Letter to the Editor

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Cerebrospinal fluid neurogranin in Alzheimer's disease studies: are immunoassay results interchangeable?

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To the Editor,

Synaptic demise is increasingly recognized as a core feature of Alzheimer's disease (AD) [1] and synaptic protein levels are reduced in AD brains [2]. Neurogranin (Ng) is a post-synaptic protein involved in the maintenance of synapses and in the regeneration of neurites. In AD patients, Ng levels are decreased in the hippocampus, the frontal and parietal cortex and are increased in the cerebrospinal fluid (CSF) as compared to non-AD dementia and to neurological controls [3,4]. Higher CSF Ng levels are associated with increased brain atrophy (hippocampal, entorhinal and parahippocampal areas) and higher CSF Tau levels [5,6]. CSF Ng concentrations could predict future rate of cognitive decline [4,5,7]. Thus, there is a growing interest in Ng as a novel biomarker candidate for AD. The measurement of CSF Ng can be performed by several assays, targeting different epitopes of the protein [8].

In this study, we compared two Ng ELISA immunoassays. Our main objectives were (1) to determine if Ng ELISA assays were substitutable allowing pooling and comparison of results from different centers, and (2) to evaluate if they exhibit the same discriminating power between AD and non-AD patients.

We conducted a retrospective cross-sectional monocentric study by including 234 consecutive patients explored in the Cognitive Neurology Center at Lariboisière hospital in Paris, from 2012 to 2015. All patients signed an informed consent, and the study was approved by the local Ethics Committee. All patients were classified according to AD positive or negative CSF results, and a consensus clinical diagnosis of MCI or dementias was made by several experts in agreement with validated clinical diagnostic criteria of the disease. Main population characteristics are summarized in Supplementary Material Table 1 including both Ng assays results and were previously reported into more details [9].

For each patient sample, CSF Ng level was assessed in duplicate using the two methods: (1) The Gothenburg University in-house Ng ELISA (monoclonal antibody Ng 36, the full methodology has been previously described [4]) (UGot), and (2) EuroImmun Ng (Trunc P75) ELISA (monoclonal antibody ADx403 and ADx451) according to the supplier's instructions [8]. Reproducibility and repeatability of both assays are similar and have been previously described [8].

Identification of any systematic difference between the measurements (i.e., fixed bias) or possible outliers was estimated using the Bland and Altman method. Intraclass correlation (ICC) estimates and their 95% confidence intervals (CI) were calculated based on single measurements, absolute agreement, and two-way mixed-effects model. Results interpretation was made according to previous guidelines [10].

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In a second step, we arbitrary recalibrated UGot levels of Ng by ADx levels of Ng ($UGot_{recalibrate}$) using a linear regression model (ADx = β1 + β2*UGot). We then reran the Bland and Altman analysis and the ICC determination between $UGot_{recalibrate}$ and ADx to assess whether the recalibration improved the comparison between both assays.

For clinical performance evaluation, we compared (1) Ng concentration means between groups of patients (Table 1, Supplementary Material) for each assay using an analysis of variance completed by a post hoc analysis using Dunn's multiple Comparison Test, and (2) the ability of each test to discriminate between AD and non-AD patient using ROC-curves analyses. Two-tailed values of $p<0.05$ were considered statistically significant. Analyses and graphics were performed using *R* (version 4.0.3).

Most of the patients had higher CSF Ng with UGot ELISA assay than ADx one (Figure 1A). The Bland and Altman diagram showed a systematic difference between both assays (ADx and UGot, bias of 73.87 [95% CI 61.2–86.6]) with UGot dosages being the highest values (data not shown). ICC analysis revealed a moderate reliability between the two measurements technics (ICC = 0.634 [95% CI 0.27–0.80]).

We then calculated corrected UGot values using the linear correction analysis ($UGot_{recalibrate}$ = 0.8817 × UGot − 41.31) and compared these data with ADx values.

