Cerebrospinal fluid biomarkers of Alzheimer’s disease in healthy elderly

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1. ABSTRACT

Numerous studies have shown that Alzheimer’s Disease (AD) pathology begins before the onset of clinical symptoms. Because therapies are likely to be more effective if they are implemented early in the disease progression, it is necessary to identify reliable biomarkers to detect AD pathology in the early stages of the disease, ideally in presymptomatic individuals. Recent research has identified three candidate cerebrospinal fluid (CSF) biomarkers that reflect AD pathology: amyloid beta, total tau protein (t-tau), and tau protein phosphorylated at AD-specific epitopes (p-tau). They are useful in supporting the AD diagnosis and have predictive value for AD when patients are in the stage of mild cognitive impairment (MCI). However, their predictive utility in cognitively healthy subjects is still being evaluated. We conducted a review of studies published between 1993 and 2011 and summarized their findings on the role of CSF biomarkers for AD in healthy elderly.

2. INTRODUCTION

The diagnosis of Alzheimer’s disease (AD) is currently based on clinical criteria, which require a patient to have dementia before a diagnosis can be made (1,2). The development of sensitive and specific biological markers has prompted the recent recommendation to incorporate biomarkers in research criteria for AD (3) and Mild Cognitive Impairment (MCI) (4). The early diagnosis and prediction of AD is especially urgent given that the testing of disease-modifying drugs will probably have a greater chance for success when administered in the early stages of AD, before neurodegeneration becomes widespread (2,5).

Biomarkers are objective measures whose presence, concentration, and activity are associated with a disease (6). An ideal biological marker would detect a fundamental feature of AD; would be reliable, inexpensive, noninvasive and simple to test; and would
have specificity greater than 75% and sensitivity greater than 85%. It should reflect a causal path between underlying pathology and clinical symptoms (7,8), and also should reflect therapeutic effects.

AD is characterized by the presence of extracellular plaques (composed of amyloid) and intracellular neurofibrillary tangles (composed of hyperphosphorylated tau protein). Deposition of plaques and tangles is believed to propagate neurodegeneration in AD (6,9,10). It is known that AD pathology begins years before clinical symptoms become evident (11).

Cerebrospinal fluid (CSF), largely produced by the choroid plexus, is found in the ventricular system and subarachnoid spaces surrounding the brain and spinal cord. Because CSF is in direct contact with the brain, its composition is affected by biochemical changes in the brain (6). CSF amyloid beta 42 (Aß42), total tau (t-tau) and phosphorylated tau (p-tau) proteins have been shown to reflect deposition of amyloid plaques, neuronal death and accumulation of tangles, respectively (12-14). These markers have been studied separately, in combination with each other, and in conjunction with information from clinical exams, laboratory tests, and brain imaging techniques like magnetic resonance imaging (MRI) and positron emission tomography (PET) in AD patients as well as in MCI and cognitively normal (NL) populations at risk for AD (15-17).

CSF biomarkers have gained an important status in the area of MCI-stage diagnosis of AD as they reflect AD-related pathological changes. However, many questions remain unanswered. One of the major unknowns is how well CSF biomarkers can identify AD pathology and predict future AD symptoms in clinically healthy NL. What is the prevalence and meaning of purportedly AD-related CSF abnormalities in NL subjects? And how well do they correlate in the NL population with imaging markers reflective of structural and functional damage? This review summarizes current studies that have addressed these issues.

Although we will focus on cognitively healthy elderly, it would be difficult to consider the meaning of biomarkers in this group without first discussing how well they discriminate between NL, MCI and AD subjects. To address this question, we analyzed CSF studies that were published in peer-reviewed journals between 1993 and 2011, and where groups had 10 or more subjects. A total of 56 studies fulfilled our criteria and will be reviewed below. We will summarize current knowledge about the relationship between age, Apolipoprotein E (ApoE) genotype and CSF biomarkers among NL elderly. We will present data concerning the prevalence of “abnormal” CSF biomarkers in normal populations and their predictive value for future cognitive decline. Finally, we will discuss the associations between neuroimaging findings and AD CSF markers in cognitively healthy subjects and their relationships with common vascular risk factors.

3. DIAGNOSTIC ACCURACY

3.1. Aß42

The extracellular plaques characteristic of AD are mainly made up of Aß peptides, which are generated by the cleavage of the amyloid precursor protein (APP) and secreted by the cells. Among several forms of Aß, the diagnostic potential of the Aß40 and Aß42 forms has been studied most extensively (18).

The majority of studies have shown that in AD subjects, CSF Aß42 and Aß40 decline markedly over time (2,6,19-22). It is hypothesized that the abnormally low CSF Aß levels in AD subjects are a reflection of increased deposition of Aß into senile plaques, resulting in reduced CSF Aß concentrations (18,23). Only a few reports found increased (24) or unchanged (25) Aß42 in AD. Interestingly, increased Aß42 was found only in subjects with early, but not late, stages of AD, allowing authors to hypothesize that Aß42 reduction is preceded by an initial increase in its concentration (24). Thus, their study group might have consisted of subjects in this particular early period. As for the report of unchanged Aß levels, a small reduction was observed; however, it did not reach statistical significance (25).

Overall, Aß42 shows an inverse relationship with the degree of cognitive impairment, with AD patients having lower levels of Aß42 than MCI and NL (15,26-28,28). In our meta-analysis of 50 studies, Aß42 levels decreased on average by 39% in AD subjects as compared to controls (Table 1). A previous literature review performed by Blennow showed that at 90% specificity, Aß42 discriminated AD from controls with 85% sensitivity (15).

