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Higher Level of Mismatch in *APOE* ε4 Carriers for Amyloid-Beta Peptide Alzheimer's Disease Biomarkers in Cerebrospinal Fluid

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Abstract

Cerebrospinal fluid (CSF) biomarkers are widely used in the diagnosis of dementia. Even though there is a causal correlation between apolipoprotein E (*APOE*) genotype and amyloid-beta (Aβ), the determination of *APOE* is currently not supported by national or international guidelines. We compared parallel measured CSF biomarkers of two independent laboratories from 126 patients who underwent clinical dementia diagnostics regarding the *APOE* genotype. *APOE* ε4 reduces Aβ1-42 ($A\beta_{42}$) and $A\beta_{42}$ to Aβ 1-40 ratio ($A\beta_{42/40}$) but not total Tau or phospho-181 Tau CSF levels. Higher discordance rates were observed for $A\beta_{42}$ and subsequently for $A\beta_{42/40}$ in *APOE* ε4 carriers compared with noncarriers, and the correlation between the two laboratories was significantly lower for $A\beta_{42}$ in *APOE* ε4 positive patients compared with patients without *APOE* ε4. These observations demonstrate that the evaluation of CSF Aβ biomarkers needs to be interpreted carefully in the clinical context. Different immunoassays, disparate cutoff values, and *APOE* should be respected.

Keywords

amyloid-beta, APOE, biomarker, discordance, immunoassay, neurochemistry

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Introduction

Biomarkers play a pivotal role in the clinical diagnosis of neurodegenerative disorders in particular for Alzheimer's disease (AD). AD biomarkers reflect the typical neuropathological hallmarks: hyperphosphorylated tangles and amyloid plaques. While increased phosphorylated tau (p181Tau) and total tau (tTau) indicate the tangle pathology in cerebrospinal fluid (CSF), amyloid-beta (Aβ) 1-42 ($A\beta_{42}$) levels and especially the decreased $A\beta_{42}$ to amyloid-β 1-40 ($A\beta_{40}$) ratio ($A\beta_{42/40}$) embody the cerebral amyloid pathology that can be verified postmortem. The importance of these biomarkers in the clinical diagnosis of AD has been reflected in national (e.g., German) and international guidelines and recommendations (Dubois et al., 2007; McKhann et al., 2011; Cummings et al., 2013; Deuschl and Maier, 2016). Novel research guidelines even more emphasize the significance of these biomarkers (Jack et al., 2018).

We have recently shown that CSF biomarkers measured in different clinically validated and certified laboratories are interpreted discordantly in up to 31.5% of cases for $A\beta_{42}$ (Vogelgsang et al., 2018), whereas $A\beta_{42/40}$ seems to be less prone to pre-analytical factors (Gervaise-Henry et al., 2017). It is not clear whether these findings are caused by pre-analytical or analytical interferences.

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Apolipoprotein E (*APOE*) ε4 is the most prominent genetic risk factor for late-onset AD (Bertram and Tanzi, 2008). Several studies have shown that *APOE* ε4 is highly associated with amyloid pathology at any cognitive stage of AD (Mattsson et al., 2018).

We aimed to analyze *APOE* ε4 as an interfering factor that leads to inconsistent CSF biomarker results under routine clinical conditions. In addition, this study aims to describe the current difficulties in clinical interpretation of CSF Aβ to make physicians aware of pitfalls. Therefore, CSF samples were sent and analyzed at two different, certified, clinical laboratories for biomarker determination.

