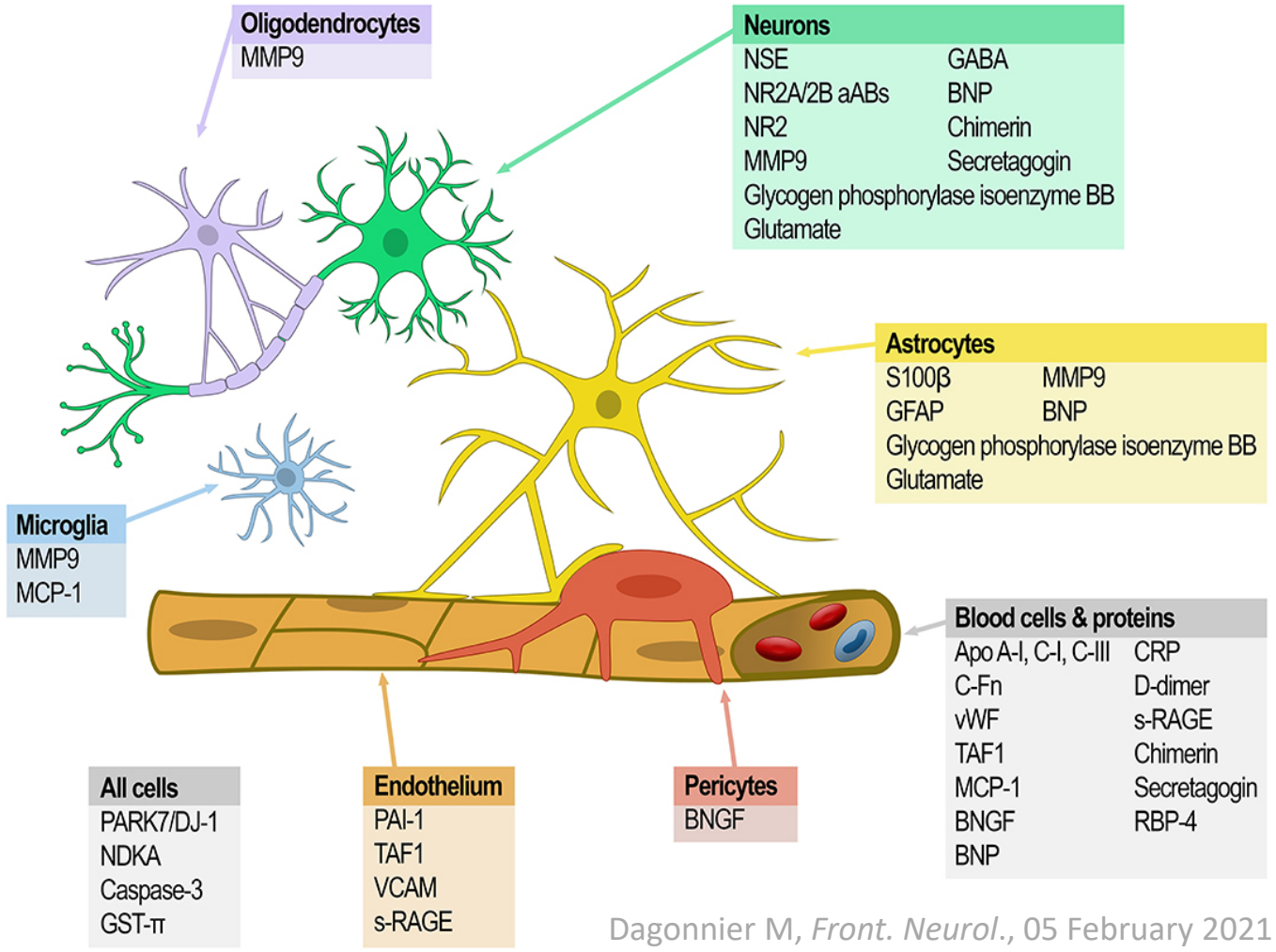
BIOMARKERS FOR IDENTIFICATION OF STROKE AND ITS RECOVERY

Joan Montaner, Anna Rosell

Madrid, 7 Junio 2022

RICORS-ICTUS | Instituto de Salud Carlos III



Dagonnier M, *Front. Neurol.*, 05 February 2021

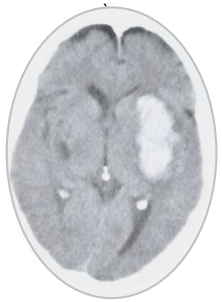
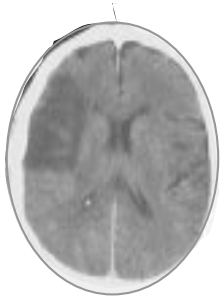
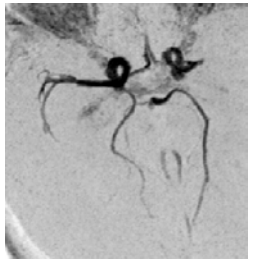
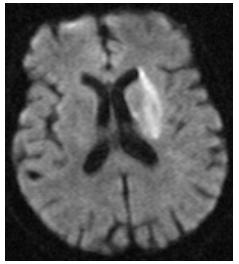
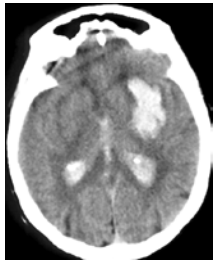
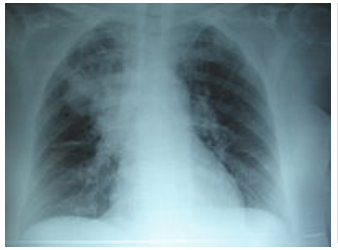
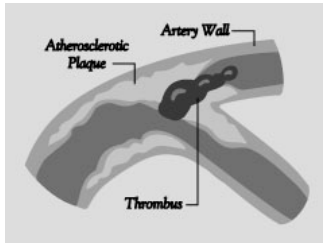
Financiado por la Unión Europea
NextGenerationEU

Plan de Recuperación, Transformación y Resiliencia

The PROMISE…

From the ambulance to the out-patients clinic, blood biomarkers based decisions will be taken soon…



