A Selected Reaction Monitoring (SRM)-Based Method for Absolute Quantification of Aβ38, Aβ40, and Aβ42 in Cerebrospinal Fluid of Alzheimer’s Disease Patients and Healthy Controls

Josef Panneea,∗, Erik Porteliusa, Madalina Oppermannb, Alan Atkinsc, Martin Hornshawc, Ingrid Zegersd, Peter Höjrupf, Lennart Minthonfg, Oskar Hanssonf, Henrik Zetterberga, Kaj Blennowg and Johan Goboma

aDepartment of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy, University of Gothenburg, Mölndal, Sweden
bThermo Scientific, Kungens Kurva, Stockholm, Sweden
cThermo Scientific, Hemel Hempstead, UK
dInstitute for Reference Materials and Measurements, Joint Research Centre, European commission, Geel, Belgium
eDepartment of Biochemistry and Molecular Biology, University of Southern Denmark, Odense M, Denmark
fDepartment of Clinical Sciences, Lund University, Lund, Sweden
gMemory Clinic, Department of Neurology, Skåne University Hospital, Malmö, Sweden
hUCL Institute of Neurology, Queen Square, London, UK

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Abstract. Cerebrospinal fluid (CSF) biomarkers for Alzheimer’s disease (AD) are increasingly used in research centers, clinical trials, and clinical settings. However, their broad-scale use is hampered by lack of standardization across analytical platforms and by interference from binding of amyloid-β (Aβ) to matrix proteins as well as self-aggregation. Here, we report on a matrix effect-resistant method for the measurement of the AD-associated 42 amino acid species of Aβ (Aβ42), together with Aβ40 and Aβ38 in human CSF based on mass spectrometric quantification using selected reaction monitoring (SRM). Samples were prepared by solid-phase extraction and quantification was performed using stable-isotope labeled Aβ peptides as internal standards. The diagnostic performance of the method was evaluated on two independent clinical materials with research volunteers who were cognitively normal and AD patients with mild to moderate dementia. Analytical characteristics of the method include a lower limit of quantification of 62.5 pg/mL for Aβ42 and coefficients of variations below 10%. In a pilot study on AD patients and controls, we verified disease-association with decreased levels of Aβ42 similar to that obtained by ELISA and even better separation was...
INTRODUCTION

Alzheimer’s disease (AD), the main cause of dementia, affects 36 million people worldwide and is a growing problem in the ageing population [1]. Neuropathologically, the disease is characterized by distinct changes in the brain including synaptic loss, aggregation of amyloid-β (Aβ) peptides into plaques, and accumulation of neurofibrillary tangles, consistent of hyperphosphorylated forms of the tau protein [2]. Since its discovery in plaques, the 42 amino acid form of Aβ (Aβ42) has been the subject of extensive research [3]. It is today widely believed that abnormal accumulation of the peptide in the brain lies at the core of AD pathogenesis [4].

Cerebrospinal fluid (CSF) abundance of Aβ42 is approximately 50% lower in AD patients, which probably reflects sequestration of the peptide in senile plaques in the brain [5]. Several studies have shown that the combined measurement of CSF Aβ42 and tau protein (phosphorylated forms and total concentrations that reflect tangle pathology and cortical axonal degeneration, respectively) provides high diagnostic accuracy of AD in cross-sectional clinical control studies and longitudinal studies of patients with mild cognitive impairment [5]. These CSF biomarkers have been incorporated in novel AD definitions and diagnostic criteria [6-9] and are increasingly used in the diagnostic workup at specialized memory clinics worldwide [5].

Currently, CSF Aβ42 is measured using several different types of immunodetecting methods such as ELISA and newer multiplexed techniques [10]. However, there is systematic bias in the concentrations determined using these techniques [11], which to a large extent depends on differences in assay calibration and sensitivity to matrix effects. These include Aβ42-interacting proteins and other factors such as oligomerization that may influence the fraction of CSF Aβ42 exposed to the antibodies used for measurements [12]. An external quality control program, launched by the Alzheimer’s Association to identify sources of variability for measurement of Aβ42 [13], concluded that while intra-laboratory CVs are generally low, the concentrations reported in different studies vary considerably, even when the same analytical platform is used, with mean Aβ42 levels in AD patients in some studies exceeding those in controls in other studies [14]. This variation presents a problem for the use of Aβ42 as biomarker in clinical routine because it is not possible to establish generally applicable cut-off values for diagnosis making, and for research because results from studies performed in different laboratories cannot be readily compared.