MCI is a clinically heterogenous group with various biomarker profiles. Despite this heterogeneity and the differing levels of risk, patients with MCI are still at increased risk for AD compared to healthy controls: The yearly rates of decline from amnestic MCI to AD were estimated at 12% as compared to 1-2% among normal controls (29). Interestingly, among non-amnestic MCI, conversion rates to AD still seem to be twice as high as in the cognitively healthy group (30). An early study showed abnormal Aß and t-tau values in MCI two years before dementia onset (31), leading to the conclusion later supported by others that these markers exhibit abnormal levels in MCI prior to an AD diagnosis (32). The MCI subjects show average biomarker values which fall between those seen in the NL and AD groups. Our analysis revealed that Aß42 levels were on average 23% lower in MCI than in NL (Table 2).

3.2. Tau

Tau is an intracellular protein that contributes to the assembly and stabilization of microtubules in neuronal axons. It was discovered in 1975 in Mark Kirschner’s laboratory at Princeton (33). The MAPT (microtubule associated protein tau) gene, which encodes tau protein, is located on chromosome 21 and has six isoforms in adult humans (34) (Figure 1). Tau is mildly phosphorylated in
Table 1. Differences between AD and NL in CSF biomarkers value across different studies

<table>
<thead>
<tr>
<th>First author, year</th>
<th>AD (n)</th>
<th>NL (n)</th>
<th>Aß42</th>
<th>T-tau</th>
<th>P-tau</th>
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<tr>
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<td></td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
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<td>50</td>
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<td>200.2%</td>
<td>158.4%(181)</td>
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<td>Buerger 2004 (43)</td>
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<td>660.5%(231)</td>
</tr>
<tr>
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<td>120.0%(181); 362.5%(231)</td>
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<tr>
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<td>Ibach 2005 (204)</td>
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<td>63.3%</td>
<td>168.1%(181)</td>
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<tr>
<td>Iqbal 2005 (204)</td>
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<td>181.8%</td>
<td>317.0%(199)</td>
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<td>Itoh 1998 (85)</td>
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<td>Maddalena 2003 (52)</td>
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<td>151.1%</td>
<td>160.7%(181)</td>
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<tr>
<td>Mattsson 2009 (193)</td>
<td>529</td>
<td>304</td>
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<td>Nishimura 1998 (212)</td>
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<td>174.5%</td>
<td>168.0%(181)</td>
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<td>170.1%</td>
<td></td>
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<td>Rosler 2001 (216)</td>
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<td>320.0%</td>
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<tr>
<td>Seppala 2011 (217)</td>
<td>56</td>
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<td>67.6%</td>
<td>156.8%</td>
<td>155.1%(181)</td>
</tr>
<tr>
<td>Shaw 2009 (128)</td>
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<td>114</td>
<td>69.9%</td>
<td>174.2%</td>
<td>168.0%(181)</td>
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<tr>
<td>Shoji 1999 (72)</td>
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<tr>
<td>Sigsgaard 2001 (42)</td>
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<tr>
<td>Sigsgaard 2001 (218)</td>
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<tr>
<td>Sunderland 2003 (219)</td>
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<tr>
<td>Tapiola 1998 (220)</td>
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<td>33</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Vanderstichele 2000 (221)</td>
<td>81</td>
<td>15</td>
<td>75.0%</td>
<td>178.0%</td>
<td></td>
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<td>Vanderstichele 2006 (222)</td>
<td>94</td>
<td>60</td>
<td>53.7%</td>
<td>347.5%</td>
<td>197.8%(181)</td>
</tr>
<tr>
<td>Vannesteelen 2000 (223)</td>
<td>41</td>
<td>17</td>
<td></td>
<td>148.0%(181)</td>
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</tbody>
</table>

Values are given as percentage of value for the control healthy group. For p-tau the numbers in parentheses indicate phosphorylation site studied. Across all studies listed in this table average Aß42 concentration in AD was 60% of that in NL group (28 studies), average p-tau199 concentration was 265% of that in NL (2 studies), p-tau231 was 977% of NL group value (3 studies), p-tau181 was 166% (14 studies) and t-tau 277% (36 studies).

the normal adult brain (18). In AD, tau becomes hyperphosphorylated and loses its ability to assemble and stabilize microtubules (35). Increase in t-tau concentration reflects the degree of neuronal degeneration and is not specific to AD. For example, a marked increase in t-tau levels has been observed after stroke (36). Furthermore, t-tau increase is accelerated in disorders with extensive and/or rapid neuronal death, such as Creutzfeldt-Jakob disease, as compared to other disorders with limited neuronal degeneration (37).

CSF t-tau concentration shows a marked increase in AD (2), reflecting the neuronal damage associated with the disease. All studies in our analysis showed elevated t-tau in AD. This increase averaged 277% in AD as compared to NL (Table 1). A previous meta-analysis of 35 studies found comparable differences, reporting t-tau levels on average 300% higher in AD than in NL. T-tau showed 80% success in discriminating between NL and AD (15,38). As with Aß42, t-tau levels in MCI subjects are intermediate between NL and AD groups (Table 2). On average t-tau levels were 153% higher in MCI than in NL.

3.3. P-tau

The tangles characteristic of AD are made up of filaments formed from an abnormally phosphorylated form of tau called phospho-tau (p-tau) (15). Tau protein contains more than 80 sites where enzymes (kinases) can attach phosphate groups. These are called phosphorylation sites (39) (Figure 2). Adding phosphate groups in different
Table 2. Differences between MCI and NL in CSF biomarkers value across different studies

