CHARACTERIZATION AND PROGNOSIS OF PATIENTS WITH MCI AND NEGATIVE AMYLOID CSF BIOMARKERS

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Abbreviations

Aβ – Amyloid β  
AD – Alzheimer’s disease  
ADL – Activities of Daily Living  
ARDRA – Alzheimer’s Disease and Related Disorders Association  
CBD – Corticobasal degeneration  
CSF – Cerebrospinal fluid  
DMN – Default Mode Network  
DTI – Diffusion tensor imaging  
FAB – Frontal assessment battery  
FTD – Frontotemporal dementia  
FIRST – FMRIB’s Integrated Registration and Segmentation Tool  
GLM – General Linear Modelling  
HV – Hippocampal volume  
IADL – Instrumental Activities of Daily Living  
LBD – Dementia with Lewy-body  
MCI – Mild cognitive impairment  
MMSE – Mini Mental State Evaluation  
MoCA – Montreal Cognitive Assessment  
MRI – Magnetic resonance imaging  
MTL – Medial temporal lobe  
NIA-AA – National Institute on Aging-Alzheimer’s Association  
NINCDS – National Institute of Neurological and Communicative Disorders and Stroke  
NFTs – Neurofibrillary tangles  
PET – Positron emission tomography  
PSP – Progressive supranuclear palsy  
PHFs – Paired helical filaments  
SNAP – Suspected non-Alzheimer’s pathophysiology  
SPECT – Single-photon emission computed tomography  
TDP43 – TAR DNA-Binding Protein  
VaD – Vascular dementia  
VBM – Voxel-based morphometry
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1 Introduction

1.1 Alzheimer’s disease vs Alzheimer’s dementia

Alzheimer’s dementia is the most common cause of dementia in older adults. It is defined as a clinical syndrome in which the cognitive impairment is severe enough to interfere with the common activities of everyday life, and it represents the latest stage of a neuropathological process defined Alzheimer’s disease (AD), which has started years or even decades before becoming clinically evident.

Conversely, Alzheimer’s disease is not defined by clinical symptoms, it is instead an “aggregate of pathophysiologic processes, thus defined in vivo by biomarkers and post-mortem by pathological changes”.¹

“Alzheimer’s disease” and “Alzheimer’s dementia” are far from being interchangeable terms. Recognizing the semantic differences between the two definitions has profound effects on the comprehension and management not only of Alzheimer’s, but also of all conditions that ultimately lead to any type of dementia.

Within this framework, Alzheimer’s dementia can be considered as the tip of the Alzheimer’s disease iceberg, a visible part of a much larger entity that becomes evident after years of undetected underlying events. Alzheimer’s dementia is not only the latest stage of this pathological process, it is “the latest stage of the latest stage”, in which the subject has lost his ability to do the common activities of everyday life.

The recognition of this difference has led scientists and clinicians to focus on earlier phases of the pathological process.

1.2 Mild Cognitive Impairment

Patients who have cognitive abilities lower than those expected for their age but who are not so impaired to be diagnosed as having dementia have been variously labelled as having age-associated memory impairments²⁻³, age-associated cognitive decline⁴, mild cognitive impairment (MCI)⁵ and the more recent definition of mild neurocognitive disorder⁶.

The change in terminology has significant effects on clinical practice and research. Unlike previous definitions, MCI is not considered an age-related event, nor is limited to patients with memory deficits. It is a broader definition that includes patients with impairment in one or more cognitive domains (memory, executive functions, language, visuospatial skills and attentional
control) not severe enough to interfere with everyday life. Similar to the concept of dementia, MCI is a clinical construct.

The more recent revision of diagnostic criteria\(^7\) for MCI, that has been done by the NIA-AA workgroups on diagnostic guidelines for Alzheimer's disease, is listed in Table 1.

### Table 1 - Diagnostic criteria for MCI

<table>
<thead>
<tr>
<th>1. <strong>Concern regarding a change in cognition</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>There should be evidence of concern about a change in cognition, in comparison to the patient’s prior level. This concern can be obtained from the patient, from an informant who knows the patient well, or from a skilled clinician observing the patient.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2. <strong>Impairment in one or more cognitive domains</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>There should be evidence of lower performance in one or more cognitive domains that is greater than would be expected for the patient’s age and educational background. If repeated assessments are available, then a decline in performance should be evident over time. This change can occur in a variety of cognitive domains, including: memory, executive function, attention, language, and visuospatial skills.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3. <strong>Preservation of independence in functional abilities</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with MCI commonly have mild problems performing complex functional tasks they used to be able to perform, such as paying bills, preparing a meal, shopping at the store. They may take more time, be less efficient, and make more errors at performing such activities than in the past. Nevertheless, they generally maintain their independence of function in daily life, with minimal aids or assistance.</td>
</tr>
</tbody>
</table>

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<tr>
<th>4. <strong>Not demented</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>These cognitive changes should be sufficiently mild that there is no evidence of a significant impairment in social or occupational functioning. It should be emphasized that the diagnosis of MCI requires evidence of intra-individual change. If an individual has only been evaluated once, change will need to be inferred from the history and/or evidence that cognitive performance is impaired beyond what would have been expected for that individual. Serial evaluations are of course optimal but may not be feasible in a particular circumstance.</td>
</tr>
</tbody>
</table>

MCI is common in the elderly population. Its prevalence increases with age – from 6.7% at ages 60-64 years to 25.2% at ages 80-84 years – and with lower educational level\(^8\).

As pointed out in the 2018 update of the MCI practical guidelines\(^8\), it is important to diagnose MCI correctly for multiple reasons:

- To investigate and treat reversible causes. Since not all cases of MCI are due to progressive and/or untreatable conditions, identifying a reversible risk factor or causative event (e.g. medication side effects, sleep apnoea, depression) can allow the
patients’ cognitive abilities to return to normality. If the cause of the cognitive impairment is irreversible, there is still room to improve the patient’s quality of life by treating behavioural and psychiatric symptoms.

- To help the patient and his family to understand the cause of the cognitive concern.
- To discuss the prognosis. Patients with MCI have a risk of developing dementia three times higher than their cognitively healthy peers. Since the progression of cognitive decline can impair decision making, it is important to educate patients with MCI to make decisions in advance on possible future scenarios (e.g. power of attorney designation, living will, and finances) while they still have mental capacity.

In order to diagnose MCI correctly, clinicians should compare the cognitive concerns referred by the patient and by an informant, and support it with validated cognitive tests (e.g. the Mini Mental State Examination, MMSE, or the Montreal Cognitive Assessment, MoCA). Because of the high sensitivity but low specificity of these tools, it is recommended to better characterize the cognitive impairment with more specific neuropsychological tests. Due to the possibility of an underlying neurodegenerative condition, clinicians should follow up the patient for changes in status and/or identify biomarkers that can stratify the risk of underlying pathology.

### 1.3 Biomarkers

The most common neurodegenerative dementia is Alzheimer’s dementia, which accounts for 2/3 of the cases of dementia. As a consequence, the concept of MCI has been mostly applied to AD, resulting in the definition of criteria for “MCI due to AD”\(^9\), in which the clinical concept of MCI (as defined above) would be supported by the presence of positive biomarkers of AD.

A “biomarker” is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. In the context of AD, biomarkers can add diagnostic and prognostic information. There is now a general consensus on the application of biomarkers in research settings, while their use in routine clinical care is still debated.

Among the several biomarkers proposed for AD up to now\(^10,11,12\), the most validated are the following:

- **CSF Aβ\(_{1-42}\)** levels decrease in AD and correlate with neuritic plaque load in the brain\(^12\). The most widely accepted explanation for the reduction in CSF Aβ\(_{1-42}\) in AD is that aggregation of Aβ into plaques (and, hence, retention of the peptide in the brain parenchyma) results in reduced availability of Aβ to diffuse into the CSF\(^11\).

