

Review

Circulating biomarkers of cognitive decline and dementia

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Abstract

Plasma and serum biochemical markers proposed for cognitive decline of degenerative (Alzheimer’s disease, AD) or vascular origin and predementia syndromes (mild cognitive impairment and other related entities) are based on pathophysiologic processes such as lipoprotein metabolism (total cholesterol, apolipoprotein E, 24S-hydroxy-cholesterol), and vascular disease (homocysteine, lipoprotein(a)); SP formation (amyloid β (A β)-protein, A β autoantibodies, platelet APP isoforms), oxidative stress (isoprostanes, vitamin E), and inflammation (cytokines). This review will focus on the current knowledge on circulating serum and plasma biomarkers of cognitive decline and dementia that are linked to cholesterol homeostasis and lipoprotein abnormalities, senile plaque formation and amyloid precursor protein (APP) metabolism, oxidative stress, and inflammatory reactions. Special emphasis will, however, be placed on biomarkers related to lipoprotein metabolism and vascular disease. Analytically, most plasma and serum proteins or metabolites lack reproducibility, sensitivity, or specificity for the diagnosis, risk and progression assessment, or therapeutic monitoring of AD and other dementing disorders. Measures linked to lipoprotein metabolism and vascular disease, APP metabolism, oxidative stress, or inflammation appear altered in AD relative to controls, but lack sufficient discriminatory power. Measures combining several biomarkers or incorporating a range of proteins in plasma and small molecule metabolites are promising approaches for the development of plasma or serum-based diagnostic tests for AD and other dementing disorders, as well as for predementia syndromes.

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1. Introduction

Projections indicate that with progressive aging of the populations, the prevalence of cognitive decline and dementia are expected to increase in the next years. Alarming predictions notwithstanding, significant advances have been made in the identification, understanding and prevention of dementia. In fact, subtle cognitive decline can precede the appearance of symptomatic Alzheimer's disease (AD) by many years [1]. As such, current clinical research has focused on the identification of early diagnostic indices of dementia.

Mild cognitive impairment (MCI) is, at present, the most widely used term to indicate nondemented aged persons with a mild memory or cognitive impairment that cannot be accounted for any recognized medical or psychiatric condition [2,3]. Different diagnostic criteria have been proposed. Terminology such as age-related cognitive decline (ARCD) [4] and aging-associated cognitive decline (AACD) [5] have been recently proposed to distinguish individuals with mild cognitive disorders associated with aging from non affected individuals. At present, it difficult to establish whether these entities are an expression of a normal aging process, are clinically distinguishable from dementigen syndromes, or are eventually a continuum with dementia. In fact, while MCI is assumed to be pathology-based and therefore amenable to intervention, ARCD and AACD are generally considered nonprogressive, a phenomenon of normal aging. Furthermore, MCI may be a prodromal phase of dementia, with estimates of 12% of MCI patients developing dementia in 1 year [2,6] and 20% over 3 years [7]. Recently, in the Italian Longitudinal Study on Aging (ILSA), a population-based study with a sample of 5632 65–84 year old subjects, we found a progression rate to dementia of MCI of 3.8/100 person-years [8]. The transitional phase between mild non-disabling cognitive decline and disabling dementia is an ambiguous diagnostic period during which it is unclear whether mild cognitive deficits predict incipient dementia [9], or not [10]. Further adding to this uncertainty are neuropathology-based studies identifying unexpectedly high burdens of vascular [11] and AD [12] types of pathology in clinically nondemented individuals. In fact, recent studies showed that many people with neuropathological changes of degenerative or vascular origin did not have cognitive impairment [11], also suggesting that MCI could not have a neuropathological basis. In fact, in a sample of nondemented elderly individuals, pathologically confirmed preclinical AD was not associated with cognitive impairment or decline, even on neuropsychological measures shown to be sensitive

to very mild AD [12]. These individuals truly are preclinical in that there is no detectable cognitive deficits despite the presence of neuropathological AD, and detectable cognitive decline already may represent “clinical” AD. In fact, a substantial body of evidence supports the suggestion that MCI largely represents very mild AD [13].

In 2001 at the “Current Concepts in MCI Conference” a definition of MCI that more broadly encompassed the clinical heterogeneity of MCI patients beyond memory impairment was proposed [7]. Three subsets of MCI were proposed: amnesic-MCI (aMCI), multiple domains slightly impaired-MCI (mdMCI), and single non-memory domain-MCI (snMCI). The tendency is to use aMCI as the earliest symptomatic phase of AD [14]. In contrast with clinical-based studies [2,6,7], where progression is more uniform, in population-based studies the MCI classification is unstable [8,15,16]. In addition to aMCI, other syndromes can progress to dementia. In the MoVIES study, 2% to 3% of previously normal individuals developed MCI over a 2-year period, between 11% and 21% of those with MCI remained MCI, and 33% to 56% became non-MCI, half of whom reverted to normal or unimpaired [15]. In the Kungsholmen Project, in a population sample of 1435 older persons, of the subjects with mild CIND 34% died, 35% progressed to dementia, 11% remained stable, and 25% improved after a 3-year follow-up [16]. Finally, in the ILSA, in a follow-up period of 3.5 years, out of the 124 cases of MCI who did not progress to dementia, 19% of patients still met criteria for MCI, and 22% of cases reverted to normal cognitive status [8].

Thus, the apparent homogeneity of MCI and its progression to AD in clinical-based samples may reflect referral patterns and selection criteria, suggesting that MCI is a heterogeneous descriptor and that the outcome at follow-up depends on which population is studied and how MCI is defined [6]. In fact, persons who have been referred to a memory clinic are likely to be at a different stage of cognitive decline compared with the cognitively impaired individuals from community samples, because they noticed a memory problem and actively sought help for it [17]. Actually, the recent subclassification of MCI according to its cognitive features (aMCI, mdMCI, and snMCI) represents an attempt to control this heterogeneity. Unfortunately, at present, the preclinical stages of dementia other than AD are not well delineated; not every case of MCI will result in a dementia syndrome, and MCI patients may be cognitively stable or reverse to normal cognition [8,15,16].

Based on current nosology, the two most common forms of dementia are AD and vascular dementia (VaD), with respective frequencies of 70% and 15% for all dementias in occidental

countries [18]. Therefore, AD is the most common dementia and primary neurodegenerative disorder in the elderly. This neurodegenerative disease gradually leads to a complete psychological and physical dependency and finally to death within one to two decades. It involves aberrant protein processing and is characterized by the presence of both intraneuronal protein clusters composed of paired helical filaments of hyperphosphorylated tau protein [neurofibrillary tangles (NFTs)], and extracellular protein aggregates [senile plaques (SPs)]. The SPs are the result of misprocessing of the amyloid precursor protein (APP) by β - and γ -secretases to form atoxic β -amyloid ($A\beta$) peptide that aggregates and initiates a pathogenic self-perpetuating cascade ultimately leading to neuronal loss and dementia. According to the “amyloid cascade hypothesis” [19], the development of SPs is thought to precede and precipitate the formation of NFTs as a result of the cellular changes invoked. A recent longitudinal clinicopathologic cohort study, using summary measures of amyloid load and NFTs, showed no correlation between amyloid load and clinical AD and global cognitive function. This finding suggested that the effect of amyloid deposition on clinical disease is mediated by NFTs [20].

The clinical presentation of VaD varies greatly depending on the causes and location of cerebral damage [21]. Large-vessel disease leads commonly to multiple cortical infarcts and a multifocal cortical dementia syndrome, whereas small-vessel disease, usually resulting from hypertension and diabetes, causes periventricular white matter ischemia and lacunar strokes characterized clinically by subcortical dementia with frontal lobe deficits, executive dysfunction, slow information processing, impaired memory, inattention, depressive mood changes, slowing of motor function, Parkinsonian features, small-step gait, urinary disturbances and pseudobulbar palsy [22]. Very recently, the term Vascular Cognitive Disorder (VCD), has been proposed by Sachdev [23]. This term would become the global diagnostic category for cognitive impairment of vascular origin [24]. VCD would include the group of syndromes and diseases characterized by cognitive impairment resulting from a cerebrovascular etiology. The main categories of VCD are Vascular Cognitive Impairment (VCI) [i.e., vascular cognitive impairment no dementia (vascular CIND), and vascular MCI], VaD, and mixed AD plus cerebrovascular disease (CVD) [23,24]. Dementia is defined as executive control deficit producing loss of function for instrumental activities of daily living, while mixed AD plus CVD is defined as pre-existing AD worsened by stroke (equivalent to prestroke dementia). Finally, VCI is a term referred to all forms of mild to severe cognitive impairment associated with CVD, including vascular CIND and vascular MCI, e.g., prodementia syndromes with a presumed primary vascular basis. VCI is considered a premonitory phase of VaD, although VCI not always proceeds to VaD [23,24]. The characteristic neuropsychological profile of VCI is believed to include frequently early impairment of attention and executive control function, with slowing of motor performance and information process-

ing, while episodic memory is relatively spared compared to that in AD [25].

