Plasma Aβ42/Aβ40, measured by a novel mass spectrometric method, identifies early amyloid deposition in individuals at risk of Alzheimer's disease (FACEHBI Cohort)

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Disclosures

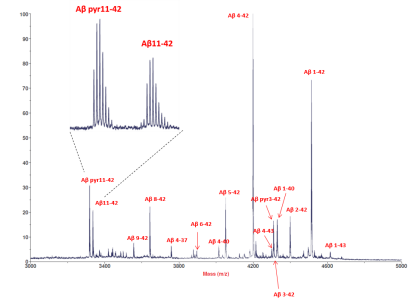JAA is a full-time employee at Araclon Biotech S.L.
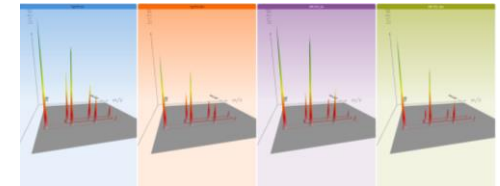
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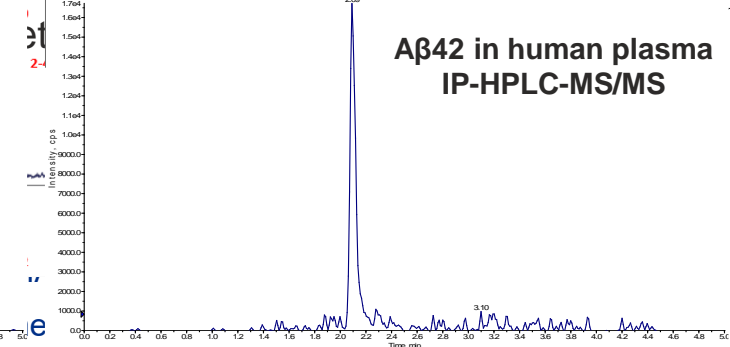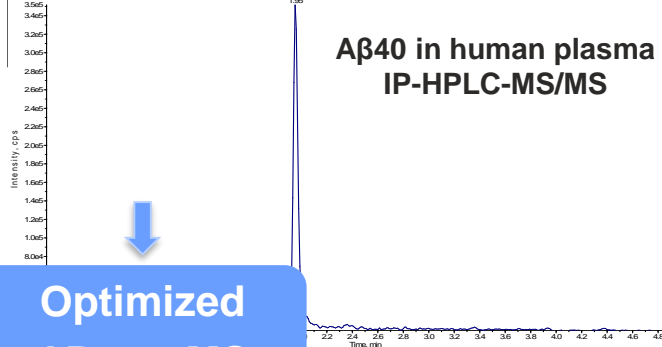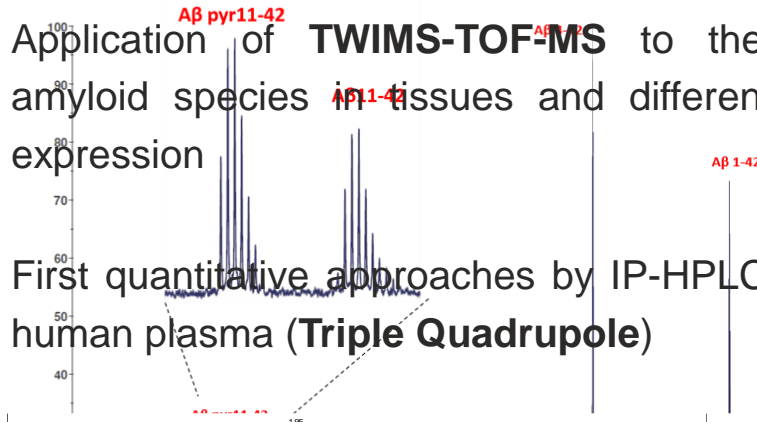# Experience with amyloid peptides in Araclon MS lab

2008

- **IP-MALDI-TOF/TOF** profiling of amyloid species in different tissues and biofluids (human and animal AD models)

- Application of **TWIMS-TOF-MS** to the study of amyloid species in tissues and differential protein expression

- First quantitative approaches by IP-HPLC-MS/MS in human plasma (**Triple Quadrupole**)

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2022

Optimized ABtest-MS

Aβ40 in human plasma
IP-HPLC-MS/MS

Aβ42 in human plasma
IP-HPLC-MS/MS

Online Poster nr. 875, Sarasa *et al.* ADPD 2022

Aβ pyr11-42

Aβ11-42

Aβ 1-42

Aβ40 in human plasma
IP-HPLC-MS/MS

Aβ42 in human plasma
IP-HPLC-MS/MS

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ABtest-MS: Method features

- **Antibody free**: Analytes are extracted directly from plasma. No immunoprecipitation is carried out, avoiding undesirable drawbacks inherent to antibodies (cross-reactivity, batch-to-batch reproducibility, cost…)

- Analysis of **intact Aβ1-40 and Aβ1-42** as no enzymatic digestion is performed

- **Micro-HPLC**: a perfect compromise between the sensitivity of Nano-HPLC and the robustness of Narrow-Bore / Analytical HPLC (0.1-1 ml/min)

- **Mass Spectrometric**: Electrospray Ionisation, followed by Ion Mobility separation and Triple Quadrupole MS/MS analysis
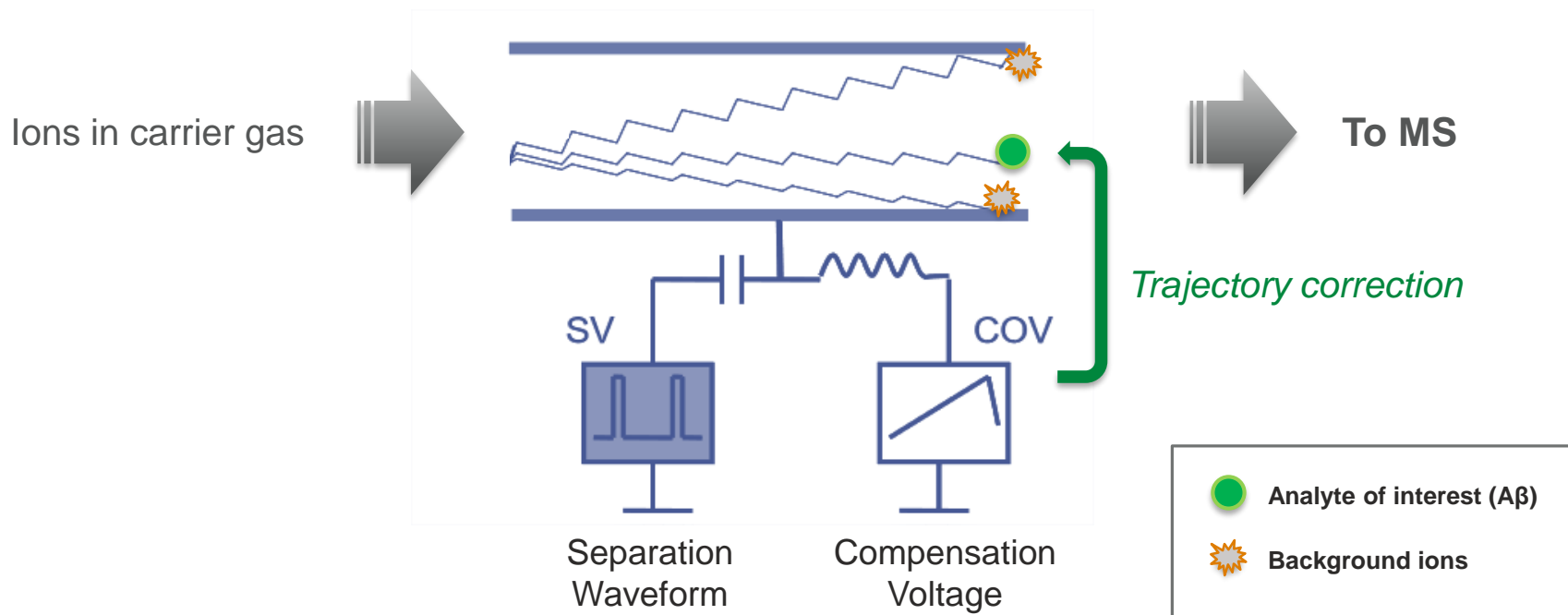
Analytical platform

- M3 Micro-HPLC system (Eksigent).