<table>
<thead>
<tr>
<th>First author, year</th>
<th>MCI (n)</th>
<th>NL (n)</th>
<th>Aß42</th>
<th>T-tau</th>
<th>P-tau</th>
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</thead>
<tbody>
<tr>
<td>Bouwman 2007 (96)</td>
<td>38</td>
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<td>76.3%</td>
<td>178.1%</td>
<td>137.7%</td>
</tr>
<tr>
<td>Brys 2009 (28,99)</td>
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<td>21</td>
<td>162.9%</td>
<td>197.3%</td>
<td>137.3%</td>
</tr>
<tr>
<td>Buerger 2005 (224)</td>
<td>59</td>
<td>28</td>
<td>172.5%</td>
<td>199.2%</td>
<td>864.2%</td>
</tr>
<tr>
<td>Buerger 2009 (197)</td>
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<td>77.7%</td>
<td>124.1%</td>
<td>118.0%</td>
</tr>
<tr>
<td>de Leon 2002 (225)</td>
<td>8</td>
<td>10</td>
<td>40%</td>
<td>199.2%</td>
<td>234%</td>
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<tr>
<td>Fellgiebel 2008 (199)</td>
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<td>10</td>
<td>194.2%</td>
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</tr>
<tr>
<td>Hansson 2006 (226)</td>
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<td>66.2%</td>
<td>175.0%</td>
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<td>Kester 2011 (100)</td>
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<td>Maruyama 2001 (211)</td>
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<td>15</td>
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<td>135.7%</td>
<td>119.6%</td>
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<tr>
<td>Okonkwo 2011 (213)</td>
<td>195</td>
<td>114</td>
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<td>17</td>
<td>18</td>
<td>129.7%</td>
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<tr>
<td>Rolstad 2011 (215)</td>
<td>170</td>
<td>60</td>
<td>78.8%</td>
<td>128.1%</td>
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<td>Schonknecht 2007 (229)</td>
<td>80</td>
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<tr>
<td>Seppala 2011 (217)</td>
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<td>8</td>
<td>103.2%</td>
<td>92.5%</td>
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</tr>
<tr>
<td>Shaw 2009 (128)</td>
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<td>89</td>
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<td>149.6%</td>
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</table>

Values are given as percentage of value for the control healthy group. For p-tau the numbers in parentheses indicate phosphorylation site studied. Across all studies listed in this table Aß42 concentration in the MCI group was 77% of that in NL group (13 studies), average p-tau181 concentration was 140% of NL group value (11 studies), p-tau231 was 357% (4 studies), and t-tau 153% (15 studies).

Figure 1. Rendering of microtubule associated protein tau (194).

positions on the amino acid chain alters tau properties. In AD tau becomes excessively phosphorylated. This process compromises tau’s capacity to stabilize microtubules and makes it more resistant to degradation, possibly contributing to the formation of fibrils and tangles (40). Even though there are multiple phosphorylation sites at serine and threonine residues, the concentration of p-tau in CSF is commonly defined based on its phosphorylation at threonine 181 or threonine 231 (2). P-tau is an important CSF marker used in the detection of AD, and is believed to reflect neurofibrillary pathology. Like t-tau, p-tau concentrations increase in AD. Moreover, the levels of t-tau and p-tau are strongly correlated both in NL and AD (41,42). This is not the case in Creutzfeldt-Jakob disease (43) or acute stroke (44), where massive increases in CSF t-tau are not accompanied by similar increases in p-tau. P-tau effectively discriminates AD from NL (45). It is more specific to AD than t-tau, so it allows for better differentiation between Alzheimer’s and frontotemporal or Lewy Body dementia (18,46), hydrocephalus (47), depression (48) or Creutzfeldt-Jakob disease (43). Our analysis showed that p-tau181 levels were an average of 166% higher in AD than in NL (Table 1). The number of p-tau 231 studies is smaller, but they also show a significant p-tau231 increase in AD as compared to NL subjects (976.71%). In an early study, p-tau231 discriminated AD from healthy controls with a sensitivity of 100% and a specificity of 90.5% (49). A 2004 review of 11 p-tau studies reported an average sensitivity of 80%, at 92% specificity (15).

In a recent meta-analysis of 51 studies, p-tau was classified as a “satisfactory” marker also for discriminating MCI from NL (45). In our review, as with Aß42 and t-tau, p-tau181 levels for MCI fell in between values for AD and controls, with p-tau181 being on average 140% higher in MCI than in NL (Table 2).
CSF biomarkers of AD in healthy elderly

Overall, all three CSF biomarkers have been reported to individually differentiate AD patients from healthy elderly individuals with 80–90% sensitivity and specificity (2). Both Aβ42 and t-tau have been shown to have a good diagnostic accuracy in discriminating NL and cognitively impaired subjects. Since the capacity of Aβ and t-tau to discriminate between AD and other dementias may not always be sufficient, adding p-tau181 and p-tau231 improves differential diagnosis (50,51).

Finally, using ratios of biomarkers like p-tau/Aβ42 and t-tau/Aβ42 can also improve diagnostic accuracy (52). Higher ratio indicates more significant pathology. Maddalena et al. (52) reported a substantial gain in specificity when differentiating NL and AD groups with p-tau/Aβ42 as compared to p-tau181 or Aβ42 alone. Individuals with higher levels of p-tau181 in combination with lower levels of Aβ42 are more likely to have significant pathology than subjects with either marker alone. Similarly, others found that sensitivity and specificity of p-tau181/Aβ42 ratio were significantly higher than those for t-tau (53), p-tau (42), Aβ42 (54) or even t-tau/Aβ42 ratio (55). Lastly, there have also been reports of better prediction of decline from NL with p-tau181/Aβ42 and t-tau/Aβ42 than with any single marker (22) (56).

4. CSF AD BIOMARKERS AND AGE IN NORMAL POPULATION

Age is the strongest risk factor for AD (57). The prevalence of medial temporal lobe atrophy (characteristic for AD) increases with age (58) (59). At post-mortem, cognitively healthy subjects have been shown to exhibit AD-like plaques and tangles (60). In PET imaging, 20–40% of NL elderly show patterns of Pittsburgh compound B (PIB) uptake characteristic of AD (61,62). PIB is a radiotracer used in PET imaging. It is a derivative of a dye, thioflavin-T, which binds to amyloid aggregates. Since PIB maintains the capacity to bind to amyloid, it is a suitable radiotracer for in vivo amyloid detection with PET (61,62). Cortical pathology, manifesting as gray matter thinning in AD-affected regions, is also evident among NL individuals, supporting the existence of a pre-clinical phase of dementia (63).