TRANSLATING BRAIN BIOMARKER RESEARCH TO CLINICAL PRACTICE

Know-how RICORS groups

Huge network

Discovery centers

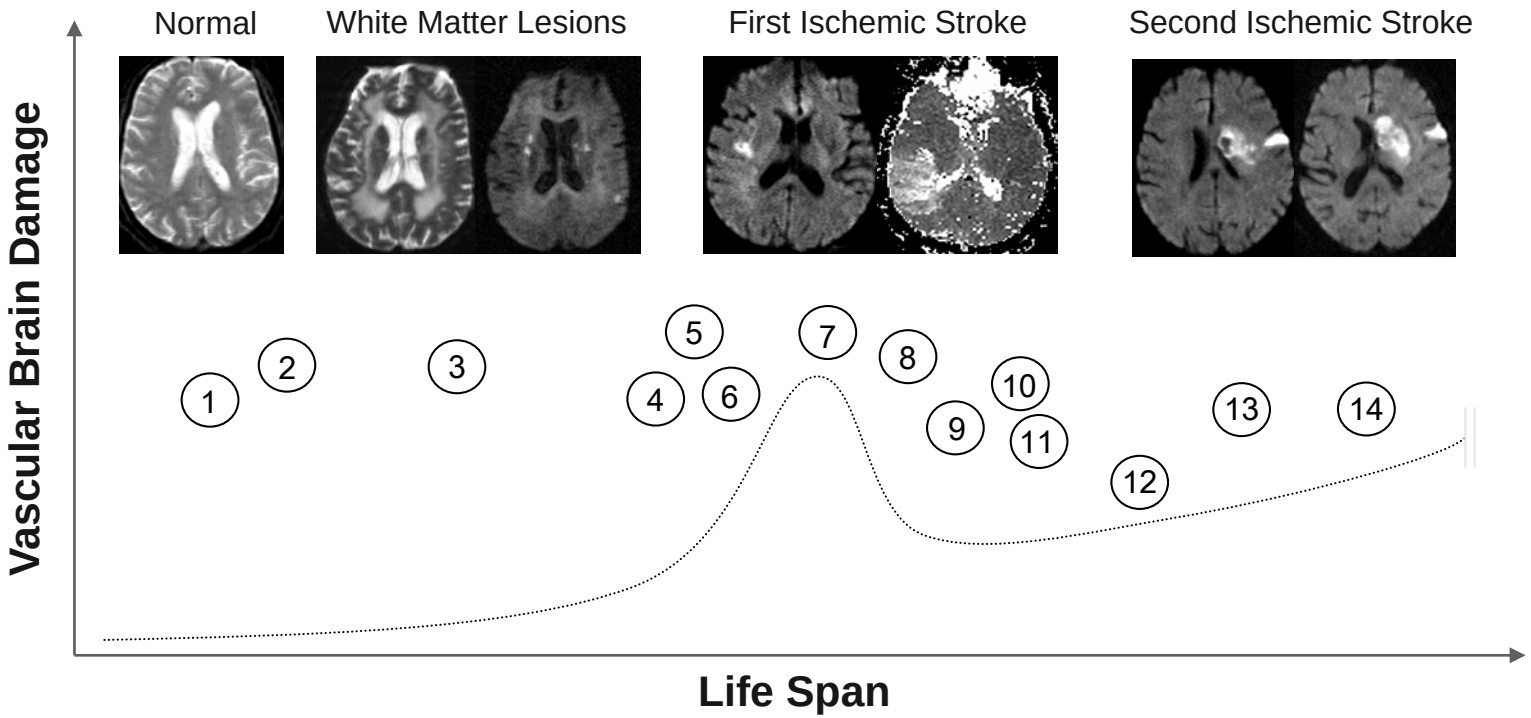
Testing centers

Biobanks

Platforms

(-) companies

Blood Biomarkers & Stroke Natural History



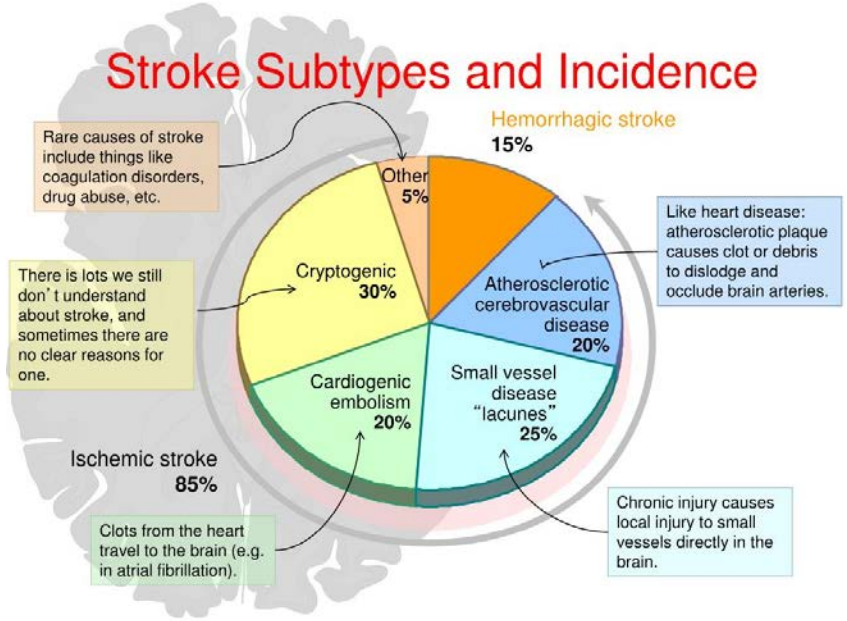
1.- Predict stroke among healthy people
2.- Predict stroke among those with vascular risk factors
3.- Identify Silent strokes
4.- Stroke vs mimics
5.- Ischemic-Hemorrhagic
6.- LVO identification
7.- Bleeding complications
8.- Futile recanalization
9.- Stroke etiology
10.- Cardiac complications
11.- Infections prediction
12.- Functional outcome & recovery
13.- Post-stroke seizures
14.- Stroke recurrence

BIOMARKERS FOR IDENTIFICATION OF STROKE AND ITS RECOVERY

- **WP1:** Biomarkers for the pre-hospital diagnosis of stroke
- **WP2:** Use of biomarkers in stroke subtypes
- **WP3:** Biomarkers for outcome (stroke complications and recovery)
- **WP4:** Biomarkers in experimental models of stroke and selected pathways