Methods

Study Design

Within the biomaterial bank of the Department of Psychiatry and Psychotherapy of the University Medical Center Goettingen, we identified 126 samples from patients between 45 and 90 years where AD-relevant CSF biomarkers were measured in two independent, clinically certified laboratories during routine clinical diagnostic procedures, as described recently (Vogelgsang et al., 2018). CSF biomarkers were measured according to the local standard operating procedures (SOPs), and both laboratories were not informed prior to the study to ensure routine procedures were maintained. No special effort was put in standardizing the SOPs. Biomarkers were measured using commercial and for *in vitro diagnostic* approved enzyme-linked immunosorbent assays (ELISAs). tTau (Fujirebio Cat# 81572, RRID:AB_2797379) and pTau (Fujirebio Cat# 81574, RRID:AB_2797380) were measured using ELISAs by Fujirebio (Ghent, Belgium), $A\beta_{40}$ (IBL Cat# RE59651, RRID:AB_2797386) was measured using ELISAs by IBL International (Hamburg, Germany), and $A\beta_{42}$ (IBL Cat# RE59661, RRID: AB_2797387 and Fujirebio Cat# 81576, RRID: AB_2797385) was measured using ELISAs by IBL and Fujirebio. CSF biomarkers were interpreted according to the corresponding cutoff values of the respective laboratory. Cutoff values were identified and adjusted during their routine validation procedures. At Center 1, cutoff values were 450 pg/ml for $A\beta_{42}$, 0.05 for the $A\beta_{42/40}$ ratio, 450 pg/ml for tTau, and 61 pg/ml for pTau. At Center 2, cutoff values were 620 pg/ml for $A\beta_{42}$, 0.05 for the $A\beta_{42/40}$ ratio, 320 pg/ml for tTau, and 50 pg/ml for pTau. Cutoff values were not adapted during the study.

A polymerase chain reaction (PCR) was performed to identify the *APOE* genotype and accordance for *APOE* ε4 carriers (E2/E4, E3/E4, and E4/E4) and non *APOE* ε4 carriers (E2/E2, E2/E3, E3/E3) were compared. We compared each CSF biomarker independently and only included data from participants where the CSF biomarkers were significantly above or below cutoff, identified by ±10% of the respective cutoff value (borderline cutoff zone). Concordance was defined as an identical interpretation of biomarkers in both laboratories (either significantly above or below cutoff), whereas discordance was defined as dissimilar biomarker interpretations in the two corresponding laboratories (above the cutoff in one center and below the cutoff in the other center).

Sample Collection

CSF was collected by a lumbar puncture and stored in polypropylene tubes during the clinical diagnostic procedure. The lumbar puncture was performed in a seated position using a traumatic *Quincke needle* (BD Diagnostics, Franklin Lakes, NJ) or an atraumatic *Sprotte cannula* (Pajunk, Geislingen, Germany), according to the preference of the treating physician. The samples were sent immediately to two independent clinical laboratories for CSF biomarker measurements of $A\beta_{42}$, $A\beta_{40}$, $A\beta_{42/40}$, p181Tau, and tTau.

APOE Measurement

APOE genotyping was performed using a quantitative real-time PCR protocol as described previously (Calero et al., 2009), with negative controls for all primer combinations and all PCR reactions run in duplicate. Measurements were carried out using a Stratagene MX3000P Real-Time PCR Cycler (Santa Clara, CA).

Statistics

Statistical analysis was performed using Prism Graph Pad 8 (RRID:SCR_002798). Age was compared using a student's *t*-test, and cohort differences for discordant CSF biomarkers and gender were analyzed using Fisher's exact test. Correlations were assessed by Spearman correlation and compared after calculating a Fisher *r*-to-*z* transformation.

Study Approval

All participants gave their informed consent for biomaterial and data collection prior to inclusion into this study. All data were pseudonymized. The study was approved by the ethics committee of the University Medical Center Goettingen (ethical vote 9/2/16). The study was conducted according to the Declaration of Helsinki.

Results

Study Cohort

In this study, 54 (42.9%) participants were *APOE* ε4 carriers with a mean age of 70.4 ± 10.0 years. No *APOE* ε4 allele was found in 72 (57.1%) participants with a mean age of 66.8 ± 10.3 years. There was a trend in distribution for age ($p = .0502$) but not for gender ($p = .8564$) between *APOE* ε4 carriers and noncarriers. CSF biomarker accordance was compared after excluding cases within the borderline cutoff zone (±10%) and age and gender were recalculated for each biomarker. No significant difference in age or gender was observed for any of the analyzed groups (Table 1).

