Reference measurement procedure for Aβ1-42 in CSF

REGION VÄSTRA GÖTALAND
SAHLGRENSKA UNIVERSITY HOSPITAL

”Highest level of expertise and a firm focus on the patient”

Sahlgrenska University Hospital

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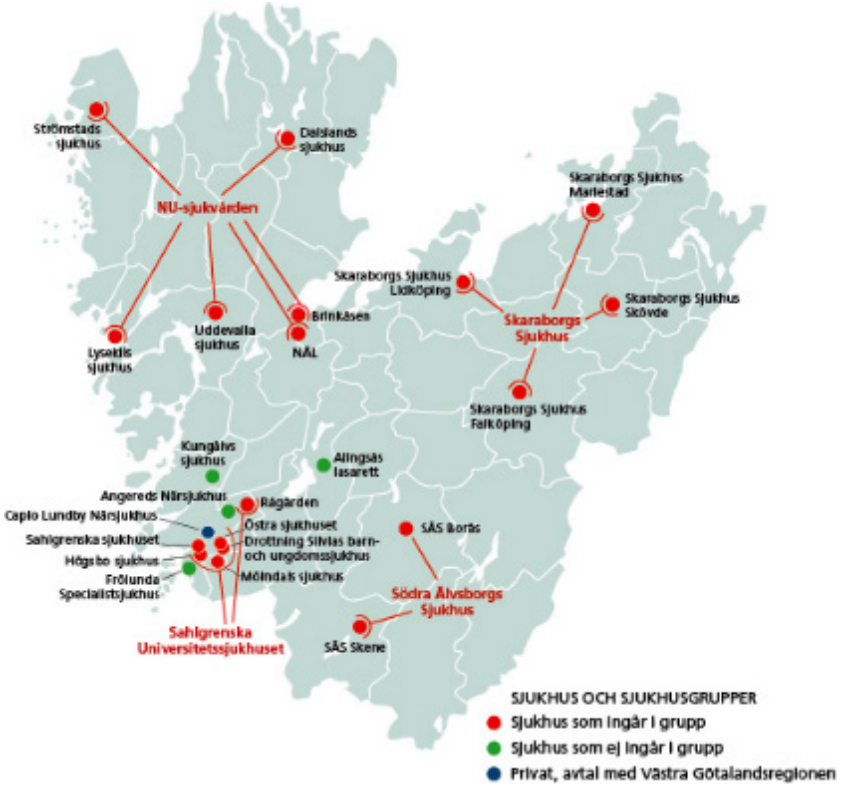
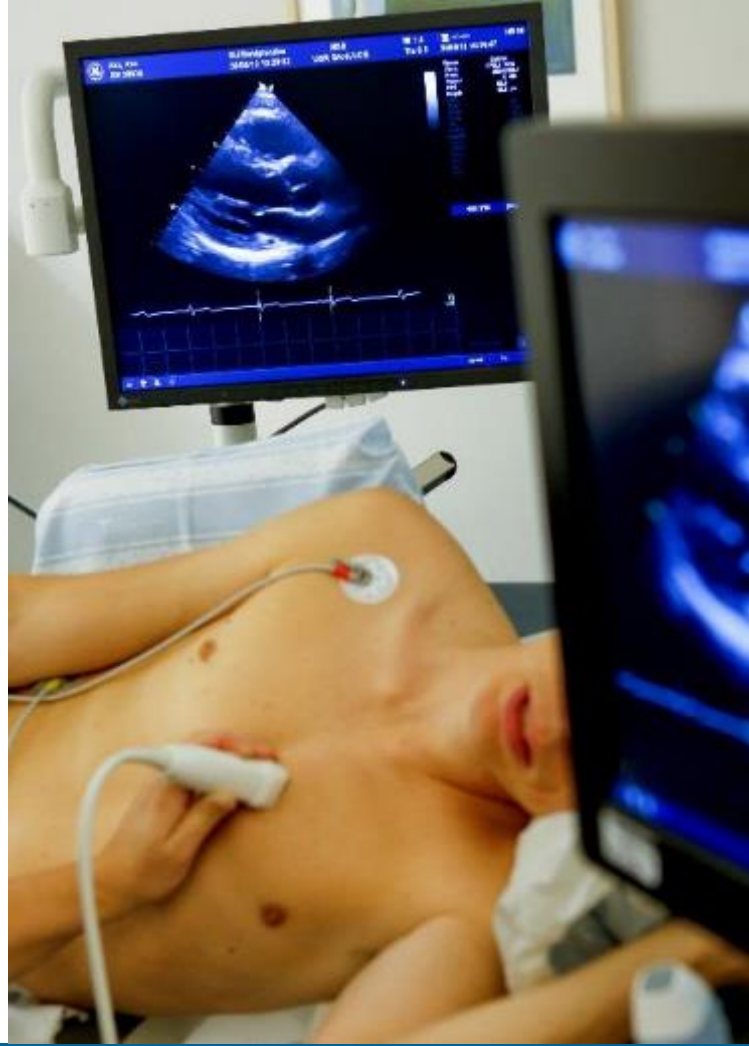
On the health care map

- One of the largest hospitals in Europe
- A specialist hospital for the Västra Götaland Region
- Seven national specialised medical care assignments
- Pioneering research conducted in collaboration with the Sahlgrenska Academy, Chalmers University of Technology, industry and other bodies



One single day at the hospital

- 30 deliveries
- 550 emergency visits
- 3 400 outpatient visits
- 17 000 laboratory analyses

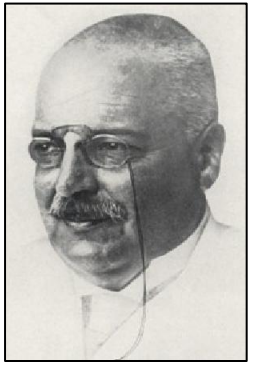


Clinical Chemistry Laboratory

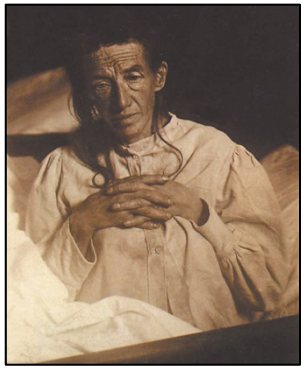
- This laboratory is accredited in accordance with the International Standards
  - ISO 15189:2012 - Medical laboratories - Requirements for quality and competence
  - ISO 22870:2006 - Point-of-care testing - Requirements for quality and competence
- First hospital laboratory in Sweden to be accredited (1992).

Alzheimer’s disease (AD)

- The most common cause of dementia
  - Accounts for 60-80% of all cases of dementia
- > 40 million people worldwide affected
- First described in 1906 by Alois Alzheimer



Alois Alzheimer
Image: public domain



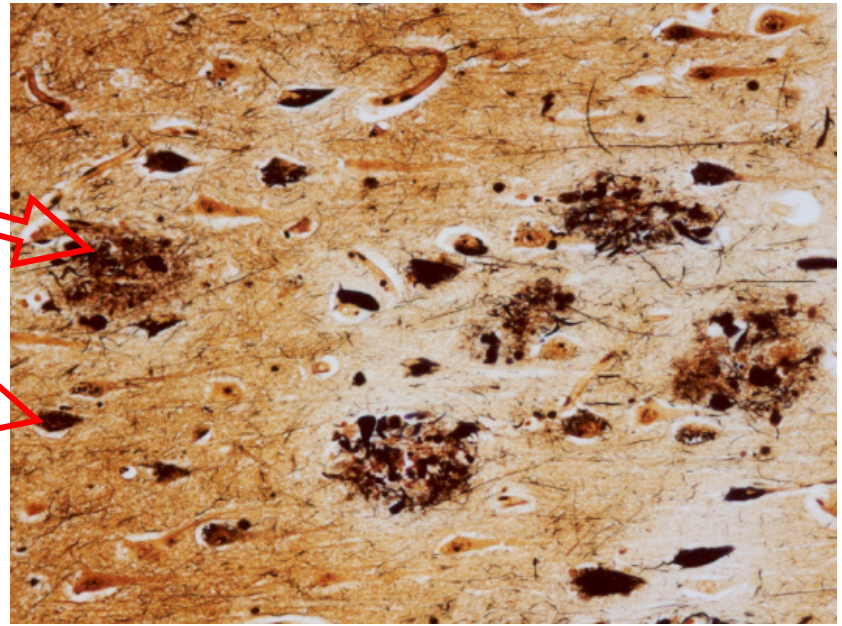
Alois Alzheimer's patient
Auguste Deter in 1902
Image: public domain

Characteristic clinical symptoms

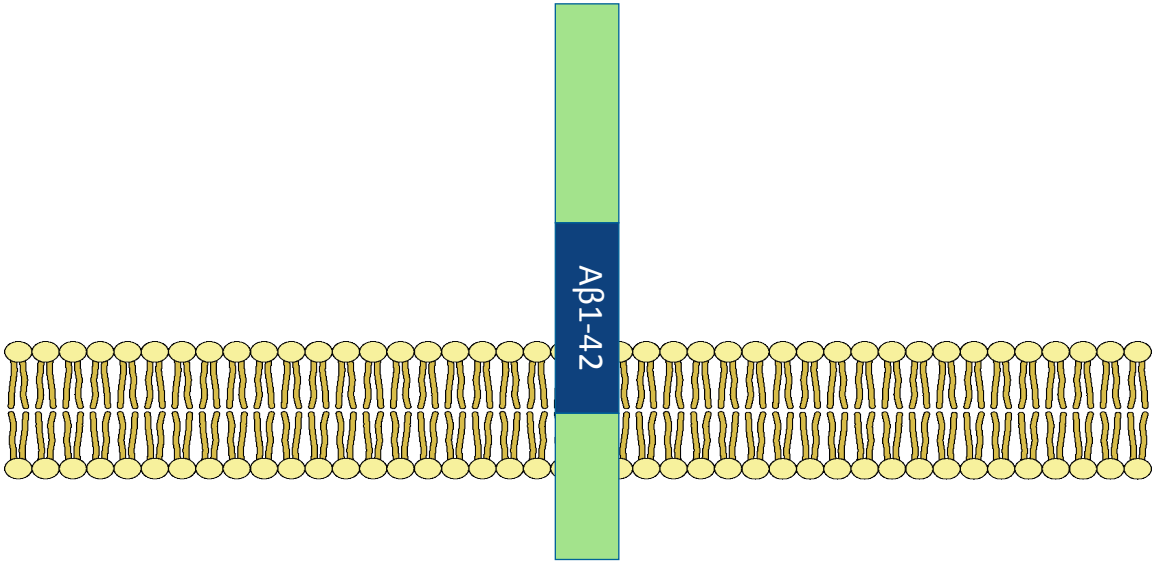
- Impaired episodic memory
- aphasia
  - disturbance in formulation and comprehension of language
- apraxia
  - loss of the ability to execute or carry out learned purposeful movements
- agnosia
  - loss of ability to recognize objects, persons, sounds, shapes, or smells
- general cognitive symptoms
  - impaired judgment, decision-making and orientation

Neuropathology

- Deposits of extracellular plaques
  - mainly Aβ peptides
- Intracellular neurofibrillary tangles
  - phosphorylated tau protein