WP1: pre-hospital

WP2: stroke subtypes

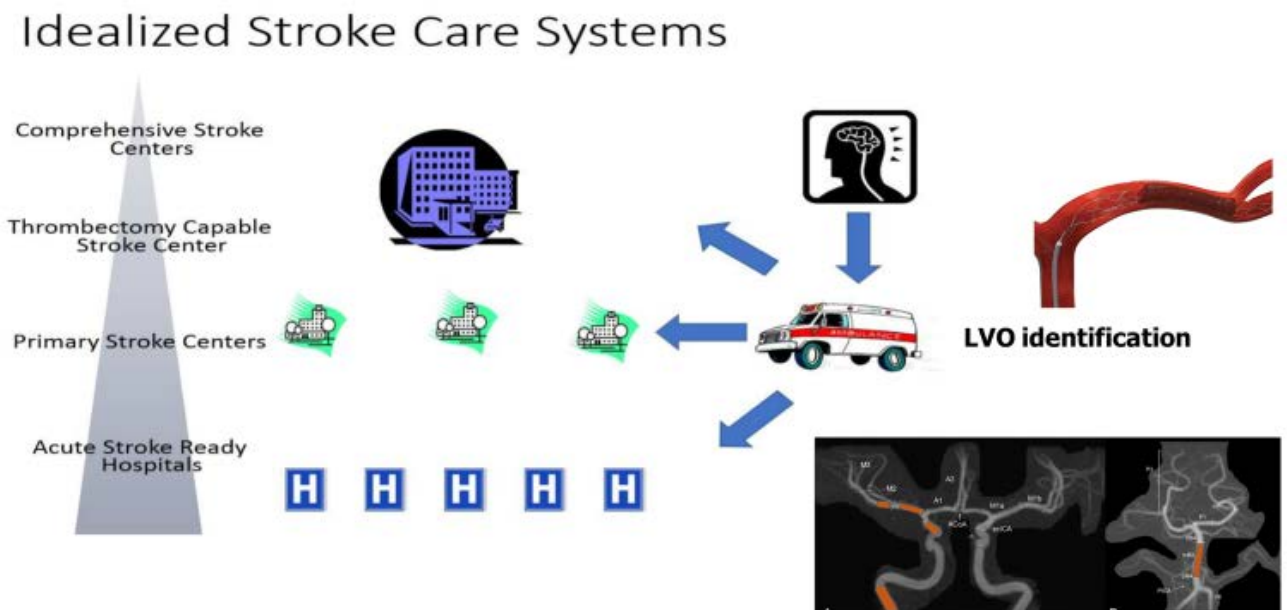


WP3: Outcome

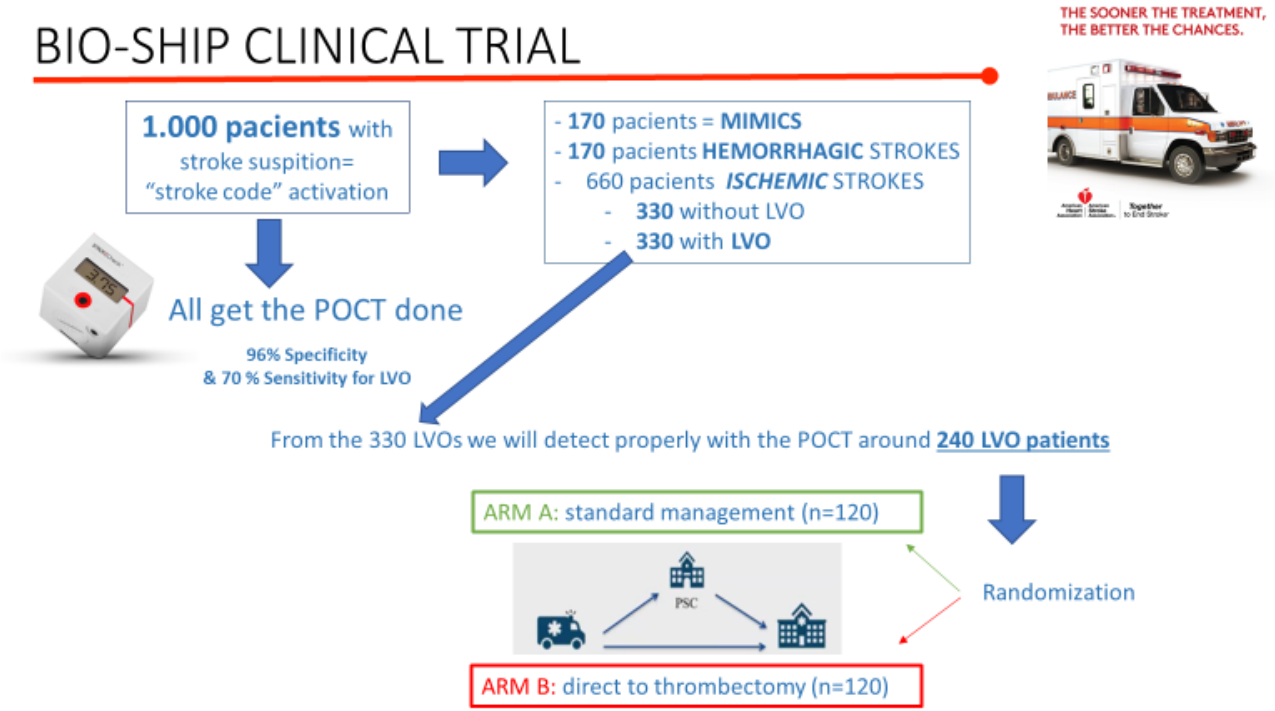
WP4: experimental

WP1.- BIOMARKERS FOR PRE-HOSPITAL DIAGNOSIS OF STROKE. As an alternative to neuroimaging, some blood-based biomarkers identified in the network have shown good predictive ability to discriminate between ischemic and hemorrhagic stroke and to identify large vessel occlusion (LVO) in the ambulances (BIO-FAST, CinicalTrials: NCT04612218). This has generated one of the largest and unique cohorts in the world in which blood samples were obtained at the ambulance that will allow discovery of new biomarkers (n=300) and brings the possibility of starting stroke therapy outside the hospital.

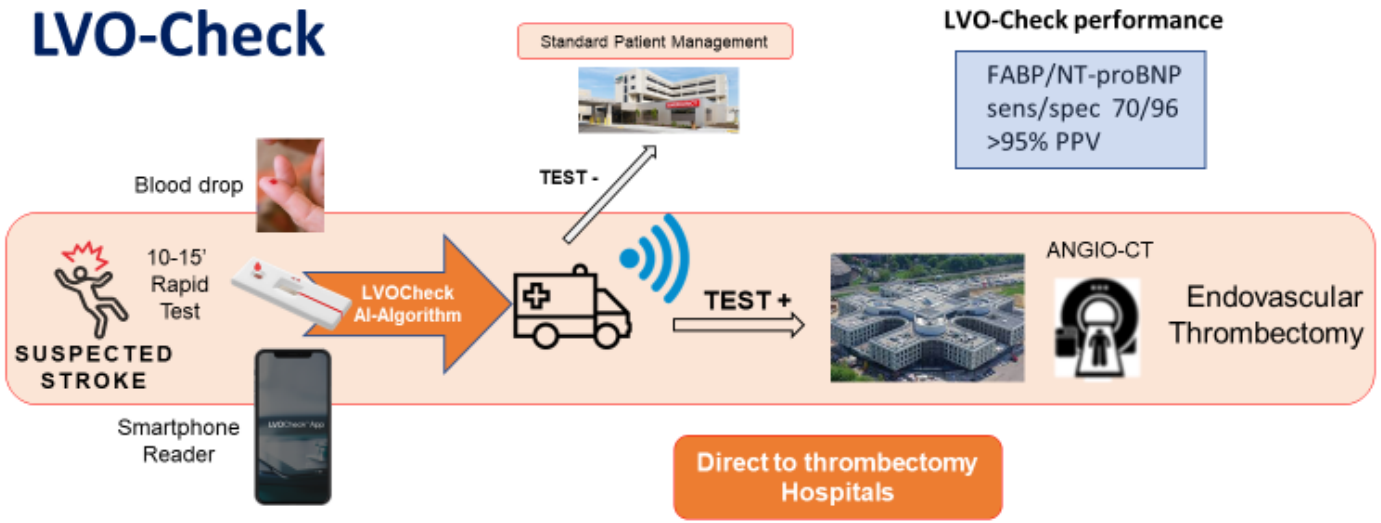
2022



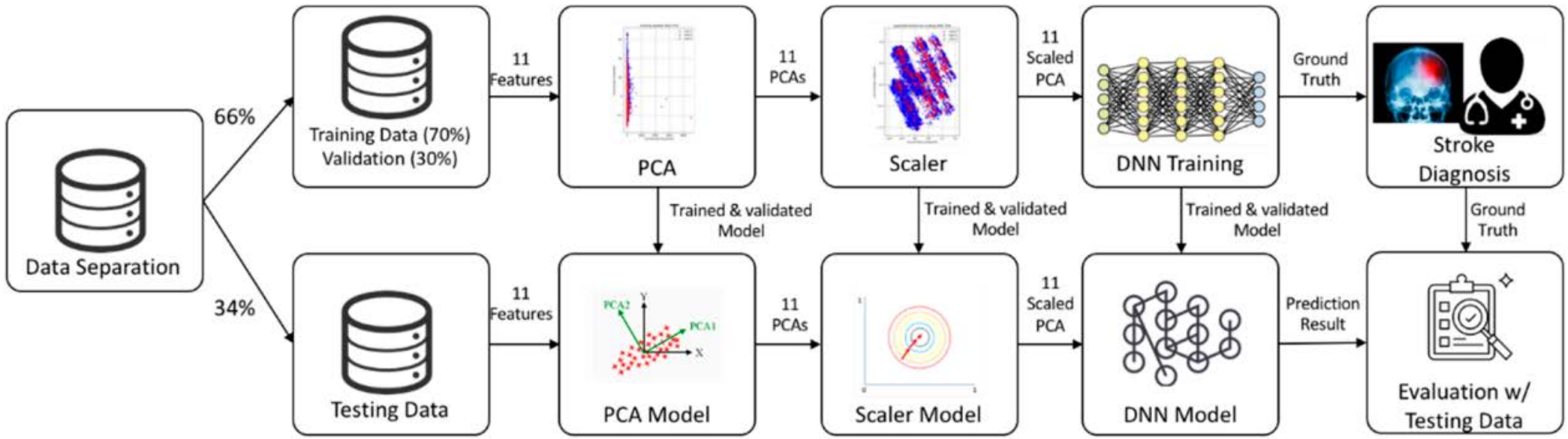
2023



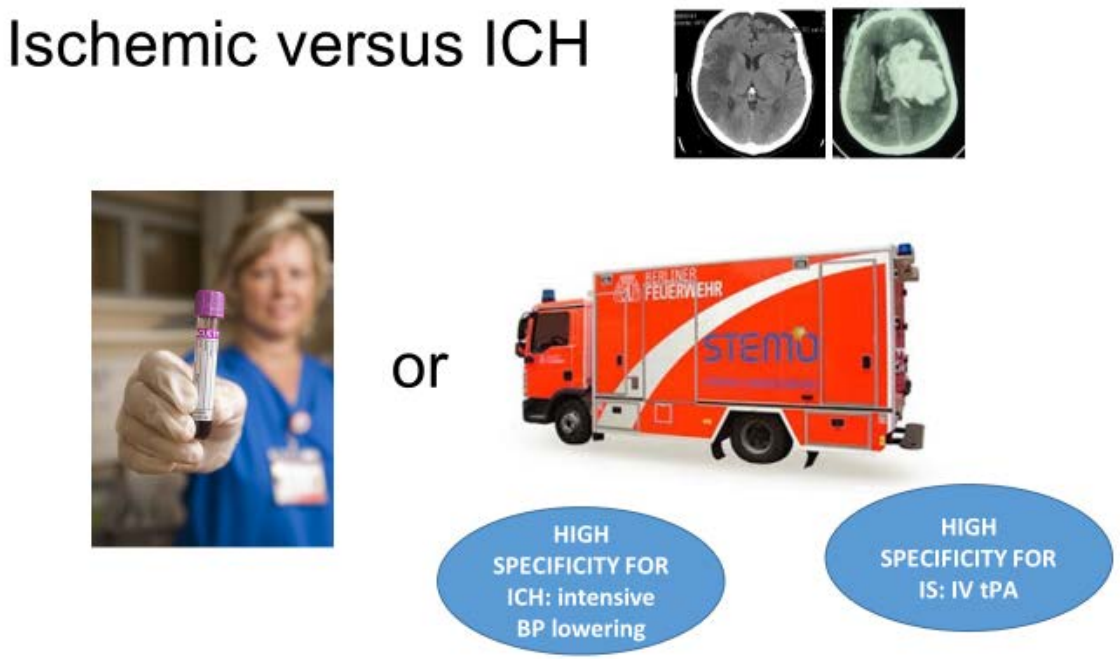
1.- Increase access to reperfusion therapies and thrombectomy centers, by detecting LVO in ambulances using blood biomarkers and clinical scales for direct transfer to the thrombectomy room. This strategy would be novel worldwide since no stroke care network is carried out such type of studies**. A multicenter study in Spain (BIOSHIP-Spain)** will be attempted in the RICORS since we already have the point-of-care technology to make these measurements. In a short time, we could carry out a study to evaluate the impact of the strategy on the prognosis of patients (our hypothesis is that this strategy will improve by 15% the percentage of patients who would achieve functional independence compared to standard managed patients).