It is still not known what causes AD and specific risk factors for the disease are difficult to isolate. However, rare mutations that occur in three genes: APP, presenilin1 (PSEN1), and presenilin2 (PSEN2) [26–28] cause familial autosomal-dominant AD with early-onset (less than 5% of all AD cases) and all result in increased production of $A\beta$. Clearly, this pathway is important. The remaining 95% of AD cases, predominantly of sporadic and late-onset nature, are neuropathologically indistinguishable from familial forms. Thus, it is possible that the disease results from a combination of hereditary and environmental factors that somehow involve the APP pathway. Attempts to identify environmental and genetic risk factors associated with AD have not, however, been conclusive. Risk factors identified thus far are intriguing, but not completely illuminating.

The apolipoprotein E (APOE) ϵ 4 allele is a major risk factor for sporadic AD. Numerous genes related to vascular disease have been shown to increase susceptibility for sporadic AD [29]. Among these genetic risk factors, APOE is the best-documented one. APOE is located on chromosome 19 and occurs in three common alleles, ϵ 2, ϵ 3, and ϵ 4. The APOE protein is a major constituent in very low density lipoproteins and plays a key role in the transport of cholesterol and other lipids among various cells of the body. The importance of APOE in the central nervous system (CNS) became evident with the association of the ϵ 4 allele of APOE with familial and sporadic LOAD [30]. The APOE ϵ 4 allele has been known to be associated with coronary artery disease (CAD) and the development of atherosclerosis well before its association with AD [31]. The association with CAD is presumably related to the higher levels of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), and apolipoprotein B in subjects homozygous for the APOE ϵ 4 genotype than in those ϵ 2- or ϵ 3-carriers. The fact that APOE is neither necessary nor sufficient to cause AD is the main reason why APOE is classified as a risk factor for AD and not a causative one. Furthermore, APOE genotyping predictive value is poor and it is not very sensitive and specific. These limitations are used to argue against the inclusion of APOE genotyping in clinical diagnosis of AD. Thus APOE testing can be performed only in patients suspected of having AD, but not in healthy people or relatives of AD patients as screening or predictive test [32]. For a person with symptoms of dementia, APOE testing may offer additional support that any dementia may be due to AD and thus potentially increase confidence to the clinical diagnosis [33]. In AD Centers, clinical diagnosis is already deemed correct over 90% of the time without APOE testing. When APOE genotyping is performed in combination with clinical disease criteria, specificity of diagnosis is increased about 4%.

The degenerative process in AD has already progressed to an advanced stage with massive loss of cell mass before diagnosis can be made. The transition from normal cognitive performance to the AD phenotype is probably gradual [34]. Considering the limited capacity of the CNS tissue to repair,

early intervention in the degenerative processes, thus in patients with MCI, will be crucial to spare as much tissue as possible. Early diagnosis may then help to increase the possibility for developing and testing new preventive strategies. In 1998, the Ronald and Nancy Reagan Research Institute, Alzheimer's Association and the National Institute on Aging (NIA) joined to assemble a working group on molecular and biochemical markers of AD [35].

Biomarkers of cognitive decline and dementia should detect the biochemical and pathological alterations of AD pathophysiology in biological fluids. In fact, the profound biochemical and pathological alterations in the AD brain result from cellular processes such as lipoprotein metabolism abnormalities, inflammation, oxidative stress, APP and A β metabolism, and tau phosphorylation. Cerebrospinal fluid (CSF) closely reflects the composition of the brain extracellular space, and is likely to have the highest yield in biomarker evaluation for dementia and MCI [36–38]. Nonetheless, CSF is not routinely collected in the evaluation of AD, and lumbar puncture is not a widespread procedure in primary care, psychiatric practices, and geriatric practices that often care for AD patients.

Therefore, the identification of biomarker molecules in blood would be more widely applicable, and reduce the need for invasive, expensive or time-consuming testing.

AD is a challenging disease for the biomarker development. Assessment of AD biomarkers is complicated by diagnostic imprecision, the long asymptomatic prodromal stages, variability in clinical features and rates of progression, complex disease genetics and multiple molecular etiologies (e.g., PS-1, PS-2, and APP mutations in familial AD; APOE polymorphisms in sporadic AD). Additional issues arise in the case of serum or plasma biomarkers of AD relative to CSF. The physiology of the blood–brain barrier may limit potential diagnostic biomarkers to small molecules, lipophilic molecules, or molecules with specific transporters. Brain proteins and metabolites that pass into the plasma become markedly diluted into a biochemically complex medium.

This review will focus on the current knowledge on circulating serum and plasma biomarkers of cognitive decline of degenerative or vascular origin that are linked to cholesterol homeostasis and lipoprotein abnormalities, oxidative stress, inflammatory reactions, SP formation and APP metabolism. Special emphasis will be placed on biomarkers related to lipoprotein metabolism and vascular disease. These processes are likely involved in the neurodegenerative processes occurring in dementia and in this respect should not be viewed as completely independent entities. This view suggests that future research will aim to combine markers for the different pathophysiologic mechanisms. We reviewed clinical and epidemiological studies from the international literature through keyword and author searches in Medline from January 1983 to May 2005. Biomarkers will be evaluated on consistency of reported changes in relation with AD and dementia, specificity of the

marker for brain-related processes and the presence of alterations at early stages of dementia or predementia syndromes.

2. Biomarkers related to lipoprotein metabolism and vascular disease

While one of the most popular theories for the pathogenesis of late-onset AD (LOAD) remains the “amyloid hypothesis”, another exciting area is rapidly developing around vascular risk factors in AD pathology [39]. This relationship between risk factors for vascular disease and AD appears at first glance contradictory, since vascular risk factors and the presence of CVD have been considered as exclusion criteria for the clinical diagnosis of AD. Furthermore, for a number of years, epidemiological studies have considered that the co-occurrence of various forms of vascular disease and dementia has been coincidental and largely dependent on the fact they are both common disorders. However, more recently, these co-occurrences, although unexplained as yet, are thought have a more pathological significance [40]. In fact, recent studies suggest that microvascular disorder may contribute to AD pathogenesis and synergistically to cognitive decline related to AD pathology, but the role of cerebrovascular pathology in AD is a matter of controversy [29,41–43]. Vascular pathology of the aging brain and AD includes cerebral amyloid angiopathy, causing lobar mass hemorrhages, small or recurrent bleeds and ischemic infarcts, microvascular degeneration, disorder of the blood–brain barrier, white matter lesions (WMLs), microinfarctions, lacunes, and cerebral hemorrhages [44]. When patients with aMCI from early AD have lacunes, these cases are diagnosed as AD although the dementing disorder is caused by vascular lesions [45]. Recent data from autopsy studies showed the strong contributions of ischemic vascular disease to AD [46,47]. Furthermore, many of the risk factors for CVD and VaD, including circulating factors such as serum/plasma TC, LDL-C, lipoprotein[a] [Lp[a]], and serum APOE levels, or vascular-related diseases such as diabetes mellitus, atrial fibrillation, hypertension, and atherosclerosis, have also been shown to increase the risk of AD. The degree to which these risk factors contribute to cognitive decline may be influenced by genetic factors, such as APOE, that have a role in both vascular disorders and AD [29,48].

Recent evidence from population-based studies [49–53] and case series [54,55] suggests that CVD and vascular factors may contribute to the heterogeneity of MCI. In fact, as seen above, factors, such as hypertension, smoking, diabetes mellitus, atrial fibrillation, and the APOE ϵ 4 allele are also associated with late-life cognitive decline [29], and so may influence the development of MCI and dementia. Because such vascular factors may be modifiable, identification and subsequent management of these possible risk factors may help to prevent, and reduce conversion rates of

MCI and dementia. In a recent study, we estimated prevalence, incidence, and rate of progression of MCI to dementia and correlated vascular risk factors with incident MCI and its progression to dementia [8]. During the 3.5-year follow-up, 113 new events of MCI were diagnosed with an estimated incidence rate of 21.5/1000 person-years. We found a progression rate to dementia (all causes) of 3.8/100 person-years. Furthermore, age was a risk factor for incident MCI, while education was protective, and serum TC evidenced a borderline non-significant trend for a protective effect. Finally, there was a non-significant trend for stroke as a risk factor of progression of MCI to dementia. Probably, a follow-up period longer than 3.5 years would reveal that other vascular factors might influence the progression of MCI to dementia. In conclusion, in our population, among those who progressed from MCI to dementia, 60% progressed to AD and 33% to VaD. A possible explanation may be that current definitions of dementia require memory loss creating a bias towards AD in detriment of VaD [24]. In fact, most studies fail to recognize that the presence of vascular risk factors in population-based studies may actually reflect higher prevalence of VaD than usually recognized. Perhaps some of the cases diagnosed as AD are actually VaD (or even mixed cases, i.e., AD plus CVD). This, of course, is highly relevant when higher than expected frequency of vascular risk factors in the “AD” population were found, suggesting that not all cases of AD are Alzheimer’s dementia [56]. Vascular risk factors may influence incident MCI and the rate of progression to dementia. Furthermore, our findings on the role of stroke in the progression of MCI patients to dementia in the ILSA sample [8], in conjunction with previous studies [57], suggest that stroke and CVD play a role in the clinical course of preclinical dementia. This may indicate new options for prevention of dementia that include CVD and stroke prevention [58].

