

# Una historia de investigación traslacional en biomarcadores: de los estudios preclínicos a los ensayos clínicos

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Director of Neurology Department, Hospital Macarena, Seville  
Coordinator of the Andalusian Healthcare Stroke Plan



# Translating brain biomarker research to clinical practice

## “Promises and pitfalls”

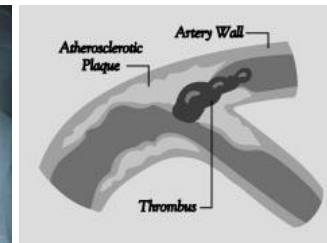
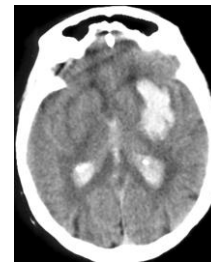
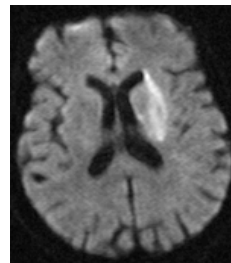
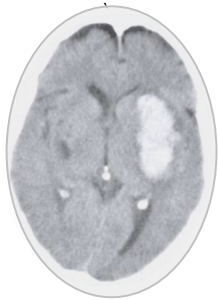
A journey through 1 failure (“biomarkers to treat stroke”) and one success (“biomarkers to triage stroke”)

& some advices for the young biomarker travelers...

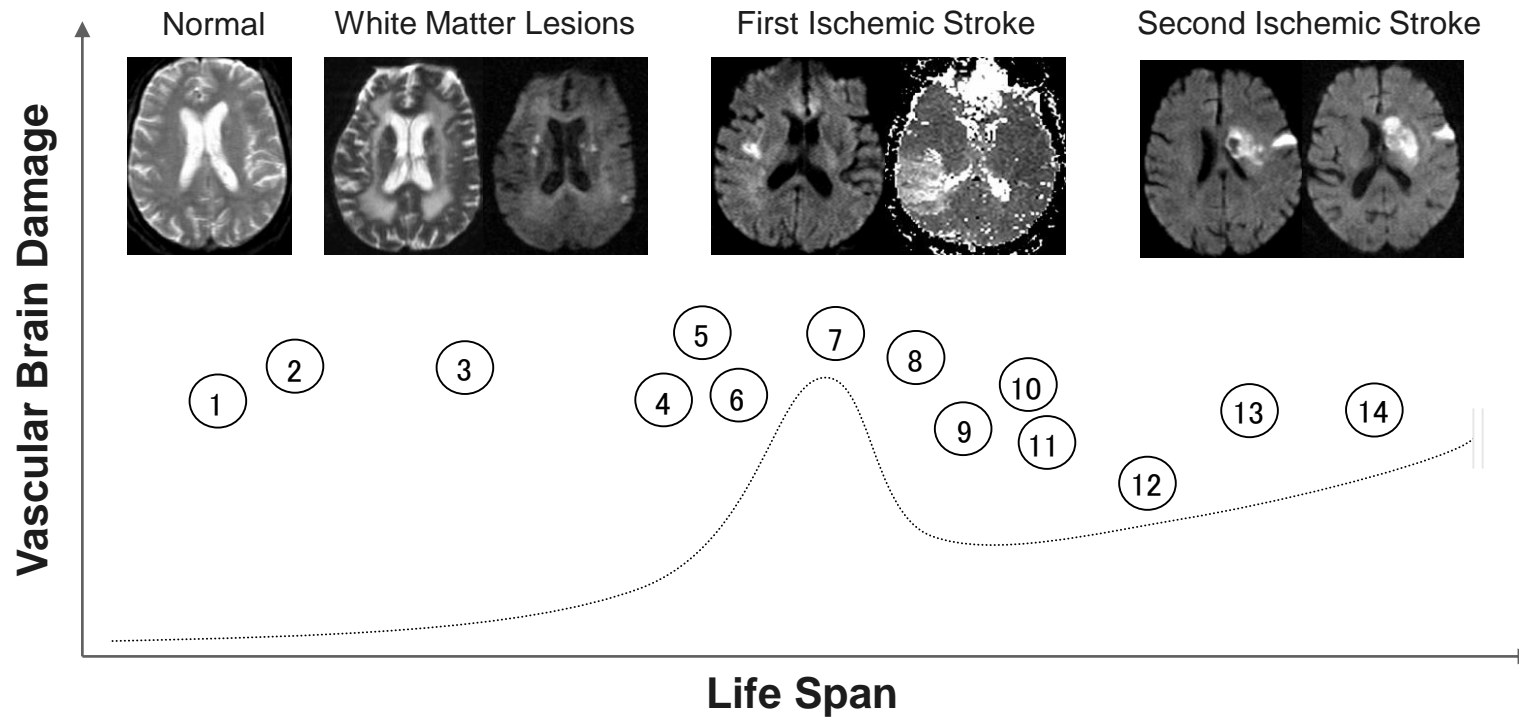


# The PROMISE...

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



# Blood Biomarkers & Stroke Natural History



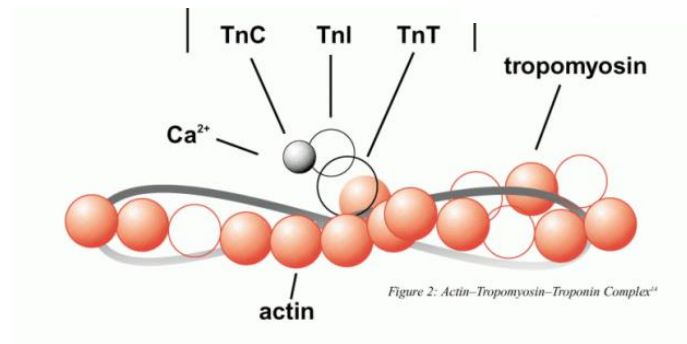
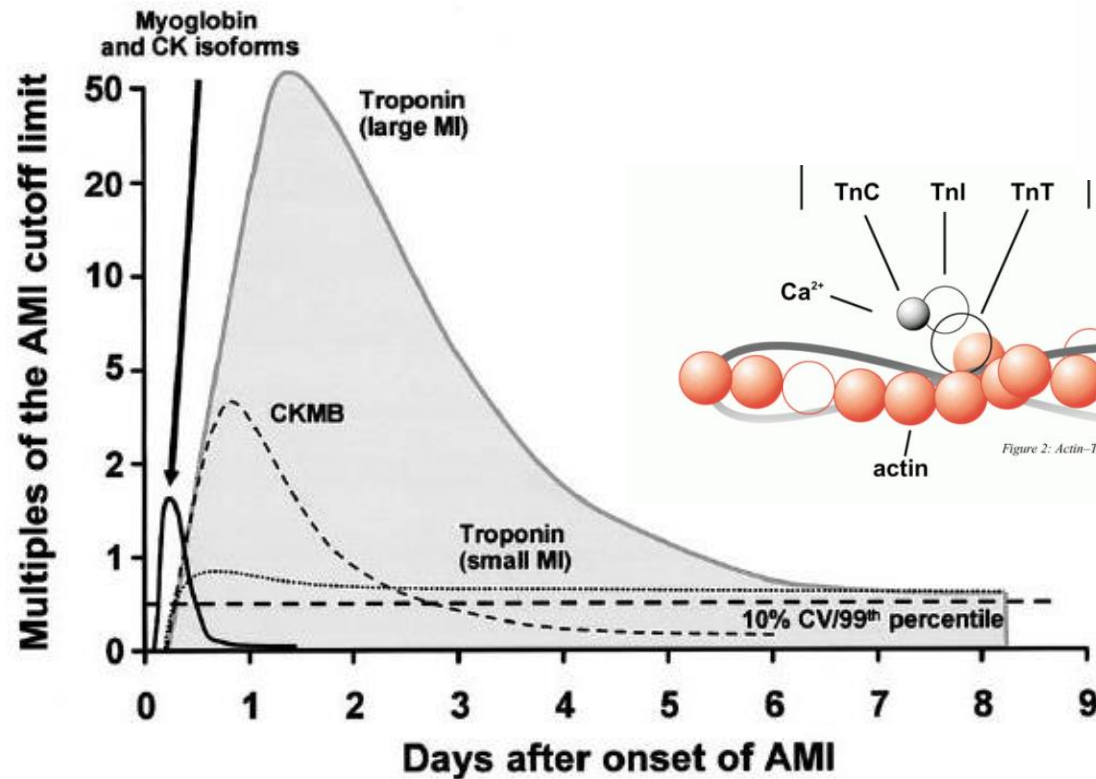
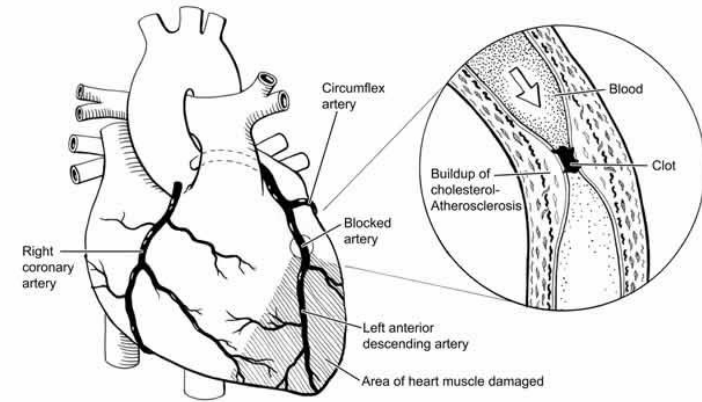
- 1.- Predict stroke among healthy people
- 2.- Predict stroke among those with vascular risk factors
- 3.- Identify Silent strokes
- 4.- Stroke vs mimics
- 5.- Ischemic-Hemorrhagic
- 6.- LVO identification
- 7.- Bleeding complications

- 8.- Futile recanalization
- 9.- Stroke etiology
- 10.- Cardiac complications
- 11.- Infections prediction
- 12.- Functional outcome & recovery
- 13.- Post-stroke seizures
- 14.- Stroke recurrence

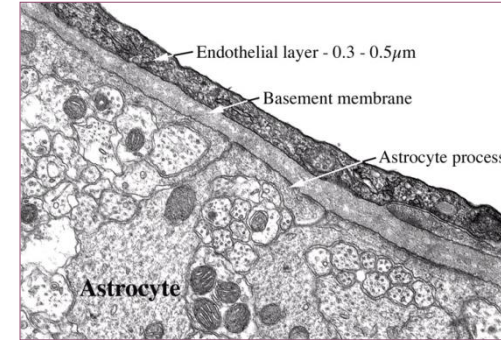
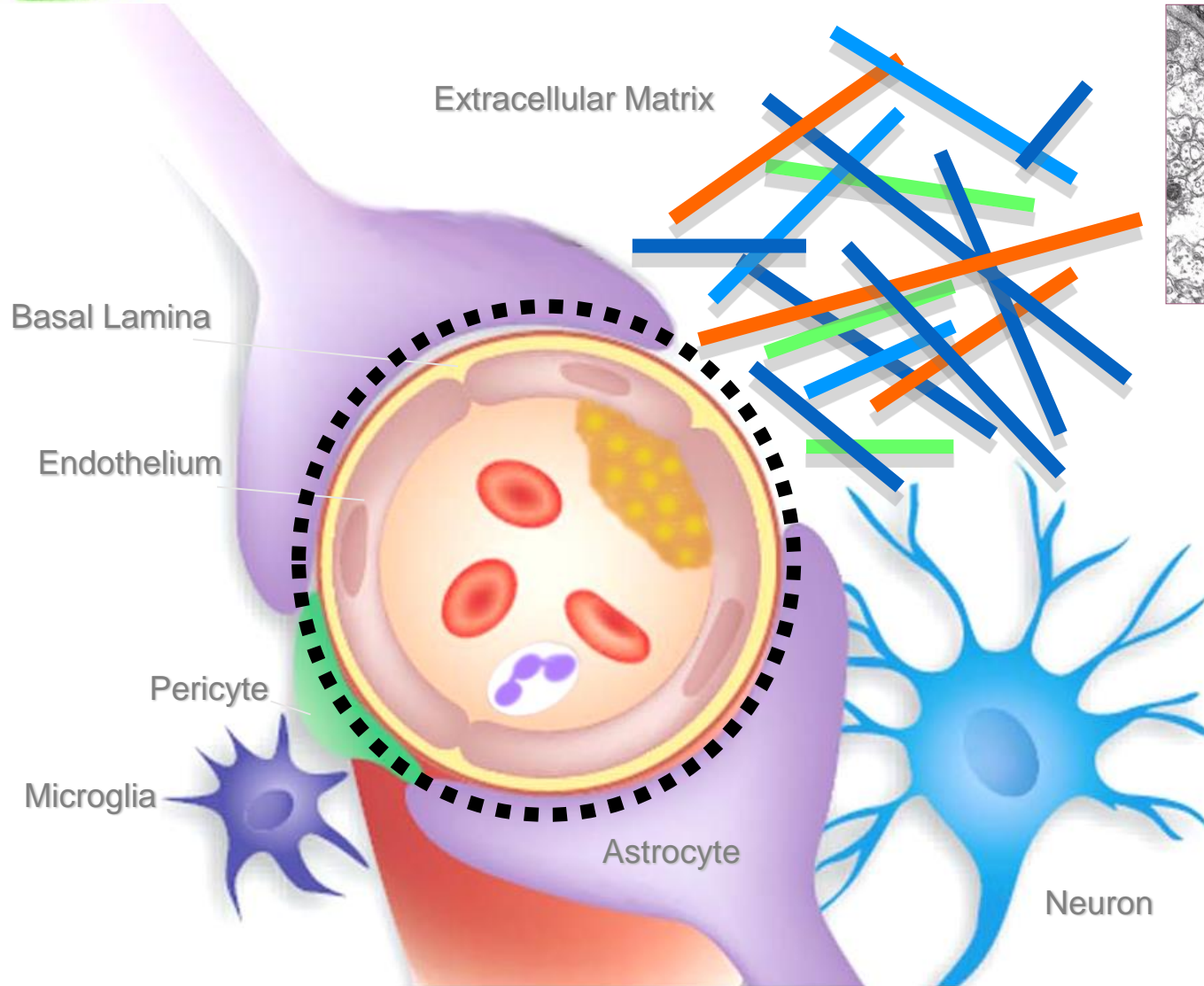
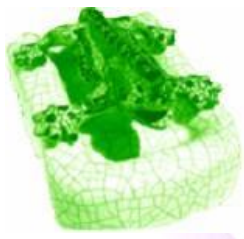
# Far away from cardiologists