No significant systematic difference was found in Bland and Altman Diagram between ADx and $UGot_{recalibrate}$ values (Figure 1B), with a bias of 0.006 [95% CI-12.57–12.58]). Significant differences yet occur for high values of Ng (>400 ng/L), mostly due to ADx higher values.

The ICC improved after the recalibration of UGot values, but remained on moderate values [95% CI 0.50–0.75], with an ICC of 0.709 [95% CI 0.64; 0.77].

For each immunoassay, analysis of variance (Figure 2A) showed significant differences between

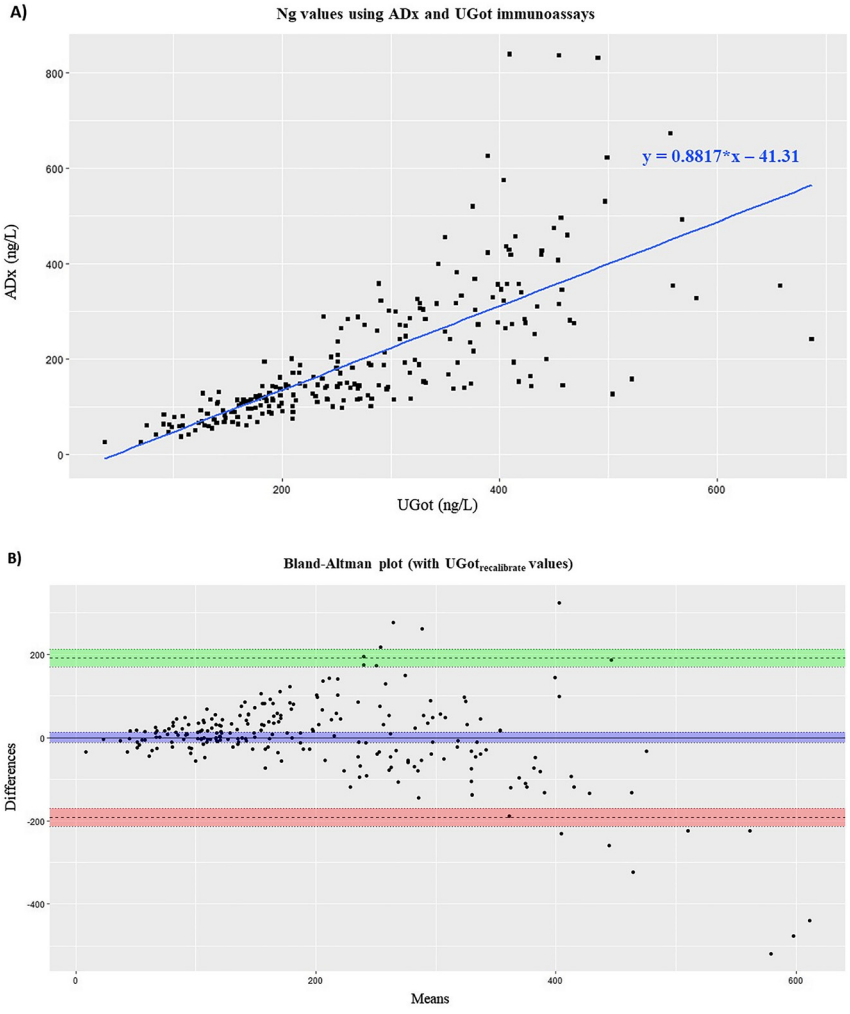

Figure 1: Measurement of agreement between ADx and UGot assays using a linear correction.
(A) Each black square represents individual CSF sample, with neurogranin value assessed with ADx and UGot assays. Blue line indicates the linear regression line, with its formula above. (B) Bland and Altman diagram using $UGot_{recalibrate}$ values. Black dots represent individual CSF sample, horizontal solid line represents bias, and dotted lines upper and lower limit of agreement (from −1.96SD to +1.96SD). Differences are calculated with $UGot_{recalibrate}$ values - ADx values.

patient's groups ($p<0.001$), and post-hoc analyses revealed significant differences in mean values of Ng between AD or MCI due to AD and other groups (MCI non due to AD, controls, others dementia). No significant difference was found between AD and MCI due to AD.

