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Improved Performance of CSF Dementia Biomarker Measurements Over Time: The Effect of Quality Control and Harmonization Programs

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Abstract

Introduction: Cerebrospinal Fluid (CSF) biomarkers Total Tau Protein (τ_T), Amyloid Beta Peptide ($A\beta_{42}$) and tau protein hyper phosphorylated at threonine 181 ($\tau_{P\text{-}181}$) are important in the (differential) diagnosis of dementia and analysis of these biomarkers is now incorporated in the diagnostic criteria of Alzheimer's disease. However, lack of standardization has led to considerable inter- and intra-laboratory variation. Various international programs have been launched aiming in the reduction of variability and harmonization of biomarker measurements.

Objectives: To explore whether experience gained from international quality control and harmonization programs had any effect on the analytical performance of our laboratory for CSF dementia biomarkers [Total Tau (τ_T), Amyloid Beta ($A\beta_{42}$) and Phospho-Tau ($\tau_{P\text{-}181}$)].

Methods: We retrospectively analyzed internal standard measurements during ELISA runs in 3 time periods: before 2010, 2010-2012 (experience from workshops and quality control programs) and after 2012 (JPND-BIOMARKAPD harmonization program).

Results: During the 1st period, coefficients of variation were 8.6%-17.1%. Subsequently, they were reduced, reaching 4.5%-6.6% at the 3rd period. Measurement error was reduced for τ_T and $A\beta_{42}$ from 9.2% and 22.1% to 1% and 3.3% respectively. Median values for $A\beta_{42}$ were significantly lower compared to the expected values during the 1st period but, came closer to (at the 2nd period) and finally reached the expected value at the 3rd period.

Conclusion: The improvement noted, indicates a beneficial effect of quality control and harmonization programs on analytical performance, by lowering measurement errors to levels which are not expected to adversely affect diagnostic performance in every day practice.

Keywords: Alzheimer's Disease; Amyloid Beta; Cerebrospinal Fluid Biomarkers; Dementia; Phospho-Tau; Tau Protein

Introduction

Cerebrospinal Fluid (CSF) levels of amyloid beta peptide with 42 Amino Acids ($A\beta_{42}$), Total Tau Protein (τ_T) and tau protein hyper phosphorylated at the threonine residue 181 ($\tau_{P\text{-}181}$), are well established biomarkers [1,2] for the discrimination among normal ageing, Alzheimer's disease and other dementing disorders in clinical practice [3-7]. The use of these biomarkers has been incorporated in current criteria for the (differential) diagnosis of Alzheimer's disease in the dementia and pre-dementia [8-10] or even the preclinical stage [11]. The development of new, disease-modifying treatments acting at a pre-dementia stage, makes the use of such biomarkers important for the selection of patients for clinical trials [12,13]. However, after almost 2 decades of research there is still a significant inter- and Intra-Laboratory variability in the Determination of CSF biomarkers, as a result of pre-analytical, analytical, post-analytical and kit-related factors [14-18]. Recently, it has been suggested that measured concentration shifts may not affect every day practice so seriously as previously thought and, indeed, the effect on AD diagnostic accuracy may be minimal (~8% or less) when deviations of ±20% are present in only one of the three biomarkers [19]. However, when deviations are present

in more than 1 biomarker and/or shifts are >20%, diagnostic performance may decrease significantly.

International Workshops and International Quality Control Programs have been organized since 2009, in order to reduce variability and harmonize the levels of biomarkers universally [14,15]. More recently, the "Biomarkers for Alzheimer's disease and Parkinson's disease" project of the Joint Programming Neurodegenerative Disease (JPND-BIOMARKAPD) was launched at June 2012 [20]. The above projects led to an ongoing research on various parameters and confounding factors of the methodologies used [14,21] and to the formulation of recommendations for (pre) Analytical Standardized Operative Procedures (SOPs) on CSF biomarker assays [22], emphasizing the need for strict adherence to these SOPs at the highest level possible. The aim of the present study was to investigate whether this strict adherence to SOPs had any effect on the quality of CSF biomarker measurements in an individual laboratory, with expertise in biomarker research.