2.1. Total, LDL cholesterol, and 24S-hydroxycholesterol

Cholesterol is the main lipid constituent of neuronal membranes and myelin. It is known that cholesterol is synthesised in the brain *in situ* [59] and that extracerebral cholesterol does not contribute significantly to brain cholesterol content [60]. Excess of brain cholesterol has to be removed into the periphery. The mechanism of transport is unclear, but may be mediated by APOE and by facilitated transport of oxidized products like 24S-hydroxycholesterol [61,62]. However an additional, as yet unknown, transport mechanism may be involved [63]. It has been hypothesised that during neurodegenerative processes an increased removal of cholesterol from the brain occurs [64]. Aberration of cholesterol homeostasis may indeed be involved in AD. For example, a decreased level of brain cholesterol led to a reversible decreased formation of A β in cultured hippocampal neurones [65]. In addition, the unesterified cholesterol to phospholipid ratio was decreased in the

temporal gyrus of AD patients, while the TC concentration was unchanged [66]. In some studies [67,68], decreased serum high density lipoprotein cholesterol (HDL-C) concentration in patients with AD was observed compared to controls, whereas others observed increased TC and LDL-C in AD patients [69,70].

Epidemiological studies showed that the onset of AD occurs earlier in APOE ϵ 4-carriers with high serum TC [71]. Moreover, high serum TC during middle age or early old age seems to confer an increased risk of AD in older age [72]. In fact, a recent population based prospective study with a 21-year follow-up in Finland found that high TC levels in middle age were associated with increased risk of AD in later life [73]. However, even if high TC often clusters with hypertension, these two entities seem to be independent risk factors of AD [74]. Furthermore, high cholesterol levels were associated with an increased risk of AD or cognitive impairment in cross-sectional and prospective studies [75,76]. On the contrary, our findings on lower TC serum levels in AD [77] confirmed the data of cross-sectional and prospective studies in which a weak but significant inverse association with AD was found, independently of APOE genotype [78,79]. Furthermore, the Hisayama study found no association between serum TC and AD over a 7-year follow-up [80], and no association was found in the Framingham cohort [81]. These findings were confirmed by another study that found no difference in serum TC or hyperlipidemia between subjects with AD, VaD, or nondemented subjects [82]. A few studies have investigated the influence of APOE genotype on the relationship between plasma lipid level and dementia risk, and they have given conflicting results [72,74,75,79]. Nonetheless, TC levels may be influenced by APOE genotype, sex, age, and stage of AD [72,75].

Our findings on lower TC serum levels in AD [77] were confirmed from data of the ILSA, in which the multivariable analysis suggested that only age was a risk factor for incident MCI, while higher levels of education and serum TC appeared to have a protective effect [8]. On the contrary, Kivipelto et al. found that midlife elevated TC serum levels (>6.5 mmol/L) increased the risk for MCI (OR: 1.9), with systolic blood pressure showing a similar trend [51]. In addition, De Carli et al. showed that elevated midlife blood pressure increased the risk for MCI [52]. We did not confirm that elevated TC is a risk factor for incident MCI [8]. This discrepancy can be explained by the fact that both TC and blood pressure were midlife determinations (with average follow-up of 21 and 25 years) in the Kivipelto et al. and De Carli et al. studies, respectively [51,52], confirming the data of cross-sectional and prospective studies [77–79] (Table 1). Very recently, Mielke et al. examined the association between cholesterol level and dementia in a population-based 70-year-old birth cohort followed for 18 years. Increasing TC levels at ages 70, 75, and 79 were associated with a reduced risk of dementia between ages 79 and 88 [83]. In this 18-year longitudinal study of 70 year olds, examination of cholesterol in quartiles showed that the reduction in risk was associated exclusively with the

Table 1

Principal population-based and case-control studies on plasma and serum total cholesterol levels in Alzheimer's disease (AD), other dementing disorders, and mild cognitive impairment

Reference	Subjects	Diagnosis	Results
Jarvik et al. (1995) [71]	206 cases 276 controls	AD	The onset of AD occurs earlier in APOE ϵ 4-carriers with high serum TC
Kuusisto et al. (1997) [78]	980 people aged 69 to 78 years (349 men, 631 women) from population-register of Kuopio, eastern Finland; 46 (4.7%) AD cases	AD	Lower serum TC was associated with an increased risk for AD in older age, independently of apoE genotype
Notkola et al. (1998) [72]	444 men, aged 70–89 years, who were survivors of the Finnish cohorts of the Seven Countries Study	AD	High serum TC during middle age or early old age seems to confer an increased risk of AD in older age
Boston et al. (1999) [82]	222 AD cases 34 VaD cases 140 nondemented	AD VaD	No difference in TC levels by diagnosis
Romas et al. (1999) [79]	1449 white, African American, and Caribbean Hispanic subjects from population-register from New York City, aged 75.8 ± 6.4 years	Incident AD	Decreased plasma TC level had an inverse association with incident AD, independently of APOE genotype
Evans et al. (2000) [75]	524 African Americans subjects older than from 65 years from door-to-door random sampling	AD	Increasing TC was associated with increased AD risk in the group with no APOE ϵ 4 alleles, whereas TC was not associated with increased AD risk in the group with one or more ϵ 4 alleles
Bonarek et al. (2000) [69]	Nested case-control study of 334 elderly French subjects aged 73 and over who participated in the PAQUID study; 37 dementia cases 297 nondemented	Dementia	No difference in TC levels by diagnosis Elevated HDL-C was associated with a significantly decreased risk of dementia, independently of APOE status
Kivipelto et al. (2001) [51]	1449 subjects aged from 65 to 79 years	Incident AD	Midlife high serum TC and raised systolic blood pressure, and in particular the combination of these risks, increased the risk of AD in later life
Kivipelto et al. (2001) [73]	50 definite AD cases 27 probable AD cases 50 possible AD cases 22 no AD subject	Incident MCI	Midlife elevated serum TC level ($> \text{or} = 6.5 \text{ mmol/L}$) was a significant risk factor for MC
Lesser et al. (2001) [70]	1449 subjects aged from 65 to 79 years	Incident AD	Serum TC and LDL-C levels were significantly higher in AD patients These associations were progressively stronger with increasing pathological certainty of AD diagnosis
Kivipelto et al. (2002) [74]	1449 subjects aged from 65 to 79 years	Incident AD	Midlife high serum TC and raised systolic blood pressure, were independent risk factors for AD in later life, independently of APOE status
Solfrizzi et al. (2002) [77]	61 cases 63 controls	AD	Lower serum TC in AD patients Lipoprotein (a) serum concentrations were significantly associated with an increased risk for AD, independently of APOE genotypes and sex and dependent on age and TC serum concentrations
Tan et al. (2003) [81]	1026 subjects from the Framingham Study original cohort, aged 78.1 ± 5.3 years	Incident AD	Serum TC levels were not associated with the risk for incident AD

Table 1 (continued)

Reference	Subjects	Diagnosis	Results
Reitz et al. (2004) [101]	4316 Medicare recipients, 65 years and older, residing in northern Manhattan, New York	AD and VaD AD and VaD Incident	Elevated levels of non-HDL-C and LDL-C and decreased levels of HDL-C were weak risk factors for VaD in either cross-sectional or prospective analyses. Higher levels of TC were associated with a decreased risk of incident AD after adjustment for demographics, APOE genotype, and cardiovascular risk factors.
Solfrizzi et al. (2004) [8]	2963 Italian subjects from population-register (including institutions) aged from 65 to 84 years	Incident MCI	Serum TC evidenced a borderline non-significant trend for a protective effect.
Dufouil et al. (2005) [100]	A population-based cohort of 9,294 subjects selected from the electoral rolls of three French cities (Bordeaux, Dijon, Montpellier)	Incident dementia, incident AD, and incident VaD	Higher TC (≥ 6.20 mmol/L) was associated with an increased odds of dementia but not AD.
Mielke et al. (2005) [83]	A total of 392 individuals, 166 men and 226 women, from the 70-year-old residents of Göteborg in 1971 to 1972	Incident dementia	In this 18-year longitudinal study of 70 year olds, an association between higher TC and a decreased risk of dementia was observed.

AD: Alzheimer's disease.

TC: total cholesterol.

APOE: apolipoprotein E.

VaD: vascular dementia.

HDL-C: high-density lipoprotein cholesterol.

LDL-C: low-density lipoprotein cholesterol.