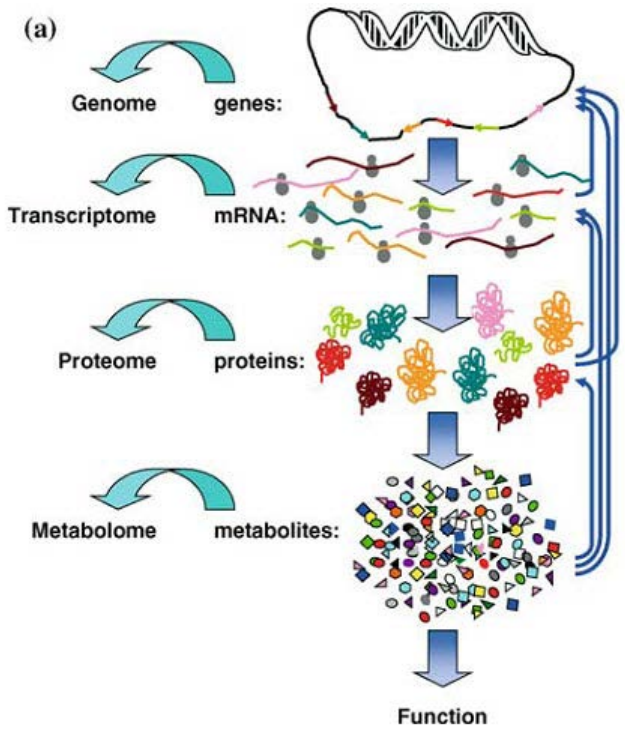
2.- Application of **machine learning techniques** on continuous hemodynamic monitoring data in patients with suspected out-of-hospital stroke and search for plasma biomarkers and clinical scale to generate predictive models of hemorrhagic vs ischemic stroke, and predictive LVO scales

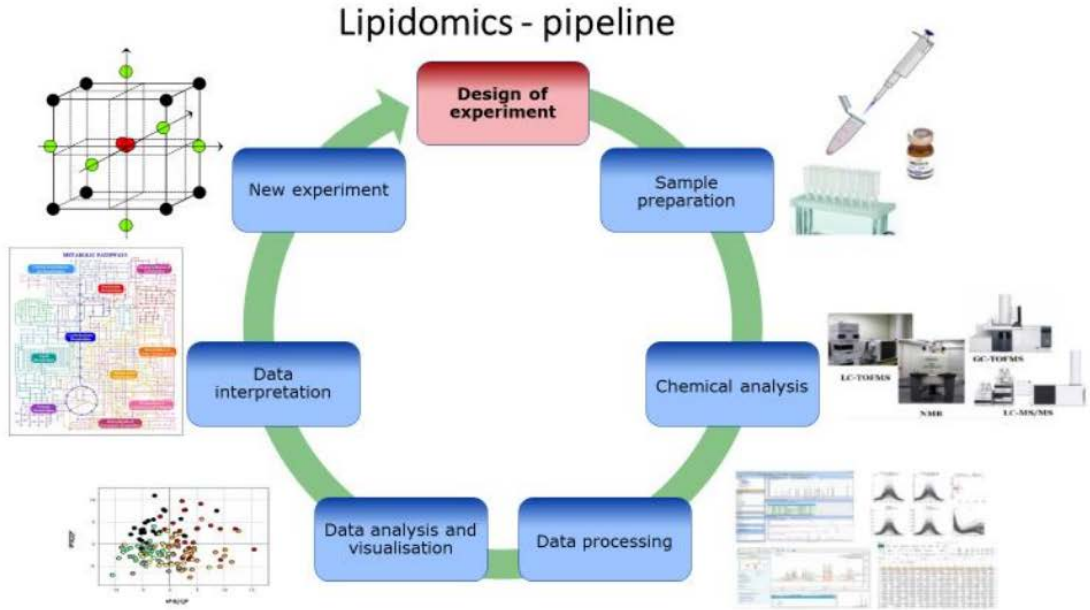


3.- Evaluation of **biomarkers through nasal exudate in stroke.** Preliminary data show metallic biomarkers (iron, copper, zinc, cadmium) in nasal exudate that allowed the differentiation between ischemic and hemorrhagic stroke (García-Cabo et al., Cerebrovasc Dis. 2020). This innovative strategy will be expanded by the network.



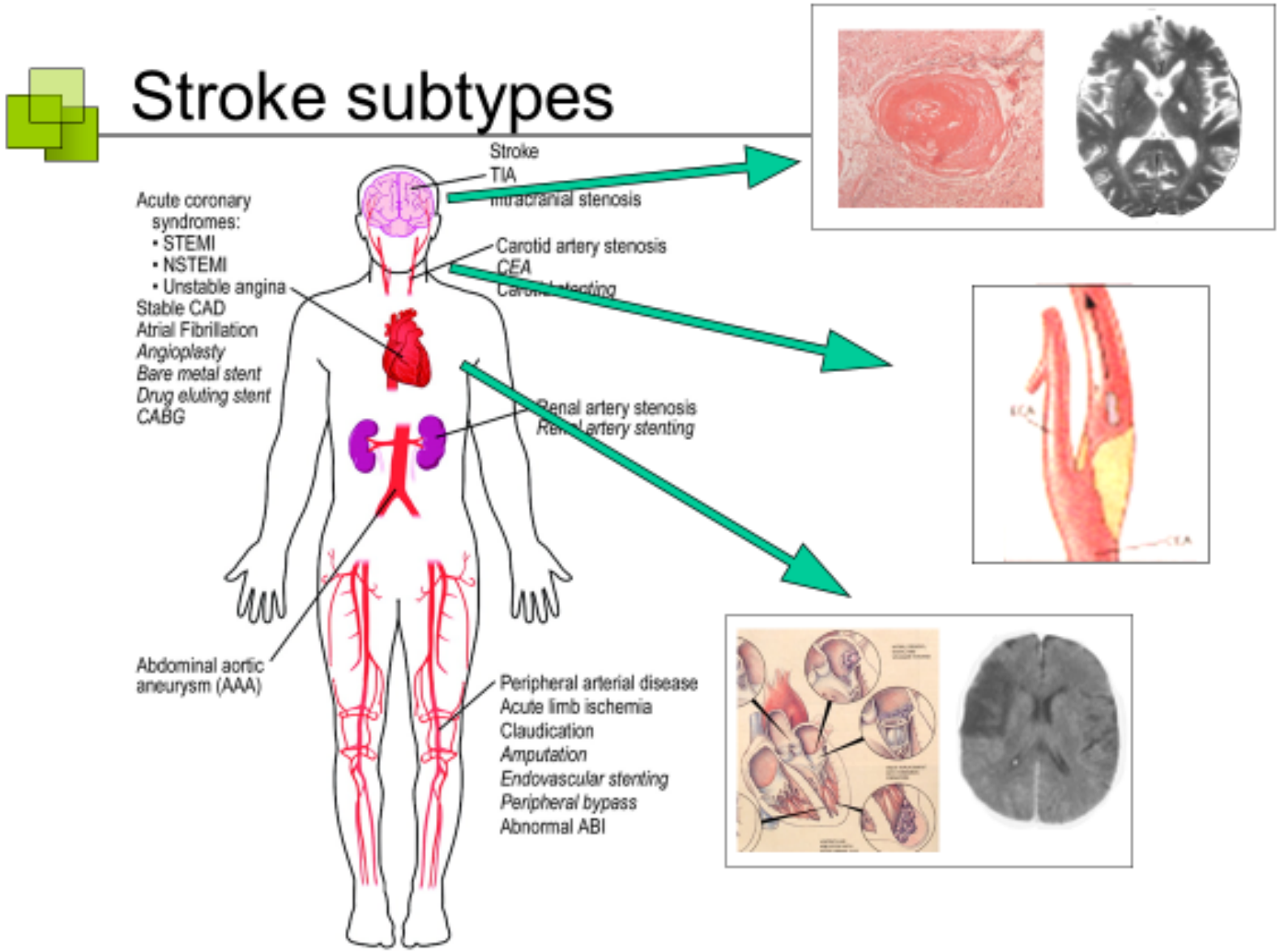
4.- Study of the **metabolomic and lipidomic profile of subjects** with suspected stroke and identification of an omics profile related to a) the certainty of the diagnosis of ischemic stroke, hemorrhagic stroke and mimic b) the presence of intracranial occlusion c) time of evolution of symptoms and the presence of penumbra at risk tissue.