4.1. Aβ

Our review of studies describing age - Aβ42 relationships in NL elderly gives somewhat conflicting results. Many have found an age-dependent linear decrease in CSF Aβ42 concentrations (22,56,64-70). However, other studies have reported no change (42,71-74) or an age-dependent increase (75,76). In a study of 92 NL subjects between 8 and 89 years old, the relationship between Aβ and age followed a u-shaped curve, with high Aβ40 and Aβ42 levels in children, lower levels in adolescents and adults, and an increase in old age (76,77). Contradictory results were found in another study of 56 subjects, which showed low Aβ40 and Aβ42 levels in children, with an increase in adulthood and a decline in old age (64). Several different factors could have contributed to these divergent findings. First, CSF Aβ fluctuates 1.5- to 4-fold throughout the day and appears to be time-of-day (78) and sleep-wake cycle dependent (79,80). Interestingly, diurnal variations in CSF Aβ42 concentrations are blunted with age and with brain amyloid accumulation (80). Second, as opposed to t-tau and p-tau, CSF Aβ42 seems to be less stable, exhibiting marked changes depending on CSF storage protocols (81) and on freeze-thaw cycles (82). Variations in the timing of lumbar puncture or CSF handling could contribute to the lack of consistency in results. In summary, CSF Aβ42 may decrease with age, but current data do not support this notion unequivocally. The possibility that the age-Aβ42 relationship may not be linear also has to be taken into account. One group observed that brain activity increases Aβ42 in the interstitial fluid (83); this finding implies that Aβ concentration may initially increase with age, and after reaching a threshold beyond which clearance mechanisms become dysfunctional, brain deposition results in Aβ sequestration and reduction of CSF levels. This hypothesis requires further corroboration.

4.2. Tau

A substantial number of studies have shown consistent negative correlations of various strengths between tau and age (22,42,56,68,72,74,84-88). Studies that have not found relationships between t-tau and age are in the minority (70,89-91). Smaller study groups (89,90) or the fact that AD patients were examined may explain their lack of positive findings (91). Finally, even with larger groups (70), intrinsic differences between populations may result in dissimilar findings.

Existing data suggest that increases in tau concentration are a common finding with increasing age. This is not surprising given that tau reflects neuronal loss and correlates well with brain atrophy, both concomitant with brain aging.

4.3. P-tau

There are a few reports indicating that like t-tau, p-tau concentrations increase with age in NL individuals. This was found for both p-tau181 (46,68,69,92) and p-tau231 (46,74,93). The much less studied p-tau199 did not show association with age in normal controls (85). Others have argued that p-tau concentrations (in this case p-tau181) are age-independent, (94) further strengthening its...
importance as a marker specific for AD. In summary, despite some contradictory studies, the majority of the available evidence (46,68,69,74,92,93) suggests that p-tau increases with age in NL. Some reservations remain, since many of the studied cohorts came from memory centers where enrichment with silent AD pathology is expected. It is thus still unclear to what extent increased p-tau levels in elderly controls are correlates of aging or reflect asymptomatic AD. Interestingly, some postulate (95) that phosphorylation of tau and formation of neurofibrillary tangles is not toxic per se, but represents a protective cellular response to oxidative stress. According to this hypothesis, increased p-tau accumulation with age (serving as an antioxidant) could reflect adaptive mechanisms (95). Surpassing the cell’s compensatory capacity could result in increased accumulation and cell death as seen in AD.

A few studies have performed longitudinal CSF examinations in NL. One study showed that Aβ42 and t-tau levels, but not p-tau, changed over a span of almost two years in NL, MCI, and AD. However, differences were seen mostly between groups rather than in individuals (96). In another 1-year study, only t-tau increased significantly in normal controls; no differences were found for Aβ42 levels, and no p-tau data were given (97). Contradicting these findings, others reported that the greatest rate of change over 3 years was observed for Aβ42 but not tau or p-tau181 (98). In a group of NL subjects studied at 2 year intervals, we did not see changes in t-tau, Aβ42/Aβ40 or p-tau231 between baseline and follow-up (28,99). The lack of change may be attributed, however, to relatively short observation period in a younger group: the mean age of our cohort was 69 vs. a mean of 75 reported in Jack et al. and Lo et al. In agreement with our findings, Kester et al. did not observe any changes in CSF markers over the span of 2.5 years in normal elderly in their sixties (100), suggesting that longitudinal dynamics in younger cohorts are slower.

Altogether, these data suggest the existence of age-related changes in CSF AD biomarkers: The probability of abnormal findings increases with age. It is tempting to assume that these abnormalities reflect a true pathological process ultimately leading to AD. However, given the lack of longitudinal data documenting the trajectory of individual subjects from normal cognition to AD, this conclusion must await further confirmation. Meanwhile, age-related dynamics of CSF biomarkers have to be taken into consideration while developing diagnostic criteria, since they influence diagnostic accuracy of biomarkers in older groups. As early as 1999, Buenger et al. observed that correct classification rate for t-tau in the young old was significantly higher than in the group of older old (101), suggesting better discriminative power of CSF tau in the younger population. Others found that the difference in CSF biomarkers between young AD patients and controls was greater than the difference between old AD patients and controls (69). Finally, a recent study found a marked decrease in diagnostic accuracy of single CSF biomarkers in older populations. Specificity was most affected, reaching only 40% for p-tau in the group older than 74 (92). As a remedy to this problem, the authors propose using a combination of markers, which still showed satisfactory sensitivity and specificity, even though analysis of Alzheimer’s Disease Neuroimaging Initiative (ADNI) data showed that the diagnostic accuracy of the tau/Aβ42 ratio was still higher in the younger (<75) than in the older group (102).