MCI: mild cognitive impairment.

highest quartile. The exclusion of lipid-lowering medication users did not attenuate the association. Furthermore, the association was found only among nonsmokers, and no association between triglyceride levels and dementia was reported. Therefore, high TC in late life was associated with decreased dementia risk, which is in contrast to previous studies suggesting high cholesterol in midlife is a risk factor for later dementia [72,73]. The conflicting results may be explained by the timing of TC measurements in relationship to age and the clinical onset of dementia [83].

In a retrospective study conducted on autopsy cases of patients older than 40 years, mild elevation in TC may be an early risk factor for the development of Alzheimer amyloid pathology in the human brain, with a significant association between plasma TC level and presence of amyloid deposition. Although a significant association was retained in the whole sample, it was interesting that no association between TC and amyloid deposition occurred at older ages (>55 years) [84]. The calculated odds for developing amyloid almost tripled with only a 10% increase in TC level in the younger group. These findings may also explain the lack of consensus regarding the association between TC and AD, suggesting that only midlife elevation in TC may be a risk factor for AD. On the other hand, recent findings from the Framingham Study showed that participants with "desirable" TC levels (<200 mg/dL) performed less well than participants with borderline-high TC levels (200–239 mg/dL) and participants with high TC levels (1240 mg/dL) on cognitive measures of verbal fluency, attention/concentra-

tion, abstract reasoning, and a composite score measuring multiple cognitive domains [85]. Finally, in a retrospective analysis of 443 AD patients from a 30-week tacrine trial, disease progression in the no-APOE $\epsilon 4$ allele/high-TC subgroup was greater than in the normal-TC subgroups with or without $\epsilon 4$ [86].

Circulating lipoproteins and lipids can be modified by dietary or pharmacologic intervention and TC is an established marker of the effects of lipid-lowering treatment. Some epidemiological studies also indicate that the prevalence of AD might be decreased in patients treated with the 3-hydroxy-3-methylglutaryl-coenzyme reductase inhibitors (statins) [87]. The relative risk of dementia in statin users was 0.21–0.29 in case-control studies from the United Kingdom and Canada [88,89]. This last evidence from a recent longitudinal observation from the Canadian Study of Health and Aging (CSHA) [89] that incidence of AD was reduced in individuals using statins further supports the hypothesis that TC may be important in AD. Moreover, one observational study in three hospitals in the United States recently found that the prevalence of AD among patients taking statin drug was 60–73% lower than in patients not on statins [90]. Statins show pleiotropic effects including regulation of eNOS activity and NO production, modulation and inflammatory processes, antioxidant activity, angiogenesis, and immunomodulation, as well as reduction of free TC and inhibition of cholesterol ester accumulation in macrophages by inhibiting LDL endocytosis and reducing mevalonate byproducts essential for cholesterol esterification. Other potential effects of statins

include reduction of atherosclerotic plaque formation, endothelial protection, and reduction of oxidized LDL [88].

However, a recent trial assessing the benefits of pravastatin in individuals ages 70 to 82 years, at high risk of cardiovascular disease and stroke, did not find any significant effect of this lipid-lowering agent on cognitive performance [91]. Furthermore, no effect on a secondary measure of incident cognitive decline occurred in a placebo-controlled study of simvastatin for cardiac disease involving 20,536 patients, despite a prominent lipid-lowering benefit [92]. Furthermore, in a prospective, cohort study of statin use and incident dementia and probable AD with a cohort of 2356 cognitively intact persons, aged 65 and older, employing time-dependent proportional hazards modelling, the authors found no significant association between statin use and incident dementia or probable AD [93]. In contrast, when the data were analyzed, inappropriately, as a case-control study, the authors found an OR of 0.55 for probable AD, falsely indicating a protective effect of statins. Therefore, study design and analytic methods may explain the discrepancy between the current null findings [93] and earlier findings [88–90].

Studies that have investigated the relationship between lipid-lowering drugs and dementia have been based on a solid rationale [94]. Laboratory studies have further shown that cholesterol might play a role in the biosynthesis of A β [95], and that simvastatin reduces the level of A β 42 and A β 40 in the brain of guinea pigs [96]. Moreover, with high cellular cholesterol levels, there is a decrease in glycosylation of mature oligosaccharides in β -secretase, which releases A β from APP, whereas in the presence of lovastatin, glycosylation progresses further. Thus, the cholesterol and statin effects are due to changes in cellular targeting induced by changed cholesterol gradients [97]. On the other hand, the real effect of statins may be the decrease risk of stroke and therefore of cognitive decline of vascular origin [58]. However, as seen above, the findings from epidemiological studies investigating the association between TC levels and dementia are inconclusive. A few of these studies, however, has taken into account lipid-lowering drug intake, which limits their interpretation. Finally, treatment of hypercholesterolemia with lovastatin or simvastatin did not cause psychological distress or substantially alter cognitive function [98,99].

Very recently, the Three-City Study, a large community-based cohort of 9294 subjects ages 65 years and older selected from the electoral rolls of three French cities (Bordeaux, Dijon, Montpellier), examined whether lipid-lowering drug use (statins and fibrates) and hyperlipidemia were associated with prevalence of dementia and, if so, if the association was modified by variants in the APOE genotype [100]. The analyses suggested that use of lipid-lowering drugs was associated with a lower prevalence of dementia. This trend was consistently found in subgroup analyses by age, gender, and center and for both statins and fibrates. It was also found when the analysis was restricted to AD cases, with no modifying effect of the APOE genotype on the association between lipid-lowering drug use and dementia. Moreover,

when TC level and lipid-lowering drug intake were studied concomitantly, an association between lipid-lowering drug use and lower prevalence of dementia was found only in lipid-lowering agent users with normal TC level [100]. High TC levels were related to higher dementia prevalence but only in non-AD cases, as already observed in another study [101] (Table 1). In fact, this very recent cross-sectional and prospective community-based cohort study found a weak relation between non-HDL-C, LDL-C, and HDL-C levels and the risk of VaD. Lipid levels and the use of agents to lower them do not seem to be associated with the risk of AD [101]. These recent findings confirmed previous data showing that elevated levels of LDL-C were associated with the risk of dementia with stroke in elderly patients [102]. Furthermore, HDL-C, but not LDL-C or TC, was associated with hippocampal volume and dementia, suggesting a possible protective effect of HDL-C on hippocampal atrophy and AD [103].

As seen above, plasma 24S-hydroxycholesterol reflects brain cholesterol homeostasis more closely than plasma TC. Excess brain cholesterol is converted to 24S-hydroxycholesterol, a brain-specific oxysterol which readily crosses the blood-brain barrier (BBB) [61,62]. In fact, there is a daily flux of about 7 mg of this oxysterol from the brain to the circulation, with the majority of this efflux apparently occurring as direct transport across the BBB [64]. 24S-hydroxycholesterol levels in plasma represent a balance between production in the brain and metabolism in the liver. Plasma levels show a weak, if any, correlation with CSF levels [104,105]. 24S-hydroxycholesterol was elevated in the CSF of AD and MCI patients [105,106]. Therefore, the elevation of 24S-hydroxycholesterol appears to occur early in the disease process, suggesting that CSF 24S-hydroxycholesterol may be a marker for monitoring the onset and progression of the disease [105]. On the contrary, the findings on a possible elevation also in plasma of 24S-hydroxycholesterol in AD patients were contrasting [104–108] and plasma 24S-hydroxycholesterol levels were reduced by statin and niacin treatment. [109–111]. 27-hydroxycholesterol is the most abundant hydroxycholesterol in human circulation [112]. The recently published observation of a strong correlation between 24S- and 27-hydroxycholesterol CSF levels [104] suggested that not only brain oxysterols such as 24S-hydroxycholesterol are altered in dementing disorders, but that also peripheral cholesterol metabolism is affected. These suggestions are supported by the recent findings of increased ratios of plasma 24S-hydroxycholesterol to 27-hydroxycholesterol in patients with dementing disorders compared to nondemented subjects [113].

2.2. Lipoprotein(a)

Lp(a) is a LDL-like particle with the plasminogen-like apolipoprotein(a) [apo(a)] linked by disulfide bridge to apolipoprotein B-100, that is believed to have atherogenic and thrombotic properties and has been associated with

vascular disease [109,114]. Unlike all other lipoproteins, plasma Lp(a) concentration is mainly determined by genetic factors at the LPA gene or other sequences located either within or near the LPA locus [29]. The LPA gene is highly polymorphic in size as a result of differences in the number of repeated kringle 4 (K4) units in apo(a) [115]. Despite the presence of LDL, apo(a) imparts to Lp(a) unique properties with respect to biological functions. Indeed, it has been shown that the majority of Lp(a) cell-to-cell interactions are mediated by its specific apo(a) moiety [29,115]. High Lp[a] levels are associated with atherosclerosis, CAD, and CVD [116]. Apo(a) was detected in primate brain, suggesting that Lp(a) particles (which can also carry APOE) are involved in cerebral lipoprotein metabolism [117]. Furthermore, a recent study found serum concentrations of Lp(a) significantly higher in patients with vascular dementia as well as patients with CVD compared with those in healthy individuals [118]. These abnormally high serum levels of Lp(a) seemed to be due to specific increase in low molecular weight (MW) apo(a) isoforms in Lp(a). Several lines of evidence linking clinical expression of AD with cerebral infarct suggest that Lp(a) could be a possible risk factor in the development of AD [119].