CSF Biomarkers

Accordance rates were compared for all four validated CSF biomarkers: $A\beta_{42}$, $A\beta_{42/40}$, tTau, and p181Tau. For $A\beta_{42}$, 26 (68.4%) *APOE* ε4 carriers obtained discordant CSF interpretations, whereas only 12 (20.7%) non *APOE* ε4 carriers received a discordant CSF interpretation ($p < .0001$). Although there were slightly less discordant cases for $A\beta_{42/40}$ than $A\beta_{42}$, there were still significantly more discordant CSF interpretations in *APOE* ε4 carriers (17 participants [40.5%]) than noncarriers (8 participants [12.9%]; $p = .0020$). We did not observe any differences in the CSF biomarker interpretation of tTau and p181Tau. tTau was discordantly interpreted in six (15.8%) and four (6.8%) cases in *APOE* ε4 and non *APOE* ε4 carriers, respectively ($p = .1762$). Similarly, two (5.4%) *APOE* ε4 carriers and two (3.4%) noncarriers received discordant p181Tau interpretations ($p = .6414$; Figure 1; Table 1).

In *APOE* ε4 carriers, the implementation of $A\beta_{42/40}$ led to a significantly reduced discordancy from 68.4% to 40.5% ($p = .0147$). Comparable discordant rates for $A\beta_{42}$ (20.7%) and $A\beta_{42/40}$ (12.9%) were observed in non *APOE* ε4 carriers ($p = .3285$).

To exclude patients with CSF Aβ concentrations slightly below or above the respective cutoff value, we widened the borderline cutoff zone to ±25%. For $A\beta_{42}$, 12 *APOE* ε4 carriers received discordant interpretations and 7 patients were interpreted concordantly. In *APOE* ε4 noncarriers, only 5 of the 31 participants received discordant biomarker interpretations ($p = .0004$; Figure 2 (a)). Noteworthy, this mismatch was not observed for $A\beta_{42/40}$. Regarding the latter Aβ peptide ratio, only 3 of the 21 *APOE* ε4 carriers and 1 of the 50 non *APOE* ε4 carriers showed discordant results ($p = .0746$; Figure 2(b)).

CSF Aβ biomarker concentrations were correlated between Center 1 and Center 2 for *APOE* ε4 carriers and noncarriers separately. Samples with Aβ concentrations above the upper limit of detection were excluded. $A\beta_{42}$ correlated with $r = .5695$ between Center 1 and Center 2 in *APOE* ε4 carriers, whereas the correlation in non *APOE* ε4 carriers was significantly higher $r = .7541$ ($z = -1.801$; $p = .036$; Figure 3(a) and 3(d)). The correlations between the two centers for *APOE* ε4 carriers and noncarriers were comparable for $A\beta_{42/40}$ and $A\beta_{40}$. $A\beta_{42/40}$ correlated with $r = .7886$ and $r = .7173$ in *APOE* ε4 carriers and non *APOE* ε4 carriers, respectively ($z = 0.858$; $p = .195$; Figure 3(b) and 3(e)). For $A\beta_{40}$, a correlation of $r = .8774$ and $r = .8980$ was calculated in *APOE* ε4 carriers noncarriers, respectively ($z = 0.523$; $p = .300$; Figure 3(c) and 3(f)).

Table 1. Analyzed CSF Biomarkers and Corresponding Patients' Data.