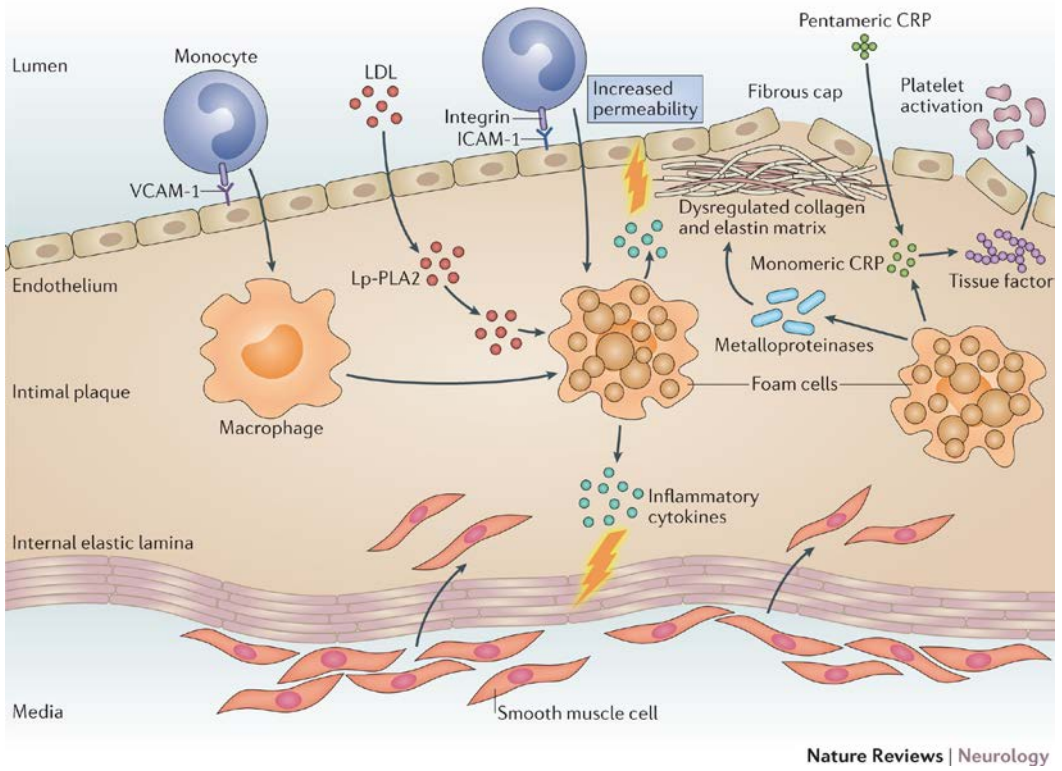


The steps involved in a lipidomic workflow. Adapted from the Swedish Metabolomics Centre (SMC).

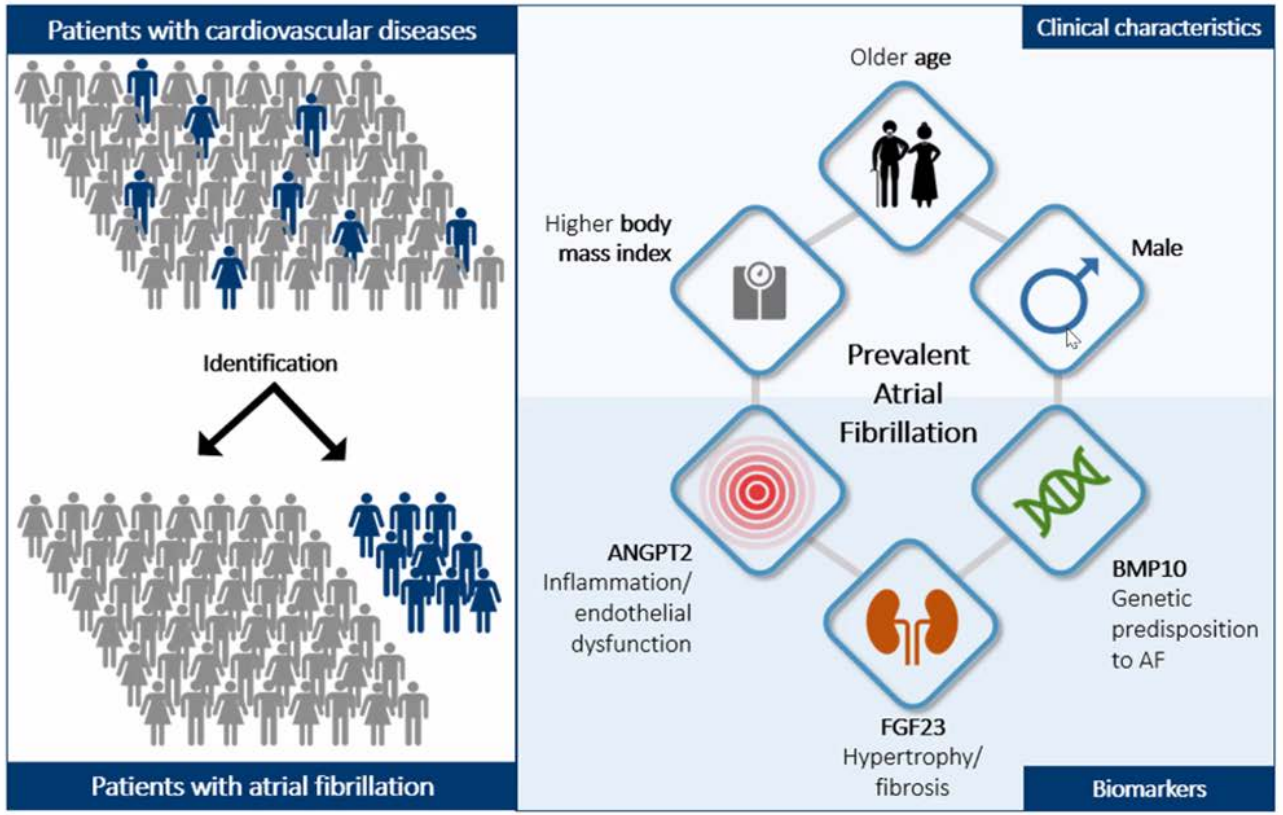
WP2.- USE OF BIOMARKERS IN STROKE SUBTYPES. We will identify new stroke markers to accelerate its diagnosis and therefore improve the treatment and prognosis of patients. Systemic biomarkers of active intracranial atherosclerosis plaque, predictive markers of vulnerable plaque and carotid angioplasty complications. Biomarkers of cardioembolic stroke to develop a predictive model for the presence of atrial fibrillation. Metabolomic, lipidomic, genetic and epigenetic markers of hemorrhagic stroke (ICH and SAH).



1.- To identify systemic biomarkers of **active intracranial atherosclerosis plaque** is an unmet need that will be addressed by the network, as well as predictive biomarkers (proteins and miRNAs) of vulnerable plaque in patients with carotid stenosis through analysis of atheroma plaques and blood (miRNAs) samples from patients with different diagnosis. Among those receiving carotid revascularization, identification of predictors of carotid angioplasty complications with the idea of improving the prognosis of patients who receive angioplasty/carotid stenting using predictive biomarkers of hyper-perfusion syndrome and restenosis, the two most feared complications among those patients, will be also attempted.

