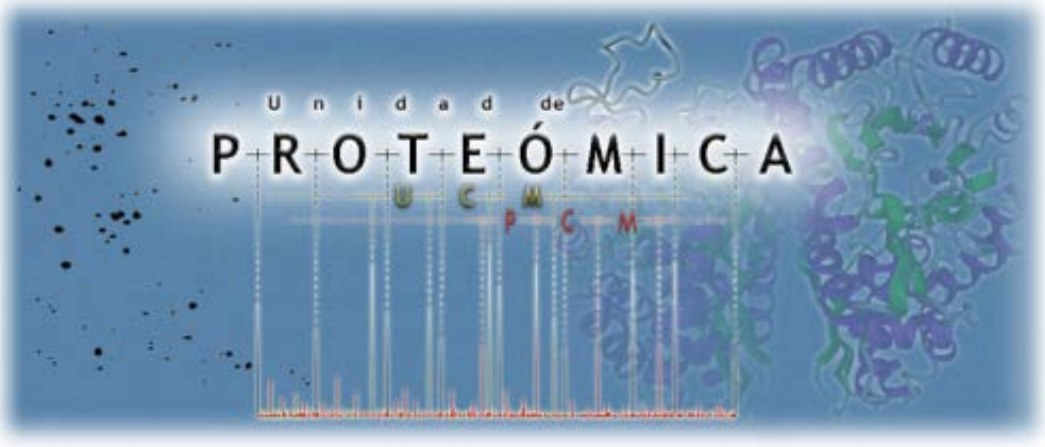
APLICACIONES DE LA PROTEÓMICA EN LA CLINICA

Fernando Vivanco-Departamento de BBM-I/IIS-FJD



CENTROS DE APOYO A LA INVESTIGACION (CAIS)

UCM

Los CAIS de la UCM son un conjunto de **instalaciones imprescindibles** (centros neurálgicos) para el desarrollo de la **actividad investigadora.**

Los CAIS (Unidad de Proteómica) son un verdadero **SERVICIO** a la comunidad científica de la UCM y de otras instituciones.

El desarrollo actual de la ciencia y de la investigación son **Instrumentación-dependiente** y esta dependencia va a incrementarse en el futuro próximo.

La Unidad de Proteómica de la UCM:

-Da servicio a los **investigadores** de la UCM y otra instituciones (OPIS, empresas).

-Pertenece a la red Nacional de Servicios de Proteómica (**PROTEORED**).

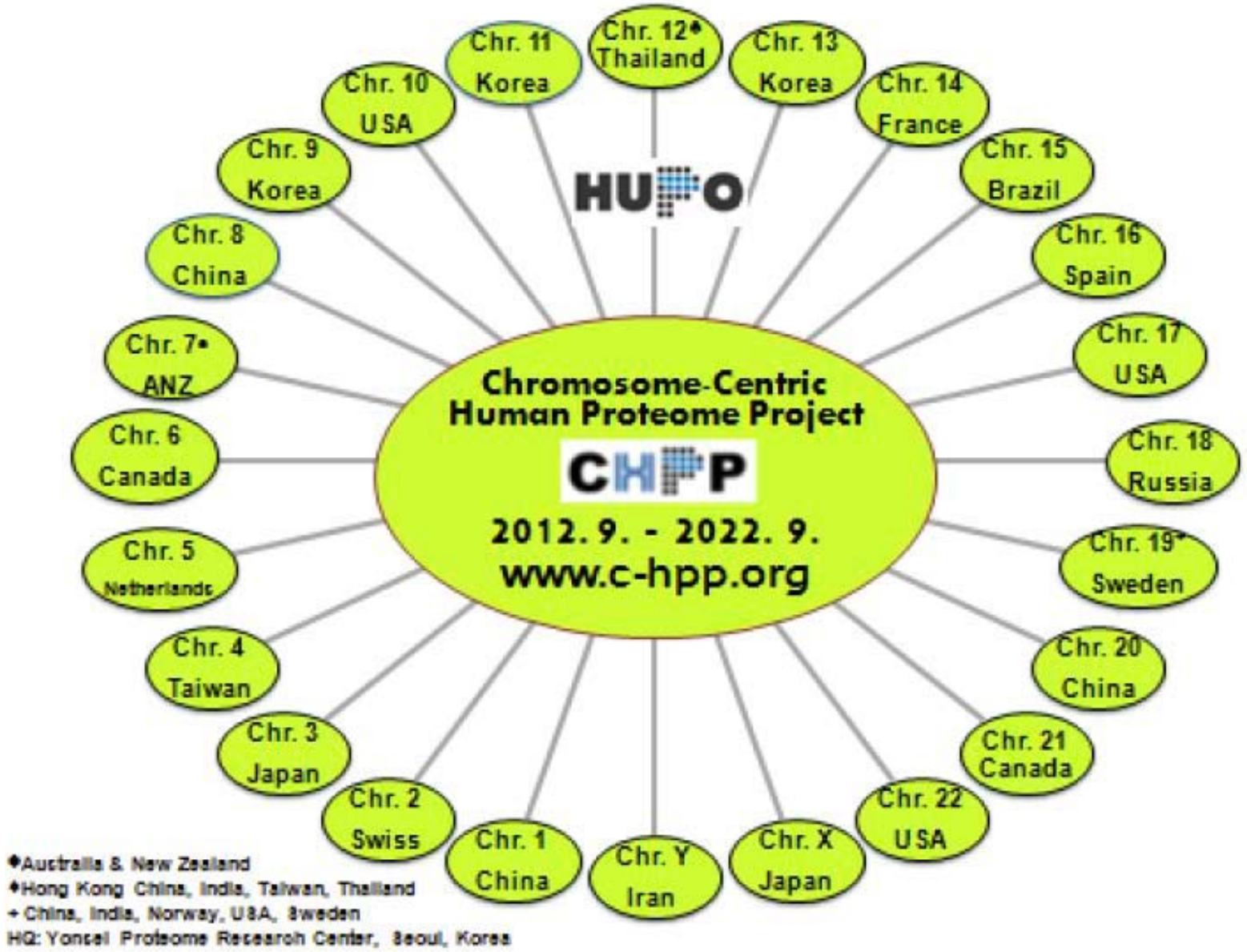
-Lleva a cabo **ensayos multicéntricos** a nivel nacional e internacional para garantizar la calidad de sus análisis.

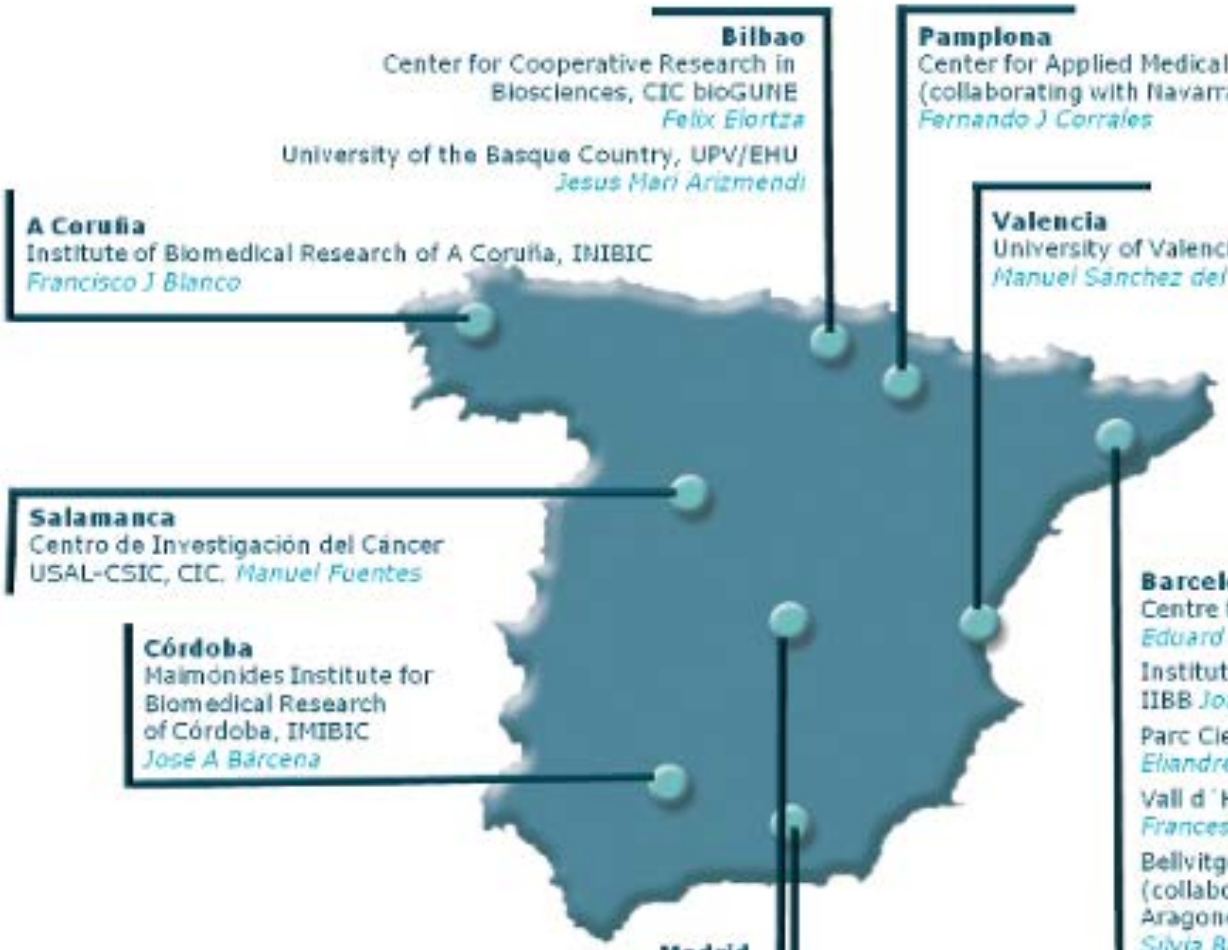
-Participa en la **docencia** de la UCM con cursos y practicas en diversos Masters.