Materials and Methods

Time Periods

We retrospectively analyzed the results of internal standard measurements during biomarker assays, performed in our laboratory from October 2000 to December 2016 divided in 3 periods: (a) From 2000 to the end of 2009. (b) From January 2010 to June 2012; during this period experience and ideas gained from quality control programs and workshops, were incorporated in routine practice and definitely influenced procedures in our laboratory. (c) From June 2012 to December 2016; during this period additional experience and guidelines from the BIOMARKAPD project were used as standard procedures.

Measurements

All measurements of τ_T, $A\beta_{42}$ and $\tau_{P\text{-}181}$ have been performed in duplicate by double sandwich, Enzyme-Linked Immunosorbent Assay (ELISA) as provided by commercially available kits according to manufacturer's instructions ("Innotest® hTau antigen", "β-amyloid$_{1\text{-}42}$" and "phospho-tau$_{181}$" respectively, provided initially by Innogenetics and then by Fujirebio Europe, Gent, Belgium). For each ELISA run a fresh in-house Internal Standard (IS) was prepared at the beginning of the assay in polypropylene tubes, using the concentrated standard provided by the manufacturer, dissolved in the provided sample diluent, exactly by the same way used to prepare the standards for the standards curve. Since, for practical reasons, assay precision has to be maximal at concentrations around the cut-off values, the IS concentrations for τ_T, $A\beta_{42}$ and $\tau_{P\text{-}181}$ were 300 pg/ml, 500 pg/ml and 62.5 pg/ml respectively, based on cut-off values suggested from previous studies in our laboratory [3,4,23]. The IS was measured in duplicate, as the last unknown sample (plate wells numbered 95 and 96). Absorbance was measured at dual wavelength (450 and 620 nm) by a Lab systems Multi scan EX ELISA reader, controlled by a computer-based program (Lab systems Genesis 3.03) which automatically calculated the concentrations by the use of a sigmoid curve.

Statistical Analysis

Both the measured concentrations of the ISs and the calculated error (%deviation) from the expected (true) concentration were analyzed. This error was calculated by the formula: error= (measured concentration -true concentration)/true concentration and expressed as the absolute value. Due to deviations from normality and/or heterogeneity of variances, non-parametrics were mainly used. Median values of the measured concentrations of ISs in each period were compared to the expected concentration by the Wilkoxon-signed rank test. Measured concentrations and errors among the studied periods were compared by the Kruskal-Wallis test followed by Bonferroni-corrected individual Mann-Whitney tests. Variances were compared by the Bartlett's test for equality of variances followed by Bonferroni-corrected individual F-tests. Statistical analyses were performed by GraphPad Prism 2.01 (GraphPad Software Inc, San Diego, CA) and StatSoft Statistica 8 (StatSoft Inc, Tulsa, OK)

Results

Results are summarized in Table 1 and Figure 1.

	1st period	2nd period	3rd period	P value
	Oct 2000-Nov 2009	Jan 2010-May 2012	Jun 2012-Dec 2016	
τ_T 300 pg/ml, n	26	14	11	
median (quartiles)	295 (270 - 324)	294 (279 -306)	297 (294 - 299)	0.980†
Min - max	171 - 380	260 - 338	259- 306	
CV	0.163[a]	0.069	0.045	<0.0001‡
Measurement error*	0.092 (0.056 -0.148)[b]	0.042 (0.014 - 0.091)	0.010 (0.003 - 0.019)	0.0127†
$A\beta_{42}$ 500 pg/ml, n	20	13	12	
median (quartiles)	389 (358 - 445)[c]	452 (431 - 471)[d]	514 (499 - 546)	<0.0001†
Min - max	289 - 582	389 - 512	452 - 581	
CV	0.171[e]	0.072	0.066	0.0069‡
Measurement error*	0.221 (0.153 - 0.285)[f]	0.097 (0.057 - 0.138)	0.033 (0.008 - 0.092)	0.0146†
$\tau_{P\text{-}181}$ 62.5 pg/ml, n	17	13	12	
median (quartiles)	63.3 (59.5 - 65.5)	62.1 (60.7 - 62.8)	62.0 (61.0 - 62.9)	0.603†
Min - max	47.8-68.7	59.9 - 69.8	52.9- 65.6	
CV	0.086[g]	0.039	0.051	0.017‡
Measurement error *	0.040 (0.020 - 0.097)[h]	0.008 (0.005 - 0.034)	0.01 (0.008-0.044)	0.031†