It has been previously suggested by Mooser et al. [120] that a high Lp(a) level may act as an additional risk factor for late-onset AD in APOE ϵ 4 carriers, while Lp(a) may protect against AD in non-carriers older than 80 years. However, the analysis of apo(a) polymorphism in this study did not show any difference in the mean size of apo(a) protein in AD patients compared with controls. In a subsequent study was tested a variant (T3888P) located in the K4 region of apo(a) in a case-control series. Overall, there were no differences between case and controls. However, in the APOE2 positive subgroup, the mutant allele was over-represented in the cases, suggesting that this polymorphism and others at the apo(a) locus be further studied in relation to AD [121].

We found that Lp(a) serum levels were significantly associated, according to a nonlinear relationship, with an increased risk for AD, independently of APOE genotypes, and dependent on age [77]. Very recently, a cross-sectional study showed an independent association of small apo(a) isoforms with both VaD and AD, suggesting that the small particle size of apo(a) may significantly increase the risk for these conditions. However, the risk difference between patients with VaD and those with AD may indicate a diversity in the role played by apo(a) in these two clinical entities [122]. Furthermore, operational null alleles, defined by absence of apo(a) isoforms from immunoblots [apo(a) null phenotype], have been described and AD patients with the null phenotype had a delayed age at onset of the disease of those who expressed at least one apo(a) band (mean age at onset: 76.8 versus 66.9 years), without APOE interaction [123]. Finally, in a group of Italian patients with frontotemporal dementia (FTD), 55.6% of the subjects had at least one apo(a) low MW isoform, compared to 29.9% of non-demented controls (OR: 2.93, 95% CI: 1.42–6.06,

$P=0.003$), suggesting a possible role in mediating susceptibility to FTD of low MW apo(a) isoforms, linked to higher plasma levels Lp(a) [124]. On the contrary, serum Lp[a] levels were not associated with cognitive decline over 3 years within an Italian elderly population [125].

The pathophysiologic mechanisms by which elevated Lp(a) could be associated with AD are, at present, unknown. Lp(a) is a LDL-like particle, and a recent study found increased levels of serum LDL-C in AD patients correlate with brain A β N-42 levels, suggesting that LDL-C may influence the expression of AD-related pathology [126]. Furthermore, clinical and epidemiological data have shown that chronic inflammation appears as a precursor of symptomatic AD [127], suggesting another possible link between elevated serum Lp(a) and AD. In fact, Lp(a) concentration has been found to be increased by a number of clinical and subclinical chronic inflammatory disorders [128]. Finally, recent studies have shown that clinical expression of AD is facilitated by cerebral ischemia. In subjects with neuropathological brain lesions typical for AD, brain infarcts, and especially lacunar infarcts, more often resulted in clinical dementia [119]. It was reported that APP activity and β A production increases in the hippocampus of rodents after severe, transient ischemia [129]. Since increased Lp(a) serum levels generally enhanced the risk of stroke [130], this may play a role in determining clinical AD.

2.3. Apolipoprotein E

The APOE ϵ 4 allele is associated with high TC, LDL-C, and apolipoprotein B levels in many populations [131], and with increased risk of AD, earlier age of AD onset, increased amyloid plaque load, and elevated levels of A β 40 in the AD brain [130–133]. Furthermore, physiological serum APOE concentrations vary between 30 and 250 mg/L [134], and have been shown to modulate lipid metabolism [135]. An increase in serum APOE levels in early-onset AD and LOAD patients in comparison with controls was also observed [136]. Increased serum APOE levels in AD could be of interest, as APOE concentration is related to vascular disease [137], and there is growing evidence that vascular factors play a role in the etiology of AD.

To date, the results on serum APOE levels in AD are controversial. In a recent study, we found that in young health subjects, age-matched controls, centenarians, and AD patients the presence of the APOE ϵ 4 allele is associated with lower, and the ϵ 2 allele with higher serum APOE levels [138]. In the normal population, serum APOE levels are higher when the APOE ϵ 2 allele is present. APOE ϵ 2/2 genotype showed the highest APOE levels [131] with the APOE ϵ 4 allele associated with less APOE protein in plasma [139]. An increase in APOE concentrations has been reported in AD patients [137]. In a recent study on late-onset AD patients from Northern Italy, serum APOE levels were similar in patients and controls [140], and a postmortem study confirmed these results [126]. Our data are in contrast to these

findings, and consistent with other, in which serum APOE level differences between AD patients and controls mainly result from the distribution of the APOE genotypes [141]. We found lower serum APOE levels, and higher $\epsilon 4$ allele frequency in our AD patients compared to age-matched controls, but these differences were statistically significant only for APOE genotypes. In fact, in a larger sample as that of the Rotterdam study, serum APOE levels were significantly lower in AD patients adjusting for age and gender and were on the borderline of statistical significance adjusting for BMI, protein, and albumin levels [141]. In another recent study, APOE concentrations were lower in AD patients and nondemented controls with $\epsilon 4$ allele than in those without the $\epsilon 4$ allele [142]. Finally, a study conducted on nine European populations showed a clear decrease in APOE serum levels in AD cases, but $\epsilon 4$ and APOE concentration seem to be independently associated with the development of AD, though without adjustment for other lipid parameters [143]. These contrasting findings in the regulatory role of APOE polymorphism on APOE serum levels in AD were explained by the authors with geographical and age differences in different studies. We suggest the first as source of variability [144], because of our data in centenarians demonstrate that the impact of APOE genotype on APOE serum levels is present across all age categories. Finally, the ratio of APOE4 protein to APOE3 protein in the plasma of heterozygous APOE $\epsilon 3/\epsilon 4$ individuals did not correlate with AD diagnosis [145].

In conclusion, the relationship between lipid metabolism and dementia remains intriguing and unresolved. In fact, epidemiological studies examining the association between TC and dementia have reported conflicting results. One potential explanation for the heterogeneous results is whether TC was assessed in midlife or late life. Multiple studies have suggested that high TC in midlife [146,147], but not late life [148,149], is associated with an increased risk of cardiovascular disease, and it is possible that a similar timing phenomenon exists for measures of cholesterol in relationship to risk of dementia. Furthermore, high TC in late-life may be an indicator of better health status [150]. Those who survive to old age with high cholesterol may be a more robust and select population and therefore relatively invulnerable to the potential adverse effects of high TC, including dementia. In addition, studies of cholesterol in old age have had short follow-ups, which leads to unclear conclusions regarding the direction of the cholesterol–dementia association. Furthermore, few studies have examined the relationship between dementia and other lipids, such as triglycerides.

Given the genetic complexity of AD, the presence of additional genes with influence on the levels of AD-related lipid risk factors or markers is very likely. In fact, a large body of evidence suggested that, at present, serum APOE levels could not be used as a biochemical marker for AD instead of APOE genotyping in neuro-epidemiologic studies. In fact, there was no consistent association of serum or plasma APOE protein levels with diagnosis when controlled for APOE genotype. Further studies are needed to investigate in depth

the role of different common APOE polymorphisms in controlling serum APOE levels in AD. In addition, there are some evidence that higher Lp(a) levels could be linked with AD, although there are studies suggesting an increased presence of low MW apo(a) in AD, VaD, and FTD, that genetically determined elevated Lp(a) levels. Larger clinical studies involving patients with predementia syndrome and non-AD dementias, as well as longitudinal studies of AD patients, are needed to confirm the relationship between Lp(a) concentrations and dementia.

Several lipid plasma or serum measures are responsive to medications, for instance statins reduce cholesterol and 24S-hydroxycholesterol levels. Several observational studies have suggested that statins lower the probability of dementia (thus far unsupported by clinical trials) and that lowering cholesterol may reduce $A\beta$ in the brain [94], but the results of these studies are inconclusive. The prevailing wisdom is that high TC is a risk factor for dementia. However, the relationship between cholesterol and dementia may vary considerably depending on when cholesterol is measured over the life course. Given that the timing of the exposure may be critical, more studies with long-term follow-up and serial assessments of TC are needed to further clarify the causal relationship between cholesterol and dementia. Therefore, although serum/plasma levels of TC, LDL-C, and 24S-hydroxycholesterol are not credible diagnostic markers for AD and cognitive decline, at present, the current evidence suggest may be modifiable risk/protective factors.