- **CSF total tau** (t-tau) levels increase in AD but also in other conditions in which neuronal and axonal damage occurs, including tissue damage (stroke or brain trauma) or rapid neuronal degeneration (Creutzfeldt–Jakob disease). Increased CSF t-tau has also been
associated with faster progression from MCI to AD and with rapid cognitive decline in AD.14

- **CSF phosphorylated tau** (p-tau) levels increase in AD and reflect both the phosphorylation state of tau and the formation of neurofibrillary tangles (NFTs) in the brain. Instead, it is normal in Creutzfeldt–Jakob disease or acute stroke. Thus, increased p-tau is considered specific for AD and differentiates AD from other dementias, including frontotemporal dementia and dementia with Lewy-bodies.15 The levels of CSF p-tau also correlate with the severity of tau paired helical filaments (PHFs) accumulation post-mortem.16,17 In addition, it has been associated with increased risk of progression from MCI to AD dementia and with rapid cognitive decline in AD.

- **Structural MRI** in patients with AD shows a typical pattern of atrophy, especially prominent in the medial temporal lobes (MTLs). By the time AD is diagnosed clinically, atrophy already involves, in addition to the MTL, the parietal and posterior superior temporal regions on the lateral cerebral surfaces, posterior portion of the cingulate gyrus on the medial surface, and cortical association areas including prefrontal cortices.18,19,20 An important challenge of structural MRI studies has been to differentiate MCI subjects who will progress to AD in the near future (converters) from those who will remain stable (no-converters). Within this framework, several studies found that lower baseline hippocampal volume is related to progression to AD, with variable sensitivity and specificity ranging between 50 and 80%. However, hippocampal volume is highly correlated with head size. Since head size tends to be larger in males,24,25 adjusted hippocampal volume has a strong relationship with male sex. Therefore, it may be preferable to use AD signature thickness in the study of AD related neurodegeneration.26

- **Positron emission tomography (PET)** with 18 fluorodeoxyglucose (18 FDG) in patients with AD shows a typical pattern of reduced cortical uptake in posterior and lateral temporal regions, medial parietal regions, particularly in the posterior cingulate gyrus and precuneus, and medial temporal lobes including hippocampus; with progression of the disease, metabolic deficit spreads to prefrontal association areas as well.27,28 FDG hypometabolism parallels cognitive function and histopathological diagnosis of AD at autopsy,29,30 and can predict conversion to MCI in the cognitively normal elderly.31

- **PET** with tracers targeting insoluble fibrillar Aβ [including the most studied Pittsburgh Compound-B (PiB), but also the newly developed fluorine-18-labelled tracers (florbetaben, florbetapir, flutemetamol)] in patients with AD shows abnormal uptake in regions of the cortical midline (medial prefrontal cortex, posterior cingulate and precuneus) and lateral temporal-parietal associative cortex. Several studies have shown that increased uptake occurs in about 95% of patients with a clinical diagnosis of AD.
dementia, 60% of patients with a clinical diagnosis of MCI, 93% of patients with MCI who then progressed to AD dementia within 3 years, and 24% of cognitively normal controls who may be in the preclinical phase of AD (percentages taken from a review study\textsuperscript{32}). Indeed, it is thought that, by being a direct surrogate for A\textsubscript{\textbeta} pathology, amyloid PET has the potential to detect the very beginnings of AD pathophysiology in the asymptomatic stage.

- PET with tracers targeting tau PHF deposits have been recently studied in humans. In vivo imaging compared to autopsy findings showed that Flortaucipir (formerly T807 and AV1451) specifically binds to tau PHF deposits, and autoradiographic studies demonstrated that it does not bind to amyloid plaques, argyrophilic grains, alpha-synuclein and TDP-43. Even if few studies have been conducted on AD patients\textsuperscript{33,34,35}, they showed a strong association between elevated tau PET binding in both the medial temporal lobe and neocortex and positive amyloid PET scans and cognitive impairment.

Different biomarkers classifications have been proposed over time. In order to better understand the study reported in this thesis as well as future directions of research, two classifications will be discussed:

- the 2011 MCI due to AD workgroup classification\textsuperscript{9}
- the 2018 NIA-AA research framework to investigate the AD continuum classification\textsuperscript{1}

1.4 MCI due to AD workgroup classification (2011)

The 2011 MCI due to AD workgroup identified two main categories of biomarkers:

- Biomarkers reflecting the neuropathological hallmarks of AD
- Biomarkers reflecting downstream events like structural and functional changes

Each class has specific pros and cons regarding the evaluation of the underlying condition (diagnosis) and the likelihood of progression (prognosis).
### Biomarkers reflecting neuropathological hallmarks of AD

- Decreased CSF \( \text{A}^\beta_{1-42} \) levels
- Increased CSF t-tau and p-tau levels
- Increased amyloid-PET ligand uptake

### Biomarkers reflecting downstream structural changes

- Hippocampal volume (HV) or medial temporal lobe (MTL) atrophy volumetric measures or visual rating
- Rate of brain atrophy
- Less well validated biomarkers: diffusion tensor imaging (DTI), voxel-based and multivariate measures

### Biomarkers reflecting downstream functional changes

- Hypometabolism in temporal-parietal regions on FDG-PET
- Hypoperfusion in temporal-parietal regions on SPECT
- Less well validated biomarkers: fMRI activation studies, resting BOLD functional connectivity, MRI perfusion, MR spectroscopy

#### 1.4.1 Biomarkers reflecting neuropathological hallmarks of AD

The neuropathological hallmarks of AD are senile plaques and neurofibrillary tangles.

The former are mostly made of \( \text{A}^\beta_{1-40} \) and \( \text{A}^\beta_{1-42} \), two by-products of the amyloid precursor protein (APP) metabolism and the major variants of the amyloid-\( \beta \) protein (A\( \beta \)). They are respectively 40 and 42 amino acids long peptides that have in common the N-terminus but differ in length by two amino acids in the C-terminal end.

The latter are primarily made of paired helical filaments of abnormally hyperphosphorylated tau protein. Tau protein is a high soluble microtubule-associated protein primarily expressed in the distal portions of axons that normally concurs to the microtubule stabilization. However, the hyperphosphorylation that occurs in AD as well as in other tauopathies, causes disruption of microtubules and alteration of the neuron’s transportation system, finally resulting in
Neuronal loss.

Neuropathological hallmarks of AD can be estimated in vivo by means of CSF and imaging biomarkers.

The main advantage of this class of biomarkers is their high specificity. Since they reflect the hallmarks of AD, these biomarkers could add to the clinical evaluation relevant information about the probable underlying condition.

The main disadvantage of this class of biomarkers is the weak correlation with the clinical disease severity. Amyloid deposition has a weak correlation with cognitive decline, which is more closely associated with tau accumulation instead. Therefore, these biomarkers could be best applied to confirm or rule out the diagnosis of AD.

1.4.2 Biomarkers reflecting downstream events like structural and functional changes

The neuropathological process underlying Alzheimer’s dementia results first in synaptic loss (undetectable in vivo and in humans) and then in neuronal dysfunction and death (both detectable in vivo and in humans). Structural and functional changes can be estimated in vivo by means of imaging biomarkers.

The main advantage of this class of biomarkers is the strong correlation with the clinical disease severity and cognitive function. Since they reflect the structural and functional substrate of the cognitive abilities, these biomarkers could add relevant information about the likelihood of the disease progression.

The main disadvantage is their low specificity. Atrophy in the MTL occurs not only in AD but also in cerebrovascular disease, epilepsy, anoxia, hippocampal sclerosis, TDP-43-opathy, primary age-related tauopathy, chronic traumatic encephalopathy, argyrophilic grain disease, progressive supranuclear palsy, and Pick disease. Temporoparietal hypometabolism also occurs in corticobasal degeneration, primary progressive aphasia, and cerebrovascular disease.

Therefore, these biomarkers could be best applied to suggest the evolution of the pathology.
In research context, the use of some of these biomarkers has been approved in order to define two categories – amyloidosis and neurodegeneration, as shown in Table 3.

