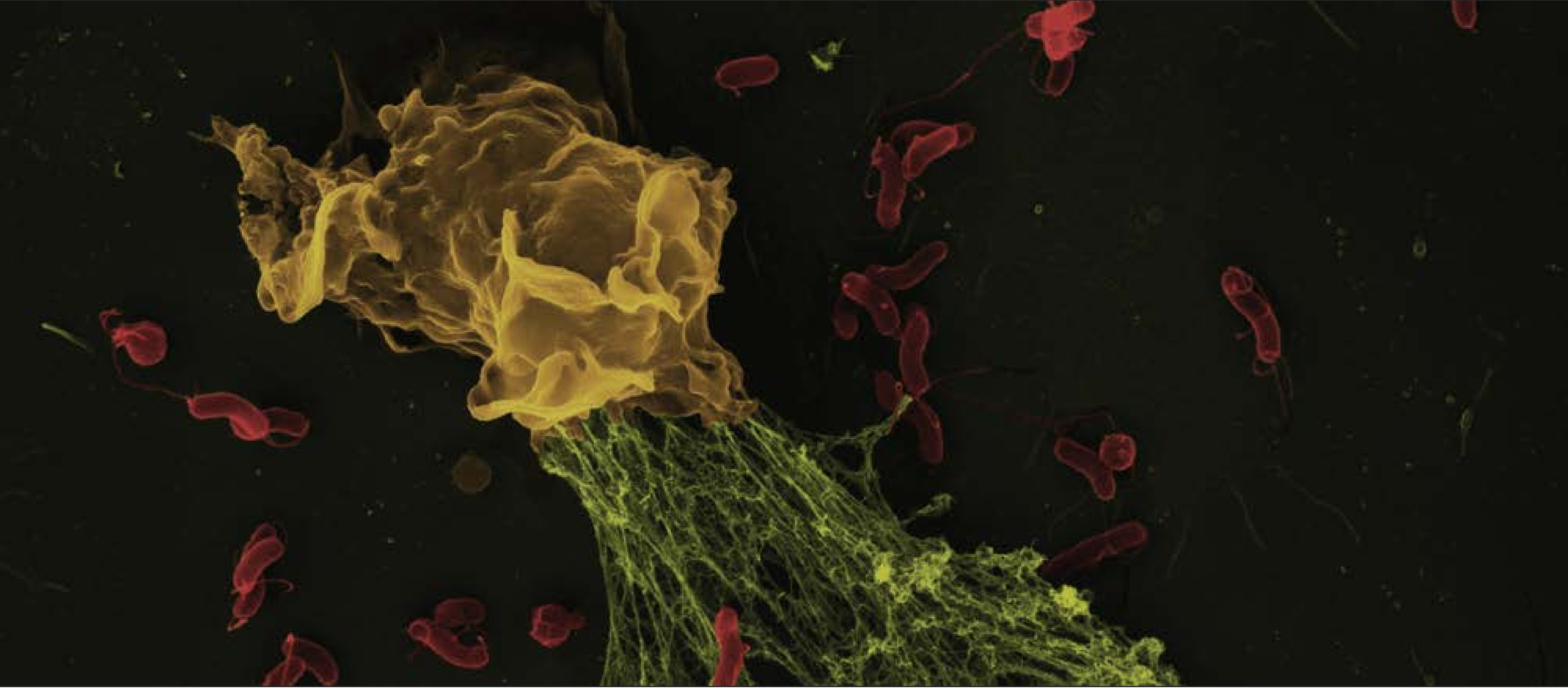
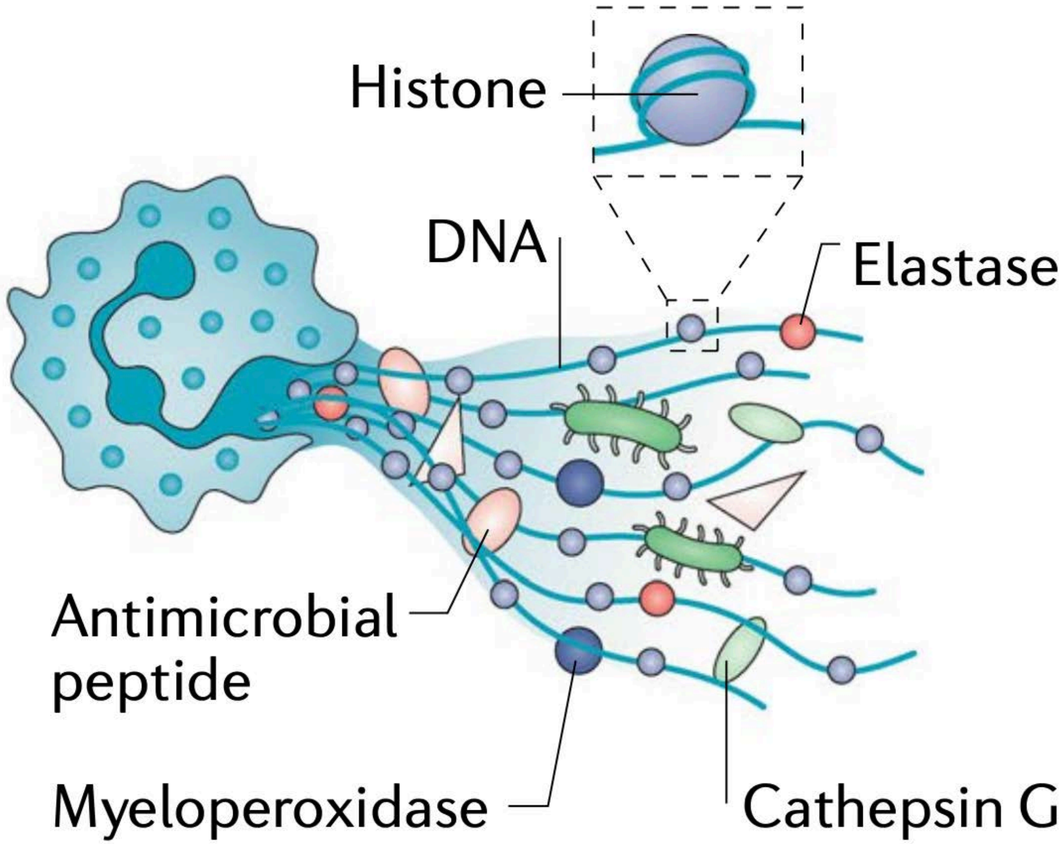


