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Increased Cerebrospinal Fluid F₂-Isoprostanes are Associated with Aging and Latent Alzheimer's Disease as Identified by Biomarkers

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Abstract

Alzheimer's disease (AD) is a common age-related chronic illness with latent, prodrome, and fully symptomatic dementia stages. Increased free radical injury to regions of brain is one feature of prodrome and dementia stages of AD; however, it also is associated with advancing age. This raises the possibility that age-related free radical injury to brain might be caused in part or in full by latent AD. We quantified free radical injury in the central nervous system with cerebrospinal fluid (CSF) F₂-isoprostanes (IsoPs) in 421 clinically normal individuals and observed a significant increase over the adult human lifespan ($P < 0.001$). Using CSF amyloid (A) β_{42} and tau, we defined normality using results from 28 clinically normal individuals < 50 years old, and then stratified 74 clinically normal subjects ≥ 60 years into those with CSF that had normal CSF A β_{42} and tau ($n=37$); abnormal CSF A β_{42} and tau, the biomarker signature of AD ($n=24$); decreased A β_{42} only ($n=4$); or increased tau only ($n=9$). Increased CSF F₂-IsoPs were present in clinically normal subjects with the biomarker signature of AD ($P < 0.05$) and those subjects with increased CSF tau ($P < 0.001$). Finally, we analyzed the relationship between age and CSF F₂-IsoPs for those clinically normal adults with normal CSF ($n=37$) and those with abnormal CSF A β_{42} and/or tau ($n=37$); only those with normal CSF demonstrated a significant increase with age ($P < 0.01$). These results show that CSF F₂-IsoPs increased across the human lifespan and that this age-related increase in free radical injury to brain persisted after culling those with laboratory evidence of latent AD.

Keywords

Alzheimer's disease; cerebrospinal fluid; biomarkers; A β_{42} ; tau; F₂-isoprostanes

INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disease and demonstrates exponential increase in prevalence with advancing age beyond 60 years. Autopsy, neuroimaging, and biomarker studies all consistently support the proposal that AD is a chronic illness that begins years if not decades prior to onset of clinically detectable cognitive impairment (Table 1) (Sonnen et al. 2008b). Thus, like all chronic diseases AD can be conceptualized in three stages: *latency* when disease has started but is asymptomatic, *prodrome* when disease has progressed and very mild clinical signs and symptoms are present, and *clinical* when disease has advanced still further and now the full clinical spectrum is expressed. The prodromal stage of AD most commonly is operationally defined by amnesic Mild Cognitive Impairment (MCI) (Petersen and Negash 2008). The stage of full clinical expression of AD is the most common contributor to the dementia syndrome while vascular brain injury (VBI), especially from small vessel disease or micro VBI (μ VBI), is the second most common chronic disease to contribute to dementia, often in combination with AD (Sonnen et al. 2007).

It is critical to realize that latent disease cannot be distinguished from absence of disease by clinical examination or neuropsychological testing, but rather requires some ensemble of laboratory-based methods to detect disease initiation in the absence of symptoms. In the past, autopsy-based comparisons of carefully documented asymptomatic individuals were the only means to investigate latent disease vs. no disease, and Dr. Markesbery was a world leader in this important area of research (Schmitt et al. 2000; Petrovitch et al. 2005; Tyas et al. 2007; Iacono et al. 2009; Markesbery et al. 2009; Price et al. 2009). However, there are clear limitations to this approach — most importantly the focus on older individuals, and the asynchrony between last clinical evaluation and autopsy. More recently, the advent of neuroimaging and biomarker technologies have provided tools that appear to be helpful in distinguishing latent disease from absence of disease in the clinical research setting (Sonnen et al. 2008b); these laboratory tools currently are more advanced for AD than μ VBI. Indeed, using decreased CSF A β ₄₂ and increased CSF tau concentrations, we and others have confirmed that an abnormal CSF tau/A β ₄₂ ratio indicates increased risk of subsequently developing MCI or AD-type dementia, strongly indicating that these two proteins may have utility as biomarkers of latent AD (Fagan et al. 2007; Li et al. 2007). Decreased soluble CSF A β ₄₂ is correlated with accumulation of fibrillar A β peptides in regions of cerebrum as insoluble aggregates (Fagan et al. 2006), including senile plaques, which arise during the latent stage of AD (Schmitt et al. 2000; Petrovitch et al. 2005; Tyas et al. 2007; Iacono et al. 2009; Price et al. 2009). It is important to stress that elevated CSF tau is not specific to AD but rather appears to derive from neuronal damage from a variety of etiologies, including VBI (Sonnen et al. 2008b).