	CSF $A\beta_{42}$			CSF $A\beta_{42/40}$			CSF tTau			CSF pTau		
	APOE ε4 carrier	Non *APOE* ε4 carrier	*p*	*APOE* ε4 carrier	Non *APOE* ε4 carrier	*p*	*APOE* ε4 carrier	Non *APOE* ε4 carrier	*p*	*APOE* ε4 carrier	Non *APOE* ε4 carrier	*p*
Excluded cases												
Within borderline cutoff zone (±10%)	16	14		12	10		16	11		17	14	
Included cases												
Concordant (*n*) (%)	12 (31.6)[a]	46 (79.3)[b]		25 (59.5)[a]	54 (87.1)[b]		32 (84.2)	57 (93.4)		35 (94.6)	56 (96.6)	
Discordant (*n*) (%)	26 (68.4)[a]	12 (20.7)[b]	<.0001	17 (40.5)[a]	8 (12.9)[b]	.0020	6 (15.8)	4 (6.8)	.1761	2 (5.4)	2 (3.4)	.6414
Age (years)	68.4 ± 8.6	67.7 ± 9.6	.5693	69.8 ± 10.1	66.6 ± 10.2	.1159	68.2 ± 10.8	66.3 ± 10.0	.3782	70.7 ± 10.7	67.5 ± 10.0	.1536
Females	25	32	.3958	23	35	>.9999	22	33	.8357	20	30	.0366
Males	13	26		19	27		16	28		17	28	

Note. Concordant and discordant cases are shown as absolute numbers and percentage. Significance for age differences were analyzed using *t* test, concordance, and gender was analyzed using Fisher's exact test. APOE = apolipoprotein E; CSF = cerebrospinal fluid; $A\beta$ = amyloid-beta; $A\beta_{42}$ = $A\beta$ 1-42; $A\beta_{42/40}$ = $A\beta_{42}$ to $A\beta$ 1-40 ratio; tTau = total tau; p181Tau = phosphorylated 181tau.

[a] Fisher's exact test comparing concordance for $A\beta_{42}$ and $A\beta_{42/40}$ in *APOE* ε4 carriers with $p = .0147$ and non *APOE* ε4 carriers.

[b] $p = .3285$.

