Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring 6 (2017) 182-187

Cerebrospinal Fluid Biomarkers

Apolipoproteins and their subspecies in human cerebrospinal fluid and plasma

Manja Koch[a,*], Jeremy D. Furtado[a], Kim Falk[b,c], Frank Leypoldt[b,c], Kenneth J. Mukamal[a,d], Majken K. Jensen[a,e]

[a]*Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA*
[b]*Neuroimmunology Unit, Christian-Albrechts-University Kiel, Kiel, Germany*
[c]*Department of Neurology, Institute of Clinical Chemistry, University Hospital Schleswig-Holstein, Kiel, Germany*
[d]*Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA, USA*
[e]*Department of Medicine, Channing Division of Network Medicine, Harvard Medical School, Brigham and Women's Hospital, Boston, MA, USA*

Abstract

Introduction: Subspecies of apolipoproteins can be defined by fractionating apolipoproteins based on the presence and absence of coexisting apolipoproteins.

Methods: We determined age- and sex-adjusted correlations of enzyme-linked immunosorbent assay–measured plasma and cerebrospinal fluid (CSF) apolipoproteins (apoA-I, apoC-III, apoE, and apoJ) or apolipoprotein subspecies (apoA-I with and without apoC-III, ApoE, or apoJ; apoE with and without apoC-III or apoJ) in 22 dementia-free participants.

Results: CSF apoE did not correlate with plasma apolipoproteins or their subspecies. CSF apoJ correlated most strongly with plasma apoA-I without apoJ ($r = 0.7$). CSF apoA-I correlated similarly strong with plasma total apoA-I and all apoA-I subspecies ($r \geq 0.4$) except for apoA-I with apoE ($r = 0.3$) or apoA-I with apoJ ($r = 0.3$). CSF apoC-III was most strongly correlated with plasma apoA-I with apoC-III ($r = 0.7$).

Discussion: CSF levels of some apolipoproteins implicated in the pathophysiology of dementia might be better approximated by specific plasma apolipoprotein subspecies than total plasma concentrations.

Keywords: Apolipoproteins; Brain; Lipoproteins; Apolipoprotein subspecies; Cerebrospinal fluid

1. Introduction

Evidence is accumulating that apolipoproteins, found in both plasma and cerebrospinal fluid (CSF), are involved in the pathophysiology of Alzheimer's disease [1]. Genetic variants of the apolipoprotein E (*APOE*) and J (clusterin, *CLU*) genes are known risk factors for Alzheimer's disease [2]. Apolipoprotein (apo) E and J have been suggested to play an important role in the deposition and clearance of neurotoxic β-amyloid [3–5]. ApoE also protects the microtubule-associated protein tau from hyperphosphorylation [6]. ApoC-III was discovered to be a β-amyloid binding protein [7] and lower plasma apoC-III levels have been related to higher Alzheimer's disease prevalence in the Alzheimer's Disease Neuroimaging Initiative [8].

CSF biomarkers are sometimes used in clinical practice to support the diagnosis of AD [9,10]. However, the identification of markers that may reflect increased risk of future disease is a major mission in the field of Alzheimer's research [11]. For purposes of such large-scale studies and use in future clinical screenings, minimally invasive measures such as those obtained from plasma

This work has been presented as a poster at the Alzheimer's Association International Conference in Toronto, Canada, July 2016.

*Corresponding author. Tel.: +1-617-432-1841; Fax: +1-617-432-2435.

E-mail address: mkoch@hsph.harvard.edu

http://dx.doi.org/10.1016/j.dadm.2017.01.007

samples are highly desirable. Plasma apolipoproteins are primarily synthesized by the liver and intestine [12]. Because of differences in local synthesis and transport across the blood-brain barrier, it is important to understand the relationships of plasma and CSF levels of specific apolipoprotein subspecies to gain insight into which plasma apolipoproteins reflect CSF levels and which apolipoproteins are best measured in CSF.

2. Methods

This cross-sectional analysis was performed in nine men and 13 women (age 20–76 years, mean age 40 years), treated at the emergency department at the University Hospital Schleswig-Holstein, Campus Kiel, Germany between 2009 and 2015 for acute headache. Patients with a history of dementia, systemic or CSF inflammatory signs, or blood-brain barrier dysfunction (CSF-to-serum albumin ratios $\geq 9 \times 10^3$) were excluded. Diagnoses were migraine and headache ($n = 8$), common cold or sinusitis ($n = 7$), skin sensation disturbance ($n = 4$), syringomyelia ($n = 1$), mild cognitive impairment ($n = 1$), and suspected pseudotumor cerebri ($n = 1$).