6,083 references on Troponin [first publications in 1987]

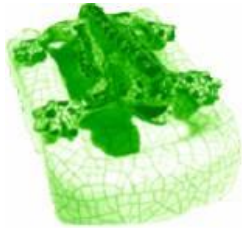
Jaffe *et al.*  
Cardiac Biomarkers: Present and Future



# Neurovascular Unit and MMPs

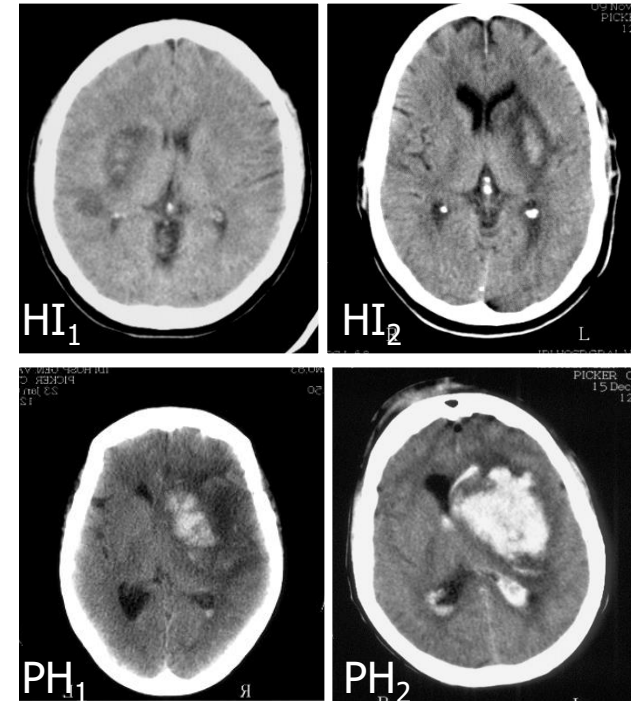
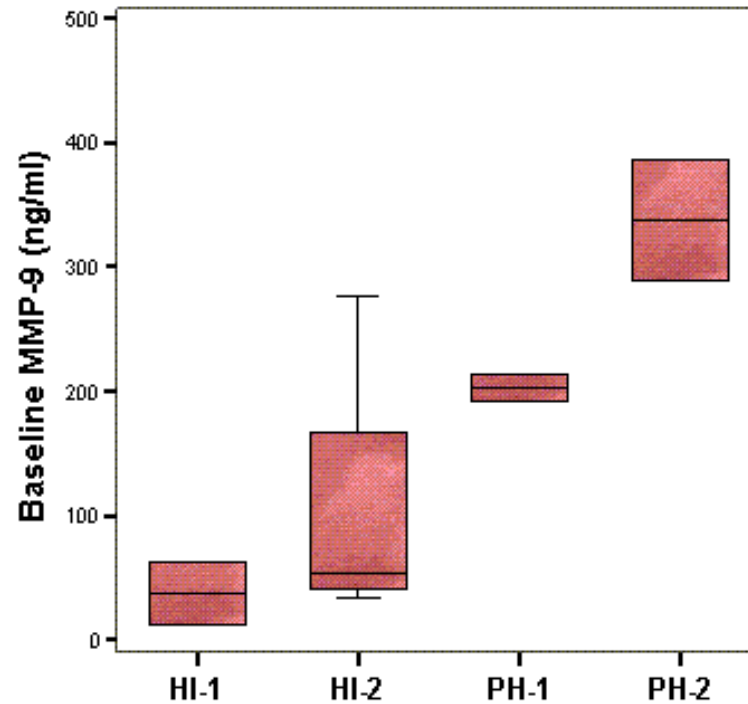


Blood Brain Barrier (BBB)



# MMP-9 and t-PA: in the plasma...

Biomarkers involved in the process

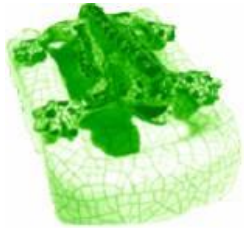


Montaner *et al.* Circulation 2003;107:598-603.

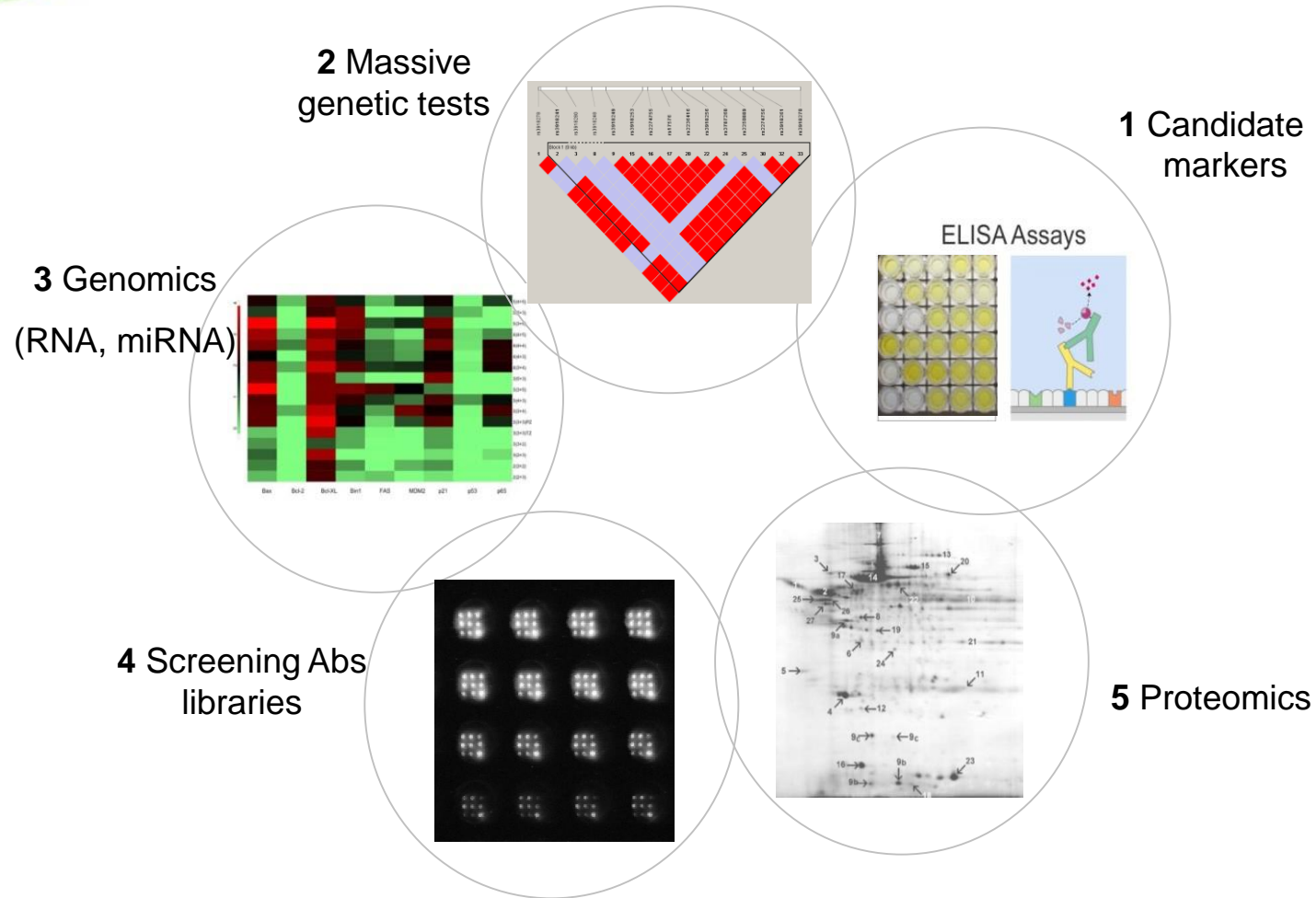


Montaner, II RICORS | Madrid 23 Marzo 2023





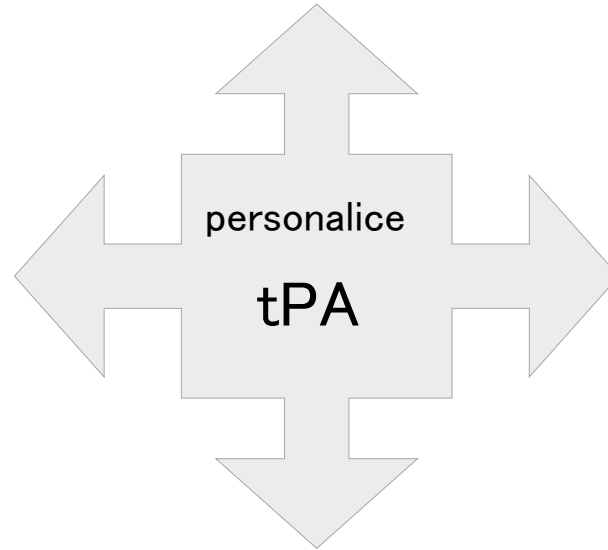
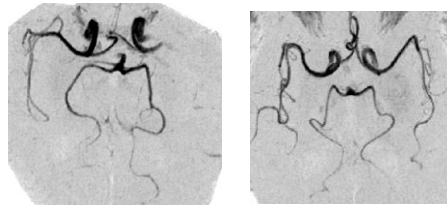
# Stroke Biomarkers discovery: How?



# Reperfusion Targeted Therapies...

## Vessel recanalization

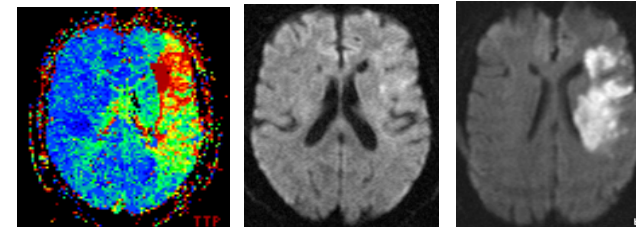
PAI-1  
TAFI  
TAT  
ADAMTS-13



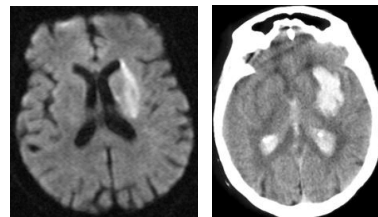
## Tissue Viability

## Futile recanalization

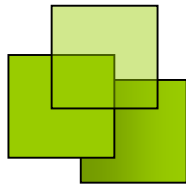
## LVO identification



## Bleeding Complications



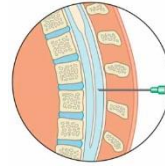
MMP-9  
FLIPS  
VAP-1  
NURR1  
Fibronectine



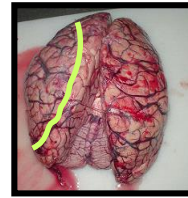
# Proteomics in human samples

## Summary of candidate stroke biomarkers

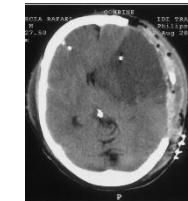
**CSF proteome**  
Lescuyer P, et al.  
*Proteomics*. 2004;4:2234-41.