Mass spectrometric quantification of Aβ42 by selected reaction monitoring (SRM) may overcome many of the problems associated with antibody-based quantification methods [15]. A central difference is the use of stable isotope labeled (heavy) Aβ42 as internal standard. The heavy peptide is added to the neat CSF prior to any sample preparation. Being chemically equivalent to endogenous Aβ42, the heavy peptide has identical yield through all sample purification steps, as well as the same ionization efficiency and fragmenta-tion behavior in the mass spectrometer, and therefore accounts for any variations in the analytical procedure. Thereby, quantification is largely unaffected by variations in the sample preparation. Furthermore, the high selectivity of detection using SRM alleviates the need for extensive sample purification. For Aβ40, Aβ40, and Aβ1-42, CSF sample preparation can be performed with a single step of solid-phase extraction (SPE) [16]. Thus, most antibody-based methods are influenced by matrix effects and measure only the so-called free fraction of Aβ42, i.e., not oligomerized or protein-bound forms, as revealed by the non-linearity of antibody-based methods upon dilution of samples [11, 12]. When SPE is performed under denaturing conditions, for example by adding guanidine hydrochloride (GdnHCl), non-linear dilution effects are much less pronounced indicating that a larger proportion of the analyte may be available for quantification (total fraction).

The aim of the current study was to develop a reliable SRM-based assay with reduced matrix effect for quantification of Aβ42, Aβ40, and Aβ1-42 in human CSF. The developed assay was evaluated with regards to linearity, coefficient of variation, limit of quantification, and correlation with an established ELISA method. Its
diagnostic accuracy was verified in a study of two independent clinical materials with 15 AD patients and 15 cognitively normal controls in each set.

MATERIAL AND METHODS

Participants and CSF collection

In the present study we analyzed CSF samples from 15 AD patients, mean age ± SD: 75.6 ± 6.6 years, and 15 healthy controls, mean age ± SD: 66.3 ± 9.8 years. A second set of CSF samples from 15 AD patients (mean age ± SD: 81 ± 5.0 years) and 15 healthy controls (mean age ± SD: 63.9 ± 9.4 years) were analyzed. All patients have undergone a thorough clinical investigation, including a medical history, physical, neurological and psychiatric examination, screening laboratory tests, and computerized tomography (CT) of the brain. AD patients fulfilled the DSM-IV criteria of dementia [17] and the criteria of probable AD defined by NINCDS-ADRDA [18]. The control individuals were cognitively normal research volunteers. The ethics committee at the University of Lund has approved analyses of Aβ variants in CSF from AD patients and controls, and all patients (or their nearest relatives) and controls gave informed consent for research, which was conducted according to the provisions of the Helsinki Declaration. The first 10–12 mL of CSF was collected in polypropylene tubes, gently mixed to avoid possible gradient effects and aliquoted in 500 μL portions in polypropylene cryo tubes. The CSF samples were stored at −80°C in 0.5 mL polypropylene tubes (Eppendorf) upon delivery.

Sample preparation

CSF was thawed at room temperature and vortexed. A 200 μL aliquot was transferred to a 1.5 mL polypropylene tube (LeBlond, Eppendorf) containing 200 μL 5 M GdnHCl for protein denaturation. The heavy peptide standards (1 μg), Aβ1-42, Aβ1-40, and Aβ1-40 uniformly labeled with 15N (peptide), were dissolved in 1 mL of 20% acetonitrile (ACN) and 1% NH4OH in water, aliquoted and stored at −80°C in 0.5 mL polypropylene tubes (Eppendorf). Upon analysis, the internal standards were diluted with 20% ACN and 1% NH4OH in water to a concentration of 12 μM. GdnHCl treated CSF samples (400 μL) were spiked with 8 μL of the diluted standard and mixed at room temperature for 45 mins followed by addition of 200 μL 4% phosphoric acid (H3PO4).