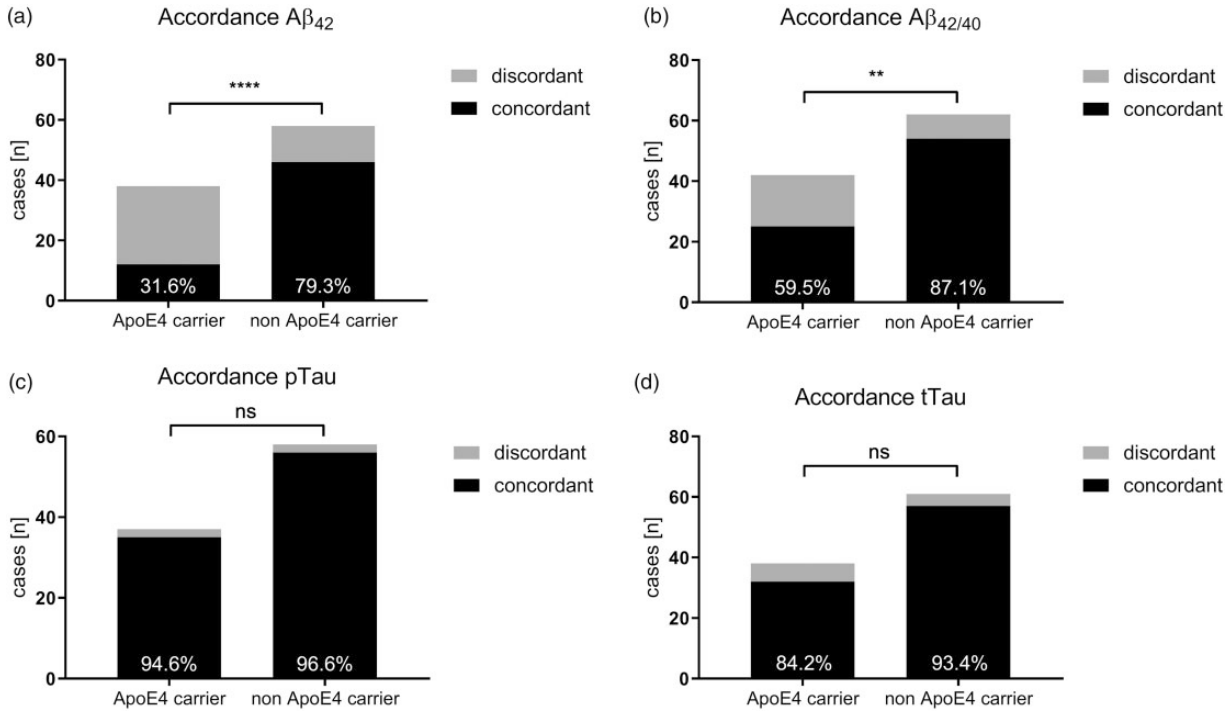

Figure 1. Presentation of concordant and discordant CSF biomarkers. $^{**}p < .01$. $^{****}p < .0001$. ns = not significant; APOE = apolipoprotein E; Aβ = amyloid-beta; $A\beta_{42}$ = Aβ 1-42; $A\beta_{42/40}$ = $A\beta_{42}$ to Aβ 1-40 ratio.

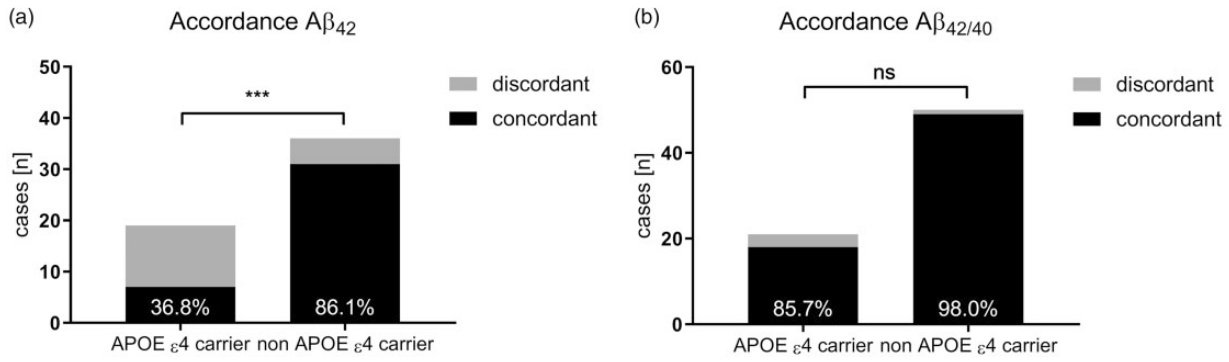

Figure 2. Accordance rate for $A\beta_{42}$ (a) and $A\beta_{42/40}$ (b) after application of a ± 25% borderline zone. $^{***}p < .001$. ns = not significant; APOE = apolipoprotein E. Aβ = amyloid-beta; $A\beta_{42}$ = Aβ 1-42; $A\beta_{42/40}$ = $A\beta_{42}$ to Aβ 1-40 ratio.

$A\beta_{42}$, and consequently $A\beta_{42/40}$, but not $A\beta_{40}$, tTau, or p181Tau CSF levels were lower in *APOE* ε4 carriers (Table 2). $A\beta_{42}$ CSF levels were 756.3 ± 295.3 pg/ml for *APOE* ε4 carriers and 1,087.0 ± 411.0 pg/ml for noncarriers at Center 1 ($p < .0001$) and 604.7 ± 324.9 pg/ml for *APOE* ε4 carriers and 860.0 ± 447.6 pg/ml for noncarriers at Center 2 ($p = .0006$). $A\beta_{42/40}$ CSF levels were 0.0721 ± 0.0363 for *APOE* ε4 carriers and 0.1021 ± 0.0396 for noncarriers at Center 1 ($p < .0001$) and 0.0473 ± 0.0189 for *APOE* ε4 carriers and 0.0658 ± 0.0176 for noncarriers at Center 2 ($p < .0001$). $A\beta_{40}$ CSF levels were 11,666 ± 3,747 pg/ml for *APOE* ε4 carriers and 11,586 ± 4,528 pg/ml for noncarriers at Center 1 ($p = .9160$) and 13,726 ± 6,099 pg/ml for *APOE* ε4 carriers and 13,338 ± 6,047 pg/ml for noncarriers at Center 2 ($p = .7231$; Table 2).

