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## Accepted Manuscript

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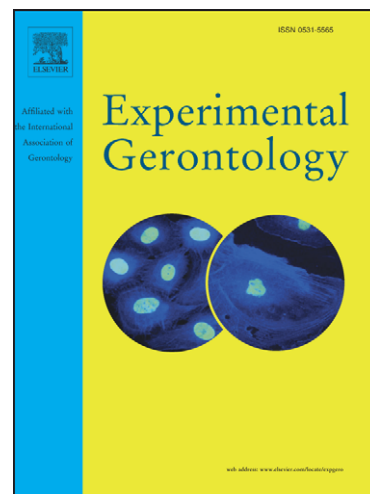
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**Biomarkers for Alzheimer's disease and other forms of dementia: clinical needs, limitations and future aspects**

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**ABSTRACT**

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4 An early diagnosis of Alzheimer's disease (AD) and other types of dementia-causing  
5 disorders is vital in order to achieve effective treatments. Fortunately, in the recent  
6 years the search for specific biomarkers has undergone a rapid evolution. New  
7 technologies in proteomics and genomics have permitted great advances in defining  
8 biochemical markers in cerebrospinal fluid (CSF) and in blood. Novel imaging  
9 techniques are also improving the diagnosis and early detection of brain changes in  
10 vivo. Furthermore, combined analysis of different biomolecules, or of biochemical  
11 and neuroimaging studies, increase diagnostic sensitivity and specificity. However,  
12 the discovery of sensitive and specific biomarkers for neurodegenerative diseases  
13 needs to overcome some important challenges. With the available technology,  
14 standardization of methods is essential to reducing inconsistency and increasing  
15 reliability. Global initiatives, multicenter studies and consensus protocols of analysis  
16 are of critical importance. The present review summarizes the results achieved in the  
17 search for an early diagnosis of neurodegenerative disorders, and reflects the  
18 limitations and the perspectives of the field.  
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42 **Keywords:** Dementia; Alzheimer's disease; proteomics; imaging; biomarkers  
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## INTRODUCTION

1  
2 A diversity of neurodegenerative disorders are associated with the development of  
3 dementia. All of them are characterized by neuronal dysfunctions that eventually lead  
4 to neuronal death. All of them are similarly complex, possibly resulting from several  
5 neuropathological mechanisms acting together. Alzheimer's Disease (AD) is the  
6 most prevalent. It has been calculated that more than half of patients with dementia  
7 have AD, and that this number will double every 20 years (Brookmeyer et al., 1998).  
8 Lewy Body dementia (LBD) is the second most common dementia after AD  
9 (Tarawneh and Galvin, 2007). Also, frontotemporal lobar neurodegenerations such  
10 as frontotemporal dementia (FTD), midbrain disorders such as Parkinson's disease  
11 (PD) or progressive supranuclear palsy (PSP) and vascular dementia (VaD) are  
12 associated with progressive cognitive impairment.

13 Mild cognitive impairment (MCI) is often, but not always, a transitional state between  
14 normal aging and dementia (Winblad et al., 2004). The concept of MCI has been  
15 essentially derived from clinical and neuropsychological settings, and its definition is  
16 continually being revised. To reduce the heterogeneous nature of MCI, a subdivision  
17 of the concept has been further proposed based on the presence of a deficit in the  
18 amnesic domain, a single domain other than the amnesic, and multiple domains  
19 (Winblad et al., 2004). The estimated annual incidence of MCI in population studies  
20 is of about 2% in population studies (Larrieu et al., 2002; Palmer et al., 2008) and  
21 about 5–9% in clinical samples (Adak et al., 2004; de Leon et al., 2001). Few  
22 longitudinal data are available at the population level on MCI subtypes and their role  
23 as a transition between normal aging and dementia. The rates of transition between  
24 MCI and AD have been calculated between one-third to one-half for the MCI-  
25 amnesic subtype (Palmer et al., 2008; Petersen et al., 1999) and two-thirds for MCI-  
26 multidomains subtype over a period of three years. However, a substantial number of  
27 MCI patients are reclassified as normal aging on follow-up (Larrieu et al., 2002) and  
28 many MCI do not progress to any sort of dementia (de Jager and Budge, 2005). In  
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1 other words, the standard clinical diagnostic evaluations, including  
2 neuropsychological tests failed to identify those people with global cognitive deficits  
3 who have high risk of progressing to dementia.  
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6 As cognitive decline advances, other specific disease-related features appear, such  
7 as visual hallucinations and Parkinsonism in LBD (McKeith et al., 1996) or  
8 personality changes in FTD (Neary et al., 2005). Although the clinical picture of these  
9 diseases is often related to the area of the brain that is primarily affected, mixed  
10 pathologies are common and clinical manifestations in early disease phases are  
11 often similar. Furthermore, a single type of pathology can produce different cognitive  
12 outcomes making the diagnose difficult.  
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22 There is evidence that the pathological changes in dementia-causing diseases begin  
23 decades before the appearance of the first clinical manifestations (Jack et al., 2009).  
24 The challenge of finding effective treatments for AD and other dementias is parallel  
25 to the prospect of detecting the preclinical changes at the earliest stage possible, and  
26 of accurately identifying the pathology (or pathologies) responsible. To develop and  
27 validate biomarkers capable to early detect and discriminate between disorders with  
28 similar clinical outcomes is one of the most important goals of neuroscience  
29 research.  
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40 The quest for biomarkers for AD and other dementias includes research in multiple  
41 fields: genomics, proteomics, structural and functional imaging and neurophysiology.  
42 The present review summarizes the most recent advances and analyses the current  
43 and future directions in the pursuit of biomarkers for AD and other dementia  
44 disorders.  
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## BIOCHEMICAL MARKERS