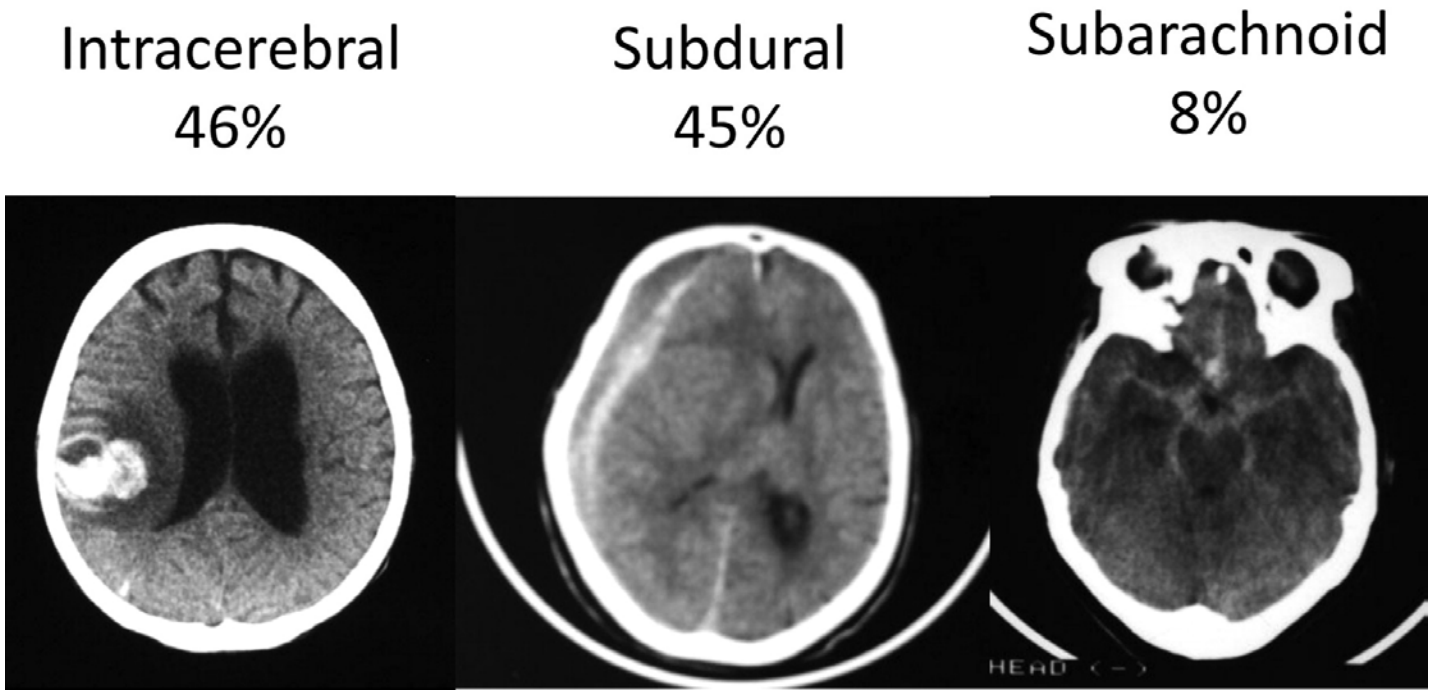


2.- Biomarkers of **cardioembolic stroke**. To develop a predictive model for the presence of atrial fibrillation in patients with recent ischemic stroke, based on clinical, biochemical, echocardiographic and neuroimaging markers, by means of a retrospective cohort study for the development and internal validation of a predictive model for the diagnosis of de novo atrial fibrillation in patients hospitalized for ischemic stroke, as well as the implementation of a screening for atrial fibrillation in patients with stroke of undetermined etiology.

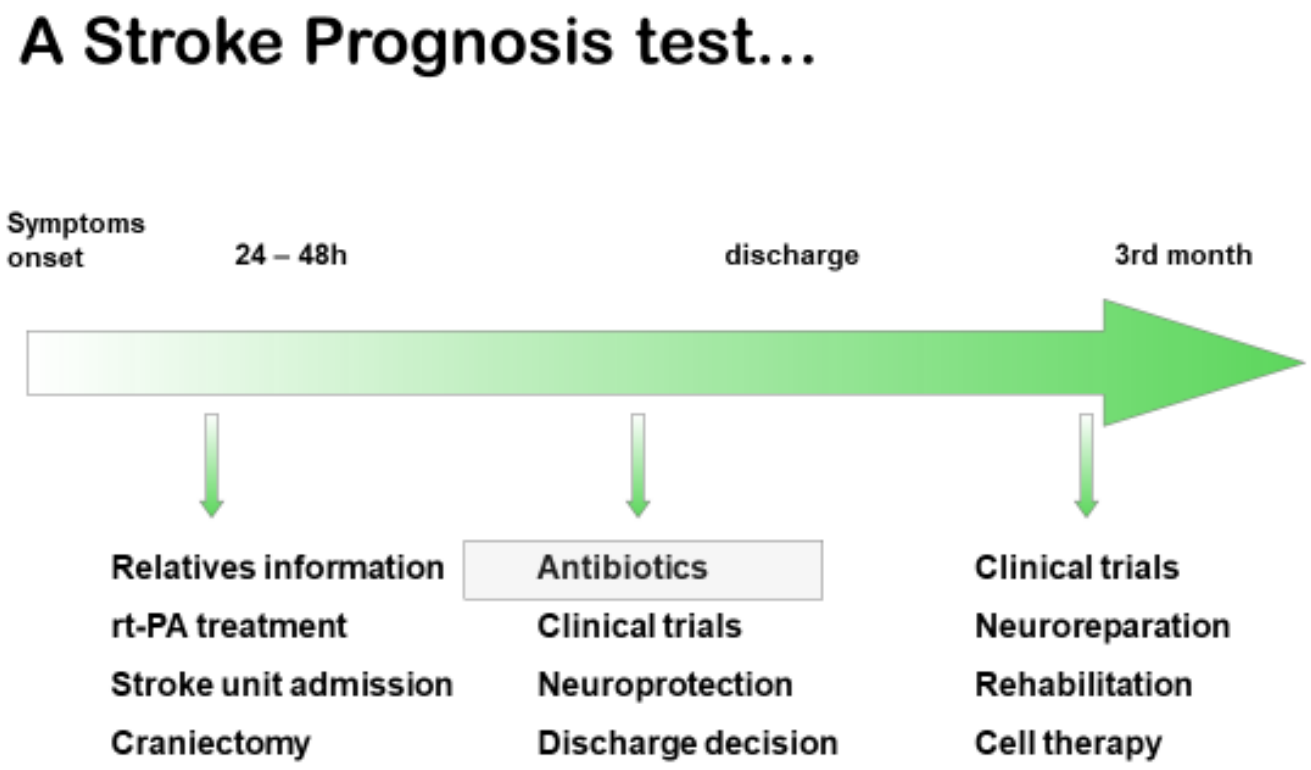


UNIVERSITY OF BIRMINGHAM | INSTITUTE OF CARDIOVASCULAR SCIENCES

3.- **Biomarkers of hemorrhagic stroke.** Study of the metabolomic and lipidomic profile related to the etiology and prognosis of patients with intracranial hemorrhage (ICH). Identification of genetic and epigenetic variants associated with ICH and its functional prognosis (GWAS). Characterization of a CSF biomarker profile in patients with amyloid angiopathy and its validation. We will also address the study of biomarkers in subarachnoidal hemorrhage (SAH), through the study of neuroimaging, blood and CSF biomarkers of early and delayed ischemic damage and functional recovery in spontaneous SAH. That will be complemented by exploring genetic and epigenetic variants associated with SAH and its functional prognosis. (GWAS, EWAS) and analyzing the complications of SAH: by means of a multi-omic analysis.

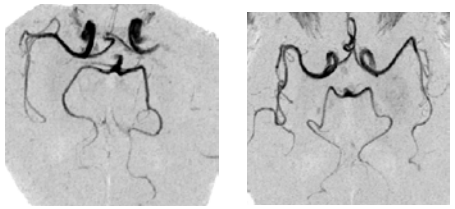


WP3.- BIOMARKERS FOR OUTCOME (STROKE COMPLICATIONS AND RECOVERY). We will study predictive biomarkers of complications associated with reperfusion therapies (HT, futile recanalization) to improve reperfusion rates and benefits/risk balance. We will also study predictive biomarkers of growth of ischemic and hemorrhagic (ICH and SAH) brain injury. The composition of the extracted thrombus and the implication on stroke outcome will be also explored to improve the diagnosis, treatment, and prognosis of those patients. Finally, we will determine different biomarkers related to functional recovery and post-stroke cognitive impairment.

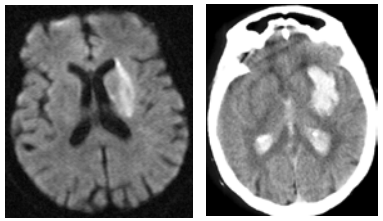


1.- **Lesion growth and tissue viability biomarkers**. Application of machine-learning techniques on continuous hemodynamic monitoring data to generate predictive models of death, re-bleeding and hemorrhagic transformation. Those will be combined with predictive blood biomarkers of complications associated with reperfusion therapies in patients with acute ischemic stroke (AIS). In fact, a great importance will be given to biomarkers of **FUTILE RECANALIZATION**, that importantly limits response to endovascular treatment in AIS (NORDICTUS network). We will try also to identify predictive biomarkers of growth of ischemic and hemorrhagic brain injury. Specifically, analyzing non-coding RNAs and cell-free DNA (cf-DNA) to identify circulating non-coding RNAs (miRNAs, lncRNAs and circRNAs) that predict hematoma growth in patients with ICH. Complementary neuroimaging markers will be also used to analyze whether the brain CT-perfusion (TCP) study increases the sensitivity in the detection of Spot-Sign (SS) and to investigate whether perihematoma hypoperfusion is associated with hematoma growth and its impact on the prognosis of patients (Deep Learning). Some specific pathways (dimetilarginines and other peri-hematoma damage biomarkers) will be explored.