Table 3 - Biomarker categories identified by the 2011 MCI due to AD workgroup classification

<table>
<thead>
<tr>
<th>Category</th>
<th>Imaging biomarkers</th>
<th>CSF biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amyloidosis (A)</td>
<td>Cortical amyloid PET ligand binding</td>
<td>Low CSF Aβ₁-42</td>
</tr>
<tr>
<td>Neurodegeneration (N)</td>
<td>• Hypometabolism on FDG-PET</td>
<td>High CSF t-tau and p-tau</td>
</tr>
<tr>
<td></td>
<td>• Atrophy on MRI</td>
<td></td>
</tr>
</tbody>
</table>

The 2011 MCI due to AD workgroup classification identifies 4 biomarker profiles (Table 4).

Table 4 - Biomarker profiles identified by the 2011 MCI due to AD workgroup classification

<table>
<thead>
<tr>
<th>A/N profiles</th>
<th>Biomarker category</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-N-</td>
<td>Normal AD biomarkers</td>
</tr>
<tr>
<td>A+N-</td>
<td>AD pathophysiology</td>
</tr>
<tr>
<td>A+N+</td>
<td>AD pathophysiology</td>
</tr>
<tr>
<td>A-N+</td>
<td>Non-AD pathophysiology</td>
</tr>
</tbody>
</table>

1.5 NIA-AA research framework to investigate the AD continuum classification (2018)

The most recent classification of the AD biomarkers is the ATN classification, which has been proposed by Jack et al. in 2016 and adopted by the 2018 NIA-AA research framework to investigate the AD continuum.

According to the ATN classification, biomarkers can be classified into three main categories reported in Table 5.
Table 5 - Biomarker categories identified by the 2018 NIA-AA research framework to investigate the AD continuum classification

<table>
<thead>
<tr>
<th>Category</th>
<th>Imaging biomarkers</th>
<th>CSF biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amyloidosis (A)</td>
<td>Cortical amyloid PET ligand binding</td>
<td>Low CSF Aβ1-42</td>
</tr>
<tr>
<td>Tauopathy (T)</td>
<td>Cortical tau PET ligand binding</td>
<td>High CSF p-tau</td>
</tr>
<tr>
<td>Neurodegeneration (N)</td>
<td>• Hypometabolism on FDG-PET</td>
<td>High CSF t-tau</td>
</tr>
<tr>
<td></td>
<td>• Atrophy on MRI</td>
<td></td>
</tr>
</tbody>
</table>

For each category, there are at least one imaging and one CSF biomarker. Thus, complete ATN biomarkers characterization is possible using either imaging or CSF, or a combination of imaging and CSF, depending on the availability of the tests and the research group’s preference.

The main innovation of this classification is to separate tauopathy from neurodegeneration. Due to an Alzheimer-centric vision, previous classifications used to consider biomarkers of tauopathy as biomarkers of neurodegeneration. While it is not a mistake to assume that in AD patients tauopathy and neurodegeneration are strictly associated, with CSF p-tau and t-tau both rising and patterns of atrophy on MRI and hypometabolism on FDG-PET similar to those of tau deposition, there are two main limits in this assumption: positivity to neurodegeneration could be due to the co-occurrence of a no-AD condition, and many no-AD conditions can cause neurodegeneration/injury, even in AD-like patterns.
The ATN classification identifies 8 biomarker profiles (Table 6).

Table 6 - Biomarker profiles identified by the 2018 NIA-AA research framework to investigate the AD continuum classification

<table>
<thead>
<tr>
<th>ATN profiles</th>
<th>Biomarker category</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-T-N-</td>
<td>Normal AD biomarkers</td>
</tr>
<tr>
<td>A+T-N-</td>
<td>Alzheimer’s pathophysiology*</td>
</tr>
<tr>
<td>A+T-N+</td>
<td>Alzheimer’s pathophysiology*</td>
</tr>
<tr>
<td>A+T+N-</td>
<td>Alzheimer’s disease*</td>
</tr>
<tr>
<td>A+T+N+</td>
<td>Alzheimer’s disease*</td>
</tr>
<tr>
<td>A-T+N-</td>
<td>Non-AD pathophysiology</td>
</tr>
<tr>
<td>A-T-N+</td>
<td>Non-AD pathophysiology</td>
</tr>
<tr>
<td>A-T+N+</td>
<td>Non-AD pathophysiology</td>
</tr>
</tbody>
</table>

*Alzheimer’s pathophysiologic continuum: umbrella term that includes both Alzheimer’s pathophysiology (earlier phase) and Alzheimer’s disease (late phase).

Patients can be classified into one of the 3 biomarker profiles: normal AD biomarkers, Alzheimer’s pathophysiologic continuum (Alzheimer’s pathophysiology and Alzheimer’s disease) and non-AD pathophysiology, also referred to as suspected non-Alzheimer’s pathophysiology or SNAP.

1.6 SNAP

Suspected non-Alzheimer’s pathophysiology (SNAP) is a biomarker-based concept that refers to individuals with signs of neurodegeneration in the absence of amyloid deposition, regardless of their clinical status. Thus, it can be applied to cognitively normal persons as well as to patients with impaired cognition (i.e. MCI and dementia).

The concept of SNAP is strictly associated with the definition of AD. Indeed, it was first described in a 2012 study by Knopman et al. who applied the NIA-AA criteria for preclinical AD to 450 cognitively normal persons. In this study, 75% of the enrolled subjects were successfully classified into one of the 4 NIA-AA preclinical stages of AD: 232 (44%) subjects had normal biomarkers and no cognitive impairment (Stage 1), 85 (16%) were A+N- and had no cognitive impairment (Stage 1), 75 (15%) were A+N+ in the absence or in the presence of cognitive
impairment (respectively Stage 2 and 3). However, 23% of subjects had positive markers of neurodegeneration without amyloidosis (A-N+). Thus, they were considered to have non-AD underlying processes and labelled as suspected non-Alzheimer’s pathophysiology.\textsuperscript{44}

Since the introduction of the term, the investigation of SNAP subjects has attracted increasing interest. Several studies applied the biomarker-based concept of SNAP to cohorts of cognitively normal and MCI persons, and one study even to patients clinically diagnosed with Alzheimer’s dementia\textsuperscript{45}.