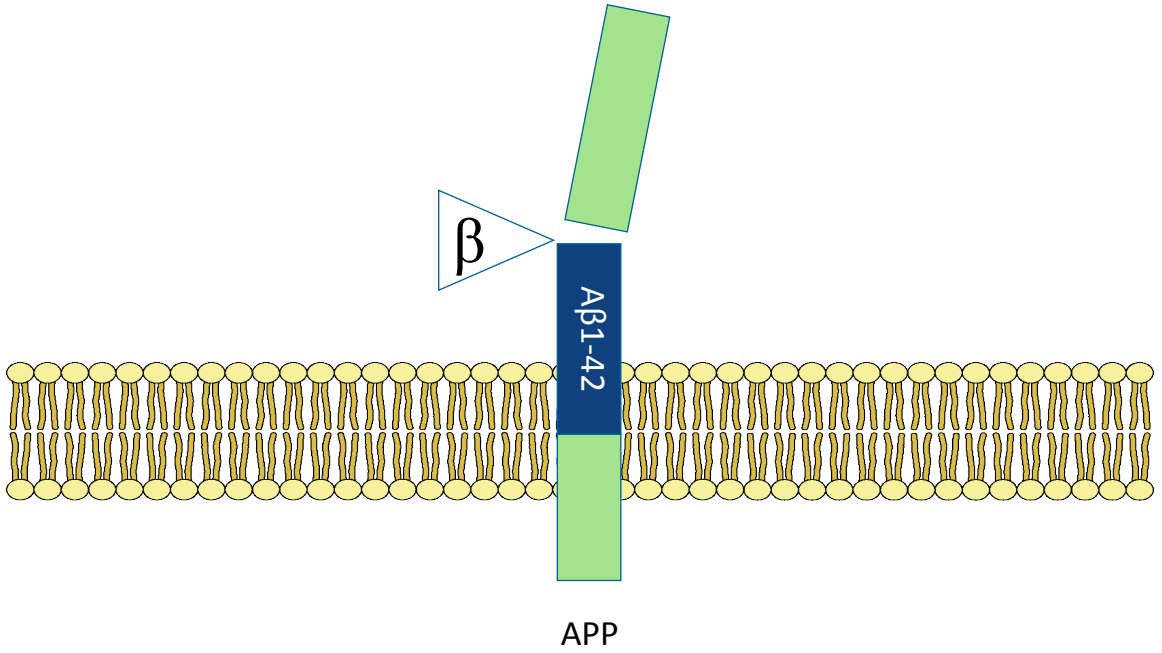
APP processing



APP

- Aβ peptides - natural metabolic products of the transmembrane glycoprotein APP.
- Generated through the amyloidogenic pathway by consecutive actions of β- & γ-secretase.

APP processing



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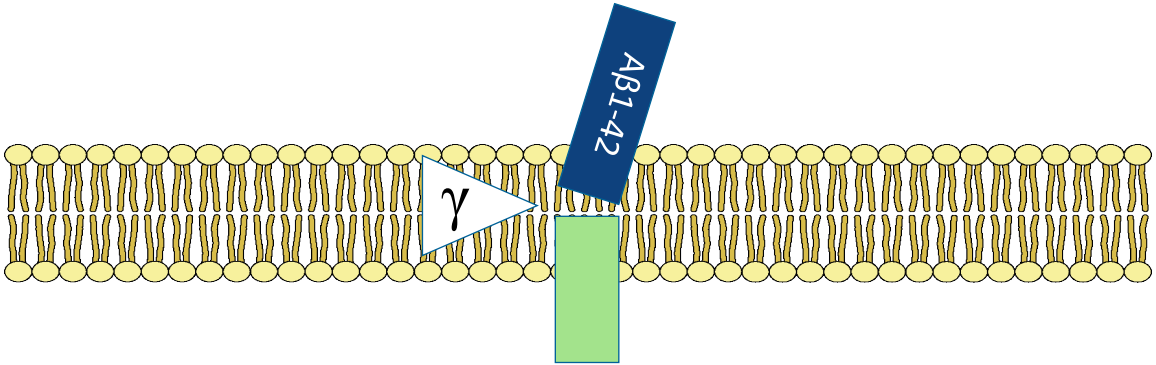
APP processing

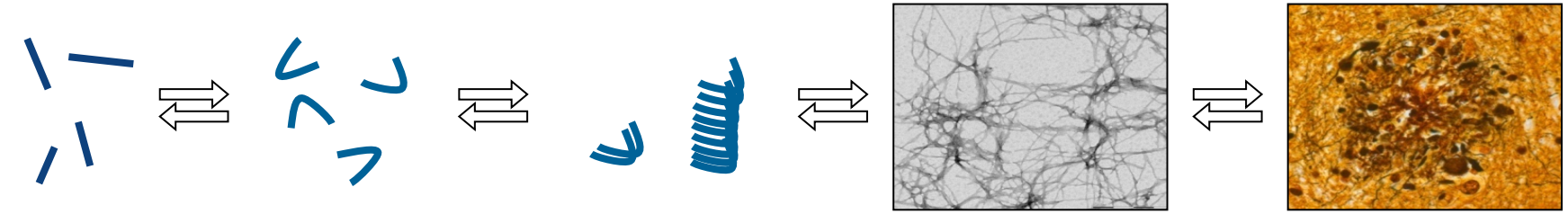


AICD (amyloid precursor protein intracellular domain)

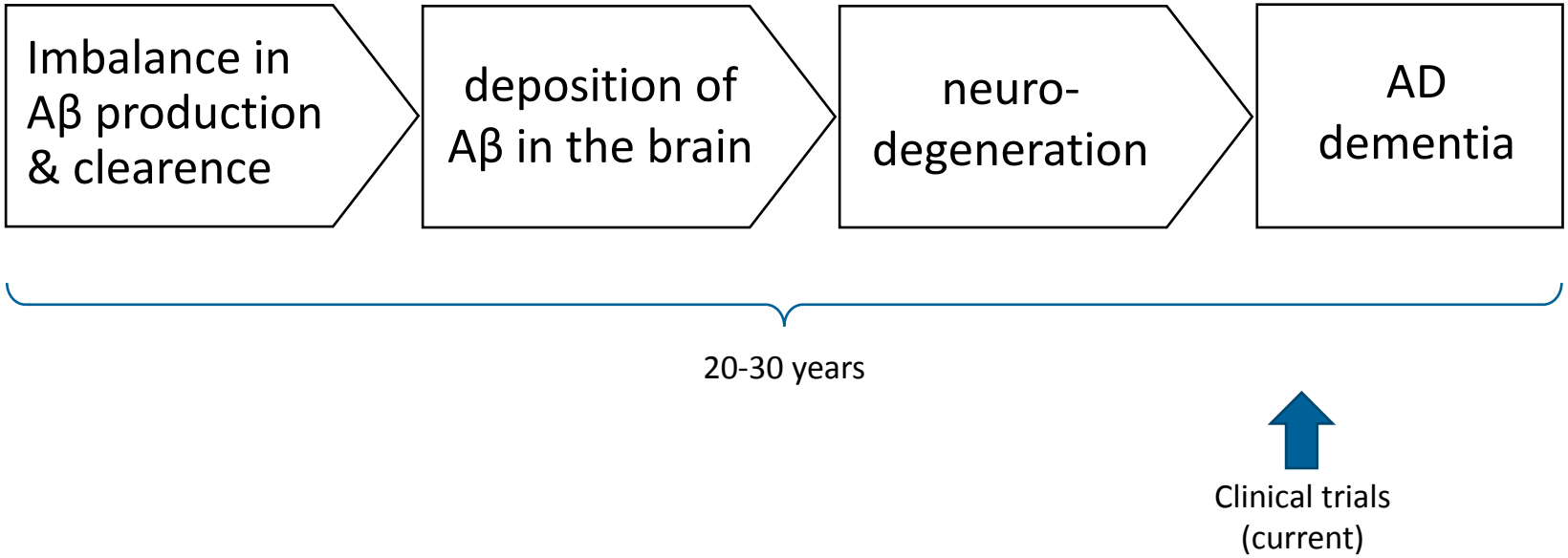
- Aβ peptides - natural metabolic products of the transmembrane glycoprotein APP.
- Generated through the amyloidogenic pathway by consecutive actions of β- & γ-secretase.

Aβ misfolding and oligomerisation

- A conformational change of Aβ into high β-sheet content is believed to increase its propensity to self-aggregate.
- Which of these forms that are neurotoxic is still uncertain, but
  - levels of soluble Aβ dimers and oligomers has been shown to correlate with clinical symptoms and synaptic loss
  - Fibrils have been shown to induce neuronal loss



The amyloid cascade hypothesis



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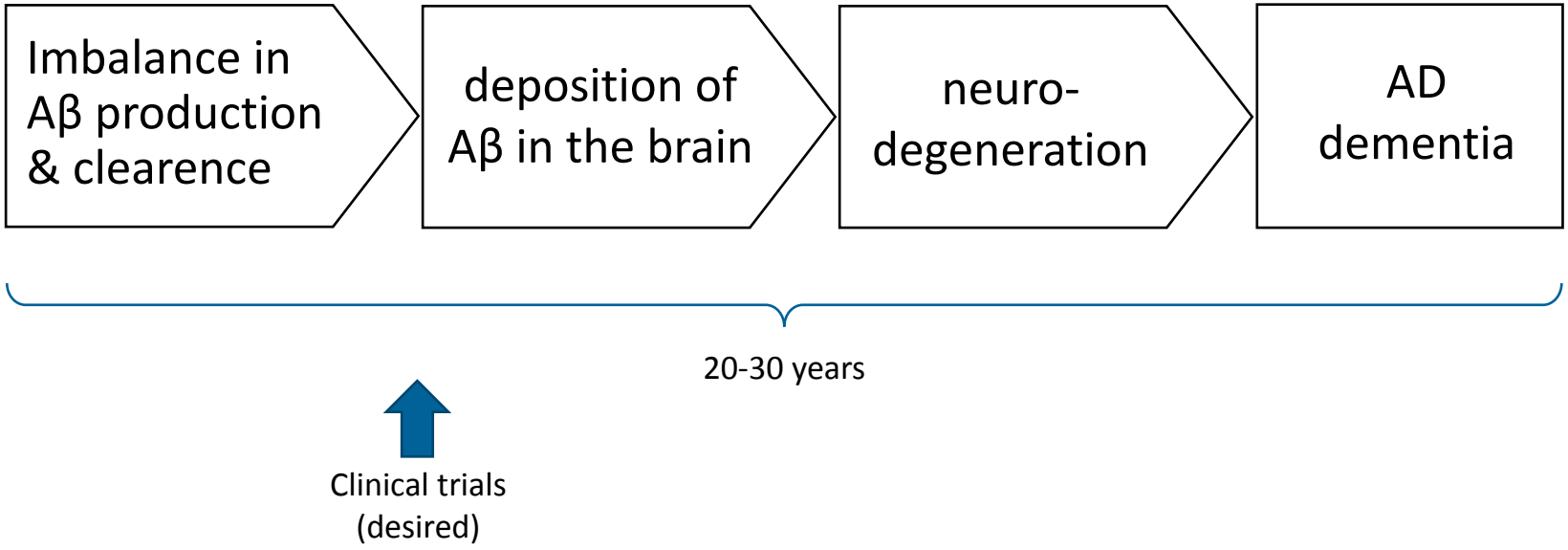


Diagnosis

- Medical history, cognitive tests and mental state exams
- Post mortem neuropathological examination required for definitive diagnosis
- Ongoing process of including biomarkers
  - The International Working Group (IWG)-2 criteria for typical AD now include
    - increased tracer retention on amyloid PET
    - decreased $A\beta_{1-42}$ together with increased tau in CSF

Biomarkers

Type	Biomarker	Change in AD
CSF	$A\beta_{1\text{-}42}$	↓ concentration
CSF	$A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio	↓ ratio
CSF	T-tau	↑ concentration
CSF	P-tau	↑ concentration
Imaging	Structural MRI	↓ volume
Imaging	Functional MRI	↓ functional connectivity
Imaging	FDG-PET	↓ glucose metabolism
Imaging	Amyloid PET	↑ Aβ retention
Imaging	Tau PET	↑ intracellular tau

Type	Biomarker	Change in AD
CSF	$A\beta_{1-42}$	↓ concentration

~50% lower concentration of $A\beta_{1-42}$ in CSF in AD patients compared to healthy controls

- Peptide accumulation in plaques in the brain → less in CSF

Type	Biomarker	Change in AD
CSF	$A\beta_{1-42}$	↓ concentration
CSF	T-tau	↑ concentration
CSF	P-tau	↑ concentration

