

## Cerebrospinal fluid tau, neurogranin and neurofilament light in Alzheimer's disease

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### Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

*Editor: Céline Carret*

1st Editorial Decision

31 May 2016

Thank you for the submission of your manuscript to EMBO Molecular Medicine. We have now heard back from the two referees whom we asked to evaluate your manuscript.

You will see that both referees find the study to be of interest, even if referee 1 is rather succinct. Referee 2 however is more detailed and while supportive, suggests improving the clinical/translational relevance of the data, which in light of our scope, we want to insist upon.

We would welcome the submission of a revised version for further consideration and depending on the nature of the revisions, this may be sent back to the referees for another round of review. Please note that it is EMBO Molecular Medicine policy to allow only a single round of revision and that, as acceptance or rejection of the manuscript may depend on another round of review, your responses should be as complete as possible.

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions, except under exceptional circumstances in which a short extension is obtained from the editor.

In order to gain time should your article be considered for publication, please carefully double check our author's guidelines and formatting details below prior to resubmitting your article.

I look forward to seeing a revised form of your manuscript as soon as possible.

\*\*\*\*\* Reviewer's comments \*\*\*\*\*

Referee #1 (Comments on Novelty/Model System):

This study allows one step further in the use of CSF biomarkers in clinical routine use, since it enlarges the panel and also links the results to the pathophysiology behind.

Referee #1 (Remarks):

Really good work and clearly written. The findings described are important.

Referee #2 (Remarks):

This is an important paper, and well performed study, showing the added value of analysing 3 different non-amyloid biomarkers in different clinical groups, in relation to amyloid positivity and negativity within the clinical groups, and the relation of the biomarkers to cognitive and MRI parameters stratified by amyloid positivity. So, very comprehensive and very clearly presented. The conclusions show that Ng does not have real added value to measuring Tau, and that NfL has added value. Also clearly shown is, due to the study design and statistical approach, that NfL is not related to amyloid pathology and thus provided other information.

I have a few suggestions for improvement, which can also be seen as discussion points. So, I would like to hear the authors answers.

Abstract: please add some results in the abstract where possible (e.g. AUCs).

Text in results section about abeta positivity could be structured a bit clearer: why compare abeta+ in AD with CNabeta-? It would help clarity if abeta+ or - is compared within diagnostic groups only, and not across diagnostic groups, since the significance of a difference in amyloid positive AD compared to CN negative is not clear to me. It reduces also the numbers of tests performed, increasing the strengths of the other results (not only in understanding but also statistically).

For all associations and group differences: given the correlations with age and sex, all associations must be corrected for these confounding factors. It is not clear from the methods if this has been done for statistical hypothesis number 3. I believe that the correction for age and sex should be stressed in the narrative of the results as well, and in the figure legends.

Since the aim is to define the added diagnostic value of combining the biomarkers, I expect also more discussion and some explanatory figures useful for the clinic, such as numbers of correctly classified patients: what is the increase in correct classification if neurofilament light is added to the diagnostic workup compared to amyloid beta and Tau alone? That will help translation to clinical practise.

The conclusion in 'paper explained' that tau and neurogranin can be used for monitoring is too hypothetical and not supported by the data, that do not include longitudinal sampling (only longitudinal clinical data).

Although I do acknowledge the tremendous and important contribution of the Gothenburg studies and of the first author to the development of the field, it would be good if external citations are used more. There are places where these would have been at least equally good papers (e.g. use of NfL for monitoring neuroinflammation; Ng papers of Fagan group).

AUC and AIC: abbreviations are not explained in the legend of table 3. Furthermore, it would be better to have confidence intervals for the AUCs, which allows the reader to judge better the significance of the increase in AUC in extended models.