- Sciex QTRAP 6500$^+$ (Hybrid Triple Quadrupole-Linear Ion Trap)

- SelexION$^+$ Differential Mobility Spectrometry Interface (Sciex)
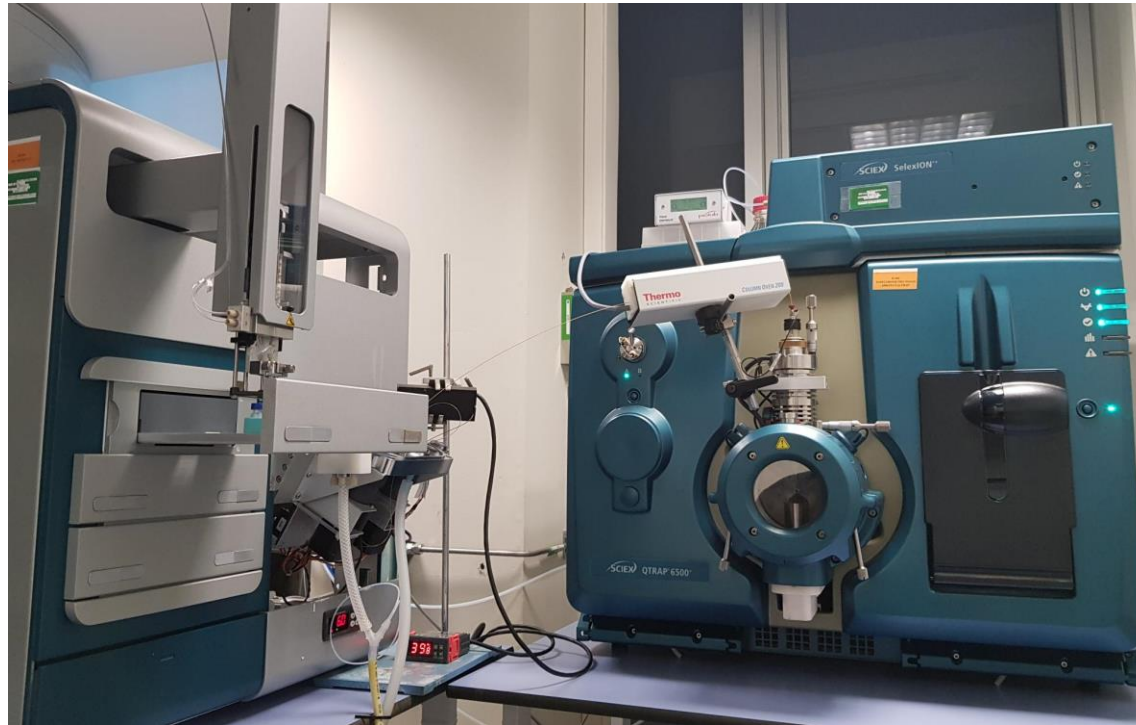
Differential Mobility Spectrometry (DMS)

- Used as a pre-filter after ionization and before mass analysis

- **Objective**: remove background noise and increase S/N

Ions in carrier gas

To MS

Trajectory correction

SV

COV

Separation
Waveform

Compensation
Voltage

● Analyte of interest (Aβ)

✴ Background ions

Only ● will pass through DMS filter

Micro HPLC

Acidic pH, reversed phase chromatography (C18)
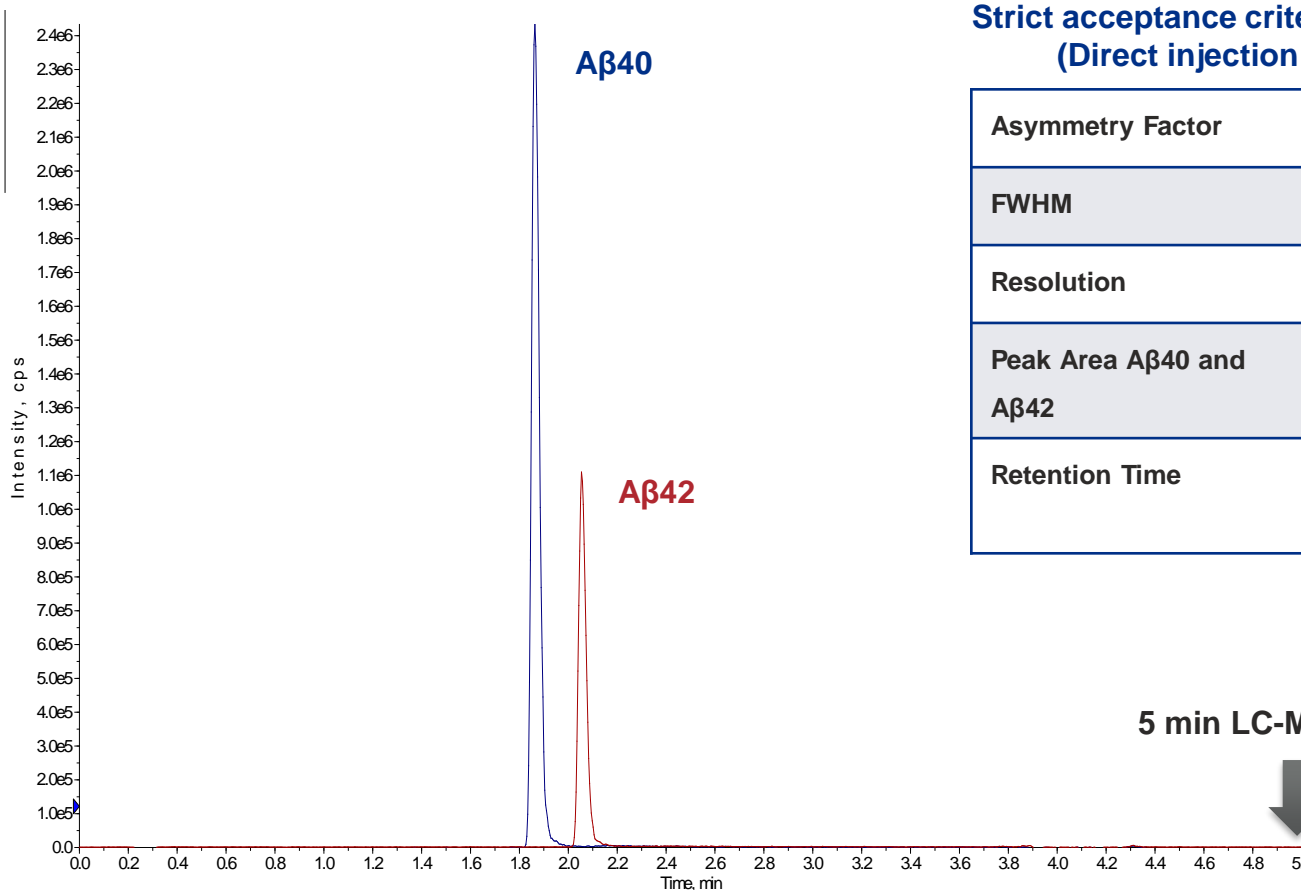
Strict acceptance criteria for HPLC columns
(Direct injection in neat solvents)