-Participa en el Consorcio Internacional encargado de descifrar el **PROYECTO PROTEOMA HUMANO**.

-**Transfiere** la Tecnología Proteómica a los **hospitales** para su implantación en la clínica humana.

CONSORCIO INTERNACIONAL: PROYECTO PROTEOMA HUMANO





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Fundación Jiménez Díaz (collaborating with Hospital Nacional de Parapléjicos) *Fernando Vivanco*

Granada
Institute of Parasitology and Biomedicine "López-Neyra" (CSIC), IPBLN
Jaime Sancho

Coordination Unit
Center for Applied Medical Research, CIMA

QUÉ ES EN REALIDAD LA UNIDAD DE PROTEÓMICA DE LA UCM para un usuario :

Un local (pequeño) en el que entras con un tubo de ensayo (conteniendo una muestra biológica) y sales con un pendrive (o CD) conteniendo un mínimo de 2 Gigas de información).

Es por tanto un centro **Generador de Datos**:

-De carácter básico (de investigación fundamental)

-De carácter aplicado (investigación aplicada/ traslacional): el ejemplo de la **Proteómica Clínica**

En los últimos 5 años (periodo 2012-2016) nuestro grupo ha producido:

Un total de 61 publicaciones, de las cuales **52** contienen datos fundamentalmente obtenidos:

- en el **CAI de Proteómica** de la UCM (**Proteínas**)

- en el **CAI de Resonancia Magnética Nuclear** de la UCM (**Metabolitos**)

Patients with calcific aortic stenosis exhibit systemic molecular evidence of ischemia, enhanced coagulation, oxidative stress and impaired cholesterol transport. *Int J Cardiol*. 2016 Sep 26;225:99-106.

Hypertensive **patients** exhibit an altered metabolism. A specific metabolite signature in urine is able to predict albuminuria progression. *Transl Res*. 2016 Jul 15. pii: S1931-5244(16)30108-6.

Plasma Molecular Signatures in Hypertensive **Patients** With Renin-Angiotensin System Suppression: New Predictors of Renal Damage and De Novo Albuminuria Indicators. *Hypertension.* 2016 Jul;68(1):157-66.

Detection of major food allergens in amniotic fluid: initial allergenic encounter during pregnancy. *Pediatr Allergy Immunol*. 2016 Jun 24. doi: 10.1111/pai.12608.

Urinary Kininogen-1 and Retinol binding protein-4 respond to Acute Kidney Injury: predictors of **patient** prognosis? *Sci Rep*. 2016 Jan 21;6:19667. doi: 10.1038/srep19667.

Urinary alpha-1 antitrypsin and CD59 glycoprotein predict albuminuria development in hypertensive **patients** under chronic renin-angiotensin system suppression. *Cardiovasc Diabetol.* 2016 Jan 16;15:8. doi: 10.1186/s12933-016-0331-7.

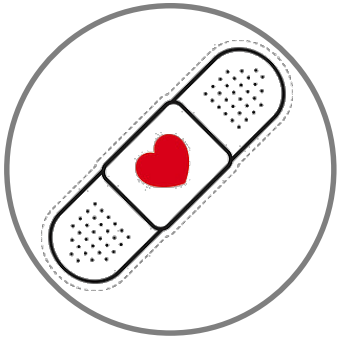
KLK1 and ZG16B proteins and arginine-proline metabolism identified as novel targets to monitor atherosclerosis, acute coronary syndrome and recovery. *Metabolomics.* 2015;11(5):1056-1067.

SINDROME CORONARIO AGUDO (aterosclerosis)