We reviewed all the published longitudinal studies that included patients with a clinical diagnosis of MCI and with A-N+ (SNAP, according to the 2011 MCI due to AD classification) and summarized them in Table 7.
<table>
<thead>
<tr>
<th>Study</th>
<th>MCI/aMCI</th>
<th>Follow-up</th>
<th>1. Amyloid biomarkers</th>
<th>Prevalence of SNAP</th>
<th>Age (SNAP)</th>
<th>Sex (% male) (SNAP)</th>
<th>Education (yrs) (SNAP)</th>
<th>MMSE scores at baseline (SNAP)</th>
<th>Cognitive decline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caroli et al. 2015</td>
<td>MCI (n=201)</td>
<td>Up to 6 years (mean follow-up of 30 months)</td>
<td>1. CSF Aβ&lt;sub&gt;42&lt;/sub&gt;</td>
<td>17%</td>
<td>70.6 (mean)</td>
<td>68</td>
<td>n/a</td>
<td>26.6 (SD 1.5)</td>
<td>Progression to dementia after 6 years: A-N: 11%; A+N: 34%; SNAP 56%; A+N+ 71%</td>
</tr>
<tr>
<td></td>
<td>ADNI cohort and a European Union Dataset (Brescia, Amsterdam, Stockholm, and Munich)</td>
<td></td>
<td>2. HV on MRI or AD-related hypometabolism on FDG-PET</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Chung et al. 2017</td>
<td>aMCI (n=40)</td>
<td>2 years</td>
<td>1. Amy PET (18F-florbetapir)</td>
<td>28%</td>
<td>77.1 (mean)</td>
<td>58</td>
<td>15.7</td>
<td>27.58 (SD 2.26)</td>
<td>Progression to dementia after 2 years: SNAP 11.5%</td>
</tr>
<tr>
<td></td>
<td>ADNI cohort</td>
<td></td>
<td>2. AD-related hypometabolism on FDG-PET</td>
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</tr>
<tr>
<td>Petersen et al. 2013</td>
<td>126 aMCI from MCSA vs 58 aMCI subjects from ADNI-1</td>
<td>a) MCSA: 96 had a follow-up up to 15 months</td>
<td>1. Amy PET (PiB)</td>
<td>a) 29%</td>
<td>a) 82 (median)</td>
<td>a) 78</td>
<td>a) 13</td>
<td>a) 25 (IQR 24.27)</td>
<td>a) Progression to dementia after 15 months: A-N: 8%; A+N: 0%; SNAP 21%; A+N+ 16%</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>b) ADNI-1: 49 aMCI subjects had a follow-up at approximately 12 months</td>
<td>b) 17%</td>
<td>b) 77 (median)</td>
<td>b) 70</td>
<td>b) 16</td>
<td>b) 28 (IQR 26.29)</td>
<td>b) Progression to dementia after 12 months: A-N: 11%; A+N: 0%; SNAP 25%; A+N+ 42%</td>
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<tr>
<td></td>
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<td>2. HV on MRI or AD-related hypometabolism on FDG-PET</td>
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</tr>
<tr>
<td>Study</td>
<td>Type</td>
<td>n</td>
<td>Clinics/Networks</td>
<td>Follow-up Duration</td>
<td>Inclusion Criteria</td>
<td>Progression to Dementia:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>------</td>
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<td>--------------------------------------------------------------------------------</td>
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<td>-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
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</tr>
</tbody>
</table>
| Prestia et al. 2013 | MCI  | 73 | 3 European memory clinics (TOMC, VUMC, KUHH)                                    | At least 12 months (mean follow-up of 28 +/- 17 months) | 1. CSF Aβ₁₋₄₂  
2. HV on MRI or AD-related hypometabolism on FDG-PET                                                                                                                                                                                      | A-N: 4.5%; A+N: 27%; SNAP 46.6%; A+N+ 100% |
| Vos et al. 2015  | MCI  | 766| DESCIRA, AddNeuroMed, German Dementia Competence Network, EADC-PET, ADNI-1       | Mean follow-up of 2.4 years (range 0.5-9)              | 1. CSF Aβ₁₋₄₂  
2. CSF tau or HV and/or MTL atrophy on MRI                                                                                                                                                                                              | A-N: 14%; A+N: 26%; SNAP 31%; A+N+ 62% |
| Wisse et al. 2015 | MCI  | 361| ADNI-GO, ADNI-2                                                                | Follow-up at 12 and 24 months                         | 1. CSF Aβ₁₋₄₂  
2. HV on MRI or AD-related hypometabolism on FDG-PET                                                                                                                                                                                      | A-N: 1.8%; A+N: 4.3%; SNAP 2.3%; A+N+ 42.4% |
As shown in Table 7, while imaging and CSF biomarkers were used for the assessment of amyloidosis in 2 studies\textsuperscript{46,47} and 4 studies\textsuperscript{48,49,50,51} respectively, all but one\textsuperscript{50} study used imaging biomarkers for the assessment of neurodegeneration.

The prevalence of patients with SNAP within the MCI group varied from 17\% to 35\% of participants. Possible causes of this variability were: (i) biomarkers used to group subjects; (ii) cut-offs of the biomarkers; (iii) cohort selection (i.e., population based or convenience cohort); and (iv) MCI inclusion criteria.

When considering the demographical characteristics, SNAP was more frequent in the male sex and in older adults compared to A-N-. Instead, no difference in educational attainment was found between individuals with SNAP and those with other biomarker profiles.

Although direct comparisons between studies outcomes cannot be made due to the variety of follow up length and end points, SNAP is a biomarker profile consistently associated with an intermediate risk of conversion to dementia in MCI patients, compared to other biomarker profiles. Among the 6 studies we examined, A-N- and A+N- individuals had the lowest risk of cognitive decline while A+N+ individuals had the highest risk, with SNAP individuals having an intermediate risk.

Since the most frequent diagnosis for converters was AD, important questions arose about the validity of the amyloid cascade hypothesis as well as the sensibility and specificity of current biomarkers and the clinical management of SNAP patients.

According to the traditional model of AD biomarkers\textsuperscript{52} (see Figure 1), the first biomarkers to become abnormal are CSF A\textsubscript{\textbeta}1-42 and amy-PET, followed by CSF tau and, finally, by structural MRI and FDG-PET. This model reflects the most widely accepted theory on the pathogenesis of AD, the amyloid cascade hypothesis. Within this framework, patients with neurodegeneration in the absence of amyloidosis who later convert to AD dementia are an unexpected finding.

\textit{Figure 1 - AD updated hypothetical model of dynamic biomarkers}
However, it must be considered that patients with MCI and SNAP who convert to AD dementia tend to have amyloid levels close to the cut-off of abnormality, putting them closer to the A+N+ group than to the SNAP group. Lack of standardization of imaging and CSF measurements as well as agreement on numeric cut-offs denoting normal and abnormal values further complicate the scenario.

It is still debated whether SNAP always represents pathologies outside the AD pathway or it could represent in some patients an early stage of AD.

1.7 Aim of the study

Previous prospective studies have shown that MCI patients with negative biomarkers of amyloidosis but abnormal markers of neurodegeneration (A-N+ or SNAP) have a risk of progression to dementia higher than MCI patients with both biomarkers negative, and lower than MCI patients with both biomarkers abnormal.

However, their results are difficult to translate into the clinical practice of Cognitive Neurology Clinics, in which PET is not routinely performed given its cost and CSF and structural MRI are usually more easily tested. In addition, previous studies were performed on convenience cohorts, selected for research purposes rather than on population-based clinical cohorts.

Therefore, diagnostic and prognostic challenges in patients with MCI remain frequent in clinical practice. While the combination of both normal or both abnormal biomarker types is relatively simple to interpret in the clinical context, the “ambiguous” combinations of biomarkers, namely normal amyloid levels associated with abnormal markers of neurodegeneration, poses interpretation difficulties.

The aim of our study was to establish the prognosis of patients with MCI with negative CSF biomarkers of amyloidosis in a clinical cohort presenting to a Cognitive Neurology Clinic.
2 Methods

2.1 Participants

Consecutive eligible patients were recruited from the Cognitive Neurology Clinic of the Ospedale Civile Sant’Agostino-Estense, a specialized centre for all the cases of suspected, atypical, and early-onset cognitive impairment in a population of about 700,000 residents of the Modena province.

We included all patients with a baseline clinical diagnosis of MCI according to Petersen criteria, namely: (i) the presence of cognitive concern reported by the patient, informant, or clinician; (ii) impairment in one or more cognitive domains established from neuropsychological assessment; (iii) normal functional activities; and (iv) absence of dementia. Additional inclusion criteria specific for this study were (i) normal CSF β-amyloid levels (according to established cut-offs, see paragraph 2.2 below) and (ii) the presence of at least one clinical follow-up assessment after at least 4 months from baseline.

Exclusion criteria were: (i) a clinical diagnosis of dementia at baseline; (ii) a clinical diagnosis of other neurological disorders that would impair cognition (as an example, multiple sclerosis or normal pressure hydrocephalus); (iii) possible vascular origin of the cognitive dysfunction and/or evidence on MRI scan of cerebral vascular encephalopathy; (iv) absence of a reliable caregiver at follow up visits; (v) and lost at follow up.

At baseline each participant underwent extended medical history interview, blood tests, neurological examination, neuropsychological assessment, structural MRI (if they did not have contraindications to MRI), and lumbar puncture. The diagnosis of MCI at baseline was established on the basis of the clinical history and neuropsychological assessment. This included the Mini Mental State Examination (MMSE), Babcock story recall test, Rey-Osterreith figure delay recall, category and letter fluency test, Stroop test and frontal assessment battery (FAB), and functional assessment with Activities of Daily Living (ADL) and Instrumental Activities of Daily Living (IADL). Importantly, the biomarker status (MRI and CSF) was not considered to establish the baseline clinical diagnosis of MCI.