**Brain proteome**  
Cuadrado E, et al.  
*J Neuropath Exp Neurol*  
2010;69:1105-15.



**Microdialysate proteome**  
Dayon L, et al.  
*Journal of Proteome Research*.  
2011;10:1043-51.

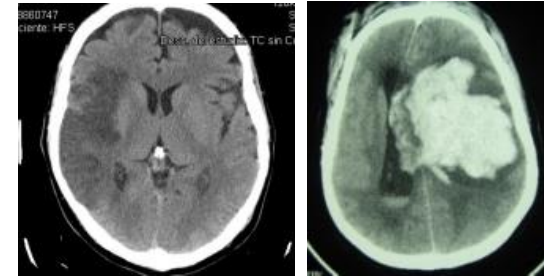


Fatty Acid Binding Protein (FABP)  
RNA-binding protein regulatory subunit (RNA-BP/PARK7)  
Nucleoside diphosphate kinase A (NDKA)  
Glutathion S Transferase P (GSTP-1)  
Ubiquitin Fusion Degradation Protein (UFD1)  
Dihydropyrimidinase-related protein 2 (DRP-2)  
Vesicle-fusing ATPase (NSF)  
Spectrin a II chain  
Heat shock cognate protein 70 pseudogene 1  
Tropomodulin 2  
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)  
Heat-shock cognate 71 kda protein (HSP7C)  
Fibrinogen  $\beta$  chain  
Apo CIII  
Monomeric form of transtirretin  
Peroxiredoxins



**If verification studies of those proteins in plasma are promising the biochemical monitoring of acute ischemic stroke might be possible.**

# Ischemic versus ICH

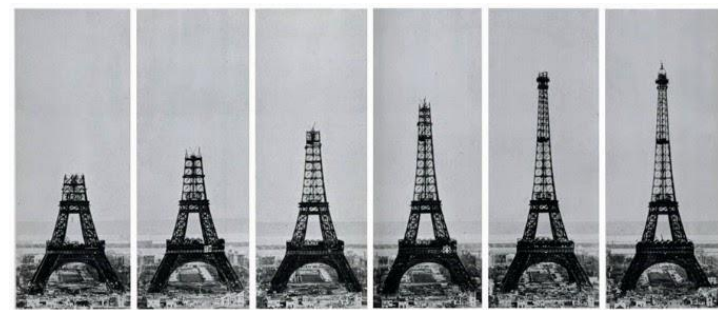
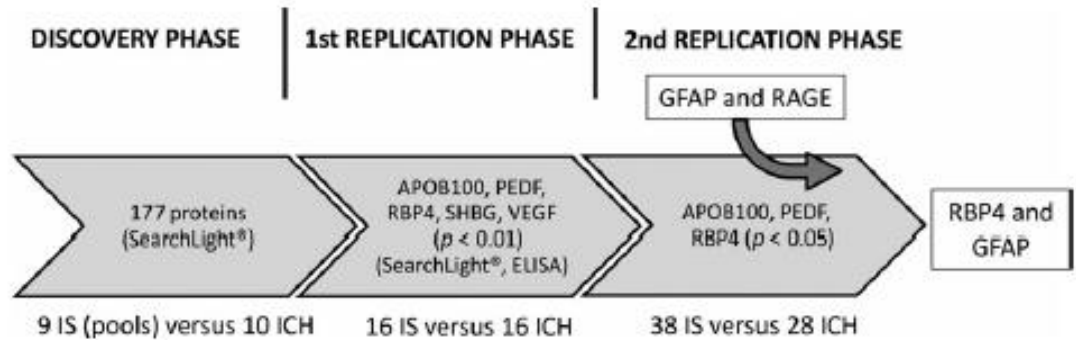


or



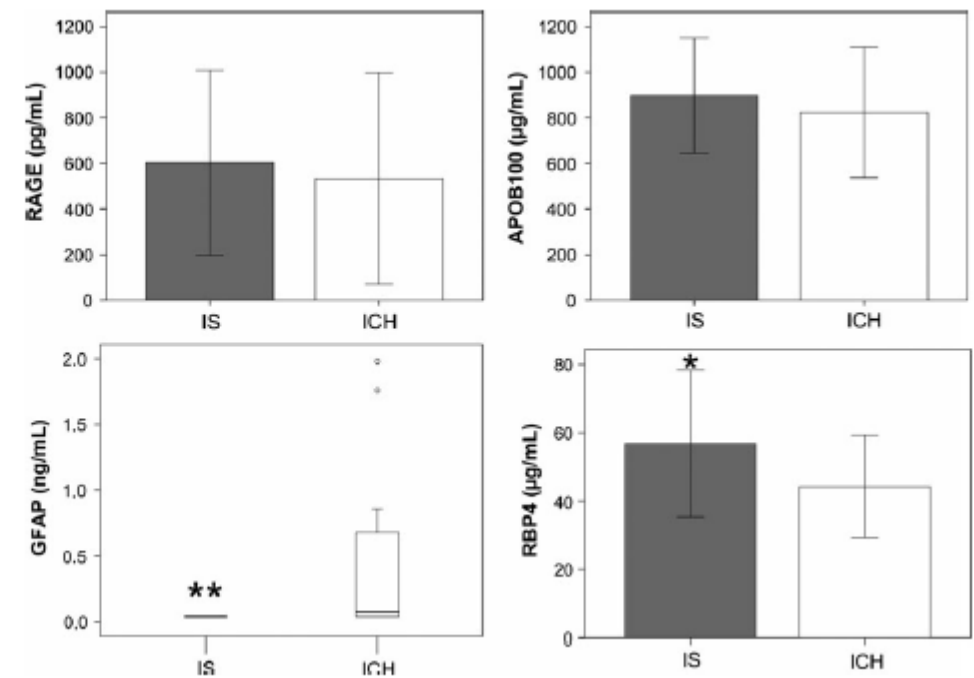
**HIGH  
SPECIFICITY FOR  
ICH: intensive  
BP lowering**

**HIGH  
SPECIFICITY FOR  
IS: IV tPA**



## Improving GFAP test

**Fig. 2** Plasma levels of RBP4, APOB100, receptor for advanced glycation end product (RAGE) and glial fibrillary acidic protein (GFAP) in IS, ischemic stroke (IS) ( $n = 38$ ) and ICH ( $n = 28$ ) patients in the second replication phase. \* $p < 0.05$ , \*\* $p < 0.0001$ . For RAGE, APOB100 and RBP4, mean and SD are indicated. For GFAP, median and interquartile range are indicated.



Llombart V, et al. *Journal of Neurochemistry* 2015;136(2):416-24.

# Can Blood Biomarker Panels be Used for Identification of Ischemic Stroke?

Quick differentiation between intracerebral hemorrhage (ICH) and ischemic stroke (IS) is vital for initiating pre-hospital thrombolysis and determining patient outcome



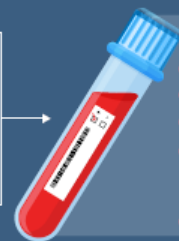
Detection of blood biomarkers may offer a fast and reliable option for differentiating between ICH and IS



This study aimed to validate and develop a panel of blood biomarkers that could accurately differentiate between ICH and IS

Patients admitted within 4.5 hours after suspected stroke

Blood samples at admission



Radioimmunoassay

- ▲ Glial fibrillary acid protein (GFAP)
- ▲ Retinol binding protein 4 (RBP-4)
- ▲ Endostatin (ES)
- ▲ N-terminal pro B-type natriuretic peptide (NT-proBNP)

High-sensitivity assay

- ▲ GFAP

Determination of cut-off points for 100% specificity

Radioimmunoassay

Biomarker levels	Radioimmunoassay	
	ICH	IS
GFAP	High	Low
RBP-4	Low	High
ES	Low	High
NT-proBNP	Low	High

Biomarker combinations that yield 100% specificity

Immunoassay

RBP-4 + NT-proBNP



29.7%

High-sensitivity assay

RBP-4 + NT-proBNP + GFAP



51.5%

Biomarker panel including RBP-4, NT-proBNP, and GFAP, can provide potentially useful sensitivity rates for IS diagnosis

doi:

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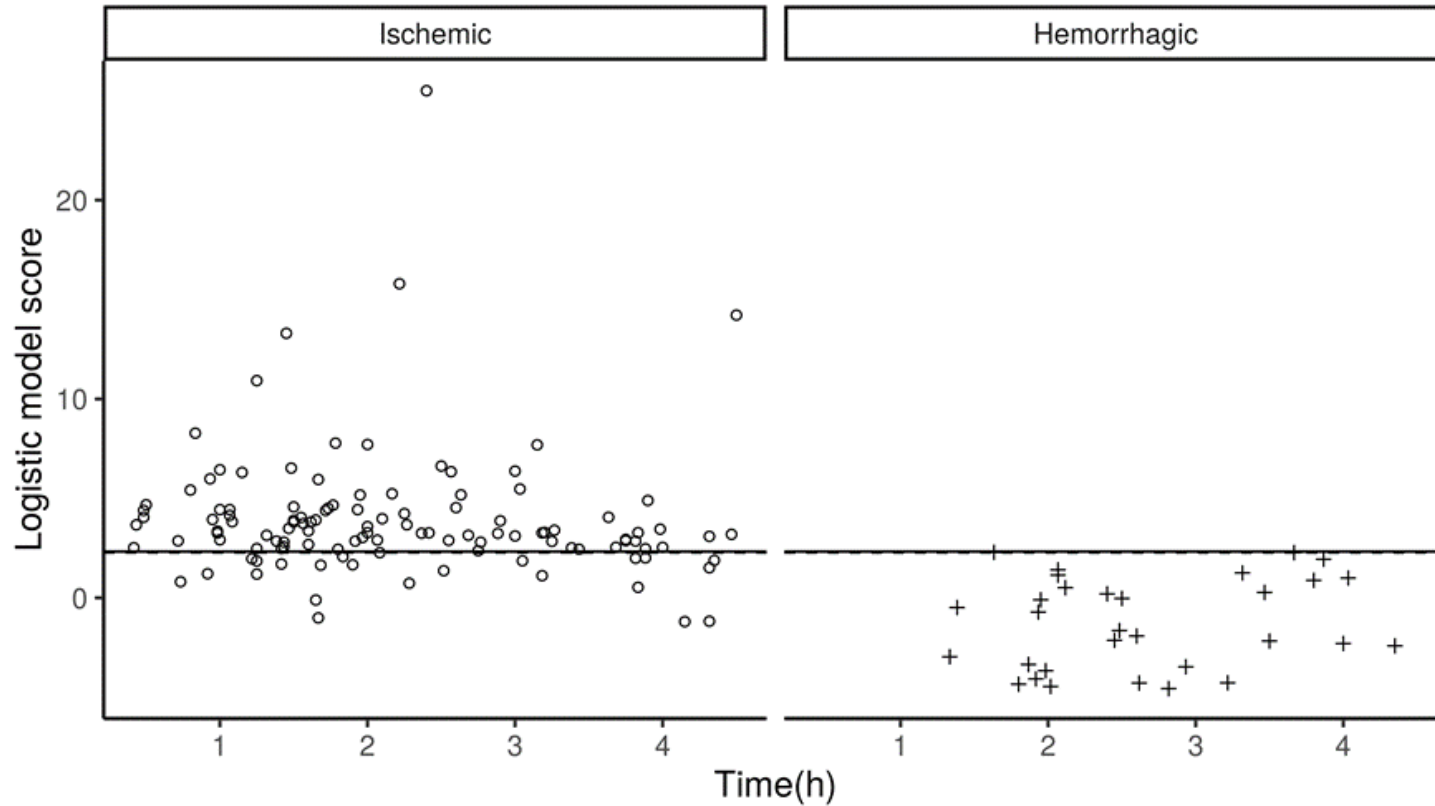
Neurology®

# N=490 patients cohort

GFAP  
+ NT-proBNP

Strokecheck 2.0 model had a sensitivity=0.60, specificity= 1.00 and accuracy =0.68

PANEL



> 2.33 spc. 1

	Ischemic	Hemorrhagic
classified as Ischemic	93	0
classified as Hemorrhagic	61	35

> 2.28 spc. 0.97

	Ischemic	Hemorrhagic
classified as Ischemic	93	1
classified as Hemorrhagic	61	34

Would you accept to treat most of ischemic strokes correctly with tPA in the ambulance and assume the risk of treating some ICH with tPA???