τ_T: Total Tau Protein. $A\beta_{42}$: Amyloid-Beta Peptide with 42 amino acids. $\tau_{P\text{-}181}$: phospho-tau protein phosphorylated at threonine-181. Quartiles: 25th-75th percentile. CV: coefficient of variation. *Absolute value [median (quartiles)]. †Kruskal-Wallis test followed by Bonferroni-corrected Mann-Whitney tests. ‡Bartlett's test for equality of variances followed by Bonferroni-corrected F-tests. [a]P=0.0075 vs 2nd period and 0.0006 vs 3rd period. [b]P=0.053 vs 2nd period and 0.0009 vs 3rd period. [c]P=0.015 vs 2nd period and 0.0001 vs 3rd period. [d]P=0.0003 vs 3rd period. [e]P=0.033 vs 2nd period and 0.074 vs 3rd period. [f]P=0.003 vs 2nd period and 0.0003 vs 3rd period. [g]P=0.03 vs 2nd period. [h]P=0.051 vs 2nd period.

Table 1: Results of the 3 internal standard determinations for CSF biomarkers during the 3 studied periods.

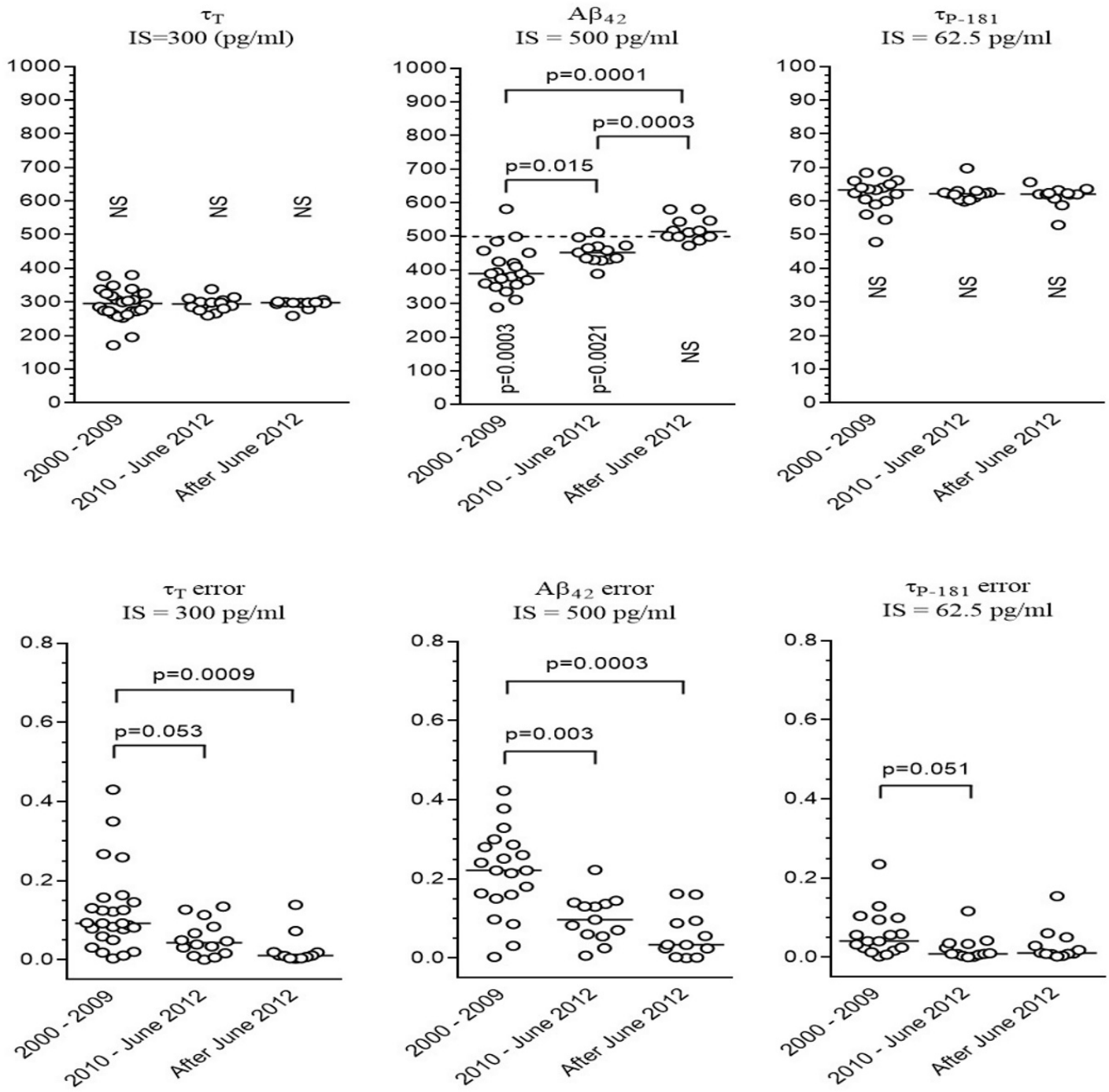

Figure 1: Measured concentrations (upper 3 scatterplots) and measurement error (lower 3 scatterplots) for the 3 internal standards in the 3 studied periods. Horizontal lines indicated expected concentrations and horizontal bars indicate median values. Upward directed P values indicate difference from the expected concentration and horizontally directed P values indicate difference between groups.