Combined with the microtubule-stabilizing tau protein → high diagnostic accuracy of AD

- t-tau – cortical axonal degeneration
- p-tau – tangle pathology

Type	Biomarker	Change in AD
CSF	$A\beta_{1-42}$	↓ concentration
CSF	T-tau	↑ concentration
CSF	P-tau	↑ concentration
CSF	$A\beta_{1-42}/A\beta_{1-40}$ ratio	↓ ratio

Low & high Aβ producers

- When using only $A\beta_{1-42}$
  - Low producers might be false positive for AD
  - High producers might be false negative for AD
- Using the ratio of $A\beta_{1-42}/A\beta_{1-40}$ improve diagnostic accuracy
  - $A\beta_{1-40}$ levels in CSF are realatively unchanged in AD compared to controls

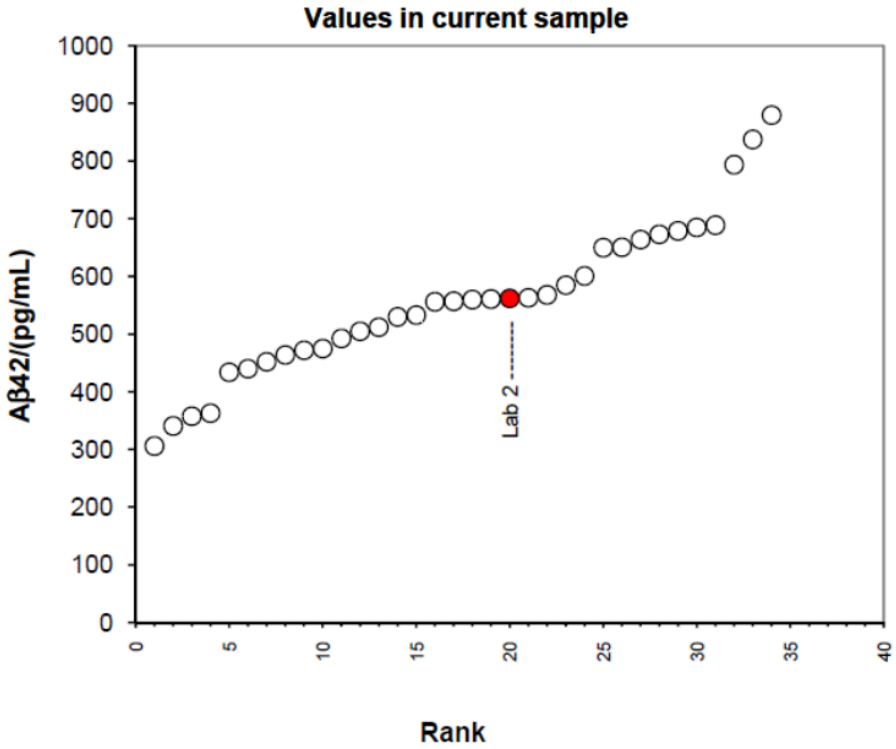
Schoonenboom, N. S. et al. Ann Neurol. 2005;58(1):139-42. Wiltfang J et al. J Neurochem. 2007;101(4):1053-9. Hansson O et al. Dement Geriatr Cogn Disord. 2007;23(5):316-20.

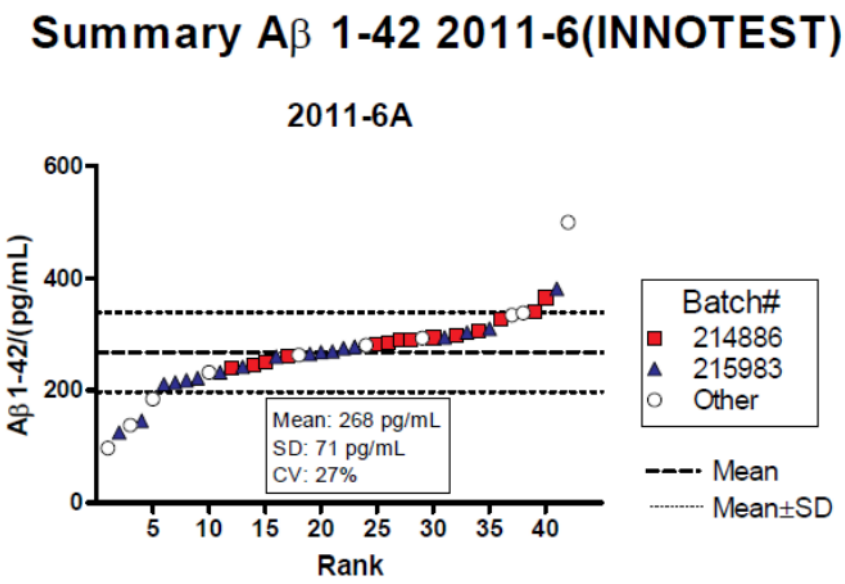
Treatment

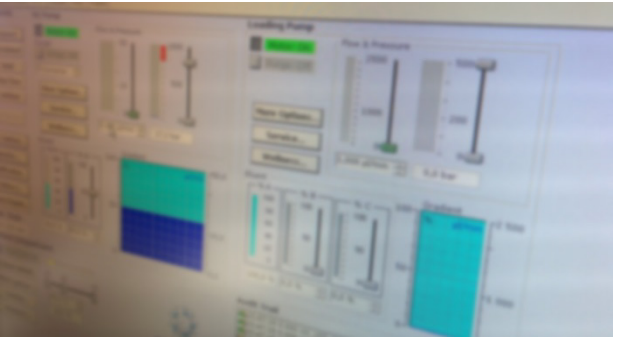
- Existing drugs temporarily improve symptoms
- There is no therapy that slows or stops the progression of AD
- Treatment strategies currently evaluated
  - Active immunotherapy: immunization with Aβ peptides
  - Passive immunotherapy: treatment with anti-Aβ antibodies
  - Inhibition of the β-secretase BACE1

BACE1 = beta-site amyloid precursor protein cleaving enzyme 1

AD QC program







Methods & results

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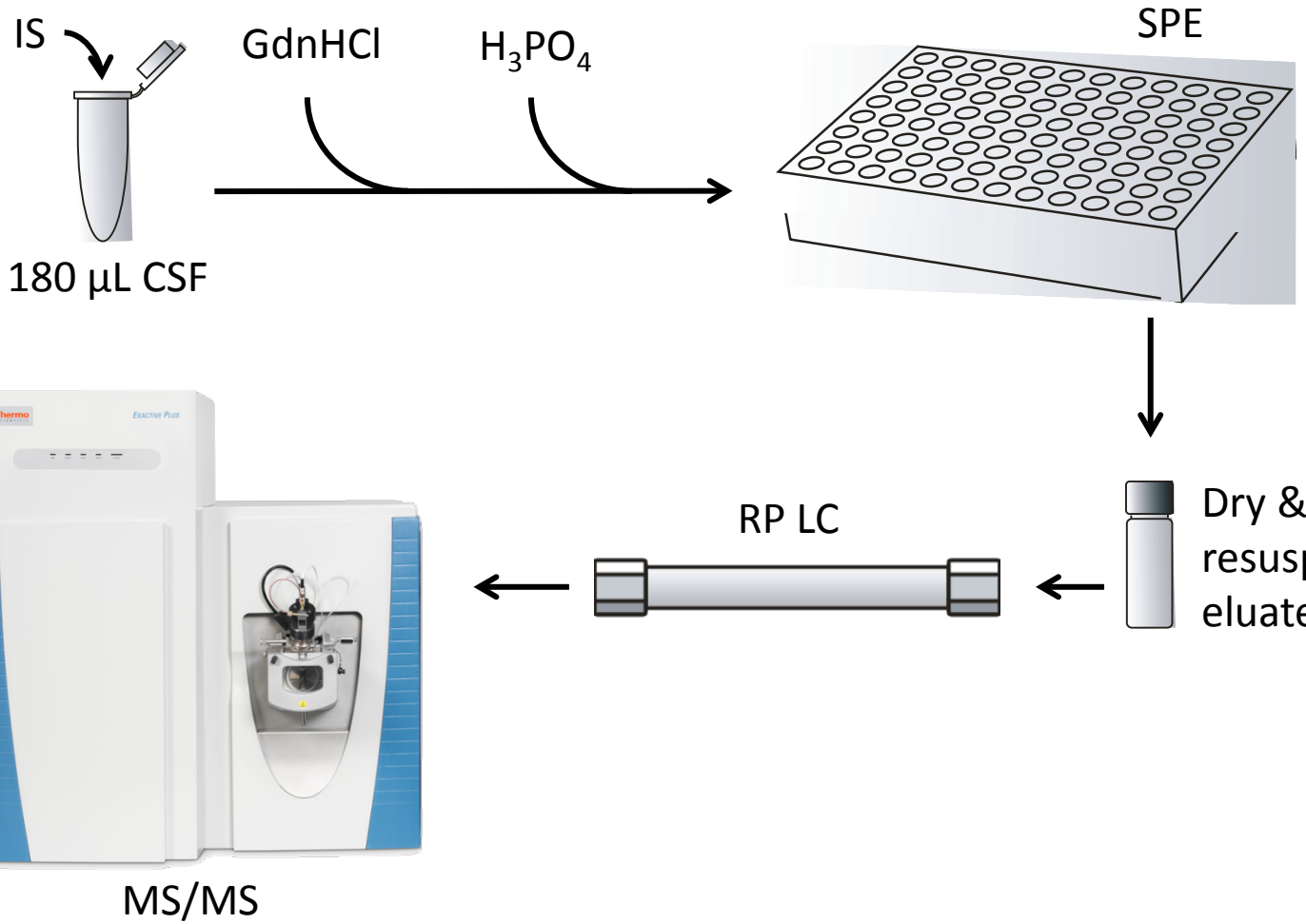
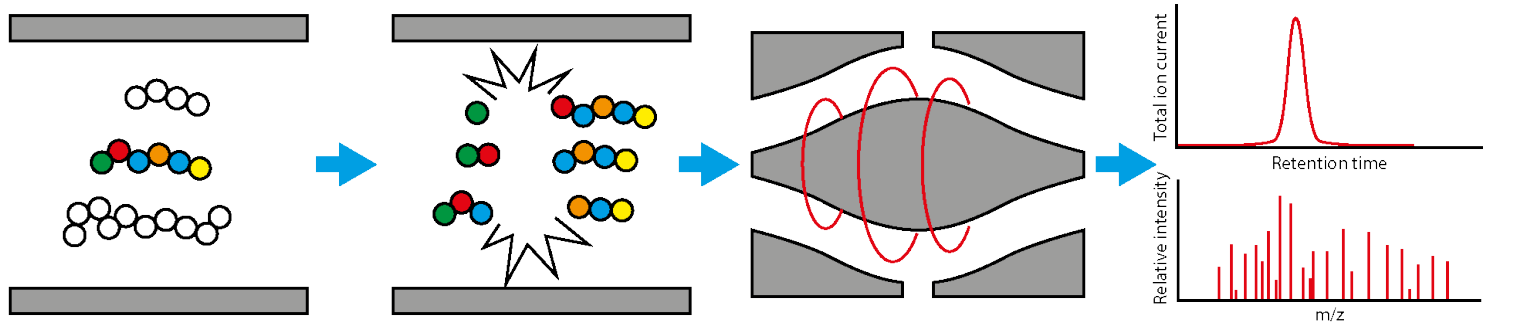

IS
GdnHCl
H_3PO_4
SPE
180 µL CSF
Dry &
resuspend
eluate
RP LC
MS/MS

Quadrupole-orbitrap hybrid MS
- parallel reaction monitoring (PRM)



