.00 €/Person	10 investigators	2,000.00 €
CONFERENCE AND PRESENTATIONS	1000.00 €/congress	2 congress x 2 investigators	4,000.00 €
TOTAL			135,450.00 €

EXPECTED RESOULTS

Taking as base the literature about this theme the results expected are the following for each variable:

- The clot composition will be a good biomarker to determine the action not only of the mechanical thrombectomy even for the rtPA effectivity. The clot with less HU in Nonenhanced-CT /Angio-CT will be more difficult to be removed.
- The collateral circulation will be a great biomarker of good outcome. As greater $cCBF_{max}$, better irrigation of collaterals and better outcome will have the patient.
- Follow-up infarct volume will be a great biomarker for the outcome. Base on the actual bibliography the big infarct volume is a good predictor of bad outcome.
- The ischemic penumbra extension is a good biomarker for selecting patients to treatment and changing the limits of nowadays treatment. Those people having a big ischemic penumbra and other bad prognostic can even be a good candidate for mechanical thrombectomy to prevent more tissue death.
- The length of arterial occlusion may not be an outcome biomarker, it will be probably an indication of direct mechanical thrombectomy rather than using the rtPA before.

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ANNEXED

Annexed 1- modified Rankin Scale (29).

Table 1 – The Modified Rankin Scale	
Description	**Score**
No symptoms.	0
No significant disability. Able to carry out all usual activities despite some symptoms.	1
Slight disability. Able to look after own affairs without assistance, but unable to carry out all previous activities.	2
Moderate disability. Requires some help, but able to walk unassisted.	3
Moderately severe disability. Unable to attend to own body needs without assistance and unable to walk unassisted.	4
Severe disability. Requires constant nursing care and attention, bedridden, incontinent.	5
Dead.	6

Annexed 2- National Institute of Health Stroke Scale (30)

1a. Level of consciousness	**0 = Alert; keenly responsive** **1 = not alert, but arousable by minor stimulation** **2 = Not alert, requires repeated stimulation** **3 = Unresponsive or responds only with reflex**
1b. Level of consciousness questions **What is the month?** **What is your age?**	0 = Answers two questions correctly 1 = Answers one question correctly 2 = Answers neither question correctly
1c. Level of consciousness commands **Open and close your eyes** **Grip and release your hand**	0 = Performs both tasks correctly 1 = Performs one task correctly 2 = Performs neither task correctly
2. Best gaze	0 = Normal 1 = Partial gaze palsy 2 = Forced deviation
3. Visual	0 = No visual lost 1 = Minor paralysis 2 = Complete hemianopia

	3 = bilateral hemianopia
4. Facial palsy	0 = Normal symmetric movements 1 = Minor paralysis 2 = Partial paralysis 3 = Complete paralysis of one or both sides
5. Motor arm **5a. Left arm** **5b. Right arm**	0 = No drift 1 = Drift 2 = Some effort against gravity 3 = No effort against gravity; limb fails 4 = No movement
6. Motor leg **6a. Left leg** **6b. Right leg**	0 = No drift 1 = Drift 2 = Some effort against gravity 3 = No effort against gravity 4 = No movement
7. Limb ataxia	0 = Absent 1 = Present in one limb 2 = Present in two limbs
8. Sensory	0 = Normal; no sensory loss 1 = Mild moderate sensory loss

	2 = Severe to total sensory loss
9. Best language	0 = No aphasia; normal 1 = Mild to moderate aphasia 2 = Severe aphasia 3 = Mute, global aphasia
10. Dysarthria	0 = Normal 1 = Mild moderate dysarthria 2 = Severe dysarthria
11. Extinction and inattention	0 = No abnormality 1 = visual, tactile, auditory, spatial, or personal inattention 2 = Profound hemi-inattention or extinction
Total score = 0-42	

Annexed 3- Subject information sheet and consentient form

SUBJECT INFORMATION SHEET

1. INTRODUCTION

We are writing to inform you about a clinical trial in which you are invited to participate. The project has been approved by the Research Ethics Committee.

Our intention is only that you receive the correct and sufficient information so that you can evaluate and judge whether or not you want to participate in this trial. For this, read this information sheet with attention and we will clarify any doubts that may arise after the explanation. In addition, you can consult with the people you consider appropriate.

2. VOLUNTARY PARTICIPATION

You should know that your participation in this project is voluntary and that you can decide not to participate and withdraw your consent at any time, without altering the relationship with your doctor or causing any harm to your treatment.

3. GENERAL DESCRIPTION OF THE RESEARCH

Nowadays stroke treatment can be performed by mechanical thrombectomy. This is a reliable treatment to remove the thrombus but we do not know which ones will have a good result and which ones will have a bad result, even the thrombus is removed.

The aim of this research is to find out which TC biomarkers (characteristics in the CT images that can be measured) are useful to determine the end result of a patient with a stroke before the treatment is performed.

This research is based in the analysis of your CT images which were performed in order to diagnosis the stroke before you receive the treatment. 574 patients are needed for this research and will be selected

The treatment you will received will not be different from another person out of this research and it will not be delayed by the imaging acquisition, all TC process is the same as a person out of this research.

4. BENEFITS AND RISK OF PARTICIPATING IN THIS RESEARCH

This research will not have any benefit for you, all process done will be the same as a non-participating patient as it has been explained to you. The future stroke patient will be benefited of this research.

With this research the unnecessary procedures will not be performed. This mean that patient will not undergo to a surgical process, patients' families will not have false expectations and money can be invested properly.

5. INSURANCE

In this project, as no extra intervention is performed, only with the civil liability insurance of the hospital is enough.

6. CONFIDENTIALITY

The treatment, communication and transfer of personal data of all participating subjects, will comply with the provisions of Organic Law 15/1999 of December 13.

The data collected will be identified by a code and only the members of the research team will be able to relate this data to you and your medical history. Your identity will not be disclosed to any person except for exceptions, in case of medical emergency or legal requirement.

Access to your personal information will be restricted to the members of the research team, the Clinical Research Ethics Committee but always maintaining the confidentiality of them according to current legislation. The access to your clinical history must be only in relation to the project.

With my signature I declare my consent to participate in this project.

I am consent that this document is not a contract I have received a copy of "Subject information sheet" and "Informed consent form". A copy will be saved in the project center.

_____

(Patient´s name and surname in capital letters)

_____ _____

(Date) (Patient´s signature)

Doctor´s certification (Professional who made the explanation)

With my signature, I give testimony that the patient participating in the project has personally signed the informed consent form.

_____ _____

(Investigator´s surname) (Hour)

_____ _____

(Date) (Signature)

Annexed 4- Data collection form

Data collection form

Age:

Biological sex:

Provenance:

Medical record number:

Date

- **Radiologic findings**

- Length of arterial occlusion:
- Composition of thrombus:
- Collateral circulation:
- Follow-up infarct volume:
- Ischemic penumbra extension:

- **Intervention process**

- Intervention time:
- Numbers of tries to remove the thrombus:
- Prior antiplatelet use:
- TICI:

- **Admission in the emergency room**

- Blood pressure in the moment:
- Baseline National Institutes of Health Stroke Scale (NIHSS):

- **Medical record variables:**

- Smoking:
- Hypertension:
- Diabetes mellitus:
- Hyperlipidemia:
- History of stroke or transient ischemic attack:
- Atrial fibrillation:
- 90 days follow-up modified Ranking Scale (mRS):

- Observations: