CORRELATES OF THE CONVERSION FROM MILD COGNITIVE IMPAIRMENT TO DEMENTIA

LUÍSA CRISTINA DA SILVA ALVES

Tese para obtenção do grau de Doutor em Medicina

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Contents

The Ethics Research Committee NMS|FCM-UNL (CEFCM) has unanimously approved the Project entitled "Correlates of the conversion from Mild Cognitive Impairment to Dementia" (nr.56/2014/CEFCM).

Papers included in the present thesis

Alves L, Cardoso S, Marôco J, De Mendonça A, Guerreiro M, Silva D. Neuropsychological predictors of long-term (10 years) mild cognitive impairment stability. *Journal of Alzheimer's Disease* 2018; 62(4): 1703-11.

Alves L, Cardoso S, Silva D, Mendes T, Marôco J, Nogueira J, Lima M, Tábuas-Pereira M, Baldeiras I, Santana I, De Mendonça A. Neuropsychological profile of amyloid-positive versus amyloid-negative amnestic Mild Cognitive Impairment. *Journal of neuropsychology* 2020; Jun 26: e12218.

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Other papers related to the topic of the thesis

Alves L, Correia AS, Miguel R, Alegria P, Bugalho P. Alzheimer's disease: a clinical practice-oriented review. *Frontiers in neurology* 2012; 3:63.

Mendonça MD, Alves L, Bugalho P. From subjective cognitive complaints to dementia: who is at risk?: a systematic review. *American Journal of Alzheimer's Disease & Other Dementias* 2016; 31(2):105-14.

Mendes T, Cardoso S, Guerreiro M, Marôco J, Silva D, Alves L, Schmand B, Gerardo B, Lima M, Santana I, de Mendonça A. Can Subjective Memory Complaints Identify Aβ Positive and Aβ Negative Amnestic Mild Cognitive Impairment Patients? *Journal of Alzheimer's Disease* 2019; 1-9.

Brief Summary

Cognitive diseases are currently very prevalent and represent an important personal, familial, social and economic burden. With increasing life expectancy worldwide, an increase in the number of people developing dementia is expected. There has been a growing interest in detecting Alzheimer's disease (AD) as soon as possible along its insidious evolution, long before the establishment of a diagnosis of dementia. The correct identification of individuals with cognitive complaints who already have an ongoing neurodegenerative process is desirable since it provides chances for these individuals to undergo interventions including clinical trials with allegedly neuroprotective drugs.

Neuropsychology is a non-invasive, relatively non-expensive ancillary diagnostic method used in the characterisation of cognitive disorders. We explored the neuropsychological evaluation of Mild Cognitive Impairment (MCI) patients and studied the kind of information it could provide, mainly in terms of etiologic diagnosis and prognosis.

We dedicated our first efforts to trying to find the neuropsychological predictors of long-term (10 years) MCI stability, through a retrospective case-control study. We observed that some patients with MCI do remain clinically and neuropsychologically stable for a decade and that better performances at baseline in memory and non-verbal abstraction tests predict long-term stability.

We then investigated the capability of neuropsychological evaluation of MCI to suggest the etiology of the syndrome. We found that neuropsychological tests have limited value in distinguishing amnestic Mild Cognitive Impairment (aMCI) patients who have amyloid pathology from those who suffer from non-amyloid disorders.

Finally, we studied the contribution of neuropsychological measures to predict time to conversion to dementia in patients with MCI due to AD. In this condition, lower performance in a test of non-verbal reasoning (Raven's Coloured Progressive Matrices) was associated with shorter interval of time to conversion to dementia. This test, that reveals little decline in the earlier phases of AD, appears to convey important information concerning prognosis of MCI due to AD.

Resumo Breve

As doenças cognitivas são actualmente muito prevalentes e onerosas do ponto de vista pessoal, familiar, social e económico. Com o aumento da longevidade a nível mundial, espera-se um aumento da prevalência da demência. Tem havido um interesse crescente na detecção da Doença de Alzheimer (DA) tão cedo quanto possível ao longo da sua evolução insidiosa, muito antes do estabelecimento do diagnóstico de demência. A identificação correcta dos indivíduos com queixas cognitivas que têm um processo neurodegenerativo subjacente é desejável pois proporciona a possibilidade de submissão a intervenções, incluindo ensaios clínicos com fármacos potencialmente neuroprotectores.

A Neuropsicologia é um método complementar de diagnóstico não invasivo, relativamente não dispendioso, usado na caracterização das doenças cognitivas. Explorámos a avaliação neuropsicológica dos indivíduos com Défice Cognitivo Ligeiro (DCL) e estudámos as informações que poderia providenciar, nomeadamente em termos de diagnóstico etiológico e prognóstico.

Dedicámos os nossos primeiros esforços a tentar encontrar os preditores neuropsicológicos da estabilidade do DCL a longo prazo (10 anos), através de um estudo retrospectivo caso-controlo. Constatámos que alguns doentes com DCL mantêm-se, de facto, clínica e neuropsicologicamente estáveis durante uma década. Por outro lado, melhor desempenho na avaliação neuropsicológica de base nos testes de memória e de abstracção não-verbal associou-se a estabilidade a longo prazo.

A seguir, investigámos a capacidade da avaliação neuropsicológica de indivíduos com DCL sugerir a etiologia da síndrome. Observámos que os testes neuropsicológicos têm um valor limitado na distinção entre doentes com DCL que têm patologia amilóide e os que não a têm.

Por fim, estudámos a contribuição das medidas neuropsicológicas para estimar o tempo de conversão para demência em indivíduos com DCL devido a DA. Nesta condição, pior desempenho num teste de raciocínio não verbal (Matrizes Coloridas Progressivas de Raven) associou-se a menor tempo de conversão para demência. Esta prova, que revela declínio ligeiro nas fases mais precoces de DA, parece transmitir informação importante no que diz respeito ao prognóstico dos doentes com DCL devido a DA.

Summary

Although the diagnosis of Mild Cognitive Impairment (MCI) corresponds to a condition likely to progress to dementia, essentially Alzheimer's disease (AD), longitudinal studies have shown that some patients may not convert to dementia and maintain the diagnosis of MCI even after many years. The objective of our first study was to determine whether patients that maintain the diagnosis of MCI in the long term (10 years) are really stable or just declining slowly, and to identify clinical and neuropsychological characteristics associated with long-term stability. The *Cognitive Complaints Cohort* (CCC) was searched for MCI cases who maintained that diagnosis for at least 10 years. For each long-term-stable MCI patient, two MCI patients that converted to dementia during follow-up, matched for age and education, were selected from the same database. The baseline and last neuropsychological evaluations for long-term-stable MCI and converter MCI were compared. Baseline neuropsychological predictors of long-term stability were searched for. Long-term-stable MCI (n=22) and converter MCI (n=44) patients did not differ in terms of gender distribution, education, age at first assessment and time between symptom onset and first evaluation. Time of follow-up was on average 11 years for long-term-stable MCI and 3 years for converter MCI. The baseline and follow-up neuropsychological tests were not significantly different in long-term-stable MCI patients, whereas a general decline was observed in converter MCI patients. Higher scores on one memory test, the Word Delayed Total Recall, and on the non-verbal abstraction test, Raven's Coloured Progressive Matrices, at the baseline predicted long-term (10 years) clinical stability. Some patients with MCI remain clinically and neuropsychologically stable for a decade. Better performances at baseline in memory and non-verbal abstraction tests predict long-term stability.

Patients diagnosed with amnestic Mild Cognitive Impairment (aMCI) are at high risk of progressing to dementia. It became possible, through the use of biomarkers, to diagnose those patients with aMCI who have AD. However, it is presently unfeasible that all patients undergo biomarker testing. Since neuropsychological testing is required to make a formal diagnosis of aMCI, it would be interesting if it could be used to predict the amyloid status of patients with aMCI. Participants with aMCI, known amyloid status (Aβ+ or Aβ-) and a comprehensive neuropsychological evaluation were selected from the CCC database for this study. Neuropsychological tests were

compared in Aβ+ and Aβ- aMCI patients. A binary logistic regression analysis was conducted to model the probability of being Aβ+. Of the 216 aMCI patients studied, 117 were Aβ+ and 99 were Aβ-. Aβ+ aMCI patients performed worse on several memory tests, namely Word Total Recall, Logical Memory Immediate and Delayed Free Recall, and Verbal Paired Associate Learning, as well as on Trail Making Test B, an executive function test. In a binary logistic regression model, only Logical Memory Delayed Free Recall retained significance, so that for each additional score point in this test, the probability of being amyloid positive decreased by 30.6%. The resulting model correctly classified 64.6% of the aMCI cases regarding their amyloid status. The neuropsychological assessment remains an essential step to diagnose and characterise patients with aMCI, however, neuropsychological tests have limited value to distinguish the aMCI patients who have amyloid pathology from those who might suffer from other clinical conditions.

Diagnosis of AD confirmed by biomarkers allows the patient to make important life decisions. However, doubt about the fleetness of symptoms progression and future cognitive decline remains. Neuropsychological measures were extensively studied in prediction of time to conversion to dementia for MCI patients in the absence of biomarker information. Similar neuropsychological measures might also be useful to predict the progression to dementia in patients with MCI due to AD. The objective of our third work was to study the contribution of neuropsychological measures to predict time to conversion to dementia in patients with MCI due to AD. Patients with MCI due to AD were enrolled from the CCC and the effect of neuropsychological performance on time to conversion to dementia was analyzed. At baseline converters scored lower than non-converters at measures of verbal initiative, non-verbal reasoning and episodic memory. The test of non-verbal reasoning (Raven's Coloured Progressive Matrices) was the only statistically significant predictor in a multivariate Cox regression model. A decrease of one standard deviation was associated with 29.0% of increase in the risk of conversion to dementia. Approximately 50% of patients with more than one standard deviation below the mean in the *z* score of that test had converted to dementia at 3 years of follow-up. In MCI due to AD, lower performance in a test of non-verbal reasoning was associated with time to conversion to dementia. This test, that reveals little decline in the earlier phases of AD, appears to convey important information concerning conversion to dementia.

Resumo

Apesar do diagnóstico de Défice Cognitivo Ligeiro (DCL) corresponder a uma condição com grande probabilidade de progressão para demência, sobretudo para Doença de Alzheimer (DA), estudos longitudinais têm mostrado que alguns doentes podem não converter para demência e manter o diagnóstico de DCL mesmo após muitos anos. O objectivo do nosso primeiro estudo foi determinar se doentes que mantêm o diagnóstico de DCL a longo prazo (10 anos) estão realmente estáveis ou apenas em declínio lento, e identificar as características clínicas e neuropsicológicas que se associam à estabilidade a longo prazo. Procurámos na *Cognitive Complaints Cohort* (CCC) casos de MCI que mantiveram esse diagnóstico ao longo de pelo menos uma década. Para cada doente com DCL estável a longo prazo, 2 indivíduos com DCL que converteram para demência durante o seguimento, emparelhados para idade e escolaridade, foram seleccionados da mesma base de dados. As avaliações neuropsicológicas inicial e final foram comparadas entre os doentes com DCL estável a longo prazo e os DCL conversores. Os preditores neuropsicológicos da estabilidade a longo termo foram procurados na avaliação inicial. Indivíduos com DCL estável a longo prazo (n=22) e DCL conversor (n=44) não diferiram em termos de distribuição de género, escolaridade, idade à data da primeira avaliação e intervalo entre início dos sintomas e primeira avaliação. O tempo de seguimento foi em média de 11 anos para os DCL estáveis a longo prazo e 3 anos para os DCL conversores. Os testes neuropsicológicos iniciais e finais não foram significativamente diferentes nos indivíduos com DCL estável a longo prazo. Observou-se um declínio global nos doentes com DCL conversor. Resultados melhores num teste de memória, Evocação de Palavras após Interferência - Total, e num teste de abstracção não verbal, Matrizes Coloridas Progressivas de Raven, previram estabilidade clínica a longo termo (10 anos). Alguns doentes com DCL permanecem clínica e neuropsicologicamente estáveis ao longo de uma década. Melhores desempenhos na avaliação inicial em provas de memória e abstracção não verbal previram estabilidade a longo termo.

Os doentes diagnosticados com Défice Cognitivo Ligeiro amnéstico (DCLa) têm risco aumentado de progressão para demência. Tornou-se possível, através do uso de biomarcadores, diagnosticar DA em doentes com DCLa. No entanto, presentemente, é impraticável submeter todos os doentes com DCLa a pesquisa de biomarcadores. Tendo em conta que a avaliação neuropsicológica é necessária para fazer um diagnóstico formal de DCLa, seria interessante que pudesse ser usada para predizer o estado amilóide dos doentes com DCLa. Participantes com DCLa, estado amilóide

conhecido (Aβ+ or Aβ-) e avaliação neuropsicológica abrangente foram seleccionados da base de dados CCC para o segundo estudo. As provas neuropsicológicas iniciais dos doentes com DCLa Aβ+ e Aβ- foram comparadas. Uma análise de regressão logística binária foi conduzida para modelar a probabilidade de ser Aβ+. Dos 216 doentes com DCLa estudados, 117 eram Aβ+ e 99 eram Aβ-. Os doentes com DCLa Aβ+ tiveram piores desempenhos em vários testes de memória, nomeadamente Evocação de Palavras - Total, Memória Lógica - Evocação Imediata e após Interferência, e Aprendizagem de Pares Verbais Associados (Pares de Palavras), assim como no Teste *Trail* B, um teste de função executiva. Num modelo de regressão logística binário, apenas a Memória Lógica - Evocação após Interferência reteve significado estatístico. Por cada ponto adicional no resultado deste teste, a probabilidade de ser Aβ+ decresceu em 30.6%. O modelo resultante classificou correctamente 64.6% dos casos DCLa no que diz respeito ao seu estado amilóide. A avaliação neuropsicológica permanece um passo fundamental no diagnóstico e caracterização dos doentes com DCLa; no entanto, os testes neuropsicológicos têm um valor limitado na distinção entre indivíduos com DCLa com patologia amilóide daqueles com outras etiologias.

O diagnóstico de DA confirmado por biomarcadores permite ao doente fazer decisões importantes acerca da sua vida. Contudo, permanecem dúvidas acerca da rapidez da progressão dos sintomas e do declínio cognitivo futuro. Medidas neuropsicológicas foram extensamente estudadas na previsão do tempo até conversão para demência em indivíduos com DCL na ausência de informação acerca de biomarcadores. Medidas neuropsicológicas semelhantes poderiam ser úteis na estimativa de tempo de progressão para demência em doentes com DCL devido a DA. O objectivo do nosso terceiro trabalho foi o de estudar a contribuição das medidas neuropsicológicas para estimar o tempo até conversão para demência em doentes com DCL devido a DA. Indivíduos com esta condição foram incluídos a partir da CCC e o efeito do desempenho neuropsicológico numa avaliação inicial no tempo até conversão para demência foi analisado.

Na avaliação inicial, os conversores tiveram pontuações mais baixas do que os não conversores em medidas de iniciativa verbal, raciocínio não verbal e memória episódica. A prova de raciocínio não verbal (Matrizes Coloridas Progressivas de Raven) foi o único indicador com significado estatístico num modelo de regressão multivariado de *Cox*. O decréscimo de um desvio-padrão associou-se a 29.0% de aumento de risco de conversão para demência. Aproximadamente 50% dos doentes com

mais de um desvio padrão abaixo da média no *z score* desta prova haviam convertido para demência aos 3 anos de seguimento. No DCL devido a DA, pior desempenho numa prova de raciocínio não verbal associou-se ao tempo até conversão para demência. Esta prova, que apresenta declínio ligeiro nas fases mais precoces da DA, parece transmitir informação importante no que diz respeito à conversão para demência.

Foreword:

When I began my specific training in Neurology, Alzheimer's disease (AD)/ Alzheimer's type Dementia was diagnosed according to DSM IV criteria. These criteria do not take into account biomarkers. They are purely clinical. As we know today [1], many other etiologies can mimic Alzheimer's disease. I had the privilege to witness the advent of biomarkers. They are important insofar as treatment (albeit "palliative") for AD is different than that for non-Alzheimer dementias. On the other hand, biomarkers help to establish prognosis. Besides, they are of utmost importance in the recruitment for disease-specific clinical trials.

In addition, two decades ago, clinicians were not yet trying to diagnose prodromal states of dementia. I was a young medical student when the term Mild Cognitive Impairment (MCI) entered our everyday lexicon. Its classical definition is that of a transitional state between the cognitive changes of aging and the earliest clinical features of dementia. It is characterised by impairment in cognition that is not severe enough to imply dependence on others in the activities of daily living. The importance of being able to identify this group of people is not just related to the need to develop interventions which ameliorate individual suffering, but also to their representing a population at high risk for developing dementia, especially AD, and are an appropriate target for dementia prevention strategies [2].

Even though evidence in systematic reviews clearly shows that cholinesterase inhibitors do not reduce progression to dementia [3,4], there are increasing numbers of trials showing the benefit of non-pharmacological treatments in MCI (for example, Straubmeier *et al.*, 2017 and McMaster *et al.*, 2020 [5,6]).

On the other hand, it is very relevant for patients and their families, when MCI is diagnosed, to be able to know their prognosis over a short and longer time window. We believe that it is of the utmost importance to be able to give patients and their families an estimate of probability of progression and of the expected time to conversion to dementia.

After having written an article on AD ("Alzheimer's disease: a clinical practice-oriented review", Alves *et al.*, 2012 [7]), I realized how important it was to continue studying cognitive diseases, so that I could more competently give advice to my patients and their families. Moreover, I

considered the cognitive field a fascinating subject and noted it was an ever growing area, which definitely contributed to its enticement.

Whereas in other centres throughout the world investigation on AD biomarkers was (and still is!) evolving at a very fast pace, in my daily clinical practice, there was no easy access to the latest scientific advances. Thus, I was in the long distance between the benchtop and the bedside, trying to figure out how to make it shorter. Neuropsychology seemed a very interesting "shortcut".

On this journey, we tried to somehow mirror the clinical experience and try to clarify the doubts that arise during everyday practice.

The initial objective of this study was to assess the cognitive, neuropsychiatric, behavioural, medical and personal history correlates of the conversion from mild cognitive impairment to dementia, in a large cohort of patients with cognitive complaints. We had decided to concentrate specifically on the occurrence of relevant medical, familial and social events during the period of transition from MCI to dementia. However, there had to be deviations from the first idea. The work in "the field" revealed that, due to their nature, the data that we intended to collect retrospectively were subjected to information bias. To obviate that, we would need to create a new, prospective cohort. However, the results obtained from such a new cohort would not be available in a timely manner. According to the known natural history of MCI, in order to get robust results, we would have to follow patients for at least three years.

Fortunately, I was given the opportunity to explore the *Cognitive Complaints Cohort* (CCC).

The CCC is a clinical cohort of non-demented patients referred for neuropsychological evaluation during the period 1999-2015 with the objective of investigating cognitive stability or evolution to dementia of patients with cognitive complaints, using a comprehensive neuropsychological evaluation. This cohort was established in the setting of a prospective study conducted at the Institute of Molecular Medicine, and approved by the local ethics committee [8]. The CCC was recruited by 3 referral centers for care of individuals presenting with cognitive complaints. CCC is a large clinical cohort, many subjects have long follow-ups and all underwent detailed neuropsychological testing. For the re-evaluations of the patients, it was possible to take advantage of the fact that most patients have regular clinical consultations at the participating

institutions to schedule the reassessments. The selection of patients to enter CCC was established according to the following criteria:

Inclusion criteria: presence of cognitive complaints; referral to neuropsychological examination, during the period 1999-2015, at Santa Maria´s Hospital, Coimbra University Hospital and a private memory clinic in Lisbon-Memoclínica; fulfillment of criteria for MCI; for different study purposes, distinct subsets of patients were used.

Exclusion criteria: presence of neurological (stroke, brain tumour, significant head trauma, epilepsy) or psychiatric disorders that might induce cognitive deficits; presence of uncontrolled systemic illness with cerebral impact (hypertension, metabolic, endocrine, toxic, and infectious diseases); history of alcohol abuse or recurrent substance abuse or dependence; and presence of dementia according to DSM-IV-TR [9] or significant impairment on activities of daily living detected by the presence of a score greater than or equal to 3 on the first part (items 1-8) of the BDRS [10] at first evaluation.

It was a privilege to be able to explore such a wealth of well collected data. Using the CCC, our endeavors turned instead to the study of the neuropsychological predictors of the evolution of MCI.

We dedicated our first efforts to a topic that is relatively new. Not much is known about stable MCI, namely about its neuropsychological predictors. The literature has been focusing on predictors of conversion from MCI to dementia and has been overlooking a relatively common, at least in community settings, outcome of MCI: MCI stability. The majority of published studies have follow-up times of only up to 5 years. However, in the CCC cohort, there were at least 20 individuals who had been classified as MCI for the last 10 years and what we proposed to do was, through a retrospective case-control study, to try to find the neuropsychological predictors of MCI stability. That work constitutes the first study of the present thesis.

We continued to explore the neuropsychological evaluation of MCI patients and on the kind of information it could provide, mainly in terms of etiological diagnosis and prognosis. Patients diagnosed with aMCI are at high risk of progressing to dementia. It became possible, through the use of biomarkers, to diagnose those patients with aMCI who have AD. However, it is presently unfeasible that all patients undergo biomarker testing. Our subsequent step was then to verify if

neuropsychological evaluation could be used to predict amyloid status of patients with aMCI. That work constitutes the second study of the present thesis.

Diagnosis of AD confirmed by biomarkers allows the patient and family to make important life decisions. However, doubts about the fleetness of symptoms progression and future cognitive decline remains. Neuropsychological measures were extensively studied in prediction of time to conversion to dementia for MCI patients in the absence of biomarker information. Similar neuropsychological measures might also be useful to predict the progression to dementia in patients with MCI due to AD. Our third stage was then to study the contribution of neuropsychological measures to predict time to conversion to dementia in patients with MCI due to AD. That work constitutes the last study of the present thesis.

The three studies were done within the CCC.

Introduction

After a brief narrative review covering the epidemiology of cognitive impairment, the history of dementia and AD, the evolution of concepts and diagnostic criteria of AD and MCI as well as neuropsychology, the questions which the present thesis intends to answer are contextualised and introduced.

-Epidemiology

Given the increased life expectancy of populations, there is an ever increasing number of individuals afflicted with AD [11]: in 2015, they were over 46 million worldwide, a number that is expected to increase to 131.5 million by 2050 [12].

Moreover, dementia and cognitive impairment are the most important contributors, among chronic diseases, to disability, dependence, and transition into residential and nursing home care [13]. In 2017, in the European Union, among all diseases, dementia was one of the three commonest causes of disability-adjusted life-years [14]. As Plum put it more than 40 years ago, "(…) by any standards, dementia represents an expanding epidemic of major proportions in terms of human suffering and societal expense" [15].

Numerous international population-based studies have been conducted to document the frequency of MCI, a condition of cognitive impairment beyond what is expected for age and education, in which the individual maintains independence in activities of daily living. These studies have estimated its prevalence to be between 15% and 20% in persons 60 years and older, making it a common condition encountered by clinicians. The annual rate in which MCI progresses to dementia varies between 8% and 15% per year, implying that it is an important condition to identify and treat [16].

-A few notes on the history of Dementia and Alzheimer´s Disease

Loss of memory with advanced age has been recognized for more than 4000 years. One of the earliest descriptions can be found in the Precepts of Ptah-hotep. Ptah-hotep was vizier under the reign of Jedkare Isesi, the eighth king of ancient Egypt's fifth Dynasty (c.2414–2375 BCE). In

reference to aging, Ptah writes "(…) the progress of age changes into senility. Decay falls upon a man and decline takes the place of youth (...) the mind decays, remembering not the day before (…)" [17].