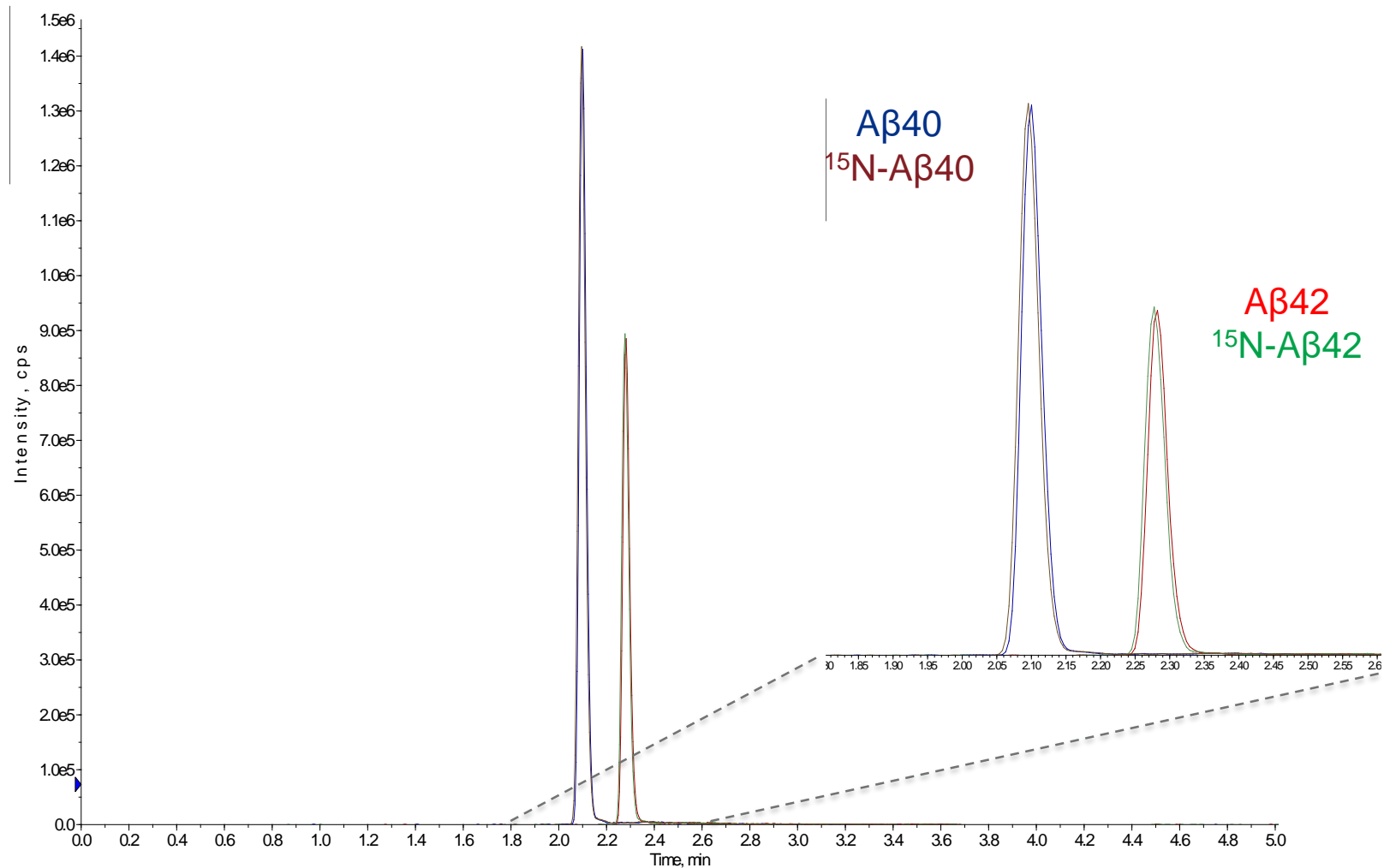|	
Asymmetry Factor	< 1.7
FWHM	< 2.7 sec
Resolution	> 2.5
Peak Area Aβ40 and Aβ42	> 4e6 / 2e6 cps
Retention Time	1.9 min ± 10% for Aβ40 2.1 min ± 10% for Aβ42

5 min LC-MS run time

Method Overview

(Validated acording to current guidelines)

- Calibration curves are made **in human plasma**, spiking ^{15}N-Aβ40 and ^{15}N-Aβ42 at seven concentration levels. Duplicated curves are used in each analytical run

- Quality control samples **in human plasma**, at three concentration levels (Low, Mid and High) are included in each analytical run

- Deuterated Internal Standards (^{2}H-Aβ40 and ^{2}H-Aβ42) are added to calibration curves, quality control samples and study samples (with the exception of *Double Blank* samples)

- **Unique** extraction procedure: **Patent filed in 2020** (EP2020382352)

- Micro-LC-ESI-DMS-MS/MS analysis

- Area ratios of the endogenous compounds (^{14}N-Aβ/^{2}H-Aβ) are interpolated in calibration curves made with ^{15}N-Aβ analogues (*surrogate analyte* approach)