2.4. Homocysteine

Homocysteine is a thiol-containing amino acid involved in the methionine cycle as the demethylation product of methionine and in the transsulfuration pathway in which it is irreversibly converted to cystathione in a vitamin B6-dependent process [151]. Vitamin B6 is also an essential homocysteine re-methylation cofactor, and its deficiency is associated with increase in blood homocysteine levels. Elevated plasma concentration of homocysteine (hyperhomocystinemia) is now recognized as an independent risk factor for cardiovascular disease, peripheral vascular disease, and CVD [152] and may also have directly toxic effects on neurons of the central nervous system [153].

Significant correlations have been observed between homocysteine concentrations and indexes of cognitive functions in case-control studies on patients with psychogeriatric conditions [154–157] and in cross-sectional, population-based studies of community-dwelling older adults [158–162]. Two studies, however, found no significant associations between homocysteine concentrations and cognitive function in either cross-sectional [163,164] or longitudinal analyses [163]. One study found negative findings in centenarians with different degrees of cognitive impairment [165]. A recent study conducted on 1789 elderly Latinos aged >60 years participating in the Sacramento Area Latino Study on Aging found only modest inverse association between

homocysteine concentrations and several indexes of cognitive function [166]. Further, in 1241 subjects aged 61 to 73 years, followed up over 4 years, cross-sectional analyses showed that higher concentrations of homocysteine were significantly related to poorer performances in several neuropsychological tests. Longitudinal analyses confirmed this finding. The odds of cognitive decline were 2.8-fold higher in subjects with homocysteine levels above 15 micromol/L compared with those with homocysteine levels below 10 micromol/L. At 2-year follow-up, 841 subjects underwent cerebral magnetic resonance imaging, and white matter hyperintensities (WMHs) were rated visually: the relationship between homocysteine and cognition was unchanged after taking into account WMHs, suggesting that the WMHs do not mediate the association between homocysteine and cognition [167].

Evidence is now accumulating for an association between AD and disturbed single-carbon metabolism. As mentioned above, elevations in plasma levels of homocysteine is an emerging risk factor for AD [153–156,168]. High plasma homocysteine levels result from a complex interaction of acquired and genetic factors, but quantitatively the most important ones are deficiencies of folate, vitamin B12, and vitamin B6 [169,170], which are associated with stroke and thrombosis [171]. In cross-sectional studies, elevated plasma homocysteine levels have been associated with either poor cognition or dementia [153]. The findings from the Rotterdam Scan Study, a population-based study involving 1077 people aged 60 to 90 years who had cerebral magnetic resonance imaging, suggested that total homocysteine levels are associated with silent brain infarcts and WMLs independent of each other and of other cardiovascular risk factors [172]. Furthermore, in another study on a sample of 1092 subjects without dementia from the Framingham Study was examined the relation of the plasma total homocysteine level measured at baseline and eight years earlier to the risk of newly diagnosed dementia on follow-up. It was found that with a plasma homocysteine level greater than 14 micromol/L the risk of AD nearly doubled. Therefore, an increased plasma homocysteine level seems to be a strong, independent risk factor for the development of dementia and AD, suggesting that the elevated homocysteine levels precede the onset of dementia, not resulting from dementia-related nutritional and vitamin deficiencies [168]. Some authors reported that elevated plasma homocysteine in patients with AD appears related to vascular disease and not AD pathology or its consequences (physiologic or behavioral) [173]. As mentioned above, several investigators have found inverse associations between objective measures of cognitive function and plasma or serum homocysteine concentrations in patients with AD, suggesting that homocysteine can serve as a predictor of cognitive performance. Nonetheless, it remains an open question whether or not interventions designed to lower plasma homocysteine concentrations will improve cognitive function or retard the rate of cognitive decline in older adults with or without AD. The most effective means

for lowering plasma homocysteine is B vitamin supplementation (a combination of folate, vitamin B12, and vitamin B6), and intervention trials are currently under way to determine if such supplements will reduce the risk for vascular disease [174]. It would be interesting to consider whether the interventions will decrease the risk for AD and cognitive decline as well.

3. Biomarkers related to APP and A β metabolism

As seen above, the major component of SP is the A β protein, a small 42 residue protein derived through proteolytic processing of the APP [175]. Secreted soluble A β is a product of normal cell metabolism, and found in various body fluids including plasma and CSF [176]. Recent studies have shown that in AD brain, A β protein ending at residue 42 (A β 42) is deposited first and is the predominant form in SP; whereas A β protein ending at residue 40 (A β 40) is deposited later in the disease and is prominent in vascular amyloid deposits [177]. The development of sensitive ELISAs for A β 40 and A β 42 enabled the detection and quantitation of A β in human blood. Studies have shown that A β levels were 100-fold lower in plasma than in the CSF [178]. Plasma total A β or A β 42 was increased in familial AD with presenilin or APP mutations [178,179] as well as in Down syndrome [180], suggesting the possibility that sporadic cases of AD might be associated with detectable and diagnostic changes in the plasma levels of A β .

Several cross-sectional studies [178,179,181–185] and two longitudinal studies investigated plasma A β measures in AD and MCI [186,187] (Table 2). One study of 78 AD and 61 control cases showed that plasma A β 40 levels are increased more in AD patients with APOE ϵ 4 allele than in those without this allele and in age-matched controls [148]. However, most groups have shown that plasma A β 40 levels are similar in AD and control groups [178–180,183,158] and A β 40 and sometimes A β 42 levels correlated strongly with age and with serum creatinine levels [185–187]. Because of the considerable overlap between the two groups, the measurement of plasma A β 40 levels is not useful as a diagnostic tool to distinguish patients with sporadic AD from elderly non-demented controls.

Although not diagnostically useful, plasma A β measures can also be evaluated in the context of AD prediction and progression. In fact, plasma A β 42 levels are similar between AD and controls in some cross-sectional studies [181,182], but two recent longitudinal studies suggested that high plasma A β 42 levels were a risk factor for developing AD [186,187]. Many cross-sectional studies have showed that CSF A β 42 levels were lower in patients with MCI than controls [188], but plasma A β levels did not correlate with measures of progression or dementia severity [182,185,189] although a longitudinal study showed that elevated plasma A β 42 levels occur before the onset of MCI in some individuals [186]. Very recently, in a sample of 88 elderly

Table 2

Plasma and serum β -amyloid levels in Alzheimer's disease and other dementing disorders and mild cognitive impairment

Reference	Subjects	Diagnosis	Results
Scheuner et al., 1996 [178]	71 cases 75 controls	AD	No difference in A β 40 and A β 42 levels by diagnosis
Tamaoka et al., 1996 [181]	28 cases 25 controls 40 neurological controls	AD	No difference in A β 40 and A β 42 levels by diagnosis
Kosaka et al., 1997 [179]	44 cases 15 controls 22 neurological controls	AD	No difference in A β 40 and A β 42 levels by diagnosis, without effect of disease stage
Mayeux et al., 1999 [186]	64 cases 105 controls	Incipient AD	Increased A β 40 and A β 42 levels with age and incipient AD, without effect of sex, APOE, or family history of AD No effect of diagnosis on rate of change
Mehta et al., 2000 [182]	78 cases 61 controls	AD	Increased A β 40 levels in AD and with age No effect of sex or MMSE
Vanderstichele et al., 2000 [183]	39 AD cases 12 controls 6 DLB cases 9 nondemented 10 other dementia	AD DLB	No difference in A β 40 and A β 42 levels by diagnosis No effect of sex or MMSE
Fukumoto et al., 2003 [185]	146 AD cases 37 MCI cases 96 PD cases 92 controls	AD PD MCI	No difference in A β 40 and A β 42 levels by diagnosis No effect of APOE, medications, and severity or duration of dementia
Mayeux et al., 2003 [187]	79 AD cases at baseline 86 incident AD cases 365 nondemented	Incident AD	Increased A β 42 levels in AD and incident AD Increased A β 40 and A β 42 levels with age Decline in A β 42 levels over 3 years in incident AD Inverse relation between A β 40 and cholesterol, without effect of APOE
Assini et al., 2004 [184]	88 MCI cases 72 controls	MCI	Increased A β 42 levels only in women with MCI No significant sex-related difference in A β 40 levels within the MCI group, as well by diagnosis No effect of APOE, education, creatinine, total cholesterol, or hemoglobin levels

AD: Alzheimer's disease.

A β : β -amyloid.

APOE: apolipoprotein E.

DLB: dementia with Lewy bodies.

MMSE: mini-mental state examination.

PD: Parkinson's disease.

MCI: mild cognitive impairment.

patients with aMCI, chosen as paradigm of preclinical sporadic AD, a significant increase of A β 42 plasma levels in women with MCI, in comparison to the affected men and 72 cognitively normal age-matched subjects was found, suggesting a possible biological explanation for the sex-dependent increased incidence of late-onset AD in women [184].