HIPERTENSIÓN

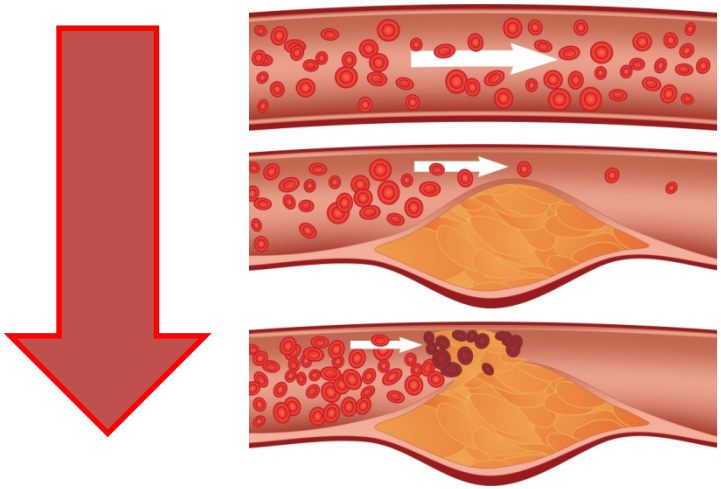
ENFERMEDAD RENAL CRÓNICA

Atherosclerosis: silent development

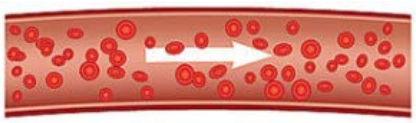


Asymptomatic

Silent

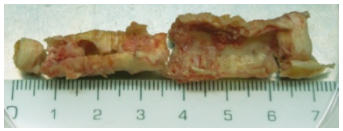


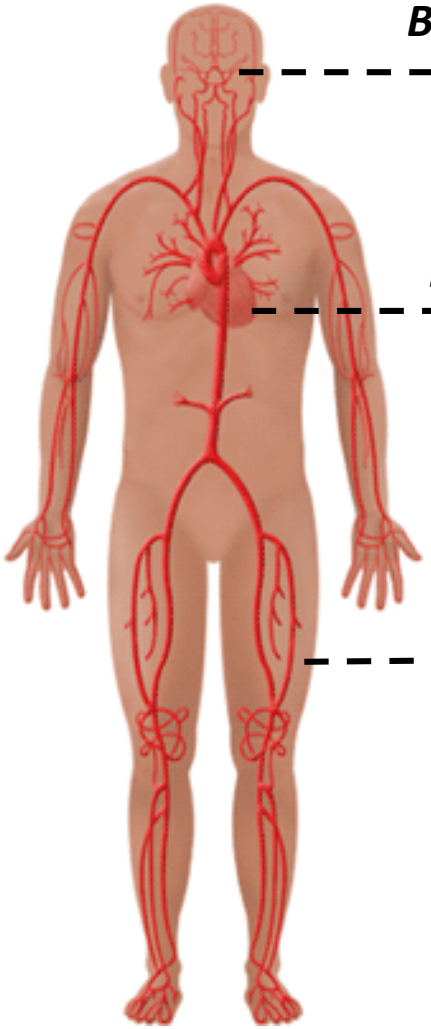
Healthy artery



Atherosclerosis

Atherosclerotic artery





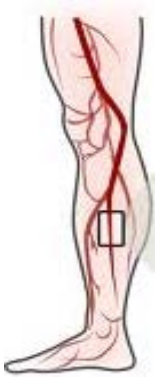
BRAIN

ISCHEMIC STROKE

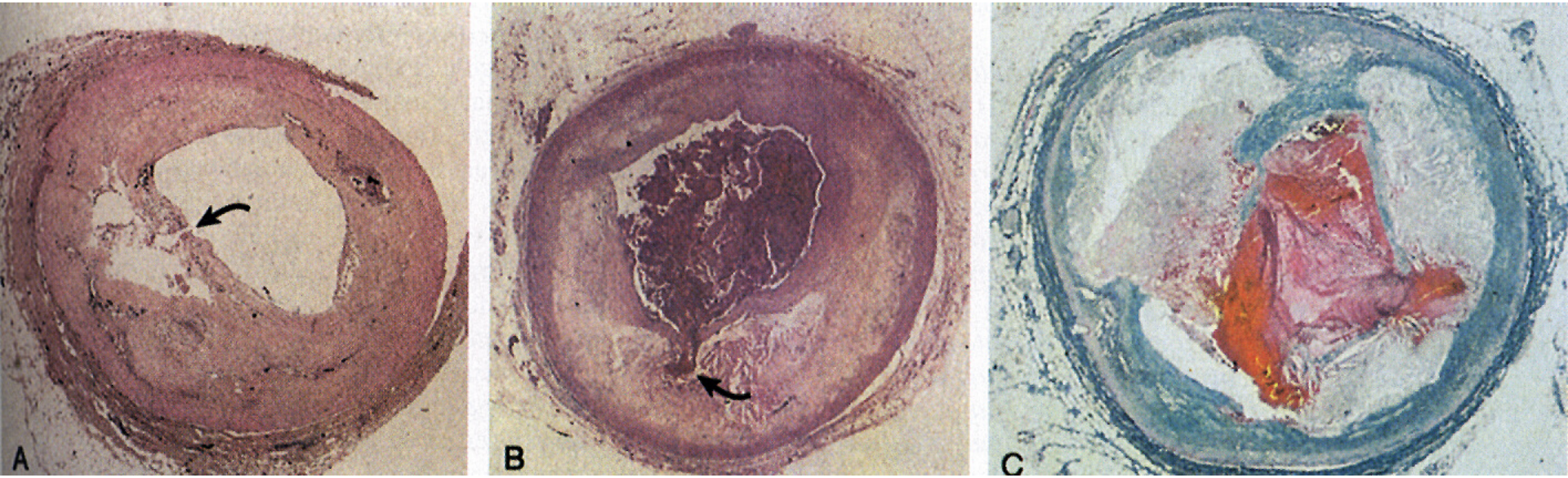
HEART

CORONARY HEART DISEASE

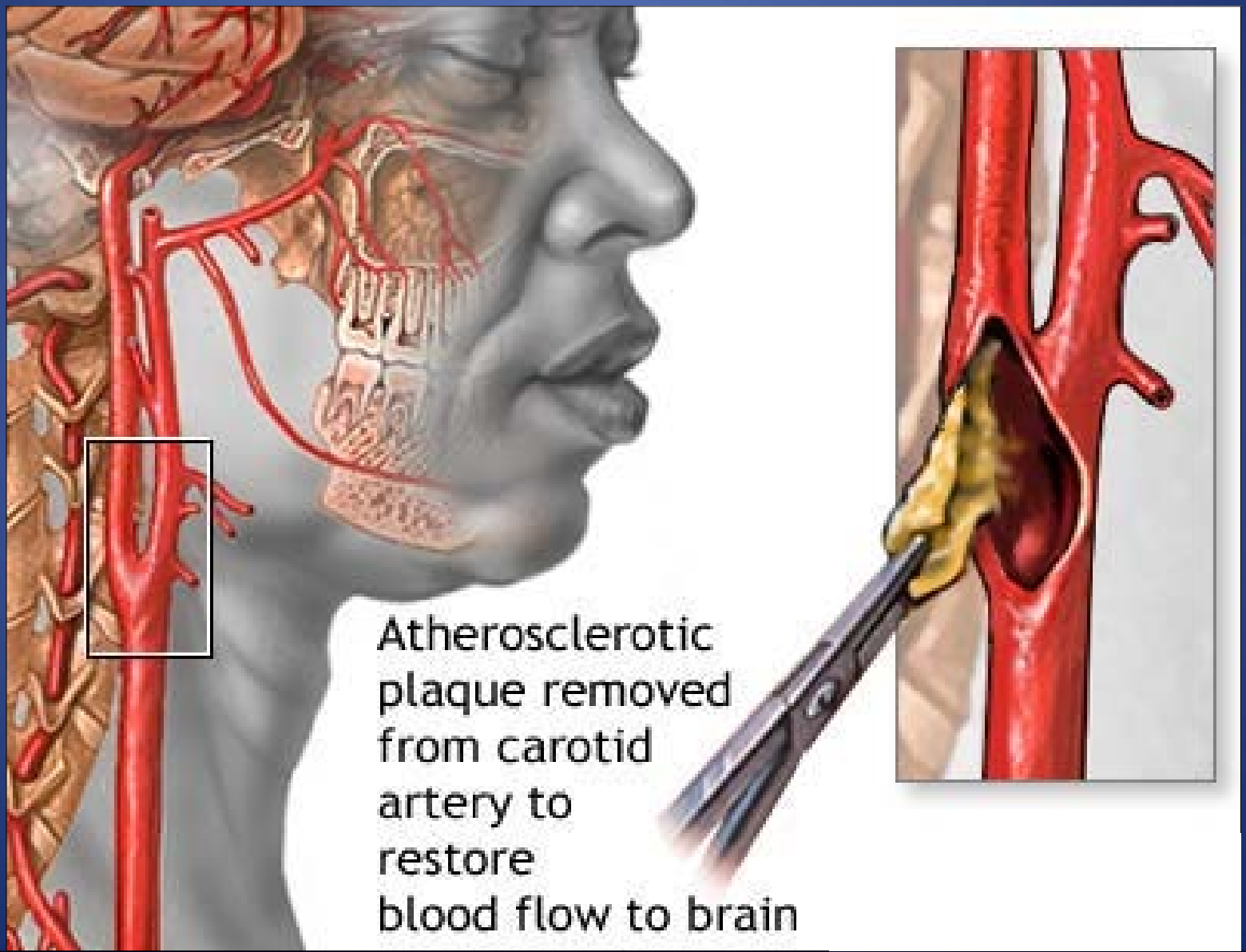
LOWER EXTREMITIES



PERIPHERAL VASCULAR DISEASE


A
B
C

ENDARTERECTOMY



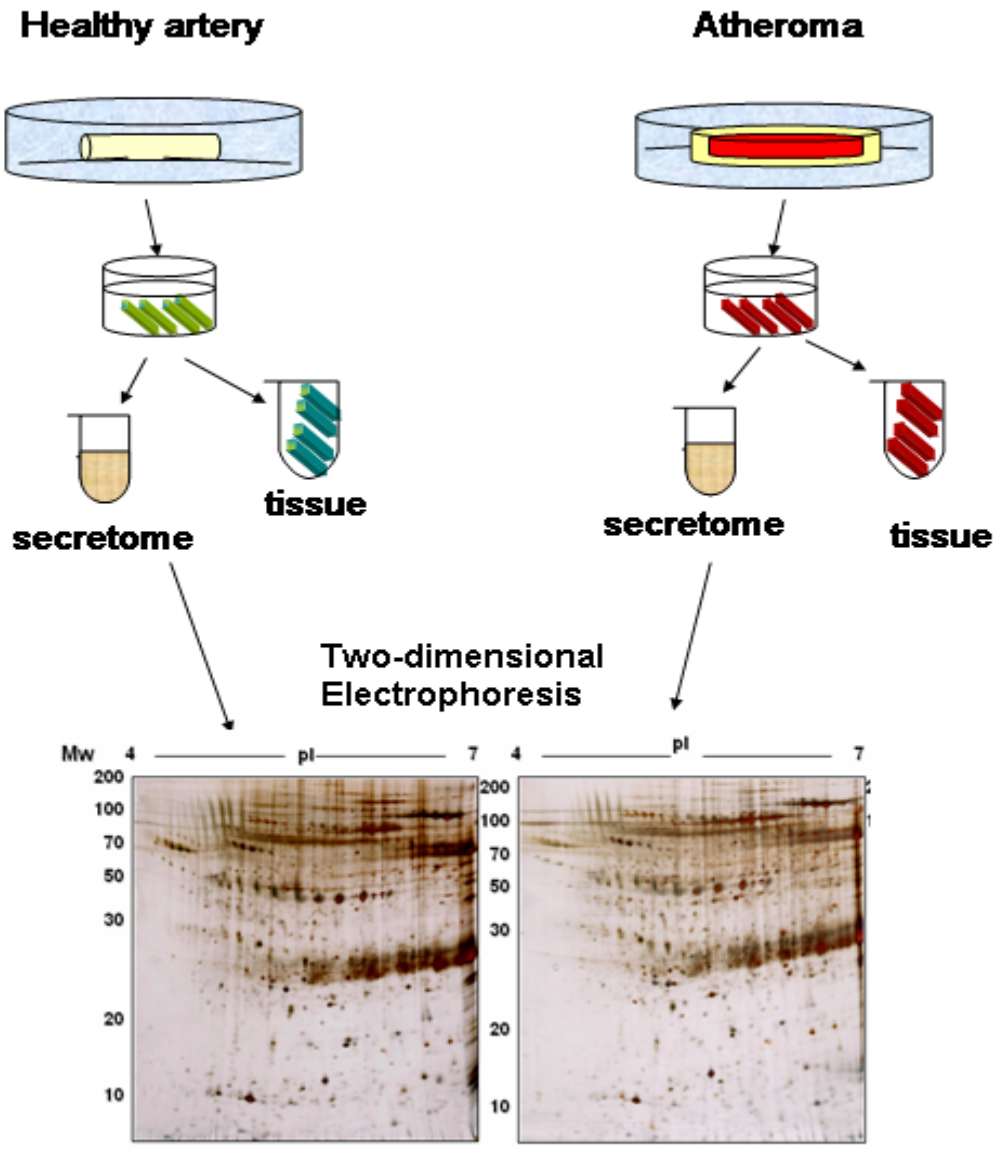
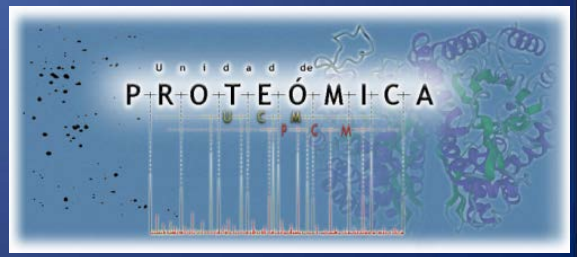