### Biomarkers in CSF

CSF is a useful diagnostic window to neurodegenerative diseases for many reasons, mainly because it reflects metabolic processes in the brain in a direct manner, due to the free exchange of molecules between the brain and the CSF (Reiber and Peter, 2001). Amyloid plaques in AD brain are mainly composed of A $\beta$ -42 and A $\beta$ -43 (Welander et al., 2009), while in cerebral amyloid angiopathy (CAA) predominantly contain A $\beta$ -40. The CSF biomarkers total tau (tTau), hyperphosphorylated tau (p-Tau), and the 42 amino acid form of A $\beta$  (A $\beta$ -42) are now established markers for AD (Zetterberg et al., 2003) and can identify AD in the early, MCI stage of the disease with high accuracy (Hansson et al., 2006). CSF of AD and MCI patients shows decreased values of A $\beta$ -42 and increased total tau (tTau) or phosphorylated tau (pTau) (Ewers et al., 2007). Recent studies have been focused in investigating if the pattern of different length A $\beta$  peptides could enhance the diagnostic power. The levels of A $\beta$  (1-37, -38, -39, -40, -42) gave a 91% sensitivity and a 64% specificity in predicting the development of AD from MCI during a follow-up study (Hoglund et al., 2008). While progressive increase of CSF tTau CSF concentrations was found along the progression of AD (Andersson et al., 2007), this is not the case for A $\beta$ -42 levels (Stefani et al., 2006). Both A $\beta$  and tau levels in CSF are influenced by other factors that participate in AD, such age and apoE. Aging and the presence of the APOE  $\epsilon$ 4 allele accelerate the deposition of A $\beta$ -42 in brain and decrease of CSF A $\beta$ -42 levels (Peskind et al., 2006). Also, CSF ApoE levels in correlate with t-tau and 24S-hydroxycholesterol (24S-OHC) in patients with cognitive disorders (Shafaati et al., 2007). The association between A $\beta$  load in the brain and CSF A $\beta$  reduction remains puzzling, but it has been suggested that aggregation of this peptide into oligomeric forms could result in concomitant reduction of soluble forms in CSF and in the brain,

1 and increased A $\beta$  deposition in plaques. Also, the relationship between CSF  
2 biomarkers (A $\beta$ -42, t-tau and p-Tau) and plaque and tangle burden in the brain is not  
3 clear. Although initial studies suggested a direct correlation between that CSF p-Tau  
4 and neurofibrillary pathology in AD patients (Buerger et al., 2006), recent reports  
5 have shown no association (Buerger et al., 2007; Engelborghs et al., 2007).  
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10 Both increased CSF tau and decreased A $\beta$  is also seen in other neurodegenerative  
11 disorders (Jellinger et al., 2008). P-tau181 differentiates AD and LBD, whereas p-  
12 tau231 differentiates AD and FTD (Hampel et al., 2004). Recent studies have also  
13 shown associations between several A $\beta$  peptides and specific dementias. For  
14 example, decreased A $\beta$ -38 levels correlated with FTD (Bibl et al., 2007b) and A $\beta$ -37  
15 (Bibl et al., 2006) with LBD.  
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26 Neurofilament light unit has been suggested as a possible CSF biomarkers to  
27 distinguish VaD from AD (Petzold et al., 2007). CSF neurofilament protein levels may  
28 also help to discriminate between FTD and early onset AD (Petzold et al., 2007).  
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34 However, the combination of tau and A $\beta$  measurements in CSF enhance the  
35 accuracy of the diagnostic power. CSF tau/A $\beta$ -42 ratio predicts cognitive decline in  
36 cognitively intact older adults (Fagan et al., 2007) and in individuals with MCI  
37 (Hansson et al., 2007; Li et al., 2007). Moreover, grouping these biomarkers is today  
38 the only biochemical tool with enough sensitivity and specificity to distinguishing AD  
39 from other dementias in the early stages (Finehout et al., 2007; Wiltfang et al., 2005),  
40 LBD (Mollenhauer et al., 2006a) and PD patients with dementia (Mollenhauer et al.,  
41 2006b).  
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52 Several other CSF biomarkers for AD and other dementia disorders have been  
53 described (Table 1). Some of these molecules have been shown to play a role in AD  
54 pathology, like the  $\beta$ -secretase BACE1 (Ewers et al., 2008; Vassar et al., 1999),  
55 cholesterol carriers (Mollenhauer et al., 2006a), cholesterol metabolites (Bjorkhem et  
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1 al., 2009; Mateos et al., 2009), or antioxidant molecules (Akterin et al., 2006), while  
2 other have an unknown relation to the disease. Comparative proteomics or  
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4 metabolomics of CSF detecting a large number of molecules may provide a further  
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6 means to diagnose and assess neurodegenerative diseases. However some  
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8 considerations need to be taken into account when designing future studies,  
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10 including the standardization of CSF extraction and the avoidance of factors like  
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12 blood contamination, CSF gradient effects, and storage differences.  
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## Biomarkers in plasma

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3 An ideal early detection of different types of dementia would require simple, non-  
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An ideal early detection of different types of dementia would require simple, non-invasive and non-expensive diagnostic tests. However, up to date, no validated peripheral diagnostic markers for the early and differential diagnosis of dementia-causing disorders are available. The structure of the blood brain barrier (BBB) limits the presence of potential biomarkers that are closely associated to brain pathogenesis to small molecules, lipophilic molecules, and molecules with specific transporters (Irizarry, 2004). Brain derived proteins and metabolites that pass into the plasma will also become markedly diluted in a biochemically complex medium. It is also possible the presence of high intra- and inter-person variability due to factors that could influence the levels of a potential biomarker. The majority of the efforts in finding plasma biomarkers for neurodegenerative disorders have been made in AD, starting more than a decade ago with the traditional approach of measuring one molecule closely related to the pathology of the disease, in this case beta-amyloid (A $\beta$ ) (Mayeux et al., 1999; Tamaoka et al., 1996; Vanderstichele et al., 2000). Plasma total A $\beta$  or A $\beta$ -42 are increased in cases of familial AD (Kosaka et al., 1997; Scheuner et al., 1996) and in Downs syndrome with amyloid precursor protein (APP) triplication (Schupf et al., 2001) but the results were not consistent with the diagnosis of sporadic AD (Assini et al., 2004; Fukumoto et al., 2003; Mayeux et al., 1999; Schupf et al., 2001; Tamaoka et al., 1996; Vanderstichele et al., 2000). Peripheral A $\beta$  is transported via receptor for advanced glycation end products (RAGE) across the BBB into the brain (Deane et al., 2004) and A $\beta$  elimination from brain across the BBB by cell surface low-density lipoprotein receptor related protein-1 (LRP) (Sagare et al., 2007). On the other hand, APP is also produced by platelets and is thus an alternative source A $\beta$  of exists in plasma.

Several studies have investigated plasma A $\beta$  levels and most groups have found no significant differences between AD and control cases (Fukumoto et al., 2003; Kosaka

1 et al., 1997; Mayeux et al., 1999; Scheuner et al., 1996; Tamaoka et al., 1996;  
2 Vanderstichele et al., 2000). Similarly, other studies showed that elevated levels of  
3 A $\beta$ -42 (van Oijen et al., 2006) low levels of A $\beta$ -40 (Sundelof et al., 2008) or a  
4 reduced A $\beta$ -42/A $\beta$ -40 ratio in plasma (Graff-Radford et al., 2007) in aging could  
5 indicate the conversion from a normal cognitive status to MCI or AD. In contrast,  
6 others reported that reduced A $\beta$ -42 plasma levels might reflect the evolution from  
7 normal aging or MCI to AD (Song et al., 2007), rather than a marker for  
8 neuropathological events occurring in the disease.  
9

10 CSF A $\beta$  levels do not correlate with plasma A $\beta$  levels in individual patients (Mehta et  
11 al., 2001; Vanderstichele et al., 2000). As previously discussed, other sources of A $\beta$ ,  
12 such as platelets, are contributing to the total A $\beta$  present in plasma. Also, it has been  
13 shown that the concentration of A $\beta$  in plasma is considerably influenced by  
14 simultaneous medication (Blasko et al., 2005), including insulin treatments (Kulstad  
15 et al., 2006).  
16

17 Decreases in plasma A $\beta$  have been shown in cerebral CAA, amyotrophic lateral  
18 sclerosis, and LBD (Jellinger et al., 2008), and a decrease in the ratio of plasma A $\beta$ -  
19 38/-40 peptides in vascular dementia (VaD) (Bibl et al., 2007a).  
20

21 Although not useful for diagnosis, plasma A $\beta$  measurement could be potentially  
22 helpful in clinical studies to evaluate the pharmacological effects of modulators of  
23 APP processing, such as  $\beta$ - or  $\gamma$ -secretase inhibitors.  
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25 Plasma homocysteine has been shown to be directly related to A $\beta$ -40 levels, while  
26 the association with A $\beta$ -42 was not significant, suggesting that homocysteine is  
27 related to aging but not specifically to AD, but it could interact to affect AD risk and  
28 cognition in PD (Irizarry, 2004; Luchsinger et al., 2007). Elevated homocysteine is a  
29 risk factor for cardiovascular disease (Boushey et al., 1995) but its relation with AD  
30 risk is unknown.  
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Chronic inflammation with microglia activation is believed to play a central role in AD pathogenesis. Whether the accumulation of inflammatory markers within the brain is also reflected in serum or plasma is unclear because many of these proteins do not easily cross the BBB. Inflammatory molecules including C-reactive protein, interleukin (IL)-1 $\beta$ , tumor necrosis factor- $\alpha$ , IL-6, IL-6 receptor complex,  $\alpha$ 1-antichymotrypsin and transforming growth factor- $\beta$  show inconsistent changes across studies, while other cytokines such as IL-12, interferon- $\alpha$ , and interferon- $\beta$  remain unchanged (Teunissen et al., 2002).

Increasing evidence has suggested that cholesterol, or cholesterol metabolism, plays a role in some neurodegenerative disorders including AD. The relation between hypercholesterolemia and AD or MCI has been confirmed repeatedly (Evans et al., 2004; Kivipelto et al., 2002; Launer et al., 2001). The presence of the E4 isoform of the apolipoprotein E (apoE), the main cholesterol transporter in the body, is the major known risk factor for AD and has been associated with either risk or severity of several other dementia-causing disorders (Cedazo-Minguez, 2007). The presence of the APOE  $\epsilon$ 4 allele has been associated with less apoE protein in plasma (Schiele et al., 2000) However, there was no consistent association of plasma apoE levels and AD. Studies have documented elevated apoE levels (Taddei et al., 1997) no difference, (Panza et al., 2003) or reduced levels relative to controls (Siest et al., 2000). Blood lipids are modifiable by dietary or pharmacologic intervention, and the lipoprotein cholesterol profile is an established marker of the effects of cholesterol-lowering medications and the associated reduction in cardiac risk. Metabolites of cholesterol such as 24S-hydroxycholesterol (24S-OHC) and 27-hydroxycholesterol (27-OH) can pass the BBB, and a large amount of 27-OH is taken up from the blood, acting as an important link between extra-cerebral and intra-cerebral pools of cholesterol (Bjorkhem et al., 2009). The central origin of 24S-OHC and its relatively constant hepatic metabolism has led several investigators to examine if 24S-OHC

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may be a marker of brain cholesterol balance. Patients with advanced AD have significantly reduced levels of 24S-OHC in plasma (Bretillon et al., 2000; Kolsch et al., 2000). In a recent work it was shown that plasma levels of 24S-OHC decrease in parallel with decreases in the volume of the caudate measured with MRI in patients with different stages of Huntington's disease (Teunissen et al., 2007). Very recent studies on patients with PD have reported a markedly decreased level of 24S-OHC in plasma, which is consistent with its possible utility as a marker of CNS neuronal mass (Bretillon et al., 2000). Although 24S-OHC is not a diagnostic marker in itself, it may be a modifiable risk factor. It has been suggested that the determination of 24S-OHC levels may complement MRI morphology as a valuable tool to follow neurodegenerative changes in the early stages of this disease.

Other proteins present in plasma with recently discovered associations to AD pathology have also been proposed as biomarkers, such ALZAS (ALzheimer ASsociated protein). Pilot studies in serum of patients with probable AD have detected an up to tenfold increase of ALZAS in patients with MCI, but only moderately increased titres in autopsy-confirmed AD (Jellinger et al., 2008).

Since the pathological processes in neurodegenerative disorders are multiple, the diagnostic accuracy may be further improved by combining several markers. Recent proteomic discovery of various plasma signaling proteins may allow the development of a simple, cost-effective test for AD (German et al., 2007; Ray et al., 2007). A multifactorial biomarker can be approached in two ways: a "knowledge-based" approach, incorporating "known" putative biomarkers (molecules with known association with the disease); or an unbiased survey of many hundreds or thousands of molecules. A few knowledge-based approaches have been attempted for AD, integrating selected molecules known to be involved in the disease (Teunissen and Scheltens, 2007). In one study a panel of 29 serum biomarkers for inflammation,

1 homocysteine metabolism, cholesterol metabolism, and brain specific proteins were  
2 evaluated. A model incorporating IL-6 receptor, cysteine, protein fraction  $\alpha$ 1 and  
3 cholesterol levels proved to be the best combination to discriminate AD from  
4 controls, although specificity to other cognitive disorders and PD was weaker  
5 (Teunissen and Scheltens, 2007). In another study, 120 different signaling proteins  
6 were evaluated and a model including 18 proteins was generated. This combination  
7 showed high accuracy (89%) to differentiate 42 AD from 39 control cases and in  
8 predicting those MCI patients that later converted to AD (Ray et al., 2007).

9 Unbiased approaches have been pursued to evaluate a broad range of proteins  
10 (proteomics), small molecule metabolites (metabolomics), or transcripts  
11 (transcriptomics) in blood. A proteomic study using 2D-PAGE identified more than 70  
12 proteins that differed between AD and control cases (Ueno et al., 2000). In another  
13 proteomic case-control study the combination of several molecules gave a sensitivity  
14 of 56% and specificity of 80% (Hye et al., 2006). The complexity of serum and  
15 plasma, and difficulties in assay standardization make these approaches challenging,  
16 but the rapid evolution in technological and computational facilities will allow greater  
17 diagnostic accuracy and broader applicability of these methods.

## 42 **GENETIC BIOMARKERS**

43 Another approach to seek for biomarkers for neurodegenerative diseases is the  
44 transcriptional profiling of genes in peripheral samples. The potential use of blood-  
45 based gene expression profiling in the diagnosis of brain disorders has been  
46 described by several independent groups (Burczynski and Dorner, 2006; Sharp et  
47 al., 2006). Expression profiling of whole blood RNA offers several advantages in  
48 deciphering aberrant patterns of gene regulation in neurodegenerative processes: it  
49 might predict much earlier changes than that measured by means of protein  
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1 alteration; the uniform chemical nature of RNA make transcriptome studies less of a  
2 challenge than both proteome and metabolome studies and it has been shown that  
3 peripheral blood cells shares significant gene expression similarities with central  
4 nervous system (CNS) tissues (Burczynski and Dorner, 2006; Scherzer et al., 2007).  
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6 A supportive example of this is a recent study that showed that the Parkinson  
7 disease-linked  $\alpha$ -synuclein gene was up regulated both in blood and in the substantia  
8 nigra of patients with Parkinson disease (Kalman et al., 2005). Some gene  
9 expression studies have been done for AD biomarker discovery using blood as the  
10 clinical sample. A global gene expression analysis on fibroblasts from 33 individuals  
11 (both healthy and demented mutation carriers as well as wild-type siblings) from  
12 three families segregating the swedish APP, artic APP and presenilin 1 H163Y  
13 mutations showed that mutation carriers share a common gene expression profile  
14 significantly different from that of their wild-type siblings (Nagasaka et al., 2005). This  
15 suggests that the disease process starts decades before the onset of cognitive  
16 decline, and that presymptomatic diagnosis of AD may be feasible in the near future.  
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18 Another study of AD patients vs. controls identified a group of 20 candidate genes  
19 (from 3200 genes investigated) that showed an altered expression in AD (Kalman et  
20 al., 2005). Transcriptional profiling of Alzheimer blood mononuclear cells by  
21 microarray identified 19 up- and 136 downregulated genes comparing to controls  
22 (Maes et al., 2007). In a recent study, the expression pattern of 33 genes (including  
23 APP, Insulin degrading enzyme, Histone cluster 1, H3e (HIST1H3E) and  
24 Cannabinoid receptor 2 (CNR2)) was investigated in whole blood samples from a  
25 population clinically diagnosed with AD and from healthy controls. These 33 genes  
26 were chosen according to previous findings of gene expression profiling in  
27 postmortem AD brains (Grunblatt et al., 2007; Jacob et al., 2007). The profiling of  
28 these genes was carried out four times in a period of one year in correlation to AD  
29 and it was found that the expression of HIST1H3E was correlated in a negative  
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1 manner to the MMSE scores, while. the expression of CNR2 showed some tendency  
2 to positively correlate with the decreased MMSE score (Grunblatt et al., 2009).

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4 The promising results point to the fact that gene expression profiling might be a  
5 promising method to seek for biomarkers for early dementia. More studies done in a  
6 longitudinal large scale manner and including multiple considerations such as  
7 diagnosis, MMSE score, and medication are need in order to establish the sensitivity  
8 and the specificity of this diagnostic tool.  
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## 20 **NEUROPHYSIOLOGICAL BIOMARKERS**

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24 In comparison with biochemical or imaging approaches little attention has been paid  
25 to the application of electroencephalograms, quantitative electroencephalography  
26 (qEEG), event-related potential (ERP), transcranial magnetic stimulation (TMS), and  
27 vagus nerve stimulation (VNS) as useful clinical markers of early disease detection  
28 or progression. A recent meta-analysis explored the diagnostic accuracy of  
29 spontaneous EEG in dementia in articles published between 1980 and 2008,  
30 concluding that this method was not sufficient for the initial diagnosis of MCI in  
31 routine clinical practice (Jelic and Kowalski, 2009)  
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42 Nonetheless, in parallel with the dramatic improvements in technology recent  
43 promising findings have been reported. Multiple different qEEG components, as well  
44 as ERP activity, are altered during the transitional stage between healthy aging and  
45 AD (Jackson and Snyder, 2008; Koenig et al., 2005). For example, abnormalities in  
46 P600 and N400, two ERP components, have been associated with the conversion  
47 from amnesic MCI to AD (Olichney et al., 2008). Recently, a novel VNS technique,  
48 vagus somatosensory-evoked potentials, was developed in an attempt to detect  
49 dementia at an early stage (Merrill et al., 2006).  
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60 The profiling of EEG, ERP or VNS markers for the diagnostic assessment of early  
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1 cognitive changes is important. These are simple and cost-effective methods that  
2 could help in the identification of patients with MCI and in the prediction of which  
3 individuals will ultimately progress from MCI to AD. This would allow the use of  
4 therapeutic interventions targeted to those persons at greatest risk for conversion to  
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ACCEPTED MANUSCRIPT

**NEUROIMAGING**

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4 Along the progression of the neuropathological processes, continuous morphological  
5 and functional changes occur in the brain, which can be detected and quantified by  
6 several brain imaging techniques. The most apparent aging-related brain change is  
7 an atrophy resulting from synaptic and neuronal loss. This atrophy is larger in  
8 individuals with dementia and often shows a regional variation depending on the form  
9 of dementia that the patient suffers (Small et al., 2008). However, there are also  
10 areas in which anatomical changes do not distinguish between these disorders.

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20 Habitually, neuroimaging techniques are classified as either structural or functional,  
21 according to the principal information that they give. Structural imaging techniques as  
22 computed tomography (CT) or magnetic resonance imaging (MRI) are often done to  
23 rule out other causes of dementia, such as normal pressure hydrocephalus or some  
24 particular lesions like tumors. Also, they can serve to clarify the diagnosis by  
25 detecting areas with atrophy or vascular lesions. While CT provides good spatial  
26 resolution (the ability to distinguish two structures from each other as separate), MRI  
27 provides comparable resolution with far better contrast resolution (the ability to  
28 distinguish the differences between two arbitrarily similar but not identical tissues).

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40 Functional imaging techniques, such as PET or SPECT, provide information about brain  
41 structure as well, but their spatial resolution is lower than structural techniques.  
42 However, their strong side is that they can be used to measure parameters of brain  
43 metabolism, like regional cerebral blood flow or regional cerebral glucose  
44 metabolism. Importantly, these parameters are often impaired in AD and other  
45 dementias before the morphological changes occur. Therefore functional imaging  
46 techniques are more suitable for helping the physicians in an earlier diagnosis. The  
47 clinical use of available neuroimaging techniques is expanding. At the same time,  
48 new technologies are now undergoing rapid evolution and novel applications of  
49 imaging techniques are being explored.  
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## Magnetic Resonance Imaging (MRI)

MRI has high-resolution and gives high-quality information on brain structure, including delineation of grey and white matters. Generally, patients with AD show atrophy of the parietal lobes and the hippocampus on MRI compared to controls (Jack et al., 1997) (Figure 1). Several structural MRI studies localize the pattern of the atrophy in early-onset AD to more posterior regions (precuneus and posterior cingulate) (Frisoni et al., 2007; Karas et al., 2007), amygdala (Krasuski et al., 1998), occipital lobes, corpus callosum and prominent posterior cortical involvement (Teipel et al., 2003). Atrophies in hippocampus and entorhinal cortex can predict memory progression (Mungas et al., 2005) and have shown to be associated with increased risk of developing AD (Apostolova et al., 2006). However, it has also been shown that these changes are not specific to AD (Rodrigue and Raz, 2004). In clinical practice, structural MRI can help to support clinical diagnosis, but is not sufficient for establishing a definitive diagnosis, as there is a substantial overlapping between atrophy associated with normal aging and several neurodegenerative disorders, including AD. Non-specific age-related changes in the white matter appear frequently in elderly patients with stroke or MCI, but are also common in healthy elderly individuals.

On the other hand, some studies have shown some discriminative diagnostic power using structural MRI. For example, patients with amnesic MCI who converted to AD showed larger atrophy in several areas including hippocampus and inferior and middle temporal gyri, compared with non-converters (Chetelat et al., 2005). With the progression of AD, the corpus callosum usually appears atrophic anteriorly, differentiating this disease from FTLN, in which the posterior area of the corpus callosum is more affected (Likeman et al., 2005).

It is expected that with the development of higher resolution volumetric MRI and new automated, computerized analytical programs (as voxel-based morphometry), the

1 capacity to identify subtle specific abnormality patterns in various forms of dementia  
2 will be greater, and the diagnostic utility of MRI improved.  
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### 6 **Functional MRI (fMRI)**