ROC curves (Figure 2B), used to discriminate AD or MCI due to AD from non-AD patients, demonstrated almost identical areas under curves (AUC) in both tests, with AUCs of 0.84 [95% CI 0.79–0.90].

In this work, we have shown that UGot ELISA assay provide higher CSF Ng levels compared to ADx ELISA, and that the reliability between the two assays was medium. The recalibration for UGot allowed to increase the comparability and the reliability between the two assays. However, it seems uneasy to use a fixed conversion factor in clinical practice, as non-negligible differences between findings were observed for high values of Ng (Figure 1B).

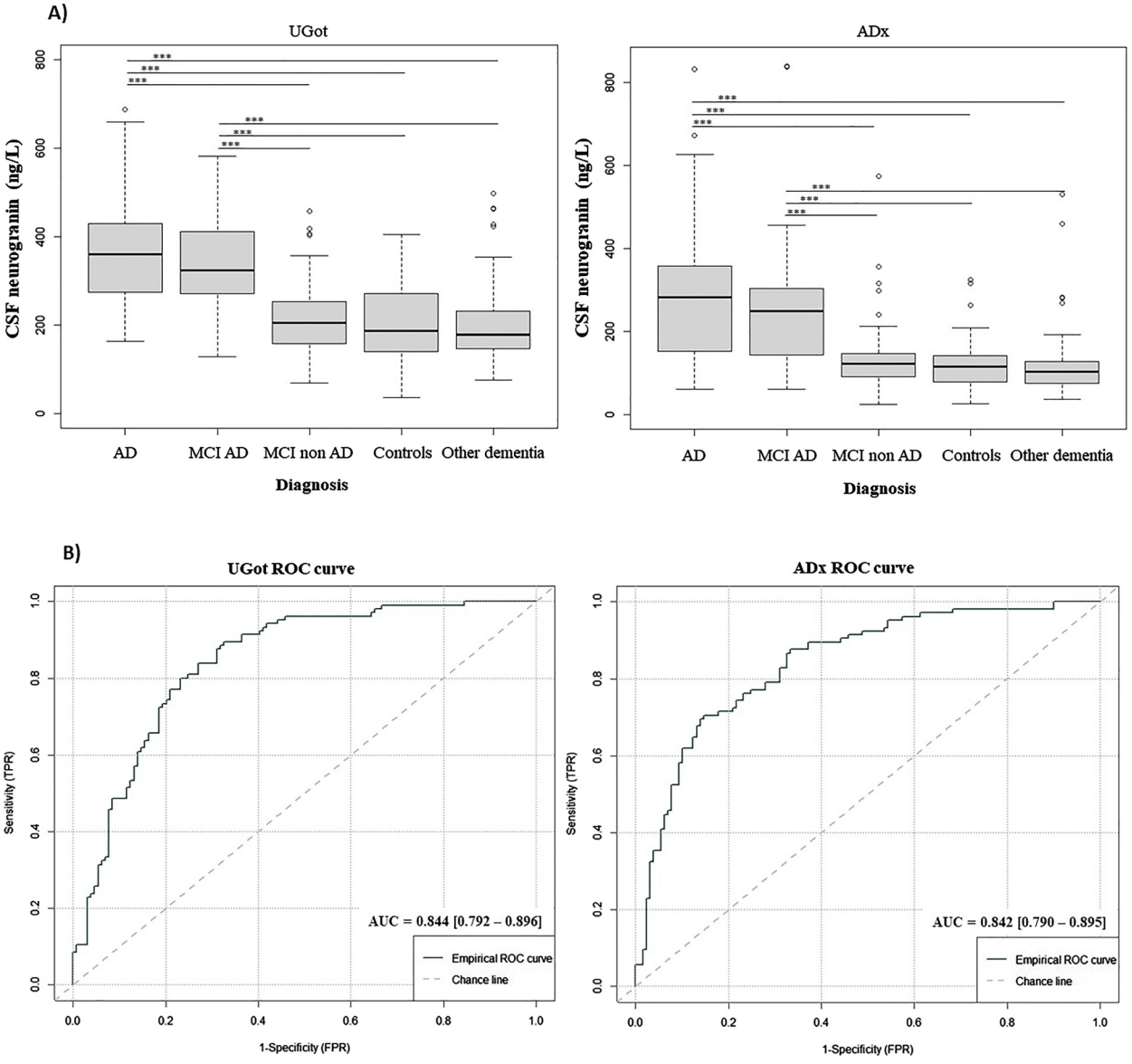

Figure 2: Neurogranin concentration comparison between diagnosis groups.
(A) Boxplots of CSF Ng median concentrations (ng/L) in differential diagnosis groups measured with UGot and ADx assays. p-Values are from Dunn test post-hoc analysis of CSF Ng mean concentrations between groups. (B) ROC curves for each assay with calculated AUC values. Discrimination was tested between AD or MCI due to AD patients (positive) and others (negative). ***p<0.001 in Dunn's multiple comparison test following one-way ANOVA.

These variations between both immunoassays could be explained by the detection of specific Ng epitopes and by their different calibration curve profiles. Indeed, as identified by Willemse et al. [8], UGot ELISA targets Ng C-terminal end while ADx ELISA target a specific C-terminally truncated form.

Also, the calibration curve of ADx assay is quite linear, allowing the detection of high values of Ng. On the other hand, the calibration curve of UGot leads to a plateau for high values. These factors may lead to poor correlation for elevated values.

In clinical practice, we showed that Ng mean concentrations with both immunoassay significantly differed between AD or MCI due to AD patients and other patients (Figure 2A). Also, these tests share a good and similar discriminatory power between AD or MCI due to AD patients, compared to controls or other types of dementia (Figure 2B). Contrasted results between moderate correlation and similar discriminatory power are likely explained by the observed findings concerning the highest values of Ng, whose consequences did not influence diagnosis accuracy.