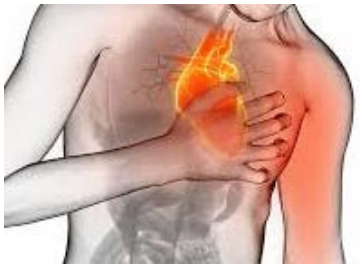
Figure 1

Duran M. C., et al. Methods Mol. Biol. 2006

ATHEROMA PLAQUES. PROTEINS

- ANTI-TRYPSIN
- FERRITIN
- TRANSFERRIN
- APO-AIV
- APO-AI
- ZINC-2-GLYCOPRO.
- CAPPING PROTEIN
- THIORED. PEROX-ASE
- CATHEPSIN D
- RETINOL BINDING
- PROT. DISUL.ISOMER.
- HEMOPEXIN
- SAP
- HSP-27
- ACTIN
- ENOLASE 1
- LEU-RICH GLYCOP.
- GELSOLIN
- GALACTOSIDASE
- FIBRINOGEN
- GLYCOPROP. HC
- HAPTOGLOBIN





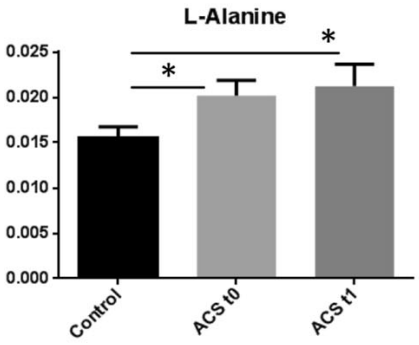
Human urine samples:

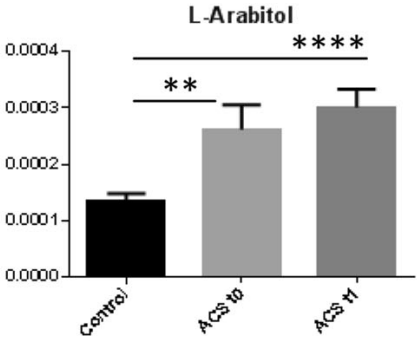
Control

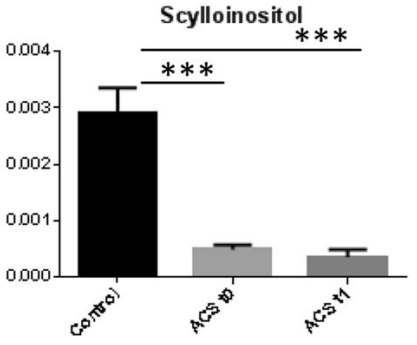
ACS (t=0)

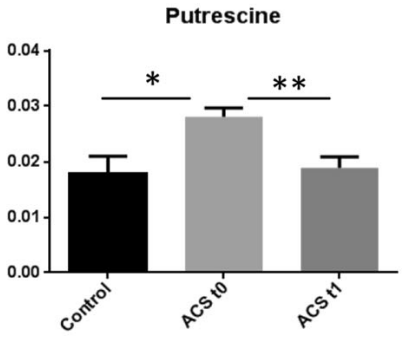
ACS (discharge, t=1)