5. BIOMARKERS AND APOE IN NORMAL POPULATION

The presence of an ApoE4 allele is the most widely accepted genetic risk factor for late-onset AD (103). It has been repeatedly shown that the frequency of the ApoE4 allele is significantly higher in AD than in NL (14,64). The presence of this allele has been associated with lower age of AD onset (104,105). The risk of developing AD is approximately 15 times greater for E4 homozygotes and 3 times greater for heterozygotes compared to non-carriers (103).

In line with the notion of the ApoE4 allele as a risk factor, multiple studies have shown that ApoE4 carriers, both NL and AD, have more abnormal CSF biomarkers than non-carriers in the same diagnostic group (106-115). A recent study of ADNI subjects showed that cognitively normal subjects classified as having an AD profile (Aβ42/p-tau181 mixture model AD feature, based on an unsupervised learning approach) were almost 7 times more likely to carry the ApoE4 allele than subjects without the AD signature (116).

Cognitively healthy ApoE4 positive (ApoE4+) subjects have lower Aβ42 levels cross-sectionally (107,114,117), greater longitudinal decreases in Aβ42 concentrations (118), and show significant elevations in Aβ40/Aβ42 ratios (119) compared with non-carriers. Others reported an interesting age-by-genotype interaction where ApoE4+ subjects exhibited a sharp decline in Aβ42 concentration beginning in their 60s, while ApoE4- subjects showed significantly less change (73). Some observed that Aβ42 levels were comparable between ApoE4+ AD patients and ApoE4+ NL (120).

Findings regarding t-tau are somewhat less consistent. Some studies showed higher CSF t-tau levels in ApoE4 carriers (74,107,121), while others did not find a significant relationship between tau levels and ApoE4 status (91,108,114). We observed that cognitively intact ApoE4+ individuals had higher t-tau and p-tau231 levels than their peers. Furthermore, p-tau231 concentration increased with age in both genotype groups, but this increase was steeper in ApoE4+ subjects (74). Increased p-tau181 levels in NL ApoE4 carriers have also been confirmed by others (107). Longitudinally, two reports analyzing ADNI data did not find significant differences in the rate of change in biomarkers between NL carriers and non-carriers (98,122). However, the follow-up periods (12 and 35 months, respectively) might not have been long enough to observe a significant change.

ApoE2 appears to be associated with lower risk of AD (123): some found that ApoE2 carriers had higher levels of Aβ, lower levels of p-tau 181, and marginally
lower levels of t-tau (124). The mechanisms of protective ApoE2 effects are not fully understood. Subjects carrying this allele seem to have less brain atrophy (125). In post-mortem studies, ApoE2 carriers without dementia had increased levels of postsynaptic density protein 95 (126). Animal studies indicate that the presence of the ApoE2 allele improves excitatory synaptic transmission impaired in the presence of ApoE4 (127), suggesting that one possible mechanism of ApoE2 protection may rely on its effects on synaptic function.

In summary, ApoE genotype seems to affect CSF biomarker levels in cognitively healthy subjects, consistent with the hypothesis that an ApoE4+ genotype confers a greater risk for AD (Table 3). Conversely, ApoE2 carriers exhibit biomarker profiles indicative of less AD pathology.

### 6. PREVALENCE of a CSF- AD “SIGNATURE” in NORMAL SUBJECTS

The issue of a threshold for “abnormality” for CSF biomarkers is a difficult one, especially in clinically healthy subjects. There are several approaches to define what is abnormal. First, abnormality may be defined based on comparison between the NL and AD groups. Replicated values best differentiating two clinical categories may be used as a threshold (128). Second, abnormality can be defined based on the distribution of values within a certain population, where subjects with values exceeding, for example, 2 standard deviations (SD) below or above the mean can be considered “abnormal”. Finally, possibly the most valid, but also the most difficult to obtain, would be a definition based on longitudinal observation of clinical progression in a group starting as healthy and declining to AD at follow-up evaluations.

A recent paper reported that 38% of normal subjects over age 60 had signs of “latent AD,” defined as abnormal Aβ42 and t-tau. 5% had normal Aβ42 and normal tau, while 12% had normal Aβ42 and elevated tau (129). Cutoff values were defined as 2 SD outside the mean of a normal group below the age of 50. In an earlier paper from the same group, 16% of controls had an elevated tau/Aβ42 ratio (defined as 2 SD outside the mean for the group of 60 and younger). The group with the elevated ratio was older (mean age 71 vs. 49 in the low tau/Aβ42 group), had increased frequency of ApoE4, and were more likely to decline to MCI during follow-up of up to 42 months (56). Shaw et al. developed a “CSF biomarker signature” for AD based on ROC analyses of autopsy-confirmed AD cases compared with controls. Application of this method generated cutoff values that classified 38% of a normal ADNI cohort (mean age of 76) as having a low Aβ42 level and 34% as having an abnormal t-tau/Aβ42 ratio (128). De Meyer et al. identified 36% of cognitively normal ADNI subjects as having an AD profile (the authors used an unsupervised learning approach to create Aβ42/p-tau181 mixture model) (116).

In our study of 115 NL subjects (mean age 63 years), we dichotomized CSF biomarker values into “high” and “low” groups based on previously published cutoff values for discrimination between AD and normal. Thirty percent of our subjects were classified as having high t-tau, 21% as having high p-tau231, and more than 40% as having a low Aβ42/Aβ40 ratio (130).

The prevalence of an abnormal CSF signature seems to be even higher among normal subjects who are at risk for AD. Subjective cognitive impairment (the presence of a memory complaint in the absence of objective impairment on neuropsychological tests) is such a risk...
factor. In the DESCRIPTA study, the CSF AD profile (calculated as: Aβ42/(240+1.8*T-tau)<1) was present in 52% of subjects with subjective cognitive impairment and in 31% of healthy controls (131).