We and others have pursued increased free radical injury as a mechanism by which AD damages neurons and perhaps other tissue elements in diseased regions of cerebrum, and have validated repeatedly the seminal observations by Dr. Markesbery and colleagues. Free radical-mediated damage to brain in AD and its transgenic mouse models (Smith et al. 1991; Sonnen et al. 2008a). Quantification of F₂-Isoprostanes (IsoPs) by a stable isotope dilution technique with gas chromatography/mass spectrometry and selective ion monitoring is a robust quantitative *in vivo* biomarker of free radical injury to tissue under a variety of biological stressors or diseases (Roberts and Morrow 2000). We adapted this methodology to investigate CSF and showed that dementia stage AD is associated with significantly increased CSF F₂-IsoPs (Montine et al. 1998); however, it is important to note that all of our studies observed approximately one-third overlap in CSF F₂-IsoPs between patients with dementia stage AD and controls with the remainder of AD patients skewing to higher CSF F₂-IsoP concentrations (Montine et al. 2007). We also demonstrated that CSF F₂-IsoPs may

be used in conjunction with CSF A β ₄₂ and tau to increase the accuracy of laboratory diagnosis of AD (Montine et al. 2001). Several groups validated our findings, and we and others expanded these observations to include individuals with MCI or some individuals with Clinical Dementia Rating 0.5 (Quinn et al. 2004), again showing overlap with controls, at least in our studies. Several publications report an increase in plasma or urine F₂-IsoPs in patients with dementia stage AD or MCI (Pratico et al. 2000; Tuppo et al. 2001; Pratico et al. 2002); however, these results are not reproducible (Montine et al. 2002; Irizarry et al. 2007; Mufson and Leurgans).

Here we undertook the first *in vivo* investigation of quantitative biomarkers of free radical injury in latent AD.

METHODS

Subjects and CSF samples

Human Subject Divisions at Oregon Health & Science University, the University of California at San Diego, and the University of Washington approved this study. Subjects were compensated community volunteers in good health with no signs or symptoms suggesting cognitive decline or neurologic disease. Exclusion criteria included heavy cigarette smoking (more than 10 packs/year), alcohol use other than social, and any psychotherapeutic use. Following informed consent, all individuals underwent evaluation that consisted of family and medical histories, physical and neurologic examinations, laboratory tests, and neuropsychological assessment. Laboratory evaluations included complete blood count, serum electrolytes, blood urea nitrogen, creatinine, glucose, vitamin B12, and thyroid stimulating hormone; all results were within normal limits for samples included in this study. The neuropsychological battery consisted of a global cognitive measure, the Mini-Mental State Examination (MMSE) (Folstein et al. 1975), memory tests including immediate and delayed (Wechsler 1987; Craft et al. 1996), and parts A and B of the Trail Making Test as a measure of attention (Reitan and Wolfson 1986). All subjects scored in the normal range for all tests.

CSF was obtained by lumbar puncture at all institutions by the same method (Peskind et al. 2005). Briefly, following written informed consent, individuals were placed in the lateral decubitus position and the L4-5 interspace was infiltrated with 1% lidocaine. Lumbar puncture was performed with a 20g or 24g spinal needle following which individuals remained at bed rest for one hour. All lumbar punctures performed in the morning to limit potential circadian fluctuation.

CSF Assays

All CSF for analysis was taken from the 15th to 25th ml collected, was stored in 0.5 ml aliquots at -80°C , and was never thawed prior to this study. CSF was analyzed for A β ₄₂ and total tau using multiplexed Luminex reagents from InnoGenetics (Alpharetta, GA), according to manufacturer's instructions and as previously described by us (Montine et al.). CSF F₂-IsoPs were quantified using a stable isotope dilution assay with gas chromatography/mass spectrometry and selective ion monitoring as described previously (Milatovic et al. 2005).

Statistical analysis was performed using GraphPad Prism (San Diego, CA).