Participants were subsequently followed up every six months. Clinical follow-up evaluation included neurological assessment, cognitive assessment with the MMSE and functional assessment with ADL and IADL. At each follow up visit patients were classified as No-converters if they still had a clinical diagnosis of MCI, i.e. their cognitive impairment had not progressed, they had remained functioning and independent on activities of daily living. They were instead classified as Converters if they had progressed to symptomatic dementia, established according to existing criteria. Converters were further classified in AD-Converters if they fit criteria for probable Alzheimer Disease according the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer’s Disease and Related Disorders Association (ADRA) criteria, or no-AD Converters if they fit criteria for probable vascular dementia (VaD) (NINDS-AIREN criteria), Frontotemporal Lobar Degeneration including Frontotemporal dementia, semantic dementia and primary...
progressive aphasia\textsuperscript{55}, Dementia with Lewy Body (LBD)\textsuperscript{56}, Progressive Sopranuclear Palsy (PSP) \textsuperscript{57}, or Corticobasal Degeneration (CBD)\textsuperscript{58}.

2.2 Lumbar puncture

Lumbar puncture was performed in the morning in fasting patients, using a standard procedure to minimize the risk of biological and chemical contamination. CSF was collected in sterile polypropylene tubes, which were transported to the adjacent laboratory within 30 min of collection, and it was centrifuged for 15 min at 2700 × g at controlled room temperature and aliquoted into polypropylene storage tubes. CSF β-amyloid, t-tau, and p-tau 181 were measured with the ELISA method following manufacturer instructions (Innogenetics, Gent, Belgium).

CSF β\textsubscript{1-42}-amyloid was used as a marker of amyloidosis while CSF t-tau was used as a marker of neurodegeneration, according to NIA-AA preclinical AD classification\textsuperscript{61}.

Since there are no universally established cut-offs of normality for CSF biomarkers, we referred to previous literature published on Italian samples and considered the following literature-based cut-offs: normal CSF β\textsubscript{1-42}-amyloid greater than 500 pg/mL\textsuperscript{49}; normal CSF t-tau less than 382 pg/mL\textsuperscript{62}.

However, since a consensus paper from the Alzheimer’s Biomarkers Standardization Initiative recently suggested that each laboratory should have its own validated cutoffs to ensure adequate sensitivity and specificity\textsuperscript{63}, we also performed follow-up analyses considering cut-offs specific for our laboratory. To establish our lab-specific cut-offs we used CSF from a sample of 40 patients with a clinical diagnosis of Alzheimer’s dementia and a sample of 46 cognitively healthy controls of similar age and sex distribution. Using the method based on ROC curve and Youden index to determine maximum sensitivity and specificity, we established the following lab-specific cut-offs: normal CSF β\textsubscript{1-42}-amyloid greater than 557 pg/mL for Aβ\textsubscript{1-42}; normal CSF t-tau less than 350 pg/mL.

Only patients with normal CSF β\textsubscript{1-42}-amyloid (i.e., negative marker of amyloidosis, A) were included in the study and were referred to as A-. They were further classified in A-N- if they also had normal CSF t-tau (i.e., negative marker of neurodegeneration, N) or A-N+ if they had abnormal t-tau. This group is also referred to as suspected non-Alzheimer’s pathophysiology (SNAP).

2.3 Statistical analyses

Analyses of behavioral and neuropsychological data were performed with SPSS version 24.0. Comparisons between Converters and No-Converters, and – among the Converters – between AD Converters and No-AD Converters were performed with independent t-test for continuous variables, and chi-square tests for dichotomous variables, using a level of statistical significance of p<.05. To assess the risk of conversion in A-N+ (i.e. those subjects with normal CSF β\textsubscript{1-42}-amyloid and abnormal CSF t-tau, also indicated as SNAP) relative to A-N- patients, we plotted survival curves and computed hazard ratios (both crude and adjusted by age, sex, and years of education).
2.4 Imaging acquisition and analyses

Patients who did not have contraindication to MRI underwent structural MRI imaging at baseline (i.e., within 1 month from the baseline clinical assessment and the lumbar puncture). Scanning was performed at Ospedale Civile Sant’Agostino Estense, Baggiovara, Modena using a 3-T Philips Intera MRI scanner equipped with a 12-channel head coil. The images acquired were high-resolution T1-weighted 3D MP-RAGE structural images (repetition time 9900 ms; echo time 4.6 ms; field of view 256x256 mm; voxel dimension 1mm³).

Structural data were analysed with FSL-VBM, a voxel-based morphometry (VBM) style analysis carried out with FSL tools (http://www.fmrib.ox.ac.uk), to detect grey matter (GM) differences. First, structural images were brain-extracted and grey matter segmented before being registered to the MNI 152 standard space using non-linear registration. The resulting images were averaged to create a study-specific template, to which the native grey matter segmented images were then non-linearly re-registered. The modulated, registered grey matter images were then smoothed with an isotropic Gaussian kernel with a sigma of 3 mm. Finally, voxel-wise General Linear Modelling (GLM) was applied using permutation-based non-parametric testing (1000 permutations). We performed voxel-based t-tests comparisons between Converters and No-Converters, and – among the Converters – between AD Converters and No-AD Converters to identify regions of significantly different grey matter atrophy between the groups of patients. We also repeated all the voxel-based comparisons including a measure of disease severity (MMSE scores) and/or CSF t-tau as covariates of no interest to control for their potential effects. VBM results were first explored at the uncorrected voxel-level using a liberal threshold of p<0.01, then also after applying correction for multiple comparisons at the p<0.1 level (cluster-correction).

From each subject’s structural MRI scan we also extracted the volumes of the left and right hippocampus using FMRIB’s Integrated Registration and Segmentation Tool (FIRST) (http://www.fmrib.ox.ac.uk). The FIRST algorithm automatically segments subcortical structures based on the shape and intensity variations, as learnt from a training set. We compared the extracted volumes between the group of interest (Converters versus No-Converters, and AD Converters versus No-AD Converters).
3 Results

3.1. Analyses on literature-based cut-offs

Among 210 consecutive patients who performed a lumbar puncture for the assessment of their cognitive impairment at the Cognitive Neurology Clinic of the Ospedale Civile Sant’Agostino Estense between January 2008 and February 2016, 92 patients fit inclusion criteria for the present study. More precisely, they had a clinical diagnosis of MCI, at least one clinical follow-up overtime, and CSF levels of $\beta_{1-42}$-amyloid > 500 pg/mL.

Table 8 reports demographical and clinical features of the 92 consecutive eligible subjects included in the analyses using literature-based cut-offs. Among them, 35 were female (38%). Their mean age at baseline was 66.5 years, mean education level was 9.9 years, mean MMSE at baseline was 26.6. 58 (63%) subjects had both CSF $\alpha$-1-42 and t-tau normal levels (A-N- group), while 34 (37%) had normal CSF $\alpha$-1-42 levels and high CSF t-tau levels (A-N+).

3.1.1 Risk of conversion

Over an average follow-up of 23.8 months (median of 17 months, total number of person-months of follow-up equal to 2190), 37 (40.2%) patients developed clinical dementia and were designated as converters, whereas 55 (59.8%) remained with a clinical diagnosis of MCI and were designated as no-converters. As shown in the flow-chart of Figure 2, among the 37 converters, 21 patients converted to Alzheimer’s dementia (AD), 10 to Frontotemporal dementia and 2 to Progressive Supranuclear Palsy (12 FTD spectrum), 2 to Lewy Body dementia and 2 to vascular dementia.

Figure 2 - Flow-chart “inclusion, conversion, and type of dementia” of the 92 subjects

Table 8 reports demographical and clinical features of converters and no-converters groups, and the level of significance of their comparisons. Age at symptoms onset ($p = 0.93$) and years of education ($p = 0.18$) were comparable between converters and no-converters. Compared with no-
converters, converters had lower baseline MMSE scores at the trend level (p=0.08). At baseline, converters had also lower CSF $A\beta_{1-42}$ concentrations at the trend level (p=0.07) and significantly higher CSF t-tau and p-tau concentrations (respectively p=0.013 and p=0.05).