To evaluate if the method is sensitive to matrix effects, a CSF pool with an Aβ42 concentration of 1000 pg/mL determined by ELISA was serially diluted with PBS four times. Four replicates of each dilution were prepared. Calibrators

The reverse curve method for calibration was used to determine the concentrations of unknown samples as described elsewhere [19]. A pool of human CSF was spiked with a mixture of heavy labeled Aβ1-42, Aβ1-40, and Aβ1-40 to final concentrations of 5000 pg/mL, 10000 pg/mL, and 2000 pg/mL, respectively. The spiked CSF samples were serially diluted (1 : 2) five times with CSF from the same pool (Fig. 1a). Three replicates of each dilution were analyzed. The performance of the method including sensitivity and linearity was evaluated by the reverse curve method with heavy Aβ1-42, Aβ1-40, and Aβ1-42 peptides spiked in at 2000 pg/mL and serially diluted down to 62.5 pg/mL.

Intra-assay coefficients of variation (CV) were determined by analyzing 6 separately prepared CSF samples from one CSF pool (1214 pg/mL) and 6 samples from another CSF pool (350 pg/mL).

Solid-phase extraction

Extraction of Aβ peptides was performed using a mixed mode cation exchange SPE 96 well plate (Oasis MCX μElution, Waters) as described elsewhere [16]. Briefly, the plate was washed with 200 μL methanol followed by 200 μL 4% H3PO4 in water before adding 600 μL pretreated CSF. After washing with 200 μL 4% H3PO4 in water followed by 200 μL 10% ACN, the samples were eluted with 2 x 50 μL 2.5% NH4OH in 75% ACN into 0.75 mL polypropylene tubes (Micronic). The extracted samples were dried using vacuum centrifugation and stored at −80°C. Prior analysis the dried samples were dissolved in 25 μL 1% NH4OH in 20% ACN, vortexed for 30 min at room temperature, and centrifuged briefly (Fig. 1b).

SRM analysis

Samples (20 μL) were injected on a reversed-phase monolith column (ProSwift RP-4 H 1 × 250 mm, Thermo Scientific) heated to 50°C. A 0.2 μm particle filter (Waters), which was backflushed between sample injections, was placed in front of the column to reduce backpressure build-up. An Accela 1250 pump (Thermo Scientific) was used for delivering the mobile phases at a flow of 300 μL/min. Mobile phase A
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Fig. 1. a) Quantification is performed using the reverse curve method for calibration. For the calibration curve, the heavy isotope labeled peptide is varied while the light (endogenous) peptide is the internal standard (IS). With unknown samples, the heavy peptide is again used as the IS. b) Overview of the method. For each sample, 200 μL GdnHCl is added to 200 μL CSF and IS are spiked in at 2000 pg/mL. The samples are then mixed in room temperature for 45 minutes and 200 μL H3PO4 is added to each sample before extraction. Each SPE plate can be loaded with 96 samples in parallel. The eluted samples are dried simultaneously using vacuum centrifugation (about 45 minutes) and stored in −80°C. Prior to analysis the dried samples are redissolved with 25 μL 1% NH4OH in 20% ACN. The samples are then injected serially on a reversed phase column which separates the peptides according to their hydrophobicity. Since the shorter Aβ peptides are less hydrophobic than the longer species, they elute earlier while co-eluting with their corresponding internal standard. The time from injection to injection is 20 minutes. The Aβ peptides are ionized using electrospray and transmitted through the first quadrupole according to their m/z values to the second quadrupole which is filled with argon. Here the peptides are fragmented as they collide with the gas atoms and only specific fragments of the Aβ peptides with predefined m/z values are transmitted through the third quadrupole and further to the detector.
RESULTS

Optimal transitions for Aβ38, Aβ40, and Aβ42 were determined through direct infusion of each peptide separately into the mass spectrometer. The most prominent precursor charge state for each of the 3 peptides was selected and followed by manual optimization of the full scan tandem mass spectrometry (MS/MS) spectra (Fig. 2a–c). For each precursor, the top 10 most prominent product ions were used for further optimization by systematically varying collision energy and collision gas pressure. The three best transitions were selected for each peptide and used for quantification (Table 1). The LC gradient was optimized for a short analysis time (20 min in total including washing and equilibration of the column) and sufficient separation for implementation of the scheduled SRM. Typical SRM chromatograms for Aβ42, Aβ40, and Aβ38 in a human CSF sample are shown in Fig. 2d.