Quadrupole:
precursor ion selection

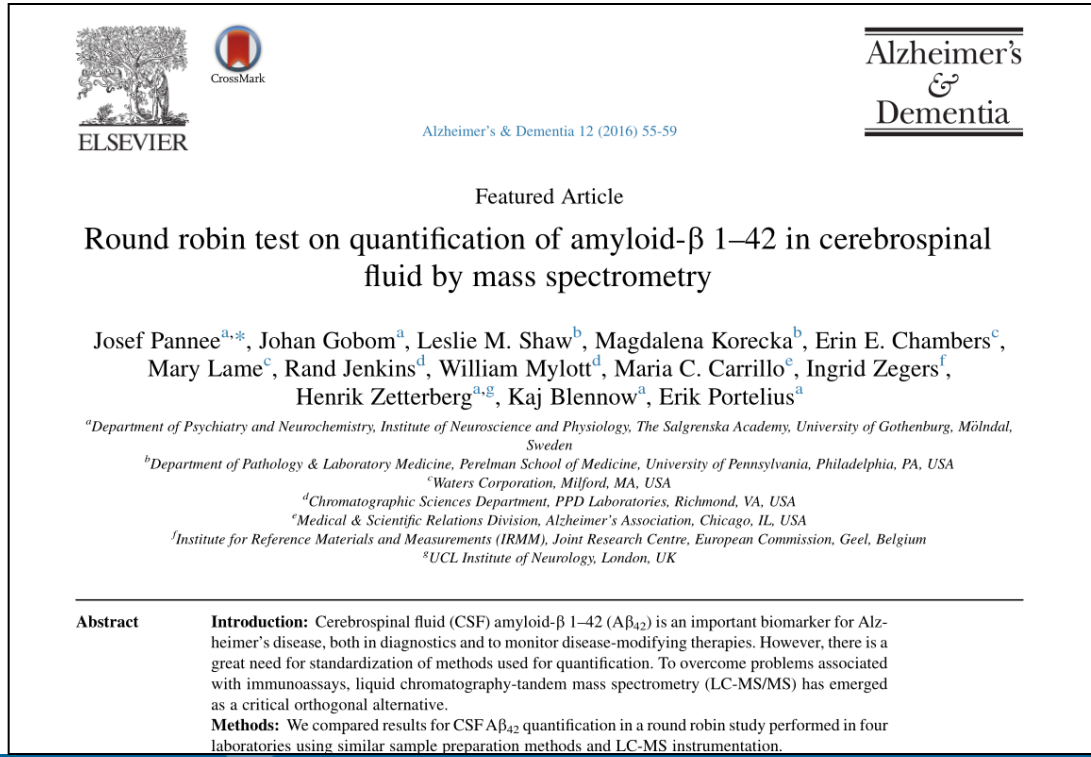
Collision cell:
fragmentation

Orbitrap:
detection

Output:
chromatogram (top)
MS/MS spectrum (bottom)

Round Robin study

- Perform an inter-laboratory study involving other laboratories using similar LC-MS methods
- Determine the inter-laboratory variation using these methods
- Examine if these methods are suitable to set the level of a certified reference material.



ELSEVIER

CrossMark

Alzheimer's & Dementia 12 (2016) 55-59

Alzheimer's & Dementia

Featured Article

Round robin test on quantification of amyloid-β 1–42 in cerebrospinal fluid by mass spectrometry

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[c]*Waters Corporation, Milford, MA, USA*
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[e]*Medical & Scientific Relations Division, Alzheimer's Association, Chicago, IL, USA*
[f]*Institute for Reference Materials and Measurements (IRMM), Joint Research Centre, European Commission, Geel, Belgium*
[g]*UCL Institute of Neurology, London, UK*

Abstract

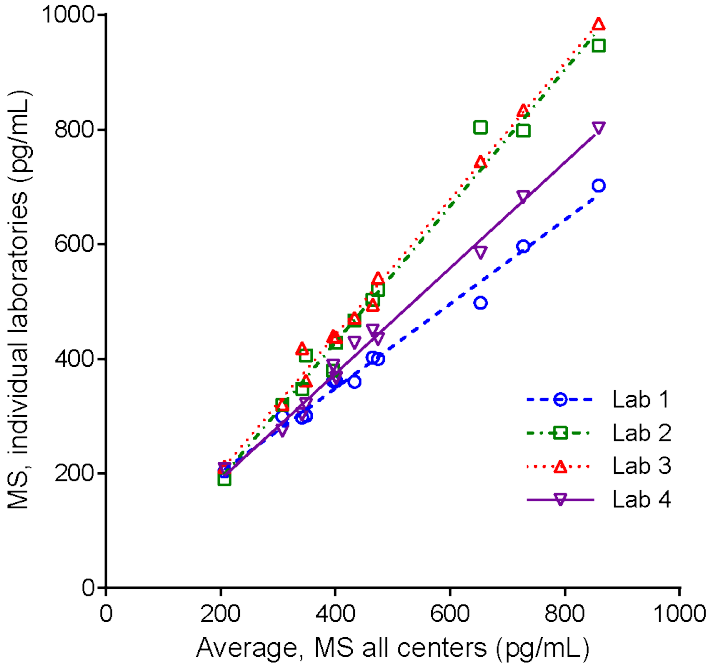
Introduction: Cerebrospinal fluid (CSF) amyloid-β 1–42 ($A\beta_{42}$) is an important biomarker for Alzheimer's disease, both in diagnostics and to monitor disease-modifying therapies. However, there is a great need for standardization of methods used for quantification. To overcome problems associated with immunoassays, liquid chromatography-tandem mass spectrometry (LC-MS/MS) has emerged as a critical orthogonal alternative.

Methods: We compared results for CSF $A\beta_{42}$ quantification in a round robin study performed in four laboratories using similar sample preparation methods and LC-MS instrumentation.

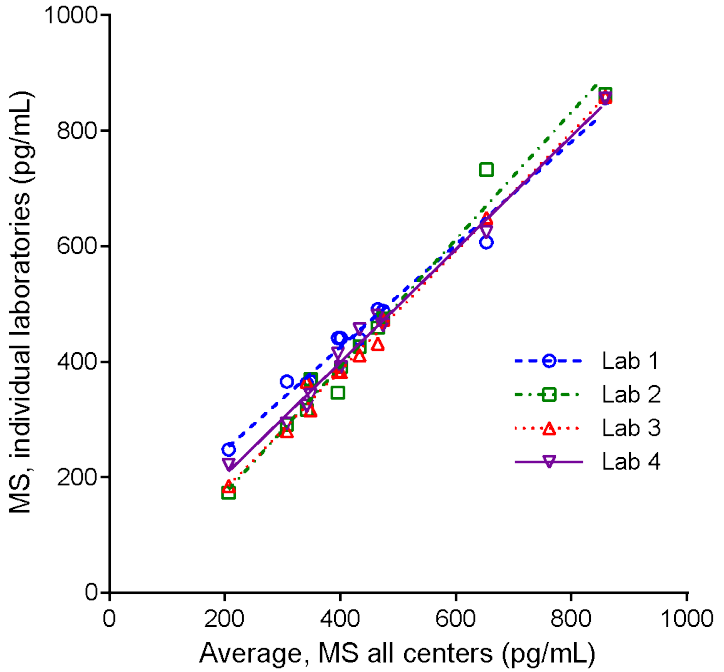
Procedures for participating laboratories

Procedure	Waters	PPD	U. Penn.	U. Got.
IS concentration, ng/mL	1	2	2	1.6
CSF volume, µL	200	100	250	200
Calbrator matrix	aCSF with 5% rat plasma	aCSF with 4 mg/mL HSA + IgG, glucose	aCSF with 4 mg/mL BSA	Human CSF
LC System	ACQUITY, 1D	ACQUITY; 2D trapping/ eluting	ACQUITY; 2D trapping/ eluting	Accela 1250
Dilution (injection)	50 µL + 25 µL H_2O (10 µL)	50 µL + 50 µL H_2O (30 µL)	50 µL + 50 µL H_2O (50 µL)	None. Dried eluate resuspended in 25 µL 79:20:1 H_2O/ACN/ NH_4OH (20 µL)
LC mobile phases	A: 0.3% NH_4OH B: 90:10 ACN/MP A	A: 0.3% NH_4OH B: 90:5:5 ACN/TFE/H_2O	A: 0.1% NH_4OH B: 75:25:5 ACN/MeOH/ TFE	A: 0.1% NH_4OH, 5% ACN B: 0.03% NH_4OH, 95% ACN
Column	Waters BEH 300 2.1 × 150 mm, 1.7 µm, 50°C	Waters BEH 300 2.1 × 150 mm, 1.7 µm, 50°C	Waters BEH 300 2.1 × 50 mm, 1.7 µm, 60°C	Thermo ProSwift RP-4H 1 × 250 mm, 50°C
Flow rate, µL/min	200	300	200	300
MS	Waters Xevo TQ-S	Waters Xevo TQ-S	ABSciex API 5000	Thermo TSQ Vantage
Transitions, m/z	1129.0→1078.5	1129.0→1078.5	1129.0→1078.5	1129.58→1054.03, 1078.79, 1107.06
Run time	8.5 minutes	8.5 minutes	12 minutes	14 minutes

Twelve pools of human CSF were analyzed at four different laboratories.



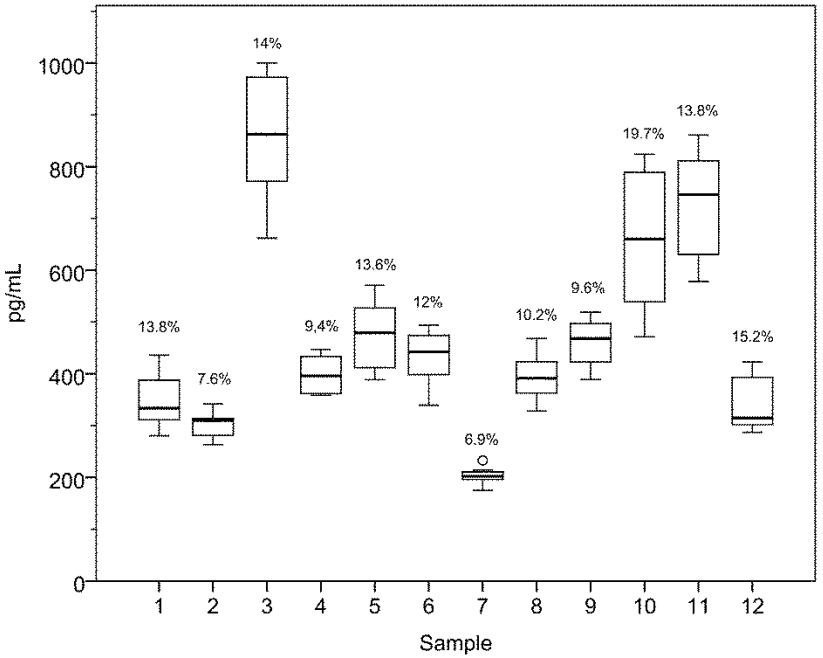
Using sample 11 as a reference, the measurements for the other samples were adjusted



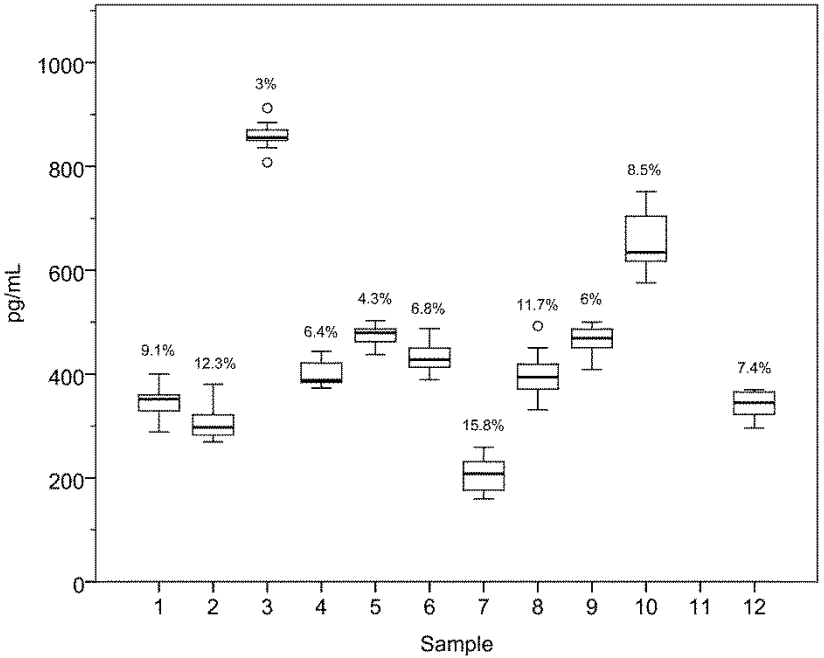
$$x \frac{\text{(sample 11 average pg/mL for all laboratories)}}{\text{(sample 11 pg/mL for each laboratory)}}$$