# 2 challenges in stroke care

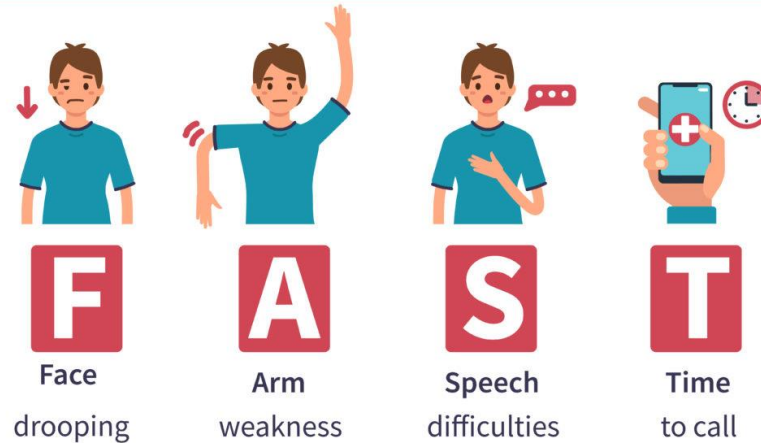
- Open the occluded artery as soon as possible
- Shift stroke patient to the right hospital

**THE SOONER THE TREATMENT,  
THE BETTER THE CHANCES.**



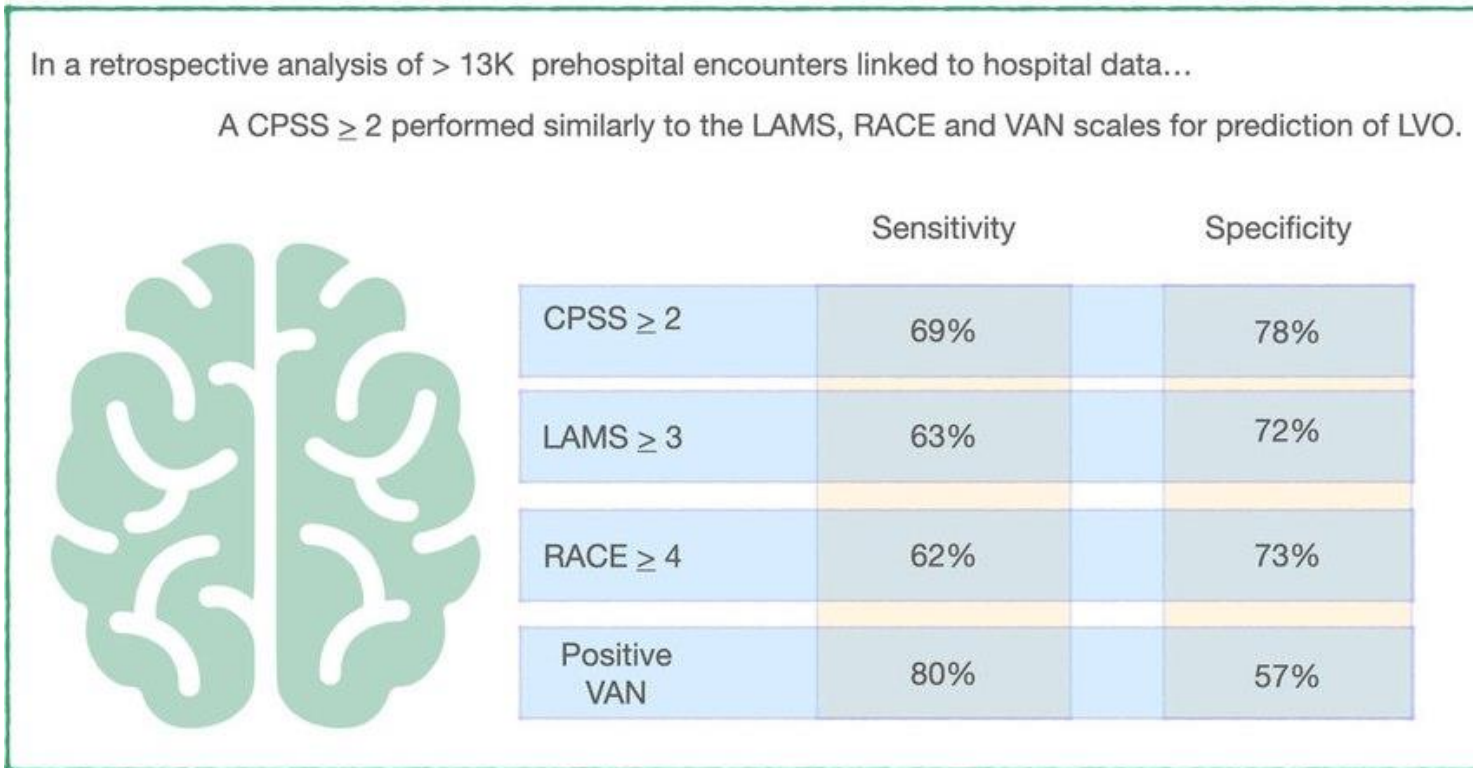
## STROKE SYMPTOMS

Remember, recognize and act fast



# Clinical Scales & LVO

Several tools such as clinical scales are being used to identify LVO patients out of the hospital and shift them directly to thrombectomy centers. However, their accuracy is suboptimal.



Remle P. Crowe, et al. The Cincinnati Prehospital Stroke Scale Compared to Stroke Severity Tools for Large Vessel Occlusion Stroke Prediction. *Prehospital Emergency Care*. 2021;25:1, 67-75.

**QUESTION** In patients experiencing suspected large-vessel occlusion (LVO) stroke in a nonurban area, is there a difference in neurological outcomes between those who are transported to the closest local stroke center vs directly to a thrombectomy-capable center?

**CONCLUSION** In nonurban areas in Catalonia, Spain, there was no significant difference in 90-day neurological outcomes between transportation to a local stroke center vs a thrombectomy-capable referral center in patients with suspected LVO stroke.

**POPULATION**

521 Men  
428 Women



Patients with a suspected acute LVO stroke who were attended by emergency medical personnel

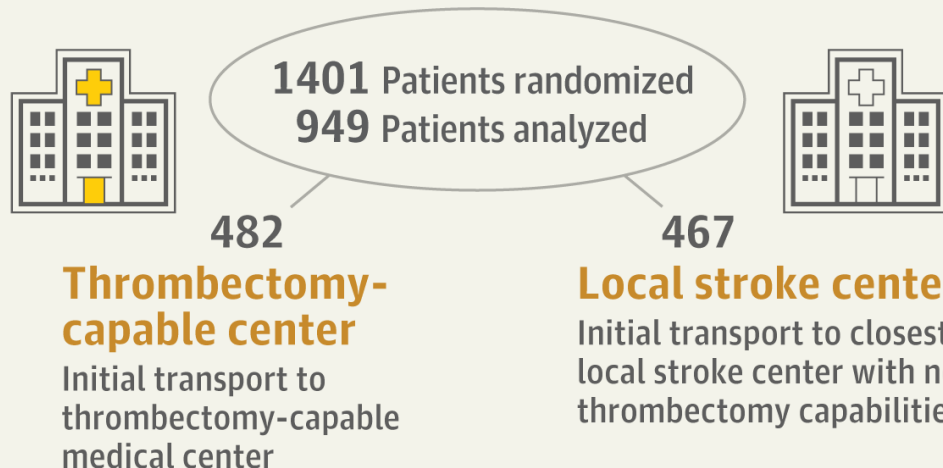
Median age: 75 years

**LOCATIONS**

28  
Medical centers  
in Catalonia, Spain



**INTERVENTION**



**PRIMARY OUTCOME**

Disability at 90 days as assessed by the modified Rankin Scale (mRS; scores, 0 [no symptoms] to 6 [death]) among patients with a final diagnosis of ischemic stroke

**FINDINGS**

Disability at 90 days

**Thrombectomy-capable center**

Median mRS score, **3** (IQR, 2-5)

**Local stroke center**

Median mRS score, **3** (IQR, 2-5)

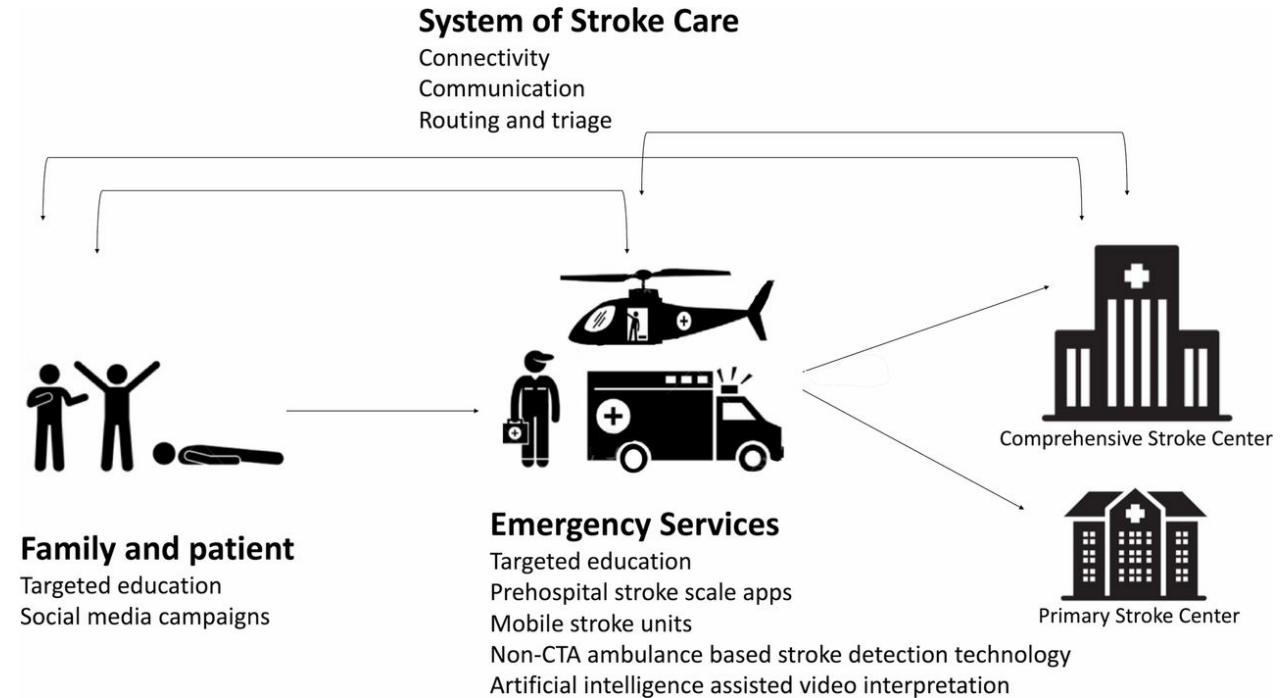
Between-group difference was not significant:

Adjusted common odds ratio, **1.03**  
(95% CI, 0.82 to 1.29)