In conclusion, a substantial number of healthy subjects over age 60 (25-40%) has CSF biomarker concentrations in ranges that can be considered abnormal, regardless of the definition used. At least a portion of this population is likely at risk for AD, but a considerable number of them may never develop the disease. One possible explanation for the lack of disease expression in this latter group is cognitive reserve, where certain characteristics of the brain or life experience delay the expression of AD despite the presence of brain pathology. The question of how to classify this group of healthy individuals with abnormal biomarker concentrations will be even more pressing should preventative therapies become available, and the decision about whether to treat subjects with an “AD CSF profile” will depend on the risk related to such therapies.

It is conceivable that the large number of individuals with abnormal biomarker levels indicates that the abnormalities are trait- rather than state-dependent. The fact that differences in metabolic brain activity are already observed in young ApoE4+ individuals (132) may also speak in favor of this explanation. In this case, different markers of imminent decline would be needed to make a decision about if and when to initiate therapy. The profile of anomalous biomarkers is also worth mentioning. Although a large group of subjects have both Aβ42 and t-tau outside the normal range, a significant number of healthy individuals express either Aβ42 or t-tau anomaly. Different pathways leading to neurodegeneration would be a tempting though unverified conclusion, since the course and the outcome of these abnormalities are yet poorly defined. There is insufficient data showing when these biomarkers become abnormal and in which order. It is not known which biomarker is the first to show changes in the pre-clinical disease progression. Even though a widely discussed work by Jack et al. (133) proposes Aβ42 as the first in the temporal ordering of biomarkers, this hypothesis still requires confirmation with longitudinal data (134).

7. PREDICTION OF DECLINE FROM NL

Most studies investigating the predictive potential of CSF biomarkers identified Aβ42 (135-137) or tau/Aβ42 ratios (22,56,138) as the best predictors of cognitive decline in NL.

In a study of 55 women followed for 8 years, baseline Aβ42 correlated negatively with change in MMSE score (135). A 3-year longitudinal study of 85-year-old demonstrated that individuals with lower CSF Aβ42 were more likely to develop dementia at follow-up. Aβ40 levels did not differ between groups, and there were no significant differences between ApoE4 carriers and non-carriers (137). Others reported that low levels of Aβ42 at baseline were associated with development of subjective memory impairment, lower MMSE score, and inability to live in regular housing 3 years later (136). A later study by the same group showed that cognitive decline at 4-year follow-up was associated with a greater longitudinal decrease in Aβ42 and a greater increase in p-tau 181 (118).

Fagan et al. reported that high tau/AB42 ratio and p-tau/AB42 ratio both predicted decline from NL, with hazard ratios (HR) of 5.21 and 4.39, respectively (22). High values were defined as the top 15% of all values, and the follow-up period was 3-4 years. A subsequent study from the same group confirmed this finding in a much bigger cohort with a follow-up period of 6 years. The HR for high (highest tertile) tau/AB42 was 5.76 (138). In line with this finding, Li et al. also reported that cognitively healthy subjects with elevated CSF tau/AB42 ratio (defined as 2 SD outside the mean for the group aged 60 and younger), were more likely to decline to MCI during follow-up of up to 42 months (56).

We observed that higher baseline p-tau231 levels predicted lower delayed recall score 2 years later. Subjects whose performance on the delayed recall test did not improve over time (lack of learning effects) also showed greater longitudinal reduction in MTL gray matter. No differences were seen for total tau or the AB42/AB40 ratio (139). Interestingly, Desikan et al. found that among 107 NL subjects, followed for 3 years, baseline low Aβ42 predicted decline only in subjects who also had high p-tau levels (140), emphasizing the importance of both markers in reflecting amyloid and neurofibrillary pathology.

Although these data suggest the potential usefulness of CSF biomarkers to predict future decline in NL populations (Table 4), the evidence gathered thus far is still unsatisfactory. Most importantly, data on individual trajectories or even their estimates are still missing. Because observation time has been limited, there is still not enough information to precisely predict outcome in younger subjects (in their fifties and sixties). Ongoing longitudinal efforts are needed in order to bridge this gap.

8. CSF BIOMARKERS AND IMAGING IN NORMAL POPULATION

Cerebral atrophy, as measured by structural MRI, indicates brain volume reductions possibly due to loss of dendrites, synapses and neurons (141). Although cerebral atrophy is not specific to AD, certain brain regions are more affected than others, giving a characteristic AD profile (for review (142)). Atrophy can therefore be considered a valid biomarker of neurodegeneration (143-145). This is especially true for the medial temporal lobe (MTL). Hippocampal atrophy predicts decline from NL to AD, and structural MTL changes can be detected before clinical symptoms are fully developed (142,146,147).

Positron Emission Tomography (PET) with 18F-Fluorodeoxyglucose (FDG) and Pittsburgh Compound B (PiB) tracers have also been extensively studied in AD and MCI subjects. FDG-PET measures brain glucose metabolism and has been validated as a marker of the synaptic dysfunction associated with AD compared to age-
matched controls (148,149). Changes in glucose metabolism, reflecting synaptic loss, have been found to occur in preclinical AD (150-153). A characteristic pattern of parieto-temporal and posterior cingulate hypometabolism is a widely accepted feature of AD (154). The application of region-of-interest based approaches to study the structures of the MTL also reveals metabolic reduction in hippocampus and entorhinal cortex (154). Furthermore, hypometabolism in these regions can predict cognitive decline in NL individuals (150,155).

PiB-PET has been shown to be a valid biomarker for AD-associated amyloid plaque load (156-159) (Figure 4). On PiB-PET, the AD profile consists of cortical PiB binding in the precuneus/posterior cingulate and frontal cortex, as well as the lateral temporal and parietal cortex, and more variably, the striatum and occipital cortex. The sensorimotor and medial temporal cortices are less affected (157). Below we present evidence for association between CSF and imaging markers of AD in cognitively healthy groups. We believe that evidence of such association will further corroborate the validity of CSF markers as early indices of AD pathology, particularly if their presence is related to atrophy or dysfunction in AD-specific brain regions.