Interlab CV%

Average inter-lab CV = 12 %



Average inter-lab CV = 8 %



Conclusions

- A good agreement was seen between the laboratories, with an average inter-laboratory CV of 12.2%
  - despite the different methods and instrumentations used
- Using a common reference sample significantly decreased the average inter-laboratory CV (to 8.3%)

Clinical Chemistry 60:7
987–994 (2014)

JCTLM approved 2015-10-21

Mass Spectrometry–Based Candidate Reference Measurement Procedure for Quantification of Amyloid-β in Cerebrospinal Fluid

Andreas Leinenbach,[1†] Josef Pannee,[2†] Thomas Dülffer,[1] Andreas Huber,[1] Tobias Bittner,[1] Ulf Andreasson,[2] Johan Gobom,[2] Henrik Zetterberg,[2,3] Uwe Kobold,[1] Erik Portelius,[2] and Kaj Blennow[2*] on behalf of the IFCC Scientific Division Working Group on CSF proteins

Isotope dilution mass spectrometry methods for amyloid beta 1-42 in other

▸ 2D-UPLC-tandem mass spectrometric method for analysis of amyloid beta 1-42 in human CSF

Applicable matrice(s)	frozen human cerebrospinal fluid (CSF)
Full description of technique(s)	Liquid chromatography tandem mass spectrometry, solid phase extraction
Quantity	Mass concentration
Applicable range	100 pg/mL to 3000 pg/mL
Expected uncertainty (level of confidence 95%)	14.3 pg/mL to 355.2 pg/mL
Reference(s)	Qualification of a surrogate matrix-based absolute quantification method for Amyloid β_{42} in human cerebrospinal fluid using 2D UPLC-Tandem Mass Spectrometry, Korecka M et al., *Journal of Alzheimer's Disease (JAD)*, 2014, **41**(2), 441-451
Comparability assessment study(ies)	Clinical comparison with immunoassay as cited in: Korecka M et al., *JAD*, 2014, **41**(2), 441-451 Round robin test on quantification of amyloid-β-1-42 in cerebrospinal fluid by mass spectrometry, Pannee J et al., *Alzheimer's and Dementia*, 2016, **12**(1), 55-59
Comment(s)	The reference measurement method, C12RMP1, for quantification of Aβ42 in cerebrospinal fluid was developed and validated by the Biomarker Research Laboratory of Perelman School of Medicine, University of Pennsylvania
JCTLM DB identification number	C12RMP1

Isotope dilution mass spectrometry methods for amyloid beta 1-42 in other

▸ Mass spectrometry-based candidate reference measurement procedure for quantification of Aβ42 in cerebrospinal fluid

Applicable matrice(s)	human cerebrospinal fluid
Full description of technique(s)	Isotope dilution mass spectrometry
Quantity	Mass concentration
Applicable range	150 pg/ml to 4000 pg/ml
Expected uncertainty (level of confidence 95%)	15.7 %
Reference(s)	Mass spectrometry-based candidate reference measurement procedure for quantification of Aβ42 in cerebrospinal fluid, A. Leinenbach et al. on behalf of the IFCC Scientific Division Working Group on CSF proteins (WG-CSF) *Clin. Chem.*, 2014, **60**(7), 987-994
Comparability assessment study(ies)	See reference cited above for comparability assessment study
Comment(s)	The reference measurement procedure, C11 RMP9, for quantification of Aβ42 in cerebrospinal fluid was developed and validated by Roche Diagnostics GmbH in collaboration with the University of Gothenburg
JCTLM DB identification number	C11RMP9

BACKGROUND: Cer
($A\beta_{42}$) is a well-
disease. Several in
in absolute conce
interference, ther
parisons and the
with the IFCC W
developed a cand
dure (RMP) for A

METHODS: The an
was based on s
dilution LC-MS/M
ently stable isotop
tion in human CSF, an important aspect since there
was no analyte-free matrix available. Because no CSF

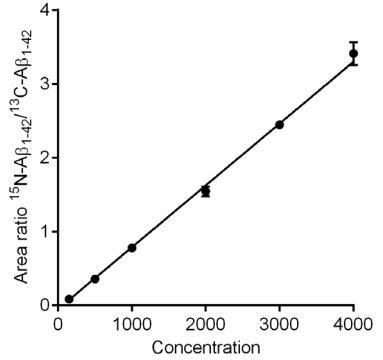
trix effects (*2, 3*). The broadscale use of immunoassays

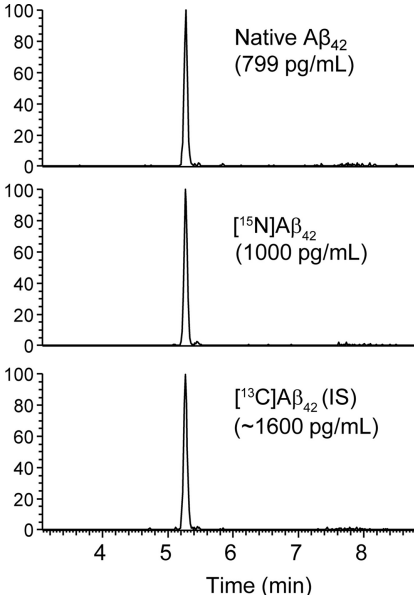
Calibration in human CSF

- Surrogate matrix such as artificial CSF might lead to low recoveries of the analyte → sensitivity issue
- Calibrators were prepared in human CSF using the surrogate analyte approach

Surrogate analyte approach

- ^{15}N-A$\beta_{1\text{-}42}$ was used as a surrogate for the native A$\beta_{1\text{-}42}$ for calibration.
- ^{13}C-A$\beta_{1\text{-}42}$ was used as IS in both calibrators and unknowns.
- A response factor (*f*) for ^{15}N-A$\beta_{1\text{-}42}$ to native A$\beta_{1\text{-}42}$ was determined in artificial CSF
- When determining the concentration of endogenous A$\beta_{1\text{-}42}$ in unknown CSF samples, the concentration of ^{15}N-A$\beta_{1\text{-}42}$ used in the calibration curve was multiplied by *f*, which was measured before and after each set of unknown samples





Imprecision

Imprecision	Intra-assay	Inter-assay
250 pg/mL	5.0%	6.4%
1000 pg/mL	2.2%	5.6%

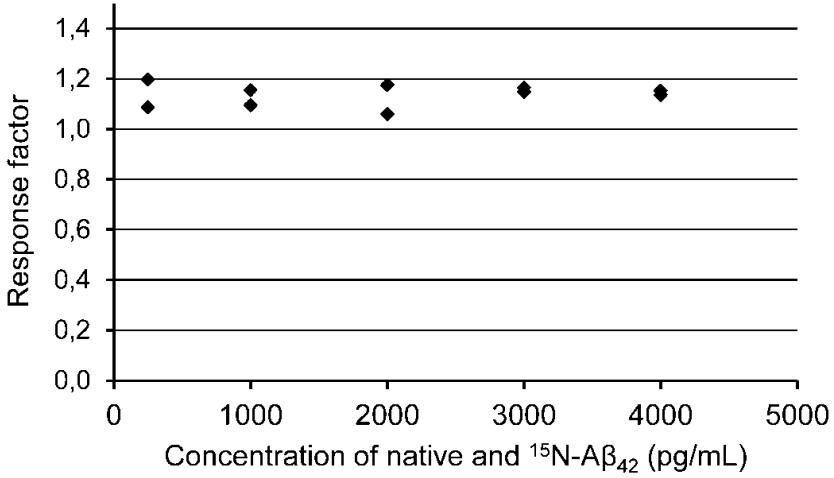
^{15}N-A$\beta_{1\text{-}42}$

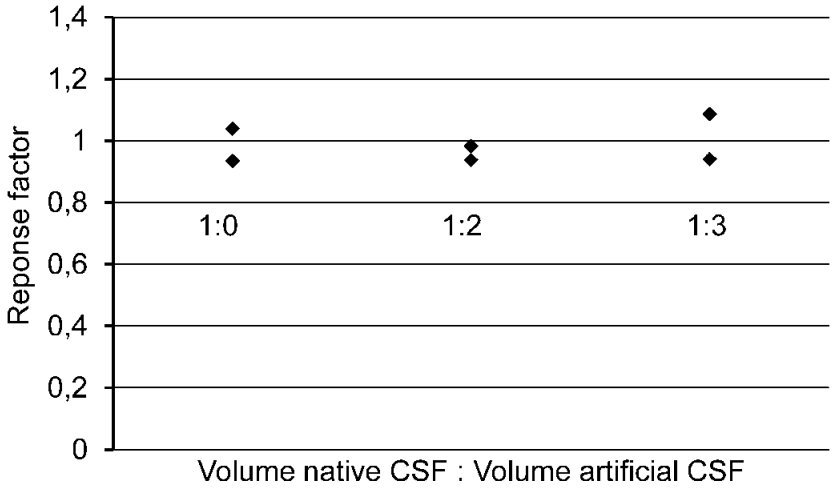
- LLOQ: 150 pg/mL
- Truenesss: 100%±15%*
- No matrix-dependent ion suppression

Native A$\beta_{1\text{-}42}$ in CSF

- Interassay imprecision for endogenous A$\beta_{1\text{-}42}$ <5.6% (6 different CSF pools over 6 days)

*Use of spiking and recovery





Response factor of native A$\beta_{1\text{-}42}$ and ^{15}N-A$\beta_{1\text{-}42}$ in artificial CSF at different concentrations (150–4000 pg/mL, n=2 at each concentration).

Relative response of endogenous A$\beta_{1\text{-}42}$ and ^{15}N-A$\beta_{1\text{-}42}$ in human CSF as well as human CSF diluted with artificial CSF (volume CSF : artificial CSF)

Clinica Chimica Acta 467 (2017) 27–33

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CSF $A\beta_{1-42}$ – an excellent but complicated Alzheimer's biomarker – a route to standardisation

Julia Kuhlmann [a], Ulf Andreasson [b,c], Josef Pannee [b,c], Maria Bjerke [b,c], Erik Portelius [b,c], Andreas Leinenbach [d], Tobias Bittner [d], Magdalena Korecka [e], Rand G. Jenkins [f], Hugo Vanderstichele [g], Erik Stoops [g], Piotr Lewczuk [h,i], Leslie M. Shaw [e], Ingrid Zegers [a], Heinz Schimmel [a], Henrik Zetterberg [b,c,j], Kaj Blennow [b,c,*], on behalf of the IFCC Working Group on Standardization of CSF proteins (WG-CSF)

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ARTICLE INFO

Article history:
Received 16 December 2015

ABSTRACT

The 42 amino acid form of amyloid β ($A\beta_{1-42}$) in cerebrospinal fluid (CSF) has been widely accepted as a central

Clinica Chimica Acta 467 (201

Contents lists available at Sc

Clinica Chimica

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CSF $A\beta_{1-42}$ – an excellent but complicated Alzhe route to standardisation