In clinical studies, plasma A β measures may be potentially useful as biomarkers in monitoring pharmacological effects of medications that affect APP processing. In fact, reduction in plasma A β levels with treatment could confirm the mechanism of action of medications that inhibit the β -secretase or γ -secretase that produces A β . Cross-sectional studies found no significant effects of statins, estrogen, non-steroidal anti-inflammatory drugs, antioxidants, or cholinesterase inhibitors on plasma A β levels [185,190]. In

contrast, in double-blind placebo-controlled studies, lovastatin reduced plasma A β levels over 3 months [191], and transdermal 17 β -estradiol was associated with a reduction of plasma A β 40 over 8 weeks [192]. However, a very recent, prospective, randomized, dose-finding 36-week treatment trial with statins was conducted on 39 outpatients who met the criteria for hypercholesterolemia randomized to oral treatment with either simvastatin or atorvastatin calcium according to the following regimen: simvastatin, 40 mg/d, or atorvastatin, 20 mg/d, for 6 weeks; followed by simvastatin, 80 mg/d, or atorvastatin, 40 mg/d, for 6 weeks; and finally, simvastatin, 80 mg/d, or atorvastatin, 80 mg/d, for 24 weeks. Treatment with both statins reduced total plasma cholesterol levels by 56% but the plasma levels of A β 40, A β 42, and total A β were stable in individual patients during the treatment period, questioning the effect of statins on the processing of

APP in humans [193]. In conclusion, plasma A β measures appear not to be sensitive or specific markers for the diagnosis of AD and further studies are needed to better clarify the possible role of plasma A β as a biomarker for predicting AD risk, tracking progression, and following the effectiveness of medications.

In APP transgenic mice or humans, plasma A β levels do not correlate with biochemical or pathological measures of cerebral A β deposition [183,189,194], although animal studies showed that A β can pass between the CSF and plasma compartments [195,196]. In APP transgenic mice, the flux of A β from the brain and CSF to the plasma increased by peripheral administration of a monoclonal anti-A β antibody [194,197]. These findings, if confirmed in humans, suggested that measures of brain-to-plasma A β efflux could be a peripheral indicator of the extent of cerebral amyloid deposition, even in preclinical AD [194].

Furthermore, passively and actively administered antibodies against A β 42 reduced the extent of SPs in the brains of APP transgenic mice expressing AD-causing mutations [198,199]. Based on these preclinical findings and after suggestive related effects in a human clinical trial of active immunization [200,201], a Phase I study in 104 AD patients demonstrated good safety and tolerability of multiple injections of aggregated A β 42 (AN1792) with QS-21 as adjuvant and elicited a detectable rise of anti-A β 42 antibodies in about 25% of patients [202]. In a successive, international, multicenter Phase II study of active immunotherapy, anti-A β antibody titers were not predictive of which patients developed subacute meningoencephalitis as a side effect of immunization (18 of 298 on active treatment) but they were evaluated as a marker of treatment effectiveness [203]. Based on these results, it was hypothesized that people possessing auto-antibodies against A β (detectable in human plasma and CSF) may be protected against AD, although titers were similar in AD and non-AD cases [204,205]. These studies focused on autoantibodies against monomeric A β ; it is possible that autoantibodies against oligomeric or aggregated A β are the more clinically relevant species [206]. In fact, in a subgroup of 30 patients from the Phase II study, patients who developed antibodies reactive to SPs in AD tissue sections performed better on neuropsychological testing and functional scales than patients who did not develop these antibodies [206].

Finally, APP is abundant in platelets, predominantly as the 770 isoform, and after the platelets are activated, soluble forms of cleaved APP are released, analogous to processing in neurons [207]. Alterations in the isoform ratios of APP in platelets in AD were reported and two research groups have measured relative amounts of two platelet isoforms in platelet membrane preparations, and reported a decrease in the amount of the higher (130 kDa) band compared to the lower (110 kDa band) (“APP isoform ratio”) [208–210]. In fact, in platelets, 150 kDa intact APP is processed into 120–130 and 110 kDa carboxy-truncated forms. Both groups used the same commercially available antibody (22C11) against APP

to visualize APP on Western blots, followed by densitometry. This method will be difficult to standardize between different laboratories and is not amenable to scaling up to analyze large numbers of samples. A relatively large volume of blood is needed (about 30 ml), with careful handling, rapid processing and preparation of platelets, and prompt freezing of samples before immunoblotting. Further limitations of the measurement include technique-related factors (tourniquet, anticoagulant, platelet activation), and the Western-blot-based procedure which precludes high through-put and consistent standardization [211]. The APP isoform ratio was reduced in AD and MCI [175,176], but not in other dementias [212], and correlated with disease severity and progression [210,213]. Sensitivities and specificities for AD diagnosis were in the 80–95% range, based on post hoc cutoff scores [209,21]. Cholesterol reduction, niacin, simvastatin, and cholinesterase inhibitors corrected the abnormally low APP isoform ratios in AD cases [213–215], suggesting quantitation of platelet APP as a promising biomarker for tracking diagnosis, progression, and treatment effects. Nonetheless, to interpret this potential biomarker more clearly, we need a better understanding of the biochemical nature of the platelet isoforms of APP, and their relationship to pathophysiological steps in AD.

4. Biomarkers for oxidative stress

During the last decade the free radical theory of aging has been established which suggests that oxidative stress is intimately related with AD. This is not surprising since the brain is especially susceptible to oxidative stress, due to the high rate of oxygen consumption, and subsequent damage to cells, including cell death. The production of free radicals species that attack neuronal lipids, proteins and nucleic acids inevitably leads to neuronal dysfunction. These neuronal alterations can be assessed using several markers such as 8-hydroxyguanosine and 8-hydroxy-2'-deoxyguanosine (nucleic acid oxidation markers), protein carbonyls and 3-nitrotyrosine (protein oxidation markers), malondialdehyde, thiobarbituric acid-reactive substances, 4-hydroxynonenal and acrolein (lipid oxidation markers), and advanced glycation end products (glyco-oxidation marker).

DNA damage is countered in cells by DNA repair and it can produce various modifications in DNA including base and sugar lesions, strand breaks, DNA-protein cross-links and base-free sites. A lesser capacity for DNA repair has been reported in AD [216] since DNA fragmentation and nicking have been found in AD subjects [217–220]. It has been speculated that the presence of A β induces the formation of purine dimers, a form of DNA damage, and that these purine dimers result from oxidative damage caused by the A β to the DNA [221]. Increased oxidative damage to RNA in neurons throughout the brain of AD subjects has been observed as well [222].

Protein oxidation that determines amino acid side-chains modifications, resulting in a diverse array of altered amino acids [223], is likely increased in AD especially in the brain regions rich in A β [224], as indexed by protein carbonyls [225] and 3-nitrotyrosine [226,227] production. Several specifically oxidized proteins have been thus far identified in AD brain which range from those involved in energy metabolism, such as creatine kinase (BB isoform), -enolase, and triosephosphate isomerase which deal with the ATP production, to damaged or aggregated proteins through the proteasome [228], to proteins involved in membrane structure and apoptosis, such as the neuropolypeptide h3 whose oxidative modification may compromise membrane phospholipid asymmetry in AD brain, and contributes to the loss of cholineacetyltransferase activity [227], to those deal with the neuronal communication as the dihydropyrimidinase-related protein 2 that modulates the activity of collapsin, and whose expression is increased in AD [229]. It means that oxidative modifications affect function of diverse proteins which, in turn, may cause some of the pathological and biochemical alterations found in AD such as aggregated accumulation, shortened dendritic lengths, excess of ubiquitination, decreased proteosomal activity. An increase of oxidized proteins has been observed also in plasma of AD subjects other than in AD brain, suggesting that such oxidized proteins may be useful as biomarkers for AD [230]. Among these oxidized proteins, isoforms of fibrinogen gamma-chain precursor protein and alpha-1-antitrypsin precursor, which are both implicated in the pathology of the disease, had a two- to six-fold greater specific oxidation index in plasma from AD subjects when compared to controls [231]. Isoforms of human transferrin, and hemopexin have also been suggested as potential biological markers of AD, since their specific oxidation indices were higher in AD plasma compared to plasma samples from normal elderly controls [232]. Among biomarkers of oxidative stress, recently, plasma IgG levels of dytyrosine have been found to be more elevated in demented patients (i.e., AD and VaD) than in controls [233].

An increase in the levels of protein carbonyls have been demonstrated in MCI patients as well. The increase was selective for the superior and middle temporal gyri and was not present in the cerebellum, stating the importance of these oxidative alterations as deterministic factors for AD in the premonitory and early phase of the disease [228].

Lipid peroxidation seems to have a major role in the pathogenesis of the disease, and can be detected by different markers. Isoprostanes (IsoPs), one group of lipid peroxidation products generated from arachidonic acid, are especially useful as *in vivo* biomarkers of lipid peroxidation. Increased levels of IsoPs have been reported in brain tissue and cerebrospinal fluid of AD patients [234–237], while conflicting results have been shown as for quantification of IsoPs in plasma and urine of AD subjects [235,238–240]. Higher levels of IsoPs were also observed in cerebrospinal fluid, plasma, and urine of subjects with MCI, suggesting that increased brain oxidative damage occurs before the

onset of symptomatic dementia [241]. Thiobarbituric acid-reactive substances (TBARS) are another one marker of lipid oxidation that, however, are not specific of membrane lipid peroxidation. Evidence of elevated levels of TBARS in various brain regions of AD and MCI patients has been reported [229,242,243].