Both MRI and CSF biomarkers are considered to reflect AD pathology, but there have been relatively few studies about the association between the two in the NL population (14). Neuropathological studies where imaging was performed prior to death showed that neurofibrillary pathology was a stronger correlate of atrophy than Aβ deposition (160,161). Previously, associations have been reported between cross-sectional CSF biomarkers and brain atrophy measures across all diagnostic categories (NL, MCI, AD) (14,162) and in AD only (14). Similarly, there were reports of associations between baseline CSF biomarkers and whole brain (163), temporal (164) or hippocampal atrophy rates (165) in AD subjects, but not NL individuals. It is likely that the relatively small number of NL subjects in these studies can explain the negative findings.

In recent years, a growing number of studies encompassing larger groups of NL indicate a relationship between CSF markers and structural or functional brain changes. Our own studies showed, that higher p-tau231 at baseline (split at the median) was related to increased MTL atrophy rate over the span of two years (139). Additionally, we reported that at cross-section, high p-tau231 was associated with less gray matter in the right MTL (entorhinal cortex) and the left inferior temporal gyrus. Subjects with high tau (above the median for the group) had less gray matter (GM) in the right hippocampus, left precuneus, anterior cingulate, and insula than the low t-tau group (130). These are regions implicated in AD pathology. We did not observe relationships between CSF Aβ and GM measures in AD-specific brain regions. Showing some similarity with our results, others found that p-tau181 was significantly associated with hippocampal radial distance (the distance from the medial core to points at the hippocampal surface) in normal controls. In addition, across all diagnostic groups, phosphorylated tau showed the strongest association, among other CSF biomarkers, with hippocampal atrophy (162). Another group reported that high p-tau181 was related to the thinning of the entorhinal cortex (166). In the same study, low Aβ42 was associated with thinning of the medial orbital frontal cortex. High p-tau181 and low Aβ42 were defined based on cutoffs proposed by Shaw et al., derived from autopsy-confirmed CSF data of control and AD subjects (128). In another study, both higher p-tau181 and lower concentrations of CSF Aβ were associated with increased rates of ventricular enlargement. Furthermore, lower Aβ was associated with cortical thinning in many AD-related brain regions at cross-section (167).

Contrary to these findings, others have reported that CSF Aβ42 levels, but not p-tau levels, are associated with brain atrophy in NL both at cross-section and longitudinally. Fagan et al. found that CSF Aβ42 levels were positively correlated with whole-brain volume in non-demented individuals (168). These results were further developed by Schott et al., who showed that NL with low Aβ42 (as defined by Shaw et al. (128)) had significantly
higher whole brain atrophy rates than their peers with high CSF Aβ42 (169). Dickerson et al. used a structural AD signature created based on the thickness of 9 AD-related cortical regions. Normal subjects with average thickness below 1 standard deviation for the normative group were classified as having high risk for AD. Subjects in this group had lower CSF Aβ42 concentrations than the remainder of the study sample (170). Others observed that Aβ42 correlated with changes in multiple brain regions (not including the MTL), only when Aβ42 reached a certain threshold (115). Some found that ventricular volume was negatively associated with CSF Aβ only in ApoE ε4 positive NL subjects (171). A small study found that NL subjects with low Aβ42 concentrations (based on the cutoff proposed by Fagan et al. (168)) had cortical thinning in many areas, including AD-specific regions (172). Interestingly, one study showed that low Aβ status was associated with entorhinal cortex atrophy rate only in subjects with high p-tau181 concentrations. A similar relationship was also observed for regions affected later in the disease process (63). Once again, high p-tau181 was defined as in Shaw et al. (128). The authors suggested that the presence of p-tau could represent a critical link between Aβ deposition and volume loss. Alternatively, these data could also imply that amyloid accumulation may be a necessary but insufficient indicator (or cause) of AD–related neurodegeneration.

Very few studies have directly examined the associations between FDG uptake or PiB accumulation and CSF biomarkers in cognitively healthy controls. We observed significant negative associations between p-tau231 and glucose metabolism in the middle occipital gyrus, parahippocampal gyrus and thalamus, as well as p-tau231/Aβ42 ratio and glucose metabolism in the parahippocampal gyrus and thalamus (173). Petrie et al. described hypometabolism in the posterior cingulate, precuneus, and parahippocampal regions in conjunction with both high tau and p-tau 181 concentrations. Lower CSF Aβ42 concentrations were associated with hypometabolism only in the inferior temporal cortex (174).

Fagan et al. reported an association between low CSF Aβ42 and high PiB uptake in a mixed group of NL and cognitively impaired subjects (23). These findings were subsequently replicated in a large group of healthy controls.
Figure 4. Schematic representation of relationships between CSF Aβ42 and p-tau and imaging findings.

Figure 5. PiB-PET of a healthy control (A) and AD patient (B). Please note non-specific accumulation of the tracer in white matter in healthy subjects (A) as opposed to cortical deposition in AD (B).

where a robust inverse relationship between cortical PiB binding and CSF Aβ42 was observed. P-tau181 also correlated linearly with cortical amyloid (175).

By and large, a picture emerges where all three AD CSF biomarkers are correlated with different aspects of imaging (Figure 5). A growing body of data challenges the older view that only tau markers are related to cortical deposition in AD (B).

that were independent of tau, even though these effects were significantly reduced after controlling for CSF tau (176). Overall, CSF and imaging markers are reasonably correlated in NL subjects, indicating that even in this stage they reflect a common process or that there may be a causal relationship between them.

9. CSF AD BIOMARKERS AND VASCULAR RISK FACTORS IN NL SUBJECTS

Vascular risk factors have been proven to increase the risk for AD. It is not yet established whether conditions related to increased cardiovascular risk also influence AD CSF markers in NL, making them “more pathological”. Available data yielded inconsistent results: Many large neuropathological studies did not show any increase in amyloid or neurofibrillary pathology with increased vascular brain damage (177), while smaller reports (178) and animal data (179,180) suggest that vascular disease and ischemia can intensify AD pathology.