RESULTS

We determined CSF F₂-IsoP concentrations from 412 volunteers who were clinically normal and who performed within normal ranges on a battery of neuropsychological tests (Figure

1). These 412 clinically normal volunteers ranged in age from 21 to 89 years old (mean $\pm$ SD = 59 ± 17 years) and had average CSF F₂-IsoPs concentration of 29.2 ± 8.4 pg/ml. Despite substantial variance across the adult human lifespan, we observed a statistically significant ($P < 0.001$) age-related increase in CSF F₂-IsoPs that could be reflected in a best-fit line with a positive slope of 0.1 pg F₂-IsoPs/ml CSF/yr and intercept of 24.3 ± 0.1 pg F₂-IsoPs/ml. It is interesting to note that the CSF F₂-IsoPs concentration in this clinically normal cohort was on average 16% greater than our previous clinically normal cohorts that had average CSF F₂-IsoPs = 25 pg/ml (Montine et al. 2007), a difference that may be related to variation in inclusion or exclusion criteria. In summary, in this largest group of clinically normal individuals published to date, our results show a positive relationship between advancing adult age and increasing CSF F₂-IsoPs but with substantial variance in CSF F₂-IsoPs across all ages.

We sought to determine the extent to which this age-related increase in CSF F₂-IsoPs was driven by latent AD by using CSF A β_{42} and tau as biomarkers. Of the 412 clinically normal individuals described above, we selected 102 subjects that also had CSF A β_{42} and tau concentrations quantified. Of these, 28 individuals were < 50 years old. Similar to our previous studies, we assumed these 28 clinically normal younger individuals to be very unlikely to harbor latent AD, and used their laboratory results to define normal cutoff values of A β_{42} , tau, and the tau/A β_{42} ratio (Table 2) (Li et al. 2007). Since A β_{42} concentrations decrease in AD, the normal cutoff for A β_{42} was the mean for these younger controls minus 2 SD. Similarly, since tau and tau/A β_{42} ratio increase in AD, normal cutoff for these were the mean of younger controls plus 2 SD. Our normal cutoff values compare well with those recently reported by the AD Neuroimaging Initiative.

We next applied these normal cutoff values to all subjects who were 60 years or older ($n=74$). We stratified our clinically normal older subjects into four groups based upon CSF A β_{42} , tau, and tau/A β_{42} results: (i) *Normal CSF* who had normal A β_{42} and tau concentrations and a normal tau/A β_{42} ratio ($n=37$), (ii) *Abnormal CSF A β_{42} & Tau* who had low A β_{42} and high Tau concentrations ($n=12$), or a high tau/A β_{42} ratio even though concentrations of both analytes were in normal ranges ($n=12$), (iii) *Low CSF A β_{42} Only* who had low A β_{42} and normal tau concentrations ($n=4$), or (iv) *High CSF Tau Only* who had normal A β_{42} and high tau concentrations ($n=9$) (Figure 2). This distribution showed that 50% ($n=37$) of clinically normal adults ≥ 60 years of age had no evidence of latent disease as determined by CSF A β_{42} and tau measurements (blue). Thirty-eight percent of clinically normal controls ≥ 60 years-old had laboratory evidence of latent AD as defined in the Abnormal A β_{42} & Tau (red) or low CSF A β_{42} Only (green) groups. Finally, 12% of clinically normal older individuals had abnormally high CSF tau with normal CSF A β_{42} concentration (purple). Given the lack of specificity of CSF tau, it is not clear if members of this last group have latent AD, latent VBI, or some other latent disease of brain.

We then compared CSF F₂-IsoPs among these four groups of clinically normal individuals who were defined by CSF A β_{42} and tau concentrations (Figure 3). Of note, the *Normal CSF* group ($n=37$) had CSF F₂-IsoPs that averaged ($\pm$ SD) 25.6 ± 0.7 pg/ml, identical to our previously reported value for normal CSF F₂-IsoPs in older adults. Analysis of variance for CSF F₂-IsoPs among these four groups had $P < 0.001$ and Bonferroni's corrected multiple comparison test had $P < 0.05$ for *Normal CSF* vs. *Abnormal A β_{42} & Tau*, and $P < 0.001$ for *Normal CSF* vs. *High CSF Tau Only*. Although the number of samples is small, especially in the *Low CSF A β_{42} Only* group, these data indicate that CSF F₂-IsoPs are increased in clinically normal individuals who show either absolute or relatively low CSF A β_{42} and high tau concentrations, and are even further increased in those who display only high CSF tau. Like the dementia stage of AD, these data are consistent with the interpretation that CSF F₂-

IsoPs are increased in some individuals with latent AD, a finding that will require validation in longitudinal studies and with different methods.