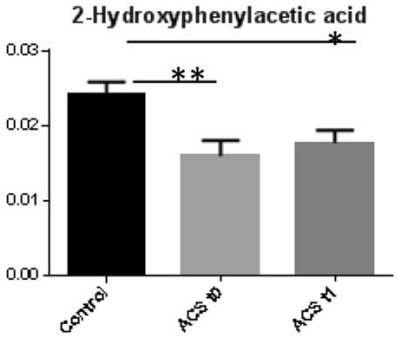
Infarction

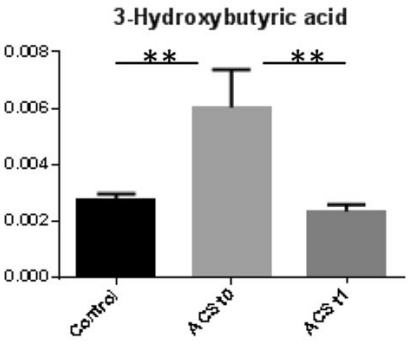


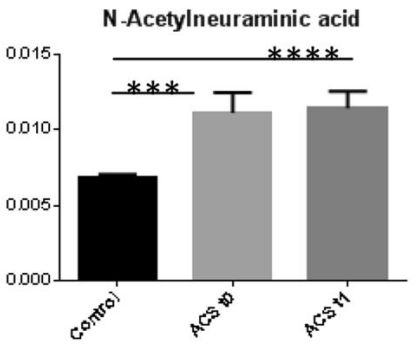


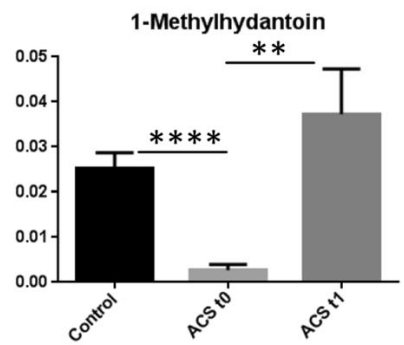


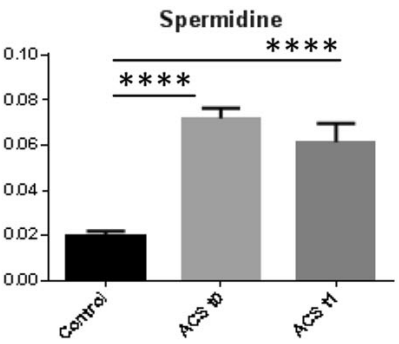


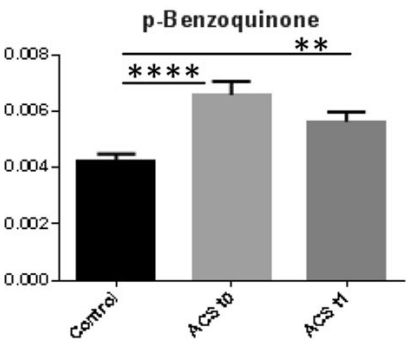


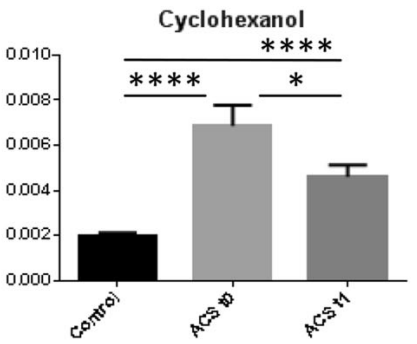












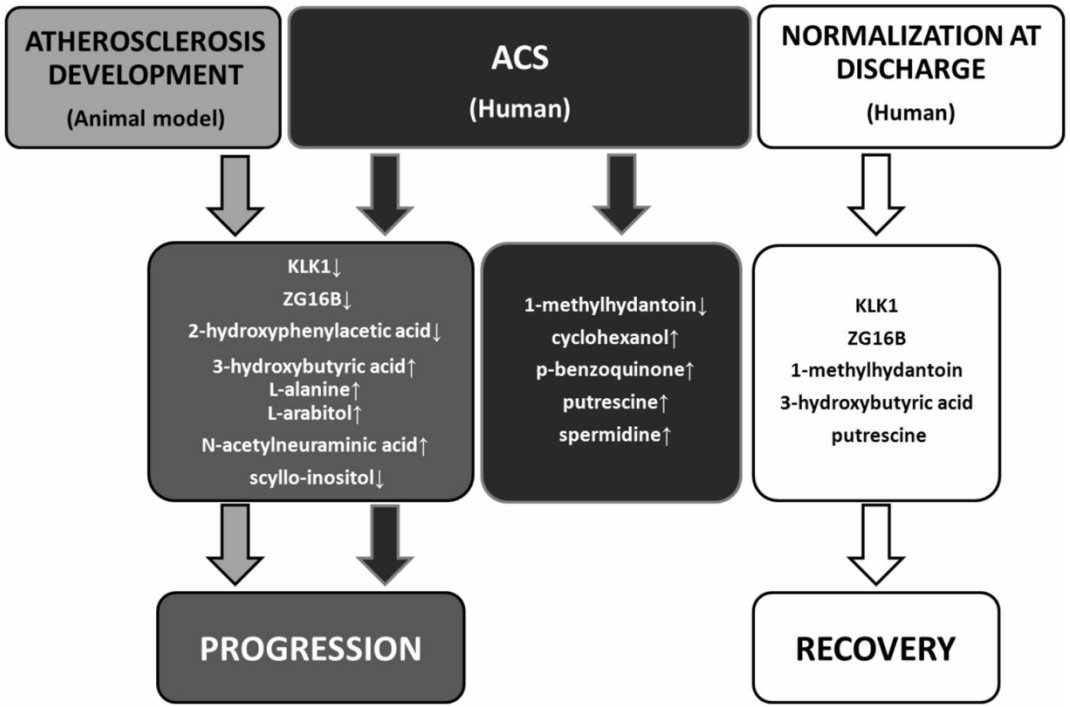
CAI RMN
Universidad Complutense de Madrid

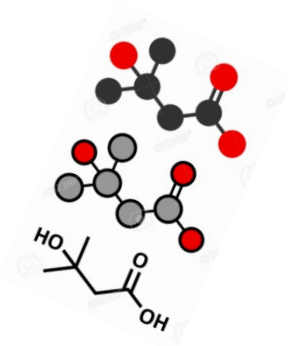
Metabolomics (2015) 11:1056–1067
DOI 10.1007/s11306-014-0761-8

ORIGINAL ARTICLE

KLK1 and ZG16B proteins and arginine–proline metabolism identified as novel targets to monitor atherosclerosis, acute coronary syndrome and recovery

Marta Martin-Lorenzo · Irene Zubiri · Aroa S. Maroto · Laura Gonzalez-Calero · Maria Posada-Ayala · Fernando de la Cuesta · Laura Mourino-Alvarez · Luis F. Lopez-Almodovar · Eva Calvo-Bonacho · Luis M. Ruilope · Luis R. Padial · Maria G. Barderas · Fernando Vivanco · Gloria Alvarez-Llamas




HO
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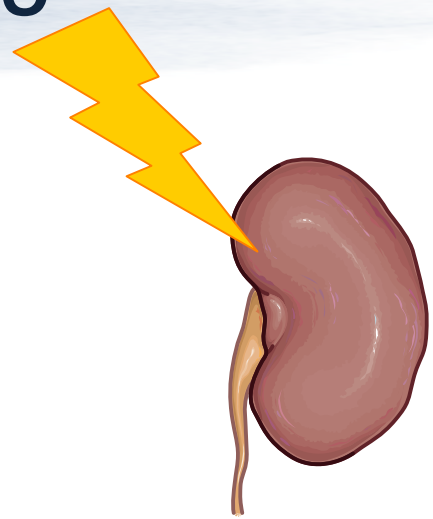
OH

ALBUMINURIA COMO MARCADOR DE DAÑO



Ya hay lesión. **NO es predictor.**

Necesidad de predictores del desarrollo de albuminuria

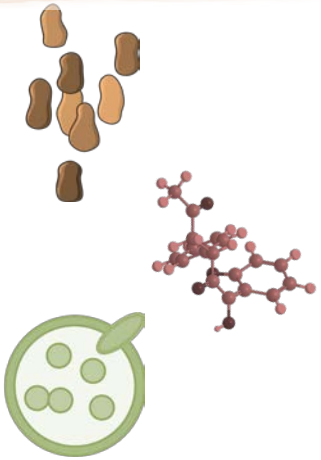
ABORDAJE MULTI-ÓMICO

Orina

Proteínas

Metabolitos

Exosomas



Gonzalez-Calero *et al. Cardiovasc Diabetol (2016) 15:8*
DOI 10.1186/s12933-016-0331-7

Cardiovascular Diabetology

ORIGINAL INVESTIGATION Open Access

Urinary alpha-1 antitrypsin and CD59 glycoprotein predict albuminuria development in hypertensive patients under chronic renin-angiotensin system suppression

Laura Gonzalez-Calero[1], Marta Martin-Lorenzo[1], Fernando de la Cuesta[2], Aroa S. Maroto[1], Montserrat Baldan-Martin[2], Gema Ruiz-Hurtado[3,4], Helena Pulido-Olmo[3], Julian Segura[3], Maria G. Barderas[2], Luis M. Ruilope[3*], Fernando Vivanco[1,5] and Gloria Alvarez-Llamas[1*]

July 2016

Original Article

Plasma Molecular Signatures in Hypertensive Patients With Renin–Angiotensin System Suppression: New Predictors of Renal Damage and De Novo Albuminuria Indicators

Montserrat Baldan-Martin, Laura Mourino-Alvarez, Laura Gonzalez-Calero, Rafael Moreno-Luna, Tamara Sastre-Oliva, Gema Ruiz-Hurtado, Julian Segura, Juan Antonio Lopez, Jesus Vazquez, Fernando Vivanco, Gloria Alvarez-Llamas, Luis M. Ruilope, Fernando de la Cuesta*, Maria G. Barderas*

Abstract—Albuminuria is a risk factor strongly associated with cardiovascular disease, the first cause of death in the general population. It is well established that renin–angiotensin system suppressors prevent the development of new-onset albuminuria in naïf hypertensive patients and diminish its excretion, but we cannot forget the percentage of hypertensive patients who develop de novo albuminuria. Here, we applied multiple proteomic strategy with the purpose to elucidate specific molecular pathways involved in the pathogenesis and provide predictors and chronic organ damage indicators. Briefly, 1143 patients were followed up for a minimum period of 3 years. One hundred and twenty-nine hypertensive patients chronically renin–angiotensin system suppressed were recruited, classified in 3 different groups depending on their albuminuria levels (normoalbuminuria, de novo albuminuria, and sustained albuminuria), and investigated by multiple proteomic strategies. Our strategy allowed us to perform one of the deepest plasma proteomic analysis to date, which has shown 2 proteomic signatures: (1) with predictive value of de novo albuminuria and (2) sustained albuminuria indicator proteins. These signatures are related to inflammation, immune as well as in the proteasome activation occurring in situations of endoplasmic reticulum stress. Furthermore, these results open the possibility of a future strategy based on anti-immune therapy to treat hypertension which could help to prevent the development of albuminuria and, hence, the progression of kidney damage. (*Hypertension*. 2016;68:00-00. DOI: 10.1161/HYPERTENSIONAHA.116.07412.) • Online Data Supplement