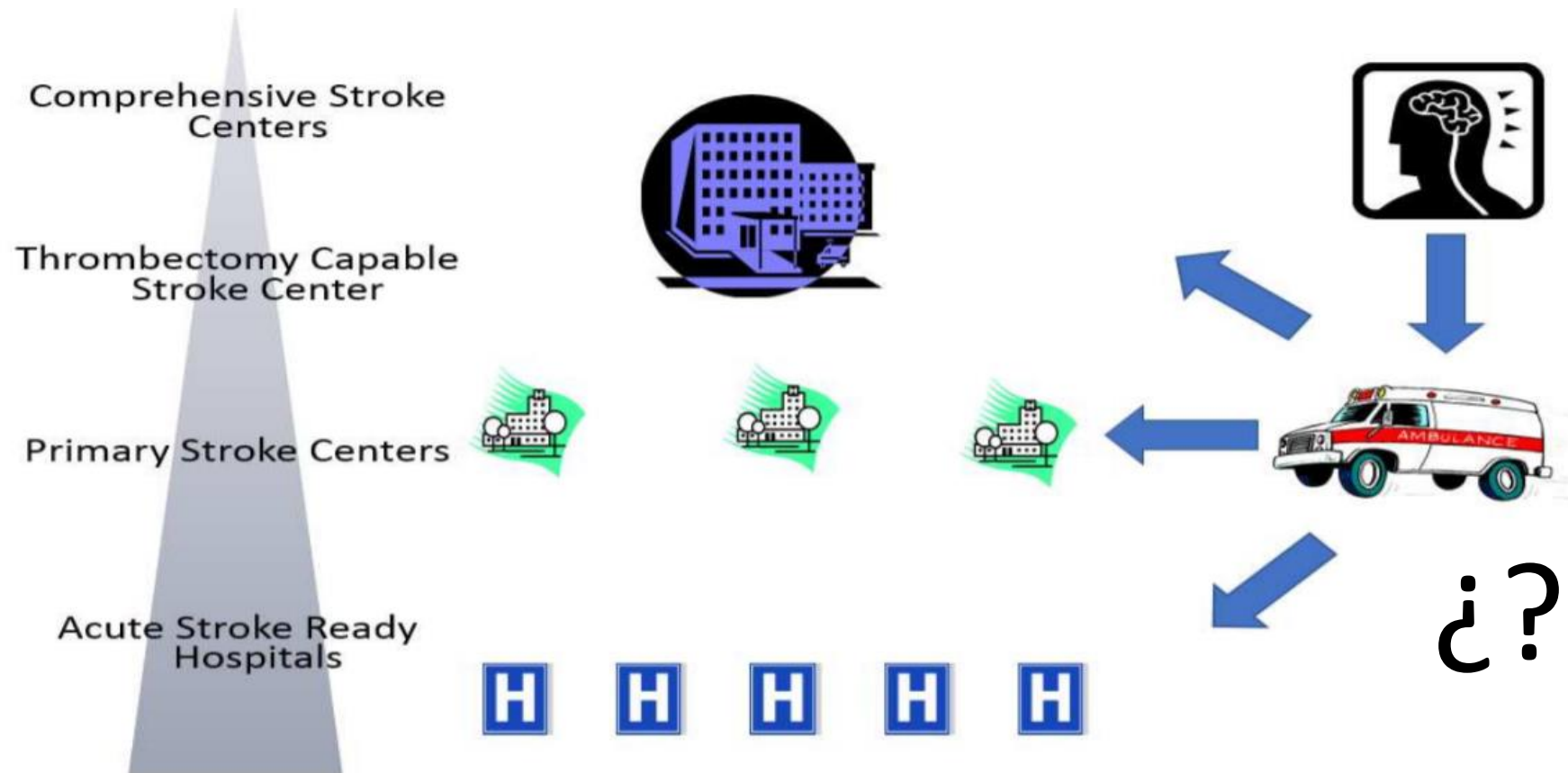
Datos recientes publicados en JAMA han demostrado que lo más adecuado es trasladar al paciente al centro mas cercano e iniciar ahí la evaluación diagnostica y terapéutica. [The RACECAT Randomized Clinical Trial]

**Este estudio ha demostrado que no hacer eso puede empeorar el pronóstico de algunos ictus (hemorragias cerebrales) y restaría eficacia a los centros comarcales y unidades de ictus en la atención de las patologías tiempo dependientes.**

La clave en el futuro en todos los países occidentales será seleccionar de forma pre-hospitalaria con diversa tecnología a los pacientes con OGV que se beneficien de acudir directamente al centro de trombectomía. Por desgracia, estas tecnologías aún no existen y no están disponibles.



# We need an efficient triage tool



Would you accept to send most of the  
LVO ischemic strokes directly to the  
thrombectomy center???



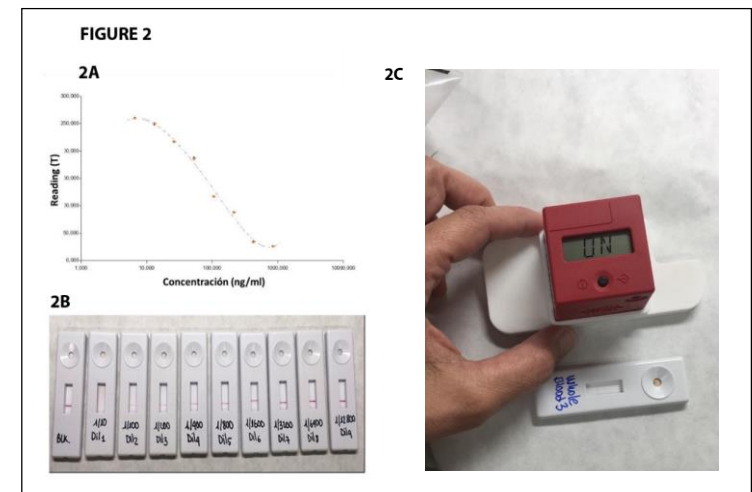
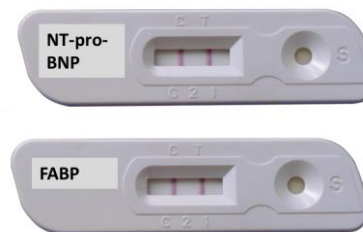
# Blood Biomarkers

Study Name	Location	Ethical approval	Sample size	Starting date	End date	Number of patients enrolled	Intend of use	Biomarkers measured
Stroke-chip	Vall d'Hebron Hospital	Yes	175	2014	Jan 2016	189	Isch-ICH & LVO Dx	21-biomarker panel & StrokeCheck
BIOFAST	Sevilla	Yes	300	2019	30th April 2021	305	Isch-ICH & LVO Dx	StrokeCheck & LVOCheck

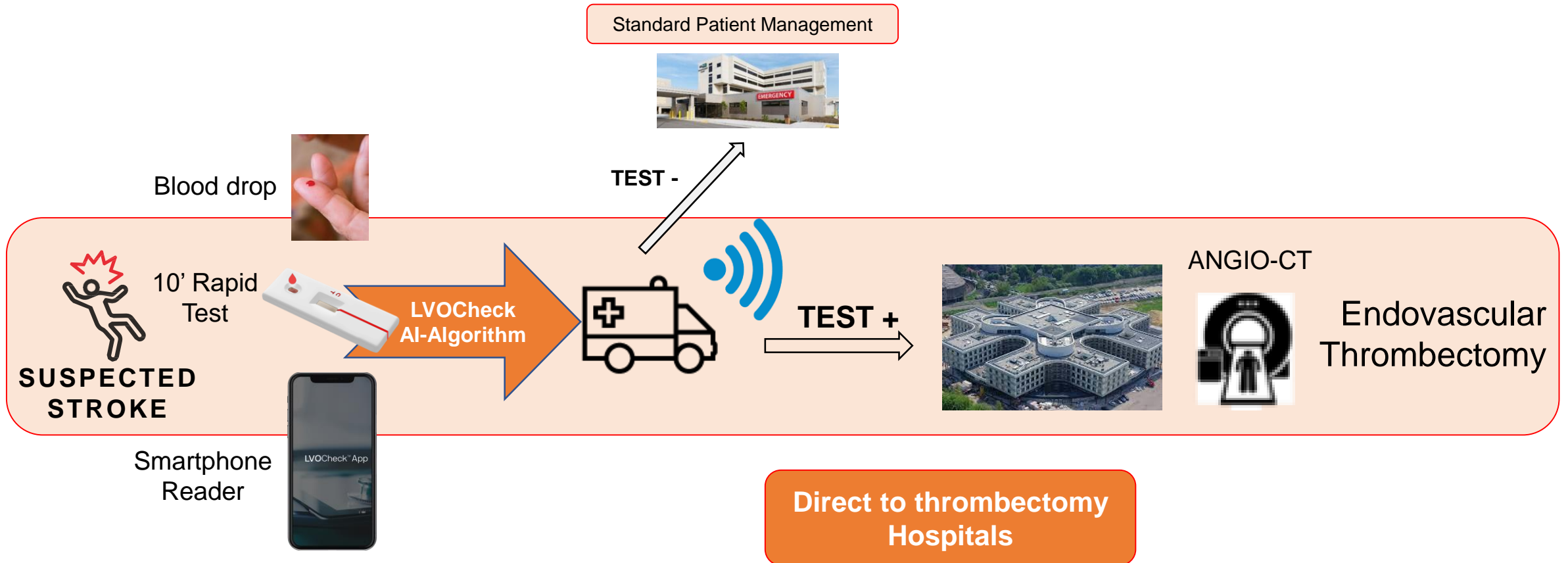
Best final candidates with available commercial POCTs were FABP/NT-proBNP in combination with simple clinical data (age, neurological scales, blood pressure, etc).

N-terminal pro B-type natriuretic peptide

h-Fatty Acid Binding Protein



# LVO-Check



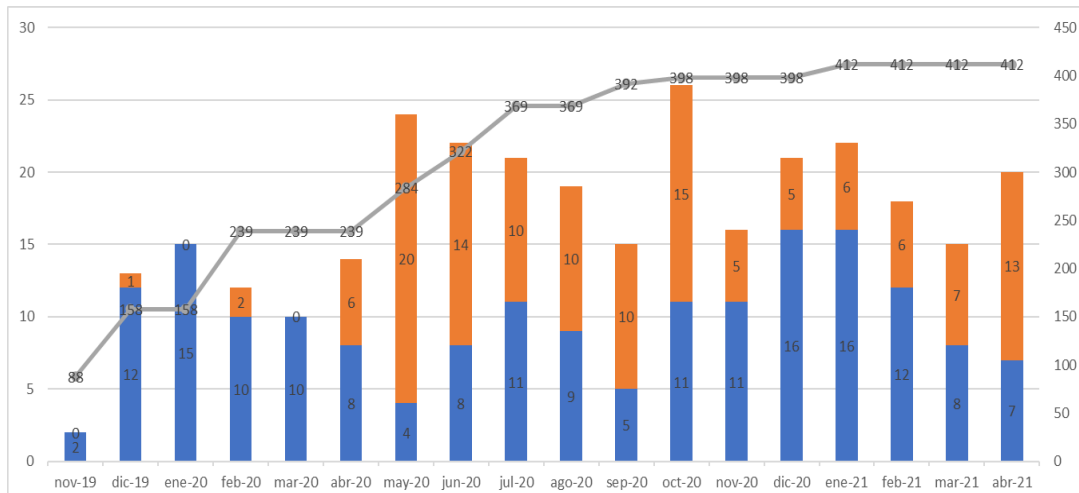


# Methodology & Results



## Biofast

Recruited stroke code suspicions <6h



- 175 patients recruited at the ambulance
- 130 patients recruited at ER
- > 400 trained staff

Trial nº →

(Biomarkers for Initiating Onsite and Faster Ambulance Stroke Therapies, ClinicalTrials.gov identifier: NCT04612218).

Web site →

<https://biofast.technology/en/home/>



### About Biofast

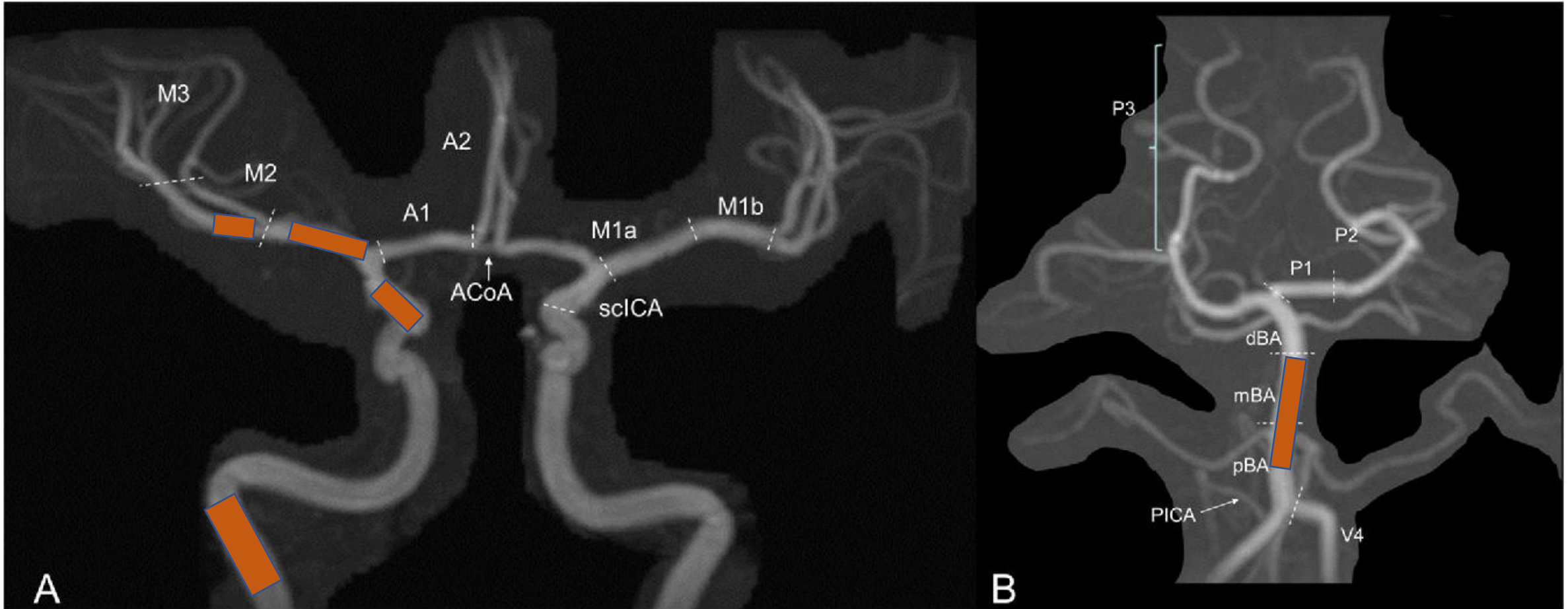
#### What is the main objective?