In line with neuropathological data, we also did not observe differences in biomarkers levels between groups of NL subjects with and without hypertension (130). Others demonstrated that the association between hypertension, t-tau and p-tau181 was modified by the presence of the ApoE genotype. Hypertension had the strongest association with t-tau and p-tau181 levels in ApoE4 homozygotes. Hypertension was not associated with Aβ42 (181).

Some have proposed that insulin resistance could play a role in AD pathogenesis (182). Several observations have driven this hypothesis: 1) impairment of brain glucose metabolism increases the risk of dementia, 2) diabetes (impaired peripheral glucose metabolism) is a risk factor for AD. In line with this theory it was found that CSF Aβ42 was inversely associated with CSF to plasma glucose ratio. This relationship was observed only in ApoE4+ subjects. No significant relationship was found between glucose levels and t-tau or p-tau (183). Others did not find any relationship between HbA1C or fasting glucose levels and t-tau, p-tau, or Aβ42 (184).
White matter lesions (WML) manifesting as hyperintense changes on T2-weighted MRI images are due to small vessel disease (185). Scandinavian researchers observed that WML are associated with low CSF Aβ42 (109). A subsequent study from the same group also indicated an interaction with ApoE4 genotype. This study divided memory clinic subjects with various degrees of subjective or objective cognitive impairment into three groups based on WML load. They found that the odds ratio of having low Aβ42 was significantly higher in the presence of ApoE4 only in the group with high WML load (186). Contrary to these findings, another study failed to reveal an association between WML and any of the CSF AD biomarkers (187).

Regarding other risk factors, it was observed that hypercholesterolemia increased the probability of low CSF Aβ42 levels (109). Others reported that lower CSF Aβ42 as well as higher t-tau and t-tau/Aβ42 were associated with lower body mass index. Somewhat counter-intuitively, the prevalence of “abnormal” biomarkers was higher in normal weight compared to overweight individuals (188). A possible explanation points to systemic energy metabolism dysfunction and sarcopenia as early indicators of AD, rather than obesity later in life as a potential risk factor for AD-type dementias. This view was confirmed in a paper using an ADNI cohort, where the authors found that more abnormal CSF biomarkers were related to lower BMI. However, this was true only across all diagnostic groups (NL, MCI, AD) (189).

Finally, emerging evidence indicates that some strategies like physical exercise aimed at counteracting negative effects of aging and vascular risk are related to favorable CSF biomarker profiles. Liang et al. reported that individuals who engaged more in physical exercise have lower t-tau, ptau181, and higher Aβ42 levels than their sedentary peers (190). Another group observed that in NL adults high intensity physical activity attenuated the changes in CSF Aβ42 induced by a diet high in saturated fat/high glycemic index (191).

The question of whether vascular risk factors contribute to the expression of AD-related pathology or diminish brain reserve, facilitating the clinical manifestation of an already pre-existing AD process, still remains a matter of debate. We believe, however, that the data presented above suggest the possibility of an association between vascular risk factors and amplification of “AD-like” pathology in CSF. This line of thinking is strengthened by work indicating beneficial effects of interventions. Moreover, the relationship between CSF markers and vascular pathology is very likely modified by the ApoE genotype, which is itself related to vascular risk. Further work is needed to reconcile the negative histopathology findings and clinical/CSF data.

10. ETHICAL IMPLICATIONS

The recent developments in biomarker research give rise to an important ethical issue. It has been suggested that the use of biomarkers to identify prodromal AD in NL individuals will not be justifiable until disease-modifying treatments become available. In particular, it has been argued that the use of biomarkers to predict AD in asymptomatic people is unwarranted in the absence of registered drugs with distinct disease-modifying effects (2,192).

There is currently no way to definitively predict who will or will not develop AD, but a patient informed of abnormal biomarker levels might interpret those results as an indication that they will develop the disease. Even when a very reliable early screen is developed, it will likely not attain 100% accuracy. There will therefore be a risk of error, meaning that an individual might be falsely told that they are at high risk for developing AD. The repercussions of such false information could be severe.

However, the prediction of AD in asymptomatic individuals also offers potential benefits, such as the opportunity for individuals to take advantage of existing drug treatments or take other measures to maintain a good quality of life as long as possible. Research and ethical analysis will be necessary to evaluate the risks and benefits of disclosing abnormal biomarker results to asymptomatic individuals.

11. CONCLUSIONS

Our analysis indicates that t-tau, p-tau, and Aβ42 are valuable as biomarkers of AD. At present, their strength relies mostly in supporting neurodegenerative etiology in research criteria for MCI and AD (3,4), and their reasonable capacity to predict the conversion from MCI to AD (1,193). A combination of biomarkers seems to be more useful in prediction than a single analyte.

In cognitively intact subjects, biomarkers reflect major risk factors for AD, such as age and genotype. They correlate with structural and functional brain changes believed to be related to AD. CSF biomarkers may also be of value for predicting cognitive deterioration in this group. However, despite an immense amount of work done so far, their predictive value in NL populations is still uncertain and awaits confirmation in studies offering long follow-up periods and repeated CSF samplings in the same individuals. Growing evidence indicates that conditions other than the “pure AD” process (i.e. vascular risk, lifestyle modifications) may influence biomarker concentrations. This opens new avenues of research into risk assessment and risk modification.

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**Abbreviations:** AD – Alzheimer’s Disease, APP – Amyloid Precursor Protein, APPs – Secreted Amyloid Precursor Protein, CBF – Cerebral Blood Flow, CDR – Clinical Dementia Rating, CSF – Cerebrospinal Fluid, FDG-PET – Positron Emission Tomography with Fluodeoxyglucose, GM- gray matter, MCI – Mild Cognitive Impairment, MMSE- Mini Mental State Examination, NL – Normal Controls, PIB – Pittsburgh Compound B, ADNI – Alzheimer’s Disease Neuroimaging Initiative

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