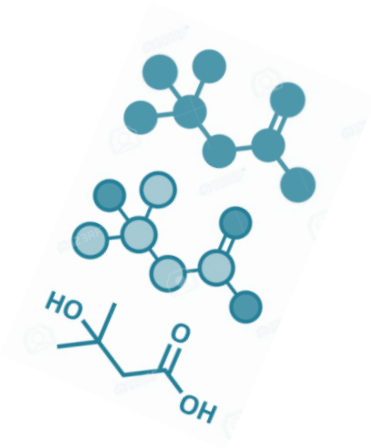
Key Words: albuminuria ■ cause of death ■ hypertension ■ immune system ■ renin-angiotensin system

Original Paper

Clinical Science (2016) **130**, 525–538 doi: 10.1042/CS20150517

Role of matrix metalloproteinase-9 in chronic kidney disease: a new biomarker of resistant albuminuria

Helena Pulido-Olmo*†, Concha F. García-Prieto†, Gloria Álvarez-Llamas§, María G. Barderas‖, Fernando Vivanco§¶, Isabel Aranguez†**, Beatriz Somoza†, Julián Segura*, Reinhold Kreutz††, María S. Fernández-Alfonso*†, Luis M. Ruilope*†‡ and Gema Ruiz-Hurtado*†


HO
O
OH

© 2013 International Society of Nephrology

Identification of a urine metabolomic signature in patients with advanced-stage chronic kidney disease

Maria Posada-Ayala[1], Irene Zubiri[1], Marta Martin-Lorenzo[1], Aroa Sanz-Maroto[1], Dolores Molero[2], Laura Gonzalez-Calero[1], Beatriz Fernandez-Fernandez[3], Fernando de la Cuesta[4], Carlos M. Laborde[4], Maria G. Barderas[4], Alberto Ortiz[3], Fernando Vivanco[1,5] and Gloria Alvarez-Llamas[1]

[1]Department of Immunology, IIS-Fundacion Jimenez Diaz, Madrid, Spain; [2]CAI-RMN, Universidad Complutense de Madrid, Madrid, Spain; [3]Department of Nephrology, IIS-Fundacion Jimenez Diaz-UAM/IRSIN, Madrid, Spain; [4]Department of Vascular Physiopathology, Hospital Nacional de Paraplejicos, SESCAM, Toledo, Spain and [5]Department of Biochemistry and Molecular Biology I, Universidad Complutense de Madrid, Madrid, Spain

OPEN

Urinary Kininogen-1 and Retinol binding protein-4 respond to Acute Kidney Injury: predictors of patient prognosis?

ived: 03 July 2015
2 December 2015
: 21 January 2016

Laura Gonzalez-Calero[1], Marta Martin-Lorenzo[1], Angeles Ramos-Barron[2], Jorge Ruiz-Criado[2], Aroa S. Maroto[1], Alberto Ortiz[3], Carlos Gomez-Alamillo[2], Manuel Arias[2], Fernando Vivanco[1,4] & Gloria Alvarez-Llamas[1]

TRANSFERENCIA DE LAS TECNICAS PROTEÓMICAS A LOS HOSPITALES: PROTEÓMICA CLINICA

Identificación de Microorganismos mediante MS

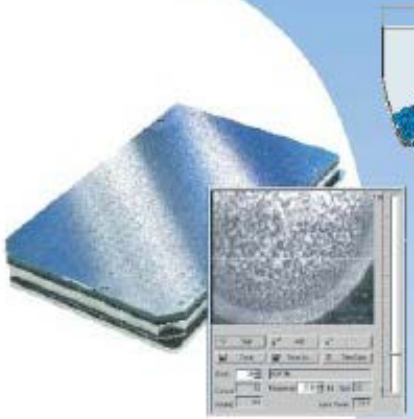
Histología Molecular (Anatomía patológica)

Profiling of Microorganisms by MALDI-TOF Mass Spectrometry

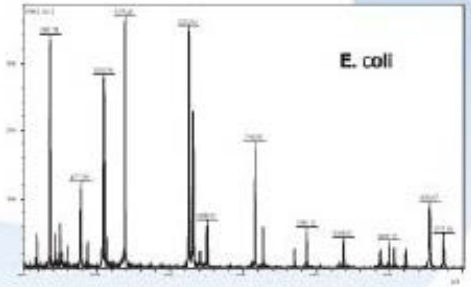
1. Select a Colony

Unknown Microorganism

2. Smear a thin-layer onto Target Plate or perform rapid organic extraction & spot supernatant

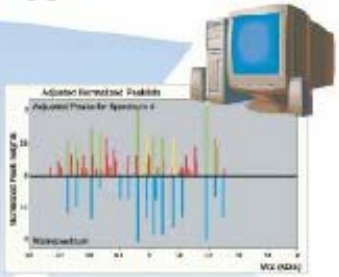


3. Add MALDI Matrix

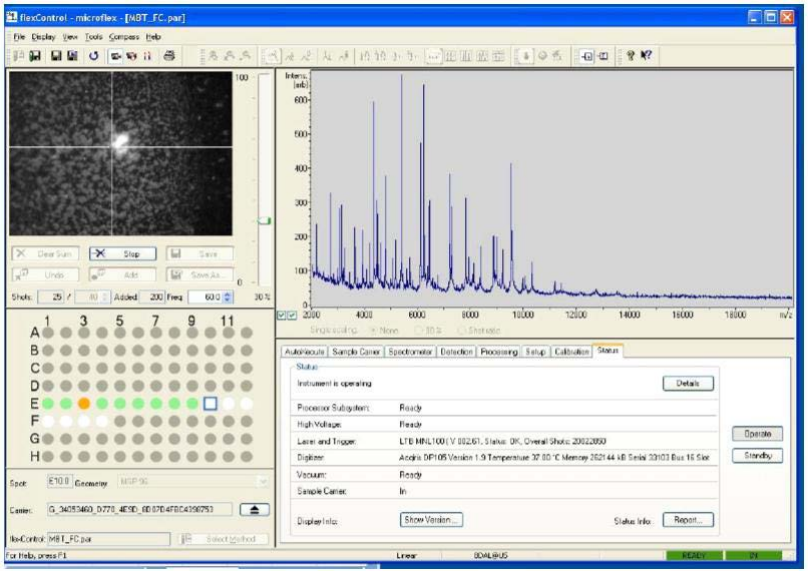


4. Generate MALDI-TOF Profile Spectrum

5. Data Interpretation



6. Match patterns to database to identify species


flexControl - microflex - [MBT_FC.par]
File Display View Tools Compass Help
Stop
Save
Shots: 25
Added: 200
Freq: 60.0
A
B
C
D
E
F
G
H
Instrument is operating
Details
Processor Subsystem: Ready
High Voltage: Ready
Laser and Trigger:
Digitizer:
Vacuum: Ready
Sample Carrier: In
Show Version...
Report...
Operate
Standby
For Help, press F1
Linear
READY