We returned to our assessment of age-related increase in CSF F₂-IsoPs but this time stratified by presence or absence of A β ₄₂ and/or tau abnormalities. We divided the individuals shown in Figure 2 into two groups: the *Normal CSF* group (n=37; blue) and all other individuals with abnormalities of CSF A β ₄₂ and/or tau concentrations (n = 37; red, green, purple) and performed linear regression for age vs. CSF F₂-IsoPs in each group. Clinically normal individuals 60 years or older with normal CSF A β ₄₂ and tau had a significantly positive slope for CSF F₂-IsoPs vs. age (Figure 4). In contrast, clinically normal individuals with abnormal CSF A β ₄₂ and/or tau concentrations or an {Montine, 1999 #35} abnormal CSF tau/A β ₄₂ ratio did not have a significant correlation between age and CSF F₂-IsoPs. We interpret these data as evidence that advancing age is associated with progressive free radical injury to brain even in the absence of disease(s) that cause abnormalities in CSF A β ₄₂ and/or tau. In contrast, disease(s) that cause abnormalities in CSF A β ₄₂ or tau concentrations or ratio are associated with increased free radical injury independent of age.

DISCUSSION

Free radical injury to diseased regions of cerebrum has been identified in autopsy samples from patients who died with AD or MCI (Smith et al. 1991; Lovell and Markesbery 2007), and, at least for measurements like F₂-IsoPs and related isoprostanoids, these assessments are not confounded by agonal events (Sonnen et al. 2009). Although important to our understanding of disease mechanism, autopsy-based studies are limited in assessing changes that occur early in the course of disease and cannot be used in longitudinal studies of the natural history of disease or response to therapeutics. It is for these reasons that in collaboration with Dr. Markesbery we developed CSF F₂-IsoPs as a means to quantify free radical injury to brain *intra vitam* (Montine et al. 1998; Montine et al. 1999). Several groups around the world have adopted this approach using different assays (Sonnen et al. 2008a). All have verified that the dementia stage of AD is associated with statistically significantly increased CSF F₂-IsoPs, although there is substantial overlap with controls in most studies. Some have reported similar findings in CSF from patients with very mild dementia (CDR 0.5) or individuals with MCI. For the first time, here we extend these investigations to latent stage AD (Montine et al. 2005).

There is as yet no widely accepted means of detecting latent AD, although two promising research tools are neuroimaging for fibrillar A β peptides and quantification of CSF A β ₄₂ and tau (Fagan et al. 2007; Li et al. 2007; Rentz et al.). We wish to stress that although we caricature chronic disease progression as an orderly transition from latent to prodrome to full expression, there is no requirement that this be so; indeed, medicine is replete with examples of patients with chronic disease that do not follow an orderly progression and others that progress only partially. Undoubtedly, the same will be true for AD and as we develop the tools necessary to probe disease mechanisms *in vivo*, we almost certainly will uncover some latent disease that does and some that does not progress to clinical expression. Clarity for these important points will come from longitudinal studies. Thus, although currently limited, here we used CSF A β ₄₂ and tau concentrations to define latent disease that at least indicates increased risk for subsequent MCI or AD.

Our results showed that 50% of individuals 60 years or older had normal CSF A β ₄₂ and tau, 33% had abnormal CSF A β ₄₂ and tau, 5% had low CSF A β ₄₂ only, and 12% had high CSF tau only. CSF F₂-IsoPs concentration among those with normal CSF A β ₄₂ and tau were identical to control values reported by us in several previous publications, and very closely

approximated the y-intercept for CSF F₂-IsoPs vs. age across the human lifespan. This prevalence for abnormal CSF A β ₄₂ and tau approximated the proportion of individuals in this age range that would be expected ultimately to develop MCI or dementia from AD, and is remarkably consistent with PET imaging for fibrillar A β peptide accumulation in brain among cognitively normal older adults (Buckner et al. 2005; Mintun et al. 2006; Aizenstein et al. 2008). Consonant with many other studies of animal models and human tissue from patients with MCI or dementia from AD (Sonnen et al. 2008a), we observed significantly increased CSF F₂-IsoPs in those clinically normal individuals $\geq$ 60 years-old who had laboratory evidence of latent AD: abnormal CSF A β ₄₂ and tau. As in our earlier studies of symptomatic subjects, there was considerable overlap in CSF F₂-IsoP concentrations between those with normal and abnormal CSF A β ₄₂ and tau concentrations. These results suggest that, like prodrome and dementia stages of AD, increased free radical injury is a feature of some individuals with latent AD.