Discussion

In this study, we identified *APOE* ε4 as a major factor leading to different Aβ CSF biomarker interpretations in two independent laboratories. However, the *APOE* genotype did not affect $A\beta_{40}$, tTau, or p181Tau CSF levels.

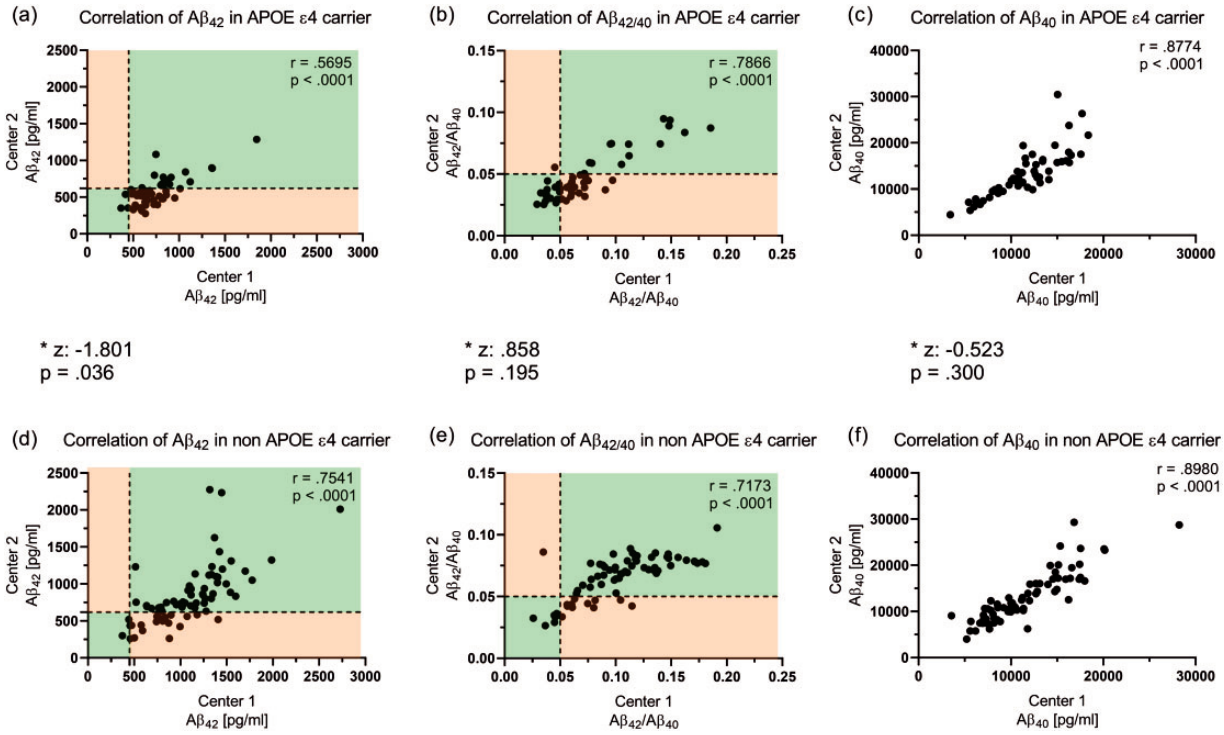

Figure 3. Correlation of Aβ_{42}, A$\beta_{42/40}$, and Aβ_{40} between the two centers for *APOE* ε4 carriers (a to c) and non *APOE* ε4 carriers (d to f). Cases with CSF concentrations above the detection limit were excluded; 53 *APOE* ε4 cases (a to c) and 71 (e and f) or 70 (e) non *APOE* ε4 cases were included. Correlation was significantly lower ($p = .036$) in *APOE* ε4 carriers for Aβ_{42} between the two centers (a and d), whereas similar correlations could be observed for A$\beta_{42/40}$ and Aβ_{40}. Concordant CSF biomarkers were defined as consistent CSF levels in both centers above or below the corresponding cutoff values. Areas with concordant cases are colored green, whereas areas with discordant cases are colored orange in Panels (a), (b), (d), and (e). *Comparison of correlation between *APOE* ε4 carriers and noncarriers for each biomarker after Fisher *r*-to-*z* transformation. APOE = apolipoprotein E; Aβ = amyloid-beta; Aβ_{42} = Aβ 1-42; A$\beta_{42/40}$ = Aβ_{42} to Aβ 1-40 ratio.