Amino acid analysis

Amino acid analysis was performed for precise determination of the concentrations of aliquoted internal standards. Polypropylene vials containing lyophilized aliquots (12.5 μg) of the heavy Aβ peptide standards were placed in separate 22 mL glass vials (Wheaton). Hydrolysis buffer (200 μL 6 N HCl, 0.1% phenol, 0.1% thioglycol acid) was added to the bottom of the glass vials. The vials were purged with argon, closed with a Minil bert valve (Parr, p<5 mbar) and the samples were incubated at 110 °C for 20 h. The dried samples were redissolved in 70 μL loading buffer (pH 2.2) containing 1 μmol NorLeu and half the sample was applied to a BioChrom 31 amino acid analyzer running the sodium-accelerated buffer system. The analyzer was calibrated to an R value of 0.999 and for every 4th sample a 1600 pmol standard was analyzed for recalibration. After analysis, integration of the chromatograms were checked manually and the integration results were analyzed using an in-house developed program AAA ver. 1.03, which corrects for intensity changes of the ninhydrin (∼3%) and performs a best linear fit of hydrolysis data to the theoretical sequence (∼1%).

Statistics

All statistical calculations were performed using Graphpad (version 5.02) software. For the AD versus control comparisons, the Mann-Whitney U-test was used. The Spearman’s rank correlation coefficient was used for analyses of correlation between methods. Estimates of diagnostic accuracy were sensitivity, specificity, and the area under the receiver operating characteristics curve (AUROC). p<0.001 was considered statistically significant.
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Fig. 2. MS/MS spectra for Aβ/H925238, Aβ/H925240, and Aβ/H925242. Transitions used for the assay are marked with corresponding ion type and charge state for Aβ/H925238 (a), Aβ/H925240 (b), and Aβ/H925242 (c). Typical SRM chromatograms for Aβ/H925238, Aβ/H925240, and Aβ/H925242 (d). Absolute concentration of endogenous Aβ peptide is calculated using the area ratio for the co-eluting internal standard of known concentration.

DISCUSSION

We here report on the development of an antibody-independent, matrix effect resistant assay for quantification of Aβ42, Aβ40, and Aβ38 using SRM-based

sensitivity of 93% and specificity of 80% compared to ELISA (Fig. 5b, c) and a sensitivity and specificity of 86.7% for set two (5b, c). The ratio of Aβ42 to Aβ40 (Aβ42/Aβ40) further improved the separation between the diagnostic groups (Fig. 5d, e and 6d, e).
Table 1
Transitions used in the SRM assay. Internal heavy standards are marked with asterisk.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Precursor ion charge state</th>
<th>Precursor ion m/z</th>
<th>Product ion</th>
<th>Product ion m/z</th>
<th>Collision Energy (eV)</th>
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<tr>
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<td>1033.897</td>
<td>b34</td>
<td>954.392</td>
<td>22</td>
</tr>
<tr>
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<td>4+</td>
<td>1045.927</td>
<td>b38</td>
<td>1000.664</td>
<td>20</td>
</tr>
<tr>
<td>1–38</td>
<td>4+</td>
<td>1033.897</td>
<td>b35</td>
<td>976.085</td>
<td>21</td>
</tr>
<tr>
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<td>1096.626</td>
<td>b38</td>
<td>1059.480</td>
<td>23</td>
</tr>
<tr>
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<td>1109.626</td>
<td>b39</td>
<td>1093.960</td>
<td>20</td>
</tr>
<tr>
<td>1–42</td>
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<td>1129.522</td>
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<td>22</td>
</tr>
<tr>
<td>1–42*</td>
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<td>20</td>
</tr>
<tr>
<td>1–42</td>
<td>4+</td>
<td>1129.522</td>
<td>b40</td>
<td>1107.000</td>
<td>20</td>
</tr>
<tr>
<td>1–42*</td>
<td>4+</td>
<td>1143.182</td>
<td>b41</td>
<td>1120.660</td>
<td>20</td>
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* m/z = mass to charge ratio.

Fig. 3. Linearity of Aβ42 measured by SRM (area ratio light/heavy) of human post-CSE, with a concentration of 1000 pg/mL measured by ELISA, serially diluted with PBS four times.