Only 5% of clinically normal individuals $\geq$ 60 years-old had low CSF A β ₄₂ and normal CSF tau concentrations. This is an interesting group that may be interpreted by some as an even earlier stage of latent AD than those with abnormal A β ₄₂ and tau, although this is speculative. A larger group, 12% of our clinically normal volunteers $\geq$ 60 years-old, had increased CSF tau and normal CSF A β ₄₂ concentrations. Increased CSF tau is not specific to AD but is characteristic of other diseases of brain, including VBI (Hesse et al. 2001). Large or territorial ischemic strokes, a type of VBI, are known to increase free radical injury to brain, and to increase CSF tau and F₂-IsoP concentrations (Montine et al. 2005). Members of our study were clinically normal and so are very unlikely to have unrecognized territorial infarcts of brain. However, μ VBI commonly is clinically silent and, based on autopsy studies, would be expected to be present in about 10 to 15% of those over 65 years of age. Moreover, we have shown that μ VBI is associated with increased free radical injury to human cerebrum (Sonnen et al. 2009). Unknown is whether free radical injury from μ VBI is reflected in increased CSF F₂-IsoPs. Our results raise this possibility. If true, then we need at least to reconsider the interpretation of increased CSF F₂-IsoPs in patients with MCI or dementia stage of AD. Free radical injury can derive from AD but perhaps may also derive, at least in part, from co-morbid μ VBI.

Our results with CSF F₂-IsoPs in clinically normal adults demonstrated that, like prodrome and dementia stages of AD, latent AD is associated with statistically significantly increased CSF F₂-IsoPs. In further analogy to symptomatic stages of AD, there was substantial overlap between clinically normal subjects with and without laboratory evidence of latent AD. This consistent pattern of overlap with controls across all stages of AD has potentially profound implications for our understanding of disease mechanism, the interpretation of previous clinical trials, and the organization of future trials. If only a subset of individuals with AD have increased free radical injury, then this raises the possibility that the mechanism(s) by which neurons are damaged may differ among patients that meet criteria for AD and implicates the need for tailored therapeutic approaches. In this specific instance, if only a subset of individuals with AD have increased free radical injury, then clinical trials of anti-oxidant interventions in subjects with AD “not otherwise specified” may have been destined to fail since about one-third of subjects would not need suppression of free radical mechanisms. Going forward it would seem prudent to use tools like CSF F₂-IsoPs to demonstrate first pharmacologic efficacy of any proposed anti-oxidant intervention, and then select for a therapeutic trial those subjects that show evidence of ongoing increased free radical injury to brain. Our results suggest that this strategy should be employed at all stages of AD.