Informed consent for scientific analysis of diagnostic remnant samples collected for clinical care was obtained at the time of specimen collection. Information on age, sex, and diagnosis was collected from anonymized medical reports.

Plasma samples and CSF samples were obtained by venipuncture and lumbar puncture, respectively. In paired CSF and serum samples, concentrations of albumin were measured immediately by particle-enhanced immunologic turbidimetry (Roche Cobas, Switzerland). Remnant samples were stored at 4°C for up to 2 weeks after collection and stored at −80°C afterward. Samples were shipped on dry ice to the lipid laboratory of the Harvard Chan School of Public Health for apolipoprotein measurements.

Concentrations of apoC-III and apoJ were measured by sandwich enzyme-linked immunosorbent assay (ELISA). For apoA-I and apoE with and without specific apolipoproteins, a patented modified sandwich ELISA approach was used. The 96-well plates were coated with antibody to the apolipoprotein by which we desired to fractionate (apoC-III, apoE, or apoJ; Academy Biomedical Company Inc, Houston TX and R&D Systems, Minneapolis, MN). Diluted samples were incubated on these plates to bind lipoproteins containing that apolipoprotein. The unbound fraction was removed and apoA-I or apoE in this fraction was measured by sandwich ELISA on a second plate coated with anti-apoA-I or anti-apoE antibody. The fraction bound to the first plate was released by dissociation of the lipoprotein complex and transferred to a third plate coated with anti–apoA-I or anti-apoE antibody to quantify the concentration of apoA-I or apoE with the apoC-III, apoE, or apoJ. Both CSF and plasma samples from an individual were assayed together on the same 96-well plate for each measurement and the coefficients of variation (CVs) were quite comparable. For instance, the average (standard deviation, SD) CV% for apoE in plasma was 4% (3%), whereas that for CSF was 5% (3%). Overall our CVs were less than 20% except for apoA-I without apoC-III in plasma (average CV 26%, SD 19%) and apoA-I with apoJ in plasma (average CV 20%, SD 14%) and in CSF (average CV 27%, SD 22%).

We examined the effect of length of time between thawing and start of assay protocol, comparing no delay, 2, 6, and 24 hours in the context of room temperature delay storage, and refrigerated (4°C) delay storage. In the room temperature storage samples, we observed a trend toward increasing concentrations of apoA-I with apoC-III with increasing delay time, along with increasing CV% for replicate measures. These effects are not found with storage at 4°C. We also examined the effect of freeze/thaw cycles comparing freshly drawn samples to aliquots frozen and assayed after one, three, and five freeze/thaw cycles. There was no effect of up to five freeze/thaw cycles on the measured concentration or CV% of the samples assayed. Samples are not affected by the act of freezing and thawing up to five times provided that they are kept chilled when in a thawed state. Partial correlation analysis was performed controlling for age and sex.

In sensitivity analysis, we determined unadjusted Pearson correlation coefficients of logarithmically transformed apolipoproteins and their subspecies. The CSF-to-serum albumin ratio correlates with the CSF-to-serum ratio of other blood-derived proteins, which also enter the brain by diffusion [13]. The CSF-to-serum albumin ratio does not correlate with the CSF-to-serum ratio of proteins with intrathecal synthesis or proteins actively transferred across the blood-CSF barrier [13,14]. Analyses were performed using SAS software, Version 9.4 (SAS Institute Inc, Cary, NC).

3. Results

ApoE and apoA-I were the quantitatively major apolipoproteins in both CSF and plasma (Table 1). Most of the apoE did not carry apoC-III or apoJ, and most of the apoA-I did not carry apoC-III, apoE, or apoJ.

CSF concentrations were approximately 10 times smaller than the plasma concentrations. Median concentrations in CSF relative to plasma concentrations were between 0.01% for apoC-III and 11.57% for apoE without apoC-III.

CSF and plasma apolipoprotein concentrations in paired samples correlated moderately with each other (all $r \geq 0.4$) except for apoE ($r = -0.01$, $P = .95$).

CSF-to-serum albumin ratios (median 4.5; interquartile range: $3.7, 5.5 \times 10^3$), a marker of blood-brain barrier integrity, correlated with the CSF-to-plasma ratio of apolipoproteins and their subspecies ($r > 0.4$) except for apoE ($r = -0.03$, $P = .89$; Table 1). Correlations were higher for apoA-I with the apoC-III ($r = 0.63$, $P \leq .01$) than total apoC-III ($r = 0.42$, $P = .06$).