International quality control survey of neurochemical dementia diagnostics

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Abstract

Currently, neurochemical dementia diagnostics (NDD) are increasingly entering routine clinical neurochemistry, offering improved early and differential diagnosis of dementias. However, there is an obvious lack of standardization in pre-analytical sample handling and systematic quality surveys. Therefore, in this study, 14 laboratories in Germany, Austria, and Switzerland were given aliquots of a human cerebrospinal fluid (CSF) sample, and were asked to measure Alzheimer’s disease (AD) biomarkers (amyloid β (Aβ) peptides, total Tau protein, and phosphorylated Tau protein (P-tau181P)) according to their routine protocols. 

Results: The inter-laboratory coefficients of variation of the results obtained by the laboratories participating in this study were in the range of 20–30%. Although the results of this quality control survey are promising, the quality of measurements has to be further optimized.

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Among the candidate biomarkers to support the diagnosis of Alzheimer’s disease (AD), the cerebrospinal fluid (CSF) concentration of amyloid β peptides (Aβ peptides) and Tau protein(s) along with hyperphosphorylated forms of the latter have been shown to fulfill the criteria for a diagnostic test according to the recently published experts’ guidance [17]. This is not surprising, because these factors are directly involved in the pathologic events of the disease, namely deposition of senile plaques and formation of neurofibrillary tangles. The CSF concentration of amyloid β peptides ending at the amino acid position 42 (Aβ42) has been reported to be decreased, and Tau protein has been reported to be increased in AD [1,5,6,10,12,15,18,19]. Moreover, recently published evidence shows that a combined analysis of biomarkers, Aβ1–42, total Tau protein, and Tau phosphorylated at the amino acid position 181 (P-tau181P), discriminated correctly a subgroup of patients with mild cognitive impairment eventually converted to AD, from those who did not [22].

Since dementias strongly affect populations of all industrialized countries, it is obvious that more and more laboratories world-wide will have to establish quality control measures to reliably analyze AD biomarkers, and to be able to compare the results between one another. To the best of our knowledge, results of an inter-laboratory quality control survey of neurochemical dementia diagnostics (NDD) have not been published so far, and hence, in collaboration with the German Society for CSF Analysis and Clinical Neurochemistry (http://www.dgln.de), we recently conducted this study to estimate the inter-laboratory imprecision of AD biomarker measurements.

Approximately 50 mL of ventricular CSF were collected from a neurosurgical patient via an external ventricular drainage. Immediately after centrifugation (1600 \( \times g \), 15 min), the sample was aliquoted into propylene test tubes (0.5 mL per portion) and frozen at \(-80^\circ C\). Each participant in this study (\( n = 14 \) clinical neurochemistry laboratories in Germany, Austria, and Switzerland) was given 2–3 of these aliquots on dry ice with a request to measure all biomarkers routinely performed. In all laboratories, the measurements were performed according to the corresponding routine protocols. The concentrations of the biomarkers were measured with commercially available ELISAs: Aβ\( \times 40 \), and Aβ\( \times 42 \) from The Genetics Co. (Zürich, Switzerland), Aβ1–42, total Tau, and P-tau181P from Innogenetics (Ghent, Belgium). Different epitope specificities of capturing and detecting antibodies used in the respective assays enable the detection of either specifically one species, namely Aβ1–42 (the assay of Innogenetics) or the ‘family’ of several Aβ peptides ending at the C-terminus of 42, but beginning at different N-termini, hence denominated in this study Aβ\( \times 42 \) (the assays of The Genetics Co.).

According to the data provided by the manufacturers of the assays, their intraassay imprecision is less than 6% (except for Aβ1–42: less than 10%, and P-tau181P: less than 5%). Corresponding interassay imprecision is less than 8% (except for Tau: less than 6%, and P-tau181P: less than 10%). The data are presented as average concentrations obtained by all participants and corresponding coefficients of variation (CV). Grubb’s test was used to search for and to eliminate possible outliers according to the guidance of the International Standardization Organization [8]. Intraassay imprecision is presented as a percentage ratio of a range to average of duplicate determinations.