IMPLICACIÓN DE LOS NETs EN EL PRONÓSTICO DEL ICTUS ISQUÉMICO

RICORS-ICTUS | Instituto de Salud Carlos III

i+12 Instituto de Investigación Hospital 12 de Octubre

- ¿Qué es un NET o trampa extracelular de neutrófilo?



Mecanismo de inmunidad innata

- Defensa contra patógenos
- Deletéreo en patologías inflamatorias

Antecedentes en ictus isquémico

- Promoción de inmunotrombosis
- Estabilización del trombo (?)

REPORTS Science

Neutrophil Extracellular Traps Kill Bacteria

Volker Brinkmann,[1] Ulrike Reichard,[1,2] Christian Goosmann,[1,2] Beatrix Fauler,[1] Yvonne Uhlemann,[2] David S. Weiss,[2] Yvette Weinrauch,[3] Arturo Zychlinsky[2]*

REVIEWS

nature reviews immunology

Neutrophil extracellular traps in immunity and disease

Venizelos Papayannopoulos

“

Las redes extracelulares de neutrófilos (NETs) tienen un papel importante en la fisiopatología del daño tisular tras ictus isquémico y por tanto, se asocian al pronóstico de la enfermedad. De esta manera, pacientes con mayores niveles de NETs tendrán una peor evolución neurológica y funcional.

- **Inclusion Criteria**
  - Age ≥18 years old
  - Patients suffering ischemic stroke
  - ≤9 hours since onset of symptoms / Wake-up stroke (established neurological symptoms assessed by NIHSS)
  - Previously independent patients (previous modified Rankin Score ≤2)
  - Patients admitted to the Stroke Unit
- **Exclusion Criteria**
  - Transitory Ischemic Attack or lacunar infarction.
  - Bleeding secondary to underlying traumatic injury or subarachnoid hemorrhage
  - Stroke, myocardial infarction, major surgery or systemic infection in the last 3 months
  - Severe systemic disease: cancer, chronic kidney disease undergoing hemodyalisis, liver failure
  - Systemic inflamatory disease that is active or under treatment
  - Pregnancy or puerperium
  - Participation in other analytical research studies such as an acute phase clinical trial

Nuestra base de datos

Episode Information

1. Date and time of onset / last time seen well
2. Known-onset / Wake-up Stroke
3. Treatment: tPA, thrombectomy
4. Infarct Volume (cc): MRI DWI (admission) or CT control scan (48h)
5. Occlusion territory
6. Etiology (atherothrombotic, cardioembolic, unknown, mixed causes)
7. ASPECTS (MCA territory)
8. TICI score (if thrombectomy)
9. Hemorrhagic transformation
10. Door-to-needle time
11. Door-to-puncture time
12. Time to reperfusion

Sample Processing information

1. Blood extraction time
2. Blood processing time

Patient Data and History

1. Age
2. Sex
3. CV Risk factors: smoking, alcohol use, hypertension, dyslipidemia, DM2, Afib
4. Previous medication: previous anticoagulant/antiplatelet agents
5. Mechanical valve prothesis
6. History of prior hemorrhagic/ ischemic stroke
7. History of myocardial Infarction
8. Peripheral arterial disease
9. CKD

Neurological Status Scores

1. NIHSS (ER)
2. NIHSS (Stroke Unit)
3. Previous mRS
4. 3 month mRS

ER Constants and Blood Tests

1. Blood Pressure Readings (ER)
2. Blood test Biochemistry panel, cell counts and coagulation parameters (ER and at Stroke Unit)
    1. Creatinine
    2. Glucemia
    3. C Reactive Protein
    4. Leukocytes
    5. Neutrophils
    6. Lymphocytes
    7. Monocytes
    8. Platelets
    9. INR

NETs Studies

1. Plasma NETosis Biomarkers
    1. Cit-Histone 3
    2. Myeloperoxidase
    3. Neutrophil Elastase
    4. Cell-free dsDNA
2. DNAse1 Polymorphism Phenotype
3. Thrombi Composition Determination: IHC-P and Proteomics