Our results are consistent with findings from Willemse et al. [8], except for the fact that we could find a significant difference in Ng mean concentration between AD or MCI due to AD and controls with the ADx assay ($p<0.05$). This can be explained either by our larger number of patients (n=234 vs 108) or by a possible difference in the groups of patients.

In conclusion, we have shown that these two assays share similar performances to discriminate AD patients from other patients. It is therefore possible to use both tests for the detection of synaptic degeneration in AD patients. Nevertheless, our results do not support the possibility to switch from one assay to the other.

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Competing interests: Mr AVENEAU (medical resident) declare no disclosure. Dr COGNAT and Dr DUMURGIER are investigator in several passive anti-amyloid immuno therapies and other clinical trials for Roche, Eisai, Lilly, Biogen, Astra-Zeneca, Lundbeck. Dr BOUAZIZ-AMAR is member of the National Advisory Board of Roche. Prof. ZETTERBERG has served at scientific advisory boards for Roche Diagnostics, Wave, Samumed and CogRx, has given open lectures sponsored by Alzecure, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. Prof. HUGON is investigator in several passive anti-amyloid immunotherapies and other clinical trials for Roche, Eisai, Lilly, Biogen, Astra-Zeneca, Lundbeck. He is a member of the advisory boards of RAMAN Health, Roche and Lilly. Prof. PAQUET is member of the International Advisory Boards of Lilly, is consultant of Fujiribio, ALZOHIS, NEUROIMMUNE, Ads Neuroscience, Roche, AgenT and GILEAD and is involved as investigator in several clinical trials for Roche, Esai, Lilly, Biogen, Astra-Zeneca, Lundbeck, Neuroimmune. Prof. BLENNOW has served as a consultant or at advisory boards for Alzheon, BioArctic, Biogen, Eli Lilly, Fujirebio Europe, IBL International, Merck, Novartis, Pfizer, and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg. HV et EM were employees of ADx neurosciences at the period of the assessments.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: The study was approved by the local Ethics Committee.

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