Suitability test before each analytical run

Equal transmission for heavy (^{15}N) and light (^{14}N) Aβ peptides is monitored
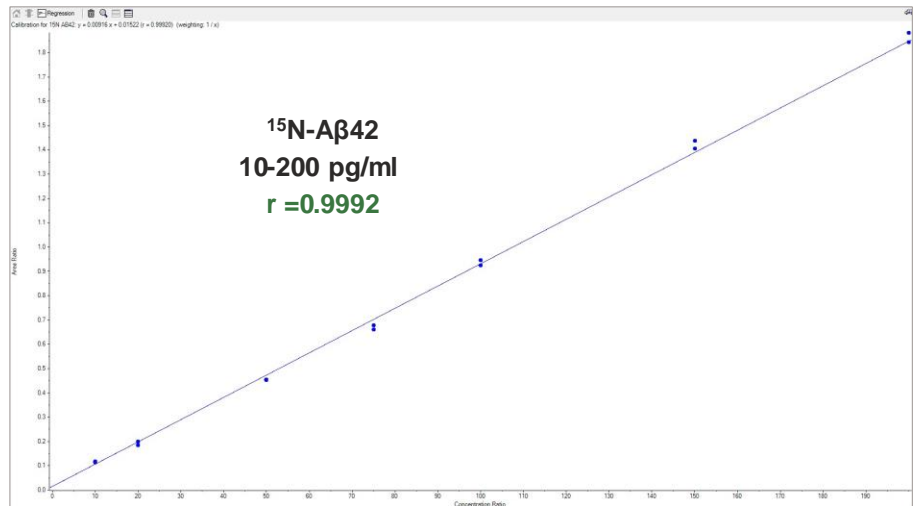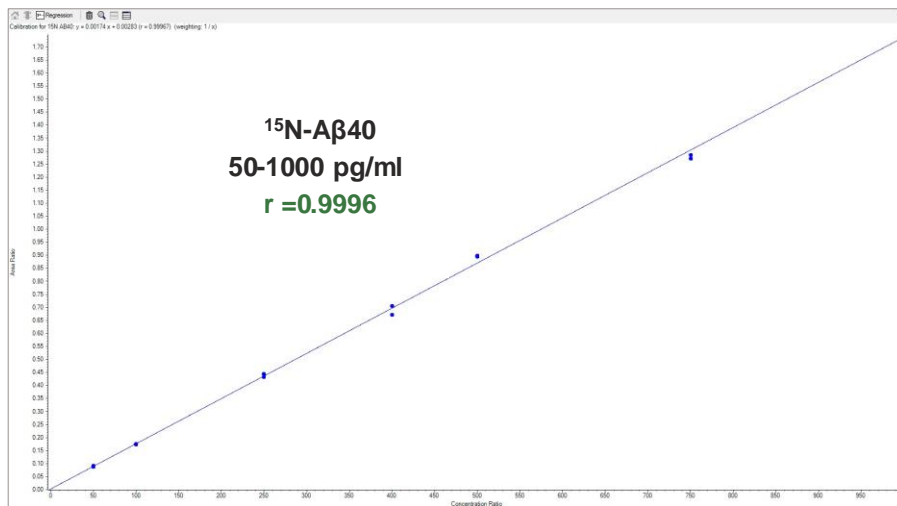
Sensitivity (LLOQ) and Linear Response
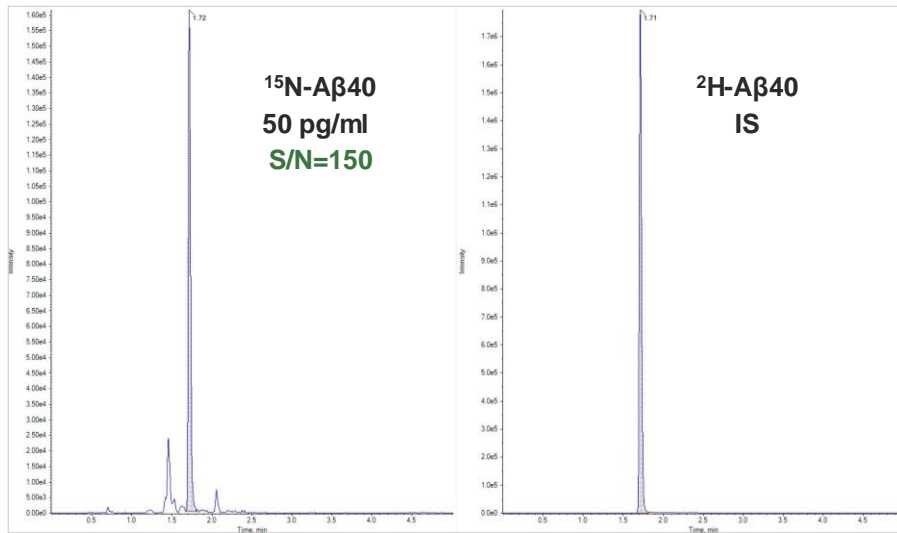

15N-Aβ40
50 pg/ml
S/N=150

2H-Aβ40
IS

15N-Aβ42
10 pg/ml
S/N=30

2H-Aβ42
IS

15N-Aβ40
50-1000 pg/ml
r =0.9996

15N-Aβ42
10-200 pg/ml
r =0.9992
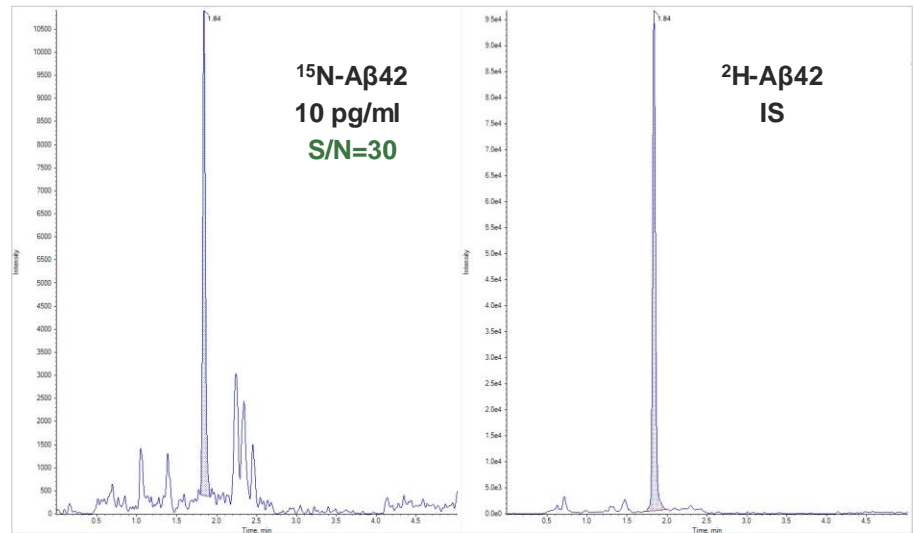
Endogenous signals interpolated in surrogate (^{15}N-Aβ) analyte curves

Example of study sample
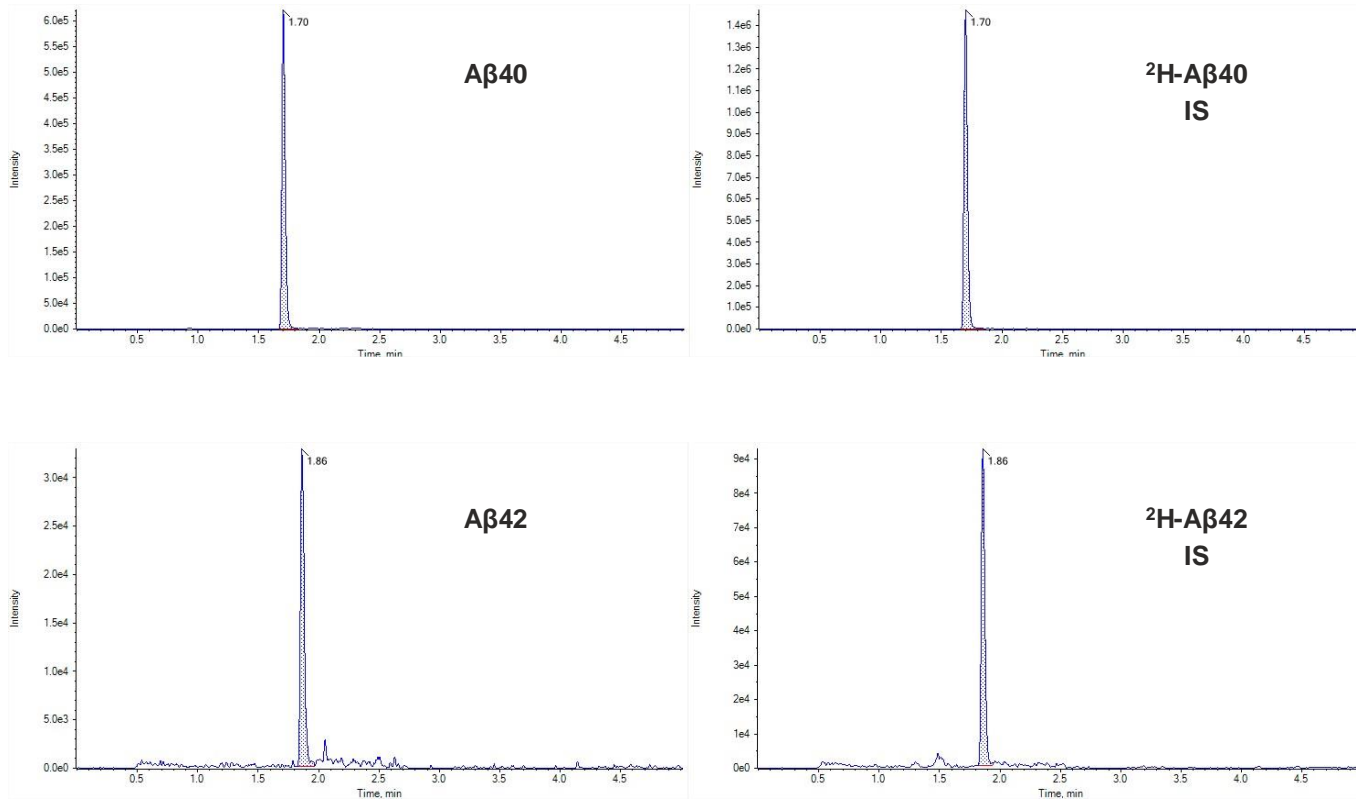
Assessment of clinical performance in the FACEHBI cohort

FACEHBI: Fundació ACE Healthy Brain Initiative

Principal Investigator: Prof. Mercè Boada MD, PhD.