Senile dementia has thus been recognized in aged individuals for a very long time. However, on account of the fact that it was considered an inexorable characteristic of aging, it was not object of specific investigation until the 19th century. Only with Pinel and Esquirol's classification of mental disorders in the mid-19th century did senile dementia begin to be differentiated from other dementias and start to be regarded as an abnormal form of aging. At the turn of the 20th century, brain pathology underlying dementia was discovered. Alzheimer's neuropathological findings in a middle-aged woman who presented with a rapidly progressive dementia led many to believe that a new disease had been discovered [11]. Alzheimer's chief and mentor, Emil Kraepelin, one of the most influential psychiatrists of the 20th century, officially coined, in 1910, the term ''Alzheimer-Krankheit'' (Alzheimer's disease), including it in the eighth edition of his authoritative Textbook of Psychiatry. For the next several decades after the publication of ''Alzheimer's disease'' in the mentioned textbook, the diagnosis of AD remained obscure and was rarely applied by those in the medical profession. AD was considered a rare condition that affected young people exhibiting presenile dementia; ''hardening of the blood vessels'' was considered the main pathology responsible for cognitive decline in the last decades of life [18].

Emil Kraepelin created thus the category of Alzheimer's disease to distinguish early-onset "presenile" cases occurring before age 65 from the much more common senile dementia occurring at later ages. It seems clear that he did not think it made sense to call a condition strongly associated with aging a disease. The pathological processes of deterioration in old age that produced senile dementia were understood to be on the extreme end of "normal," while dementia occurring at earlier ages, as in the case Alzheimer presented, even though ostensibly associated with the same brain pathology and clinical symptoms, seemed to suggest some kind of disease process. Kraepelin's reluctance to view age-associated deterioration as a disease apparently justifies his creation of this new entity [19].

In the fifties, Kral noted through clinical observation that a decline in memory function commonly occurred with, and was significantly correlated to, advancing age [20]. Kral described two types of "senescent memory impairment": "benign" and "malignant". Malignant senescent memory

impairment was characterised by the inability of the subject to recall events of the recent past, whereby not only relatively unimportant data and parts of an experience but the experience as such could not be recalled. He observed that the loss of recent memories led to two important consequences: disorientation, at first in time and place and later also to disorientation as to personal data; and secondly, because of the missing cues, to retrograde loss of remote memories. He considered that malignant senescent memory impairment bore the essential characteristics of the amnestic syndrome, namely, loss of recent memories, retrograde loss of remote memories, disorientation and confabulation. He thought it was identical to the senile amnestic syndrome, and formed the axis syndrome of what used to be called senile dementia and was then being termed chronic brain syndrome associated with senile brain disease. Kral considered that "the most important factor responsible for both the benign and the malignant type of senescent memory dysfunction was the process of ageing *per se* as it affects the brain and perhaps also the endocrine glands. The nature of this process still remains a mystery" [21].

The riddle began to be unravelled when Tomlison *et al.* examined brains from 50 elders with proven dementia. Several features were assessed and compared with brains of non-demented old people. Statistically significant differences were found between the two groups in relation to cortical atrophy, ventricular dilatation, senile plaque formation, Alzheimer's neurofibrillary change, granulo-vacuolar degeneration and the quantity of cerebral softening (corresponding to arteriosclerotic disease). Half of the demented subjects' brains were considered to be cases of senile dementia, showing the histological features of AD, the majority with no or small ischaemic lesions [22].

Later, Plum noted that "(…) the condition [dementia] does not predominantly reflect the effects of cerebral vascular disease as once was believed. Although widespread among the elderly, some persons altogether escape the disorder, suggesting that the abnormal cell changes reflect a specific inherited or acquired disease and not merely that the subject has outlived the foreordained life expectancy of the human race" [15].

Nowadays, the term AD is used to denominate a neurodegenerative disorder defined by neuronal degeneration and death, associated with deposition of the amyloid β1–42 peptide (Aβ42) and the hyperphosphorylated tau protein (p-tau), initially involving the hippocampus and other medial temporal lobe structures, whose prevalence increases with age [23].

-The evolution of Concepts and Diagnostic Criteria of Alzheimer´s Disease and Mild Cognitive Impairment

The diagnosis of AD is, still nowadays, frequently based on the criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition [9] and on the National Institute of Neurologic and Communicative Disorders and Stroke – Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) criteria [24]. Both sets of criteria require deficits in memory and at least one other cognitive domain. The DSM-IV-TR criteria additionally stipulate that there must be an impact of the cognitive impairment on social function or activities of daily living (ADL). According to the NINCDS-ADRDA criteria, the AD diagnosis is classified as definite (clinical diagnosis with histologic confirmation), probable (typical clinical syndrome without histologic confirmation), or possible (atypical clinical features but no alternative diagnosis apparent; no histologic confirmation). The NINCDS-ADRDA criteria have been reasonably reliable for the diagnosis of probable AD: across more than a dozen clinical-pathological studies, they have had a sensitivity of 81% and a specificity of 70% [25].

However, using the DSM-IV-TR and the 1984 NINCDS-ADRDA recommendations, the AD cases are discovered late in the disease process. It is known that neurodegenerative diseases begin years before the onset of clinical symptoms, and that standard clinical practice may be insensitive in the identification of early neurodegenerative states. Therefore, substantial efforts have been made to create criteria for the clinical stage preceding dementia.

Even though the American Psychiatric Association's DSM-III [26] had already identified an early dementia stage, it was in 1982 that the terms “questionable dementia” and “mild cognitive decline” were introduced in the context of, respectively, the Clinical Dementia Rating (CDR) and the Global Deterioration Scale (GDS), as dementia antecedents. The CDR 0.5 “questionable dementia” stage encompasses both mild dementia and earlier antecedents [27]. GDS stage 3 describes a predementia condition termed “mild cognitive decline” or, alternatively, beginning in 1988, “mild cognitive impairment” [28].

The construct of MCI thus emerged as a response to the need to identify an insidious clinical condition that would reliably predict progression to dementia, particularly AD. The MCI concept pinpoints the clinical parameters that define the earliest stages of the neurodegenerative process. The narrow concept of MCI as an early form of AD has been broadened by research that

established the existence of alternative forms of the condition that may presage other forms of dementia [29].

The first clinical criteria for MCI were proposed by a group of investigators from the Mayo Clinic in 1999. The diagnosis of MCI was made if the patient met the following criteria: (1) memory complaint, (2) normal activities of daily living, (3) normal general cognitive function, (4) objective deficits on tests of episodic memory, and (5) absence of dementia [30].

The criteria were derived from the clinical observation of signs and cognitive performance in patients in a longitudinal study of aging and dementia in the community. While the study was designed to characterise normal ageing and dementia, it became apparent that a sizeable group of subjects were 'in between' corresponding to the concept of MCI. This first definition of MCI was clearly focused on memory problems that were regarded as prodromal signs of incipient AD (with the purpose of early detection). Mild deficits in cognitive domains other than memory were allowed, but isolated deficits in nonmemory domains were not taken into account. When these criteria were investigated by other researchers and in other settings, it became clear that not all forms of MCI evolved into AD and that other underlying causes could lead to MCI. Thus, a broader conceptualization became necessary. To reach an agreement on the clinical characterisation of MCI, an international consensus conference was held in 2003. The discussion at the first Key Symposium on MCI led to the formulation of revised core criteria for this condition [31]. The expanded Mayo Clinic criteria for MCI were no longer focused on memory impairment alone but were broadened to include impairment in other areas of cognitive functioning. MCI was subclassified into amnestic and non-amnestic MCI, each category being subdivided into single and multiple domain. In the international criteria, MCI became thus a construct with a wider scope, referring to a clinical syndrome with multiple clinical profiles, due to a variety of etiologies. The assumption was that the new criteria would identify all individuals at the intermediate cognition stage and have a greater clinically utility. In the new definition, the initial purpose of MCI, directed specifically towards the detection of underlying AD, was restricted to a subtype of MCI. The clinical characterisation could integrate information coming from anamnesis as well as from laboratory tests and neuroimaging, when available, to guide the clinician in formulating hypotheses regarding the progression of the cognitive impairment syndromes. Specifically, the central idea was that, through the combination of clinical subtypes and putative etiologies, it could be possible to predict the type of dementia that patients with MCI would develop [32,33].

In 2004, Dubois and Albert proposed the concept of prodromal AD in order to identify patients with AD, the most important subgroup of patients with MCI, before the appearance of the fully developed clinical dementia syndrome [34]. The core principle of the research criteria for the diagnosis of AD later outlined [35] is based upon the presence of consistent episodic memory disturbance which, together with biomarker positivity, recognizes AD across the full spectrum of the clinical disease. To fulfill criteria for probable AD, a patient must meet the cornerstone clinical criterion A and at least one of the supportive biomarker criteria. Criterion A specifies that there must be an episodic memory deficit within test conditions of encoding specificity. The presence of a biological footprint of the disease is established either by criterion B (structural imaging), criterion C (cerebrospinal fluid), criterion D (molecular imaging), or criterion E (dominant mutation within the immediate family). Apart from the incorporation of biomarkers, two relevant innovations characterise the Dubois criteria: (1) the presence of a progressive memory deficit is considered sufficient to make a diagnosis of AD, even if it is the patient's only cognitive deficit; (2) the declarative memory impairment necessary for diagnosis is of the "medial temporal lobe type" [36].

In 2011, the NIA-AA workgroup published recommendations concerning the definition of the preclinical stages of AD [37], the diagnosis of MCI due to AD (MCI-AD) [38] and the diagnosis of dementia due to AD (AD dementia) [39], which also integrated biomarker information. According to the NIA-AA workgroup, the major AD biomarkers can be divided into those related to the process of brain Aβ42 protein deposition, comprising low cerebrospinal fluid (CSF) Aβ42 and positive positron emission tomography (PET) amyloid imaging, and those related to downstream neuronal degeneration or injury: elevated CSF tau, both total tau (t-tau) and phosphorylated tau (p-tau); decreased 18-fluorodeoxyglucose (FDG) uptake on PET in the temporo–parietal cortex; and disproportionate atrophy on structural magnetic resonance imaging (MRI) in medial, basal and lateral temporal lobe, and medial parietal cortex [39].

According to the NIA-AA recommendations [38], in the presence of a change in cognition, objective impairment in at least one cognitive domain, preservation of independence in ADL (and inherent absence of dementia) and clinical syndrome suggestive of AD, an individual is classified as having MCI due to AD-core clinical criteria in the following situations: (1) in the absence of information on biomarkers; (2) in the event that they are uninformative (neither clearly negative nor positive); or (3) in the case that their information is conflicting (e.g., low Aβ42 and normal tau in CSF). The

"suggestive" clinical syndrome typically involves a prominent impairment in episodic memory, but other patterns, such as visuo-spatial impairment, are also possible manifestations of underlying AD pathology and, as such, are compatible with a diagnosis of MCI due to AD. A subject is attributed a diagnosis of MCI due to AD with intermediate likelihood if he/she has one positive biomarker either reflecting Aβ42 deposition or neuronal injury. A person is diagnosed with MCI-AD with a high likelihood if both biomarkers are positive. An individual is attributed a diagnosis of MCI unlikely due to AD if both biomarkers are negative.

In 2013, the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) [40] was published. It provides a common framework for the diagnosis of neurocognitive disorders, first by describing the main cognitive syndromes, and then by defining criteria to delineate specific etiological subtypes of mild and major neurocognitive disorders. The DSM-5 allows the diagnosis of neurocognitive disorders based on three syndromes: delirium, mild neurocognitive disorder and major neurocognitive disorder. The category of mild neurocognitive disorder corresponds broadly to the concept of mild cognitive impairment. Major neurocognitive disorder is mostly synonymous with dementia, although the criteria have been modified so that impairments in learning and memory are not necessary for diagnosis. DSM-5 describes criteria to delineate specific etiological subtypes of mild and major neurocognitive disorder. The diagnostic certainty of an aetiological diagnosis is based on clinical features and biomarkers, and can be qualified as probable or possible [41].

Meanwhile, both the International Working Group (IWG) and the US National Institute on Aging–Alzheimer's Association (NIA-AA) contributed criteria for the diagnosis of AD that better defined clinical phenotypes and integrated biomarkers into the diagnostic process, covering the full staging of the disease. In 2014, a Position Paper was published that considered the strengths and limitations of the IWG research diagnostic criteria and proposed advances to improve the diagnostic framework. On the basis of these refinements, the diagnosis of AD could then be simplified, requiring the presence of an appropriate clinical AD phenotype (typical or atypical) and a pathophysiological biomarker consistent with the presence of Alzheimer's pathology. They proposed that downstream topographical biomarkers of the disease, such as volumetric MRI and fluorodeoxyglucose PET (FDG-PET), might better serve in the measurement and monitoring of the course of disease. The paper also elaborated on the specific diagnostic criteria for the preclinical states of AD [42].

In 2015, Vos *et al.* compared three sets of research criteria that were then available for diagnosis of AD in subjects with mild cognitive impairment: the IWG-1, IWG-2, and NIA-AA criteria. Their findings supported the use of the proposed research criteria to identify AD at the MCI stage. They concluded that, in clinical settings, the use of both amyloid and neuronal injury markers as proposed by the NIA-AA criteria offered the most accurate prognosis. They considered that, for clinical trials, selection of subjects in the NIA-AA high Alzheimer's disease likelihood group or the IWG-2 prodromal Alzheimer's disease group should be privileged [43].

In 2016, the ATN classification emerged. The NIA-AA Alzheimer's Diagnostic Framework introduced a new classification system in 2016 to apply validated biomarkers for the separation of AD from non-AD causes of impaired cognition [44]. The classification uses three types of biomarkers to determine the extent of pathology typical of AD: A (amyloid, represented either by cerebrospinal fluid (CSF) levels of Aβ42 or amyloid plaque deposition in brain as seen with amyloid-PET); T (tau, measured as the level of CSF p-tau or tangle-formation as seen by tau-PET); and N (neurodegeneration as shown by structural MRI, CSF levels of t-tau, or brain metabolism as measured with FDG-PET. The framework thereby characterises the AD spectrum by its biological presentation and is independent of clinical assessment of cognitive status. It has been designated the A/T/N classification system and individuals can be classified as positive (+) or negative (–) for A, T and N, resulting in 8 possible A/T/N profiles [44, 45].

In a study by Eckman *et al.*, a positive "A" biomarker was represented in more than ninety percent in both individuals with MCI that progressed to dementia and with AD dementia. However, a positive "A" biomarker was also present in subjects with stable MCI (59%) and healthy controls (39%), but to a lesser degree. Thus, there was a considerable amount of individuals in these two groups with evidence of AD-like pathology [46]. Other studies noted that the ATN scheme identified different biomarker profiles with overlapping baseline features and patterns of cognitive decline. The authors concluded then that the large number of profiles poses a challenge to the application of the ATN scheme, since its prognostic value depends on clinical status [45,47].

In conclusion, the concept of MCI draws attention to cognitive changes not severe enough to warrant the diagnosis of dementia. It covers different pathological entities and characterises diverse populations of patients. It is thus an heterogenous entity. In order to make it possible to identify the underlying pathological disorders before the affected patients meet the criteria of

dementia, specific neuropsychological assessments, neuroimaging, and biomarkers have been proposed. In particular, patients with AD, the most important subgroup of patients with MCI, can already be identified before appearance of the fully developed clinical dementia syndrome [34].

It may then be said that MCI can be conceptualized from two perspectives. One of them sees MCI as a disease *per se*. The other considers MCI a stage in the continuum of AD. We believe that, if aMCI as proposed by Petersen *et al.* (1999) [30] is considered, Alzheimer´s disease can be identified at its early clinical phase. We used thus Petersen criteria in the first and the second study of the present thesis. The third included participants according to the diagnostic criteria of MCI due to AD, as proposed by the NIA-AA workgroups [38], because the focus of this last work was specifically on amyloid positive MCI and because they offer the most accurate prognosis in clinical settings [43].

-Contribution of Neuropsychology

Neuropsychological assessment is essential in early detection, differential diagnosis, and measuring progression of cognitive impairment [48].

Alzheimer described memory, visuo-spatial, and language problems in Auguste D., which were brought to light in neuropsychological evaluation performed by himself [49,50].

Petersen's MCI criteria (1999) included "normal general cognitive function" and "abnormal memory for age" [30]. They therefore imply the performance of a thorough neuropsychological evaluation.

As mentioned before, the core principle of the research criteria for the diagnosis of AD proposed by Dubois *et al.* (2007) [35] is based upon the presence of the amnestic syndrome of the medial temporal type, defined by the Free and Cued Selective Recall Reminding Test [51]. In these criteria, once again, the importance of neuropsychology is highlighted.

The NIA-AA criteria for MCI due to AD [38] include objective impairment in at least one cognitive domain, which obviously requires neuropsychological evaluation.

In DSM-5, whilst no definite neuropsychological cut-off scores are recommended, there is the implication that neuropsychological testing can be very helpful in making the diagnosis of Mild Neurocognitive Disorder [40].

The IWG-2 specifies the criteria of "objective evidence of an amnestic syndrome of the hippocampal type, based on significantly impaired performance on an episodic memory test with established specificity for AD, such as cued recall with control of encoding test" for typical AD [42], enhancing once more the value of neuropsychology in the early diagnosis of AD.

-Context of the present work

Detailed information on the progression process from MCI to dementia will be essential when effective treatment that hinders development of the disease becomes available. Society as a whole will also benefit since a 5-year delay in dementia onset has been estimated to reduce the number of dementia cases by 57% [37].

It is groundbreaking to be able to make the diagnosis of AD with a great degree of precision *pre mortem*. It is nonetheless very disappointing that such little progress has been made in more than a century: what can now be detected through sophisticated methods are the same neurofibrillary tangles and amyloid plaques described by Alois Alzheimer at the beginning of the twentieth century [49].

Molecular and imaging biomarkers of AD represent a big step in the field of etiological diagnosis and management of cognitive impairment. However, these are not widely available, are expensive and some are invasive. Also, recent work shows that biomarkers are not sufficient to estimate prognosis [45].

Therefore, it would be interesting to explore if neuropsychology, which is essential in diagnosis and characterisation of patients, is non-invasive and relatively inexpensive, could give some information on prognosis of MCI and also distinguish between amyloid positive and negative subjects. On the other hand, in MCI patients who are known to be amyloid positive, it is of utmost importance to be able to estimate time until conversion to dementia and neuropsychology could also be useful for this purpose.

Objectives/Aims

The global objective of the present study was to elucidate the neuropsychological predictors of the evolution of MCI. For this purpose, three specific aims were pursued.

1. To determine the neuropsychological predictors of long-term MCI stability.

2. To compare the neuropsychological profiles in amyloid positive and amyloid negative MCI patients.

3. To evaluate the contribution of neuropsychological assessment to the prognosis of amyloid positive MCI patients.

Study 1

Neuropsychological predictors of long-term (10 years) MCI stability

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Luísa Alves' roles: conception of the work; data analysis and interpretation; drafting the article; final approval of the version to be published.

Introduction

Petersen *et al.* defined MCI as a condition characterised by subjective memory complaints, objective memory deficit, normal general cognitive performance and maintained activities of daily living [30]. From the beginning, the concept of MCI assumed a continuum between normality and Alzheimer's disease and corresponded to a condition likely to progress to dementia, essentially AD [30]. The rate of conversion of MCI to dementia in a clinical setting was about 10% to 15% per year [52]. Nevertheless, it soon became apparent that a few patients, despite fulfilling MCI criteria, might not convert to dementia and would maintain the diagnosis of MCI even after many years [53].

As research progressed, several MCI subtypes were recognized, confirming that aMCI, involving predominant impairment of the memory domain, was indeed the subtype associated with a higher conversion to AD [32,54].

Another important observation was that high rates of conversion to dementia were observed in clinical studies, enrolling participants from clinical sources, such as memory clinics, whereas in epidemiologic studies, recruiting participants in the community, the conversion rates were lower [55]. A meta-analysis of 41 MCI studies found an adjusted annual progression rate to dementia from MCI of 9.6% in clinical settings and of 4.9% in community settings [56]. Thus, in epidemiological studies, a substantial proportion of MCI patients does not convert to dementia and may even revert to normal [57], which is not the case in a clinical setting.

An important aspect is that most studies about conversion or stability of MCI had relatively short follow-up times, rarely exceeding 5 years [57]. On the other hand, the annual rate of conversion of aMCI patients to AD or dementia is not fixed along time [58]. Visser and colleagues showed, in their 10 year-follow-up study in a clinical setting, that the annual conversion rate was highest during the first years of follow-up and decreased at longer follow-up intervals. The annual conversion rate in subjects with aMCI was on average 10.8% during the first 2 years of follow-up, 4.5% during the next 3 years, and 2.5% during the last 5 years [58]. It is thus possible that patients with MCI who appear stable will still convert to dementia if the study follow-up is long enough. In a similar vein, patients who sustain the diagnosis of MCI for long periods may show some cognitive decline, albeit not severe enough to induce major changes in activities of daily living and justify the diagnosis of dementia. Only studies with a long follow-up and detailed neuropsychological testing

may reliably answer the question of whether there is a proportion of patients with MCI in a clinical setting who really remain stable in the long term, or whether they are deemed to decline cognitively at a faster or slower pace. This question has relevant implications for the prognosis of patients suffering from MCI, and theoretical consequences for the pathophysiological meaning of MCI.

In the present study, we identified, within the CCC, 22 patients that maintained the diagnosis of MCI for at least 10 years. These stable patients we compared to a group of MCI patients that converted earlier to dementia, matched for age and education, selected from the same cohort. The objectives of this study were to ascertain whether patients that maintain the diagnosis of MCI in the long term (10 years) are really stable or just declining slowly, and to identify clinical and neuropsychological characteristics associated with long-term stability in MCI patients.

Materials and Methods

Participants

Participants were selected from the CCC. The study was conducted in accordance with the Declaration of Helsinki, and was approved by the local ethics committee.

Inclusion Criteria

The inclusion criteria for the diagnosis of MCI were adapted from Petersen *et al*. (1999) [30], corresponding to aMCI:

1) presence of memory complaints;

2) abnormal memory function, documented by impairment in the Logical Memory A test score. Logical Memory is a subtest of the Bateria de Lisboa para Avaliação das Demências (BLAD) [59,60] (see below). For the memory function to be considered abnormal, we set the cutoff score of the Logical Memory A test at 1 SD below the age and education norms. Busse *et al*. [61], have observed, in the cohort of the Leipzig Longitudinal Study of the Aged, that the "MCI modified, 1.0 SD" criteria had the highest relative predictive power for the development of dementia. Interestingly, the DSM-5 considers a rather broad range to establish the cognitive deficit in mild neurocognitive disorder, namely 1–2 standard deviations below age- and education-adjusted norms [40].

3) normal general cognitive function, determined by the Mini Mental State Examination (MMSE) [62] (see below) within normal values for the Portuguese population [60].

4) no or a minimal impairment in activities of daily living determined by the Instrumental Activities of Daily Living Scale (IADL) [63] (see below) - that is to say, no more than one item from the IADL scale was altered.

Exclusion Criteria

a) Presence of neurological (stroke, brain tumor, significant head trauma, epilepsy) or psychiatric disorders that may induce cognitive deficits; patients with major depression according to DSM-IV-TR [9] or serious depressive symptoms, indicated by a score >10 in Geriatric Depression Scale short version (GDS_{15}) [64-66](see below);

b) Presence of systemic illness with cerebral impact (hypertension, metabolic, endocrine, toxic, and infectious diseases);

c) History of alcohol abuse or recurrent substance abuse or dependence;

d) Medication use with possible cognitive side effects;

e) Seriously reduced vision or other sensory deficits likely to interfere with assessment;

f) Presence of dementia according to DSM-IV-TR [9]

The diagnosis of MCI was made by an experienced neurologist (AdeM), after multidisciplinary consensus using all available information.

Long-term-stable MCI

Patients with long-term-stable MCI must fulfil the criteria for MCI during at least 10 years. The database of the CCC was thoroughly searched for these patients.

Converter MCI

For each long-term-stable MCI patient, the first two MCI patients that converted to dementia during follow-up, matched for age and education, were selected in the CCC database, and considered converter MCI.

Neuropsychological assessment

The comprehensive neuropsychological assessment at each CCC visit is carried out by the same team of trained neuropsychologists, following a standard protocol and including several instruments:

(1) Mini-Mental State Examination (MMSE) [62]. The Portuguese version of the test adapted from Guerreiro *et al.* [60] was used. Normal values for the Portuguese population are >27 for more than 11 years of education and >22 for 11 or less years of education [60].