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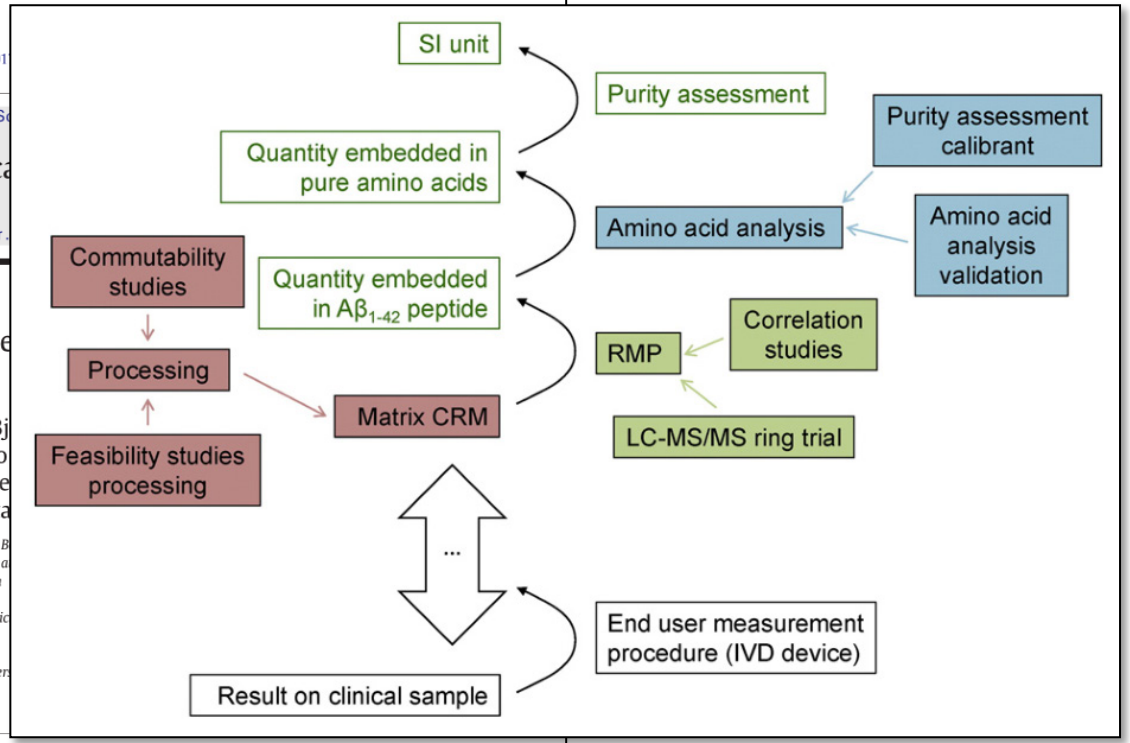
ARTICLE INFO

Article history:
Received 16 December 2015

ABSTRACT

The 42 amino acid form of amyloid β ($A\beta_{1-42}$) in cerebrospinal fluid (CSF) has been widely accepted as a central



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ABSTRACT

The 42 amino acid form of amyloid β ($A\beta_{1-42}$) in cerebrospinal fluid (CSF) has been widely accepted as a central

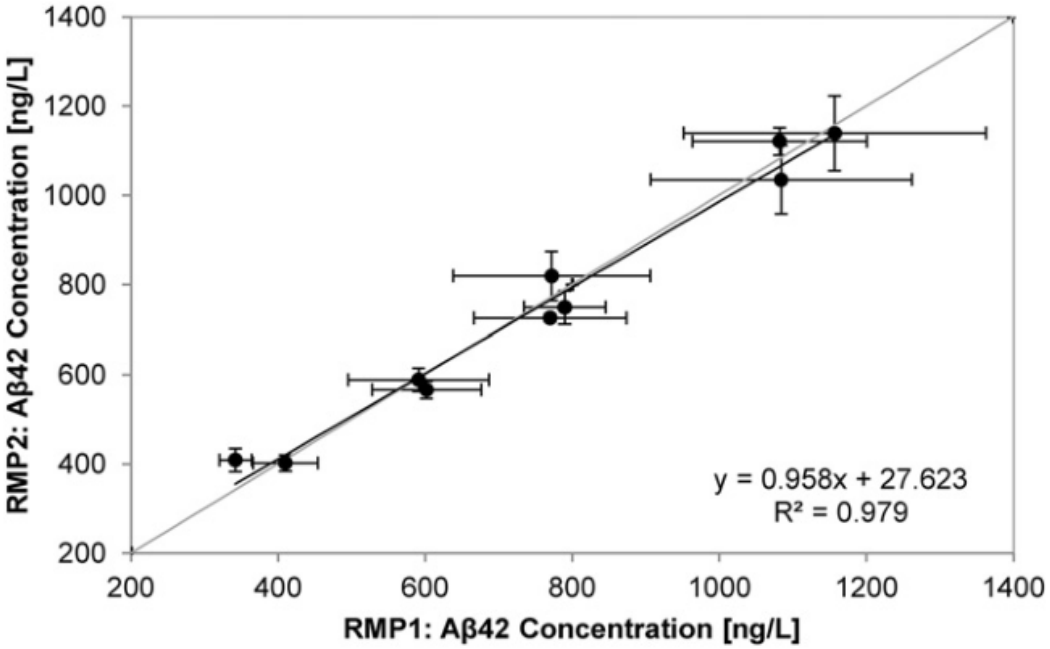
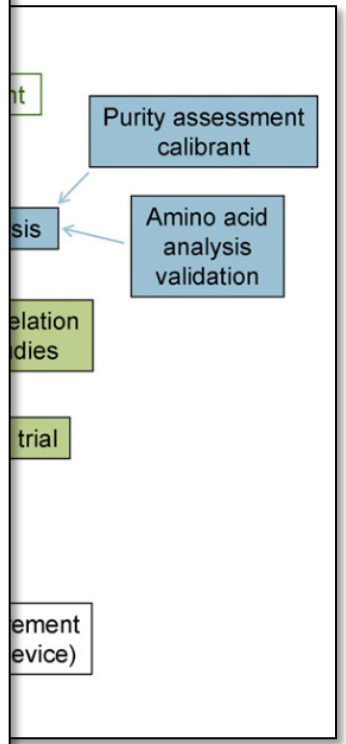


Fig. 3. Correlation of the results on 10 CSF pools measured with the 2 reference measurement procedures (RMPs) for CSF $A\beta_{1-42}$ quantification by LC-MS/MS. Over the course of 3 days, 2 aliquots per CSF pool were measured in duplicate. Both procedures were calibrated with a common $A\beta_{1-42}$calibrator provided by JRC-IRMM. Error bars indicate standard deviations of the daily averages measured with RMP2.



CRM released 1/DEC/2017

High, middle and low concentrations

Amyloid β_{1-42} peptide in human CSF[1)]	Mass concentration	
	Certified value [2)] [µg/L]	Uncertainty [3)] [µg/L]
ERM-DA480/IFCC	0.45	0.07
ERM-DA481/IFCC	0.72	0.11
ERM-DA482/IFCC	1.22	0.18

[1)] As obtained by solid phase extraction and subsequent quantification by liquid chromatography with mass spectrometry detection, according to the reference methods (Leinenbach *et al.* Clin. Chem. 60 (2014) 987-94; Korecka *et al.* J. Alzheimers Dis. 41 (2014) 441-451) [5,6].

[2)] Certified values are values that fulfil the highest standards of accuracy and represent the unweighted mean value of the means of 5 accepted sets of data, each set being obtained in a different laboratory. The certified value and its uncertainty are traceable to the International System of Units (SI).

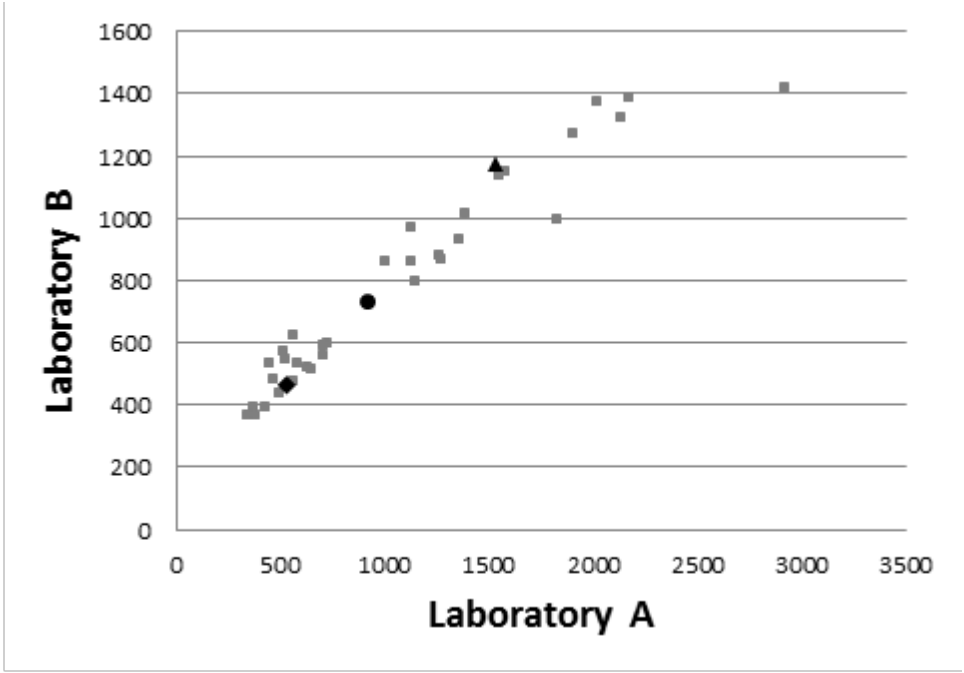
[3)] The uncertainty is the expanded uncertainty of the certified value with a coverage factor $k = 2$ corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008 [4].

Commutability

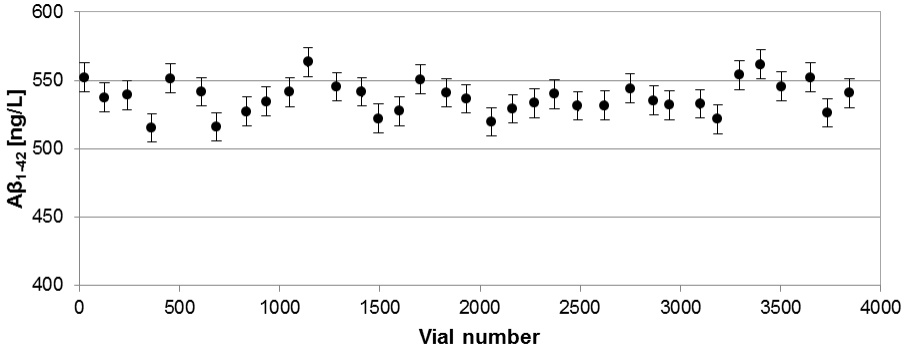
"A property of a reference material, demonstrated by the equivalence of the mathematical relationships among the results of different measurement procedures for a reference material and for representative samples of the type intended to be measured.“

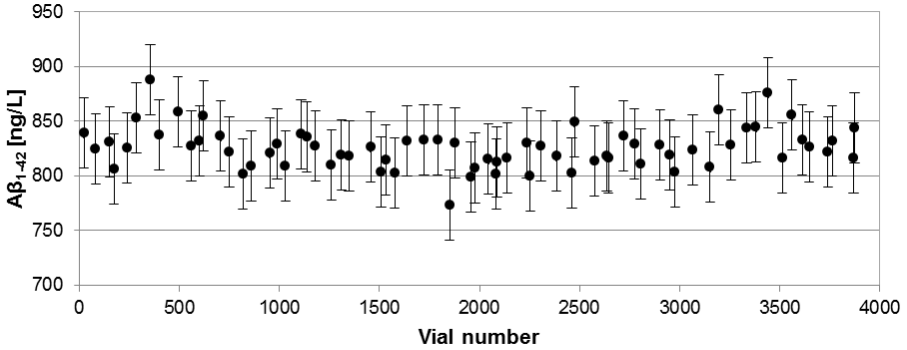
Three commutability studies show good commutability of the three materials