- 200 cognitively unimpaired individuals with subjective cognitive decline (SCD)

- Eight-year follow-up planned

- Results corresponding to baseline (V0) and two-year follow-up (V2) visits are presented

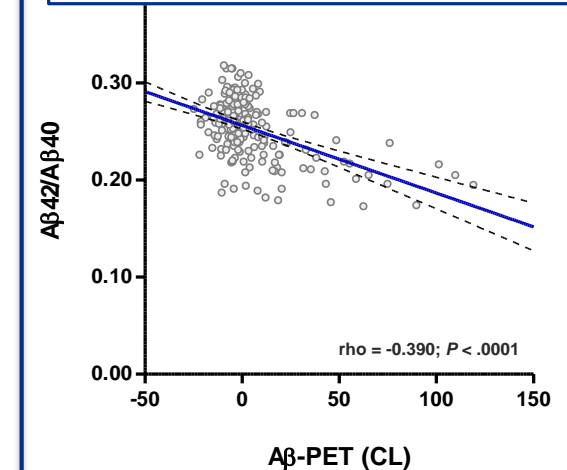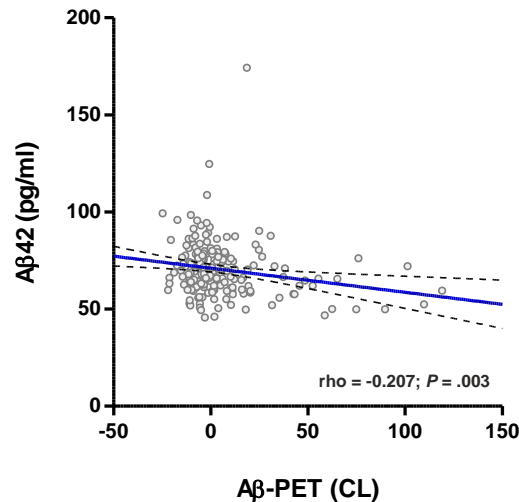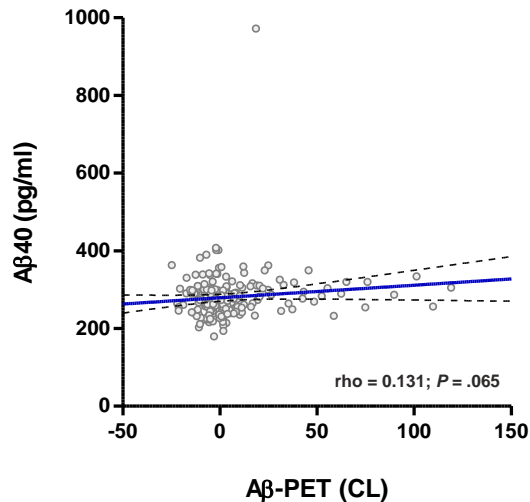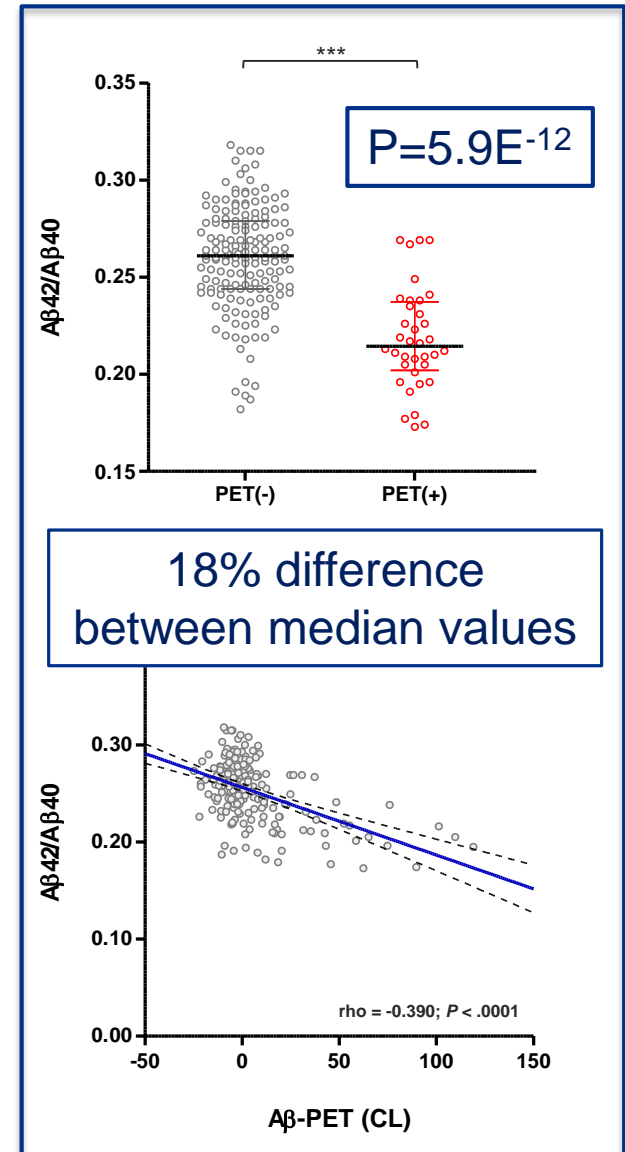- Analysis of five-year follow-up visit (V5) ongoing

Manuscript in Preparation

Demographic and clinical characteristics at baseline

	Aβ-PET (-)	Aβ-PET (+)[&]	*P* value
Participants	164 (82%)	36 (18%)	
Age, years	66.0 (60.0-69.5)	70.0 (67.0-73.0)	**.0003**
Female	110 (67%)	16 (44%)	**.0185**
APOE ε4, number of alleles			**.0002**
0 alleles	131 (80%)	17 (47%)	
1 alleles	29 (18%)	18 (50%)	
2 alleles	4 (2%)	1 (3%)	
Duration of education, years	15.0 (12.0-18.0)	16.0 (10.0-18.0)	.9796
FBB-PET, centiloids	-3.7 (-7.9-1.7)	34.1 (20.3-57.0)	**< .0001**
MMSE, score	29 (29-30)	30 (29-30)	.1321
S-FNAME, score	33.5 (22.0-46.0)	24.0 (16.0-33.0)	**.0013**
SFN-O, score	-0.01 (-0.72-0.86)	-0.16 (-0.82-0.40)	.1238
SFN-N, score	-0.03 (-0.61-0.65)	-0.88 (-1.05--0.22)	**< .0001**
Ventricular volume	24959.5 (19776.4-32028.2)	29061.4 (21966.7-38034.6)	.1079
Hippocampal volume	3606.1 (3421.0-3820.8)	3581.8 (3284.2-3792.7)	.3611
Plasma Aβ40, pg/ml	269.3 (243.9-294.2)	297.0 (267.2-317.6)	**.0009**
Plasma Aβ42, pg/ml	70.2 (63.1-77.3)	63.4 (57.8-72.1)	**.0041**
Plasma Aβ42/Aβ40, ratio	0.261 (0.244-0.279)	0.215 (0.203-0.236)	**< .0001**

Data are median values (interquartile range) or number of cases (%). Mann-Whitney and χ^2 tests, as appropriate.