Our aim is to develop a point-of-care (POC) device to validate a panel of biomarkers that differentiate ischemic and hemorrhagic stroke using a blood sample.



# “STRICT” LVO DEFINITION

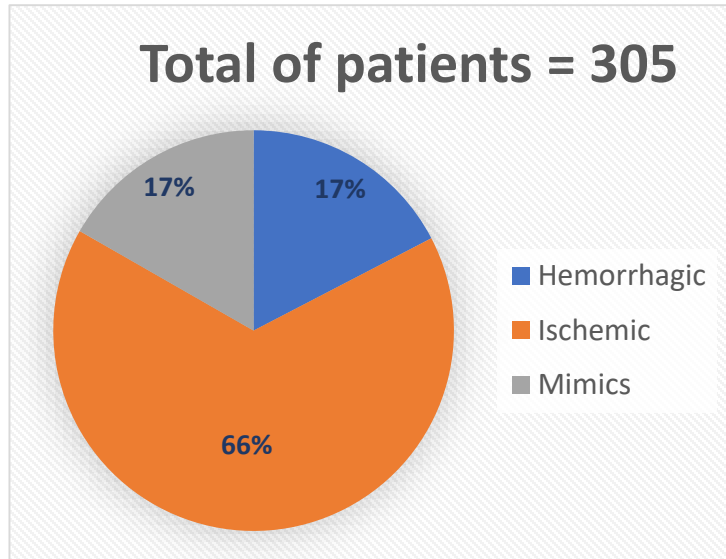
\* In most studies, it was defined as occlusion of the ICA, M1, M2, or BA



Naggara O, et al. “T2\* “Susceptibility Vessel Sign” Demonstrates Clot Location and Length in Acute Ischemic Stroke.” *PLoS ONE* 8 (2013).

\* Waqas M, et al. Effect of definition and methods on estimates of prevalence of large vessel occlusion in acute ischemic stroke: a systematic review and meta-analysis. *J Neurointerv Surg.* 2020;12:260-265.

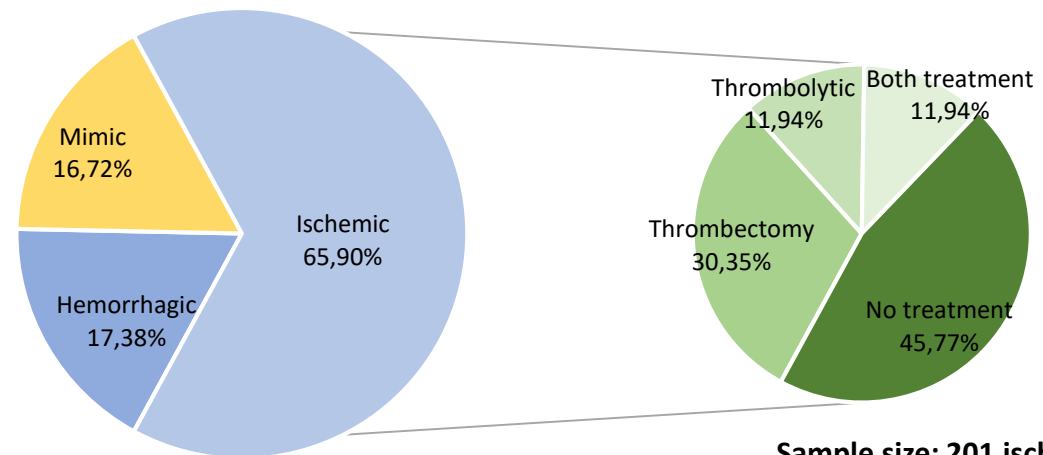
# BIOFAST: clinical data results



Total LVO patients	THROMBECTOMY	NO THROMBECTOMY
n=100	n=78	n=22

- 33% of the BIOFAST patients were LVOs
- 50% of the ischemic patients were LVOs

AMBULANCES	Total = 175
Ischemic	112
Hemorrhagic	33
Mimics	30
ER hospital	Total = 130
Ischemic	89
Hemorrhagic	20
Mimics	21



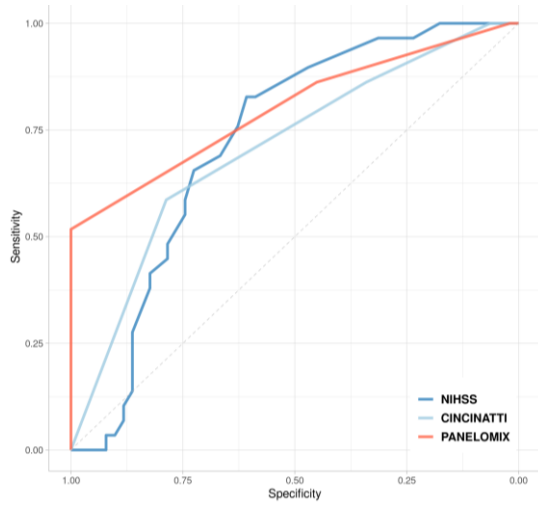
Sample size: 201 ischemic strokes

# LVO-Check adds value on top of scales

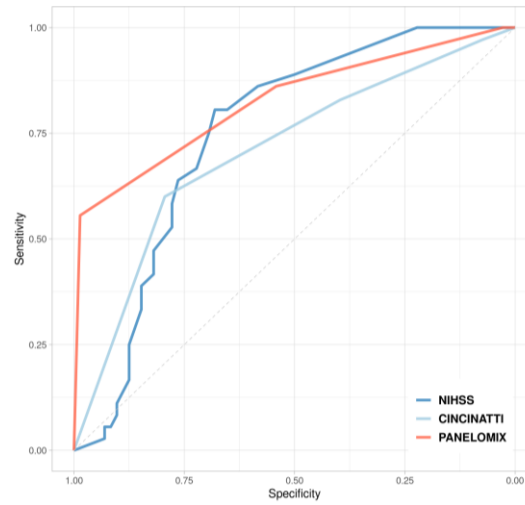
Hospital + Ambulances

Ambulances

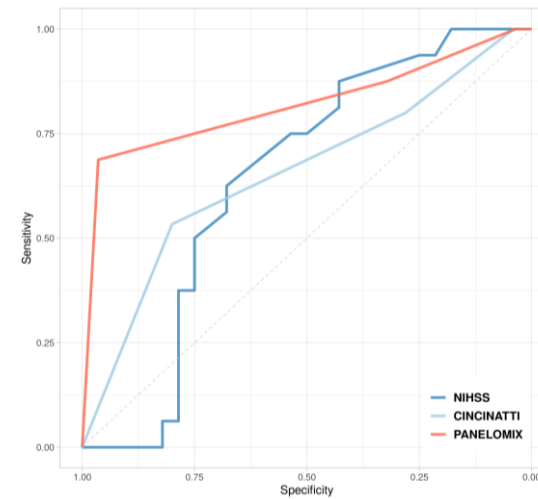
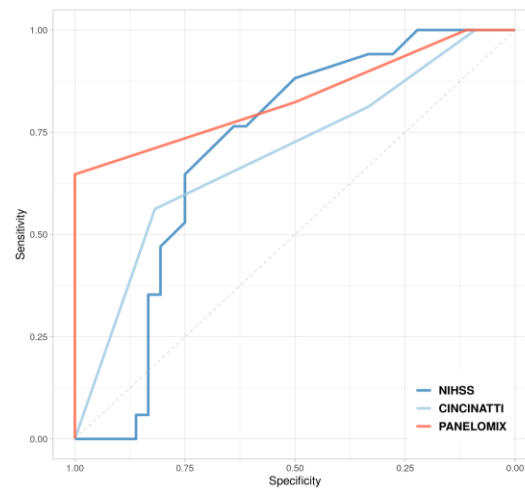
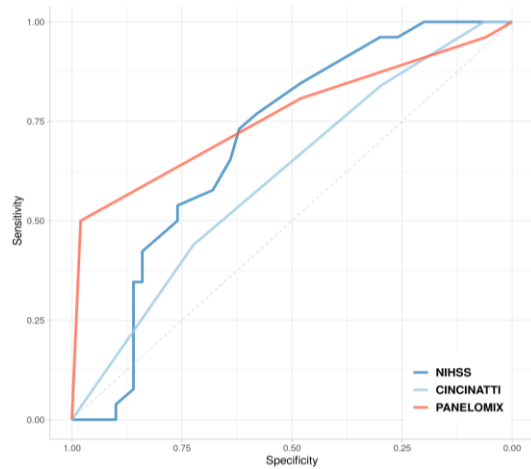
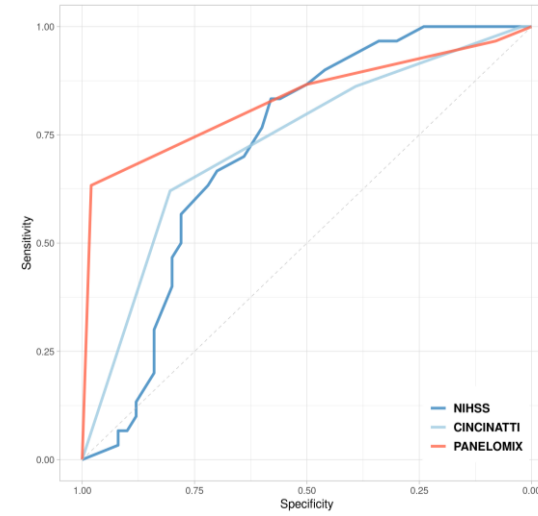
All times



<6 hours



<3 hours



# LVO-Check results

Accuracy of LVO-Check (FABP/NT-proBNP) in combination with simple clinical data (age, blood pressure, etc) and **without NIHSS scores**

## Ambulance

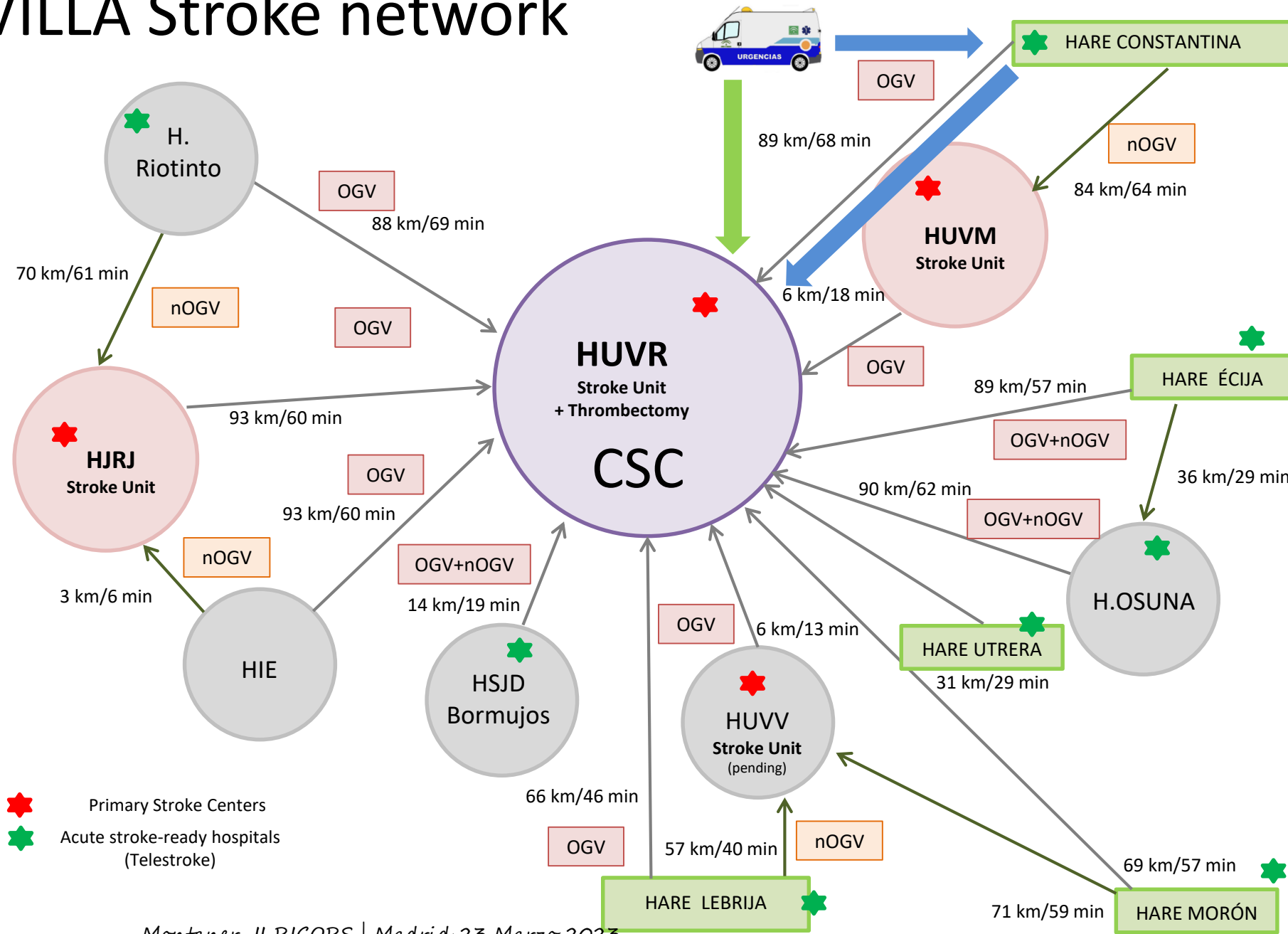
	Sensitivity	Specificity	PPV	Accuracy
All	40	98	91%	0.76
<6h	50	100	100%	0.77
<3h*	62	97	91%	0.71