(2) Battery of Lisbon for the Assessment of Dementia (BLAD) [59,60]: The BLAD is a comprehensive neuropsychological battery, including tests from the Wechsler Memory Scale (WMS) [67], that evaluates multiple cognitive domains and has been validated for the Portuguese population. For the present study, the following tests were considered:

a) Memory and Learning: Digit Span Forward from WMS, Word Delayed Total Recall (delayed recall of 5 non-related words), Logical Memory (immediate and delayed free recall) from WMS, Verbal Paired Associate Learning from WMS (difficult verbal pairs)

b) Attention and Executive Functions: Cancellation task (cross out 16 letters "A" from a set of 100 letters), Digit Span Backward from WMS, Clock Draw (free drawing of a clock), Verbal Semantic Fluency (supermarket food items)

c) Abstract Thought: Raven's Coloured Progressive Matrices (Ab series), Interpretation of Proverbs (3 proverbs)

d) Orientation: Temporal Orientation (7 questions concerning temporal orientation)

e) Calculation: Basic Written Calculation (4 additions, 2 subtractions,3 multiplications)

f) Visuo-Constructional Abilities: Cube Copy (drawing of a cube with perspective)

(3) Geriatric Depression Scale (GDS) [64-66]: For this study a short-form (15 items) of the self-report instrument, in the Portuguese version, adapted from Barreto *et al.* [64], was used. The presence of depression was defined as clinical history of depression or $GDS_{15}>5$ [68].

(4) Instrumental Activities of Daily Living Scale (IADL) [63]. The Portuguese version, done in the context of the LADIS project, was used [69].

Conversion to dementia

The diagnosis of dementia and AD was established according to the DSM-IV-TR criteria [9], in a consensus meeting with the neurologist and the neuropsychologists.

Data Analysis

Neuropsychological test raw scores of the baseline and last assessments of all long-term-stable MCI and matched converter MCI individuals were registered; *z* scores were also calculated according to the age and education norms for the Portuguese population with the equation [$z=(x-mean)/SD$]. In the case of converter MCI, the visit in which the diagnosis of dementia was made was considered the last assessment.

Statistical analysis was performed using IBM SPSS Statistics 23 for Windows (2015 SPSS Inc., an IBM Company, Chicago, IL) package. Significance was set at $p<0.05$.

Baseline demographic data were compared between groups using Student's *t* test for numerical variables and χ^2 Pearson test for categorical data.

The baseline and follow-up neuropsychological test scores for long-term-stable MCI and converter MCI were compared with One-Way ANOVA, followed by the *post hoc* Tukey's test. Binomial logistic regression was used to identify baseline neuropsychological predictors of long-term stability.

Results

Twenty-two patients in the CCC met criteria for long-term-stable MCI, representing 3.4% of the 655 MCI patients having follow-up in the CCC. Forty-four converter MCI patients matched in terms of age and education at baseline were selected. As expected from the matching procedure, the two groups did not differ in terms of age and education. Gender distribution was also similar. They did not differ in terms of the presence of depression either. Time between symptom onset and baseline was similar for both groups. Mean time to conversion to dementia was 3.5±2.1 (median 3.0, range 1-9) years in converter MCI patients, whereas the mean time of follow-up in the long-term-stable MCI group was 11.1±2.1 (median 11.0, range 10-14) years (Table 1).

Table 1. Demographic and clinical characterisation

	Long-term-stable MCI (n=22)	**Converter MCI (n=44)**	***p*-Value**
Gender, male/female, n (% female)	8/14 (63.6%)	16/28 63.6%)	1.000[a]
Education, years, mean (SD)	9.5 (5.0)	9.1 (4.8)	0.780[b]
Age of first symptoms, years, mean (SD)	64.1 (8.3)	64.5 (7.5)	0.856[b]
$Time_{between}$ symptoms' $onset_{and}$ $first_{assessment}$, y, mean (SD)	1.6 (1.2)	1.9 (1.2)	0.326[b]
Age at first evaluation, years, mean (SD)	65.5 (8.3)	66.4 (7.7)	0.678[b]
Presence of depression[c], n (%)	7(31.8%)	14 (31.8%)	1.000[a]
Duration of follow-up, years, mean (SD)	11.1 (2.1)	3.5 (2.1)	<0.001[b]

[a] χ^2 Pearson test.
[b] Independent samples Student's *t* test.
[c] Presence of depression was defined as clinical history of depression or GDS>5.

Long-term-stable MCI patients maintained performances at the 10-year follow-up in all neuropsychological tests (there were no significant differences between baseline and follow-up scores) (Table 2).

On the contrary, converter MCI patients declined significantly between baseline and follow-up assessments in the domains of attention and executive functions, abstract thought, temporal orientation, calculation and visuo-constructional abilities. They already scored low at the baseline

and did not further decline significantly in the domain of memory and learning, except for the Word Delayed Total Recall test, in which a decay was noticed (Table 2).

Interestingly, at the baseline assessment, MCI patients that would remain stable for 10 years already performed consistently better than MCI patients deemed to convert to dementia in all the tests, the difference being statistically significant for Word Delayed Total Recall, Logical Memory - Immediate Free Recall and Raven's Progressive Matrices (Table 2).

In order to identify neuropsychological predictors of long-term stability, the neuropsychological tests that were different at the baseline between long-term-stable MCI and converter MCI patients, namely Word Delayed Total Recall, Logical Memory - Immediate Free Recall and Raven's Progressive Matrices, entered the binomial logistic regression model. Univariate logistic regression analysis revealed a significant association between high scores at baseline in all three tests and long-term MCI stability. In multivariate logistic regression analysis, only Word Delayed Total Recall and Raven's Progressive Matrices retained significance in the prediction of long-term stability. For each additional score point in the Word Delayed Total Recall, the odds were 1.7, that is the probability of long-term stability increased by 70%. Each additional point in the total score of Raven's Progressive Matrices increased the probability of long-term stability about 2 fold (Table 3).

Discussion

The present work confirms that some patients with MCI maintain this diagnosis in the long term (10 years) and shows that they are able to maintain stable neuropsychological performance in all studied cognitive domains for an extended period.

Proportion of long-term-stable MCI patients

It should be noted that only a small proportion of patients with MCI (3.4% of MCI patients with follow-up in this cohort) maintain this diagnosis for a long period, presumably due to continual conversion to dementia [52] as well as persistent attrition of the cohort over the 10-year follow-up.

Table 2. Neuropsychological tests in long-term-stable and converter MCI patients

	Baseline		**Follow-up**	
Cognitive domain Neuropsychological tests	**Long-term stable MCI** mean (SD) ***z* scores (SD)**	**Converter MCI** mean(SD) ***z* scores (SD)**	**Long-term stable MCI** mean (SD)	**Converter MCI** mean (SD)
Memory and Learning				
Digit span forward	5.27(0.83) **0.29 (0.97)**	4.77(0.61) **-0.37 (0.75)**	5.43(1.08) #	4.24(1.22)
Word delayed total recall	9.80(1.51) **-0.70 (0.83)**	8.05(2.26) § **-1.54 (1.36)**	9.16(2.36) #	5.86(2.85) †
Logical memory (immediate free recall)	9.68(4.11) **-0.73 (1.42)**	6.07(3.97) § **-1.81 (1.32)**	9.73(4.36) #	3.85(3.13)
Logical memory (delayed free recall)	6.80(4.84) **-1.67 (1.48)**	4.12(4.29) **-2.49 (1.06)**	9.14(4.92) #	1.48(1.96)
Difficult Verbal Paired Associate Learning	3.67(2.78) **-1.21 (1.84)**	1.81(2.72) **-1.83 (1.51)**	4.35(3.20) #	1.16(2.08)
Attention and executive functions				
Cancellation task-total	4.63(1.49) **0.16 (1.15)**	3.86(1.49) **-0.25 (1.08)**	3.92(1.55) #	2.74(1.24) †
Digit span backward	3.68(0.89) **0.18 (1.15)**	3.50(0.73) **-0.03 (0.97)**	3.76(0.89) #	2.34(1.29) †
Clock draw	2.70(0.47) **0.295 (0.86)**	2.49(0.74) **-0.01 (1.56)**	2.65(0.59) #	1.59(1.05) †
Verbal semantic fluency	16.23(3.82) **0.00 (1.12)**	14.45(4.36) **-0.49 (1.38)**	16.82(4.25) #	9.93(4.43) †
Abstract Thought				
Raven's Progressive Matrices	9.57(1.75) **0.36 (1.03)**	7.31(2.33) § **-0.49 (1.26)**	9.40(2.01) #	5.33(2.48) †
Interpretation of Proverbs	7.00(1.54) **0.79 (1.25)**	6.75(1.77) **0.52 (1.14)**	6.90(1.73) #	4.46(2.19) †
Orientation				
Temporal orientation	6.55(0.83) **-0.26 (1.16)**	6.02(1.12) **-1.49 (2.01)**	6.15(1.14) #	3.00(2.49) †
Calculation				
Basic written calculation	13.41(1.00) **0.38 (0.40)**	12.13(2.24) **-0.06 (1.24)**	11.74(2.85) #	9.46(4.27) †
Visuo-constructional abilities				
Cube copy	2.53(0.70) **0.45 (1.21)**	1.97(1.06) **-0.35 (1.79)**	2.41(0.71) #	1.39(1.23)

Raw test scores are shown. In the baseline neuropsychological results, *z* scores (SD) values are shown below raw scores.
Long-term-stable MCI, MCI patients that have maintained this diagnosis for 10 years or more. Converter MCI, MCI patients that converted to dementia during follow-up.
§ Converter MCI patients had worse scores than long-term-stable MCI patients at baseline, One-Way ANOVA, Tukey *post-hoc* test
# Follow-up tests were not significantly different from baseline in long-term-stable MCI patients, One-Way ANOVA, Tukey *post-hoc* test
† Follow-up tests worsened from baseline in converter MCI patients, One-Way ANOVA, Tukey *post-hoc* test

Table 3. Neuropsychological predictors of long-term MCI stability

Neuropsychological tests	B	SE	Wald	Significance	Exp(B)
Word Delayed Total Recall	0.555	0.236	5.516	0.019	1.743
Logical memory (immediate free recall)	0.091	0.108	0.718	0.397	1.096
Raven's Progressive Matrices	0.665	0.205	10.459	0.001	1.944

Multivariate binary logistic regression analysis

Neuropsychological stability

This study is the first, to our knowledge, to ascertain real neuropsychological stability in all the studied cognitive domains in stable MCI individuals over a decade of follow-up, that is, no statistically significant worsening was observed in any cognitive domain. In contrast, the converter MCI patients worsened in all neuropsychological tests, although this decline was not statistically significant in tests where they already had a low score at baseline, namely Digit Span Forward, Logical Memory-Free Immediate and Free Delayed Recall, Difficult Verbal Paired Associate Learning and Cube Copy tests, presumably due to a floor effect.

Demographic factors as predictors

Several factors influencing conversion of MCI to dementia have been recognized; namely older age [58] and lower level of education increase the risk of conversion ([70], but see [71]). These factors were *a priori* controlled for by the design of the study, matching long-term-stable with converter MCI patients by educational level and age at baseline evaluation. Time between symptom onset and baseline evaluation as well as distribution of gender were also similar between groups.

Neuropsychological tests as predictors

The neuropsychological performance at baseline, in particular, tests measuring memory, were shown to predict conversion of MCI to dementia in previous clinical studies with shorter follow-up durations. Arnáiz *et al.* [53], in a clinical sample of 303 MCI patients, followed up for 3 years on average, found that tests assessing learning and retention, specifically Wechsler Memory Scale-Revised delayed recall, were the best predictors of conversion to AD. Sarazin *et al.* [51] observed, in a cohort of 251 patients with MCI, followed for up to 3 years, that the most sensitive and specific test for diagnosis of prodromal AD was the Free and Cued Selective Recall Reminding Test (FCSRT). Silva *et al.* [72] found that four commonly used verbal memory tests were able to predict conversion

to dementia in 272 non-demented patients reporting subjective cognitive complaints followed up for 3 years on average, and that the California Verbal Learning Test had the highest predictive value, which was not improved by adding other memory tests. In a similar vein, Gómez-Tortosa *et al.* [73] followed up for 48 months on average a cohort of 210 cases with aMCI. They were divided into two groups according to their initial recognition memory discrimination index (DI) on the Hopkins Verbal Learning Test, and conversion to dementia occurred significantly later in cases with higher DI. A multivariate regression model revealed DI and delayed recall as the strongest predictors of dementia. A recent systematic review [74], covering data for a total of 2365 participants with MCI at entry, followed over an average of 31 months, found that Paragraph Delayed Recall, Word-list Free Delayed Recall with and without oriented encoding were tests with excellent overall accuracy for predicting progression to Alzheimer's type dementia. Another recent literature review [75] found that the majority of studies on the prediction of conversion from MCI to AD dementia report delayed recall as the most sensitive neuropsychological measure.

It is also clear that other tests, namely assessing attentional and executive capabilities, may also contribute as predictors of MCI conversion to dementia. For instance, in a work by Tabert *et al.* [76], with 148 MCI patients followed up for almost 4 years, the percent savings from immediate to delayed recall on the Selective Reminding Test and the Wechsler Adult Intelligence Scale–Revised Digit Symbol Test score were the strongest predictors of time to conversion. Also, in Fleisher *et al.* study [77], 539 participants with aMCI were followed during 3 years and it was found that progression from MCI to Alzheimer dementia was best determined by combining distinct cognitive measures, namely Delayed Paragraph Recall Test, Delayed 10-Word List Recall, Symbol Digit Modalities Test and the ADAS-cog total score. Along the same lines, Li *et al.* [78] studied 139 persons patients with aMCI enrolled in the Alzheimer's Disease Neuroimaging Initiative and observed that, not only the baseline Memory composite (scores on the Logical Memory and Rey Auditory Verbal Learning tests), but also the executive function composite (scores on the Trail Making, Digit Symbol Substitution, and spontaneous Clock drawing tests) could predict progression to AD after 3 years.

In the present work, with 10 years of follow-up, although one verbal memory delayed measure at baseline was associated with long-term stability, we found that performance on the Raven's Coloured Progressive Matrices at the baseline was the stronger neuropsychological predictor of

long-term clinical stability. Remarkably, each additional point in the total score of the Raven's Progressive Matrices increased the probability of long-term stability about 2-fold. This finding may assume clinical relevance, but must be replicated in other MCI cohorts.

Raven's Coloured Progressive Matrices [79] are considered a non-verbal reasoning measure of fluid-type intelligence [80]. So-called fluid intelligence tests are most predictive of a general ability to do well, calling for novel problem solving with simple visual or other kind of materials, reflecting current ability for abstract thought and reasoning. Interestingly, Elias *et al*. [81], in a prospective study in healthy community participants in the Framingham cohort, found poor abstract reasoning to be a strong predictor of conversion to dementia in the long run. Fluid intelligence has been considered a good proxy for cognitive reserve [82]. Cognitive reserve is associated with lower risks for incident dementia [83]. As mentioned above, in our study, education, a variable known to influence prevalence rates of dementia [84], was controlled for. However, other factors thought to contribute to cognitive reserve, such as occupation, premorbid IQ and mental activities, were not specifically analyzed [83].

Presumed etiology of long-term-stable MCI

The question of whether these patients with long-term-stable MCI have AD pathology, that is, suffer from prodromal AD from the start, or not, is rather intriguing. Nowadays, the AD biomarkers are commonly used to detect AD pathology and diagnose prodromal AD or MCI due to AD (e. g. [38, 42, 85, 86]). However, when these patients were recruited, at least 10 years ago, AD biomarkers were not routinely used in clinical practice.

It is known that there can be a very long interval, about 20 years, between first development of amyloid positivity and onset of dementia [87]. Clinical cohort studies suggest that there may be very subtle cognitive alterations that are detectable a decade or more before meeting criteria for MCI [37]. Thus, the AD pathophysiological process may course with a long preclinical stage [37]. Based on our findings, we could speculate that the disorder might also be quiescent for long periods at the MCI stage, at least in some patients. However, factors that might assume a neuroprotective role at this stage are still largely unknown [88].

Limitations and strengths

This cohort is constituted mainly by memory clinic patients, and the findings may not apply to clinical settings with different patient characteristics. The absence of data on Apolipoprotein E genotype is a limitation of the present study, since the *ε4* allele is an important risk factor for AD [89].

The strengths of this study were that it was carried out in the context of a large cohort, in which the patients underwent comprehensive standardized neuropsychological assessments, and a long term 10-year follow-up was achieved.

Conclusions

We found that, in some MCI patients, real neuropsychological stability over a decade is possible and that long-term stability could be predicted on the basis of neuropsychological tests measuring memory and non-verbal abstract reasoning.

Study 2

Neuropsychological profile of amyloid positive versus amyloid negative amnestic Mild Cognitive Impairment

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Introduction

As a consequence of the ageing of the population, the number of people affected by neurodegenerative disorders, particularly AD, is increasing dramatically worldwide [12]. There has been a growing interest in detecting AD as soon as possible along its insidious evolution, before the establishment of the diagnosis of dementia. The correct identification of patients with memory complaints who already have an ongoing neurodegenerative process is desirable, since it offers patients the possibility to make important life decisions, anticipate future care, start symptomatic drugs, initiate cognitive rehabilitation therapy, and eventually participate in clinical trials with putative neuroprotective drugs [23]. About 2 decades ago, the Mayo Clinic group fostered an important advance by proposing the concept of aMCI, as a condition characterised by subjective memory complaints, objective memory deficit, normal general cognitive performance and maintained activities of daily living [30]. Patients diagnosed with aMCI in a clinical setting have about 10% annual progression rate of conversion to dementia, usually AD [56]. However, aMCI can have other etiologies [90], and some aMCI patients actually remain stable for as long as a decade [91].

In recent years, the use of biomarkers has allowed the possibility of diagnosing AD in vivo in patients that present with aMCI. These biomarkers are surrogates of pathological alterations in the brain characteristic of AD [92]. The presence of amyloid pathology may be determined by measuring Aβ42 concentrations in the CSF, and/or quantifying brain deposits of Aβ with amyloid PET [93].

In spite of the remarkable advance that the development of biomarkers represents both from an investigational and a clinical perspective, and the rapid acceptance of these methods by reference centres [93], the generalization of biomarker testing to other settings has been more sluggish. Several explanations might be advanced, for instance lumbar puncture, used to obtain CSF, is an invasive procedure with contra-indications and side effects, and amyloid PET is quite expensive and not widely available. Bearing this in mind, it would be important to discover non-invasive and affordable methods that could discriminate between amyloid positive (Aβ+) and amyloid negative (Aβ-) aMCI patients.

Since neuropsychological testing is not invasive and is required to make a formal diagnosis of aMCI, it would be very interesting if it could be used to identify the amyloid status in patients with aMCI [94]. In other words, Aβ+ aMCI patients might have a particular neuropsychological profile that

would distinguish them from Aβ- aMCI patients. Several studies compared global cognition, attention, executive functions, visuospatial functions, language, visual memory and verbal memory between Aβ+ and Aβ- aMCI patients.

Since patients with AD typically have deficits in episodic memory as a consequence of early and marked hippocampal neurodegeneration, it is not surprising that Aβ+ aMCI patients consistently presented more prominent episodic memory deficits than Aβ- aMCI patients in several different studies [94-101]. However, regarding attention and executive functions, different studies produced less consistent results, possibly depending on the kind of test used to measure these abilities as well as the number of patients recruited. In the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort, Aβ+ aMCI patients took longer to complete the Trail Making Tests A and B, when compared to Aβ- aMCI patients [96]. These results were not corroborated by other studies, that did not observe significant differences in the Trail Making Tests A and B between Aβ+ and Aβ- aMCI patients [95,100,101]. Regarding another commonly used executive test, Verbal Semantic Fluency, Aβ+ aMCI patients had worse performance in one study [96] but not in other work [95].

We now reappraise neuropsychological testing in Aβ+and Aβ- aMCI patients, particularly concerning performances on executive tests, as well as cognitive domains so far scarcely analysed, like abstract reasoning and calculation. Furthermore, we aim to test whether a statistical model involving different neuropsychological variables could be valuable to help identify the amyloid status of patients with aMCI.

Materials and methods

Participants

Participants belong to the CCC. The study was approved by the local ethics committee and conducted according to the declaration of Helsinki. Informed consent was obtained from patients before any procedure.

Inclusion criteria

1) Diagnosis of aMCI. The criteria for the diagnosis of aMCI were adapted from Petersen *et al.*(1999) [30]:

a) Presence of memory complaints;

b) Abnormal memory function, documented by impairment in the Logical Memory A test Immediate Free Recall score. Logical Memory is a subtest of the Bateria de Lisboa para Avaliação das Demências (BLAD) [59,60] (see below). For the memory function to be considered abnormal, we set the cut-off score of the Logical Memory A Immediate Free Recall at 1 SD below the age and education norms. Busse et al. (2006) [61] observed, in the cohort of the Leipzig Longitudinal Study of the Aged, that the "MCI modified, 1.0 SD" criteria had the highest relative predictive power for the development of dementia;

c) Normal general cognitive function, determined by the Mini-Mental State Examination (MMSE) [62] (see below) within normal values for the Portuguese population [60];

d) No or a minimal impairment in activities of daily living, determined by the Instrumental Activities of Daily Living Scale (IADL) [63] (see below), that is to say, no more than one item from the IADL scale was altered.

2) Known amyloid status, determined by CSF Aβ42 measurement and/or cortical uptake of the Pittsburgh compound B (^{11}C-PiB) on the PET scan.

Exclusion criteria

1) Presence of neurological (stroke, brain tumor, significant head trauma, epilepsy) or psychiatric disorders that may induce cognitive deficits; patients with major depression according to DSM-IV-TR [9] or serious depressive symptoms, indicated by a score > 20 in Geriatric Depression Scale (GDS30) or >10 in Geriatric Depression Scale short version (GDS15) [64-66] (see below);

2) Presence of systemic illness with cerebral impact (uncontrolled hypertension, metabolic, endocrine, toxic, and infectious diseases);

3) History of alcohol abuse or recurrent substance abuse or dependence;

4) Medication use with possible cognitive side effects;

5) Seriously reduced vision or other sensory deficits likely to interfere with assessment;

6) Presence of dementia according to DSM-IV-TR [9];

7) Interval between neuropsychological assessment and knowledge of amyloid status longer than 12 months.

The diagnosis of aMCI was made by an experienced neurologist, after multidisciplinary consensus using all available clinical, neuropsychological and neuroimaging information available from the diagnostic workup.

Biomarker analysis

The amyloid biomarker status was based on cerebrospinal fluid Aβ42 level and/or cortical uptake on ^{11}C-PiB PET, and the aMCI patients were classified as Aβ+ or Aβ-. Both sources of amyloid status were considered interchangeable since a high agreement between Aβ42 concentrations in the CSF and amyloid PET scan results in aMCI and AD disease patients was confirmed by previous studies [102].

The levels of Aβ42 were measured using commercially available enzyme-linked immunosorbent assays (INNOTEST® β-amyloid (1–42); Innogenetics, Ghent, Belgium) according to the established protocols on participating centers [103]. The levels of Aβ40 and the ratio Aβ42 over Aβ40 were not determined routinely, only in exceptional cases where a discrepancy was found between CSF and PET scan amyloid results. The expected site assay variability present in multicenter studies was acknowledged [104] and positivity was determined using locally available cut-off values.

The cortical uptake with ^{11}C-PiB PET was performed only in one center using the same scanner (Philips PET/CT Gemini GXL), preceded by a low-dose brain computed tomography (CT) acquisition for attenuation correction (Institute of Nuclear Science Applied to Health, ICNAS, University of Coimbra). ^{11}C-PiB PET images were classified as amyloid positive or negative based on a support vector machines (SVM) local classifier, which uses the voxelwise brain grey matter standardized uptake value ratio (SUVR) and the cerebellar grey matter as reference region [105].