[&] Cutoff for FBB-PET positivity was established at > 13.5 CL corresponding to early amyloid deposition (Bullich *et al*, Alzheimer's Research & Therapy 2021)

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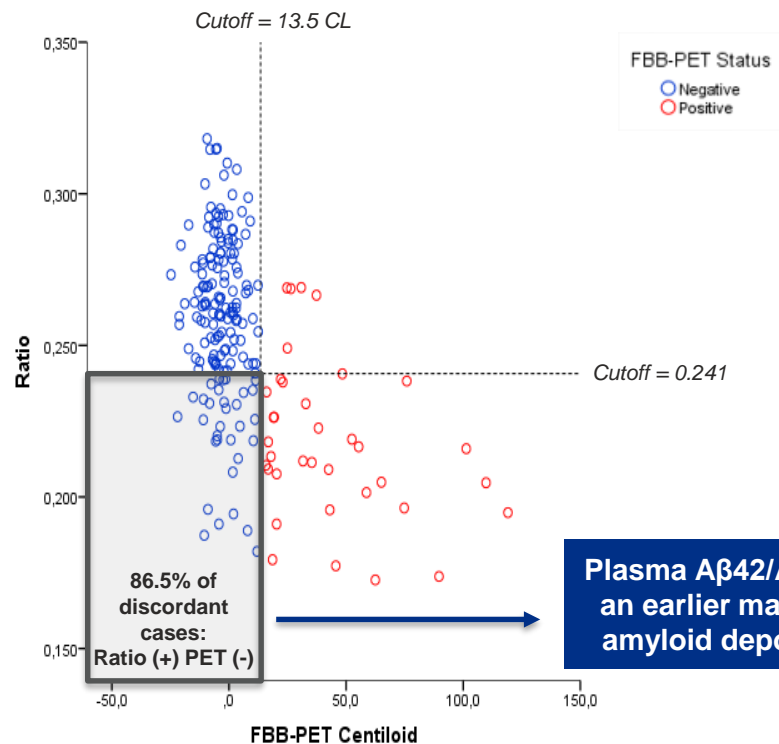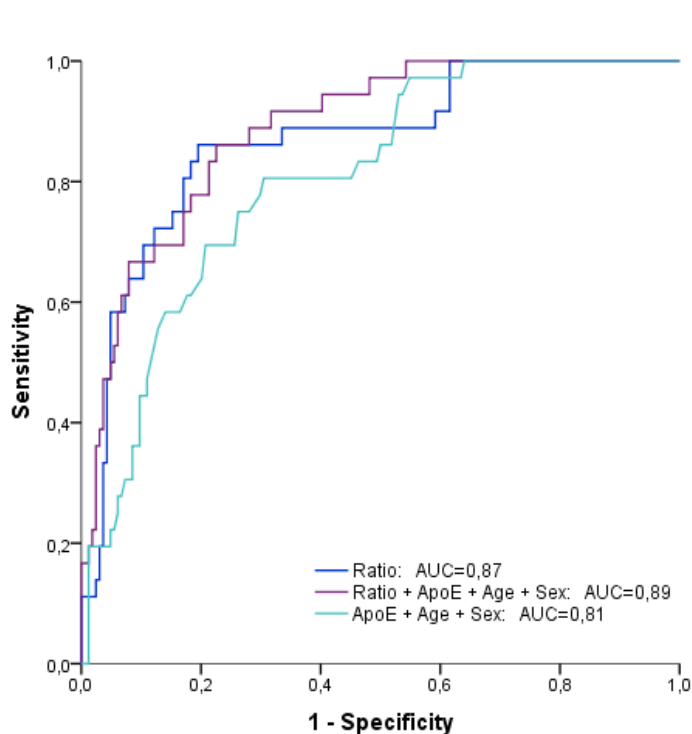# Association of plasma Aβ levels with brain amyloid deposition
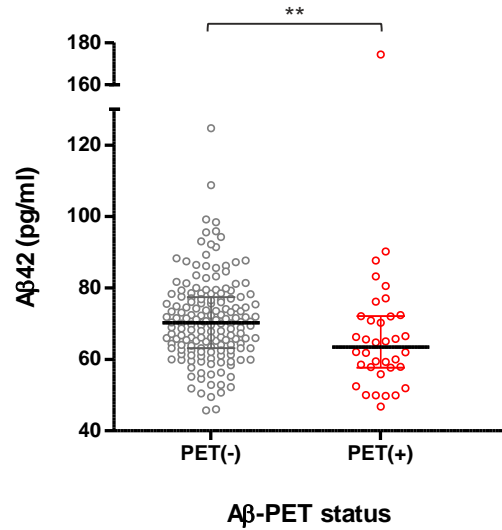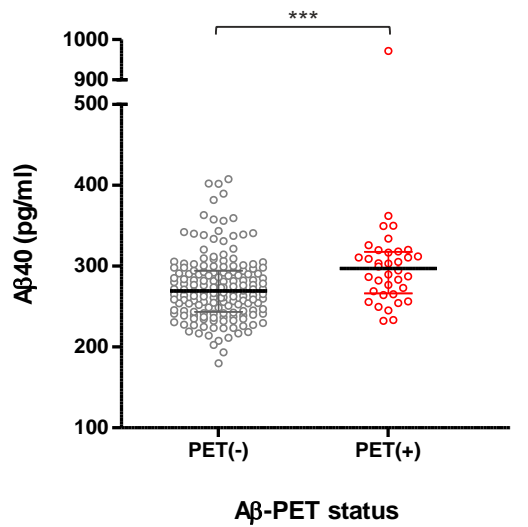
Median and IQR. Mann-Whitney test. ** P < 0.01 *** P < 0.001

Plasma Aβ42/Aβ40 discriminative ability of Aβ-PET status

ROC curve legend:
- Ratio: AUC=0,87
- Ratio + ApoE + Age + Sex: AUC=0,89
- ApoE + Age + Sex: AUC=0,81

Scatter plot: *Cutoff = 13.5 CL*, *Cutoff = 0.241*

FBB-PET Status
- Negative
- Positive

86.5% of discordant cases: Ratio (+) PET (-)

Plasma Aβ42/Aβ40 as an earlier marker of amyloid deposition

Model	AUC	95% CI	Se	Sp	PPV	NPV	Acc
Ratio	0.87	0.80-0.93	86.1%	80.5%	49.2%	96.4%	81.5%
Ratio+ApoE+Age+Sex	0.89[&]	0.83-0.94	86.1%	77.4%	45.6%	96.2%	79.0%
ApoE+Age+Sex	0.81	0.74-0.88	80.6%	69.5%	36.7%	94.2%	71.5%

& P = 0.005 vs base model. DeLong´s test.