REDCap
Research Electronic Data Capture

Excel

Access

Nuestros ensayos

326
PATIENTS

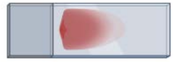
Blood Samples
(Admission and 24h)
N= 220

Thrombi (N=185)

Buffy Coat

Plasma

Blood Smear



Fresh

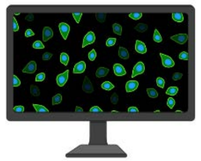
4% PFA + sucrose

DNA Genotype

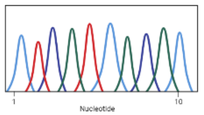
NETs
biomarkers (ELISA)

Proteomics

Immunohistochemistry
(IHC-P)



DNAse1 polymorphism
220 sequenced
patients

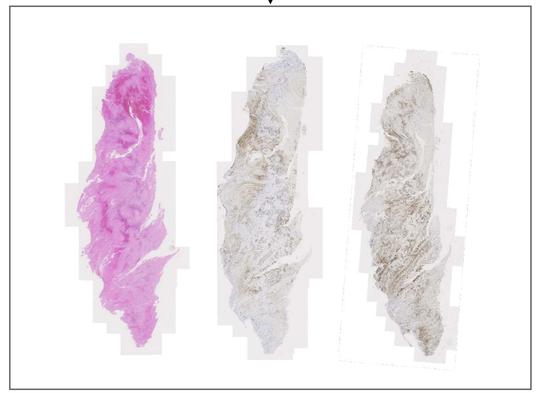


211 assays of cfDNA

211
assays of Neutrophil
Elastase

211 assays of MPO

159 assays of cit-H3



NIGHT (6:01 pm -5:59 am)

60 pacientes

DAY (6:00 am - 6pm)

159 pacientes

WAKE-UP STROKE

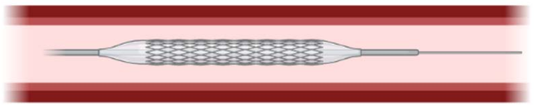
61 pacientes

73 pacientes
<65 años

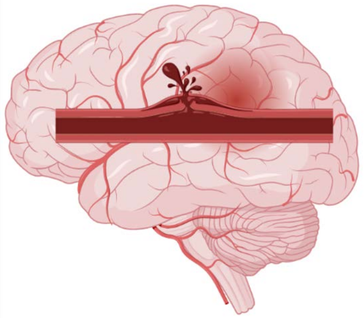
252 pacientes
≥65 años

151 pacientes TICI 2c-3

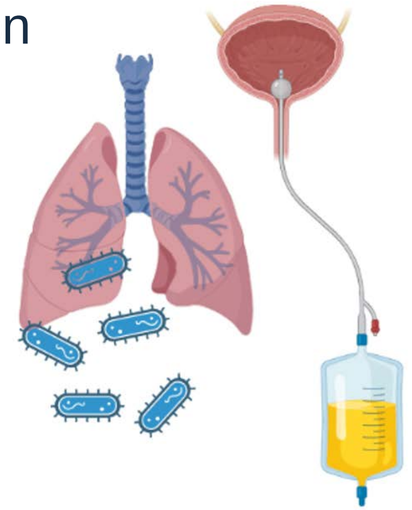
52 pacientes TICI 0-2b

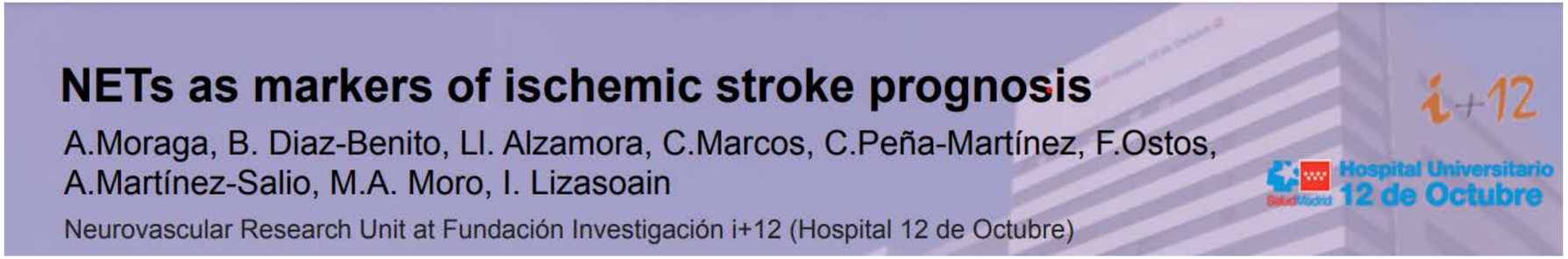


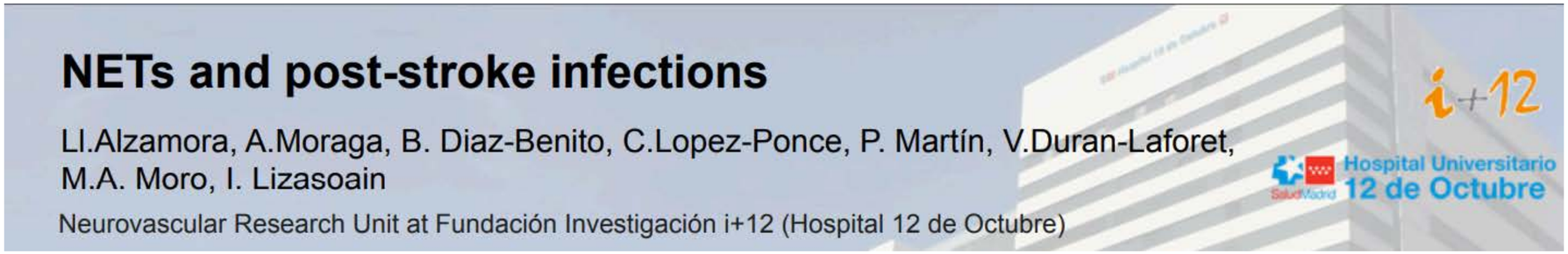
34 pacientes con transformación hemorrágica