Median values of ISs for τ_T did not differ significantly among each period and as compared to the expected concentrations (300 pg/ml) at any time period. However, a significant reduction by ~70% in the coefficient of variation was noted, accompanied by a gradual and significant drop of the measurement error in both the 2nd and 3rd periods, compared to the 1st period. Although the difference between 2nd and 3rd periods did not reach statistical significance, numerically the reductions were more obvious at the 3rd period.

Median value of the IS for $A\beta_{42}$ in the 1st period was significantly lower from the expected (500 pg/ml) concentration by 22% (P = 0.0003). At the 2nd period the median value increased significantly, but it was still significantly lower, compared to the expected concentration (P = 0.0021). Median value of the 3rd period did not differ significantly from the expected and, it was significantly higher compared to the median value of the 1st and 2nd periods. A significant reduction by ~ 60% in the coefficient of variation was noted, that was accompanied by a significant reduction of the measurement error in both the 2nd and 3rd periods compared to the 1st period. Again, the difference between 2nd and 3rd periods did not reach statistical significance; however, the reductions were numerically more obvious at the 3rd period.

Median values of ISs for $\tau_{P\text{-}181}$ did not differ significantly from the expected concentrations. Additionally, measured concentrations and errors did not differ among each other in the 3 periods. However, a significant reduction in the coefficient of variation and measurement error was noted after the 1st period.