ABtest-MS performance confirmed at two-year follow-up visit (V2)

V0
N = 200

Model	AUC	95% CI	Se	Sp	PPV	NPV	Acc
Ratio	0.87	0.80-0.93	86.1%	80.5%	49.2%	96.4%	81.5%
Ratio + ApoE + Age + Sex	0.89[&]	0.83-0.94	86.1%	77.4%	45.6%	96.2%	79.0%
ApoE + Age + Sex	0.81	0.74-0.88	80.6%	69.5%	36.7%	94.2%	71.5%

Cutoff = 0.241

V2
N = 165

Model	AUC	95% CI	Se	Sp	PPV	NPV	Acc
Ratio	0.86	0.80-0.93	75.7%	86.7%	62.2%	92.5%	84.2%
Ratio + ApoE + Age + Sex	0.90[$]	0.85-0.96	89.2%	82.0%	58.9%	96.3%	83.6%
ApoE + Age + Sex	0.81	0.74-0.88	70.3%	80.5%	51.0%	90.4%	78.2%

Cutoff = 0.238

1.2% difference

ROC curve of V0

Ratio: AUC=0,87
Ratio + ApoE + Age + Sex: AUC=0,89
ApoE + Age + Sex: AUC=0,81

ROC curve of V2

Ratio: AUC=0,86
Ratio + ApoE + Age + Sex: AUC=0,90
ApoE + Age + Sex: AUC=0,81
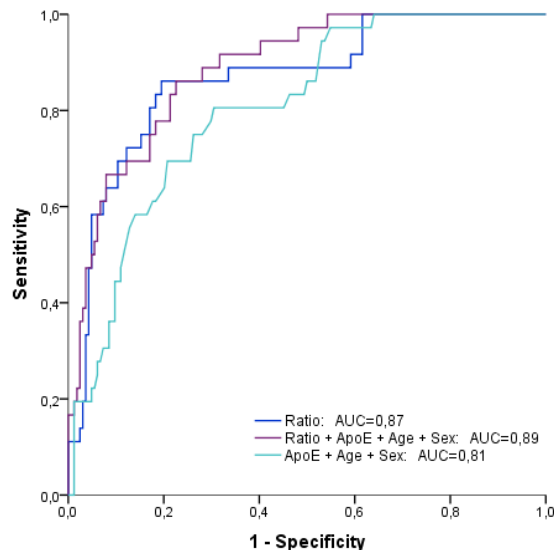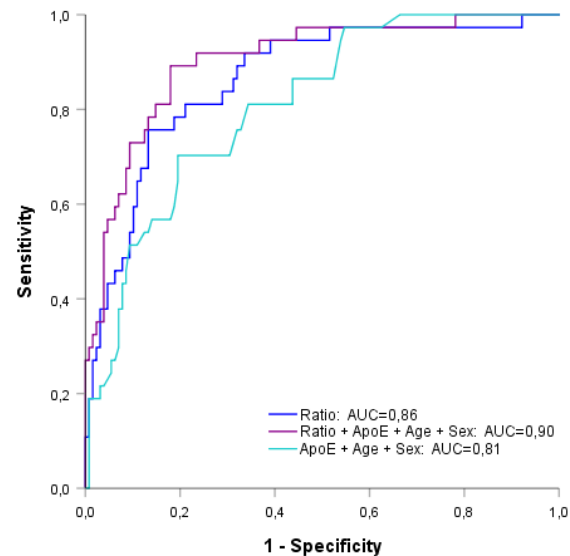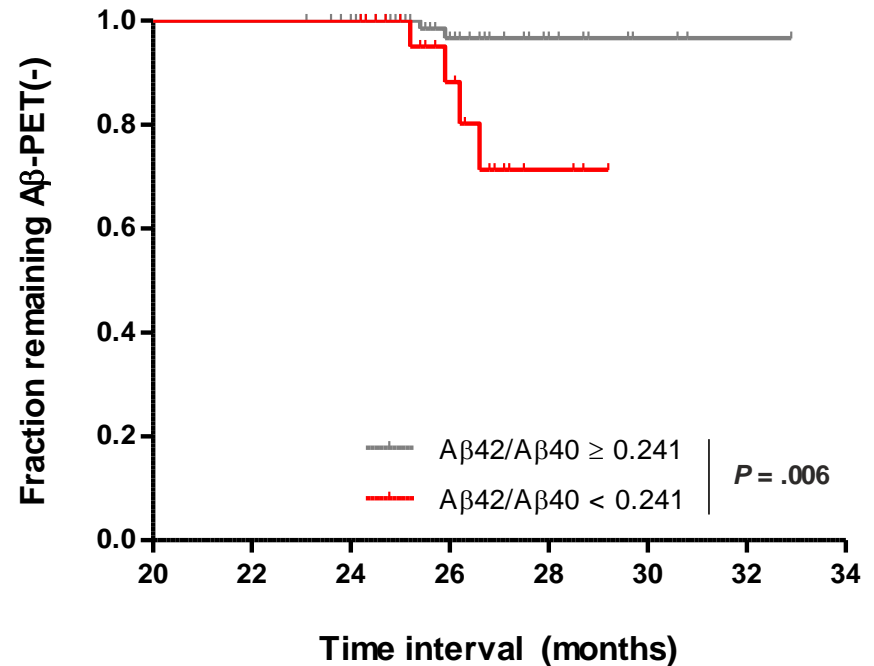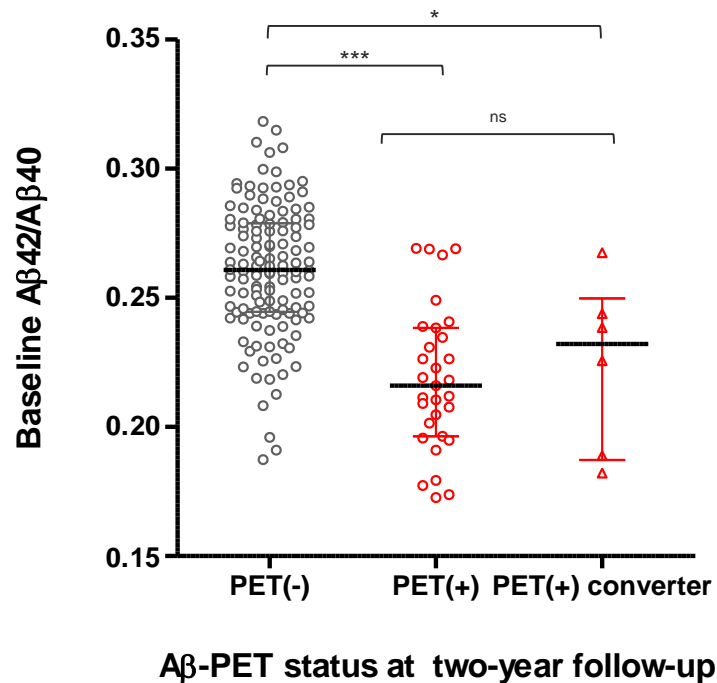
[&] P = 0.005 vs base model. DeLong´s test.
[$] P = 0.006 vs base model. DeLong´s test.
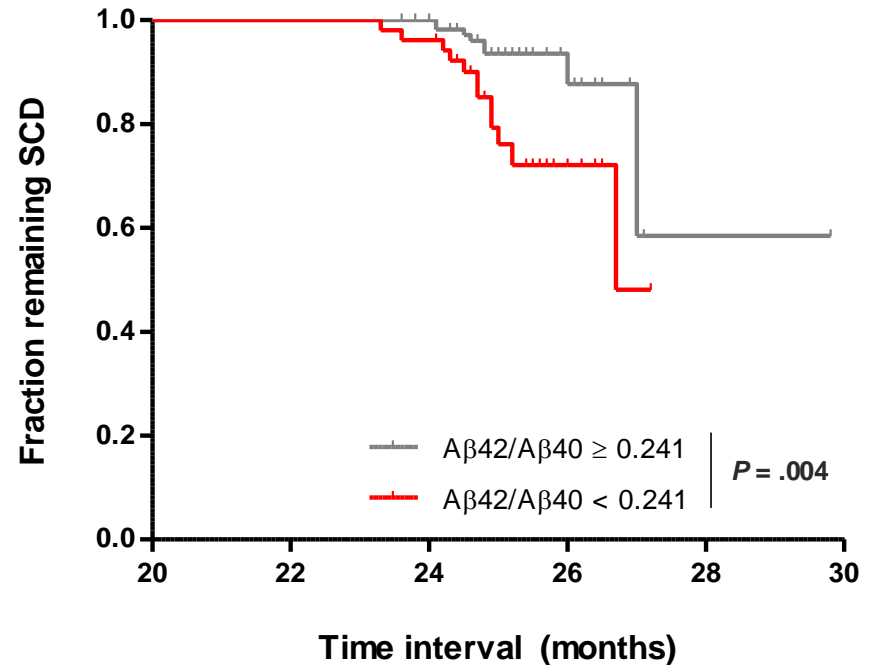
Plasma Aβ42/Aβ40 at baseline and conversion to Aβ-PET(+)