Table 2. CSF Biomarker Comparison Between *APOE* ε4 Carriers and Noncarriers for Each Center.

	CSF Aβ_{42}		CSF A$\beta_{42/40}$		CSF Aβ_{40}		CSF tTau		CSF pTau	
	Concentration, pg/ml (*SD*)	*p*	Concentration, pg/ml (*SD*)	*p*	Concentration, pg/ml (*SD*)	*p*	Concentration, pg/ml (*SD*)	*p*	Concentration, pg/ml (*SD*)	*p*
Center 1										
APOE ε4 carrier	756.3 (295.3)	<.0001	0.0721 (0.0363)	<.0001	11,666 (3,747)	.9160	503.6 (325.4)	.0521	71.69 (35.91)	.0452
Non *APOE* ε4 carrier	1087.0 (411.0)		0.1021 (0.0396)		11,586 (4,528)		392.3 (307.1)		58.68 (35.56)	
Center 2										
APOE ε4 carrier	604.7 (324.9)	.0006	0.0473 (0.0189)	<.0001	13,726 (6,099)	.7231	515.1 (370.3)	.0122	67.63 (35.22)	.0645
Non *APOE* ε4 carrier	860.0 (447.6)		0.0658 (0.0176)		13,338 (6,047)		364.0 (296.2)		56.04 (34.01)	

Note. *APOE* ε4 significantly reduces Aβ_{42} and subsequently A$\beta_{42/40}$ but not Aβ_{40}, tTau, or pTau. Due to multiple comparison, *p* values should be considered as $\alpha < .005$. APOE = apolipoprotein E; CSF = cerebrospinal fluid; Aβ = amyloid-beta; Aβ_{42} = Aβ 1-42; A$\beta_{42/40}$ = Aβ_{42} to Aβ 1-40 ratio; *SD* = standard deviation; tTau = total tau; p181Tau = phosphorylated 181tau.

The ELISAs used for tTau (Fujirebio), p181Tau (Fujirebio), and Aβ_{40} (IBL) were identical in both centers, whereas the ELISAs used for Aβ_{42} were different (IBL and Fujirebio). This could be one reason for a higher discordance in Aβ_{42} compared with A$\beta_{40,}$ tTau, and p181Tau. However, this study reflects the real life in clinical dementia diagnostics, where physicians have limited impact on the used assays but need to rely on the best laboratory praxis in the corresponding centers. Even though it is not unexpected that different immunoassay show less concordance than identical immunoassays, the impact of *APOE* ε4 on the accordance level is surprising. This effect was consistent even after excluding more some samples with CSF biomarkers close to the

corresponding cutoff. Despite a substantially broader borderline cutoff zone (±25%), we still observe a significant discordant $A\beta_{42}$ interpretation in *APOE* ε4 carriers compared with *APOE* ε4 noncarriers. This finding indicates that a molecular interaction of *APOE* ε4 with $A\beta_{42}$ or one of the two ELISAs significantly contributes to the observed interlaboratory mismatch for the measurement of $A\beta_{42}$ in CSF. Accordingly, our observation is unlikely explained only by interlaboratory differences in cutoff values.

A further comparison between both immunoassays could improve the diagnostic accuracy in clinical laboratories; however, it is not trivial to determine the exact Aβ levels in CSF and determine whether one immunoassay is superior to the other one.