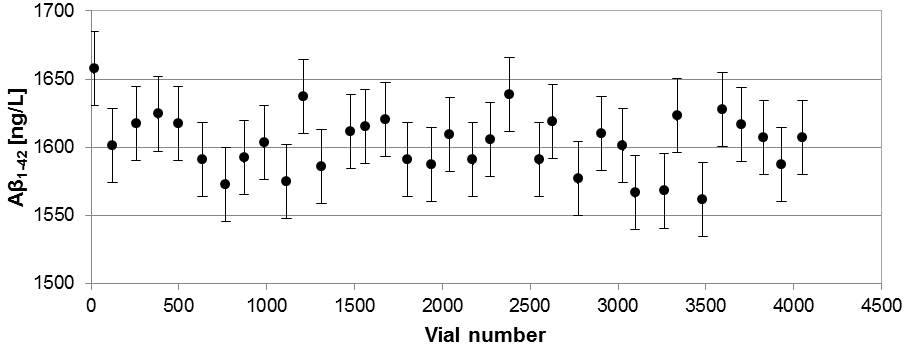
- Bjerke M et al. Clin Chem Lab Med 2016 (I & II)
- Manuscript in preparation (III)



Homogeneity

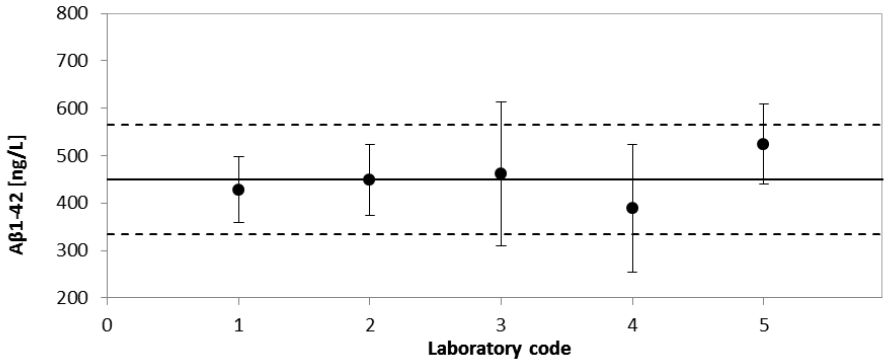


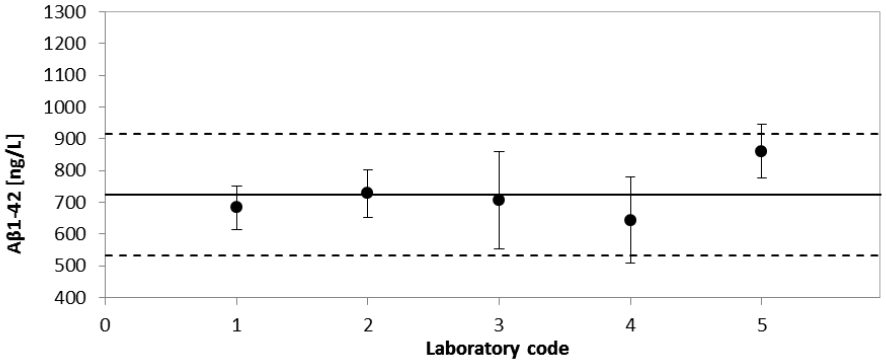


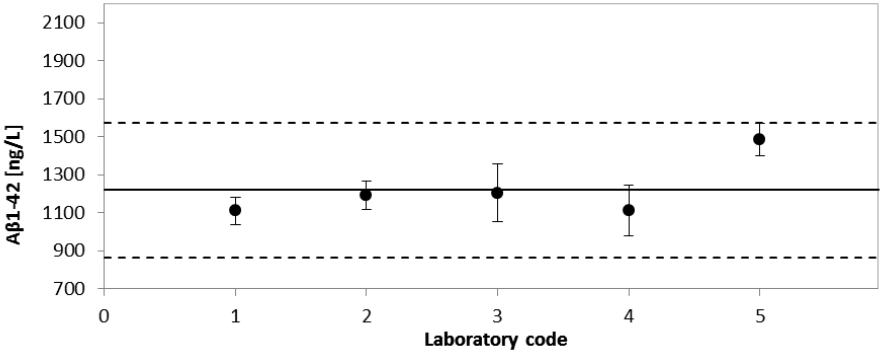


Averages / vial number and their 95 % CI

based on the within-group standard deviation as derived from a one-way ANOVA of all data grouped by vial number after correction of the analysis trend.

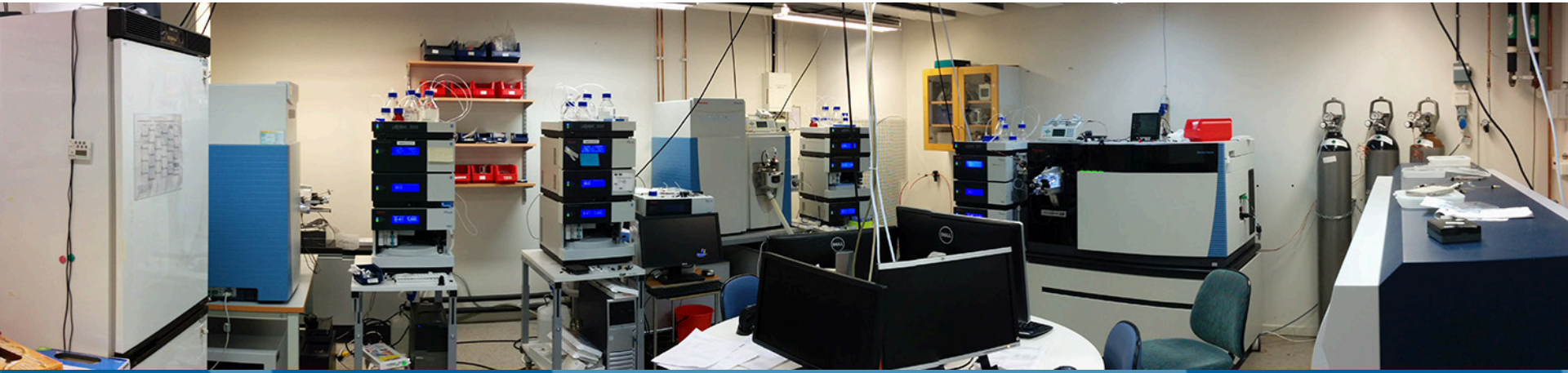






Average Aβ1-42 concentrations in CSF the three CRMs as measured with the RMPs.

Bars = laboratory means ± 2s.
Full line = mean of the means
Dotted lines = the mean of the means ± 2s.



Next steps

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Concordance between CSF Aβ & amyloid PET

Research

JAMA Neurology | Original Investigation

Concordance Between Different Amyloid Immunoassays and Visual Amyloid Positron Emission Tomographic Assessment

Shorena Janelidze, PhD; Josef Pannee, PhD; Alvydas Mikulskis, PhD; Ping Chiao, PhD; Henrik Zetterberg, MD, PhD; Kaj Blennow, MD, PhD; Oskar Hansson, MD, PhD

Supplemental content

IMPORTANCE Visual assessment of amyloid positron emission tomographic (PET) images has been approved by regulatory authorities for clinical use. Several immunoassays have been developed to measure β-amyloid (Aβ) 42 in cerebrospinal fluid (CSF). The agreement between CSF Aβ42 measures from different immunoassays and visual PET readings may influence the use of CSF biomarkers and/or amyloid PET assessment in clinical practice and trials.

OBJECTIVE To determine the concordance between CSF Aβ42 levels measured using 5 different immunoassays and visual amyloid PET analysis.

DESIGN, SETTING, AND PARTICIPANTS The study included 262 patients with mild cognitive impairment or subjective cognitive decline from the Swedish BioFINDER (Biomarkers for Identifying Neurodegenerative Disorders Early and Reliably) cohort (recruited from September 1, 2010, through December 31, 2014) who had undergone flutemetamol F 18 ([^{18}F]flutemetamol)-labeled PET. Levels of CSF Aβ42 were analyzed using the classic INNOTEST and the newer modified INNOTEST, fully automated Lumipulse (FL), EUROIMMUN (EI), and Meso Scale Discovery (MSD) assays. Concentrations of CSF Aβ were assessed using an antibody-independent mass spectrometry–based reference measurement procedure.

MAIN OUTCOMES AND MEASURES The concordance of CSF Aβ42 levels and Aβ42:Aβ40 and Aβ42:tau ratios with visual [^{18}F]flutemetamol PET status.

Brain Advance Access published July 7, 2016

doi:10.1093/brain/aww160 BRAIN 2016: Page 1 of 14 | 1

BRAIN
A JOURNAL OF NEUROLOGY

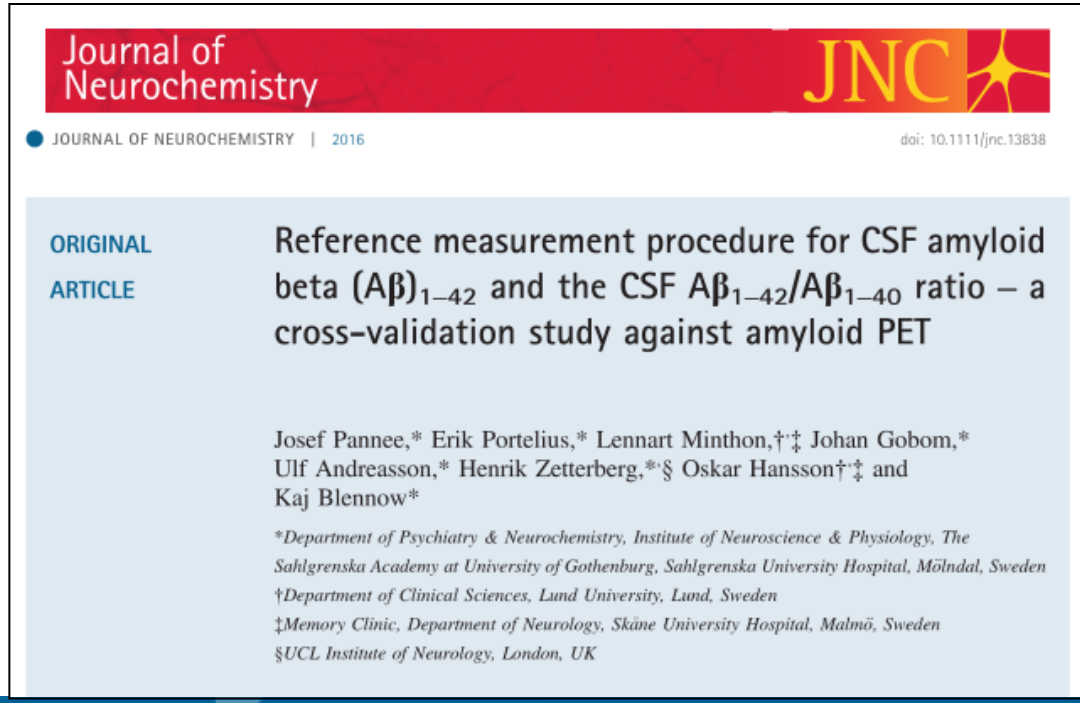
Pittsburgh compound B imaging and cerebrospinal fluid amyloid-β in a multicentre European memory clinic study

Antoine Leuzy,[1] Konstantinos Chiotis,[1] Steen G. Hasselbalch,[2] Juha O. Rinne,[3,4] Alexandre de Mendonça,[5] Markus Otto,[6] Alberto Lleó,[7,8] Miguel Castelo-Branco,[9,10] Isabel Santana,[11,12] Jarkko Johansson,[4] Sarah Anderl-Straub,[6] Christine A. F. von Arnim,[6] Ambros Beer,[13] Rafael Blesa,[7,8] Juan Fortea,[7,8] Sanna-Kaisa Herukka,[14] Erik Portelius,[15] Josef Pannee,[15] Henrik Zetterberg,[15,16] Kaj Blennow[15] and Agneta Nordberg[1,17]