The results are presented in Table 1. Out of all participants, three laboratories performed Aβ\( \times 40 \) and Aβ\( \times 42 \) measurements. Thirteen participants performed Aβ1–42 assays, all fourteen participants measured total Tau, and 11 laboratories measured P-tau181P, however, in the latter case the result from one participant was identified as an outlier, and had to be excluded from the further analysis. For almost all biomarkers, the results show imprecision lower than 30%, except for the higher imprecision of Aβ\( \times 42 \), which can be attributed to the small number of participants performing this assay.

In this study, we present the results of an international quality control survey on neurochemical dementia diagnostics. Since results of quality control studies (intra-, and inter-laboratory) might be falsified when ‘artificial CSF’ is used, for example due to lack of CSF-specific proteins and significant discrepancies between different fractions of proteins, we conducted this study, in accordance with experts’ suggestion, with a real human CSF sample [14]. Moreover, since the CSF concentration of brain-derived proteins does not depend on their blood concentration [13], the biomarkers in this study, as in routine practice, were measured exclusively in the CSF. For two reasons we decided to conduct our study with ventricular CSF instead of pooled lumbar CSF, as postulated by Reiber et al. [14]: (a) the overall pattern of the results (decreased Aβ42, mildly increased Tau, and normal P-tau181P) corresponds well to neurodegeneration and/or neuroinflammation and, with the exception of P-tau181P being in the normal range, perfectly reflects the pattern of biomarkers in Alzheimer’s disease [20], and (b) the pooling of the lumbar CSF samples from a large number of subjects may lead to unpredictable interactions between Aβ peptides (and perhaps Tau proteins) during extended storage time necessary for collecting a sufficient volume of the material.

Several pre-analytical and biological confounding factors may influence the analytical outcome of CSF analyses, such as a tendency to stick to the walls of test tubes made of polystyrene [9] or extended storage time [2]. Therefore, inter-laboratory quality control surveys are necessary to assure the results obtained in different laboratories are reliable. The results

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>n</th>
<th>Average concentration (pg/mL) and CV (%)</th>
<th>Average intraassay imprecision in % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ( \times 40 )</td>
<td>3</td>
<td>3725 (21%)</td>
<td>2.2% (2)</td>
</tr>
<tr>
<td>Aβ( \times 42 )</td>
<td>3</td>
<td>335 (38%)</td>
<td>2.1% (2)</td>
</tr>
<tr>
<td>Aβ1–42</td>
<td>13</td>
<td>334 (29%)</td>
<td>7.5% (9)</td>
</tr>
<tr>
<td>Total tau</td>
<td>14</td>
<td>603 (26%)</td>
<td>5.3% (10)</td>
</tr>
<tr>
<td>P-tau181P</td>
<td>10</td>
<td>33.4 (27%)b</td>
<td>3.3% (7)</td>
</tr>
</tbody>
</table>

a Calculated as range-to-average from the results of the laboratories that performed the assays in duplicate.
b Results from one participant have been eliminated according to Grubb’s test for outliers.
of this study show inter-laboratory differences of results in the range of 20–30%, i.e. somewhat higher than the imprecision of basic CSF parameters (concentrations of total protein, albumin, or immunoglobulins) [14], which can result from a relatively small number of laboratories performing specialized analysis of dementia biomarkers (three to fourteen in this study) compared to those performing a basic CSF program (approximately two hundred in the quality control surveys coordinated in Germany by Reiber et al. [14]). However, this leads to the conclusion that the direct comparison of concentrations obtained in different laboratories might cause false interpretation of the results. Indeed, in the reports from different groups, cut off values of AD biomarkers differ significantly [7,12,21]. Certainly, further measures should be undertaken to further optimize and control performance of biomarker analysis.

Although the dementia biomarkers currently routinely measured characterize with reasonable diagnostic sensitivity and specificity, it is obvious that novel, even more specific biomarkers need to be searched for. This especially holds true for specificity, it is obvious that novel, even more specific biomarkers characterize with reasonable diagnostic sensitivity and specificity.

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