Neuropsychological assessment

The comprehensive neuropsychological assessment was carried out by the same team of trained neuropsychologists, following a standard protocol and comprised the following instruments and scales:

•Mini-Mental State Examination (MMSE [60, 62]) - the MMSE is a brief screening instrument to assess global cognitive performance. The Portuguese version was applied, and normative data were >27 for individuals with more than 11 years of education and >22 for patients with 11 or less years of education [60].

•Battery of Lisbon for the Assessment of Dementia (BLAD [59,60]) – the BLAD is a comprehensive neuropsychological battery that includes some tests from the Wechsler

Memory Scale (WMS [67]) and has been validated for the Portuguese population. This battery includes tests for the following cognitive domains: immediate memory (Digit Span forward); verbal memory (Word Total Recall, a 5-words 1-minute delayed recall test, in which the total score contemplates spontaneous and cued recall); logical memory (Logical Memory Immediate and Delayed Recall; for this test, the score is based on the combination of 7 literal elements and 17 meaningful elements); associate learning (Verbal Paired Associate Learning); general information (General Information, consisting of 20 questions on subjects of general knowledge); working memory (Digit Span backward); attention (Cancellation Task); verbal initiative (Verbal Semantic Fluency); verbal and non-verbal abstraction (Raven's Coloured Progressive Matrices – Ab series-B and Interpretation of Proverbs); calculation (Basic Written Calculation);

•Trail Making Test (part A and part B [106, 107]) – the TMT task measures sustained attention, visuomotor processing speed (part A), visuospatial working memory and cognitive flexibility (part B). The part A consists of 25 circles numbered 1 – 25 distributed over a sheet of paper and the patient should draw lines to connect the numbers in ascending order. In Part B there are 25 circles as well, but the circles include both numbers (1 – 13) and letters (A – M) and the patient has to draw lines to connect them all in an ascending pattern with the added task of alternating between the numbers and letters (i.e., 1-A-2-B-3-C, etc.).

•Geriatric Depression Rating Scale (GDS [64-66]) - the GDS is a self-report instrument used specifically to identify depressive symptomatology in the elderly. For this study the Portuguese versions of GDS30 and GDS15 were used [64].

•Blessed Dementia Rating Scale is a clinical rating scale with 22 items that measures changes in performance of everyday activities (8 items), self-care habits (3 items), and changes in personality, interests, and drives (11 items). Ratings are based on information from relatives or friends and concern behaviour over the preceding 6 months [10,108].

For the present work, the neuropsychological assessment closest to the knowledge of the amyloid status was used.

Statistical analysis

For comparison of demographic and clinical data between groups the independent samples 2-tailed Student's *t* test and the χ^2 Pearson test were used, for numerical and nominal data, respectively. The neuropsychological assessments were standardized according to the age and education norms for the Portuguese population [59,60] and *z* scores were calculated with the equation [*z*=(x-mean)/SD]. The comparison of neuropsychological results between Aβ+ and Aβ- groups was done with the independent samples 2-tailed Student's *t* test. To check if the differences that were found between groups still held when controlling for the MMSE score, a general linear model analysis was performed considering the MMSE as a covariate. A binary logistic regression analysis was conducted to assess if the neuropsychological tests scores could predict amyloid positivity. The tests that were significantly different between the groups entered the model. The Enter method (that is, standard regression analysis) was used. Receiver Operating Characteristic (ROC) curves were obtained when appropriate. In order to control for an eventual redundancy in the tests comprising the neuropsychological battery, a principal component analysis using a rotated varimax component matrix was performed.

Statistical analyses were performed using IBM SPSS Statistics 25 for Windows (2017 SPSS Inc., an IBM Company) package. A probability value of <0.05 was assumed to be statistically significant.

Results

A total of 216 patients with aMCI were enrolled from the CCC for the present study, of whom 117 were Aβ+ and 99 were Aβ-. The two groups did not differ in terms of gender, education, age of first symptoms and time between symptoms onset and neuropsychological assessment. They did not differ in terms of the presence of depressive symptoms either. Regarding the Blessed Dementia Rating Scale scores, aMCI patients in the two groups had similar global levels of severity (Table 1).

Neuropsychological evaluation (Table 2) showed that Aβ+ aMCI patients had lower MMSE scores than Aβ- aMCI patients. MMSE values for Aβ+ aMCI patients were 26.8 (SD 2.2, skewness -0.3, range 23-30) and for Aβ- aMCI patients 27.6 (SD 2.0, skewness -0.7, range 23-30).

Table 1. Demographic and clinical characterisation

	Aβ+ aMCI (*n*=117)	**Aβ- aMCI** (*n*=99)	***p* value**
Gender, male/female, *n* (% female)	53/64,(54.7%)	41/58,(58%)	0.676[a]
Education, years, mean (SD)	10.6 (4.6)	9.8 (4.7)	0.204[b]
Age of first symptoms, years, mean (SD)	64.0 (7.7)	61.8 (10.8)	0.117[b]
Time$_{between}$symptoms'onset$_{and}$neuropsychological$_{assessment}$, y, mean (SD)	2.8 (2.5)	3.3 (2.9)	0.163[b]
Presence of depressive symptoms [c], %	34.5%	42.3%	0.317[a]
Blessed Dementia Rating Scale, mean (SD)	3.3 (2.0)	3.5 (2.0)	0.439[b]

a. X^2 Pearson's test
b. Independent samples Student's *t* test
c. Presence of depressive symptoms was considered when GDS_{15} score was higher than 5 points or when GDS_{30} score was higher than 10 points

Aβ+ aMCI patients also performed worse on several memory tests, namely the Word Total Recall, Logical Memory Immediate and Delayed Free Recall and Verbal Paired Associate Learning, as compared to Aβ- aMCI patients. To check if the differences on these memory tests still held when the groups were controlled for the MMSE, a general linear model analysis was performed considering the distinct neuropsychological tests as dependent variables and the MMSE score as a covariate. Aβ+ aMCI patients essentially kept poorer performances in the same tests as previously found: Word Total Recall (F=6.181, p=0.003); Logical Memory, Immediate Free Recall (F=3.077, p=0.052); Logical Memory, Delayed Free Recall (F=7.651, p=0.001); and Verbal Paired Associate Learning (F=12.281, p<0.001).

Regarding attention and executive functions, there were no differences in the Digit Span Backward, in the Trail Making Test A, in the Cancellation Task nor in the Verbal Semantic Fluency test, however, the Aβ+ aMCI patients performed significantly worse on the Trail Making Test B. Using the Trail Making Test B over A ratio, we found no significant differences between groups (p=0.905). For the Aβ+ aMCI patients, the mean value of the ratio was 2.9 (SD 1.1), for the Aβ- aMCI patients the mean was 2.9 (SD 1.2). Finally, there were no statistically significant differences between the two groups in the abstract reasoning and calculation domains.

A binary logistic regression model was built in order to predict the amyloid status of aMCI patients. In general, the tests in which there were significant differences between the two groups entered the model. Regarding Logical Memory, the Delayed Free Recall measure was chosen. Only Logical Memory Delayed Free Recall retained statistical significance to determine the amyloid status of

aMCI patients. For each additional score point in the Logical Memory Delayed Free Recall z score, the odds ratio was 0.694, that is, the probability of being Aβ+ decreased by 30.6% (Table 3).

The resulting model correctly classified 64.6% of the aMCI cases regarding their amyloid status. Only 17.7% of the variation in the dependent variable (amyloid positivity) was explained by the present model. The ability of Logical Memory Delayed Free Recall (z score) to discriminate between Aβ+ and Aβ- aMCI patients was checked with a ROC curve, producing an Area Under the Curve (AUC) of 0.633.

Since there might be some redundancy in the tests comprising the neuropsychological battery that was applied, a principal component analysis was performed. The rotated varimax component matrix pointed out 5 factors. Of these, there were significant differences between Aβ+ and Aβ- aMCI patients in factor 2 (Memory factor, comprising Logical Memory Immediate Free Recall, Logical Memory Delayed Free Recall, and Verbal Paired Associate Learning; F=9.546, p=0.003) and in factor 3 (Executive factor, comprising Trail Making Test A time and Trail Making Test B, as well as Raven´s Coloured Progressive Matrices, F=5.881, p=0.017). These results confirmed that Aβ+ and Aβ- aMCI patients essentially differed in memory test as well as in executive tests.

Discussion

The main finding of the present study is that aMCI patients who are Aβ+ have more deficits in general cognition, memory tests and executive functions as compared to Aβ- aMCI patients. A few points deserve consideration.

Table 2. Neuropsychological tests in Aβ+ and Aβ- aMCI patients (n=216)

Cognitive domain Neuropsychological tests	**Aβ+ aMCI** (n=117) Mean (SD)	**Aβ- aMCI** (n=99) Mean (SD)	***p* value**
Global cognition			
Mini-Mental State Examination (MMSE)	26.8 (2.2)	27.6 (2.0)	**0.004**
Memory and Learning			
Digit Span Forward, *z* score	0.51 (1.27)	0.24 (1.19)	0.122
Word Total Recall, *z* score	-1.70 (1.53)	-0.96 (1.27)	**<0.001**
Logical Memory, Immediate Free Recall, *z* score	-1.49 (1.73)	-0.89 (1.25)	**0.005**
Logical Memory, Delayed Free Recall, *z* score	-2.21 (1.23)	-1.61 (1.25)	**0.001**
Verbal Paired Associate Learning, *z* score	-1.56 (1.40)	-0.71 (1.31)	**<0.001**
General Information, *z* score	-0.34 (1.34)	-0.39 (1.26)	0.811
Digit Span Backward, *z* score	-0.06 (1.14)	-0.04 (1.26)	0.886
Trail Making Test A time, *z* score	-1.49 (2.19)	-0.91 (1.70)	0.054
Trail Making Test B time, *z* score	-2.57 (2.54)	-1.50 (2.28)	**0.005**
Cancellation Task, total, *z* score	0.02 (1.29)	0.33 (1.64)	0.139
Verbal Semantic Fluency, *z* score	-0.56 (1.67)	-0.44 (1.39)	0.610
Abstract Reasoning			
Raven´s Coloured Progressive Matrices, *z* score	-0.39 (1.42)	-0.12 (1.30)	0.151
Interpretation of Proverbs, *z* score	0.53 (1.62)	0.84(1.56)	0.162
Calculation			
Basic Written Calculation, *z* score	-0.37 (1.25)	-0.34 (1.46)	0.917

Table 3. Neuropsychological predictors of amyloid positivity

Neuropsychological tests	B	SE	Wald	*p* value	Exp(B)	95% C.I. for Exp(B)	
						Lower	Upper
Word Total Recall, *z* score	-0.240	0.146	2.709	0.100	0.787	0.592	1.047
Logical Memory Delayed Free Recall, *z* score	-0.366	0.172	4.549	**0.033**	0.694	0.495	0.971
Verbal Paired Associate Learning, *z* score	-0.085	0.155	0.301	0.583	0.918	0.677	1.245
Trail Making Test B time, *z* score	-0.114	0.080	2.058	0.151	0.892	0.763	1.043

Binary logistic regression analysis

In the first place, we confirmed that Aβ+ aMCI patients are more impaired in memory tests as compared to Aβ- aMCI patients, as previously reported by several studies [94-101]. As patients with aMCI patients who are Aβ+ suffer from AD [92], the observed memory deficits correspond to the typical cognitive profile of AD, reflecting the hippocampal atrophy observed early in the course of the disease. In the present work, Word Total Recall, Logical Memory (Immediate and Delayed Free Recall) and Verbal Paired Associate Learning were significantly worse in Aβ+ aMCI patients.

The second point is that we contributed to clarify the controversial issue whether Aβ+ aMCI patients are more affected in executive functions and attention, which has not been clear from previous studies. We showed that tests assessing executive functions, namely the Trail Making Test B, were more affected in Aβ+ aMCI patients. It could be argued that the worse performance on the Trail Making Test part B in Aβ+ when compared to Aβ- aMCI patients was due to impairment of visuospatial abilities in the first group. However, the observation that there were no significant differences between the Aβ+ and Aβ- aMCI patients in the Raven's Coloured Progressive Matrices, a visuospatially very demanding test, suggests that differences in the Trail Making Test part B are probably not attributable to visuospatial difficulties. The results concerning Trail Making Tests are in accordance with Kandel *et al.* (2015) [96] reports in aMCI patients from the ADNI cohort, who also found significantly worse results in both Trail Making Tests in Aβ+ patients. We did not observe differences in Verbal Semantic Fluency between Aβ+ and Aβ- aMCI patients, similarly as reported in a previous study [100]. However, another study found that Verbal Semantic Fluency was significantly worse in Aβ+ as compared to Aβ- aMCI patients [96]. This last study used animal category for the task, while we used supermarket food items, which might explain the discrepancy of the results. Regarding attention, we found no significant differences between the two groups in the Cancellation Task, no previous studies having previously compared, to the best

of our knowledge, Aβ+ and Aβ- aMCI patients on this test. More studies are certainly needed to further investigate how the amyloid status influences performances in different tests of executive functions and attention in patients with aMCI. It should be added that patients with aMCI who are Aβ+ showed less global cognitive performance, albeit within the normative range, assessed by the MMSE, as compared to Aβ- aMCI patients, probably reflecting the more pronounced alteration in several cognitive domains, particularly memory and executive functions, as described above.

A third point has to do with the value of neuropsychological tests to predict the patients with aMCI who have amyloid pathology. In the present study, the statistical model could only correctly classify 64.6% of the aMCI cases regarding their amyloid status. The only test that remained in the model was the Logical Memory Delayed Free Recall.

It is noteworthy that in the present study the Logical Memory Immediate Recall score was chosen to classify patients as aMCI and the Logical Memory Delayed Recall score for analysis, in order to avoid circularity bias. However, it could be argued that both measures were rather equivalent. This did not seem to be the case, as there was no significant collinearity between these neuropsychological test variables, with a variance inflation factor (VIF) value relating Logical Memory Immediate Recall and Logical Memory Delayed Recall of 1.199.

The Logical memory Delayed Recall score produced a modest area under the curve (AUC; 0.633). In a previous study in aMCI patients, the 30-minute delayed recall score of the Rey Auditory Verbal Learning Test was the best predictor of Aβ status among the psychometric tests, but it produced an AUC of only 0.67 [96]. Using a 16-word list, Tomadesso et al. (2018) [100] calculated slightly better AUC values for the free recall (0.73) and recognition (0.74) tasks in classifying the aMCI cases according to the amyloid status. It thus seems that neuropsychological tests have a limited ability to identify the aMCI cases who are Aβ+ and those who are Aβ-, not attaining the values of 80% recommended for AD biomarkers [109]. Of course, these results do not exclude that neuropsychological tests could add predictive value to determine the amyloid status in conjunction with other clinical or neuroimaging biomarkers.

Finally, the intriguing question of the etiology of aMCI cases who are Aβ- certain merits further research. Depressive symptoms were not more frequent in Aβ- than in Aβ+ aMCI patients. Patients with history of stroke or relevant cerebrovascular disease in brain imaging were excluded in the present study. It is possible that Aβ- aMCI patients might be at an initial stage of a

neurodegenerative disorder other than AD, for instance frontotemporal dementia or the Lewy body dementia-Parkinson's disease continuum [110]. To be sure, a long follow-up of these Aβ- aMCI patients might be needed.

The main strength of this study is that it was carried out in the context of a large prospective cohort, in which the participants underwent comprehensive standardized neuropsychological assessment. Several limitations of the study must be recognized. Participants were patients who attended a memory clinic or a general hospital outpatient clinic, and the findings may not applicable to different clinical settings. Certainly, only a proportion of patients with aMCI undergo a comprehensive AD biomarker workout, and these are probably different from those patients with aMCI who do not.

In conclusion, the neuropsychological assessment remains an essential step to diagnose and characterise patients with aMCI. However, neuropsychological tests have limited value to distinguish the aMCI patients who have amyloid pathology and AD, from those who might suffer from other clinical conditions.

Study 3

Neuropsychological contribution to predict conversion to dementia in patients with Mild Cognitive Impairment due to Alzheimer's disease

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Luísa Alves' roles: contribution to conceptualization; discussion of results; critical revision of the article.

Introduction

Nowadays, the development and clinical application of biomarkers has dramatically changed the framework of Alzheimer's disease (AD) diagnosis. It is now possible to diagnose AD at an early pre-dementia stage, that is, before the patient has symptoms severe enough to be considered demented [39,111]. Different diagnostic criteria with slight differences were advanced, namely prodromal AD [35,42,112] and Mild Cognitive Impairment (MCI) due to AD [38], that rely on biomarkers reflecting pathological alterations in the brain typical of AD, namely: (1) decline in episodic memory, confirmed by neuropsychological testing, (2) atrophy of the hippocampus and other medial temporal lobe structures shown by magnetic resonance imaging, (3) detection of abnormal CSF biomarkers, namely low amyloid Aβ42 concentrations, increased p-tau or t-tau concentrations, (4) abnormal brain deposits of Aβ and tau, as well as reduced glucose metabolism in temporoparietal regions, by PET scan. The use of biomarkers for diagnosis of MCI due to AD quickly spread to AD reference centers [93] and more sluggishly to routine clinical practice.

Uncertainties remain about the possible benefits and disadvantages of obtaining and communicating a specific diagnosis of prodromal AD, or MCI due to AD, to an individual patient. On the one hand, it should be relevant for the patient to make life decisions and prepare the near future, engage in a cognitive rehabilitation program, start appropriate pharmacological therapy, and eventually participate in a clinical trial. On the other hand, it might upset patients and caregivers, leading to emotional distress and concerns about progression of symptoms and the fleetness of future cognitive decline [113]. One important present limitation of obtaining and communicating a specific diagnosis of MCI due to AD is that the actual pace of disease progression, attainment of important clinical milestones, and in particular conversion to dementia, are presently impossible to predict in an individual basis. This point could not be made more clearly than by the patient's sentence when receiving the diagnosis of MCI due to AD: *Yes, I hope for the best. It will definitely evolve. I don't think it will stay like that, but is that within 5 years?* [113].

Importantly, prediction of time to conversion to dementia has already been extensively studied in MCI without the information of biomarkers, namely using neuropsychological assessments. These studies showed that memory tests, as well as executive function and verbal fluency tests, are able to predict with accuracy the time to conversion to dementia [76, 114-129]. We hypothesize that similar neuropsychological measures may also be useful to predict the progression to dementia in MCI due to AD. It should be very important to provide the individual patient diagnosed with MCI due to

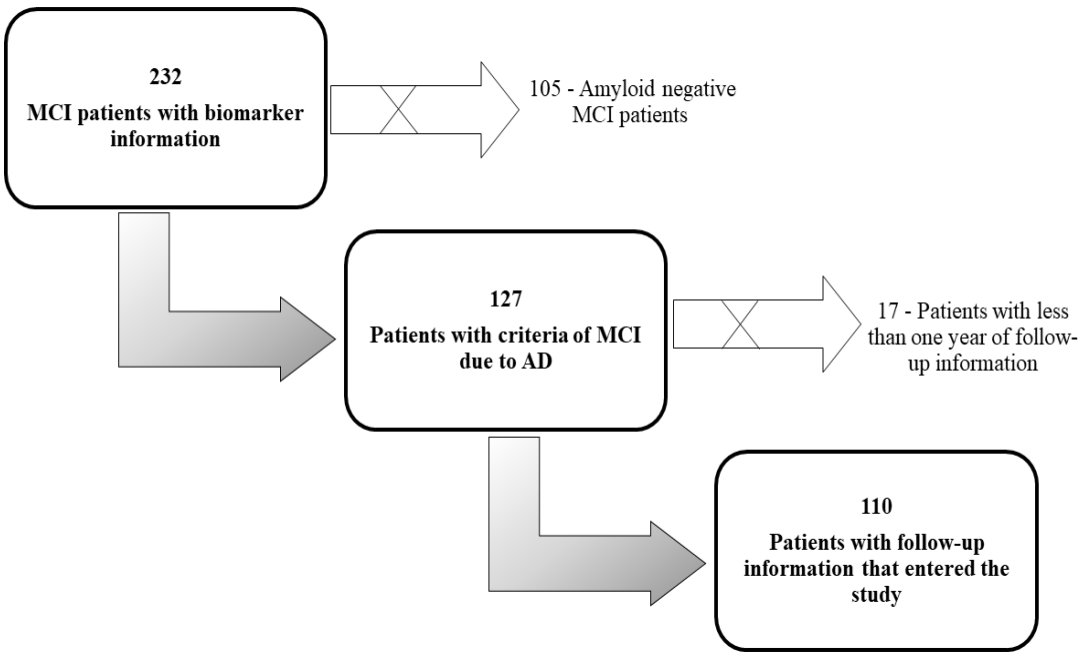
AD with reliable information on the prediction of stability or conversion to dementia at a clinically relevant time window.

Methods

Participants

A cohort of 232 patients, part of the CCC, who attended neurologic consultation in a private memory clinic in Lisbon (Memoclínica) and Coimbra University Hospital, in Coimbra, from 2006 to 2017, performed a comprehensive neuropsychological evaluation and were tested for biomarkers of brain amyloidosis and neuronal injury. From these, 127 had the diagnosis of MCI due to AD and were included in the present study. Patients had to have associated follow-up information and to be followed for at least one year, thus only 110 patients were analyzed for the present study (Figure 1).

Figure 1. Flow-chart of patient selection for the study.



Diagnostic criteria

The diagnostic criteria of MCI due to AD, as proposed by the NIA-AA workgroups [38], offer the most accurate prognosis in clinical settings [43]. Specifically, the criteria of MCI due to AD–High Likelihood

[38] were considered in the present study since they provide the highest degree of certainty that the patient will progress to AD dementia:

1. Clinical and cognitive criteria
    a. Cognitive concern reflecting a change in cognition reported by patient, informant or clinician
    b. Objective evidence of impairment in one or more cognitive domains, typically including memory
    c. Preservation of independence in functional abilities
    d. Not demented
2. Etiology of MCI consistent with AD pathophysiological process
    a. Vascular, traumatic and medical causes of cognitive decline were ruled out
    b. Evidence of longitudinal decline in cognition (when feasible)
3. Biomarkers of Aβ deposition
    a. Low CSF Aβ42
        and/or
    b. Positive amyloid PiB-PET imaging
4. Biomarkers of neuronal injury (at least one present)
    a. High CSF t-tau or p-tau
        and/or
    b. Medial temporal atrophy by volumetric measures or visual rating
        and/or
    c. Temporoparietal hypometabolism by FDG-PET imaging

Both sources of amyloid status (CSF and PiB-PET) were considered interchangeable since a high agreement between Aβ42 concentrations in the CSF and amyloid PiB-PET scan results in MCI and Alzheimer's disease patients was confirmed by previous studies [102]. All procedures were performed according to the established protocols on participating centers [103, 105, 130-132]. The levels of Aβ42, t-tau, and p-tau were measured using commercially available enzyme-linked immunosorbent assays (INNOTEST® Aβ42, INNOTEST hTAU Ag and INNOTEST PHOSPHO-TAU (181P); Innogenetics, Ghent, Belgium). The expected site assay variability present in multicenter studies was acknowledged [104] and positivity was determined using locally available cut-off values. Amyloid PET scans used the Pittsburgh Compound B (11C-PIB) and were performed in the same

scanner (Philips PET/CT Gemini GXL), preceded by a low-dose brain computed tomography (CT) acquisition for attenuation correction (Institute of Nuclear Science Applied to Health, ICNAS, University of Coimbra). PiB-PET images were classified as amyloid positive or negative based on a support vector machines (SVM) local classifier, which uses the voxel wise brain grey matter standardized uptake value ratio (SUVR) and the cerebellar grey matter as reference region [131].

Conversion to dementia

At follow-up, the patients were classified as "non-converter" if the diagnosis persisted until last assessment or "converter" in the presence of a dementia diagnosis established according to the DSM-IV-TR criteria [9], in a consensus meeting with the team of neurologists and neuropsychologists that followed the patients.