65 pacientes con infecciones





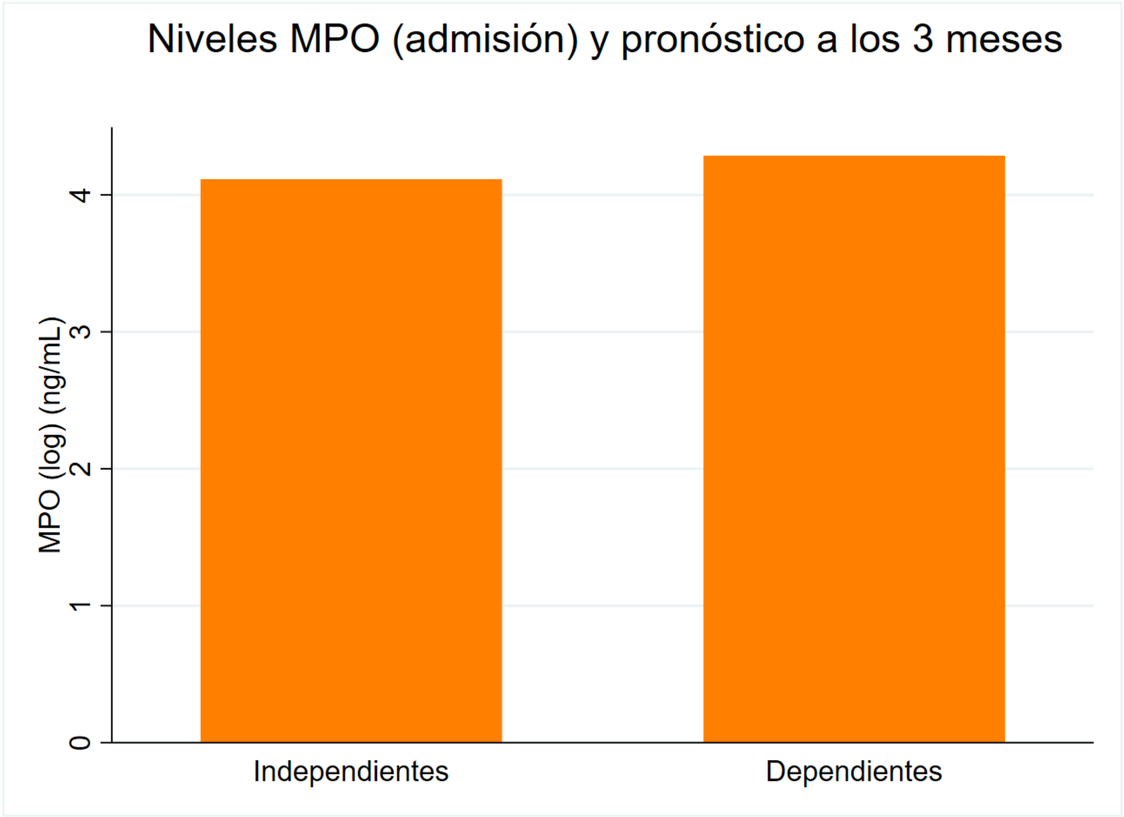


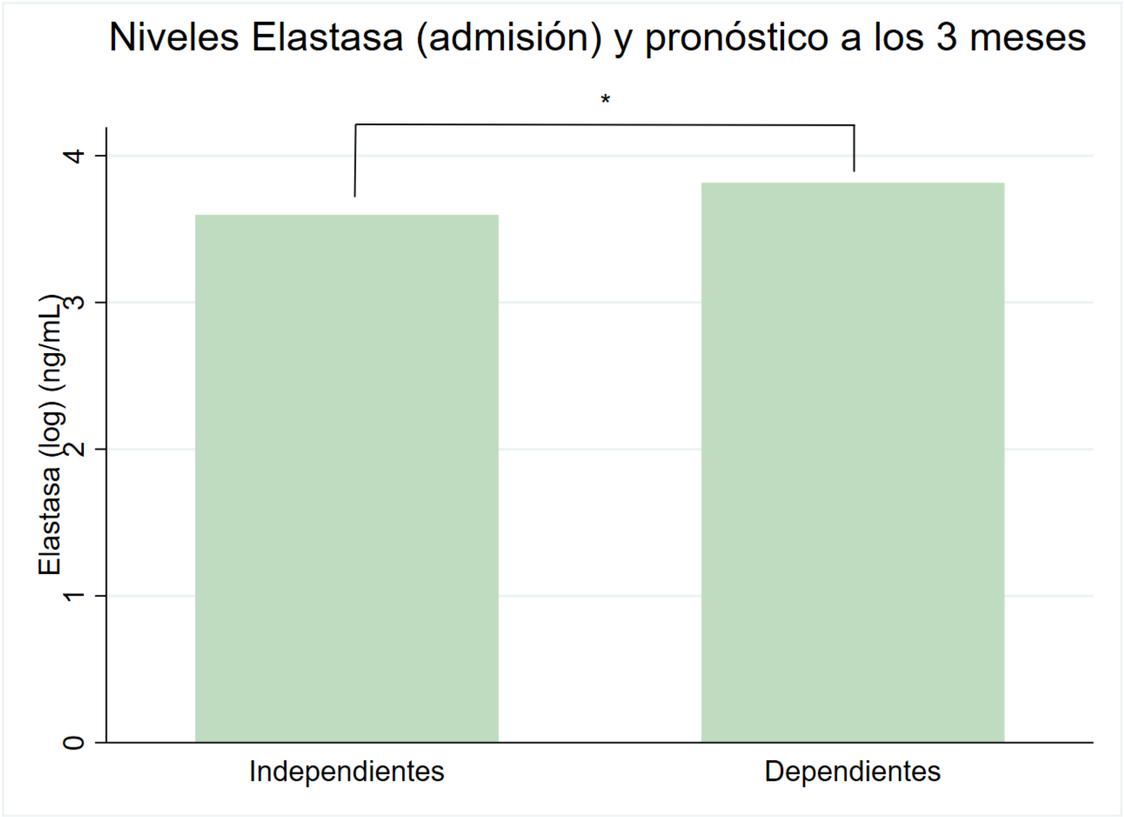
ESOC 2022
4-6 MAY 2022
LYON, FRANCE
The Voice of Stroke in Europe