\*LVO-Check, Age, Sampling times & NO neurological scales at all

## LVO-Check + Cincinnati



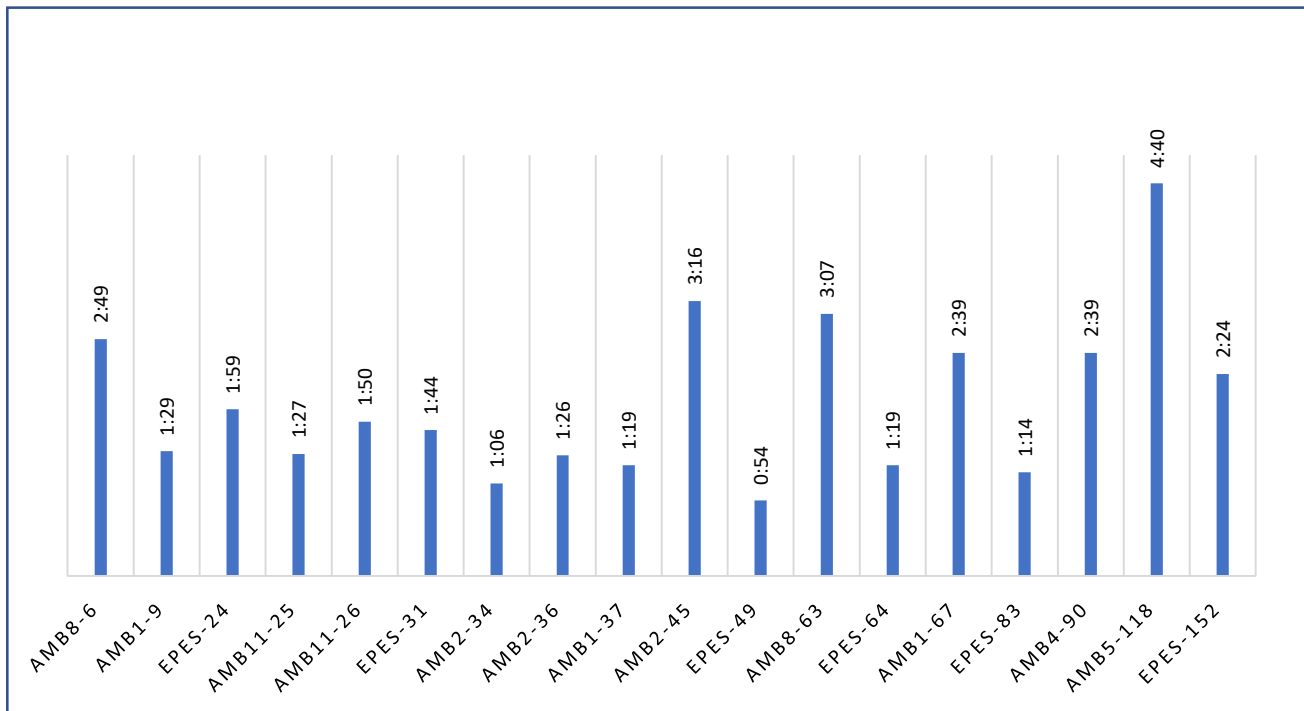
# SEVILLA Stroke network



- Following standard practice patients were driven to the closest t-PA-capable center and diagnosis was confirmed at arrival.
- Strict LVO definition including ICA, M1, M2 and basilar occlusion.
- Times from blood-drawn and rapid test performance were recorded as well as thrombectomy onset.

# Theoretical saved time

Saved time = [Real groin-puncture time - (Sample extraction time + 15 minutes of POCT Reading + time of transfer to CSC from the different origen points)]

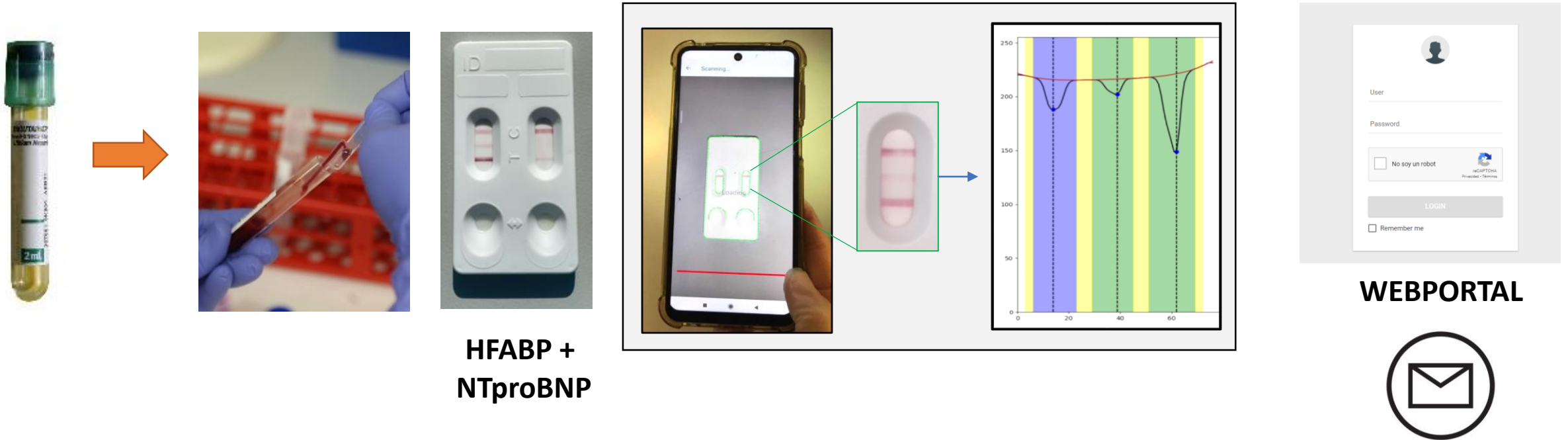


Time (hours) saved in each individual patient transferred for thrombectomy (n=18)

Max	4:40 h
Min	0:54 h
Median	1:47 h
Mean	2:04 h



# LVO-Check next steps...



An easy tool to launch large multicenter clinical trials is ready



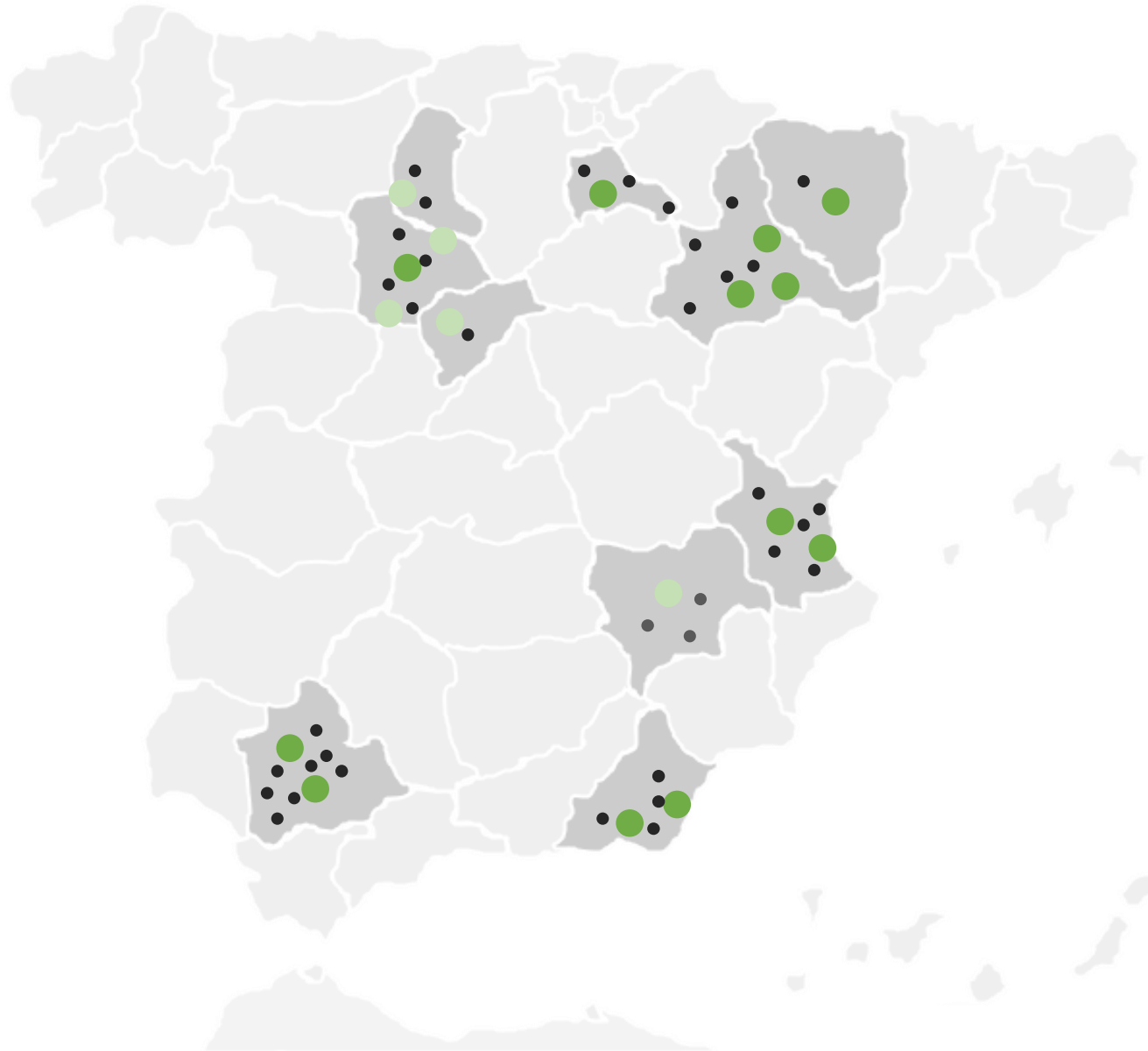
# BIO-SHIP TRAINING

## Confirmed

- Hospital ●
- Ambulance ●

## To be confirmed

- Hospital ●
- Ambulance ●



# BIO-SHIP TRAINING

## Confirmed

- Hospital ●
- Ambulance ●

## To be confirmed

- Hospital ●
- Ambulance ●



# BIO-SHIP TRAINING

## Confirmed

- Hospital ●
- Ambulance ●

## To be confirmed

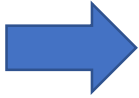
- Hospital ●
- Ambulance ●



# BIO-SHIP CLINICAL TRIAL



**1.000 patients** with stroke suspicion = "stroke code" activation



- 170 patients = **MIMICS**
- 170 patients **HEMORRHAGIC STROKES**
- 660 patients **ISCHEMIC STROKES**
  - 330 without LVO
  - 330 with **LVO**

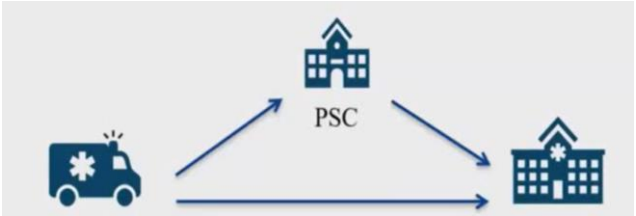
All get the POCT done

96% Specificity & 70 % Sensitivity for LVO



From the 330 LVOs we will detect properly with the POCT around **240 LVO patients**