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8 Functional MRI (fMRI) measures brain function over time during rest, or more  
9 commonly, in association with a cognitive task or stimulus that require the  
10 involvement of related brain areas and networks (Wagner et al., 1998). Blood  
11 Oxygen Level Dependent fMRI is the most common functional imaging method and  
12 measures alterations in blood flow associated with neuronal activity, which indirectly  
13 reflects brain activity. Currently, the high inter- and intra-individual variability of the  
14 signals and its inherent dependency of hemodynamics has precluded the use of this  
15 technique for differential diagnostic of dementias. However, fMRI could be useful to  
16 characterize the functional abnormalities specific to every disease. For example, in  
17 comparison to controls, individuals with AD show lowered brain activity in parietal  
18 and hippocampal regions and higher activity in primary cortices unaffected by the  
19 disease. Some studies found differential fMRI activation responses to a specific  
20 paradigms in MCI compared to healthy controls (Teipel et al., 2003). Recent  
21 advances in fMRI have helped to define intrinsic functional networks in the human  
22 brain (Fox and Raichle, 2007). The study of cognitive-behavioral functions in early  
23 phases of neurodegenerative disorders could identify the neuroanatomical networks  
24 affected by these diseases (Sperling, 2007) and it is possible that function-critical  
25 neural networks could be affected differentially by variant neurodegenerative  
26 diseases (Seeley et al., 2009). This novel perspective has opened new exciting  
27 possibilities for the use on fMRI in the differential diagnosis of dementia-causing  
28 disorders.  
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### 58 **Positron Emission Tomography (PET) and Single Photon Emission Computed** 59 **Tomography (SPECT)** 60 61 62 63 64 65

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Molecular imaging by PET or SPECT with radiopharmaceuticals permits quantitative evaluation of physiological functions, protein pharmacokinetics and distribution of receptors with high sensitivity. PET and SPECT have been extensively evaluated as diagnostic procedures for dementia, since they have demonstrated diagnostic and prognostic utility for evaluating patients with cognitive impairment and in distinguishing among primary neurodegenerative disorders.

Studies often use the radiolabelled glucose [18F]FDG-PET to measure cerebral glucose metabolism, which indirectly indicates synaptic activity. In AD, FDG-PET studies have shown distinctive symmetric cortical hypometabolism from posterior cingulate and parietal regions, spreading to the temporal and prefrontal cortex (Figure 1) (Pakrasi and O'Brien, 2005; Silverman et al., 2001). These alterations in AD have been shown to be distinctive from controls and from other types of dementia and to correlate with cognitive impairment in MCI patients (Small et al., 2008). In terms of diagnostic accuracy, PET assessment of glucose metabolism has been found to have high sensitivity (94%) but low specificity (73–78%) (Silverman et al., 2001). Similar specificity for diagnosis has been found with SPECT studying regional blood flow with Tc-hexamethylpropyleneamine oxime (O'Brien, 2007).

Several low-molecular-weight probes have been developed to measure A $\beta$  deposits with PET in vivo. The most studied is the Pittsburgh compound B (PIB) that shows significantly greater cortical binding in patients with AD compared with controls (Figure 1) (Klunk et al., 2004). A 2-year longitudinal follow-up showed PIB not significant binding increase in AD patients despite declines in glucose metabolism and cognitive function (Engler et al., 2006). PIB shows retention in patients with CAA, low binding with variable pattern in LBD, and absent binding in FTD (Rowe et al., 2007). PIB binding in MCI patients appeared bimodal, with approximately 50% of MCI patients showing high binding (like AD patients), and 50% low binding (as cognitive normal controls). Amyloid binding studies in vivo may serve also to better

1 define the role of A $\beta$  in AD. Recently, correlation of PIB binding with brain atrophy  
2 (determined by MRI) has been shown. Higher amyloid deposition was generally not  
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4 associated with more severe gray matter atrophy, except in the medial temporal  
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6 lobes (amygdala and hippocampus), suggesting that different brain areas have  
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8 diverse susceptibility to amyloid toxicity, or that amyloid is nonessential for  
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10 neurodegeneration (Frisoni et al., 2009).  
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13 New PET ligands, such as 2-(1-6-[(2-[F-18]fluoroethyl)(methyl)amino]-2-  
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15 naphthyl)ethylidene)malononitrile ([18F]FDDNP), are being developed to measure  
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17 amyloid plaques and tau inclusions that may facilitate the investigation of this  
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19 pathologies in living humans (Braskie et al., 2008). When comparing binding  
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21 potential of PIB and [18F]FDDNP it was found low correlation between them, with  
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23 differences in regional binding and discrepant findings in MCI, suggesting that they  
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25 measure related, but different, characteristics of the disease (Tolboom et al., 2009).  
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## CONCLUSION

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2 The current biochemical and neuroimaging markers have very limited predictive  
3 value. Combining neuroimaging studies with the analysis of some CSF proteins  
4 increase diagnostic sensitivity and specificity, especially in AD, but the predictive  
5 significance is still narrowed to the final stages of the disease (Figure 2).  
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9 A major limitation in the field is that markers discovered by one group cannot be  
10 reproduced by other groups. Most of dementia-causing diseases are complex and  
11 heterogeneous, but it is also true that diagnostic criteria and analytical methodology  
12 varies among studies.  
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15 Standardization of methods seems critical to reducing inconsistency and increasing  
16 reliability. It is necessary to implement common protocols for sample preparation,  
17 experimental design and generation proteomics data. Global initiatives of  
18 standardization are of critical importance. A good example is a recent study coming  
19 from the Swedish Brain Power initiative. Mattsson et al (Mattsson et al., 2009)  
20 evaluated the utility of the classical CSF biomarkers for AD (A $\beta$ 42, T-tau and P-tau)  
21 by including a large number of samples from several European centers. The study  
22 showed that levels A $\beta$ 42, T-tau and P-tau in CSF could predict to some extent the  
23 outcome of individuals with MCI. However, the sensitivity and specificity of these  
24 markers were sufficient to be used for screening but not as an early diagnostic test.  
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26 The inclusion of other molecules could improve the power of diagnostics.  
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29 An early detection of different types of dementia using non-invasive diagnostic tests  
30 is the ultimate dream. However, the potential use of proteomic analysis of serum or  
31 plasma faces increased difficulties in assay standardization, and would require  
32 additional multilateral efforts.  
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35 The rapid evolution of technological and computational advances will eventually  
36 permit the discovery of biomarkers for an early and differential diagnosis of  
37 dementia-causing disorders. Identifying the individual risk for having AD or related  
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disorders will allow the development of preventive / delaying strategies.