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References

- Aizenstein HJ, Nebes RD, Saxton JA, Price JC, Mathis CA, Tsopelas ND, Ziolkowski SK, James JA, Snitz BE, Houck PR, Bi W, Cohen AD, Lopresti BJ, DeKosky ST, Halligan EM, Klunk WE. Frequent amyloid deposition without significant cognitive impairment among the elderly. *Arch Neurol* 2008;65:1509–17. [PubMed: 19001171]
- Buckner RL, Snyder AZ, Shannon BJ, LaRossa G, Sachs R, Fotenos AF, Sheline YI, Klunk WE, Mathis CA, Morris JC, Mintun MA. Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. *J Neurosci* 2005;25:7709–17. [PubMed: 16120771]
- Craft S, Newcomer J, Kanne S, Dagogo-Jack S, Cryer P, Sheline Y, Luby J, Dagogo-Jack A, Alderson A. Memory improvement following induced hyperinsulinemia in Alzheimer's disease. *Neurobiol Aging* 1996;17:123–30. [PubMed: 8786794]
- Fagan AM, Mintun MA, Mach RH, Lee SY, Dence CS, Shah AR, LaRossa GN, Spinner ML, Klunk WE, Mathis CA, DeKosky ST, Morris JC, Holtzman DM. Inverse relation between in vivo amyloid imaging load and cerebrospinal fluid Aβ42 in humans. *Ann Neurol* 2006;59:512–9. [PubMed: 16372280]
- Fagan AM, Roe CM, Xiong C, Mintun MA, Morris JC, Holtzman DM. Cerebrospinal fluid tau/β42-amyloid(42) ratio as a prediction of cognitive decline in nondemented older adults. *Arch Neurol* 2007;64:343–9. [PubMed: 17210801]
- Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975;12:189–98. [PubMed: 1202204]
- Hesse C, Rosengren L, Andreasen N, Davidsson P, Vanderstichele H, Vanmechelen E, Blennow K. Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. *Neurosci Lett* 2001;297:187–90. [PubMed: 11137759]
- Iacono D, Markesbery WR, Gross M, Pletnikova O, Rudow G, Zandi P, Troncoso JC. The Nun study: clinically silent AD, neuronal hypertrophy, and linguistic skills in early life. *Neurology* 2009;73:665–73. [PubMed: 19587326]
- Irizarry MC, Yao Y, Hyman BT, Growdon JH, Pratico D. Plasma F2A isoprostane levels in Alzheimer's and Parkinson's disease. *Neurodegener Dis* 2007;4:403–5. [PubMed: 17934322]
- Li G, Sokal I, Quinn JF, Leverenz JB, Brodey M, Schellenberg GD, Kaye JA, Raskind MA, Zhang J, Peskind ER, Montine TJ. CSF tau/Aβ42 ratio for increased risk of mild cognitive impairment: a follow-up study. *Neurology* 2007;69:631–9. [PubMed: 17698783]
- Lovell MA, Markesbery WR. Oxidative damage in mild cognitive impairment and early Alzheimer's disease. *J Neurosci Res* 2007;85:3036–40. [PubMed: 17510979]
- Markesbery WR, Jicha GA, Liu H, Schmitt FA. Lewy body pathology in normal elderly subjects. *J Neuropathol Exp Neurol* 2009;68:816–22. [PubMed: 19535990]
- Milatovic D, VanRollins M, Li K, Montine KS, Montine TJ. Suppression of murine cerebral F2-isoprostanes and F4-neuroprostanes from excitotoxicity and innate immune response in vivo by alpha- or gamma-tocopherol. *J Chromatogr B Analyt Technol Biomed Life Sci* 2005;827:88–93.
- Mintun MA, Larossa GN, Sheline YI, Dence CS, Lee SY, Mach RH, Klunk WE, Mathis CA, DeKosky ST, Morris JC. [11C]PIB in a nondemented population: potential antecedent marker of Alzheimer disease. *Neurology* 2006;67:446–52. [PubMed: 16894106]
- Montine TJ, Kaye JA, Montine KS, McFarland L, Morrow JD, Quinn JF. Cerebrospinal fluid Aβ42, tau, and F2-isoprostane concentrations in patients with Alzheimer disease, other dementias, and in age-matched controls. *Arch Pathol Lab Med* 2001;125:510–2. [PubMed: 11260625]
- Montine TJ, Markesbery WR, Morrow JD, Roberts LJ 2nd. Cerebrospinal fluid F2-isoprostane levels are increased in Alzheimer's disease. *Ann Neurol* 1998;44:410–3. [PubMed: 9749613]