Neuropsychological assessment

The baseline and follow-up comprehensive neuropsychological assessment was carried out by the same team of trained neuropsychologists, following a standard protocol and comprised the following instruments and scales:

-Mini-Mental State Examination (MMSE) [62,133] - the MMSE is a brief screening instrument to assess global cognitive performance. The Portuguese version was applied, and normative data was > 27 for more than 11 years of education and >22 for 11 or less years of education [133].
-Battery of Lisbon for the Assessment of Dementia (BLAD) [59,60] - the BLAD is a comprehensive neuropsychological battery that includes some tests from the Wechsler Memory Scale [67] and has been validated for the Portuguese population. This battery includes tests for the following cognitive domains: attention (Cancellation Task); verbal initiative (Semantic Fluency), motor and graphomotor initiatives; verbal comprehension (a modified version of the Token Test); verbal and non-verbal reasoning (Interpretation of Proverbs and the Raven's Coloured Progressive Matrices – Ab series); orientation (Personal, Spatial, and Temporal Orientation); visuo-constructional abilities (Cube Copy); planning and visuospatial/praxis abilities (Clock Draw); calculation (Basic Written Calculation); immediate memory (Digit Span Forward); visual memory (Visual Reproduction Test); working memory (Digit Span Backward); learning and verbal memory (Verbal Paired-Associate Learning, Logical Memory and Word Recall).

-California Verbal Learning Test (CVLT) [134,135] - the CVLT measures verbal learning and assesses constructs such as repetition learning, serial position effects, semantic organization, intrusion, and proactive interference. The word lists (List A and List B) are made up of 16 items from 4 different categories of "shopping list" items. The trial of interest (better discriminating ability for different stages of cognitive decline) [136] considered for the present study was the total number of words from List A correctly recalled on the first 5 learning trials (CVLT 5 Trials Total Recall).

-Trail Making Test (part A and part B) [106,107] - the TMT task measures sustained attention, visuomotor processing speed (part A), visuospatial working memory and cognitive flexibility (part B). The part A consists of 25 circles numbered 1 – 25 distributed over a sheet of paper and the patient should draw lines to connect the numbers in ascending order. In Part B there are 25 circles as well, but the circles include both numbers (1 – 13) and letters (A – M) and the patient has to draw lines to connect them all in an ascending pattern with the added task of alternating between the numbers and letters (i.e., 1-A-2-B-3-C, etc.).

-Geriatric Depression Rating Scale (GDS) [64-66] - the GDS is a self-report instrument used specifically to identify depressive symptomatology in the elderly. For this study a Portuguese version of a short form (15 items) was applied [64].

-Subjective Memory Complaints Scale (SMC) [137,138] - the SMC scale comprises 10 individual questions for the assessment of subjective memory complaints, with total scores ranging from 0 (absence of complaints) to 21 (maximal complaints score).

-Blessed Dementia Rating Scale (BDRS) [10,108] - the BDRS is a brief behavioral scale based on the interview of a close informant. This scale is composed of 22 items that address daily life activities, habits and changes in personality.

Statistical analysis

For baseline comparison of demographic and clinical data between groups the Student's *t* test and Pearson's χ^2 test were used, for numerical and nominal data, respectively. All tests were 2-tailed and a *p*-value<0.05 was assumed to be statistically significant. The neuropsychological assessments were standardized according to the age and education norms for the Portuguese population [59,60] and *z* scores were calculated. The comparison of neuropsychological results between the group that progressed to dementia during follow-up and the group that remained with MCI was conducted using Student's *t* test. To explore the effect of impairment in

neuropsychological tests on the time to conversion to dementia during follow-up, first the proportional hazards assumption for neuropsychological predictors was tested by adding time dependent covariates (interaction of predictors and a function of survival time) and then a Cox Proportional Hazards Regression model was conducted. The hazard or risk of conversion to dementia for the neuropsychological tests that were significantly different between converter and non-converter groups was computed. Time to event was calculated as the interval from the initial baseline evaluation to the diagnosis of dementia. For cases that remained non-demented, time was censored at the date of the last clinical/neuropsychological assessment. Kaplan-Meier curves analyzing the incidence of dementia according to the *z* scores of the lowest and the highest tercile were depicted. For comparison of curves, we opted for the Gehan-Breslow test since one group had a higher risk of conversion due to the significantly lower cognitive performance at baseline. Statistical analyses were performed using IBM SPSS Statistics 25 for Windows (2017 SPSS Inc., an IBM Company) package.

Results

One hundred and ten patients with MCI due to AD were enrolled. During the follow-up period (2.69±1.56 years for converters and 2.67±1.39 for non-converters), 63 patients (56%) progressed to dementia and 50 (44%) did not. Demographic and clinical data are reported in Table 1. The converters at the baseline assessment were younger than the non-converters, however, for mean follow-up time, education level, gender, depressive symptomatology, cognitive complaints and independence at daily activities, no statistically significant differences were found (Table 1).

Table 1. Baseline demographic and clinical characteristics of Non-converters and Converters.

	Non-converter *n*= 49	**Converter** *n*=61	***p*-value**
Age at first assessment, years, mean (SD)	70.1 (6.2)	65.4 (7.3)	**<0.001**[*]
Formal education, years, mean (SD)	10.7 (4.6)	10.2 (4.8)	0.591
Gender, female/male, *n*	28/22	35/27	1.000[#]
Follow-up time, years, mean (SD)	2.7 (1.4)	2.7 (1.6)	0.921
Time between onset of symptoms and first neuropsychological assessment, mean (SD)	2.4 (1.5)	2.2 (1.2)	0.576
Geriatric Depression Scale, mean (SD)	5.1 (3.4)	5.8 (4.5)	0.420
Subjective Memory Complaints Scale, mean (SD)	10.3 (4.6)	10.2 (4.1)	0.959
Blessed Dementia Rating Scale, mean (SD)	3.1 (1.9)	3.4 (2.0)	0.528
Mini-Mental State Examination, mean (SD)	26.4 (2.2)	25.6 (2.4)	0.084

Group comparisons were performed with parametric Student's *t* test (or χ^2 Pearson test when appropriate[#]);
[*]Statistically significant $p < 0.05$;
Abbreviations: SD – standard deviation.

The results of a comprehensive neuropsychological assessment showed the presence of impairment (*z* score<-1) in measures of attention and executive functions (Trail Making Test A and B), orientation, verbal learning and episodic memory (Word Recall; Logical Memory immediate recall; Logical Memory delayed recall; Verbal Paired-Associate Learning; California Verbal Learning Test 5 Trials Total Recall) for both groups. In a measure of language comprehension (Token Test) only the converters showed impairment. Moreover, converters scored significantly lower than non-converters at measures of verbal initiative (Semantic Fluency), non-verbal reasoning (Raven's Coloured Progressive Matrices) and episodic memory (Logical Memory immediate recall). Noteworthy, a trend towards statistical significance was found for the delayed recall condition of the Logical Memory test with converters scoring lower than non-converters at baseline assessment (Table 2).

Table 2. Baseline neuropsychological performances of Non-converters and Converters.

Cognitive domain Neuropsychological Test	Non-converter (*n*=49)	Converter (*n*=61)	*p*-value	Cohen's d
Attention and Executive Functions				
Cancellation Task	0.26 (1.17)	0.04 (1.37)	0.406	0.14
Digit Span Backward	0.06 (0.90)	-0.09 (1.20)	0.488	0.12
Clock Draw	0.05 (1.49)	-0.37 (1.53)	0.216	0.28
Trail Making Test A	-1.31 (1.70)#	-1.36 (1.85)#	0.896	0.02
Trail Making Test B	-1.97 (1.84)#	-1.63 (1.79)#	0.413	-0.18
Initiative				
Semantic Fluency	-0.07 (1.33)	-0.86 (1.48)	**0.004***	0.54
Motor Initiative	-0.27 (1.80)	-0.70 (1.90)	0.238	0.23
Graphomotor Initiative	0.05 (0.76)	-0.13 (1.00)	0.319	0.21
Reasoning				
Raven´s Coloured Progressive Matrices	0.05 (1.06)	-0.60 (1.43)	**0.009***	0.48
Interpretation of Proverbs	0.73 (1.23)	0.34 (1.82)	0.211	0.21
Orientation				
Personal, Spatial and Temporal Orientation	-2.32 (2.45)#	-2.23 (2.35)#	0.846	-0.04
Calculation				
Basic Written Calculation	-0.47 (1.00)	-0.59 (1.17)	0.582	0.08
Visuo-constructional abilities				
Cube Copy	1.54 (1.95)	1.33 (2.37)	0.656	0.11
Language				
Token Test	-0.59 (1.11)	-1.17 (1.83)#	0.113	0.36
Memory and Learning				
Visual Reproduction	1.45 (1.30)	0.58 (0.99)	0.150	0.77
Digit Span Forward	0.55 (1.30)	0.42 (1.34)	0.622	0.08
Word Recall	-1.25 (1.44)#	-1.77 (1.57)#	0.093	0.35
Logical Memory (immediate recall)	-1.17 (1.13)#	-1.92 (1.53)#	**0.005***	0.53
Logical Memory (delayed recall)	-1.99 (1.40)#	-2.64 (0.93)#	0.056	0.53
Forgetting Index (1)	-1.23 (2.38)#	-1.79 (2.78)#	0.266	0.26
Verbal Paired-Associate Learning	-1.18 (1.20)#	-1.58 (1.54)#	0.139	0.25
CVLT 5 Trials Total Recall	-3.14 (1.36)#	-3.69 (0.95)#	0.077	0.42

Means of *z* scores calculated according to the equation [*z* = (*x*-mean)/SD];
Group comparisons were performed with independent samples Student's *t* test
*Statistically significant $p < 0.05$
#Presence of impairment (*z* score <-1)
(1)Forgetting Index = [(LM delayed recall –LM immediate)/LM immediate)]*100
Abbreviations: CVLT – California Verbal Learning Test

A multivariate Cox proportional hazards regression model was applied to identify the independent predictors associated with time to conversion. The proportional hazards assumption was tested for each predictor (Age: Hazard Ratio [HR]=1.020, CI: 0.990-1.052, *p*=0.192; Semantic Fluency: HR=0.965, CI: 0.804-1.159, *p*=0.704; Logical Memory (immediate recall): HR=0.981, CI: 0.834-1.155, *p*=0.821; Raven´s Coloured Progressive Matrices: HR=1.217, CI: 1.005-1.475, *p*=0.045). Only the clinical and neuropsychological measures that differentiate the groups were included as

predictors. In the first model only the clinical predictor (age) by the method enter was included. Age at baseline was not associated with time to event (conversion to dementia). Neuropsychological predictors were subsequently subjected to multivariate Cox proportional hazards regression analysis (Table 3). The Semantic Fluency was added to the model and was a significant predictor (HR=0.762, CI: 0.634-0.916, p=0.004), whereas the Logical Memory (immediate recall) in the presence of Semantic Fluency did not reach significance as predictor (HR=0.852, CI: 0.704-1.031, p=0.099) (Table 3). However, the Logical Memory (immediate recall) was a significant predictor if entered first in the model (data not shown in Table 3; HR=0.797, CI: 0.663-0.957, p=0.015). When the Raven´s Coloured Progressive Matrices was added to the model the other predictors lost their significance (Semantic Fluency: HR=0.835, CI: 0.691-1.009, p=0.062; Logical Memory (immediate recall): HR=0.898, CI: 0.738-1.092, p=0.281). In the final model only the Raven´s Coloured Progressive Matrices, a test of non-verbal reasoning, remained significant as a predictor of time to conversion to dementia (HR=0.712, CI: 0.566-0.894, p=0.004). A decrease of one unit (z score) in Raven´s Coloured Progressive Matrices was associated with a 29% increase in the risk of conversion to dementia (Table 3).

For the Kaplan-Meier curves the comparison was between the highest and the lowest terciles of the Raven´s Coloured Progressive Matrices scores to assess the differences in time to conversion to dementia. Because at baseline both groups showed normative results, the presentation of Kaplan-Meier curves comprised the lowest and the highest terciles, instead of impaired and unimpaired z scores, to offer a more balanced sample size curves (Figure 2).

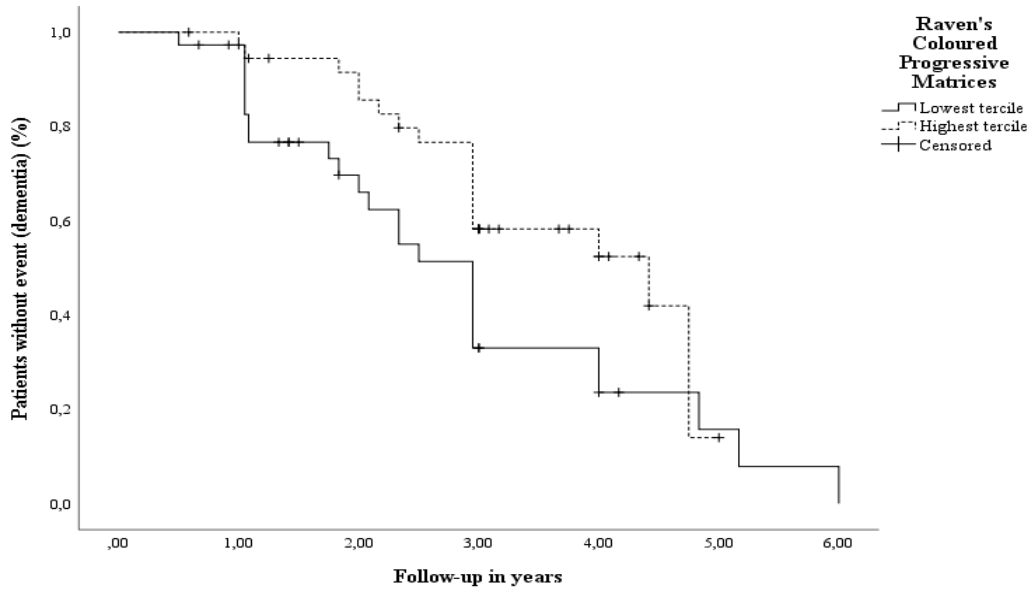
According to the Kaplan-Meier curves, for z scores in the lowest tercile (z score range: -2.88 to -0.96) after 3 years of follow-up approximately 50% of patients had converted to dementia, whereas for the highest tercile (z score range: 0.59 to 1.82) the conversion of approximately 50% of patients occurred later, after 4 years of follow-up. Accordingly, a significant difference between Kaplan-Meier curves was found ($\chi^2(1) = 6.131$; p=0.013).

Table 3. Multivariate Cox proportional-hazard regression models for predictors of conversion to dementia.

Predictors (n= 110; event/conversion to dementia=61; censured=49)	HR	95%CI	*p*-value
Multivariate analysis			
Model 1 – demographic variable (enter method)			
Age (mean, years)	0.984	0.950-1.019	0.376
Model 2 – cognitive predictors (enter method)			
Semantic Fluency (mean, *z* score)	0.762	0.634-0.916	**0.004***
Model 3 – cognitive predictors (enter method)			
Semantic Fluency (mean, *z* score)	0.804	0.664-0.974	**0.026***
Logical Memory (immediate recall) (mean, *z* score)	0.852	0.704-1.031	0.099
Model 4 – cognitive predictors (enter method)			
Semantic Fluency (mean, *z* score)	0.835	0.691-1.009	0.062
Logical Memory (immediate recall) (mean, *z* score)	0.898	0.738-1.092	0.281
Raven's Coloured Progressive Matrices (mean, *z* score)	0.712	0.566-0.894	**0.004***

Abbreviations: CI – Confidence Interval, HR – Hazard Ratio; *Statistically significant ($p < 0.05$).

Figure 2. Kaplan-Meier analysis of the incidence of dementia among patients in the lowest and in the highest tercile of the z scores



Number at risk						
	1 year	2 years	3 years	4 years	5 years	6 years
Lowest tercile (n=37)	33	18	8	6	1	0
Highest tercile (n=37)	35	30	18	9	0	0

Discussion

Patients with MCI due to AD that converted to dementia during the follow-up period were more impaired at the baseline in neuropsychological tests assessing verbal fluency, non-verbal reasoning and episodic memory, as compared to non-converters. An interesting result is that only non-verbal reasoning, assessed through Raven´s Coloured Progressive Matrices, remained significant as a predictor of time to conversion to dementia in a multivariate model.

Several studies have previously evidenced the predictive value of neuropsychological measures to assess time to conversion to dementia in MCI patients with unknown biomarker status [126,139-141]. Noteworthy, some studies highlighted that not only episodic memory performance but also other cognitive areas, namely executive functions and language tests are associated with a higher likelihood of progression from MCI to dementia during follow-up [74,119,125,142,143]. Thus, it would be plausible to expect a similar contribution of neuropsychological testing for patients with MCI due to AD.

According to our results, cognitive areas associated with reasoning and fluid intelligence, that reveal little decline until more advanced phases of Alzheimer's disease, as can be seen in the normative results of our MCI patients, can contribute significantly to predict time to conversion. As previously mentioned, only non-verbal reasoning, assessed through Raven´s Coloured Progressive Matrices, remained significant as a predictor of time to conversion to dementia in a multivariate model. For each standard deviation reduction in the *z* score of Raven´s Coloured Progressive Matrices score the risk of conversion to dementia increased approximately 30%. This test is a measure of fluid intelligence that demands several abilities as visual-perceptual, process integration, logical reasoning and cognitive flexibility [144]. The contribution of the Raven´s Coloured Progressive Matrices to predict time to conversion to dementia has been, to the best of our knowledge, largely neglected in the literature. Fluid intelligence has been addressed as a proxy of cognitive reserve [82]. In AD patients a higher cognitive reserve was associated with slower clinical progression in predementia stages, but after the onset of dementia it appears to have the opposite effect and accelerate the cognitive decline [145]. Interestingly, in the first study of the present thesis, in aMCI patients without amyloid status information, an association of performance in RCPM with long-term (10 years) diagnostic stability was also found [91]. Likewise, a large community-based study with non-demented subjects, the Framingham cohort prospective

study, showed that a test of abstract reasoning was a strong predictor of long-term (22 years) conversion to dementia [81]. In the present study, the Raven´s Coloured Progressive Matrices test was found to be the stronger predictor of conversion to dementia at a shorter (3 years) term in patients with MCI due to AD.

As foreseeable most of MCI due to AD patients converted during the follow-up period. Remarkably patients that converted to dementia during follow-up were younger at baseline than patients that did not convert, no differences being found in duration of symptoms, presence of depressive symptoms and years of formal education. This result seems to be in contradiction to longitudinal studies of conversion from MCI to AD that commonly report higher risk of conversion to dementia for the older patients [146,147]. However, the influence of age in cognitive decline for AD patients is not straightforward and some studies have revealed that AD patients starting the symptoms earlier had a less benign course with higher rate of cognitive decline [148]. Notwithstanding the difference at baseline, age was not a significant predictor of time to conversion.

The present study has some limitations that might be addressed in future studies. Obtaining a longer follow-up would be important. Replication of the present findings in other studies recruiting patients at a similar clinical stage would be needed. The genotyping of Apolipoprotein E (APOE) ε4 is not recommended in a clinical context [149] and for that reason was not available, and this is a limitation of the present study. Patients did not undergo all neuronal injury biomarkers, so it was not possible to assess their predictive value on time to future conversion to dementia. Not all patients with MCI undergo the diagnostic procedures with biomarkers, which are costly and invasive, thus the patients diagnosed with MCI due to AD are not representative of the AD population in a memory clinic.

The major strengths of the present study are the sample high likelihood of having Alzheimer's disease neurodegeneration according to the diagnostic criteria and the minor loss to follow-up of the cohort. As future perspectives, predicting conversion of MCI due to AD to dementia might be improved by machine learning techniques, namely by a feature selection ensemble approach to automatically choose the best neuropsychological predictors of future conversion, as was already done for MCI patients without amyloid status information [150]. Anticipating a precision medicine approach, it would important to refine risk models that can provide reliable prognostic information to the individual patient with MCI due to AD [151].

It has been an extraordinary recent advance being able to diagnose AD at an early clinical stage. Still, after being diagnosed with MCI due to AD, patients and families need to make important life decisions and future planning, and expectedly wish to get a reliable estimation of the disease progression. To the best of our knowledge, the present study is the first to explore the differential contribution of routine neuropsychological tests to predict time to conversion to dementia among patients diagnosed with MCI due to AD. Neuropsychological tests, namely assessing verbal fluency, episodic memory, and particularly non-verbal reasoning assessed with the Raven´s Coloured Progressive Matrices, may contribute to predict stability or conversion to dementia at a clinically meaningful time window.

Discussion and Final Conclusions

Investigation on Alzheimer´s disease has so far made extraordinary advances. In the last quarter of century, much effort has been devoted to the early diagnosis of cognitive disorders with the aim of identifying signs and symptoms that could be used as reliable predictive markers of disease development. Such identification would allow research to ascertain whether and which interventions at the early stages could change the natural history of the disorder. Mild Cognitive Impairment has been proposed to capture the intermediate phase between healthy ageing with slight cognitive changes and dementia [55,114,152,153]. MCI was introduced as a clinical entity more than 25 years ago, and since then, groups of individuals with this diagnosis have been intensively investigated from clinical, neuropsychological, imaging, genetic, pathological and epidemiological perspectives [154].

Not all those who experience cognitive decline, especially in advanced ages, will develop dementia, and some classified as having MCI will not even progress to clinically defined dementia. Rates of progression from MCI to dementia are consistently lower in community settings than in specialty clinical and research programs where individuals with MCI seek services, despite using the same criteria. Notably, all population-based studies find substantial proportions of individuals with variously defined MCI remaining stable or even reverting to normal during follow-up [155].

Several studies have shown that the characterisation of subjects with MCI and their outcome can be influenced by the setting in which the criteria are applied. For example, subjects attending a dementia clinic are likely to have significant cognitive impairment at the time of assessment. That is, the likelihood that they will be cognitively impaired with either MCI or dementia is much higher than if subjects from the community were assessed in an epidemiological study. As such, MCI prevalence is much higher in referral clinics than in the general population, and the rates of progression to greater degrees of cognitive impairment or dementia are also much higher. Positive predictive value is strongly influenced by this prevalence. In general, the progression rate to dementia in many referral clinics is in the range of 10–15% per year, whereas progression rate in the general population, prospectively sampled in epidemiological studies, tends to be around 5–10% per year. In the clinic setting, the subset of individuals with MCI is more likely to be on the AD pathophysiological spectrum, whereas in the community setting, MCI due to any etiology is more likely to be found. Causes other than neurodegeneration include depression, anxiety, drug use,

medical comorbidities and other treatable conditions. In other words, it seems that the MCI construct identifies different clinical syndromes, depending on the populations to which the criteria are applied. At the community level, a larger spectrum, probably of the same clinical syndrome, is captured by MCI criteria and this obviously will affect prognostic outcomes. Furthermore, in the specialized clinical setting, it is likely that the reference bias of MCI subjects is driven not only by severity of the symptoms, but also by other factors such as presence of other dementia cases in the family, educational level of the subjects and presence of serious comorbidities [33].

Neuropathological population-based studies have long shown the development of considerable pathology in individuals who do not express clinical dementia, and these findings are being suggested by emerging biomarker studies [33].

Postmortem studies indicate that past 80 years of age, presence of Aβ plaques (and neurofibrillary tangles) has less value in the discrimination between clinical dementia and nondementia cases. In a cohort of oldest old, Aβ deposition was not associated with the incidence of dementia during a 2 years'study among the highest-risk participants with MCI (although it was associated with incidence of dementia among all participants). However, more sensitive cognitive outcomes reported for a 12-year follow-up indicate that, despite frequent occurrence of Aβ deposition at this age (56.0%), Aβ is associated with long-term cognitive decline compared with its absence. The same finding was observed for the frequent occurrence of hippocampal volume reduction (57.7%) *vs.* its absence, although cognitive decline was restricted to visual memory. Both biomarkers appear to have measurable cognitive consequences and are hallmarks of decline, even among the oldest old. Although this study does not refute the notion of a diminished role of Aβ with advanced age, results support the hypothesis that Aβ remains functionally consequential in advanced aging and thus remains an important, if not sufficient, pathophysiologic process [156].

Nowadays, it is possible to detect the biological fingerprints of AD *in vivo*. We have at our disposal biomarkers of the disease like CSF AD biomarkers, amyloid PET imaging and evidence of hippocampal atrophy on MRI. These biomarkers, reflecting both amyloid deposition and neuronal injury, have been incorporated into diagnostic criteria, like those proposed by the NIA-AA for AD dementia [39], mild cognitive impairment (MCI) [38] or preclinical states [37].