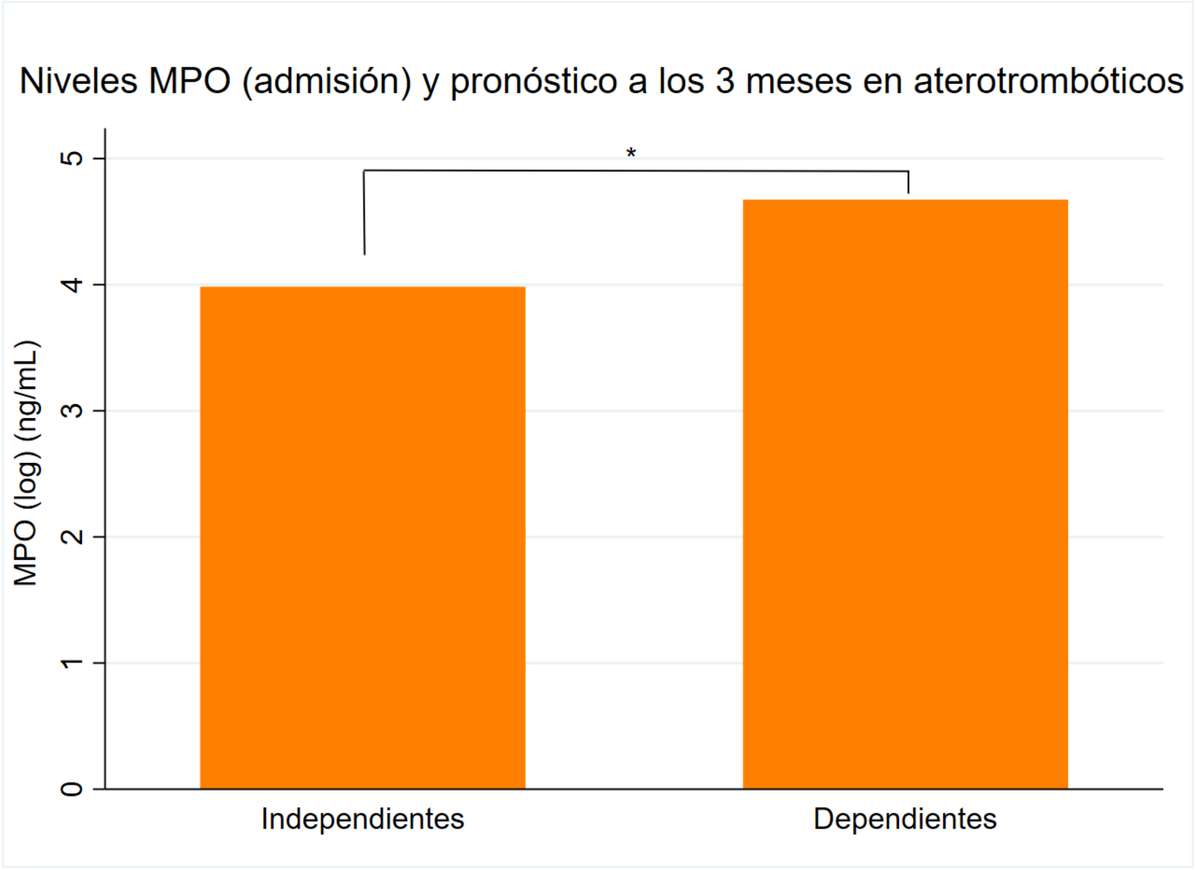
NETs como biomarcadores de pronóstico

Variables	Cases	N	3-month mRS ≤2 (Functionally Independent)	N	3-month mRS ≥3<6 (Functionally Dependent)	p-value
Admission NETs markers (Plasma), Mean (SE)						
Citrullinated-Histone H3 (log)	**Total**	92	0.67 (0.12)	41	0.455 (0.12)	0.229
	Atherothrombotic	30	0.17 (0.17)	9	0.55 (0.24)	0.256
	Cardioembolic	40	0.62 (0.17)	18	0.70 (0.17)	0.785
cfDNA (log)	**Total**	131	6.67 (0.01)	51	6.62 (0.02)	0.084
	Atherothrombotic	43	6.67 (0.02)	13	6.68 (0.04)	0.847
	Cardioembolic	50	6.64 (0.02)	24	6.64 (0.03)	0.990
Neutrophil Elastase (log)	**Total**	128	3.59 (0.05)	53	3.81 (0.10)	**0.042**
	Atherothrombotic	44	3.48 (0.07)	14	3.81 (0.22)	0.083
	Cardioembolic	51	3.66 (0.08)	24	3.74 (0.17)	0.626
Myeloperoxidase (log)	**Total**	132	4.11 (0.06)	49	4.28 (0.12)	0.173