Different functions of *APOE* have been described within the pathologic pathway of AD (reviewed in Bertram and Tanzi, 2008). Besides assisting the transportation of Aβ through the blood brain barrier, there is strong evidence that *APOE* interacts with Aβ peptides (Naslund et al., 1995; Tiraboschi et al., 2004) and promotes conformational changes into β sheets (Wisniewski and Frangione, 1992). As described by Strittmatter et al., *APOE* in general (Strittmatter et al., 1993a), but *APOE* ε4 even faster than *APOE* ε3 (Strittmatter et al., 1993b), binds to Aβ peptides. This could affect the CSF biomarker measurements using enzyme-based immunoassays. We observed a reduced correlation between the ELISAs by IBL and Fujirebio in *APOE* ε4 carriers compared with noncarriers, supporting the hypothesis that *APOE* ε4 interacts with $A\beta_{42}$ or one of the corresponding ELISAs.

Different studies have compared blood and CSF *APOE* levels. Wahrle et al. (2007) reported an age-dependent effect on general *APOE* levels in CSF, whereas the dementia stage (as measured by the clinical dementia rating), the *APOE* genotype, gender, and race did not affect CSF *APOE* levels. Interestingly, CSF but not plasma *APOE* levels showed an *APOE genotype* independent association with $A\beta_{42}$ concentrations in CSF (Cruchaga et al., 2012).

According to the study by Mayeux et al. (1998), without additional CSF or positron emission tomography (PET) biomarkers, *APOE* has a sensitivity of 65% and specificity of 68% for the detection of AD. Due to the limited diagnostic significance, national and international guidelines do not include *APOE* genotyping in the clinical diagnostics (Deuschl and Maier, 2016). However, with the knowledge of a significant interference of *APOE* and Aβ biomarker in the CSF, the determination of patients *APOE* genotype should be considered more important.

This study does not intend to explain any causal relation between *APOE* and Aβ but aims to call attention to a critical interpretation of Aβ CSF biomarkers in routine patient care and research. As CSF biomarkers are gaining importance in the etiological diagnosis of dementia, misinterpreted biomarkers have a significant impact on the clinical and therapeutic procedure (e.g., medication). Thus, false-negative CSF biomarker results could lead to insufficient treatment of AD patients.

Novel data suggest higher reproducibility CSF biomarkers using fully automated analyzers (Hansson et al., 2018). However, further validation studies are needed to support the superiority of fully automated analyzers compared with classical ELISAs.

The determination of the *APOE* genotype could be a diagnostic benefit not only as a risk factor for AD but also as an interfering factor for CSF Aβ biomarker measurements, which should be handled and interpreted carefully, in particular in *APOE* ε4 positive patients. Due to the lacking gold standard (post mortem analysis), it is difficult to predict the superiority of one ELISA in this study. However, CSF Aβ concentration slightly above or below the corresponding cutoff value should be questioned even more in *APOE* ε4 positive patients. We recommend additional diagnostic procedures, for example, amyloid-PET if CSF biomarkers and clinical or neuropsychological examinations are conflicting. Moreover, this study strengthens the diagnostic use of $A\beta_{42/40}$ to reduce insecure CSF biomarker interpretations.

Different immunoassays and cutoff points can render discordance between different laboratories. *APOE* ε4 should be taken into account when applying round robin studies to harmonize cutoff values between different centers.

Strengths and Limitations of the Study

The major limitation of this study is the missing of harmonized SOPs, different infrastructures, and immunoassays. However, we did not address these aspects to outline the practical difficulties in the real-life clinical usage of biomarkers. The strength of this study is the naturalistic character of this analysis.

Summary

CSF biomarker misinterpretations are a widely known problem in clinical practice. This study shows that, besides different immunoassays and cutoff points, the *APOE* status contributes significantly to discordant CSF Aβ biomarkers. *APOE* ε4 increases the risk of misinterpreting CSF $A\beta_{42}$.

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Authors' Contributions

J. V. and J. W. designed the study, analyzed the data, and wrote the manuscript. D. W. and R. V. supported with the interpretation, read and approved the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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