Cerebrospinal fluid biomarkers for AD are Aβ42, which is found in low concentrations in AD, probably reflecting brain amyloid deposition, t-tau, detected at high concentrations representing cortical neuronal loss, and phosphorylated tau, also present in high concentrations, reflecting cortical tangle formation [157]. These markers have shown high diagnostic accuracy for established AD [158], and they may also be used to identify AD before onset of dementia at the stage of MCI, as shown in both single-centre studies [159,160] and large-scale heterogeneous multicentre studies [104,161,162]. The NIA-AA guidelines for MCI due to AD propose categorizing MCI according to the individual likelihood of underlying AD pathophysiology, according to their biomarker profile [38]. In these guidelines, the highest likelihood category is characterised by biomarker findings pointing to the presence of AD pathophysiology, whereas the lowest likelihood category is characterised by findings not typical for AD. This categorization also includes subgroups of conflicting biomarker results, namely patients with biomarkers positive for amyloidosis but negative for neurodegeneration and patients with normal amyloid markers but positive for neurodegeneration. It is consensual that the risk of progression to AD is higher in patients with all biomarkers positive for AD and lowest in patients with no positive biomarkers for AD. However, the biological significance and the prognosis of patients who fall into conflicting biomarker categories are still controversial [163].

The starting point for the studies presented here was precisely this: how can we estimate prognosis in MCI patients who do not have biomarkers, in those whose biomarkers are conflicting and even in those who have clear biomarkers indicative of Alzheimer´s disease? Neuropsychology could be useful in all these groups of patients.

Personalised diagnosis is desirable to help the clinician interpret biomarker findings in individual patients with MCI. Practical models could support clinical decision making and facilitate application of magnetic resonance imaging and cerebrospinal fluid biomarkers in daily practice. Van Maurik *et al*. (2017) designed a cohort modelling study with the goal of constructing biomarker-based prognostic models (cerebrospinal fluid model, magnetic resonance imaging model, and a combined model) that could be applied in individual patients with MCI, taking into account patient characteristics (age, sex, and Mini-Mental State Examination score). The resulting models showed particularly high negative predictive values, and external validation showed the models to be highly robust [164]. A model including more detailed neuropsychological parameters, namely the ones used in our studies, would certainly deliver more precise results.

Although the construct of aMCI represents a great advance in the early diagnosis of Alzheimer´s disease, it contains a great deal of uncertainty. MCI, namely aMCI, is a concept which has flaws. We believe one of our contributions was to demonstrate that MCI classification even using meticulous and comprehensive neuropsychological evaluation is not satisfactory:

1) There is a group of patients with MCI who do not convert. We have proved that it can persist for ten years, not evolving into dementia during this period. Therefore, MCI is not always a prodrome of dementia. In study 1, it was shown that not only do these patients maintain the diagnosis of aMCI, but also their performance in the several neuropsychological tests during a decade remains globally similar.

2) There are no clear-cut differences between amyloid positive and negative MCI, so we do need biomarkers. Concerning the concept of aMCI corresponding specifically to the first clinical stage of Alzheimer´s dementia, it seems inaccurate to the extent that amyloid negative patients in study 2 met criteria for aMCI. In this line, a study has recently shown that subjective memory complaints (SMC) profile is also very similar between amyloid positive and negative patients. Evaluating SMC does not seem helpful to identify, among patients with aMCI, those who have AD [165].

3) Nonetheless, among amyloid positive MCI patients, neuropsychological evaluation revealed to be very informative regarding estimation of time to conversion to dementia. Study 3 showed that neuropsychology is able to give important prognostic information about aMCI patients, beyond the data provided by amyloid biomarkers alone.

Addressing neuropsychology in particular, study 1 and 3 demonstrated that cognitive areas associated with reasoning and fluid intelligence, which reveal little decline until more advanced phases of Alzheimer's disease, can contribute significantly to estimation of prognosis of MCI.

In aMCI patients without amyloid status information, an association of performance in Raven´s Coloured Progressive Matrices with long-term (10 years) diagnostic stability was found. The better the subjects scored, the higher the probability was of remaining stable in the long term (10 years). In study 3, the Raven´s Coloured Progressive Matrices test was found to be the stronger predictor of conversion to dementia at a short (3 years) term in patients with MCI due to AD. The worse the patients scored, higher was the probability of conversion in the short term.

Raven´s Coloured Progressive Matrices is a test of non-verbal reasoning. It measures fluid intelligence, which demands several abilities, including visual-perceptual, process integration, logical reasoning and cognitive flexibility. It seems to us that the contribution of the Raven´s Coloured Progressive Matrices to inform about prognosis of aMCI has been overlooked. Fluid intelligence has been addressed as a proxy of cognitive reserve [82], and, considering this assumption, it makes sense that individuals with more cognitive reserve sustain neuronal destruction during a larger period than those who have less, therefore maintaining MCI status and not converting to dementia during longer terms.

Replication of our findings is warranted. Nevertheless, we believe to have contributed to the advance of knowledge on MCI and its prognosis. Our results suggest that there are different subgroups inside the category of aMCI, with meaningful prognostic implications. Estimates of the time window until conversion to dementia, for instance, are useful for the future plans of patients and family. It allows individuals to adjust expectations regarding their future. Our studies are also important since there is the possibility that some non-pharmacological interventions can delay conversion to dementia, and those treatments may have differential impact depending on the neuropsychological profile of the aMCI subject, mainly concerning non-memory domains. Lastly, our data are important in the recruitment of MCI patients for clinical trials. For instance, it may be pertinent to distinguish among aMCI groups with and without significant impairment on tests of non-verbal reasoning as their trajectory seems to be distinct.

Even though our research was based in a cohort study, it has very strong naturalistic features, close to reality in memory clinics worldwide. Also, the neuropsychological tests which were used have been validated for the Portuguese population and have been applied by a group of experient neuropsychologists in a standardized approach. As drawbacks, we can list the absence of evaluations at regular pre-determined intervals for every individual and the fact that patients were recruited from tertiary centres and, as such, do not represent aMCI in general.

Plans for the future

We have been able to answer some questions, but, as to be expected, many more have arisen from our results. New avenues of research have been opened. Advances, albeit small, always lead to new challenges.

We intend to increase the predictive value of our model. Would it add to its diagnostic and prognostic precision the inclusion of tau biomarkers, apart from the neuropsychological evaluation and the amyloid biomarkers?

In many recent studies, most of the progression to dementia was predicted by the amyloid plus neuronal injury or neuronal injury alone. Correlative clinicopathological research has revealed the existence of multiple pathologies that contribute to determining cognitive function during the life course [166-168]. It is therefore conceivable to expect that a combination of different biomarkers performs better in terms of diagnostic classification accuracy at the group but also at the individual level when interpreted in the context of presenting clinical symptoms [33,169].

We would like to construct a prognostic model similar to the one proposed by van Maurik *et al.* (2017) [164], providing a framework for a precision medicine approach by allowing personalised identification of clinical progression in patients with MCI using an equation based on patient characteristics, namely neuropsychological performances, and continuous biomarker values.

It is known that even amyloid negative MCI patients convert to dementia. We plan to study those amyloid negative patients in order to determine the underlying pathology in these cases.

We also envisage reviewing the cases who were stable for a decade, studying biomarkers in them and looking for protective factors, namely lifestyle elements.

We are also looking forward to study the extraordinary possibility that long-term stability might happen in a few cases of aMCI amyloid positive patients.

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Appendixes

Facsimiles of the published articles

-Neuropsychological predictors of long-term (10 years) MCI stability

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Neuropsychological Predictors of Long-Term (10 Years) Mild Cognitive Impairment Stability

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Abstract.
Background: Although the diagnosis of mild cognitive impairment (MCI) corresponds to a condition likely to progress to dementia, essentially Alzheimer's disease, longitudinal studies have shown that some patients may not convert to dementia and maintain the diagnosis of MCI even after many years.
Objectives: To determine whether patients that maintain the diagnosis of MCI in the long term (10 years) are really stable or just declining slowly, and to identify clinical and neuropsychological characteristics associated with long-term stability.
Methods: The Cognitive Complaints Cohort (CCC) was searched for MCI cases who maintained that diagnosis for at least 10 years. For each long-term-stable MCI patient, two MCI patients that converted to dementia during follow-up, matched for age and education, were selected from the same database. The baseline and last neuropsychological evaluations for long-term-stable MCI and converter MCI were compared. Baseline neuropsychological predictors of long-term stability were searched for.
Results: Long-term-stable MCI ($n = 22$) and converter MCI ($n = 44$) patients did not differ in terms of gender distribution, education, age at first assessment and time between symptom onset and first evaluation. Time of follow-up was on average 11 years for long-term-stable MCI and 3 years for converter MCI. The baseline and follow-up neuropsychological tests were not significantly different in long-term-stable MCI patients, whereas a general decline was observed in converter MCI patients. Higher scores on one memory test, the Word Delayed Total Recall, and on the non-verbal abstraction test, Raven's Progressive Matrices, at the baseline predicted long-term (10 years) clinical stability.
Conclusions: Some patients with MCI remain clinically and neuropsychologically stable for a decade. Better performances at baseline in memory and non-verbal abstraction tests predict long-term stability.

Keywords: Amnestic, follow-up, long-term, mild cognitive impairment, neuropsychological tests, prediction, stability

INTRODUCTION

Although the term "mild cognitive decline" had been previously used in the literature [1], this diagnosis became widely disseminated after a set of studies by a group of investigators at the Mayo Clinic in 1999. Petersen et al. defined mild cognitive impairment (MCI) as a condition characterized by subjective memory complaints, objective memory deficit, normal general cognitive performance, and maintained activities of daily living [2]. From the beginning, the concept of MCI assumed a continuum

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highest relative predictive power for the development of dementia. Interestingly, the DSM-5 considers a rather broad range to establish the cognitive deficit in mild neurocognitive disorder, namely 1–2 standard deviations below age- and education-adjusted norms [15].

3) normal general cognitive function, determined by the Mini-Mental State Examination (MMSE) [16] (see below) within normal values for the Portuguese population [13].
4) no or a minimal impairment in activities of daily living, determined by the Instrumental Activities of Daily Living Scale (IADL) [17] (see below)—that is to say, no more than one item from the IADL scale was altered.

Exclusion criteria

1) Presence of neurological (stroke, brain tumor, significant head trauma, epilepsy) or psychiatric disorders that may induce cognitive deficits; patients with major depression according to DSM-IV-TR [18] or serious depressive symptoms, indicated by a score >10 in Geriatric Depression Scale short version (GDS_{15}) [19–21] (see below);
2) Presence of systemic illness with cerebral impact (hypertension, metabolic, endocrine, toxic, and infectious diseases);
3) History of alcohol abuse or recurrent substance abuse or dependence;
4) Medication use with possible cognitive side effects;
5) Seriously reduced vision or other sensory deficits likely to interfere with assessment;
6) Presence of dementia according to DSM-IV-TR [18]

The diagnosis of MCI was made by an experienced neurologist (AdeM), after multidisciplinary consensus using all available information.

Long-term-stable MCI

Patients with long-term-stable MCI must fulfil the criteria for MCI during at least 10 years. The database of the CCC was thoroughly searched for these patients.

Converter MCI

For each long-term-stable MCI patient, the first two MCI patients that converted to dementia during follow-up, matched for age and education, were selected in the CCC database, and considered converter MCI.

Neuropsychological assessment

The comprehensive neuropsychological assessment at each CCC visit is carried out by the same team of trained neuropsychologists, following a standard protocol and including several instruments:

1) MMSE [16]. The Portuguese version of the test adapted from Guerreiro et al. [13] was used. Normal values for the Portuguese population are >27 for more than 11 years of education and >22 for 11 or less years of education [13].
2) BLAD [12, 13]: The BLAD is a comprehensive neuropsychological battery, including tests from the Wechsler Memory Scale (WMS) [22], that evaluates multiple cognitive domains and has been validated for the Portuguese population. For the present study, the following tests were considered:

a) Memory and Learning: Digit Span Forward from WMS, Word Delayed Total Recall (delayed recall of 5 non-related words), Logical Memory (immediate and delayed free recall) from WMS, Verbal Paired Associate Learning from WMS (difficult verbal pairs);
b) Attention and Executive Functions: Cancellation task (cross out 16 letters "A" from a set of 100 letters), Digit Span Backward from WMS, Clock Draw (free drawing of a clock), Verbal Semantic Fluency (supermarket food items);
c) Abstract Thought: Raven's Colored Progressive Matrices (Ab series), Interpretation of Proverbs (3 proverbs);
d) Orientation: Temporal Orientation (7 questions concerning temporal orientation);
e) Calculation: Basic Written Calculation (4 additions, 2 subtractions, 3 multiplications);
f) Visuo-Constructional Abilities: Cube Copy (drawing of a cube with perspective);

3) GDS [19–21]: For this study a short-form (15 items) of the self-report instrument, in the Portuguese version, adapted from Barreto et al. [19], was used. The presence of depression was defined as clinical history of depression or GDS_{15} >5 [23].
4) IADL [17]. The Portuguese version, done in the context of the LADIS project, was used [24].

Conversion to dementia

The diagnosis of dementia and AD was established according to the DSM-IV-TR [18] criteria, in a consensus meeting with the neurologist and the neuropsychologists.

Data analysis

Neuropsychological test raw scores of the baseline and last assessments of all long-term-stable MCI and matched converter MCI individuals were registered; *z* scores were also calculated according to the age and education norms for the Portuguese population with the equation [$z = (x\text{-mean})/SD$]. In the case of converter MCI, the visit in which the diagnosis of dementia was made was considered the last assessment.

Statistical analysis was performed using IBM SPSS Statistics 23 for Windows (2015 SPSS Inc., an IBM Company, Chicago, IL) package. Significance was set at $p < 0.05$.

Baseline demographic data were compared between groups using Student's *t* test for numerical variables and χ^2 Pearson test for categorical data.

The baseline and follow-up neuropsychological test scores for long-term-stable MCI and converter MCI were compared with One-Way ANOVA, followed by the *post hoc* Tukey's test. Binomial logistic regression was used to identify baseline neuropsychological predictors of long-term stability.

RESULTS

Twenty-two patients in the CCC met criteria for long-term-stable MCI, representing 3.4% of the 655 MCI patients having follow-up in the CCC. Forty-four converter MCI patients matched in terms of age and education at baseline were selected. As expected from the matching procedure, the two groups did not differ in terms of age and education. Gender distribution was also similar. They did not differ in terms of the presence of depression either. Time between symptom onset and baseline assessment was similar for both groups. Mean time to conversion to dementia was 3.5 ± 2.1 (median 3.0, range 1–9) years in converter MCI patients, whereas the mean time of follow-up in the long-term-stable MCI group was 11.1 ± 2.1 (median 11.0, range 10–14) years (Table 1).

Long-term-stable MCI patients maintained performances at the 10-year follow-up in all neuropsychological tests (there were no significant differences between baseline and follow-up scores) (Table 2).

On the contrary, converter MCI patients declined significantly between baseline and follow-up assessments in the domains of attention and executive functions, abstract thought, temporal orientation, calculation, and visuo-constructional abilities. They already scored low at the baseline and did not further decline significantly in the domain of memory and learning, except for the Word Delayed Total Recall test, in which a decay was noticed (Table 2).

Interestingly, at the baseline assessment, MCI patients that would remain stable for 10 years already performed consistently better than MCI patients deemed to convert to dementia in all the tests, the difference being statistically significant for Word Delayed Total Recall, Logical Memory – Immediate Free Recall and Raven's Progressive Matrices (Table 2).

In order to identify neuropsychological predictors of long-term stability, the neuropsychological tests that were different at the baseline between long-term-stable MCI and converter MCI patients, namely Word Delayed Total Recall, Logical Memory – Immediate Free Recall and Raven's Progressive Matrices, entered the binomial logistic regression model.

Table 1
Demographic and clinical characterization

	Long-term-stable MCI (n = 22)	Converter MCI (n = 44)	*p*-value
Gender, male/female, *n* (% female)	8/14 (63.6%)	16/28 (63.6%)	1.000[a]
Education, y, mean (SD)	9.5 (5.0)	9.1 (4.8)	0.780[b]
Age of first symptoms, y, mean (SD)	64.1 (8.3)	64.5 (7.5)	0.856[b]
Time between symptoms onset and first assessment, y, mean (SD)	1.4 (1.2)	1.9 (1.2)	0.326[b]
Age at first evaluation, y, mean (SD)	65.5 (8.3)	66.4 (7.7)	0.678[b]
Presence of depression[c], *n*(%)	7(31.8%)	14 (31.8%)	1.000[a]
Duration of follow-up, y, mean (SD)	11.1 (2.1)	3.5 (2.1)	<0.001[b]

[a] χ^2 Pearson test. [b] Independent samples Student's *t* test. [c] Presence of depression was defined as clinical history of depression or GDS ≥5.

Table 2
Neuropsychological tests in long-term-stable and converter MCI patients

Cognitive domain / Neuropsychological tests	Baseline		Follow-up	
	Long-term stable MCI: mean (SD) / z scores (SD)	Converter MCI: mean(SD) / z scores (SD)	Long-term stable MCI: mean (SD)	Converter MCI: mean (SD)
Memory and Learning				
Digit span forward	5.27(0.83)	4.77(0.61)	5.43(1.08)[#]	4.24(1.22)
	0.29(0.97)	−0.37 (0.75)		
Word delayed total recall	9.80(1.51)	8.05(2.26)[†]	9.16(2.36)[#]	5.86(2.85)[†]
	−0.70(0.83)	−1.54 (1.36)		
Logical memory (immediate free recall)	9.68(4.11)	6.07(3.97)[†]	9.73(4.36)[#]	3.85(3.12)
	−0.77(1.42)	−1.81 (1.32)		
Logical memory (delayed free recall)	6.80(4.84)	4.12(4.29)	9.14(4.92)[#]	1.48(1.96)
	−1.67(1.48)	−2.49 (1.06)		
Difficult Verbal Paired Associate Learning	3.67(2.78)	1.83(2.72)	4.35(3.20)[#]	1.16(2.08)
	−1.21(1.84)	−1.83 (1.53)		
Attention and executive functions				
Cancellation task-total	4.63(1.48)	3.86(1.49)	3.82(1.55)[#]	2.74(1.24)[†]
	0.16(1.15)	−0.25 (1.08)		
Digit span backward	3.68(0.89)	3.56(0.71)	3.76(0.89)[#]	2.34(1.29)[†]
	0.18(1.15)	−0.69 (0.97)		
Clock draw	2.70(0.47)	2.49(0.74)	2.65(0.59)[#]	1.59(1.05)[†]
	0.295(0.86)	−0.01 (1.56)		
Verbal semantic fluency	16.23(3.82)	14.45(4.36)	16.82(4.25)[#]	9.93(4.43)[†]
	0.00(1.12)	−0.49 (1.38)		
Abstract Thought				
Raven's Progressive Matrices	9.57(1.75)	7.31(2.33)[†]	9.40(2.01)[#]	5.33(2.48)[†]
	0.36(1.08)	−0.49 (1.36)		
Interpretation of Proverbs	7.00(1.54)	6.75(1.77)	6.90(1.73)[#]	4.46(2.19)[†]
	0.79(1.25)	0.52 (1.14)		
Orientation				
Temporal orientation	6.55(0.83)	6.02(1.12)	6.15(1.14)[#]	3.00(2.49)[†]
	−0.26(1.18)	−1.49 (2.01)		
Calculation				
Basic written calculation	13.41(1.00)	12.13(2.24)	11.74(2.85)[#]	9.46(4.27)[†]
	0.28(0.48)	−0.06 (1.24)		
Visuo-constructional abilities				
Cube copy	2.53(0.70)	1.93(1.08)	2.41(0.71)[#]	1.39(1.22)
	0.45(1.21)	−0.35 (1.78)		

Raw test scores are shown. In the baseline neuropsychological results, z scores (SD) values are shown below raw scores. Long-term-stable MCI, MCI patients that have maintained this diagnosis for 10 years or more. Converter MCI, MCI patients that converted to dementia during follow-up. [†]Converter MCI patients had worse scores than long-term-stable MCI patients at baseline, One-Way ANOVA, Tukey *post-hoc* test. [#]Follow-up tests were not significantly different from baseline in long-term-stable MCI patients, One-Way ANOVA, Tukey *post-hoc* test. [†]Follow-up tests worsened from baseline in converter MCI patients, One-Way ANOVA, Tukey *post-hoc* test.

Univariate logistic regression analysis revealed a significant association between high scores at baseline in all three tests and long-term MCI stability. In multivariate logistic regression analysis, only Word Delayed Total Recall and Raven's Progressive Matrices retained significance in the prediction of long-term stability. For each additional score point in the Word Delayed Total Recall, the odds were 1.7, that is the probability of long-term stability increased by 70%. Each additional point in the total score of Raven's Progressive Matrices increased the probability of long-term stability about 2 fold (Table 3).

DISCUSSION

The present work confirms that some patients with MCI maintain this diagnosis in the long term (10 years) and shows that they are able to maintain stable neuropsychological performance in all studied cognitive domains for an extended period.

Proportion of long-term-stable MCI patients

It should be noted that only a small proportion of patients with MCI (3.4% of MCI patients with follow-up in this cohort) maintain this diagnosis for

Table 3
Neuropsychological predictors of long-term MCI stability

Neuropsychological tests	B	SE	Wald	Significance	Exp(B)
Word Delayed Total Recall	0.555	0.236	5.516	0.019	1.743
Logical memory (immediate free recall)	0.091	0.108	0.718	0.397	1.096
Raven's Progressive Matrices	0.665	0.205	10.459	0.001	1.944

Multivariate binary logistic regression analysis.

a long period, presumably due to continual conversion to dementia [3] as well as persistent attrition of the cohort over the 10-year follow-up.

Neuropsychological stability

This study is the first, to our knowledge, to ascertain real neuropsychological stability in all the studied cognitive domains in stable MCI individuals over a decade of follow-up, that is, no statistically significant worsening was observed in any cognitive domain. In contrast, the converter MCI patients worsened in all neuropsychological tests, although this decline was not statistically significant in tests where they already had a low score at baseline, namely Digit Span Forward, Logical Memory-Free Immediate and Free Delayed Recall, Difficult Verbal Paired Associate Learning and Cube Copy tests, presumably due to a floor effect.

Demographic factors as predictors

Several factors influencing conversion of MCI to dementia have been recognized; namely older age [10] and lower level of education increase the risk of conversion ([25], but see [26]). These factors were *a priori* controlled for by the design of the study, matching long-term-stable with converter MCI patients by educational level and age at baseline evaluation. Time between symptom onset and baseline evaluation as well as distribution of gender were also similar between groups.

Neuropsychological tests as predictors

The neuropsychological performance at baseline, in particular, tests measuring memory, were shown to predict conversion of MCI to dementia in previous clinical studies with shorter follow-up durations. Amáiz et al. [4], in a clinical sample of 303 MCI patients, followed up for 3 years on average, found that tests assessing learning and retention, specifically Wechsler Memory Scale-Revised delayed recall, were the best predictors of conversion to AD. Sarazin et al. [27] observed, in a cohort of 251 patients with MCI, followed for up to 3 years, that the most sensitive and specific test for diagnosis of prodromal AD was the Free and Cued Selective Recall Reminding Test (FCSRT). Silva et al. [28] found that four commonly used verbal memory tests were able to predict conversion to dementia in 272 non-demented patients reporting subjective cognitive complaints followed up for 3 years on average, and that the California Verbal Learning Test had the highest predictive value, which was not improved by adding other memory tests. In a similar vein, Gómez-Tortosa et al. [29] followed up for 48 months on average a cohort of 210 cases with amnestic MCI. They were divided into two groups according to their initial recognition memory discrimination index (DI) on the Hopkins Verbal Learning Test, and conversion to dementia occurred significantly later in cases with higher DI. A multivariate regression model revealed DI and delayed recall as the strongest predictors of dementia. A recent systematic review [30], covering data for a total of 2,365 participants with MCI at entry, followed over an average of 31 months, found that Paragraph Delayed Recall, Word-list Free Delayed Recall with and without oriented encoding were tests with excellent overall accuracy for predicting progression to Alzheimer's type dementia. Another recent literature review [31] found that the majority of studies on the prediction of conversion from MCI to AD dementia report delayed recall as the most sensitive neuropsychological measure.