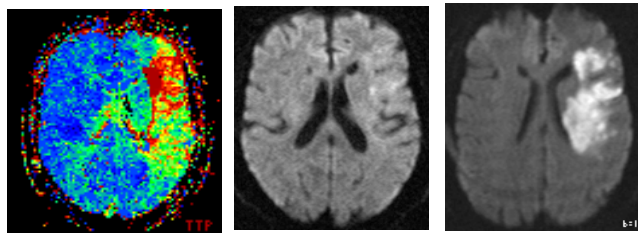
Vessel recanalization



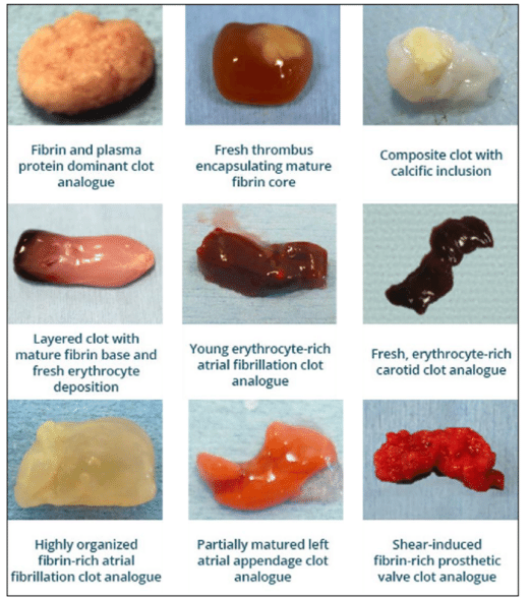
Bleeding Complications

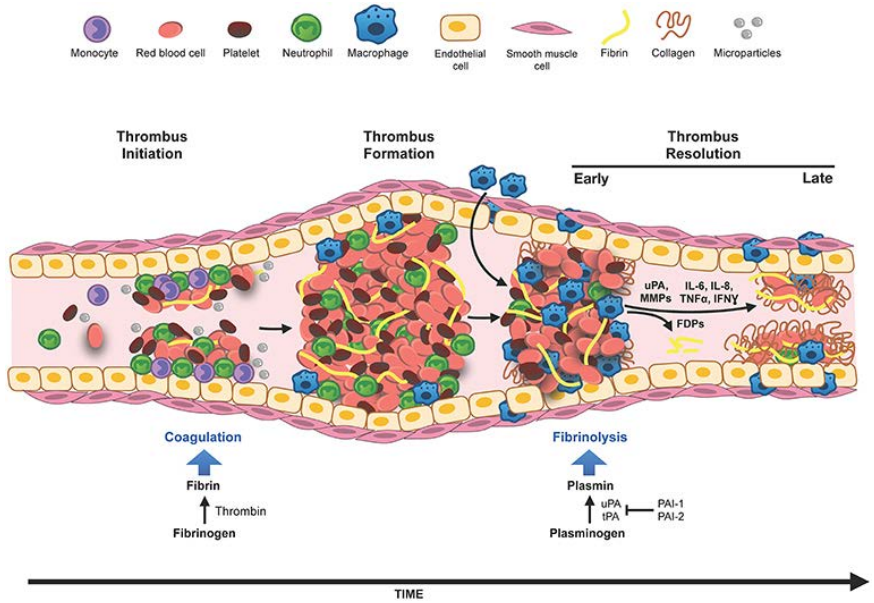


Tissue Viability, biological clock



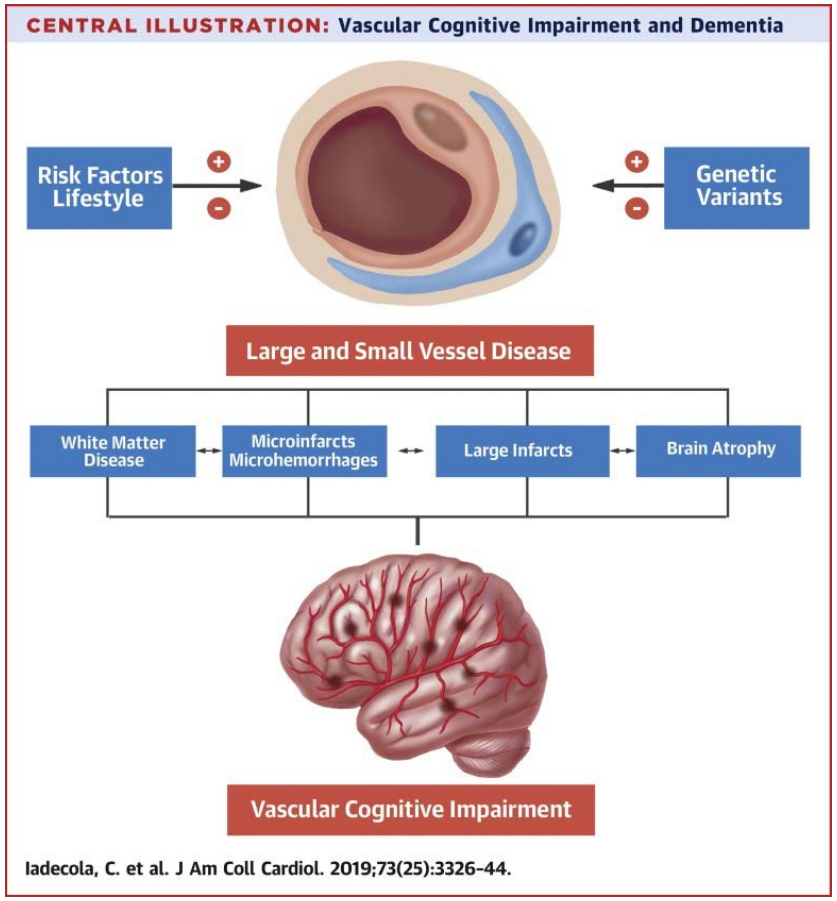
2.- Biomarkers **of the extracted thrombus**. The network will explore associations between macroscopic and radiological images, thrombus composition, blood biomarkers and angiographic/neurofunctional result; attempting the identification of a molecular/cellular signature with diagnostic/prognostic potential. Association of adherence and composition of the Mediterranean diet with the composition of thrombi in patients with AIS treated by mechanical thrombectomy will be explored. Thrombus molecular and cellular level to establish biomarkers of etiology and resistance to recanalization that allow improving their diagnosis, treatment and prognosis of patients will be explored through the study the transcriptome of extracellular vesicles (EVs) from thrombi and plasma samples. Also, redox proteomics applied to the diagnosis of ischemic stroke biomarkers to identify protein biomarkers by proteomic analysis, in thrombi obtained from thrombectomies and peripheral blood.





3.- **Global FUNCTIONAL OUTCOME biomarkers**. In this objective, we intend to identify new predictive biomarkers of brain damage and biomarkers of post-rehabilitation functional recovery, using different lab techniques. This will involve the identification and monitoring of both brain and peripheral markers. Baseline imaging markers predictors of recovery after stroke will be implemented in forecasting algorithms based on AI. Specifically, we intend to identify genetic and epigenetic variants associated with stroke and its functional prognosis. To analyze temporal variation in DNA methylation after a stroke, and its association with its severity and clinical evolution.

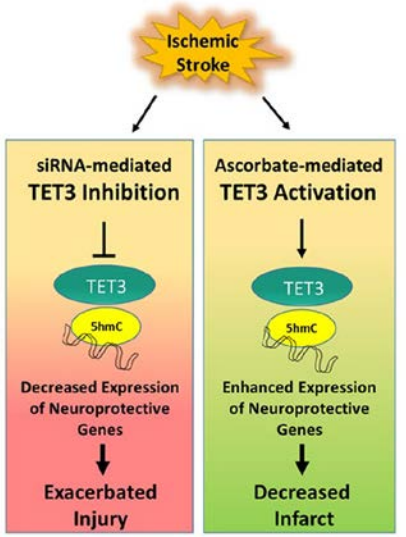
4.- **Biomarkers of cognition decline following stroke**. Study of post-stroke cognitive deficit markers by a metabolomic study and neuroimaging in animal models and in patients. Those will be complemented by the study on the value of functional near infrared spectroscopy (fNIRS) as a prognostic biomarker in patients with cerebral infarction and executive dysfunction.



WP 4.- BIOMARKERS IN EXPERIMENTAL MODELS OF STROKE AND SELECTED PATHWAYS FOR DIAGNOSIS AND THERAPY.