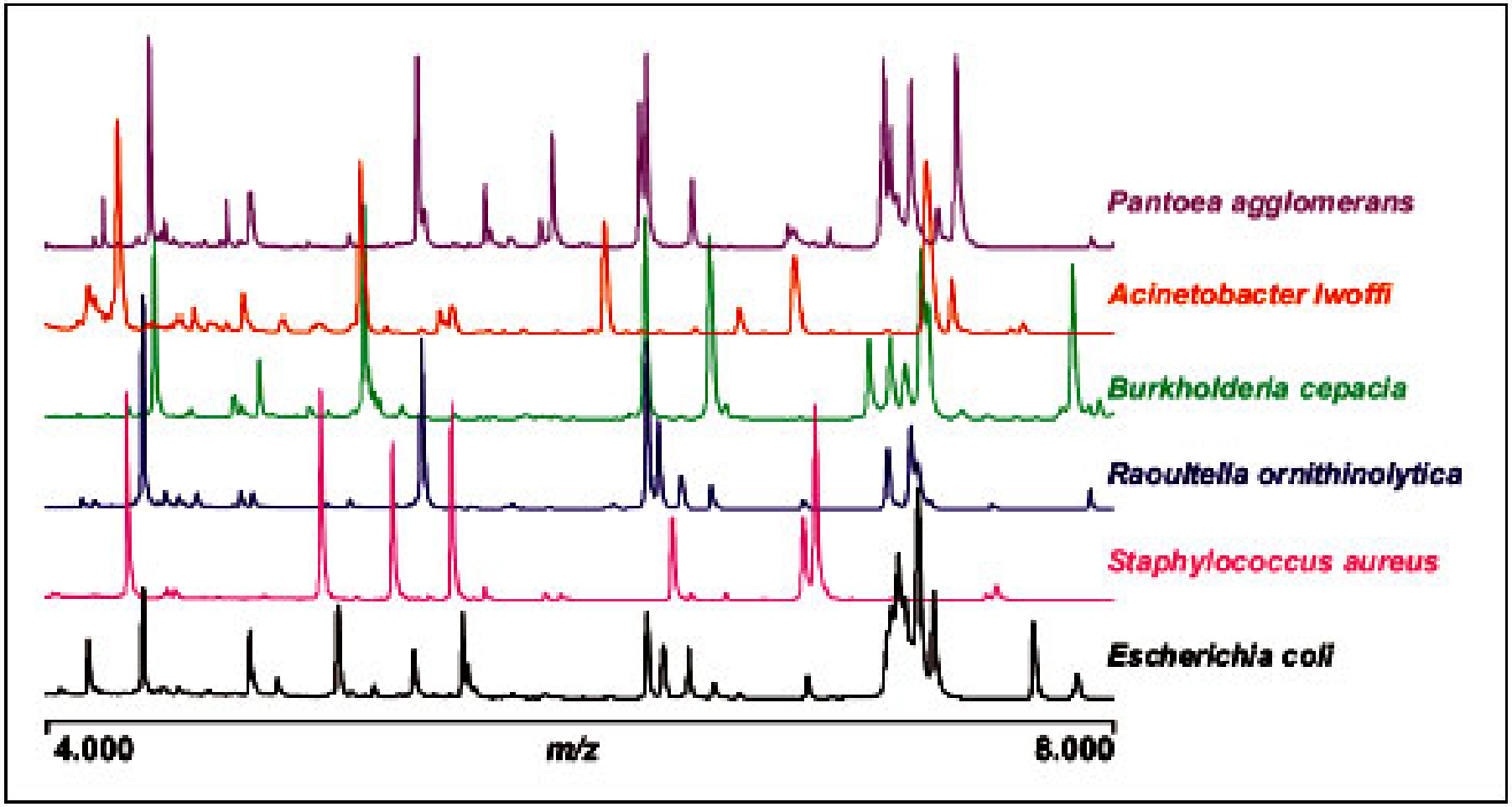


Figura 1. Diferentes perfiles de proteínas obtenidas para diferentes bacterias por MALDI-TOF MS (cortesía bioMerieux).

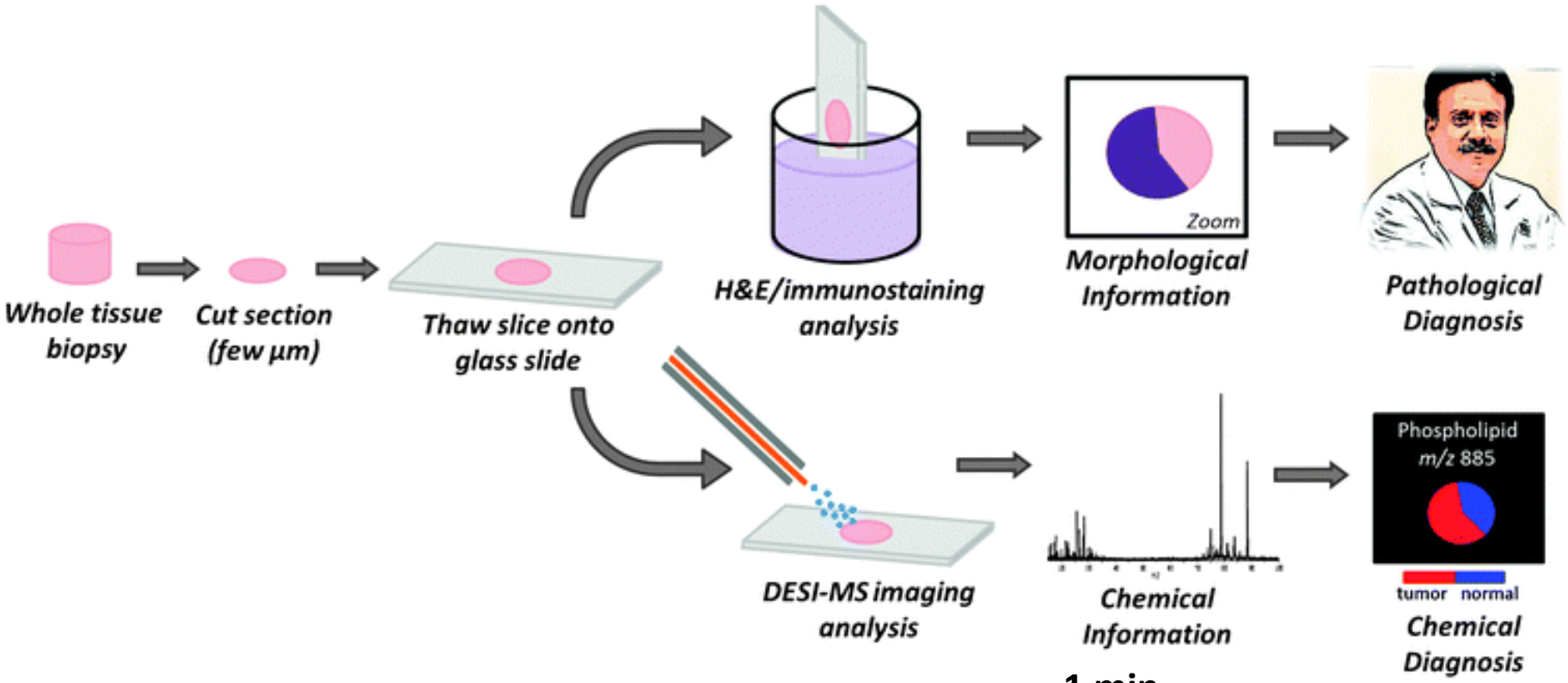
ANATOMIA PATOLOGICA CLÁSICA

Ab 1º: 1hr
Lavado: 15´
Ab 2º: 1hr

Observación:
Microscopía
Fluorescencia:
10-30´

1 Marcador

Subjetivo



ANATOMIA PATOLOGICA MOLECULAR (MS)