Table 8 - Demographical, clinical and biomarkers features of total sample, Converters and No-converters

<table>
<thead>
<tr>
<th>Study</th>
<th>TOTAL</th>
<th>Converters</th>
<th>No-Converters</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N°</td>
<td>92</td>
<td>37</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>66.5±7.0</td>
<td>66.4±7.1</td>
<td>66.6±7.0</td>
<td>0.873</td>
</tr>
<tr>
<td>Female Sex (%)</td>
<td>35 (38.0%)</td>
<td>12 (32.4%)</td>
<td>23 (41.8%)</td>
<td>0.363</td>
</tr>
<tr>
<td>A-N+ (%), others A-N-</td>
<td>34 (36.9%)</td>
<td>19 (51.4%)</td>
<td>15 (27.3%)</td>
<td>0.19</td>
</tr>
<tr>
<td>Years of education</td>
<td>9.9±4.6</td>
<td>10.6±4.6</td>
<td>9.3±4.5</td>
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</tr>
<tr>
<td>Age symptoms onset</td>
<td>64.5±7.0</td>
<td>64.4±7.5</td>
<td>64.6±6.7</td>
<td>0.932</td>
</tr>
<tr>
<td>Length of follow up</td>
<td>23.8±19.3</td>
<td>16.1±11.5</td>
<td>29.0±21.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MMSE baseline</td>
<td>26.6±8.9</td>
<td>26.2±1.9</td>
<td>27.0±7.4</td>
<td>0.079</td>
</tr>
<tr>
<td>CSF $A\beta_{1-42}$</td>
<td>771.2±255.3</td>
<td>713.2±222.3</td>
<td>810.2±270.2</td>
<td>0.074</td>
</tr>
<tr>
<td>CSF t-tau</td>
<td>366.0±242.2</td>
<td>447.3±277.7</td>
<td>311.4±199.7</td>
<td>0.013</td>
</tr>
<tr>
<td>CSF p-tau</td>
<td>64.9±31.7</td>
<td>72.8±39.5</td>
<td>59.6±24.2</td>
<td>0.050</td>
</tr>
<tr>
<td>L Hippocampal volume</td>
<td>3233.3±592.6</td>
<td>3067.5±545.9</td>
<td>3321.1±605.2</td>
<td>0.144</td>
</tr>
<tr>
<td>R Hippocampal volume</td>
<td>3293.3±687.9</td>
<td>2923.9±636.8</td>
<td>3489.5±639.1</td>
<td>0.004</td>
</tr>
</tbody>
</table>

**Legend**: N°, sample size; A, amyloidosis; N, neurodegeneration; CSF, cerebrospinal fluid; $A\beta_{1-42}$ levels of amyloid; t-tau, levels of total tau; p-tau, levels of phosphorylated tau; L, left; R, right. In **bold**, significant differences between converters and no-converters.

Among 58 A-N- MCI patients, 18 (38%) converted to dementia. Among 34 A-N+ MCI patients, 19 (55.9%) converted to dementia. In a Cox proportional hazards model (see Figure 3), A-N+ subjects had double the risk of developing dementia (hazard ratio 2.2, p=0.017; 95% CI: 1.15-4.19) compared to A-N- subjects. This increased when adjusting for sex, age at baseline, and years of education (HR 2.6, p=0.004; 95% CI: 1.35-5.17), and persisted when considering the 81 patients who did not convert in the first 6 months from baseline (HR 2.3, p=0.035; 1.0-4.8, adjusted model).
Table 9 reports demographical and clinical features of AD converters and no-AD converters, and the level of significance of their comparisons. The two groups had comparable age at onset of symptoms (p = 0.81), years of education (p = 0.54) and baseline MMSE scores (p = 0.13). At baseline, those who converted to AD had significantly lower CSF Aβ1-42 concentrations (p < 0.001) and significantly higher CSF t-tau and p-tau concentrations (respectively p = 0.001 and p < 0.001) compared with no-AD converters.
Table 9 - Demographical, clinical and biomarkers features of AD converters and No-AD converters

<table>
<thead>
<tr>
<th>Study</th>
<th>AD converters</th>
<th>No-AD converters</th>
<th>p-value</th>
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<tbody>
<tr>
<td>N°</td>
<td>21</td>
<td>16</td>
<td></td>
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<tr>
<td>Age at diagnosis</td>
<td>66.2±7.0</td>
<td>66.6±7.4</td>
<td>0.892</td>
</tr>
<tr>
<td>Female Sex (%)</td>
<td>8 (38.1%)</td>
<td>4 (25.0%)</td>
<td>0.399</td>
</tr>
<tr>
<td>A-N+ (%), others A-N-</td>
<td>15 (71.4%)</td>
<td>4 (25.0%)</td>
<td><strong>0.005</strong></td>
</tr>
<tr>
<td>Years of education</td>
<td>10.3±4.3</td>
<td>11.1±5.1</td>
<td>0.591</td>
</tr>
<tr>
<td>Age symptoms onset</td>
<td>64.7±7.1</td>
<td>64.1±8.3</td>
<td>0.808</td>
</tr>
<tr>
<td>Length of follow up</td>
<td>15.7±10.7</td>
<td>16.6±12.7</td>
<td>0.805</td>
</tr>
<tr>
<td>MMSE baseline</td>
<td>25.6±2.1</td>
<td>26.8±2.0</td>
<td>0.127</td>
</tr>
<tr>
<td>CSF Aβ1-42</td>
<td>606.3±197.8</td>
<td>853.5±171.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CSF t-tau</td>
<td>573.9±294.0</td>
<td>281.1±133.8</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>CSF p-tau</td>
<td>90.5±42.6</td>
<td>49.6±17.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>L Hippocampal volume</td>
<td>2850.6±482.7</td>
<td>3408.3±484.1</td>
<td><strong>0.030</strong></td>
</tr>
<tr>
<td>R Hippocampal volume</td>
<td>2848.7±718.3</td>
<td>3042.0±512.3</td>
<td>0.516</td>
</tr>
</tbody>
</table>

**Legend:** N°, sample size; A, amyloidosis; N, neurodegeneration; CSF, cerebrospinal fluid; Aβ1-42 levels of amyloid; t-tau, levels of total tau; p-tau, levels of phosphorylated tau; L, left; R, right.

In **bold**, significant differences between AD converters and no-AD converters.

Among the 18 A-N- MCI patients converted to dementia, 6 converted to AD and 12 to non-AD dementia (10 FTD, 1 LBD and 1 VaD). Among the 19 A-N+ MCI patients converted to dementia, 15 converted to AD and 4 to non-AD dementia (2 FTD, 1 LBD and 1 VaD). In a Cox proportional hazards model, A-N+ subjects had double risk of developing AD type dementia relative to other dementias, which however was only significant at the trend level (HR 2.3, p=0.07; 95% CI: 0.9-6.0).

3.1.2 Neuroimaging

Group comparisons on hippocampal volumes and patterns of atrophy were performed on the subsample of 52 subjects who had available T1-weighted high-resolution MRI data.
Converters had significantly smaller right hippocampal volumes relative to no-converters (p= 0.004), whereas there were no significant differences in left hippocampal volume (p= 0.144).

Results of the voxel-wise comparison with VBM consistently showed that at baseline converters already had greater grey matter atrophy in the anterior part of the right medial temporal lobe (cluster-corrected P<0.1). In addition, they also showed that converters had greater grey matter atrophy in the right medial temporal lobe (see Figure 5)

In red, regions of greater atrophy in converters relative to no-converters (cluster-corrected p<0.1)
When adding t-tau as covariate of no interest, converters still showed atrophy in regions of the right temporal lobe but also in regions of the anterior medial prefrontal cortex compared with no-converters (see Figure 6).