*Referee #1 (Comments on Novelty/Model System):*

*This study allows one step further in the use of CSF biomarkers in clinical routine use, since it enlarges the panel and also links the results to the pathophysiology behind.*

*Referee #1 (Remarks):*

*Really good work and clearly written. The findings described are important.*

**\* We appreciate these kind words about our paper.**

*Referee #2 (Remarks):*

*This is an important paper, and well performed study, showing the added value of analysing 3 different non-amyloid biomarkers in different clinical groups, in relation to amyloid positivity and negativity within the clinical groups, and the relation of the biomarkers to cognitive and MRI parameters stratified by amyloid positivity. So, very comprehensive and very clearly presented. The conclusions show that Ng does not have real added value to measuring Tau, and that NfL has added value. Also clearly shown is, due to the study design and statistical approach, that NfL is not related to amyloid pathology and thus provided other information.*

*I have a few suggestions for improvement, which can also be seen as discussion points. So, I would like to hear the authors answers.*

*Abstract: please add some results in the abstract where possible (e.g. AUCs).*

**\* We have added results in the abstract (page 3).**

*Text in results section about abeta positivity could be structured a bit clearer: why compare abeta+ in AD with CNabeta-? It would help clarity if abeta+ or - is compared within diagnostic groups only, and not across diagnostic groups, since the significance of a difference in amyloid positive AD compared to CN negative is not clear to me. It reduces also the numbers of tests performed, increasing the strengths of the other results (not only in understanding but also statistically).*

**\* We have restructured the section about Abeta (AB) positivity to make these results clearer (page 7, line 9 – page 8, line 8). We did the comparisons between CN AB- and CN AB+, MCI AB- /AB+, and AD AB-/AB+ because we wanted we examine if CSF injury biomarkers are differently related to different disease trajectories. As we explain in the methods section (page 16, lines 12-14), and as we now also mention in this paragraph in the results section, we believe that AD follows a sequence of events during its development in humans. First, CN AB- convert to AB+ without developing cognitive decline (CN AB+). Later, CN AB+ convert to MCI AB+ and finally AD AB+. In contrast, people who develop non-AB-dependent cognitive decline may also start as CN AB- but convert to MCI AB- and AD AB- (note that we consider these clinical “AD” diagnoses likely to be erroneous). We found that CSF injury biomarkers differentiate between these two trajectories as shown in Figure 2 and Table 5. For example, MCI AB+ have significantly higher CSF Ng, CSF T-tau and CSF NFL compared to CN AB-, while MCI AB- only have higher CSF NFL compared to CN AB-, and do not differ from CN AB- in CSF Ng or T-tau levels. This supports the idea that CSF Ng and CSF T-tau are associated with development of AD, while CSF NFL is associated with cognitive decline independent of AB.**

*For all associations and group differences: given the correlations with age and sex, all associations must be corrected for these confounding factors. It is not clear from the methods if this has been done for statistical hypothesis number 3. I believe that the correction for age and sex should be stressed in the narrative of the results as well, and in the figure legends.*

**\*Hypothesis 3 tests were also adjusted for age and sex. We now explain this in the statistical methods section (page 16, line 17), and in the narrative and in the figure legends.**

*Since the aim is to define the added diagnostic value of combining the biomarkers, I expect also more discussion and some explanatory figures useful for the clinic, such as numbers of correctly classified patients: what is the increase in correct classification if neurofilament light is added to the diagnostic workup compared to amyloid beta and Tau alone? That will help translation to clinical practise.*

**\*We have now included classification data generated from the logistic regression models (page 6, line 20 – page 7, line 7). The data are shown in a new table (Table 4) and provide numbers for classifications of AD, CN, PMCI and SMCI with different combinations of biomarkers, and when adjusting for Ab42 and demographic covariates. For the basic models, these results show a relative increase of 12% for classification of AD when combining biomarkers compared to when only using T-tau alone. In the more complex models the effects of combining biomarkers were smaller.**

*The conclusion in 'paper explained' that tau and neurogranin can be used for monitoring is too hypothetical and not supported by the data, that do not include longitudinal sampling (only longitudinal clinical data).*

**\*We have changed “monitor” to “detect” to make the conclusion compatible with our cross-sectional results (page 19, line 20).**

*Although I do acknowledge the tremendous and important contribution of the Gothenburg studies and of the first author to the development of the field, it would be good if external citations are used more. There are places where these would have been at least equally good papers (e.g. use of NFL for monitoring neuroinflammation; Ng papers of Fagan group).*