- Montine TJ, Montine KS, McMahan W, Markesbery WR, Quinn JF, Morrow JD. F2-isoprostanes in Alzheimer and other neurodegenerative diseases. *Antioxid Redox Signal* 2005;7:269–75. [PubMed: 15650414]
- Montine TJ, Quinn J, Kaye J, Morrow JD. F(2)-isoprostanes as biomarkers of late-onset Alzheimer's disease. *J Mol Neurosci* 2007;33:114–9. [PubMed: 17901555]
- Montine TJ, Quinn JF, Milatovic D, Silbert LC, Dang T, Sanchez S, Terry E, Roberts LJ 2nd, Kaye JA, Morrow JD. Peripheral F2-isoprostanes and F4-neuroprostanes are not increased in Alzheimer's disease. *Ann Neurol* 2002;52:175–9. [PubMed: 12210787]
- Montine TJ, Shi M, Quinn JF, Peskind ER, Craft S, Ghingina C, Chung KA, Kim H, Galasko DR, Jankovic J, Zabetian CP, Leverenz JB, Zhang J. CSF A β ₄₂ and tau in Parkinson's disease with cognitive impairment. *Movement disorders*. 2010 in press.
- Montine TJ, Sidell KR, Crews BC, Markesbery WR, Marnett LJ, Roberts LJ 2nd, Morrow JD. Elevated CSF prostaglandin E2 levels in patients with probable AD. *Neurology* 1999;53:1495–8. [PubMed: 10534257]
- Mufson EJ, Leurgans S. Inability of plasma and urine F2A-isoprostane levels to differentiate mild cognitive impairment from Alzheimer's disease. *Neurodegener Dis* 2010;7:139–42. [PubMed: 20197693]
- Peskind ER, Riekse R, Quinn JF, Kaye J, Clark CM, Farlow MR, Decarli C, Chabal C, Vavrek D, Raskind MA, Galasko D. Safety and acceptability of the research lumbar puncture. *Alzheimer Dis Assoc Disord* 2005;19:220–5. [PubMed: 16327349]
- Petersen RC, Negash S. Mild cognitive impairment: an overview. *CNS Spectr* 2008;13:45–53. [PubMed: 18204414]
- Petrovitch H, Ross GW, Steinhorn SC, Abbott RD, Markesbery W, Davis D, Nelson J, Hardman J, Masaki K, Vogt MR, Launer L, White LR. AD lesions and infarcts in demented and non-demented Japanese-American men. *Ann Neurol* 2005;57:98–103. [PubMed: 15562458]
- Pratico D, Clark CM, Lee VM, Trojanowski JQ, Rokach J, FitzGerald GA. Increased 8,12-iso-iPF2alpha-VI in Alzheimer's disease: correlation of a noninvasive index of lipid peroxidation with disease severity. *Ann Neurol* 2000;48:809–12. [PubMed: 11079549]
- Pratico D, Clark CM, Liun F, Rokach J, Lee VY, Trojanowski JQ. Increase of brain oxidative stress in mild cognitive impairment: a possible predictor of Alzheimer disease. *Arch Neurol* 2002;59:972–6. [PubMed: 12056933]
- Price JL, McKeel DW Jr, Buckles VD, Roe CM, Xiong C, Grundman M, Hansen LA, Petersen RC, Parisi JE, Dickson DW, Smith CD, Davis DG, Schmitt FA, Markesbery WR, Kaye J, Kurlan R, Hulette C, Kurland BF, Higdon R, Kukull W, Morris JC. Neuropathology of nondemented aging: presumptive evidence for preclinical Alzheimer disease. *Neurobiol Aging* 2009;30:1026–36. [PubMed: 19376612]
- Quinn JF, Montine KS, Moore M, Morrow JD, Kaye JA, Montine TJ. Suppression of longitudinal increase in CSF F2-isoprostanes in Alzheimer's disease. *J Alzheimers Dis* 2004;6:93–7. [PubMed: 15004331]
- Reitan, RM.; Wolfson, D. The neuropsychology handbook: behavioral and clinical perspectives. W. D. New York: Springer Publishing Co; 1986. The Halstead-Reitan neuropsychological test battery; p. 134-160.
- Rentz DM, Locascio JJ, Becker JA, Moran EK, Eng E, Buckner RL, Sperling RA, Johnson KA. Cognition, reserve, and amyloid deposition in normal aging. *Ann Neurol* 2010;67:353–64. [PubMed: 20373347]
- Roberts LJ, Morrow JD. Measurement of F(2)-isoprostanes as an index of oxidative stress in vivo. *Free Radic Biol Med* 2000;28:505–13. [PubMed: 10719231]
- Schmitt FA, Davis DG, Wekstein DR, Smith CD, Ashford JW, Markesbery WR. "Preclinical" AD revisited: neuropathology of cognitively normal older adults. *Neurology* 2000;55:370–6. [PubMed: 10932270]
- Smith CD, Carney JM, Starke-Reed PE, Oliver CN, Stadtman ER, Floyd RA, Markesbery WR. Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. *Proc Natl Acad Sci U S A* 1991;88:10540–3. [PubMed: 1683703]