Converters to Aβ-PET(+) in V2 show lower values of Aβ42/Aβ40 at baseline (V0)

Participants with lower Aβ42/Aβ40 values present higher risk of conversion to Aβ-PET(+)

Plasma Aβ42/Aβ40 at baseline and conversion to MCI

Diagnosis at two-year follow-up

Time interval (months)

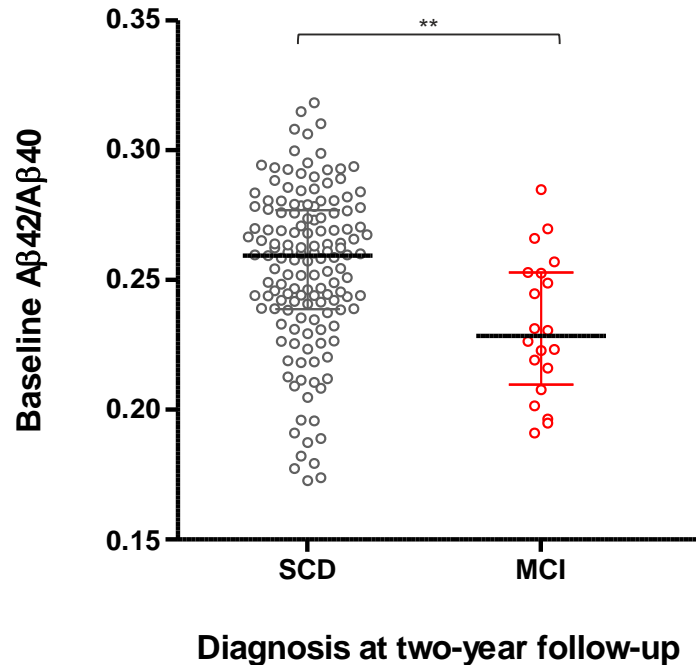**Converters to MCI in V2 show lower values of Aβ42/Aβ40 at baseline (V0)**

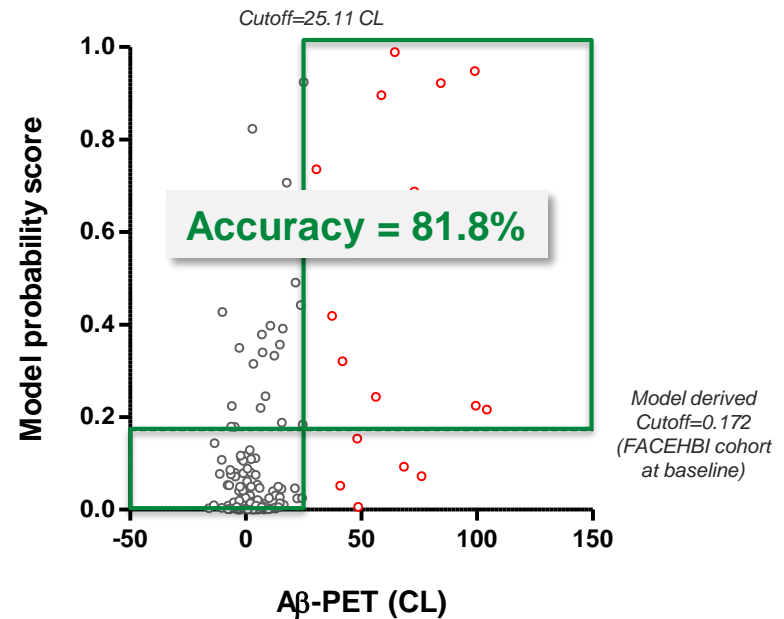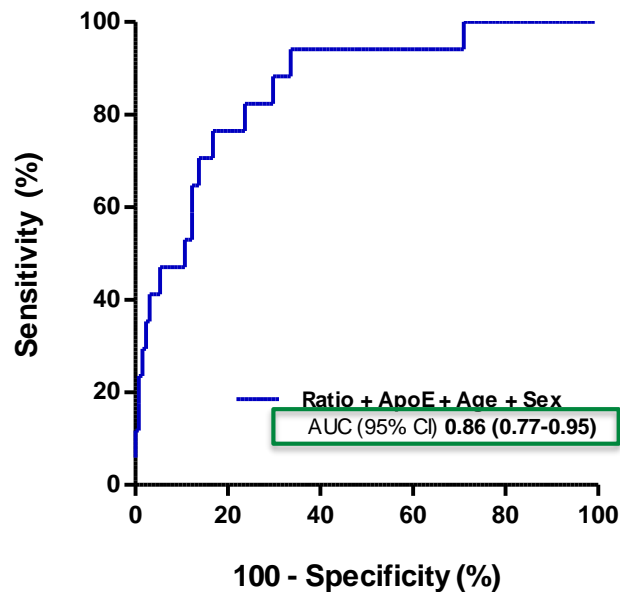**Participants with lower Aβ42/Aβ40 values present higher risk of conversion to MCI**

Cross-validation in an independent cohort (DPUK-Korea)[1]

148 CU individuals

SAMSUNG MEDICAL CENTER

SAMSUNG

	Aβ-PET (-)	Aβ-PET (+)	P value
Participants	131 (89%)	17 (11%)	
Age, years	69.0 (64.5-75.0)	74.0 (72.0-79.0)	**.0036**
Female	88 (67%)	10 (59%)	.6800
APOE ε4, number of alleles			**.0030**
0 alleles	102 (78%)	7 (41%)	
1 alleles	26 (20%)	8 (47%)	
2 alleles	3 (2%)	2 (12%)	
Duration of education, years	12.0 (6.0-16.0)	12.0 (6.0-12.0)	.4210
Aβ-PET, centiloids	2.8 (-2.3-7.3)	58.6 (41.8-76.0)	**< .0001**
MMSE	28 (27-30)	28 (27-29)	.2329

Ratio + ApoE + Age + Sex
AUC (95% CI) **0.86 (0.77-0.95)**

Cutoff=25.11 CL

Accuracy = 81.8%

Model derived
Cutoff=0.172
(FACEHBI cohort
at baseline)

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Conclusions

- We have set up and validated a new method (ABtest-MS) which allows **direct quantitation** of Aβ1-40 and Aβ1-42 in plasma **independently of any immunoprecipitation and / or enzymatic digestion procedure,** thus maintaining all the advantages of MS but with a simplified workflow

- ABtest-MS has demonstrated **good clinical performance** in a cohort of 200 cognitively unimpaired individuals with subjective cognitive decline (FACEHBI Cohort)

- This performance has been **confirmed** after the analysis of the two year follow-up visit (V2)

- Plasma Aβ42/Aβ40, as measured with ABtest-MS, may be an useful tool for **prediction of conversion** to PET(+) and MCI (**ongoing validation** with data from V5 and successive visits)

- **Robustness** of the predictive model has been **validated** in an independent cohort with 148 cognitively unimpaired individuals (DPUK-Korea Cohort)

Aknowledgements

Araclon Biotech
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Sang Won Seo
Hyemin Jang

CAEBi.

Mª Eugenia Sáez

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In Memoriam

Prof. Dr. Manuel Sarasa (1957-2020)

Thanks for your attention

Q & A