**\*We have added additional references for CSF NFL in relation to neuroinflammation (Christensen et al) and frontotemporal lobe dementia (Petzold et al). We cite the two papers on neurogranin from the Fagan group (Tarawneh et al, and Kester et al).**

*AUC and AIC: abbreviations are not explained in the legend of table 3.*

**\*The abbreviations are now explained.**

*Furthermore, it would be better to have confidence intervals for the AUCs, which allows the reader to judge better the significance of the increase in AUC in extended models.*

**\*We have added 95 % confidence intervals for the AUCs to the tables.**

Acceptance

08 July 2016

Please find enclosed the final report on your manuscript. We are pleased to inform you that your manuscript is accepted for publication and is being sent to our publisher to be included in the next available issue of EMBO Molecular Medicine.

Congratulations on your interesting work!

\*\*\*\*\* Reviewer's comments \*\*\*\*\*

Referee #2 (Comments on Novelty/Model System):

It is well done!

Referee #2 (Remarks):

Thanks for the careful explanations!

**YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓**

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Niklas Mattsson

Journal Submitted to: EMBO Molecular Medicine

Manuscript Number: EMM-2016-06540

### Reporting Checklist For Life Sciences Articles (Rev. July 2015)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

#### A- Figures

##### 1. Data

##### The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if  $n \leq 5$ , the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

##### 2. Captions

##### Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple  $\chi^2$  tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

#### B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	All available ADNI-1 subjects with available CSF Aβ42, T-tau, Ng and NFL data were included.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	We excluded two subjects who were significant outliers in Ng measurements (CSF Ng > 2000 ng/L) because we suspected technical errors in these measurements.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	NA, no treatments
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	The investigators running the assays were blinded to the clinical diagnoses
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes. We checked model assumptions by inspecting residuals (correlations with fitted values and predictors of interest, histograms and q-q plots).
Is there an estimate of variation within each group of data?	Variations for the CSF injury biomarkers are shown in Figure 2 for different groups of diagnosis and Aβeta pathology.
Is the variance similar between the groups that are being statistically compared?	Variations are overall similar.

#### C- Reagents

#### USEFUL LINKS FOR COMPLETING THIS FORM

<http://www.antibodypedia.com>

<http://1degreebio.org>

<http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo>

<http://grants.nih.gov/grants/olaw/olaw.htm>

<http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm>

<http://ClinicalTrials.gov>

<http://www.consort-statement.org>

<http://www.consort-statement.org/checklists/view/32-consort/66-title>

<http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun>

<http://datadryad.org>

<http://figshare.com>

<http://www.ncbi.nlm.nih.gov/gap>

<http://www.ebi.ac.uk/ega>

<http://biomodels.net/>

<http://biomodels.net/miriam/>

<http://jil.biochem.sun.ac.za>

[http://oba.od.nih.gov/biosecurity/biosecurity\\_documents.html](http://oba.od.nih.gov/biosecurity/biosecurity_documents.html)

<http://www.selectagents.gov/>

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	NA
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

\* for all hyperlinks, please see the table at the top right of the document

#### D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

#### E- Human Subjects

11. Identify the committee(s) approving the study protocol.	ADNI subjects have been recruited from over 50 sites across the U.S. and Canada. For up-to-date information, see <a href="http://www.adni-info.org">www.adni-info.org</a> . Regional ethical committees of all institutions approved of the study.
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	All subjects provided informed consent.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	ADNI samples may be available by formal request to the ADNI steering committee.
15. Report the clinical trial registration number (at <a href="http://ClinicalTrials.gov">ClinicalTrials.gov</a> or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

#### F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	NA
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the Journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	All ADNI data is open to all researchers after registering an account at the LONI homepage
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	All ADNI data is open to all researchers after registering an account at the LONI homepage
21. As far as possible, primary and referenced data should be formally cited in a Data Availability section. Please state whether you have included this section.  Examples: <b>Primary Data</b> Wetmore KM, Deuschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in <i>Shewanella oneidensis</i> MR-1. Gene Expression Omnibus GSE39462 <b>Referenced Data</b> Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	All ADNI data is open to all researchers after registering an account at the LONI homepage
22. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

#### G- Dual use research of concern

23. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	NA
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