- Sonnen JA, Breitner JC, Lovell MA, Markesbery WR, Quinn JF, Montine TJ. Free radical-mediated damage to brain in Alzheimer's disease and its transgenic mouse models. *Free Radic Biol Med* 2008a;45:219–30. [PubMed: 18482592]
- Sonnen JA, Larson EB, Crane PK, Haneuse S, Li G, Schellenberg GD, Craft S, Leverenz JB, Montine TJ. Pathological correlates of dementia in a longitudinal, population-based sample of aging. *Ann Neurol* 2007;62:406–13. [PubMed: 17879383]
- Sonnen JA, Larson EB, Gray SL, Wilson A, Kohama SG, Crane PK, Breitner JC, Montine TJ. Free radical damage to cerebral cortex in Alzheimer's disease, microvascular brain injury, and smoking. *Ann Neurol* 2009;65:226–9. [PubMed: 19259965]
- Sonnen JA, Montine KS, Quinn JF, Kaye JA, Breitner JC, Montine TJ. Biomarkers for cognitive impairment and dementia in elderly people. *Lancet Neurol* 2008b;7:704–14. [PubMed: 18635019]
- Tuppo EE, Forman LJ, Spur BW, Chan-Ting RE, Chopra A, Cavalieri TA. Sign of lipid peroxidation as measured in the urine of patients with probable Alzheimer's disease. *Brain Res Bull* 2001;54:565–8. [PubMed: 11397549]
- Tyas SL, Snowdon DA, Desrosiers MF, Riley KP, Markesbery WR. Healthy ageing in the Nun Study: definition and neuropathologic correlates. *Age Ageing* 2007;36:650–5. [PubMed: 17906306]
- Wechsler, W. Wechsler Memory scale. San Antonio, TX: The Psychological Corporation; 1987. Paragraph recall as well as category fluency and letter fluency.

Figure 1. Clinically normal adults who had normal scores on a battery of neuropsychological tests volunteered for lumbar puncture (n=412). Data are cerebrospinal fluid (CSF) F₂-isoprostanes (IsoPs) for each subject plotted against age. Regression yielded a statistically significant line ($P < 0.001$) plotted with 95% confidence intervals.

Figure 2.

Data from all clinically normal subjects with cerebrospinal fluid (CSF) CSF $A\beta_{42}$ and tau determinations (n=28) were used to define normal cutoff values that were then applied to all clinically normal subjects 60 years or older (n=74). This pie chart shows the stratification of these 74 older controls based on results of CSF $A\beta_{42}$ and tau levels: (i) Normal CSF (n=37), (ii) Abnormal CSF $A\beta_{42}$ & Tau (n=24), (iii) Low CSF $A\beta_{42}$ Only (n=4), or (iv) High CSF Tau Only (n=9).

Figure 3. Concentrations of cerebrospinal fluid (CSF) F₂-isoprostanes (IsoPs) among 74 clinically normal subjects aged 60 years or more were stratified by CSF Aβ₄₂ and tau results into four groups ($P < 0.001$). Bonferroni's corrected multiple comparison test had $P < 0.05$ for Normal CSF vs. Abnormal Aβ₄₂ & Tau, and $P < 0.001$ for Normal CSF vs. High CSF Tau Only.

Figure 4. Concentrations of cerebrospinal fluid (CSF) F₂-isoprostanes (IsoPs) among 37 clinically normal subjects aged 60 years or more with normal CSF A β ₄₂ and tau concentrations were analyzed by linear regression ($P < 0.01$). Best-fit line and 95% confidence intervals are plotted.

Table 1

Chronic disease model of Alzheimer's disease (AD) and Vascular Brain Injury (VBI)

Stage of Chronic Disease	Terms used in AD or VBI Research	Disease	Laboratory Evidence*	Clinical Signs and Symptoms
No disease		None	None	Normal
Latent	Pre-clinical or Pre-symptomatic	+	+	Normal
Prodrome	MCI or CIND	+ to ++	+ to ++	+ Abnormal
Full clinical expression	Dementia	++ to +++	++ to +++	++ to +++ Abnormal

* Biomarkers, neuroimaging, or autopsy. Mild (+), Moderate (++), or Severe (+++).

Abbreviation: Cognitive Impairment Not Dementia (CIND), Mild Cognitive Impairment (MCI)

Table 2

Clinically Normal Individuals Younger than 50 Years-Old (n=28).

	Age (yr)	CSF A β ₄₂ (ng/ml)	CSF Tau (ng/ml)	CSF Tau/A β ₄₂ (ng/ml)
Minimum	25	200.0	32.0	0.13
Maximum	49	362.4	85.6	0.26
Mean	34	279.9	54.2	0.19
SD	8	47.0	14.3	0.04
Normal	---	> 185.9	< 82.8	< 0.27