For Aβ, previous studies have shown that while there is no significant change in the CSF Aβ40 concentration between AD and controls, there is already a decrease in the Aβ42/Aβ40 in the mild cognitive impairment stage of AD. This decrease is more pronounced than the reduction in CSF Aβ42 alone [20, 21]. Using SRM-based quantification of Aβ42 and Aβ40, we here replicate the finding that the CSF Aβ42/Aβ40 ratio increases the separation performance between AD and controls as compared to Aβ42 alone. Further studies will be aimed to elucidate whether the ratio is a prognostic AD marker which can be used for AD-diagnosis already at the pre-symptomatic stage of the disease. An important advantage over immunassays is the ability to include additional peptides such as other isoforms of Aβ or other endogenous peptides to an already existing SRM assay to assemble a panel of biomarkers tailored to specific clinical questions which can be analyzed in a single run.

Several studies suggest that Aβ-binding proteins may have an impact on the measured concentration of Aβ42 since there might be a difference in free Aβ42 and total Aβ42 in CSF [11, 12]. Recently, it was shown that denaturation of proteins in CSF with GdnHCl before analysis resulted in increased concentration of Aβ42 by ELISA [12]. In the present study, using GdnHCl in the sample purification, the CSF Aβ42 concentrations obtained with the SRM assay were approximately twice as high as the concentrations determined by ELISA, while the separation between AD patients and controls remained in spite of the denaturing conditions. Furthermore, increasing the amount of GdnHCl added to the CSF did not increase the free Aβ42 measured in our study.
There are several aspects to consider including high specificity as well as robustness and stability of the method when analyzing large series of samples. While SPE purification in the 96-well format is performed in parallel with many samples, the subsequent LC-MS analysis is serial. Thus, the extracted samples will have different time delay for LC-MS. Using the described SRM method, dried extracted samples were analyzed after being stored in freezer (−20 °C) for four months with minimal loss in signal (less than 1%).

A matrix effect-resistant method is important to reduce the bias in CSF Aβ42 measurements between commercially available assays. An essential component of such a method is that, e.g., the calibration of the method is performed in a matrix identical to the sample-matrix to be analyzed. To achieve a matrix effect-resistant method, we used the reverse curve method for calibration which previously has been shown to give equal or better trueness and precision compared to traditional calibration curves or single reference point calibration [19]. For a reverse curve calibration, the heavy peptide is varied while the light (endogenous) peptide is the internal standard and is held constant. With unknown samples, the heavy peptide is again used as the internal standard by adding at a fixed amount to the samples, and the light amount is measured by reverse-calculating against the calibration curve. The method also works with calibration in artificial CSF and gives similar quantitation results as the reversed curve method in human CSF. The latter method was chosen because it avoids the risk of matrix effects that may distort results when constructing a calibration curve in a matrix different from that of the unknowns.

SRM-based quantification of Aβ40 and Aβ42 in human AD and control CSF was first reported by Oe et al. [22]. Their method relied on antibodies for sample purification, followed by LC-MS in a basic buffer system, and the authors showed higher concentrations of CSF Aβ42 obtained with SRM compared to ELISA, which is in agreement with this study [22]. However, electrospray was performed in the negative ion mode, resulting in low efficiency of fragmentation, and only one transition per peptide was used for quantification, which may make the assay vulnerable to interference from other sample components (cross-talk) [23]. The current practice is to select two or more transitions per peptide for reliable quantification [24]. Furthermore, the transition chosen was neutral loss of H2O. While sensitive, this is a non-peptide-specific loss, which increases the risk of cross-talk. In a further development, Lame et al. showed that antibody-dependent enrichment of CSF Aβ38, Aβ40, and Aβ42 could be replaced by SPE without loss of detection sensitivity or precision. They also demonstrated that while using a basic buffer system, operating the mass spectrometer in the positive ion mode resulted in lower detection sensitivity compared to negative mode. This approach was still preferable because of increased selectivity for Aβ peptides together with increased stability of the Aβ signal [16]. They did not perform AD-control comparisons to test the diagnostic accuracy of the method.