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**FIGURE LEGENDS.**

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4 **Figure 1. Representative examples of brain images (MRI, and PET) from age**  
5 **matched cognitive intact individuals and Alzheimer's disease patients.**

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7 MRI pictures of brains from a control and an AD patient. AD brain shows (symmetric)  
8 hippocampus atrophy and enlargement of lateral ventricles.

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13 PET pictures of AD brains showed reduced glucose metabolism mainly in parietal  
14 areas (determined with the radiolabelled glucose [18F]FDG) and increased cortical  
15 A $\beta$  load (determined with the Pittsburgh compound B (PIB)).  
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22 **Figure 2. Diagnostic and treatment possibilities along the evolution to**  
23 **Dementia.**  
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**TABLE 1: Molecules that showed differential levels in CSF from Alzheimer's Disease patients as compared to controls.**

PROTEIN	Up / Downregulated and references
Albumin	↑(Abdi et al., 2006; Zhang et al., 2005)
Amyloid $\beta$ A4 protein	↑(Abdi et al., 2006; Simonsen et al., 2007; Zhang et al., 2005)
Angiotensinogen	↑(Zhang et al., 2005)
Apolipoprotein AI	(Castano et al., 2006; Davidsson et al., 2002; Puchades et al., 2003)↓
Apolipoprotein AII	↑(Abdi et al., 2006; Zhang et al., 2005)
Apolipoprotein E	(Davidsson et al., 2002; Puchades et al., 2003)↓
BACE 1	↑(Finehout et al., 2007)
24S-Hydroxycholesterol	↑(Ewers et al., 2008; Zhong et al., 2007)
C3a	↑(Leoni et al., 2006)
C3a	↑(Simonsen et al., 2007; Simonsen et al., 2008)
C4a	↑(Abdi et al., 2006; Simonsen et al., 2007; Zhang et al., 2005)
Cystatin C	↑(Carrette et al., 2003; Hu et al., 2005)
Cystatin C, 8 amino acid N-terminal truncation	↑(Simonsen et al., 2007; Simonsen et al., 2008)
Immunoglobulin heavy chain	↓(Abdi et al., 2006; Zhang et al., 2005)
Leucine-rich repeat-containing protein 4B	↑(Abdi et al., 2006; Zhang et al., 2005)
Leucine-rich repeat-containing protein 4B	↓(Abdi et al., 2006; Zhang et al., 2005)
N-acetyllactosamine	↓(Abdi et al., 2006; Zhang et al., 2005)
Neuronal pentraxin-1	↑(Abdi et al., 2006; Finehout et al., 2007; Zhang et al., 2005)
Prostaglandin-H2 D-isomerase	↓(Korolainen et al., 2007; Puchades et al., 2003)
	↑(Hu et al., 2005)
Retinol-binding protein	↑(Abdi et al., 2006; Davidsson et al., 2002; Zhang et al., 2005)
	↓(Jung et al., 2008; Puchades et al., 2003)
Thioredoxin	↑(Abdi et al., 2006; Hu et al., 2005)
Transthyretin	↓(Castano et al., 2006; Korolainen et al., 2007; Puchades et al., 2003)
	↑(Abdi et al., 2006; Davidsson et al., 2002; Finehout et al., 2007; Zhang et al., 2005)
VGF	↓(Carrette et al., 2003; Simonsen et al., 2007; Simonsen et al., 2008)
$\alpha$ -1-Antitrypsin	↑(Abdi et al., 2006; Finehout et al., 2007; Zhang et al., 2005)
	↓(Puchades et al., 2003)
$\alpha$ -1 $\beta$ Glycoprotein	↑(Hu et al., 2005; Puchades et al., 2003)
$\alpha$ -2HS Glycoprotein	↑(Zhang et al., 2005)
	↓(Puchades et al., 2003)
$\beta$ Fibrinogen	↑(Abdi et al., 2006; Finehout et al., 2007)
$\beta$ -2-Microglobulin	↑(Abdi et al., 2006; Carrette et al., 2003; Davidsson et al., 2002; Hu et al., 2005; Simonsen et al., 2007)
	↓(Puchades et al., 2003; Simonsen et al., 2008)