Comparisons on patterns of atrophy showed that AD converters had atrophy in the posterior midline regions and in the right parietal lobe (cluster-corrected p<0.1, Figure 7) relative to non-AD converters.
3.2. Follow-up analyses on lab-specific cut-offs

Table 10 - Demographical, clinical and biomarkers features of total sample, Converters and No-converters - Follow-up analyses using lab-specific cut-offs

<table>
<thead>
<tr>
<th>Study</th>
<th>TOTAL</th>
<th>Converters</th>
<th>No-Converters</th>
<th>p-value</th>
</tr>
</thead>
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<td>N°</td>
<td>72</td>
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<td>48</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>66.4±7.0</td>
<td>67.1±6.9</td>
<td>66.1±7.0</td>
<td>0.553</td>
</tr>
<tr>
<td>Female Sex (%)</td>
<td>30 (41.7%)</td>
<td>8 (33.3%)</td>
<td>22 (45.8%)</td>
<td>0.310</td>
</tr>
<tr>
<td>A-N+ (%), others A-N-</td>
<td>24 (33.3%)</td>
<td>8 (33.3%)</td>
<td>16 (33.3%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Years of education</td>
<td>9.7±4.5</td>
<td>11.0±4.6</td>
<td>8.9±4.3</td>
<td>0.063</td>
</tr>
<tr>
<td>Age symptoms onset</td>
<td>66.4±7.0</td>
<td>65.1±7.6</td>
<td>64.2±6.8</td>
<td>0.627</td>
</tr>
<tr>
<td>Length of follow up</td>
<td>25.0±20.9</td>
<td>15.4±11.3</td>
<td>29.9±22.9</td>
<td>0.001</td>
</tr>
<tr>
<td>MMSE baseline</td>
<td>26.8±2.2</td>
<td>26.7±2.1</td>
<td>26.7±2.2</td>
<td>0.733</td>
</tr>
<tr>
<td>CSF Aβ1-42</td>
<td>840.6±246.9</td>
<td>818.1±211.1</td>
<td>851.9±264.4</td>
<td>0.587</td>
</tr>
<tr>
<td>CSF t-tau</td>
<td>309.9±187.6</td>
<td>341.0±214.3</td>
<td>294.3±173.1</td>
<td>0.323</td>
</tr>
<tr>
<td>CSF p-tau</td>
<td>56.5±21.8</td>
<td>54.8±21.2</td>
<td>57.4±22.3</td>
<td>0.648</td>
</tr>
<tr>
<td>L Hippocampal volume</td>
<td>3226.6±559.4</td>
<td>3135.2±536.6</td>
<td>3266.2±573.4</td>
<td>0.488</td>
</tr>
<tr>
<td>R Hippocampal volume</td>
<td>3228.9±647.9</td>
<td>2809.7±633.0</td>
<td>3410.6±573.8</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Legend: N°, sample size; A, amyloidosis; N, neurodegeneration; CSF, cerebrospinal fluid; Aβ1-42 levels of amyloid; t-tau, levels of total tau; p-tau, levels of phosphorylated tau; L, left; R, right. In bold, significant differences between converters and no-converters.

When considering the more restrictive cut-off of normality for CSF amyloid (>557 pg/dL), calculated specifically for our laboratory, only 72 patients would fit the inclusion criteria of having a clinical diagnosis of MCI, at least one clinical follow-up overtime, and normal CSF levels of β1-42-amyloid > 557 pg/mL.

Table 10 reports demographical and clinical features of the whole sample of the 72 subjects included in the follow-up analyses, and of the converters and no-converters among them.

Age at symptoms onset was comparable between converters and no-converters (p=0.63). Compared with no-converters, converters had fewer years of education (p= 0.063) at the trend level. MMSE scores (p= 0.73), CSF Aβ1-42 concentrations (p= 0.59), CSF t-tau and p-tau concentrations (respectively p= 0.32 and p= 0.65) were comparable between converters and no-converters.

Among the 72 A- patients, 48 (66.7%) had both CSF Aβ1-42 and t-tau normal levels (A-N-), while 24 (33.3%) patients had normal CSF Aβ1-42 levels and high CSF t-tau levels (A-N+). Of the 72 A- MCI patients, 24 (33.3%) converted to dementia while 48 (66.7%) maintained the diagnosis of MCI. Of the 48 A-N- MCI patients, 16 (33.3%) converted to dementia. Of the 24 A-N+ MCI patients, 8 (33.3%) converted to dementia.
There were no differences in the number of converters between A-N+ and A-N- when considering the new cutoff. In fact, in a Cox proportional hazards model (see Figure 8), A-N+ subjects had a similar risk of developing dementia (HR 1.1, p=0.4; 95% CI: 0.6-3.4) compared to A-N- subjects.

Table 11 reports demographical and clinical features of AD converters and no-AD converters included in the follow-up analyses.

Overall, of the 24 converters, 8 (33.3%) converted to AD while 16 (66.7%) converted to a non-AD type of dementia (12 FTD, 2 LBD, and 2 VaD). The more restrictive cut-off of CSF amyloid normality made the number of AD converters fall to near a third of what resulted when using the 500 pg/dL cut-off, while the number of no-AD converters did not change.
Table 11 - Demographical, clinical and biomarkers features of AD converters and No-AD converters - Follow-up analyses using lab-specific cut-offs

<table>
<thead>
<tr>
<th>Study</th>
<th>AD converters</th>
<th>No-AD converters</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N°</td>
<td>8</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>68.3±6.1</td>
<td>66.6±7.4</td>
<td>0.584</td>
</tr>
<tr>
<td>Female Sex (%)</td>
<td>4 (50.0%)</td>
<td>4 (25.0%)</td>
<td>0.221</td>
</tr>
<tr>
<td>A-N+ (%), others A-N-</td>
<td>4 (71.4%)</td>
<td>4 (25.0%)</td>
<td>0.221</td>
</tr>
<tr>
<td>Years of education</td>
<td>10.9±3.8</td>
<td>11.1±5.2</td>
<td>0.904</td>
</tr>
<tr>
<td>Age symptoms onset</td>
<td>67.0±6.0</td>
<td>64.1±8.3</td>
<td>0.391</td>
</tr>
<tr>
<td>Length of follow up</td>
<td>12.9±8.0</td>
<td>16.6±12.7</td>
<td>0.457</td>
</tr>
<tr>
<td>MMSE baseline</td>
<td>26.5±2.6</td>
<td>26.8±2.0</td>
<td>0.807</td>
</tr>
<tr>
<td>CSF Aβ1-42</td>
<td>747.1±273.3</td>
<td>853.5±171.5</td>
<td>0.253</td>
</tr>
<tr>
<td>CSF t-tau</td>
<td>460.8±296.5</td>
<td>281.1±133.8</td>
<td>0.139</td>
</tr>
<tr>
<td>CSF p-tau</td>
<td>65.3±24.8</td>
<td>49.6±17.8</td>
<td>0.088</td>
</tr>
<tr>
<td>L Hippocampal volume</td>
<td>2816.7±428.5</td>
<td>3408.3±484.1</td>
<td>0.041</td>
</tr>
<tr>
<td>R Hippocampal volume</td>
<td>2538.7±695.1</td>
<td>3042.0±512.3</td>
<td>0.161</td>
</tr>
</tbody>
</table>

Legend: N°, sample size; A, amyloidosis; N, neurodegeneration; CSF, cerebrospinal fluid; Aβ1-42 levels of amyloid; t-tau, levels of total tau; p-tau, levels of phosphorylated tau; L, left; R, right.

In bold, significant differences between AD converters and no-AD converters

Of the 16 A-N- MCI patients converted to dementia, 4 converted to AD and 12 to a no-AD type of dementia (10 FTD, 1 LBD, and 1 VaD). Of the 8 A-N+ MCI patients converted to dementia, 4 converted to AD and 4 to a no-AD type of dementia (2 FTD, 1 LBD, and 1 VaD). Overall, no differences were found in the number of AD converters between A-N- and A-N+ MCI patients.

This finding can be explained considering that with the new cut-off 20 patients out of the total number (92) were considered as having amyloid pathology and, thus, were excluded from our cohort. But, of these 20 patients, 13 (65%) were patients who later converted to AD dementia – 62% of the AD dementia converters.

Only a subsample of 43 patients out of the 72 with negative amyloid according to lab-specific cut-offs had MRI. Comparisons between converters and no-converters on the hippocampal volumes showed that Converters had significantly smaller right hippocampal volumes relative to non-converters (p= 0.004), whereas there were no significant differences in left hippocampal volume (p= 0.49).
4. Discussion

In this study we showed that patients with a clinical diagnosis of MCI and negative CSF marker of amyloid pathology (A-) presenting to a cognitive neurology clinic had an overall rate of conversion to dementia of 40% in 2-years. Those who later converted to dementia at baseline had higher levels of CSF t-tau and p-tau, lower levels of CSF Aβ1-42, lower MMSE scores, and lower right hippocampal volumes than those who did not develop dementia. The presence of abnormal CSF t-tau as marker of neurodegeneration despite normal CSF marker of amyloid (A-N+, also defined as SNAP) doubled the risk of conversion to dementia, specifically to AD dementia.