It is also clear that other tests, namely assessing attentional and executive capabilities, may also contribute as predictors of MCI conversion to dementia. For instance, in a work by Tabert et al. [32], with 148 MCI patients followed up for almost 4 years, the percent savings from immediate to delayed recall on the Selective Reminding Test and the Wechsler Adult Intelligence Scale–Revised Digit Symbol Test score were the strongest predictors of time to conversion. Also, in Fleisher et al. study [33], 539 participants with amnestic MCI were followed during 3 years and it was found that progression from MCI to AD dementia was best determined by com-

bining distinct cognitive measures, namely Delayed Paragraph Recall Test, Delayed 10-Word List Recall, Symbol Digit Modalities Test and the ADAS-cog total score. Along the same lines, Li et al. [34] studied 139 patients with amnestic MCI enrolled in the Alzheimer's Disease Neuroimaging Initiative and observed that, not only the baseline Memory composite (scores on the Logical Memory and Rey Auditory Verbal Learning tests), but also the executive function composite (scores on the Trail Making, Digit Symbol Substitution, and spontaneous Clock drawing tests) could predict progression to AD after 3 years.

In the present work, with 10 years of follow-up, although one verbal memory delayed measure at baseline was associated with long-term stability, we found that performance on the Raven's Progressive Matrices at the baseline was the stronger neuropsychological predictor of long-term clinical stability. Remarkably, each additional point in the total score of the Raven's Progressive Matrices increased the probability of long-term stability about 2 fold. This finding may assume clinical relevance, but must be replicated in other MCI cohorts.

Raven's Progressive Matrices [35] are considered a non-verbal reasoning measure of fluid-type intelligence [36]. So-called fluid intelligence tests are most predictive of a general ability to do well, calling for novel problem solving with simple visual or other kind of materials, reflecting current ability for abstract thought and reasoning. Interestingly, Elias et al. [37], in a prospective study in healthy community participants in the Framingham cohort, found poor abstract reasoning to be a strong predictor of conversion to dementia in the long run. Fluid intelligence has been considered a good proxy for cognitive reserve [38]. Cognitive reserve is associated with lower risks for incident dementia [39]. As mentioned above, in our study, education, a variable known to influence prevalence rates of dementia [40], was controlled for. However, other factors thought to contribute to cognitive reserve, such as occupation, premorbid IQ, and mental activities, were not specifically analyzed [39].

Presumed etiology of long-term-stable MCI

The question of whether these patients with long-term-stable MCI have AD pathology, that is, suffer from prodromal AD from the start, or not, is rather intriguing. Nowadays, the AD biomarkers are commonly used to detect AD pathology and diagnose prodromal AD or MCI due to AD (e.g., [41–44]). However, when these patients were recruited, at least 10 years ago, AD biomarkers were not routinely used in clinical practice.

It is known that there can be a very long interval, about 20 years, between first development of amyloid positivity and onset of dementia [45]. Clinical cohort studies suggest that there may be very subtle cognitive alterations that are detectable a decade or more before meeting criteria for MCI [46]. Thus, the AD pathophysiological process may course with a long preclinical stage [46]. Based on our findings, we could speculate that the disorder might also be quiescent for long periods at the MCI stage, at least in some patients. However, factors that might assume a neuroprotective role at this stage are still largely unknown [47].

Limitations and strengths

This cohort is constituted mainly by memory clinic patients, and the findings may not apply to clinical settings with different patient characteristics. The absence of data on Apolipoprotein E genotype is a limitation of the present study, since the ε4 allele is an important risk factor for AD [48].

The strengths of this study were that it was carried out in the context of a large cohort, in which the patients underwent comprehensive standardized neuropsychological assessments, and a long term 10-year follow-up was achieved.

Conclusion

We found that, in some MCI patients, real neuropsychological stability over a decade is possible and that long-term stability could be predicted on the basis of neuropsychological tests measuring memory and non-verbal abstract reasoning.

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-Neuropsychological profile of amyloid positive versus amyloid-negative amnestic MCI

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Neuropsychological profile of amyloid positive versus amyloid negative amnestic Mild Cognitive Impairment

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Introduction. Patients diagnosed with amnestic mild cognitive impairment (aMCI) are at high risk of progressing to dementia. It became possible, through the use of biomarkers, to diagnose those patients with aMCI who have Alzheimer's disease. However, it is presently unfeasible that all patients undergo biomarker testing. Since neuropsychological testing is required to make a formal diagnosis of aMCI, it would be interesting if it could be used to predict the amyloid status of patients with aMCI.

Methods. Participants with aMCI, known amyloid status (Aβ+ or Aβ−) and a comprehensive neuropsychological evaluation, were selected from the Cognitive Complaints Cohort database for this study. Neuropsychological tests were compared in Aβ+ and Aβ− aMCI patients. A binary logistic regression analysis was conducted to model the probability of being amyloid positive.

Results. Of the 216 aMCI patients studied, 117 were Aβ+ and 99 were Aβ−. Aβ+ aMCI patients performed worse on several memory tests, namely Word Total Recall, Logical Memory Immediate and Delayed Free Recall, and Verbal Paired Associate Learning, as well as on Trail Making Test B, an executive function test. In a binary logistic regression model, only Logical Memory Delayed Free Recall retained significance, so that for each additional score point in this test, the probability of being amyloid positive decreased by 30.6%. The resulting model correctly classified 64.6% of the aMCI cases regarding their amyloid status.

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Conclusions. The neuropsychological assessment remains an essential step to diagnose and characterize patients with aMCI; however, neuropsychological tests have limited value to distinguish the aMCI patients who have amyloid pathology from those who might suffer from other clinical conditions.

As a consequence of the ageing of the population, the number of people affected by neurodegenerative disorders, particularly Alzheimer disease (AD), is increasing dramatically worldwide (Prince, 2015). There has been a growing interest in detecting AD as soon as possible along its insidious evolution, before the establishment of the diagnosis of dementia. The correct identification of patients with memory complaints who already have an ongoing neurodegenerative process is desirable, since it offers patients the possibility to make important life decisions, anticipate future care, start symptomatic drugs, initiate cognitive rehabilitation therapy, and eventually participate in clinical trials with putative neuroprotective drugs (de Mendonça, 2012). About 2 decades ago, the Mayo Clinic group fostered an important advance by proposing the concept of amnestic mild cognitive impairment (aMCI), as a condition characterized by subjective memory complaints, objective memory deficit, normal general cognitive performance, and maintained activities of daily living (Petersen *et al.*, 1999). Patients diagnosed with aMCI in a clinical setting have about 10% annual progression rate of conversion to dementia, usually AD (Mitchell & Shiri-Feshki, 2009). However, aMCI can have other aetiologies (Hanfelt, Peng, Goldstein, & Lah, 2018), and some aMCI patients actually remain stable for as long as a decade (Alves *et al.*, 2018).

In recent years, the use of biomarkers has allowed the possibility of diagnosing AD *in vivo* in patients who present with aMCI. These biomarkers are surrogates of pathological alterations in the brain characteristic of AD (Jack *et al.*, 2018). The presence of amyloid pathology may be determined by measuring amyloid Aβ1–42 concentrations in the cerebrospinal fluid (CSF) and/or quantifying brain deposits of Aβ with amyloid positron emission tomography (PET) (Bocchetta *et al.*, 2015).

In spite of the remarkable advance that the development of biomarkers represents both from an investigational and a clinical perspective and the rapid acceptance of these methods by reference centres (Bocchetta *et al.*, 2015), the generalization of biomarker testing to other settings has been more sluggish. Several explanations might be advanced; for instance, lumbar puncture, used to obtain CSF, is an invasive procedure with contra-indications and side effects, and amyloid PET is quite expensive and not widely available. Bearing this in mind, it would be important to discover non-invasive and affordable methods that could discriminate between amyloid-positive (Aβ+) and amyloid-negative (Aβ−) aMCI patients.

Since neuropsychological testing is not invasive and is required to make a formal diagnosis of aMCI, it would be very interesting if it could be used to identify the amyloid status in patients with aMCI (Bahar-Fuchs *et al.*, 2013). In other words, Aβ+ aMCI patients might have a particular neuropsychological profile that would distinguish them from Aβ− aMCI patients. Several studies compared global cognition, attention, executive functions, visuospatial functions, language, visual memory, and verbal memory between Aβ+ and Aβ− aMCI patients.

Since patients with AD typically have deficits in episodic memory as a consequence of early and marked hippocampal neurodegeneration, it is not surprising that Aβ+ aMCI patients consistently presented more prominent episodic memory deficits than Aβ− aMCI patients in several different studies (Bahar-Fuchs *et al.*, 2013; Huijbers *et al.*,

2015; Kandel, Avants, Gee, Arnold, & Wolk, 2015; Kim *et al.*, 2018; Reijs *et al.*, 2017; Tomadesso *et al.*, 2018, 2019; Wolk *et al.*, 2009). However, regarding attention and executive functions, different studies produced less consistent results, possibly depending on the kind of test used to measure these abilities as well as the number of patients recruited. In the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort, Aβ+ aMCI patients took longer to complete the Trail Making Tests A and B, when compared to Aβ− aMCI patients (Kandel *et al.*, 2015). These results were not corroborated by other studies, that did not observe significant differences in the Trail Making Tests A and B between Aβ+ and Aβ− aMCI patients (Tomadesso *et al.*, 2018, 2019; Wolk *et al.*, 2009). Regarding another commonly used executive test, Verbal Semantic Fluency, Aβ+ aMCI patients had worse performance in one study (Kandel *et al.*, 2015) but not in other work (Wolk *et al.*, 2009).

We now reappraise neuropsychological testing in Aβ+ and Aβ− aMCI patients, particularly concerning performances on executive tests, as well as cognitive domains so far scarcely analysed, like abstract reasoning and calculation. Furthermore, we aim to test whether a statistical model involving different neuropsychological variables could be valuable to help identify the amyloid status of patients with aMCI.

Materials and methods

Participants

Participants belong to the Cognitive Complaints Cohort (CCC). The CCC was established in a prospective study to evaluate the cognitive evolution of patients with cognitive complaints and no dementia, based on a comprehensive neuropsychological evaluation and other biomarkers. Detailed information concerning CCC establishment was provided in a previous publication (Maroco *et al.*, 2011). The study was approved by the local ethics committee and conducted according to the declaration of Helsinki. Informed consent was obtained from patients before any procedure.

Inclusion criteria

1. Diagnosis of amnestic MCI (aMCI). The criteria for the diagnosis of aMCI were adapted from Petersen *et al.* (1999):
   a. Presence of memory complaints;
   b. Abnormal memory function, documented by impairment in the Logical Memory A test Immediate Free Recall score. Logical Memory is a subtest of the Bateria de Lisboa para Avaliação das Demências (BLAD) (Garcia, 1984; Guerreiro, 1998) (see below). For the memory function to be considered abnormal, we set the cut-off score of the Logical Memory A Immediate Free Recall at 1 *SD* below the age and education norms. Busse, Hensel, Gühne, Angermeyer, and Riedel-Heller (2006) observed, in the cohort of the Leipzig Longitudinal Study of the Aged, that the 'MCI modified, 1.0 *SD*' criteria had the highest relative predictive power for the development of dementia;
   c. Normal general cognitive function, determined by the Mini-Mental State Examination (MMSE) (Folstein, Folstein, & McHugh, 1975) (see below) within normal values for the Portuguese population (Guerreiro, 1998);
   d. No or a minimal impairment in activities of daily living, determined by the Instrumental Activities of Daily Living Scale (IADL) (Lawton & Brody, 1969)

(see below), that is to say, no more than one item from the IADL scale was altered.

2. Known amyloid status, determined by CSF Aβ1–42 measurement and/or cortical uptake of the Pittsburgh compound B (^{11}C-PiB) on the PET scan.

Exclusion criteria

1. Presence of neurological (stroke, brain tumour, significant head trauma, epilepsy) or psychiatric disorders that may induce cognitive deficits; patients with major depression according to DSM-IV-TR (APA, 2000) or serious depressive symptoms, indicated by a score >20 in Geriatric Depression Scale (GDS30) or >10 in Geriatric Depression Scale short version (GDS15) (Barreto, Leuschner, Santos, & Sobral, 2008; Yesavage *et al.*, 1982; Yesavage & Sheikh, 1986) (see below);
2. Presence of systemic illness with cerebral impact (uncontrolled hypertension, metabolic, endocrine, toxic, and infectious diseases);
3. History of alcohol abuse or recurrent substance abuse or dependence;
4. Medication use with possible cognitive side effects;
5. Seriously reduced vision or other sensory deficits likely to interfere with assessment;
6. Presence of dementia according to DSM-IV-TR (APA, 2000);
7. Interval between neuropsychological assessment and knowledge of amyloid status longer than 12 months.

The diagnosis of aMCI was made by an experienced neurologist, after multidisciplinary consensus using all available clinical, neuropsychological, and neuroimaging information available from the diagnostic workup.

Biomarker analysis

The amyloid biomarker status was based on CSF Aβ1–42 level and/or cortical uptake on ^{11}C-PiB PET, and the aMCI patients were classified as Aβ+ or Aβ−. Both sources of amyloid status were considered interchangeable since a high agreement between Aβ1–42 concentrations in the CSF and amyloid PET scan results in aMCI and AD disease patients was confirmed by previous studies (Leuzy *et al.*, 2016).

The levels of Aβ1–42 were measured using commercially available enzyme-linked immunosorbent assays (INNOTEST® β-amyloid (1–42); Innogenetics, Ghent, Belgium) according to the established protocols on participating centres (Teunissen, Tumani, Engelborghs, & Mollenhauer, 2014). The levels of Aβ1–40 and the ratio Aβ1–42 over Aβ1–40 were not determined routinely, only in exceptional cases where a discrepancy was found between CSF and PET scan amyloid results. The expected site assay variability present in multicentre studies was acknowledged (Mattsson *et al.*, 2009), and positivity was determined using locally available cut-off values.

The cortical uptake with ^{11}C-PiB PET was performed only in one centre using the same scanner (Philips PET/CT Gemini GXL), preceded by a low-dose brain computed tomography (CT) acquisition for attenuation correction (Institute of Nuclear Science Applied to Health, ICNAS, University of Coimbra). ^{11}C-PiB PET images were classified as amyloid positive or negative based on a support vector machines local classifier, which uses the voxelwise brain grey matter standardized uptake value ratio and the cerebellar grey matter as reference region (Oliveira *et al.*, 2018).

Neuropsychological assessment

The comprehensive neuropsychological assessment was carried out by the same team of trained neuropsychologists, following a standard protocol and comprised the following instruments and scales:

1. Mini-Mental State Examination (Folstein *et al.*, 1975; Guerreiro, 1998) – the MMSE is a brief screening instrument to assess global cognitive performance. The Portuguese version was applied, and normative data were >27 for individuals with more than 11 years of education and >22 for patients with 11 or less years of education (Guerreiro, 1998).
2. Battery of Lisbon for the Assessment of Dementia (Garcia, 1984; Guerreiro, 1998) – the BLAD is a comprehensive neuropsychological battery that includes some tests from the Wechsler Memory Scale (WMS; Wechsler, 1969) and has been validated for the Portuguese population. This battery includes tests for the following cognitive domains: immediate memory (Digit Span forward); verbal memory (Word Total Recall, a five words 1-min delayed recall test, in which the total score contemplates spontaneous and cued recall); logical memory (Logical Memory Immediate and Delayed Recall; for this test, the score is based on the combination of 7 literal elements and 17 meaningful elements); associate learning (Verbal Paired Associate Learning); general information (General Information, consisting of 20 questions on subjects of general knowledge); working memory (Digit Span backward); attention (Cancellation Task); verbal initiative (Verbal Semantic Fluency); verbal and non-verbal abstraction (Raven's Coloured Progressive Matrices – Ab series-B and Interpretation of Proverbs); and calculation (Basic Written Calculation);
3. Trail Making Test (part A and part B; Cavaco *et al.*, 2013; Reitan, 1958) – the TMT task measures sustained attention, visuomotor processing speed (part A), visuospatial working memory, and cognitive flexibility (part B). The part A consists of 25 circles numbered 1–25 distributed over a sheet of paper, and the patient should draw lines to connect the numbers in ascending order. In part B, there are 25 circles as well, but the circles include both numbers (1–13) and letters (A–M), and the patient has to draw lines to connect them all in an ascending pattern with the added task of alternating between the numbers and letters (i.e., 1-A-2-B-3-C).
4. Geriatric Depression Rating Scale (GDS; Barreto *et al.*, 2008; Sheikh *et al.* 1986; Yesavage *et al.*, 1982) – the GDS is a self-report instrument used specifically to identify depressive symptomatology in the elderly. For this study, the Portuguese versions of GDS30 and GDS15 were used (Barreto *et al.*, 2008).
5. Blessed Dementia Rating Scale is a clinical rating scale with 22 items that measures changes in performance of everyday activities (8 items), self-care habits (3 items), and changes in personality, interests, and drives (11 items). Ratings are based on information from relatives or friends and concern behaviour over the preceding 6 months.

For the present work, the neuropsychological assessment closest to the knowledge of the amyloid status was used.

Statistical analysis

For comparison of demographic and clinical data between groups, the independent samples two-tailed Student's *t*-test and the chi-squared Pearson test were used, for

numerical and nominal data, respectively. The neuropsychological assessments were standardized according to the age and education norms for the Portuguese population (Garcia, 1984; Guerreiro, 1998), and *z* scores were calculated with the equation [$z = (x - \text{mean})/SD$]. The comparison of neuropsychological results between Aβ+ and Aβ− groups was done with the independent samples two-tailed Student's *t*-test. To check whether the differences that were found between groups still held when controlling for the MMSE score, a general linear model analysis was performed considering the MMSE as a covariate. A binary logistic regression analysis was conducted to assess whether the neuropsychological tests scores could predict amyloid positivity. The tests that were significantly different between the groups entered the model. The Enter method (i.e., standard regression analysis) was used. Receiver operating characteristic (ROC) curves were obtained when appropriate. In order to control for an eventual redundancy in the tests comprising the neuropsychological battery, a principal component analysis using a rotated varimax component matrix was performed.

Statistical analyses were performed using IBM SPSS Statistics 25 for Windows (2017 SPSS Inc., an IBM Company) package. A probability value of $<.05$ was assumed to be statistically significant.

Results

A total of 216 patients with aMCI were enrolled from the CCC for the present study, of whom 117 were Aβ+ and 99 were Aβ−. The two groups did not differ in terms of gender, education, age of first symptoms, and time between symptoms onset and neuropsychological assessment. They did not differ in terms of the presence of depressive symptoms either. Regarding the Blessed Dementia Rating Scale scores, aMCI patients in the two groups had similar global levels of severity (Table 1).

Neuropsychological evaluation (Table 2) showed that Aβ+ aMCI patients had lower MMSE scores than Aβ− aMCI patients. MMSE values for Aβ+ aMCI patients were 26.8 (*SD* 2.2, skewness −0.3, range 23–30) and for Aβ− aMCI patients 27.6 (*SD* 2.0, skewness −0.7, range 23–30).

Aβ+ aMCI patients also performed worse on several memory tests, namely the Word Total Recall, Logical Memory Immediate and Delayed Free Recall, and Verbal Paired Associate Learning, as compared to Aβ− aMCI patients. To check whether the differences

Table 1. Demographic and clinical characterization

	Aβ+ aMCI (n = 117)	Aβ− aMCI (n = 99)	p value
Gender, male/female, *n* (% female)	53/64 (54.7%)	41/58 (58%)	.676[a]
Education, years, mean (SD)	10.6 (4.6)	9.8 (4.7)	.204[b]
Age of first symptoms, years, mean (SD)	64.0 (7.7)	61.8 (10.8)	.117[b]
Time between symptoms onset and neuropsychological assessment, years, mean (SD)	2.8 (2.5)	3.3 (2.9)	.163[b]
Presence of depressive symptoms [c],%	34.5%	42.3%	.317[a]
Blessed Dementia Rating Scale, mean (*SD*)	3.3 (2.0)	3.5 (2.0)	.439[b]

Note. [a]Chi-squared Pearson's test.; [b]Independent samples Student's *t*-test.; [c]Presence of depressive symptoms was considered when GDS_{15} score was higher than 5 points or when GDS_{30} score was higher than 10 points.

Table 2. Neuropsychological tests in Aβ+ and Aβ− aMCI patients (n = 216)

Cognitive domain Neuropsychological tests	Aβ+ aMCI (n = 117) Mean (SD)	Aβ− aMCI (n = 99) Mean (SD)	p value
Global cognition			
Mini-Mental State Examination	26.8 (2.2)	27.6 (2.0)	**.004**
Memory and learning			
Digit Span Forward, z score	0.51 (1.27)	0.24 (1.19)	.122
Word Total Recall, z score	−1.70 (1.53)	−0.96 (1.27)	**<.001**
Logical Memory Immediate Free Recall, z score	−1.49 (1.73)	−0.89 (1.25)	**.005**
Logical Memory Delayed Free Recall, z score	−2.21 (1.23)	−1.61 (1.25)	**.001**
Verbal Paired Associate Learning, z score	−1.56 (1.40)	−0.71 (1.31)	**<.001**
General Information, z score	−0.34 (1.34)	−0.39 (1.26)	.811
Attention and executive functions			
Digit Span Backward, z score	−0.06 (1.14)	−0.04 (1.26)	.886
Trail Making Test A time, z score	−1.49 (2.19)	−0.91 (1.70)	.054
Trail Making Test B time, z score	−2.57 (2.54)	−1.50 (2.28)	**.005**
Cancellation Task, total, z score	0.02 (1.29)	0.33 (1.64)	.139
Verbal Semantic Fluency, z score	−0.56 (1.67)	−0.44 (1.39)	.610
Abstract reasoning			
Raven's Coloured Progressive Matrices, z score	−0.39 (1.42)	−0.12 (1.30)	.151
Interpretation of Proverbs, z score	0.53 (1.62)	0.84 (1.56)	.162
Calculation			
Basic Written Calculation, z score	−0.37 (1.25)	−0.34 (1.46)	.917

on these memory tests still held when the groups were controlled for the MMSE, a general linear model analysis was performed considering the distinct neuropsychological tests as dependent variables and the MMSE score as a covariate. Aβ+ aMCI patients essentially kept poorer performances in the same tests as previously found: Word Total Recall (F = 6.181, p = .003); Logical Memory, Immediate Free Recall (F = 3.077, p = .052); Logical Memory, Delayed Free Recall (F = 7.651, p = .001); and Verbal Paired Associate Learning (F = 12.281, p < .001).

Regarding attention and executive functions, there were no differences in the Digit Span Backward, in the Trail Making Test A, in the Cancellation Task nor in the Verbal Semantic Fluency test; however, the Aβ+ aMCI patients performed significantly worse on the Trail Making Test B. Using the Trail Making Test B over A ratio, we found no significant differences between groups (p = .905). For the Aβ+ aMCI patients, the mean value of the ratio was 2.9 (*SD* 1.1); for the Aβ− aMCI patients, the mean was 2.9 (*SD* 1.2). Finally, there were no statistically significant differences between the two groups in the abstract reasoning and calculation domains.