The aim of this study was to assess the agreement between data on cerebral amyloidosis, derived using Pittsburgh compound B positron emission tomography and (i) multi-laboratory INNOTEST enzyme linked immunosorbent assay derived cerebrospinal fluid concentrations of amyloid-β_{42}; (ii) centrally measured cerebrospinal fluid amyloid-β_{42} using a Meso Scale Discovery enzyme linked immunosorbent assay; and (iii) cerebrospinal fluid amyloid-β_{42} centrally measured using an antibody-independent mass spectrometry-based reference method. Moreover, we examined the hypothesis that discordance between amyloid biomarker measurements may be due to interindividual differences in total amyloid-β production, by using the ratio of amyloid-β_{42} to amyloid-β_{40}. Our study population consisted of 243 subjects from seven centres belonging to the Biomarkers for Alzheimer's and Parkinson's Disease Initiative, and included subjects with normal cognition and patients with mild cognitive impairment, Alzheimer's disease dementia, frontotemporal dementia, and vascular dementia. All had Pittsburgh compound B positron emission tomography data, cerebrospinal fluid INNOTEST amyloid-β_{42} values, and cerebrospinal fluid samples available for reanalysis. Cerebrospinal fluid samples were reanalysed (amyloid-β_{42} and amyloid-β_{40}) using Meso Scale Discovery electrochemiluminescence enzyme linked immunosorbent assay technology, and a novel, antibody-independent, mass spectrometry reference method. Pittsburgh compound B standardized uptake value ratio results were scaled using the Centiloid method. Concordance between Meso Scale Discovery/mass spectrometry reference measurement procedure findings and Pittsburgh compound B was high in subjects with mild cognitive impairment and Alzheimer's disease, while more variable results were observed for cognitively normal and non-Alzheimer's disease groups. Agreement between Pittsburgh compound B classification and Meso Scale Discovery/mass spectrometry reference measurement procedure findings was further improved when using amyloid-$\beta_{42/40}$.

$A\beta_{1-38,}$ $A\beta_{1-40}$ and the $A\beta_{1-42}/A\beta_{1-40}$ ratio

- Validation of LC-MS $A\beta_{1-38}$ & $A\beta_{1-40}$
- Comparison to amyloid PET



Journal of Neurochemistry JNC

JOURNAL OF NEUROCHEMISTRY | 2016 doi: 10.1111/jnc.13838

ORIGINAL ARTICLE

Reference measurement procedure for CSF amyloid beta $(A\beta)_{1-42}$ and the CSF $A\beta_{1-42}/A\beta_{1-40}$ ratio – a cross-validation study against amyloid PET

Josef Pannee,* Erik Portelius,* Lennart Minthon,†‡ Johan Gobom,* Ulf Andreasson,* Henrik Zetterberg,*§ Oskar Hansson†‡ and Kaj Blennow*

*Department of Psychiatry & Neurochemistry, Institute of Neuroscience & Physiology, The Sahlgrenska Academy at University of Gothenburg, Sahlgrenska University Hospital, Mölndal, Sweden

†Department of Clinical Sciences, Lund University, Lund, Sweden

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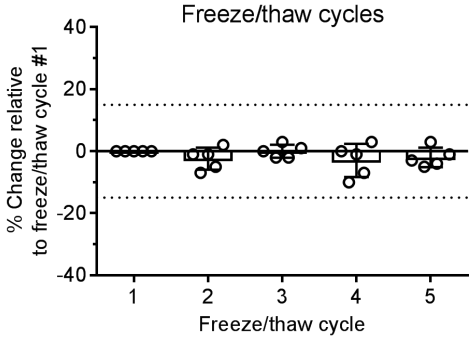
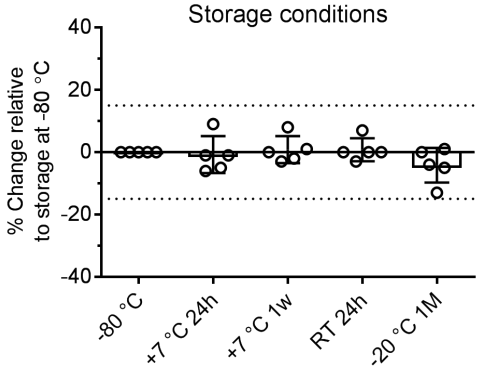
§UCL Institute of Neurology, London, UK

Validation results - $A\beta_{1-40}$

Sample	Average concentration (pg/mL)	s_r (pg/mL)	CV_r (%)	s_{RW} (pg/mL)	CV_{RW} (%)
HIGH	4197	172	4.1	252	6.0
LOW	2738	94	3.4	166	6.1

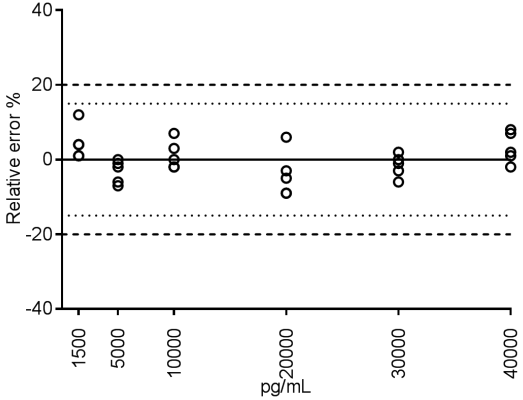
Imprecision - Repeatability (CV_r) <10% and reproducibility (CV_{RW}) <15% for high & low QC samples.





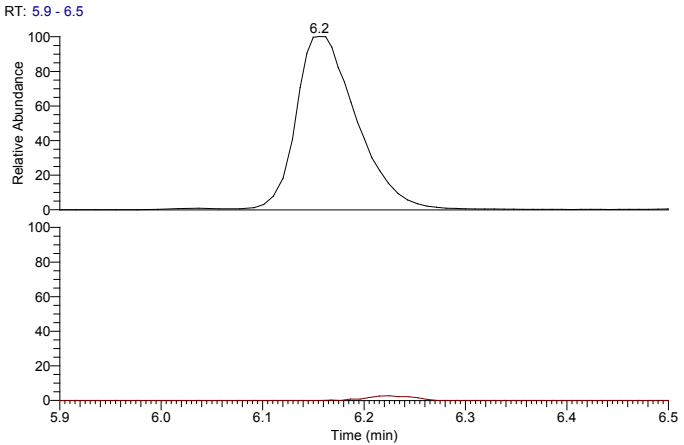
Sample stability - A sample can go through up to five freeze/thaw cycles with no statistically significant effect on the measured concentration of the analyte.

Storage in -80 ºC is preferred while storage in -20 ºC is acceptable.



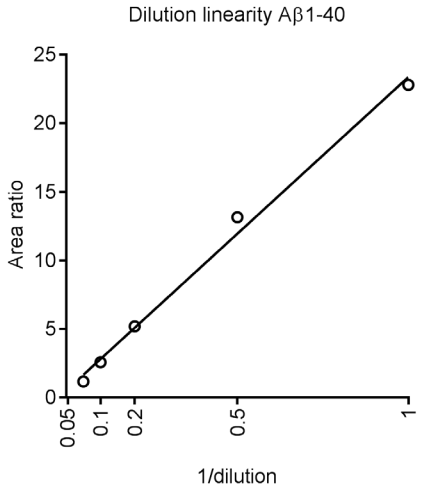
Measurement range - The relative errors for the back-calculated calibrators <15% in the whole measurement range defined by the calibrator curve (1 500 – 40 000 pg/mL).

Validation results - $A\beta_{1\text{-}40}$

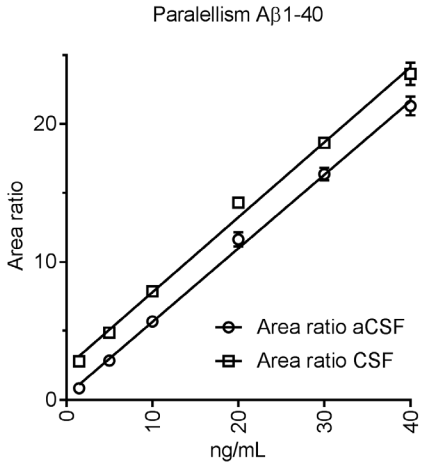


Carry over - No carry-over was detected.

Highest calibrator (top panel) followed by a blank injection (bottom panel, Y-axis range adjusted to typical LLOQ-level). No analyte was detected in the blank injection.

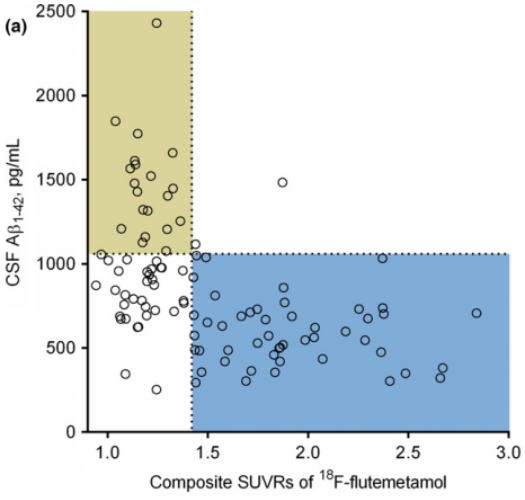


Dilution linearity - Human CSF serially diluted with a-CSF (2, 5, 10 & 20 fold)

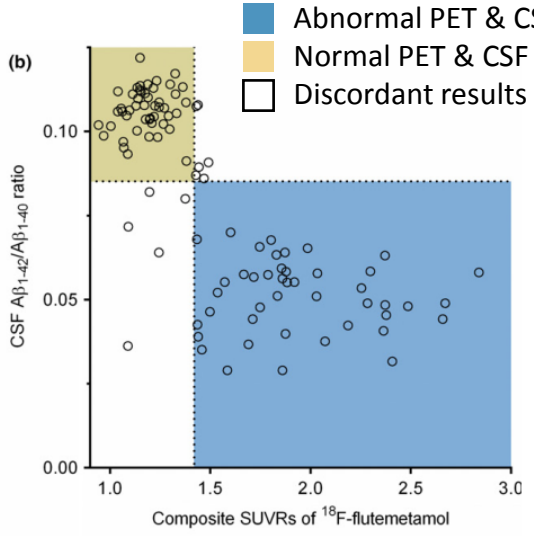


Parallelism - Calibrators prepared in human CSF & artificial CSF.

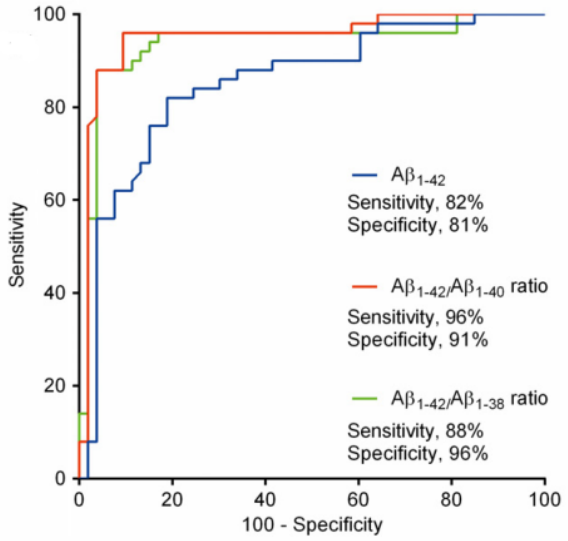
Both matrices can be used



CSF $A\beta_{1–42}$ concentrations with an unbiased cutoff determined to 1059 pg/mL (horizontal dashed line)



CSF $A\beta_{1–42}/A\beta_{1–40}$ ratios with a cutoff determined at 0.0852 (horizontal dashed line)



CSF $A\beta_{1–42}/A\beta_{1–40}$ ratios with a cutoff determined at 0.0852 (horizontal dashed line)

Results show that the CSF $A\beta_{1–42}/A\beta_{1–40}$ ratio using LC-MS is strongly associated with cortical Aβ fibrils measured by ^{18}F-flutemetamol PET.

Thank you for your attention

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