Other reactive products of lipid peroxidation are the alkenals, 4-hydroxynonenal (HNE) and 2-propenal (acrolein), which result from the attack of free radical on polyunsaturated fatty acids of phospholipids. These reactive aldehydes can structurally modify proteins by covalent interaction and inhibit enzyme function, and are both elevated in multiple brain regions of AD subjects [244–251]. An interplay of transition metals, A β peptide and lipid peroxidation has been suggested to be responsible for increased oxidative stress and cell damage in AD. In fact, oxidative stress in the nervous system is highly associated with amyloid deposits. Amyloid peptide induces oxidative stress, and inflammation and oxidative stress generate more A β , determining a vicious cycle between A β and free radical formation.

Since oxidative stress results from an imbalance between formation and neutralization of pro-oxidants, diverse anti-oxidant compounds have been examined in AD, such as the intracellular enzymes superoxide dismutase (SOD), and glutathione peroxidase, the endogenous molecule glutathione (GSH), the essential nutrients vitamin C, and vitamin E, and some dietary compounds (bioflavonoids, proanthocyanidans). Decreased levels of any of these antioxidants have been reported in brains and plasma of AD subjects, and MCI patients as well [252]. The evidence accumulated so far indicates that oxidative imbalance and subsequent oxidative stress are early events during the evolution of AD, and improved oxidative balance in the nervous system might help protect for the development of AD, likely by decreasing A β formation.

5. Biomarkers of inflammation

In recent years, there has been a significant increase of interest in the role that inflammation plays in the development and progress of AD. The major evidence of the involvement of inflammation in the pathogenesis of AD derives from the presence in the brains of AD patients, especially close to SPs, of large numbers of activated microglia and numerous inflammatory mediators including complement factors (C1q, C3b, C3c, C3d, and C4), acute phase proteins (e.g., α 1 chymotrypsin), chemokines such as the monocyte chemoattractant protein-1 and IP-10, chemokine receptors (CXCR2, CCR3 and CCR5), key enzymes of inflammation such as nitric oxide synthase (NOS) and cyclooxygenase (COX), and inflammatory cytokines [e.g., interleukin (IL)-1, tumor necrosis factor (TNF)- α , and IL-6] [253].

It has been shown that virtually all the inflammatory molecules occur at abnormal levels in brain regions affected by the disease [254–256], and it is supposed that cytokines or chemokines upregulation promote the production and

deposition of A β . On the other hand, interestingly, A β and APP seem to induce the production of various inflammatory cytokines and chemokines in the astrocytes, microglia, and in some cases, neurons, which sets off a vicious cycle whereby A β production stimulates cytokine synthesis and cytokines, in turn, stimulate A β synthesis and amyloid formation [257].

As well as in the brain, similar molecules and patterns of events have been observed in the periphery of AD patients. This implies that analysis of any of these inflammation markers in CSF and plasma or serum may help and improve the discrimination between AD patients and healthy subjects, although it is not clear if the accumulation of inflammation molecules within brain is reflected in serum or plasma. In fact, results of cytokine and acute phase reactants levels in the plasma or serum are somewhat controversial. In particular, IL-6 has been widely examined, and some studies showed an increase of IL-6 in serum or plasma of AD patients [258–263], whereas others did not report any change in the concentration of the interleukin between AD and healthy subjects [264–267]. Similar contrasting results have been obtained for other chemokines or interleukins as well, such as IL-1 β , TNF- α , α 1-antichymotrypsin, transforming growth factor- β .

It is possible that the severity of dementia influenced these differences in results. In fact, one study observed an increase in IL-6 serum levels only in the most severely affected patients compared to controls [268]. Unfortunately, not all studies measured the duration or the severity of dementia. The low number of patients included in several studies may also limit the possibilities to establish a relation with severity. In summary, the definite statements concerning inflammation biomarker differences between control and AD patients require the use of sensitive assays, large patient groups, in combination with the measurement of the duration and the severity of dementia. Of course, this is imperative not only for measuring very low concentrations of interleukins but for all possible AD and dementia markers. Therefore, although signs similar to chronic inflammation have been found in brain regions affected by AD, and brain inflammation might be a possible trigger of the disease, plasma and serum biomarkers are unlikely to be reproducible, sensitive, or specific for AD diagnosis and progression.

6. Conclusions

While diagnostic accuracy for AD and other dementing disorders has improved, the differential diagnosis for these disorders is still problematic. In the very early stages of disease, frequently classified as MCI or other predementia syndromes [6,269], delineating disease process from “normal ageing” may be difficult; in later stages of the disease distinguishing AD from a number of neurodegenerative diseases associated with dementia may also be difficult. Furthermore, the disease progression is slow and there is variability of

performance on clinical measures, making it difficult to monitor change effectively. Since disease modifying therapy is likely to be most effective early in the course of disease, early diagnosis is highly desirable before neurodegeneration becomes severe, and therefore, there is a great need for biomarkers that could substantially aid early diagnosis of AD.

In 1998, criteria for an ideal biomarker of AD have been proposed by the Ronald and Nancy Reagan Research Institute, Alzheimer’s Association and the NIA joined to assemble a working group on molecular and biochemical markers of AD [35]. For this consensus group the ideal biomarker for AD would be directed at the fundamental CNS pathophysiology of AD and be validated in neuropathologically-confirmed cases, it would mark more than increased risk for the disease, marking the presence of the disease process itself; it would track disease severity at early, or pre-clinical stages of AD; it should have a diagnostic sensitivity >80% for detecting AD and a specificity of >80% for distinguishing other dementias; it should be reliable, reproducible, non-invasive, simple to perform, and inexpensive [35]. At present, studies on cognitive decline and dementia have not yielded a consistent, easily reproducible, sensitive, or specific plasma or serum biomarker marker for diagnosis, risk, progression, or treatment effects.

Serum or plasma investigations may be more promising for a population-screening test, but alterations observed in the periphery may be less specific for CNS disorders [270]. Measures related to lipoprotein metabolism and homocysteine homeostasis, APP metabolism, oxidative stress, and inflammation appear to be altered in dementia and MCI relative to controls, but without sufficient discriminatory power.

The diagnostic accuracy of biomarker measurements may be improved by determination of the individual genetic background. For example, several studies have demonstrated the dependence of APOE serum levels upon the APOE genotype. Given the genetic complexity of AD, the presence of additional genes with influence on the levels of AD-related biomarkers is very likely. Thus, future research should include genetic information in the establishment of AD-related biomarkers [271]. One possible flaw in most biomarker studies is the heterogeneity of AD and dementing disorders, not only on the pathobiochemical or pathological level, but also at the clinical level, for example, the difference between early-onset and late-onset of the disease, differences in the presence or absence of vascular lesions or the difference in rates of cognitive decline between AD patients [272]. Nevertheless, most of the studies included too few patients to stratify for or to identify differences in the rate of cognitive decline. Another pitfall in studies investigating biological markers is that most studies are designed to compare AD patients to normal healthy controls, while for use in clinical practice studies should be designed to discriminate AD patients from patients with other neurodegenerative diseases [273]. The recently defined STARD initiative provides guidelines for future studies on diagnostic markers [274].

Furthermore, given the multiplicity of pathophysiological processes implicated in AD and other dementing disorders, the diagnostic accuracy of biomarkers may be improved by combining several serum or plasma markers [275], thereby creating a more robust biomarker profile characteristic of AD. Large-scale unbiased approaches evaluate a broad range of proteins (“proteomics”) or small molecule metabolites (“metabolomics”) in biological fluids. Approaches to biomarker profiling can be “knowledge-based,” incorporating the range of known putative AD biomarkers, or “unbiased,” surveying hundreds or thousands of biomolecules using proteomic or metabolomic methods to discover novel molecular profiles representative of AD [276].

Beyond these criteria for early and accurate diagnosis [35], it would be especially useful if the biomarker could also capture the beneficial effect of disease modifying therapy. For these reasons the NIA commissioned a working group on biomarkers as part of its Initiative on Neuroimaging in AD with the mission to provide the NIA with a list of biological measures suitable for a multicenter, longitudinal study of AD, with special consideration given to MCI [277]. These measures are considered to have potential value in diagnosis, prognosis, or assessing the beneficial effects of treatment. Several plasma or serum measures are responsive to medications, for instance statins reduce cholesterol and 24S-hydroxy-cholesterol levels, folate reduces homocysteine levels, A β immunization produces anti-A β antibodies, and lipid-lowering drugs and cholinesterase inhibitors corrected the abnormally low APP isoform ratios in AD. However, confirmation in larger populations and a consistent correlation with clinical benefit are needed before any measure can be recommended as a putative plasma or serum biomarker in clinical trials on predementia syndromes and dementing disorders.

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