The main goal of this WP is to use experimental models to identify new outcome biomarkers and confirm the ones identified in previous WP. Identification of new predictive biomarkers of brain damage and worsening in murine models of cerebral ischemia by comparing those who die/survive at different timepoints after MCAO will bring new candidate biomarkers to the stroke field. We use a dynamic study of cerebral infarction and reperfusion in a humanized model of stroke in pigs through endovascular access, using imaging markers.

Those biomarkers identified in a cohort of stroke patients, corresponding to **functional polymorphisms of genes**, identified as key in experimental studies evaluating the balance between brain damage and repair in murine models of ischemia and hemorrhage will be further explored in vitro and in vivo. Functional studies will be performed in an in vitro model of atherosclerosis to elucidate its role in the progression and instability of atherosclerosis. Functional studies will also be carried out in an in vitro model of ICH to provide information on the role of the identified non-coding RNAs in controlling the expression of their targets and in cell viability and permeability. Proteomic studies to identify biomarkers involved in brain damage and recovery after ischemic stroke, focused on biological sub-compartments, addressing studies limited to known targets and of potential interest. Finally, evaluation of neuroinflammation **biomarkers in preclinical ischemic stroke** will be carried out. Study of brain and peripheral receptors as potential experimental therapeutic targets will be addressed and evaluation of neuroimaging biomarkers for PET/MRI imaging in preclinical ischemic stroke conducted together with the study of brain receptors and lipidomics.



• **WP1:** Biomarkers for the pre-hospital diagnosis of stroke

1. BIOSHIP
IBIS-Sevilla
VHIR-Barcelona
HUCA- Asturias
HCU Valladolid
Germans Trias-Badalona
IRB-Lleida
GCA-Aragón Associated clinical Group (Dr. Javier Marta)

2. Machine Learning
IBIS-sevilla
HCU Valladolid
IIS IP-UCM (HU Princesa, Madrid): data integration and machine learning techniques for the joint analysis of pre-hospital biomarkers
Patricia Calleja. Hospital Universitario Doce de Octubre. (Proyecto La Princesa Vivancos)
IDIPAZ-Madrid: escala Madrid-Direct y estudio promovido por el hospital de la Princesa para la identificación de otros biomarcadores prehospitalarios.
Germans Trias-Badalona
IRB-Lleida
GCA-Aragón Associated clinical Group (Dr. Javier Marta)
IIS IP-UCM: data modeling techniques (HU Princesa, Madrid)

3. Nasal Biomarkers
HUCA- Asturias
Alberto Alcázar. Grupo Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Hospital Ramón y Cajal, Madrid. (CSF biomarker profile)

4. Metabolomic/lipidomic
HUCA- Asturias
Patricia Calleja. Hospital Universitario Doce de Octubre
IRB-Lleida
GCA-Aragón Associated clinical Group (Dr. Javier Marta)

• **WP2:** Use of biomarkers in stroke subtypes

1. Intracranial atherosclerosis
HUCA- Asturias
HCU Valladolid
HOSPITAL UNIVERSITARIO PUERTA DE HIERRO. DR J CARNEADO RUIZ.
Germans Trias-Badalona
IRB-Lleida
GCA Málaga – Carlos de la Cruz Cosme
GCA-Aragón Associated clinical Group (Dr. Javier Marta)
IDIBGI-Serena
IS La Fe Valencia RD21/0006/0014: identification of predictors of carotid angioplasty complications
IBIS-sevilla (SHP)

2. Cardioembolic
IBIS-Sevilla
VHIR-Barcelona
HUCA- Asturias
TORRECÁRDENAS - Almería
HCU Valladolid
HOSPITAL UNIVERSITARIO PUERTA DE HIERRO. DR J CARNEADO RUIZ.
IdiPAZ-Madrid: Tenemos un proyecto activo en colaboración con el Servicio de Cardiología del Hospital Universitario La Paz,
Germans Trias-Badalona
IRB-Lleida
Patricia Calleja. Centro de Ictus. Hospital Doce de octubre.
GCA Málaga – Carlos de la Cruz Cosme.
GCA-Aragón Associated clinical Group (Dr. Javier Marta)

3. ICH
HUCA- Asturias
HCRB-IDIBAPS - Barcelona (SAH studies: neuroimaging, blood and CSF biomarkers and genetic studies)
IdiPAZ-Madrid. papel de los exosomas como biomarcador en HC, con estudios de proteómica de su contenido.
GCA Málaga – Carlos de la Cruz Cosme
GCA-Aragón Associated clinical Group (Dr. Javier Marta)
IIS La Fe Valencia RD21/0006/0014: biomarkers in subarachnoidal hemorrhage (SAH)
IRB-Lleida

• **WP3:** Biomarkers for outcome (stroke complications and recovery)

1. Growth/FUTILE…
IBIS-sevilla
HUCA- Asturias
i+12 Madrid
IDIS - Santiago
Futile recanalization: HCU Valladolid (NORDICTUS)
HOSPITAL UNIVERSITARIO PUERTA DE HIERRO. DR J CARNEADO RUIZ.
IdiPAZ-Madrid: Estamos desarrollando el estudio GLIAS-TM (financiación ISCIII AES 2021) para el estudio de microRNA/glicemia en el daño por isquemia-reperfusión
Germans Trias-Badalona
GCA Málaga – Carlos de la Cruz Cosme
GCA-Aragón Associated clinical Group (Dr. Javier Marta)
IIS IP-UCM: data modeling techniques (HU Princesa, Madrid)
IDIBGI-Serena
IRB-Lleida

2. Thrombus
IBIS-Sevilla (trombo/plasma correlación biomarkers)
HUCA- Asturias
i+12 Madrid
HCU Valladolid (thrombectomy bank)
HOSPITAL UNIVERSITARIO PUERTA DE HIERRO. DR J CARNEADO RUIZ.
IdIPAZ-Madrid: Tenemos una colección de trombos con los que podríamos colaborar.
Alberto Alcázar. Grupo Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Hospital Ramón y Cajal, Madrid
Patricia Calleja. Hospital Universitario Doce de Octubre.
GCA Málaga – Carlos de la Cruz Cosme
GCA-Aragón Associated clinical Group (Dr. Javier Marta)
IIS Princesa-Santa Cristina (Martínez Ruiz & Marina) - Redox proteomics (HU Princesa, Madrid)
IDIBGI-Serena
IIS La Fe Valencia RD21/0006/0014: Association of adherence and composition of the Mediterranean diet with the composition of thrombi
IRB-LLeida

• **WP3:** Biomarkers for outcome (stroke complications and recovery)

3. Global Outcome
IBIS-Sevilla
VHIR-Barcelona
HUCA- Asturias
i+12 Madrid
HOSPITAL UNIVERSITARIO PUERTA DE HIERRO. DR J CARNEADO RUIZ.
HCRB-IDIBAPS - Barcelona (in collaboration with Sant Pau): Study of the influence of intestinal microbiota on the evolution of ischemic stroke.
Measurable criteria: functional prognosis at 3 months.
Alberto Alcázar. Grupo Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Hospital Ramón y Cajal, Madrid
GCA Málaga – Carlos de la Cruz Cosme
IRB-Lleida

4. Cognition
HUCA- Asturias (pacientes)
i+12 Madrid
TORRECÁRDENAS - Almería
GCA Málaga – Carlos de la Cruz Cosme
GCA-Aragón Associated clinical Group (Dr. Javier Marta)
IRB-Lleida

- **WP4:** Biomarkers in experimental models of stroke and selected pathways

1. Experimental & targets
IBIS-sevilla
i+12 Madrid
TORRECÁRDENAS - Almería
IDIS - Santiago
HCRB-IDIBAPS - Barcelona: Blood biomarkers of severity (including infection and inflammation) and time course evolution according to the progression of damage and recovery. We are interested in collaborative studies of proteomics and lipidomics.
IdiPAZ-Barcelona: estamos trabajando con modelos animales de hemorragia cerebral para identificación de biomarcadores.
Germans Trias-Badalona
Alberto Alcázar. Grupo Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Hospital Ramón y Cajal, Madrid
IIS-IP-Santa Cristina Javier Egea & Antonio Martínez: **(HU Princesa, Madrid)**
IDIBGI-Serena
IIS La Fe Valencia RD21/0006/0014: Identification of new predictive biomarkers of brain damage and worsening in murine models of cerebral ischemia
IRB-Lleida

- Identificar proyectos claramente colaborativos
- Concentrar esfuerzos en esos grandes proyectos con alta participación de grupos de la red ¿?
- Propuestas mas concretas de los lideres de esos proyectos
- Visibilizar esos proyectos en web etc
- Cronograma para alinear esos proyectos con *deliverables* y *milestones* del WP
- Conexiones con las otras líneas RICORS-ICTUS