**ARM A: standard management (n=120)**



**ARM B: direct to thrombectomy (n=120)**



Randomization

## European Innovation Council

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### EIC Accelerator

Funding and investments through the EIC Fund for individual start-ups and small companies to develop and scale up game changing innovations

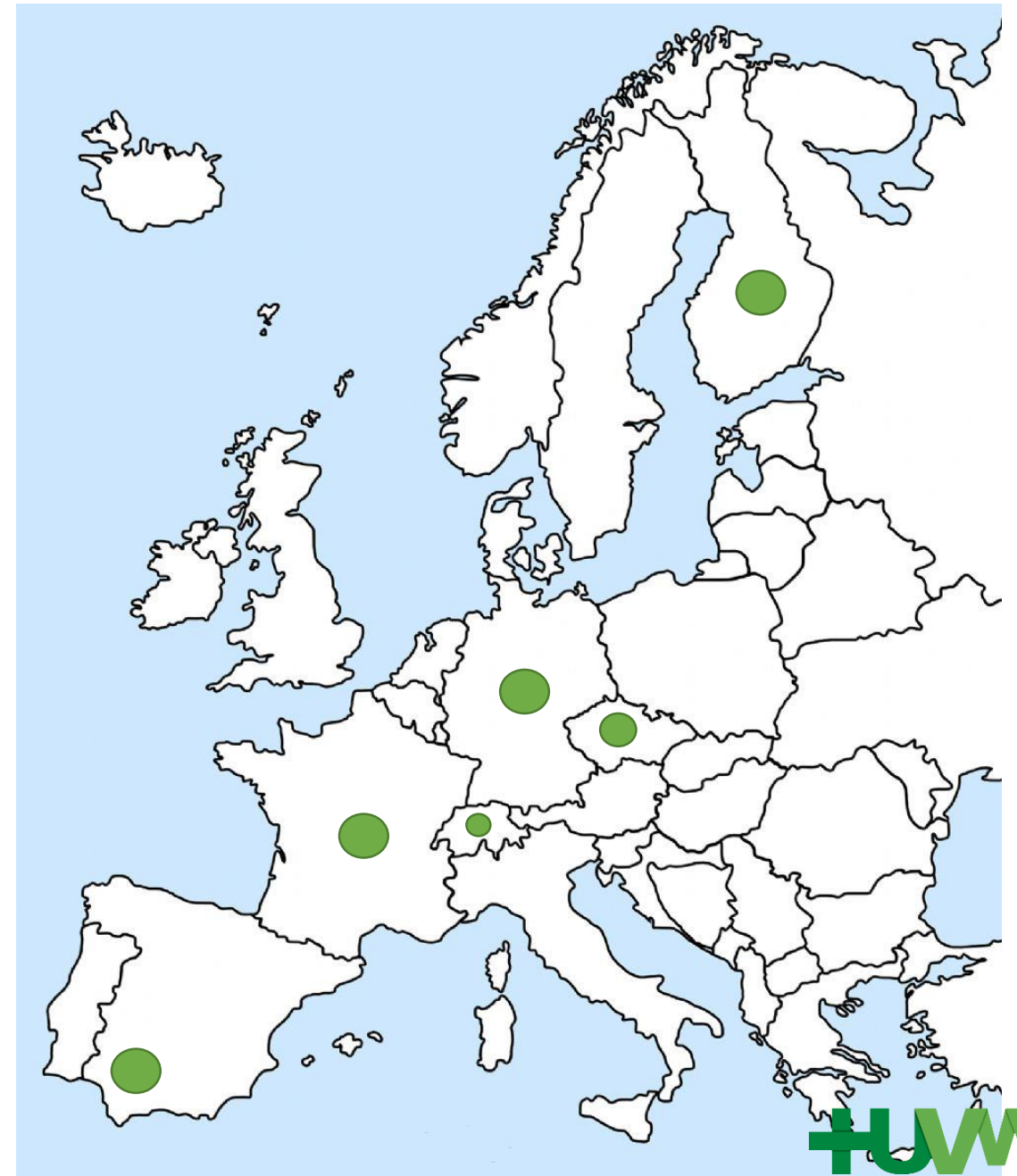
European  
Innovation  
Council





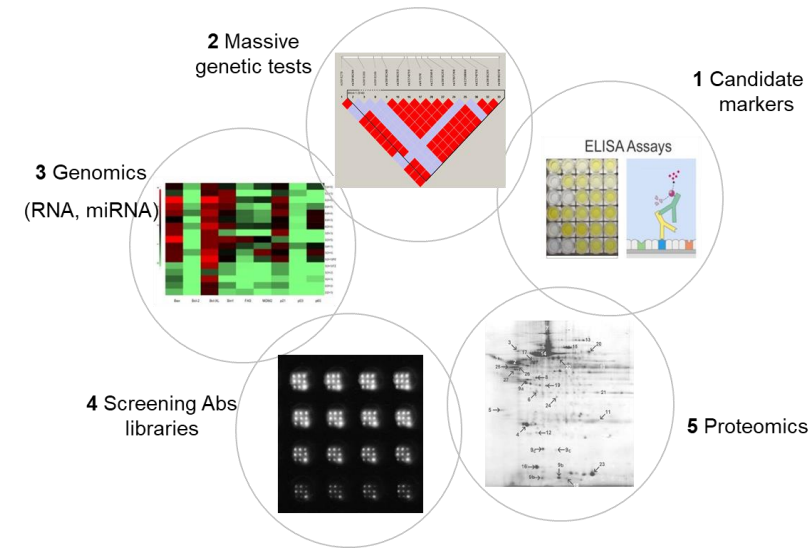
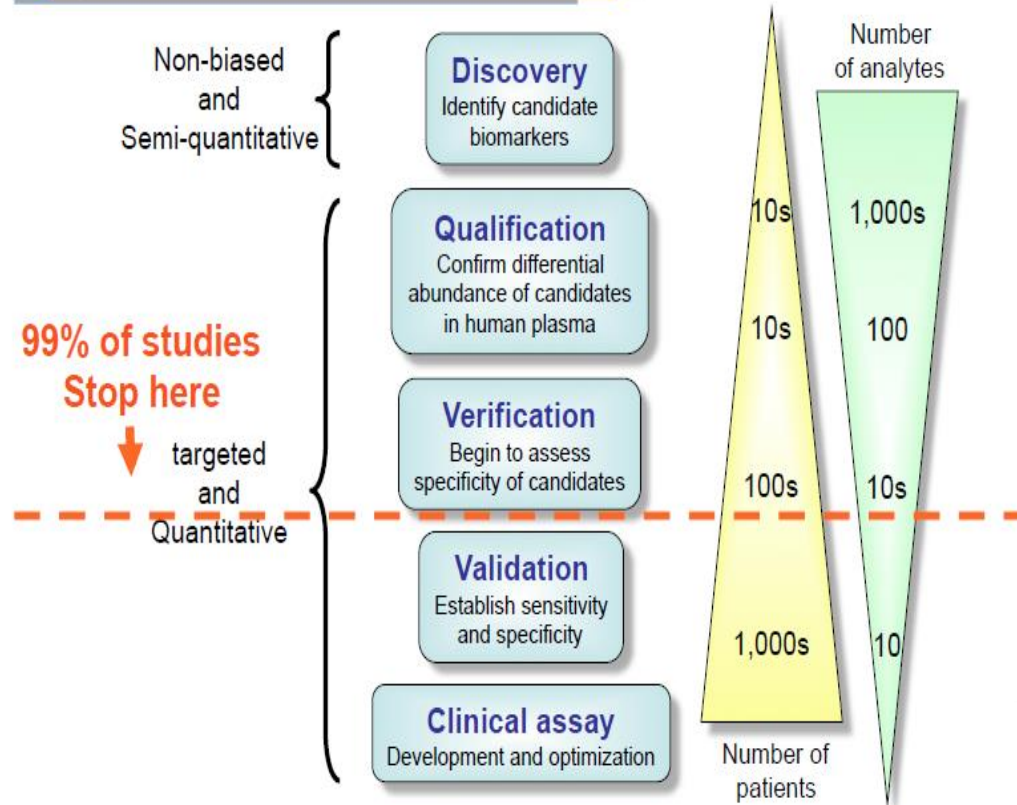
2,7 M€ grant

1,6 M€ para hacer BIOSHIP-Europa



- Excellent markers come clearly in a discovery but use to be suboptimal and need to be complemented with other biomarkers or clinical data
- Do your discovery in the final samples you would like to use those biomarkers
- And in a relevant number of samples
- Do we have a gold standard to compare?
- If not, is hard that you get a biomarker that diagnose or predicts an unstable phenotype
- Escape those situations

## Biomarker discovery and validation



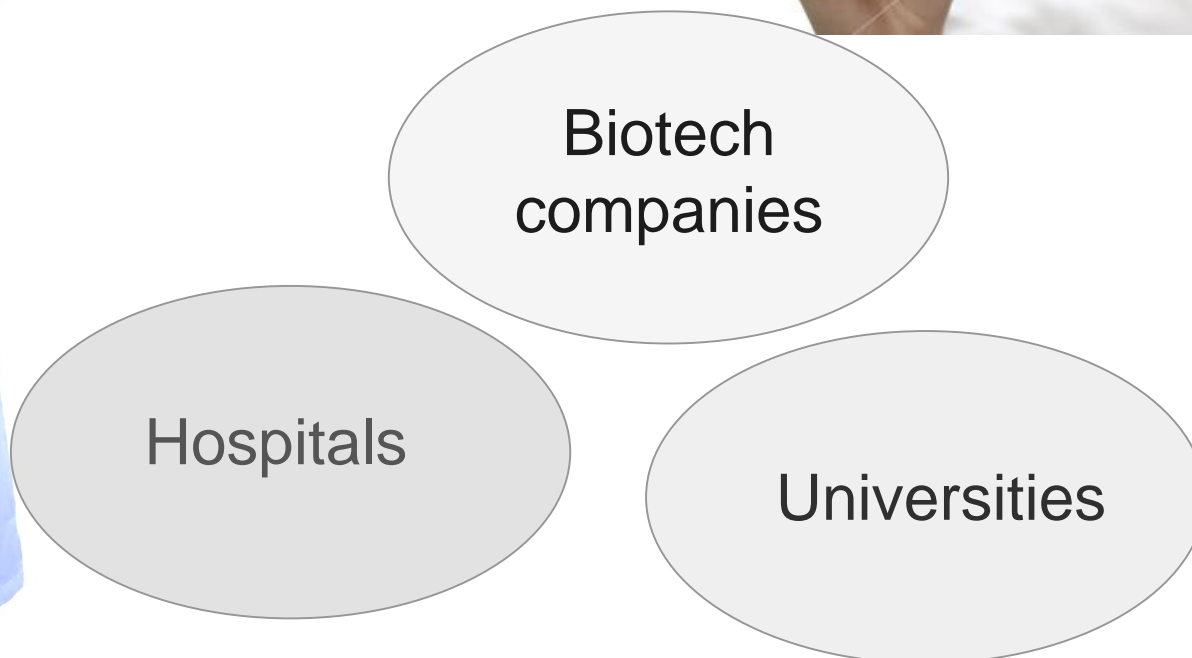
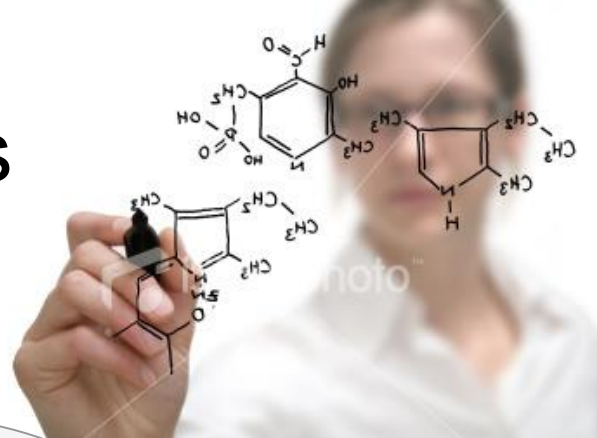
New protein arrays



- Lab talk to clinicians before expts design
- Both talk with biotech companies
- Grants are not ready for a full development
- Companies should go back to the clinicians
- Do we really need a POCT
- What is the desired sens/specif



# Stroke Biomarkers Implementation









# BIOSHIP-training



“Estudio del uso potencial de un dispositivo rápido de Point-of-Care para el traslado directo de pacientes con ictus desde la ambulancia al centro de trombectomía”

PIN-0139-2020

## REALIZACIÓN DEL TEST DE BIOMARCADORES

Grupo de investigación de Neurovascular



Financiado por:





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