	Atherothrombotic	45	3.98 (0.09)	12	4.67 (0.32)	**0.006**
	Cardioembolic	50	4.18 (0.10)	23	4.15 (0.16)	0.900

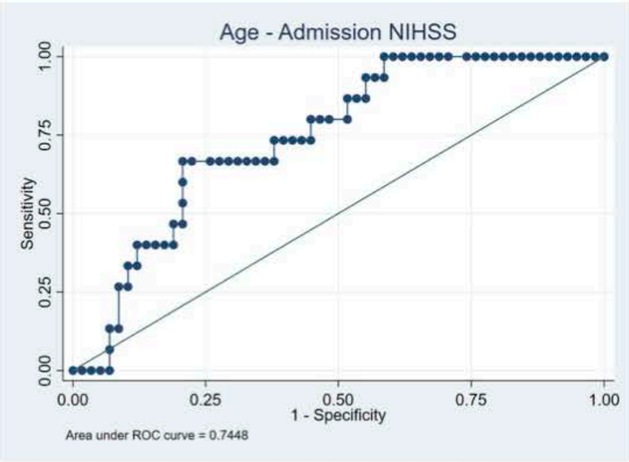
Student's t-test (p*≤0.05)


Niveles MPO (admisión) y pronóstico a los 3 meses
MPO (log) (ng/mL)
0
1
2
3
4
Independientes
Dependientes

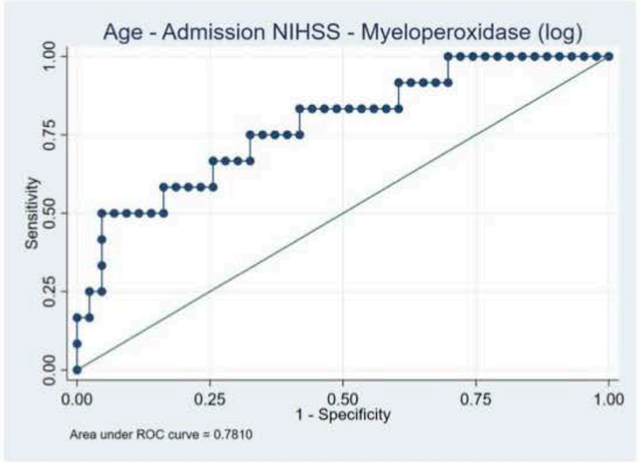

Niveles Elastasa (admisión) y pronóstico a los 3 meses
*
Elastasa (log) (ng/mL)
0
1
2
3
4
Independientes
Dependientes


Niveles MPO (admisión) y pronóstico a los 3 meses en aterotrombóticos
MPO (log) (ng/mL)
0
1
2
3
4
5
*
Independientes
Dependientes

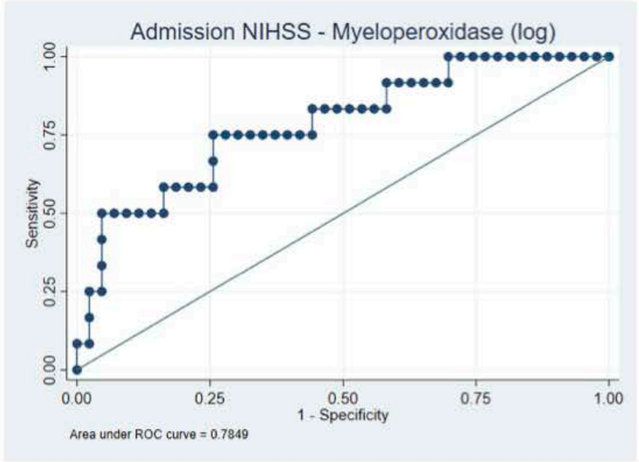
Modelos multivariables para predecir dependencia a los 90 días en ictus aterotrombótico



Variable	Odds Ratio	95% CI	p-value
Age	1.012	0.95 – 1.07	0.668
NIHSS (admission)	1.105	1.01 – 1.19	**0.015**
C-statistic: 0.7448			