To achieve high specificity and stability over time, we analyzed the samples in the positive ion mode, and monitored three b ions per peptide using scheduled SRM, thereby reducing the signal-to-noise ratio while the limit of detection was improved [25]. With the one-step SPE procedure described here, the Aβ42 samples loaded on the LC-MS system are quite crude, containing many other peptides and larger proteins, as well as aggregates and particles. These sample impurities may contaminate the system and cause degradation.
Fig. 5. Set one of 15 AD patients and 15 controls. Correlation between SRM Aβ38 and ELISA Aβ38 assays (a). Separation of AD and control groups for ELISA Aβ42 (p < 0.0001) (b) and SRM Aβ42 (p = 0.0011) (c) and separation of AD and control groups for SRM using the Aβ42/Aβ40 ratio (p < 0.0001) (d). The solid line represents median value for each group while the dashed line represents the optimal sensitivity and specificity for separation of AD and control groups. The sensitivity and specificity of the different methods is summarized in an ROC curve: AUC ELISA Aβ42 = 0.92, SRM Aβ42 = 0.85, SRM Aβ42/Aβ40 = 0.97 (e).
Fig. 6. Set two of 15 AD patients and 15 controls. Correlation between SRM $A\beta_{42}$ and ELISA $A\beta_{42}$ assays (a). Separation of AD and control groups for ELISA $A\beta_{42}$ ($p=0.0004$) (b) and SRM $A\beta_{42}$ ($p=0.0004$) (c) and separation of AD and control groups for SRM using the $A\beta_{42}/A\beta_{40}$ ratio ($p=0.0002$) (d). The solid line represents median value for each group while the dashed line represents the optimal sensitivity and specificity for separation of AD and control groups. The sensitivity and specificity of the different methods is summarized in an ROC curve. AUC ELISA $A\beta_{42} = 0.88$, SRM $A\beta_{42} = 0.88$, SRM $A\beta_{42}/A\beta_{40} = 0.91$ (e).
of the analytical performance over time. Trapping of insoluble materials on the particle filter resulted in loss of mass sensitivity. By implementing a routine for backflushing the filter in every six samples, the trapped particles were removed, and minimal pressure build-up over the filter was observed.

When using a silica-based C18 column for peptide separation, as described in previous studies [16, 17, 26, 27], we noticed a gradual back pressure build-up over the separation column following each consecutive sample injection. This is most likely caused by irreversible binding of sample impurities to the column and resulted in the column needing to be replaced after less than 10 injections. This may have severe implications for the applicability of the method to routine work: it increases the cost per sample by over $100 and causes down-time because each new column has to be tested before use. We therefore evaluated the use of a monolith separation column based on a polystyrene dimethylbenzene copolymer bed. Possessing weaker hydrophobic retention characteristics than particle-based C18 stationary phases, this chromatographic medium is less prone to contamination by proteins and other hydrophobic molecules. The separation column used in this study has been used for over 200 injections of human CSF samples without any increase in back pressure or degradation of performance.

While SRM has been used for over three decades, its main area of application has been small-molecule analysis, and only in recent years has the technique emerged as an alternative to immunoassays for protein and peptide quantification [27]. In this respect, the technique has distinct advantages. For one, molecular mass-based selective peptide quantification allows one to discriminate between modified forms of a target molecule, which would be indistinguishable in an immunoassay. Assay development is generally quicker and panels of peptides with relatively low sequence identity can be assessed in one analysis. For Aβ1-42, it is plausible that SRM-based assays will overcome the problem of inter-laboratory variation discussed above, because mass spectrometric quantification using stable isotope labeled internal standards enables absolute quantification and is influenced by matrix effects. This notion is currently being evaluated in the collaborative effort involving several research laboratories within the Global Consortium for Biomarker Standardization (GCBS) of the Alzheimer’s Association. A positive evaluation, however, does not necessarily imply that SRM assays will likely replace immunoassays in clinical routine in the near future; LC-MS analysis is a serial, relatively time-consuming process that cannot match the sample throughput of state-of-the-art immunoassay methods. A more likely scenario is that SRM will be used as a reference technique to determine the absolute Aβ1-42 levels in reference materials. Such materials have been used as ‘gold standards’ in other areas of laboratory medicine to reduce measurement variability and harmonize analytical results obtained in different laboratories [28].

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REFERENCES