Discussion

Before 2010, in our laboratory, two problems were noted: high CVs and high measurement error for $A\beta_{42}$. The CV of $A\beta_{42}$ was the highest (~17%), followed by τ_T (~16%) and $\tau_{P\text{-}181}$ (8.6%). This is in agreement with other observations suggesting higher intra-laboratory variability for $A\beta_{42}$, as compared to τ_T and $\tau_{P\text{-}181}$ [18]. Such a high CV is not surprising, since even higher CVs have been reported in multicenter studies, even in reference laboratories. In a world-wide multicenter study, intra-laboratory coefficients of variation for $A\beta_{42}$ and τ_T as high as 25% and 18% were reported in 2008 [24]. During 2009-2010 the intra-laboratory CVs in reference laboratories participating in a quality control program, ranged at levels of 6.4%-19% for $A\beta_{42}$, 3.2%-24% for τ_T and 3.8%-14% for $\tau_{P\text{-}181}$ [15]. Based on cumulative results of the same quality control program, the mean CVs for all laboratories participated were 19% for $A\beta_{42}$, 16% for τ_T and 12% for $\tau_{P\text{-}181}$ [17]. Thus, it has been suggested that not only the inter-laboratory, but also the intra-laboratory variation still remains high, sometimes contributing to diagnostic uncertainty, especially at levels near the cut-off value, which may form a "grey zone" [17]. However, such studies have mainly focused on the inter-laboratory variation and, although presenting intra-laboratory data, they did not compare the intra-laboratory accuracy and variance in different periods over time. To our knowledge, this is the 1st study directly addressing this question.

Additionally, before 2010, the median measurement error for $A\beta_{42}$ was 22%. For τ_T the median error was much lower (9.2%); however, for some individual ELISA runs, the error was higher, even reaching 40%. Such a deviation from true values may be expected to affect clinical diagnostic accuracy [19].

Three types of change have been observed from 2010 onwards. First, a reduction of the inter-assay variation was noted, regarding the ISs of all three markers. At the 3rd period all CVs were at the range of 4.5%-6.6%. Second, the measurement error was reduced, being <3.3% for all 3 biomarkers during the 3rd period, which is not expected to significantly affect diagnostic accuracy in clinical practice [19]. Third, median values for $A\beta_{42}$ came closer to (at the 2nd period) and finally reached the expected value at the 3rd period. Thus, an improvement was noted since 2010 that was ongoing and reached maximal levels after June 2012.

The present study deals neither with stored standards nor samples, but with freshly prepared ISs. Thus, it evaluates analytical accuracy and, the improvement noted, reflects an improvement in (post)analytical performance. The reason of this improvement may involve analytical and post-analytical causes (Table 2), whilst a lot to lot variability or improvement of kits over time should also be considered.

2002 - 2009	2010 - June 2012	After June 2012
τ_T		
Point to point sigmoid curve	Sigmoid (4-parameter logistic) curve fitting	Sigmoid (4-parameter logistic) curve fitting
Single channel pipettes	Single channel pipettes	Multichannel pipettes
Room temperature 18-26°C	Stable room temperature 25±2°C	Stable room temperature 25±2°C
$A\beta_{42}$		
Point to point sigmoid curve	Sigmoid (4-parameter logistic) curve fitting	Sigmoid (4-parameter logistic) curve fitting
Single channel pipettes	Single channel pipettes	Multichannel pipettes
Rare use of uncoated polypropylene plate	Always uncoated polypropylene plate	Always uncoated polypropylene plate
Room temperature 18-26°C	Stable room temperature 25±2°C	Stable room temperature 25±2°C
$\tau_{P\text{-}181}$		
Sigmoid (4-parameter logistic) curve fitting	Sigmoid (4-parameter logistic) curve fitting	Sigmoid (4-parameter logistic) curve fitting
Single channel pipettes	Single channel pipettes	Multichannel pipettes
Room temperature 18-26°C	Stable room temperature 25±2°C	Stable room temperature 25±2°C
τ_T: Total Tau Protein. $A\beta_{42}$: Amyloid-Beta Peptide with 42 amino acids. $\tau_{P\text{-}181}$: Phospho-Tau Protein Phosphorylated At Threonine-181.		

Table 2: Analytical parameters in our laboratory changed after the end of 2009.

Conclusion

Strict adherence to SOPs according to current suggestions [25], is necessary in order to reduce variability of CSF biomarker determination and increase diagnostic performance in the era of biomarker-supported AD diagnosis.

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