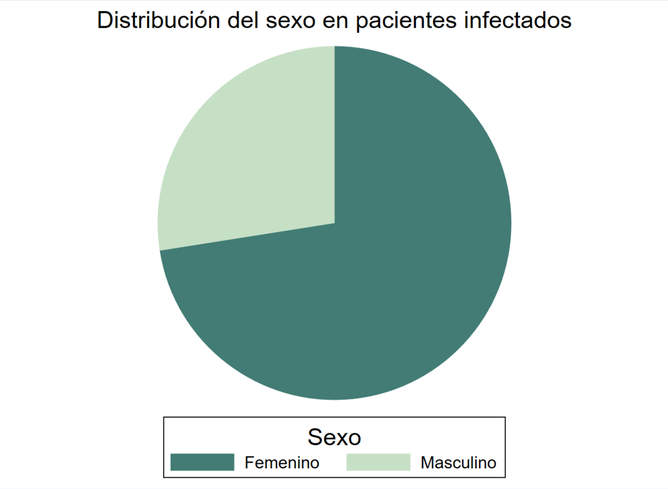
Variable	Odds Ratio	95% CI	p-value
Age	1.006	0.94 – 1,07	0.838
NIHSS (admission)	1.093	0.98 – 1.21	0.090
MPO (log)	3.077	1.22 – 7.71	**0.017**
C-statistic: 0.7810			



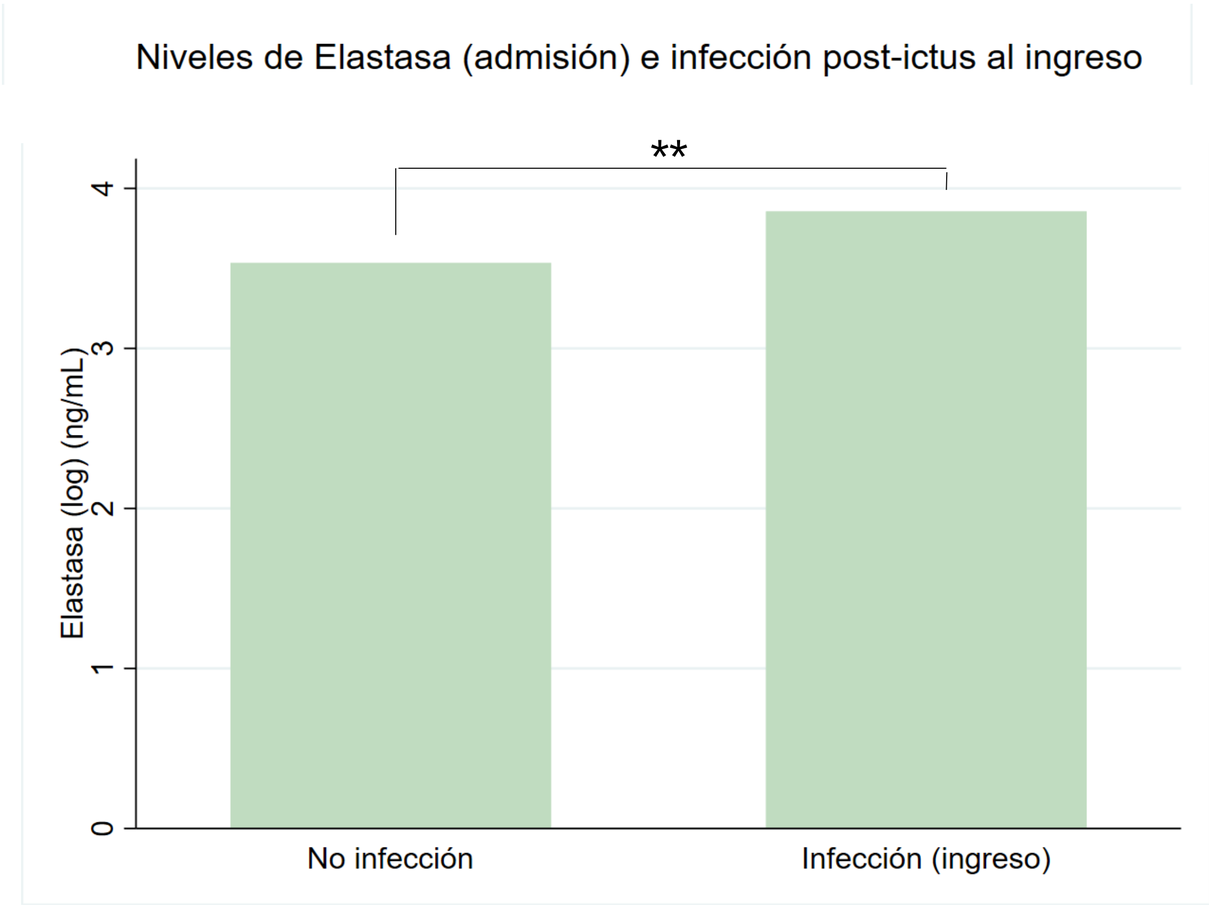
Variable	Odds Ratio	95% CI	p-value
NIHSS (admission)	0.991	0.95 – 1.21	0.073
MPO (log)	3.078	1.21– 7.77	**0.017**
C-statistic: 0.7849			