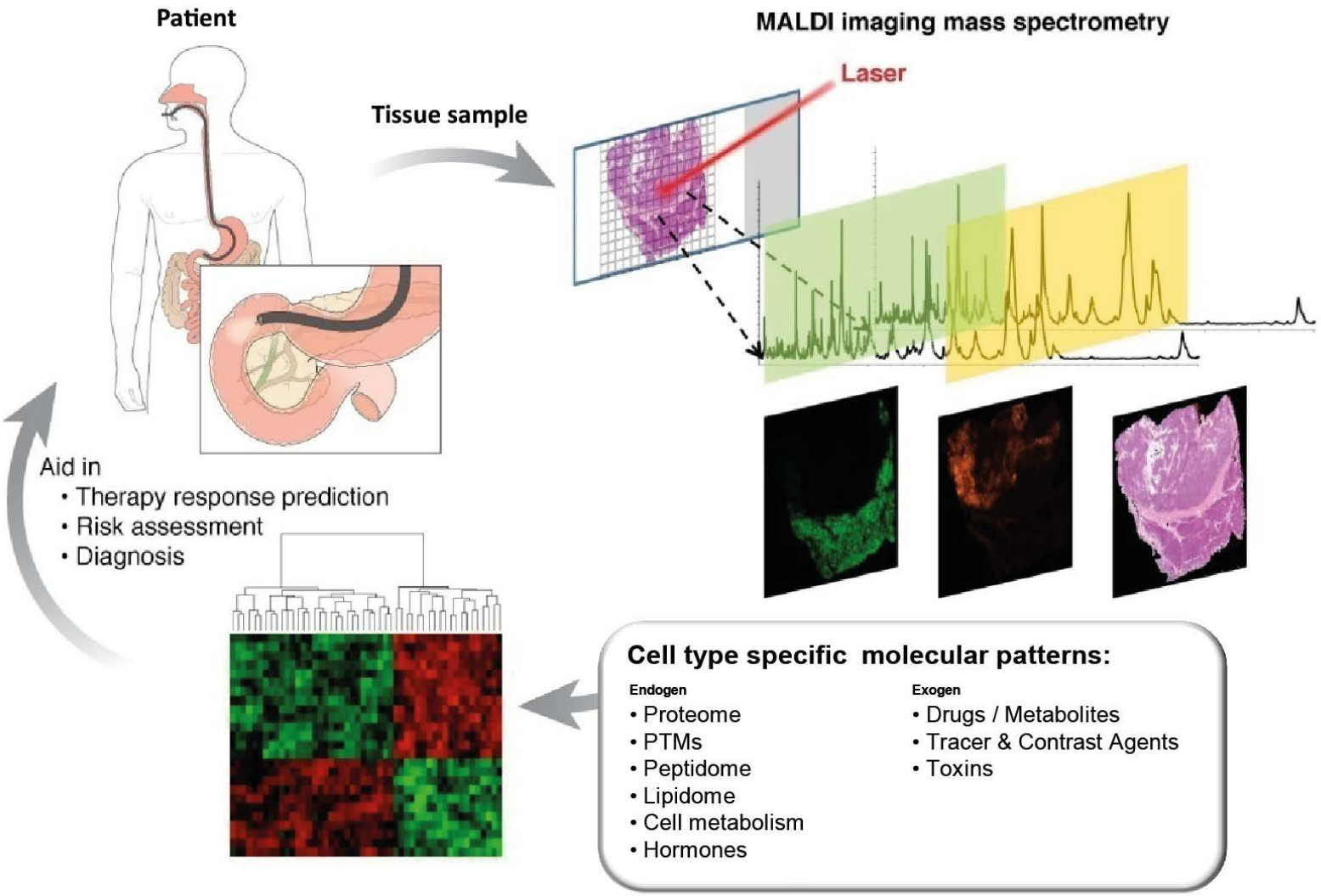
Barrido MS: 10´

1 min
Automático

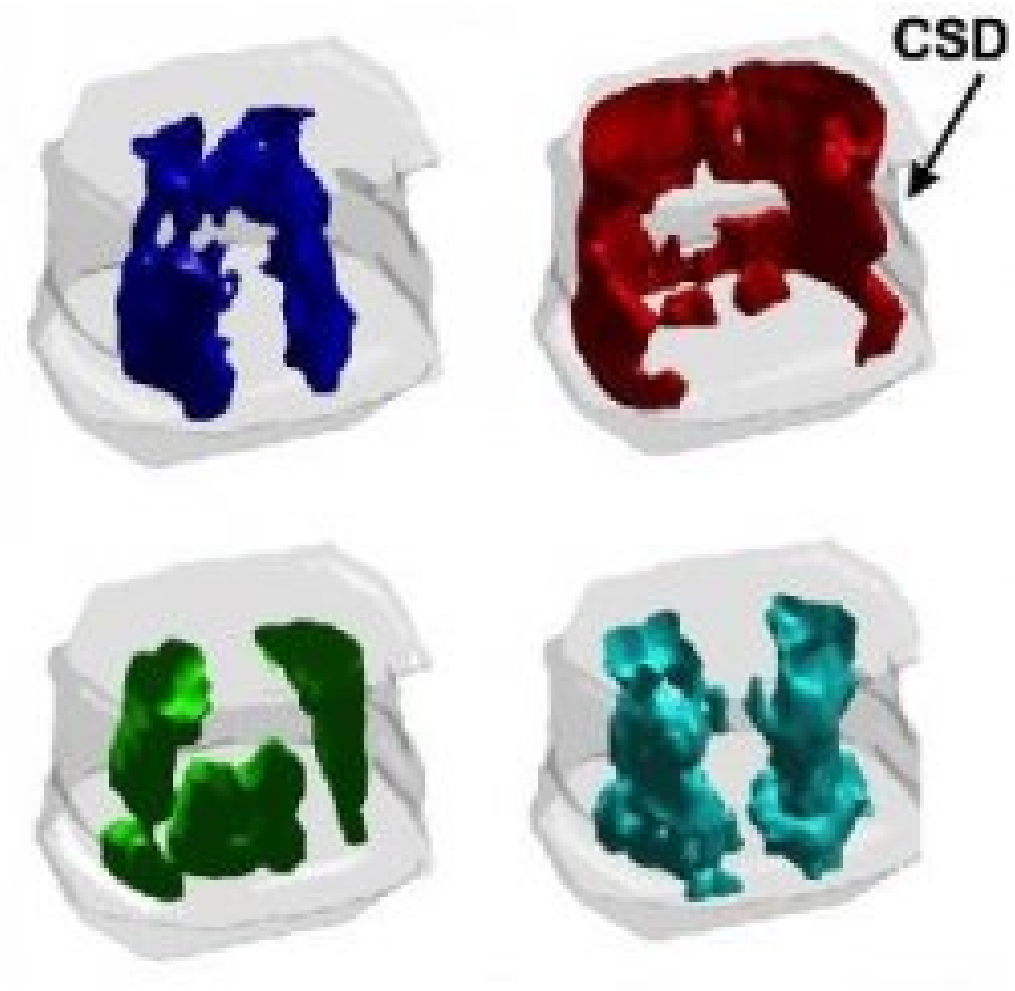
Objetivo

500-1500 Proteínas
Perfil proteico

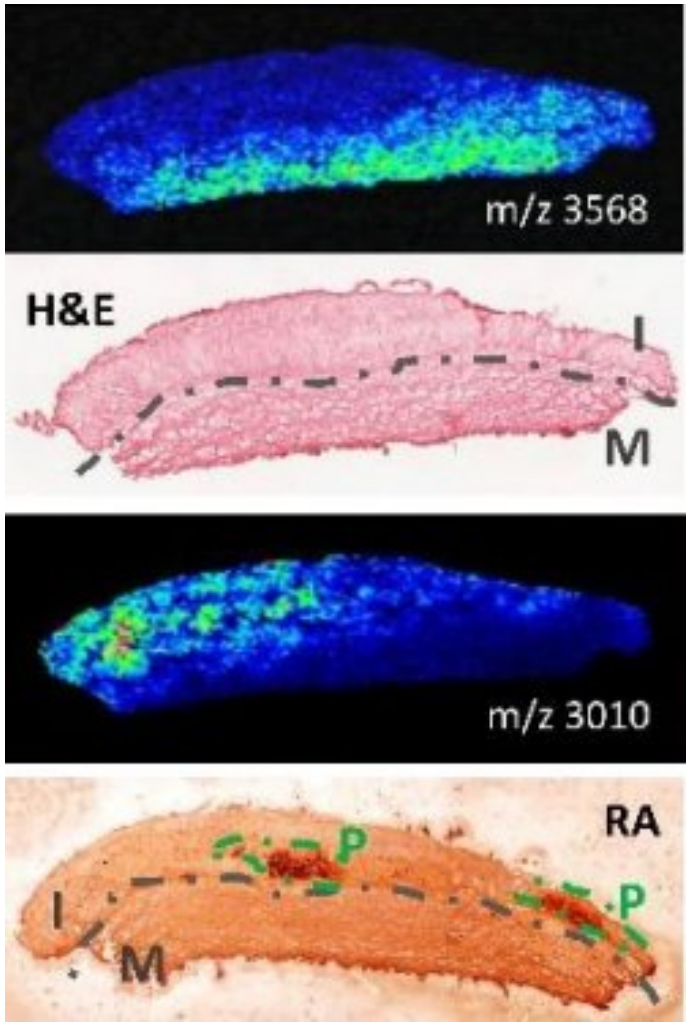
Molecular tissue analysis by MALDI imaging mass spectrometry



3D BRAIN



2D- ARTERIES



GRACIAS ¡