These findings are in line with the previous literature, which confirms that SNAP is a biomarker profile characterized by an intermediate risk of progression to dementia relative to the other biomarker profiles (i.e. A-N-, A+N-, and A+N+).

However, the fact that the profile characterised by normal CSF amyloid and abnormal t-tau (SNAP) was specifically associated with higher risk of AD rather than to other dementia syndromes was somehow surprising, because, whereas CSF amyloid is specifically associated with AD, t-tau is instead considered a generic biomarker of neurodegeneration associated with multiple causes of neuronal loss.

We reasoned that the cut-off that we had used for CSF amyloid may have been not enough restrictive in identifying subjects with truly normal amyloid levels. Consequently, subjects with borderline levels of CSF amyloid may have been those who more likely converted to AD, driving our results. In line with this, a previous prospective study on MCI reported that subjects with SNAP who progressed to AD dementia had CSF Aβ1-42 levels closer to the cut-off than subjects who did not progress or progressed to a no-AD type of dementia.

We therefore re-run the analyses using a more restrictive cut-off of normality for CSF amyloid, calculated specifically for our laboratory, as suggested by recent guidance on CSF biomarkers standardization. With this more restrictive cut-off the rate of conversion to dementia of MCI subjects with normal amyloid decreased to 33%, because several subjects previously identified amyloid negative who had converted to AD were now excluded, further confirming that borderline levels of CSF amyloid still identify those who are at greater risk of converting to AD dementia. In the MCI subjects with “truly” normal amyloid identified with the more restrictive lab-specific cut-off, the presence of abnormal CSF t-tau as marker of neurodegeneration (A-N+ or SNAP) did not increase the risk of conversion to dementia, nor increased the risk of conversion to any specific type of dementia (either AD or other dementia syndromes). Similarly, the significant differences in CSF markers and MMSE between converters and no-converters disappeared when using the more restrictive cut-off. The only significant difference between converters and no-converters was the baseline right hippocampal volume.

These results suggest that excluding those patients whose CSF amyloid levels are closer to the cut-off of abnormality results in a higher specificity with regard to patients with pure SNAP. However,
this comes at the cost of a higher sensitivity with regard to subjects in the AD pathway, thus leading to a higher risk of false positives.\textsuperscript{[63]}

In this respect, the choice of the CSF amyloid cut-off can be seen as the weight that tips the scale in favour of a more sensitive diagnosis of AD (higher cut-offs lead to higher false positives and lower false negatives) or a more specific diagnosis of AD (lower cut-offs lead to lower false positives and higher false negatives). But, if the former is undoubtedly necessary for clinical trials – which aim to test a potential drug well before persons reach the full clinical syndrome of AD, the latter is probably the best option when it comes to clinical settings. In fact, as also pointed out by Molinuevo et al.\textsuperscript{[63]}, when it comes to neurodegenerative diseases such as AD, for which disease-modifying therapies are still lacking, in clinical settings it is preferable for ethical reasons to underdiagnose some persons with AD (i.e., accepting to have some false negative but greater specificity) rather than to risk to diagnose AD to persons who do not have it (i.e., having false positives and greater sensitivity). Thus, the literature-based less restrictive cut-off of CSF amyloid abnormality should still be used in the clinical context when it comes to give a formal diagnosis of MCI due to AD. However, our results suggest that subjects with borderline levels of amyloid who would not be considered normal using the more restrictive cut-off should be clinically monitored with more frequent assessment, as they are at greater risk of conversion. Nonetheless, it should be noted that CSF results should always be interpreted according to clinical presentation, neuropsychological assessment and, when feasible, neuroimaging techniques.

Our finding that MCI subjects with normal CSF amyloid levels who later converted to dementia at baseline already had smaller right hippocampal volumes relative to those who did not convert supports the role of MRI in the prognostication process. Importantly, the significant difference in hippocampal volume between converters and no-converters survived the more restrictive cut-off, that excluded subjects with borderline amyloid levels. This suggests that hippocampal volume measured on structural MRI, which is considered a biomarker of neurodegeneration similar to t-tau, adds indeed more prognostic information than t-tau.

Voxel-wise analysis of the structural MRI data with VBM also showed that at baseline converters already had higher degrees of atrophy not only in the right temporal lobe but also in the medial prefrontal cortex, over and above the effect of baseline CSF t-tau, suggesting that MRI-detectable atrophy may add on the clinical prognosis of MCI patients with normal CSF levels of CSF amyloid (with either normal or abnormal CSF t-tau). Thus, in patients whose CSF results are ambiguous or borderline in relation to underlying AD pathology, analysing hippocampal volumes could help to lower the risk of false positives. The early involvement of specifically the right hippocampus (and not the left) is in line with the findings of a previous study from Tondelli et al.\textsuperscript{[67]} in which the authors showed that atrophy of the right hippocampus could be detected in cognitively healthy subjects up to ten years before they developed Alzheimer’s dementia. These converging findings in different populations at different stages of the disease confirm a key role of right hippocampal atrophy as an early feature in the AD pathological process.
One of the strengths of our study is that, unlike previous studies, we compared AD converters to no-AD converters (i.e. FTD, LBD, and VaD). With the literature-based cut-off of normal CSF amyloid, AD dementia converters had lower baseline levels of CSF Aβ1-42, higher baseline levels of CSF t-tau and p-tau, and smaller left hippocampal volume compared to no-AD dementia converters. When using more restrictive lab-based cut-offs, only differences in the left hippocampal volume persisted between those who developed AD relative to other dementia syndromes. Interestingly, while we found that the right hippocampal volume distinguishes converters from no-converters, we then found that, among the converters, the left but not the right hippocampus distinguishes AD from no-AD. Whereas the relative small number of subjects who converted suggests caution in the interpretation and needs replication with larger samples, these results are in line with a previous study suggesting that, while the right hippocampal atrophy may have an early role along the dementia process, injury to the left hippocampus may mark the final transition to AD.

On the VBM analyses the pattern of atrophy in posterior medial and lateral parietal regions distinguished those who developed AD dementia from those who developed other types of dementias. The posterior medial and lateral parietal regions are involved in the Default Mode Network (DMN), which previous fMRI studies demonstrated to be disrupted in persons in the AD pathway. Our results suggests that a loss of volume in regions important for the DMN is already present before patients develop the full clinical syndrome of AD dementia.

Another strength of our cohort study is that we included all consecutive eligible patients presenting to a Cognitive Neurology Clinic. Several studies have analysed the conversion rate to AD in MCI patients but they were mostly conducted on convenience cohorts recruited for research purposes (such as the AD Neuroimaging Initiative, ADNI) rather than on clinical cohorts with patients seeking medical attention. As such, our results are more clinically useful and more easily translatable into clinical settings. In addition, population-based cohorts are better suited at validating the prognostic role of clinical biomarkers.

Most of the previous studies used positron emission tomography (PET) for the assessment of amyloidosis and neurodegeneration, which is more expensive – and therefore less used in clinical settings – than the assessment of CSF biomarkers. Not only is PET less used in clinical settings, but it requires different tracers depending on what the examiner wants to study (i.e. amyloidosis, neurodegeneration, or tauopathy).

This study has several limitations, which we plan to address in the near future by expanding the sample and improving its clinical and biomarker characterization by including variables from the neuropsychological assessment as well other biomarkers such as CSF p-tau into the analyses.

In conclusion, our study established the risk of conversion to dementia of patients presenting to a cognitive neurology clinic with MCI and normal CSF amyloid markers, thereby allowing direct translation of our results into clinical practice.
References


65) Andersson, Jenkins M., et Smith S.M., Non-linear registration, aka Spatial normalization. FMRIB technical report TR07JA2 from www.fmrib.ox.ac.uk/analyses/techrep


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