Regresión logística binaria

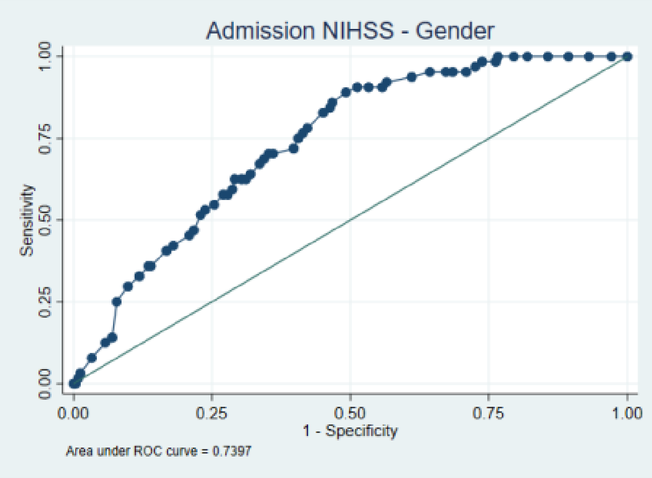
NETs como biomarcadores de infección

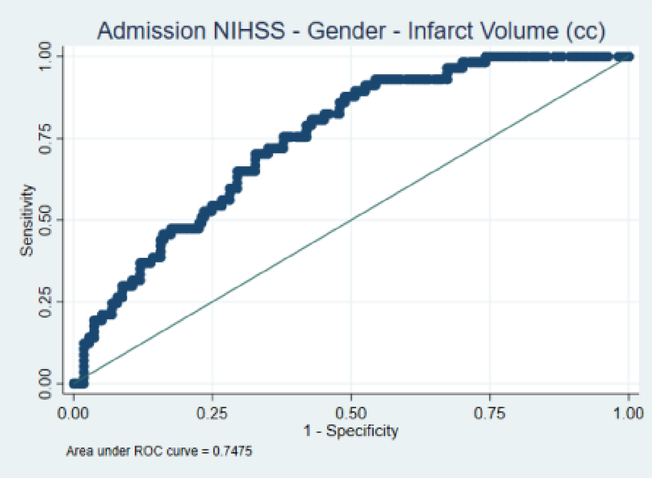


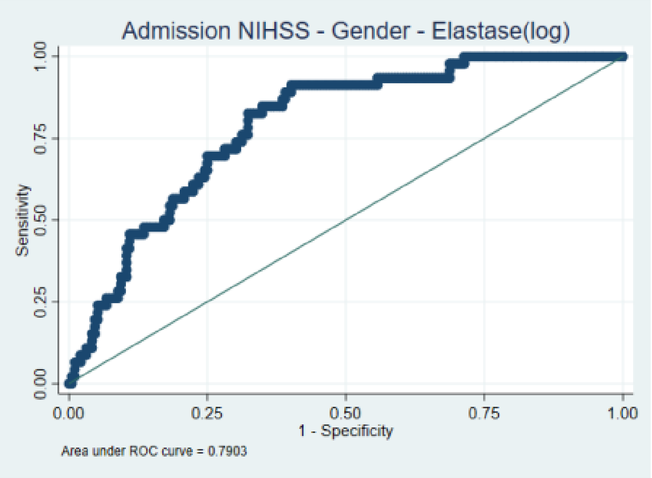
	Varones N=152	Mujeres N=177	p-value
Infecciones	18 (12.33%)	47 (27.49%)	**<0.001**
Tipo de infección			0.40
ITUs	5 (27.78%)	9 (19.57%)	
Neumonía	10 (55.56%)	20 (43.48%)	
Otras infecciones	1 (5.56%)	10 (21.74%)	
Múltiples infecciones	2 (11.11%)	7 (15.22%)	



Modelos multivariables para predecir infección post-ictus en el ingreso







Variable	Odds Ratio	95% CI	p-value
NIHSS (admission)	1.09	1.05 -1.14	**<0.001**
Female Gender	2.10	1.12 - 3.94	**0.02**
C-statistic: 0.7397			

Variable	Odds Ratio	95% CI	p-value
NIHSS (admission)	1.09	1.05 -1.14	**<0.001**
Female Gender	2.10	1.12 - 3.94	**0.02**
Infarct Volume (cc)	1.002854	0.99 - 1.00	0.189
C-statistic: 0.7475			

Variable	Odds Ratio	95% CI	p-value
NIHSS (admission)	1.10	1.04 -1.15	**<0.001**
Female Gender	3.32	1.46 - 7.51	**0.04**
Elastase (log)	1.80	1.05 - 3.08	**0.032**
C-statistic: 0.7903			

Biomarcadores

- **MPO y Elastasa** están probando ser mejores marcadores de NETosis en plasma en nuestra cohorte (vs. cfDNA y citH3)

Dependencia a los 90 días

- **Los NETs** son mejores **marcadores pronósticos** en ictus **aterotrombótico** (vs. Cardioembólico)

Infección durante el ingreso

- Niveles de **Elastasa** al ingreso podrían predecir el desarrollo de **infecciones** durante la estancia hospitalaria

Conclusiones

- De esta manera, se confirma su importancia en la fisiopatología del ictus isquémico

Aumentar número de muestras (colaboración con otros hospitales)

Publicar resultados

Análisis proteómico de trombos

Análisis histopatológico de trombos

Medición de NETs mediante citometría y Western Blot

Plantear estudios clínicos

GRACIAS POR SU ATENCIÓN

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