A binary logistic regression model was built in order to predict the amyloid status of aMCI patients. In general, the tests in which there were significant differences between the two groups entered the model. Regarding Logical Memory, the Delayed Free Recall measure was chosen. Only Logical Memory Delayed Free Recall retained statistical significance to determine the amyloid status of aMCI patients. For each additional score point in the Logical Memory Delayed Free Recall z score, the odds ratio was 0.694, that is, the probability of being Aβ+ decreased by 30.6% (Table 3). The resulting model correctly classified 64.6% of the aMCI cases regarding their amyloid status. Only 17.7% of the variation in the dependent variable (amyloid positivity) was explained by the present

Table 3. Neuropsychological predictors of amyloid positivity

Neuropsychological tests	B	SE	Wald	p value	Exp(B)	95% C.I. for Exp(B) Lower	Upper
Word Total Recall, z score	−0.240	.146	2.709	.100	0.787	0.592	1.047
Logical Memory Delayed Free Recall, z score	−0.366	.172	4.549	**.033**	0.694	0.495	0.971
Verbal Paired Associate Learning, z score	−0.085	.155	0.301	.583	0.918	0.677	1.245
Trail Making Test B time, z score	−0.114	.080	2.058	.151	0.892	0.763	1.043

Note. Binary logistic regression analysis.

model. The ability of Logical Memory Delayed Free Recall (z score) to discriminate between Aβ+ and Aβ− aMCI patients was checked with a ROC curve, producing an area under the curve (AUC) of 0.633.

Since there might be some redundancy in the tests comprising the neuropsychological battery that was applied, a principal component analysis was performed. The rotated varimax component matrix pointed out 5 factors. Of these, there were significant differences between Aβ+ and Aβ− aMCI patients in factor 2 (Memory factor, comprising Logical Memory Immediate Free Recall, Logical Memory Delayed Free Recall, and Verbal Paired Associate Learning; $F = 9.546$, $p = .003$) and in factor 3 (Executive factor, comprising Trail Making Test A time and Trail Making Test B, and Raven's Coloured Progressive Matrices, $F = 5.881$, $p = .017$). These results confirmed that Aβ+ and Aβ− aMCI patients essentially differed in memory test as well as in executive tests.

Discussion

The main finding of the present study is that aMCI patients who are Aβ+ have more deficits in general cognition, memory tests, and executive functions as compared to Aβ− aMCI patients. A few points deserve consideration.

In the first place, we confirmed that Aβ+ aMCI patients are more impaired in memory tests as compared to Aβ− aMCI patients, as previously reported by several studies (Bahar-Fuchs *et al.*, 2013; Huijbers *et al.*, 2015; Kandel *et al.*, 2015; Kim *et al.*, 2018; Reijs *et al.*, 2017; Tomadesso *et al.*, 2018, 2019; Wolk *et al.*, 2009). As patients with aMCI patients who are Aβ+ suffer from AD (Jack *et al.*, 2018), the observed memory deficits correspond to the typical cognitive profile of AD, reflecting the hippocampal atrophy observed early in the course of the disease. In the present work, Word Total Recall, Logical Memory (Immediate and Delayed Free Recall), and Verbal Paired Associate Learning were significantly worse in Aβ+ aMCI patients.

The second point is that we contributed to clarify the controversial issue whether Aβ+ aMCI patients are more affected in executive functions and attention, which has not been clear from previous studies. We showed that tests assessing executive functions, namely the Trail Making Test B, were more affected in Aβ+ aMCI patients. It could be argued that the worse performance on the Trail Making Test part B in Aβ+ when compared to Aβ− aMCI patients was due to impairment of visuospatial abilities in the first group. However, the observation that there were no significant differences between the Aβ+ and Aβ− aMCI patients in the Raven Progressive Matrices, a visuospatially very demanding test, suggests that differences in the Trail Making Test part B are probably not attributable to visuospatial

difficulties. The results concerning Trail Making Tests are in accordance with Kandel *et al.* (2015) reports in aMCI patients from the ADNI cohort, who also found significantly worse results in both Trail Making Tests in Aβ+ patients. We did not observe differences in Verbal Semantic Fluency between Aβ+ and Aβ− aMCI patients, similarly as reported in a previous study (Tomadesso *et al.*, 2018). However, another study found that Verbal Semantic Fluency was significantly worse in Aβ+ as compared to Aβ− aMCI patients (Kandel *et al.*, 2015). This last study used animal category for the task, while we used supermarket food items, which might explain the discrepancy of the results. Regarding attention, we found no significant differences between the two groups in the Cancellation Task, no previous studies having previously compared, to the best of our knowledge, Aβ+ and Aβ− aMCI patients on this test. More studies are certainly needed to further investigate how the amyloid status influences performances in different tests of executive functions and attention in patients with aMCI. It should be added that patients with aMCI who are Aβ+ showed less global cognitive performance, albeit within the normative range, assessed by the MMSE, as compared to Aβ− aMCI patients, probably reflecting the more pronounced alteration in several cognitive domains, particularly memory and executive functions, as described above.

A third point has to do with the value of neuropsychological tests to predict the patients with aMCI who have amyloid pathology. In the present study, the statistical model could only correctly classify 64.6% of the aMCI cases regarding their amyloid status. The only test that remained in the model was the Logical Memory Delayed Free Recall.

It is noteworthy that in the present study, the Logical Memory Immediate Recall score was chosen to classify patients as aMCI and the Logical Memory Delayed Recall score for analysis, in order to avoid circularity bias. However, it could be argued that both measures were rather equivalent. This did not seem to be the case, as there was no significant collinearity between these neuropsychological test variables, with a variance inflation factor value relating Logical Memory Immediate Recall and Logical Memory Delayed Recall of 1.199.

The Logical memory Delayed Recall score produced a modest AUC (0.633). In a previous study in aMCI patients, the 30-min delayed recall score of the Rey Auditory Verbal Learning Test was the best predictor of Aβ status among the psychometric tests, but it produced an AUC of only 0.67 (Kandel *et al.*, 2015). Using a 16-word list, Tomadesso *et al.* (2018) calculated slightly better AUC values for the free recall (0.73) and recognition (0.74) tasks in classifying the aMCI cases according to the amyloid status. It thus seems that neuropsychological tests have a limited ability to identify the aMCI cases who are Aβ+ and those who are Aβ−, not attaining the values of 80% recommended for AD biomarkers (The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group, 1998). Of course, these results do not exclude that neuropsychological tests could add predictive value to determine the amyloid status in conjunction with other clinical or neuroimaging biomarkers.

Finally, the intriguing question of the aetiology of aMCI cases who are Aβ− certain merits further research. Depressive symptoms were not more frequent in Aβ− than in Aβ+ aMCI patients. Patients with history of stroke or relevant cerebrovascular disease in brain imaging were excluded in the present study. It is possible that Aβ− aMCI patients might be at an initial stage of a neurodegenerative disorder other than AD, for instance frontotemporal dementia or the Lewy body dementia–Parkinson's disease continuum (Ye *et al.*, 2014). To be sure, a long follow-up of these Aβ− aMCI patients might be needed.

The main strength of this study is that it was carried out in the context of a large prospective cohort, in which the participants underwent comprehensive standardized

neuropsychological assessment. Several limitations of the study must be recognized. Participants were patients who attended a memory clinic or a general hospital outpatient clinic, and the findings may not applicable to different clinical settings. Certainly, only a proportion of patients with aMCI undergo a comprehensive AD biomarker workout, and these are probably different from those patients with aMCI who do not.

In conclusion, the neuropsychological assessment remains an essential step to diagnose and characterize patients with aMCI. However, neuropsychological tests have limited value to distinguish the aMCI patients who have amyloid pathology and AD, from those who might suffer from other clinical conditions.

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Conflicts of interest

All authors declare no conflict of interest.

Author contributions

Luísa Alves, M.D. (Conceptualization; Writing – original draft); Sandra Cardoso (Data curation; Investigation); Dina Silva (Data curation; Investigation); Tiago Mendes (Visualization); João Maróco (Formal analysis); Joana Nogueira (Investigation; Visualization); Marisa Lima (Investigation; Visualization); Miguel Tábuas-Pereira (Investigation; Visualization); Inês Baldeiras (Investigation; Resources; Visualization); Isabel Santana (Data curation; Investigation; Writing – review and editing); Alexandre de Mendonça (Conceptualization; Formal analysis; Investigation; Methodology; Project administration; Supervision; Writing – original draft; Writing – review and editing); Manuela Guerreiro (Conceptualization; Investigation; Methodology; Supervision).

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Neuropsychological Contribution to Predict Conversion to Dementia in Patients with Mild Cognitive Impairment Due to Alzheimer's Disease

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Abstract.
Background: Diagnosis of Alzheimer's disease (AD) confirmed by biomarkers allows the patient to make important life decisions. However, doubt about the fleetness of symptoms progression and future cognitive decline remains. Neuropsychological measures were extensively studied in prediction of time to conversion to dementia for mild cognitive impairment (MCI) patients in the absence of biomarker information. Similar neuropsychological measures might also be useful to predict the progression to dementia in patients with MCI due to AD.
Objective: To study the contribution of neuropsychological measures to predict time to conversion to dementia in patients with MCI due to AD.
Methods: Patients with MCI due to AD were enrolled from a clinical cohort and the effect of neuropsychological performance on time to conversion to dementia was analyzed.
Results: At baseline, converters scored lower than non-converters at measures of verbal initiative, non-verbal reasoning, and episodic memory. The test of non-verbal reasoning was the only statistically significant predictor in a multivariate Cox regression model. A decrease of one standard deviation was associated with 29% of increase in the risk of conversion to dementia. Approximately 50% of patients with more than one standard deviation below the mean in the *z* score of that test had converted to dementia after 3 years of follow-up.

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Conclusion: In MCI due to AD, lower performance in a test of non-verbal reasoning was associated with time to conversion to dementia. This test, that reveals little decline in the earlier phases of AD, appears to convey important information concerning conversion to dementia.

Keywords: Alzheimer's disease (AD), amyloid-β, cognitive impairment, dementia, mild cognitive impairment due to AD, neuropsychological assessment, prodromal AD, Raven Coloured Progressive Matrices

INTRODUCTION

Nowadays, the development and clinical application of biomarkers has dramatically changed the framework of Alzheimer's disease (AD) diagnosis. It is now possible to diagnose AD at an early pre-dementia stage, that is, before the patient has symptoms severe enough to be considered demented [1, 2]. Different diagnostic criteria with slight differences were advanced, namely prodromal AD [3–5] and mild cognitive impairment (MCI) due to AD [6], that rely on biomarkers reflecting pathological alterations in the brain typical of AD, namely: 1) decline in episodic memory, confirmed by neuropsychological testing, 2) atrophy of the hippocampus and other medial temporal lobe structures shown by magnetic resonance imaging, 3) detection of abnormal cerebrospinal fluid (CSF) biomarkers, namely low amyloid-β ($A\beta_{42}$) concentrations, increased phosphorylated tau or total tau concentrations, 4) abnormal brain deposits of Aβ and tau, as well as reduced glucose metabolism in temporoparietal regions, by positron emission tomography (PET scan). The use of biomarkers for diagnosis of MCI due to AD quickly spread to AD reference centers [7] and more sluggishly to routine clinical practice.

Uncertainties remain about the possible benefits and disadvantages of obtaining and communicating a specific diagnosis of prodromal AD, or MCI due to AD, to an individual patient. On the one hand, it should be relevant for the patient to make life decisions and prepare the near future, engage in a cognitive rehabilitation program, start appropriate pharmacological therapy, and eventually participate in a clinical trial. On the other hand, it might upset patients and caregivers, leading to emotional distress and concerns about progression of symptoms and the fleetness of future cognitive decline [8]. One important present limitation of obtaining and communicating a specific diagnosis of MCI due to AD is that the actual pace of disease progression, attainment of important clinical milestones, and in particular conversion to dementia, are presently impossible to predict in an individual basis. This point could not be made more clearly than by the patient's sentence when receiving the diagnosis of MCI due to AD: *Yes, I hope for the best. It will definitely evolve. I don't think it will stay like that, but is that within 5 years?* [8].

Importantly, prediction of time to conversion to dementia has already been extensively studied in MCI without the information of biomarkers, namely using neuropsychological assessments. These studies showed that memory tests, as well as executive function and verbal fluency tests, are able to predict with accuracy the time to conversion to dementia [9–25]. We hypothesize that similar neuropsychological measures may also be useful to predict the progression to dementia in MCI due to AD. It should be very important to provide the individual patient diagnosed with MCI due to AD with reliable information on the prediction of stability or conversion to dementia at a clinically relevant time window.

METHODS

Participants

A cohort of 232 patients who attended neurologic consultation in a private memory clinic in Lisbon (Memoclínica) and Coimbra University Hospital, in Coimbra, from 2006 to 2017, performed a comprehensive neuropsychological evaluation and were tested for biomarkers of brain amyloidosis and neuronal injury. From these, 127 had the diagnosis of MCI due to AD and were included in the present study. Patients had to have associated follow-up information and to be followed for at least one year, thus only 110 patients were analyzed for the present study (Fig. 1).

Ethical guidelines

The study was conducted in accordance with the Declaration of Helsinki, and the local ethics committee approved the study. All patients provided their written informed consent before any procedure.

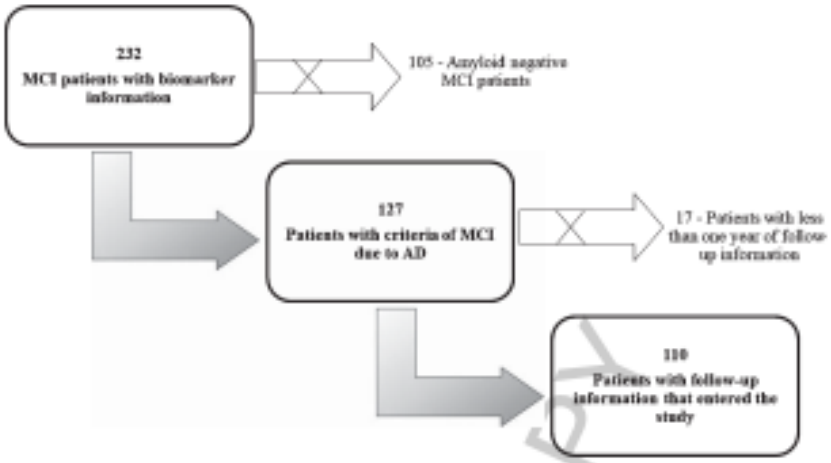


Fig. 1. Flow-chart of patient selection for the study.

Diagnostic criteria

The diagnostic criteria of MCI due to AD, as proposed by the National Institute on Aging - Alzheimer's Association workgroups [6], offer the most accurate prognosis in clinical settings [26]. Specifically, the criteria of MCI due to AD–High Likelihood [6] were considered in the present study since they provide the highest degree of certainty that the patient will progress to AD dementia:

1. Clinical and cognitive criteria
   a. Cognitive concern reflecting a change in cognition reported by patient, informant, or clinician
   b. Objective evidence of impairment in one or more cognitive domains, typically including memory
   c. Preservation of independence in functional abilities
   d. Not demented
2. Etiology of MCI consistent with AD pathophysiological process
   a. Vascular, traumatic and medical causes of cognitive decline were ruled out
   b. Evidence of longitudinal decline in cognition (when feasible)
3. Biomarkers of Aβ deposition
   a. Low CSF $A\beta_{42}$ and/or
   b. Positive amyloid PiB-PET imaging.
4. Biomarkers of neuronal injury (at least one present)
   a. High CSF total tau or hyperphosphorylated tau, and/or
   b. Medial temporal atrophy by volumetric measures or visual rating, and/or
   c. Temporoparietal hypometabolism by FDG-PET imaging

Both sources of amyloid status (CSF and PiB-PET) were considered interchangeable since a high agreement between $A\beta_{42}$ concentrations in the CSF and amyloid PiB-PET scan results in MCI and AD patients was confirmed by previous studies [27]. All procedures were performed according to the established protocols on participating centers [28–32]. The levels of $A\beta_{42}$, total tau (t-tau), and hyperphosphorylated tau (p-tau) were measured using commercially available enzyme-linked immunosorbent assays (INNOTEST® Aβ42, INNOTEST hTAU Ag and INNOTEST PHOSPHO-TAU(181P); Innogenetics, Ghent, Belgium). The expected site assay variability present in multicenter studies was acknowledged [33] and positivity was determined using locally available cut-off values. Amyloid PET scans used the Pittsburgh Compound B (^{11}C-PIB) and were performed in the same scanner (Philips PET/CT Gemini GXL), preceded by a low-dose brain computed tomography (CT) acquisition for attenuation correction (Institute of Nuclear Science Applied to Health, ICNAS, University of Coimbra). PiB-PET images were classified as amyloid positive or negative based on a support vector machines (SVM) local classifier, which uses the voxel wise brain grey matter standardized uptake value ratio

(SUVR) and the cerebellar grey matter as reference region [31].

Conversion to dementia

At follow-up, the patients were classified as "non-converter" if the diagnosis persisted until last assessment or "converter" in the presence of a dementia diagnosis established according to the DSM-IV-TR [34] criteria, in a consensus meeting with the team of neurologists and neuropsychologists that followed the patients.

Neuropsychological assessment

The baseline and follow-up comprehensive neuropsychological assessment was carried out by the same team of trained neuropsychologists, following a standard protocol and comprised the following instruments and scales:

- Mini-Mental State Examination (MMSE) [35, 36] - the MMSE is a brief screening instrument to assess global cognitive performance. The Portuguese version was applied, and normative data was >27 for more than 11 years of education and >22 for 11 or less years of education [36].
- Battery of Lisbon for the Assessment of Dementia (BLAD) [37, 38] - the BLAD is a comprehensive neuropsychological battery that includes some tests from the Wechsler Memory Scale [39] and has been validated for the Portuguese population. This battery includes tests for the following cognitive domains: attention (Cancellation Task); verbal initiative (Semantic Fluency), motor and graphomotor initiatives; verbal comprehension (a modified version of the Token Test); verbal and non-verbal reasoning (Interpretation of Proverbs and the Raven's Coloured Progressive Matrices – Ab series); orientation (Personal, Spatial, and Temporal Orientation); visuo-constructional abilities (Cube Copy); planning and visuospatial/praxis abilities (Clock Draw); calculation (Basic Written Calculation); immediate memory (Digit Span Forward); visual memory (Visual Reproduction Test); working memory (Digit Span Backward); learning and verbal memory (Verbal Paired-Associate Learning, Logical Memory and Word Recall).
- California Verbal Learning Test (CVLT) [40, 41] - the CVLT measures verbal learning and assesses constructs such as repetition learning, serial position effects, semantic organization, intrusion, and proactive interference. The word lists (List A and List B) are made up of 16 items from 4 different categories of "shopping list" items. The trial of interest (better discriminating ability for different stages of cognitive decline) [42] considered for the present study was the total number of words from List A correctly recalled on the first 5 learning trials (CVLT 5 Trials Total Recall).
- Trail Making Test (part A and part B) [43, 44] - the TMT task measures sustained attention, visuomotor processing speed (part A), visuospatial working memory and cognitive flexibility (part B). The part A consists of 25 circles numbered 1–25 distributed over a sheet of paper and the patient should draw lines to connect the numbers in ascending order. In Part B there are 25 circles as well, but the circles include both numbers (1–13) and letters (A–M) and the patient has to draw lines to connect them all in an ascending pattern with the added task of alternating between the numbers and letters (i.e., 1-A-2-B-3-C, etc.).
- Geriatric Depression Rating Scale (GDS) [45–47] - the GDS is a self-report instrument used specifically to identify depressive symptomatology in the elderly. For this study, a Portuguese version of a short form (15 items) was applied [47].
- Subjective Memory Complaints Scale (SMC) [48, 49] - the SMC scale comprises 10 individual questions for the assessment of subjective memory complaints, with total scores ranging from 0 (absence of complaints) to 21 (maximal complaints score).
- Blessed Dementia Rating Scale (BDRS) [50, 51] - the BDRS is a brief behavioral scale based on the interview of a close informant. This scale is composed of 22 items that address daily life activities, habits and changes in personality.

Statistical analysis

For baseline comparison of demographic and clinical data between groups the Student's t test and Pearson's χ^2 test were used, for numerical and nominal data, respectively. All tests were 2-tailed and a p-value <0.05 was assumed to be statistically significant. The neuropsychological assessments were standardized according to the age and education

norms for the Portuguese population [37, 38] and z scores were calculated. The comparison of neuropsychological results between the group that progressed to dementia during follow-up and the group that remained with MCI was conducted using Student's t test. To explore the effect of impairment in neuropsychological tests on the time to conversion to dementia during follow-up, first the proportional hazards assumption for neuropsychological predictors was tested by adding time dependent covariates (interaction of predictors and a function of survival time) and then a Cox Proportional Hazards Regression model was conducted. The hazard or risk of conversion to dementia for the neuropsychological tests that were significantly different between converter and non-converter groups was computed. Time to event was calculated as the interval from the initial baseline evaluation to the diagnosis of dementia. For cases that remained non-demented, time was censored at the date of the last clinical/neuropsychological assessment. Kaplan-Meier curves analyzing the incidence of dementia according to the z scores of the lowest and the highest tercile were depicted. For comparison of curves, we opted for the Gehan-Breslow test since one group had a higher risk of conversion due to the significantly lower cognitive performance at baseline.

Statistical analyses were performed using IBM SPSS Statistics 25 for Windows (2017 SPSS Inc., an IBM Company) package.

RESULTS

One hundred and ten patients with MCI due to AD were enrolled. During the follow-up period (2.69 ± 1.56 years for converters and 2.67 ± 1.39 for non-converters), 61 patients (55.5%) progressed to dementia and 49 (44.5%) did not. Demographic and clinical data are reported in Table 1. The converters at the baseline assessment were younger than the non-converters; however, for mean follow-up time, education level, gender, depressive symptomatology, cognitive complaints, and independence at daily activities, no statistically significant differences were found (Table 1).

The results of a comprehensive neuropsychological assessment showed the presence of impairment (z score<−1) in measures of attention and executive functions (Trail Making Test A and B), orientation, verbal learning and episodic memory (Word Recall; Logical Memory immediate recall; Logical Memory delayed recall; Verbal Paired-Associate Learning; California Verbal Learning Test 5 Trials Total Recall) for both groups. In a measure of language comprehension (Token Test), only the converters showed impairment. Moreover, converters scored significantly lower than non-converters at measures of verbal initiative (Semantic Fluency), non-verbal reasoning (Raven's Coloured Progressive Matrices), and episodic memory (Logical Memory immediate recall). Noteworthy, a trend toward statistical significance was found for the delayed recall condition of the Logical Memory test with converters scoring lower than non-converters at baseline assessment (Table 2).

A multivariate Cox proportional hazards regression model was applied to identify the independent predictors associated with time to conversion. The proportional hazards assumption was tested for each predictor (Age: Hazard Ratio [HR] = 1.020, CI: 0.990–1.052, $p = 0.192$; Semantic Fluency: HR = 0.965, CI: 0.804–1.159, $p = 0.704$; Logical Memory (immediate recall): HR = 0.981, CI:

Table 1
Baseline demographic and clinical characteristics of non-converters and converters

	Non-converter $n = 49$	Converter $n = 61$	p
Age at first assessment, y, mean (SD)	70.1 (6.2)	65.4 (7.3)	**<0.001***
Formal education, y, mean (SD)	10.7 (4.6)	10.2 (4.8)	0.591
Gender, female/male, n	28/22	35/27	1.000#
Follow-up time, y, mean (SD)	2.7 (1.4)	2.7 (1.6)	0.921
Time between onset of symptoms and first neuropsychological assessment, mean (SD)	2.4 (1.5)	2.2 (1.2)	0.576
Geriatric Depression Scale, mean (SD)	5.1 (3.4)	5.8 (4.5)	0.420
Subjective Memory Complaints Scale, mean (SD)	10.3 (4.6)	10.2 (4.1)	0.959
Blessed Dementia Rating Scale, mean (SD)	3.1 (1.9)	3.4 (2.0)	0.528
Mini-Mental State Examination, mean (SD)	26.4 (2.2)	25.6 (2.4)	0.084

Group comparisons were performed with parametric Student's t test (or χ^2 Pearson test when appropriate#); *Statistically significant $p < 0.05$; SD, standard deviation; y, years.

Table 2
Baseline neuropsychological performances of non-converters and converters

Cognitive domain Neuropsychological test	Non-converter ($n=49$)	Converter ($n=61$)	p	Cohen's d
• **attention and executive functions**				
Cancellation Task	0.26 (1.17)	0.04 (1.37)	0.406	0.14
Digit Span Backward	0.06 (0.90)	−0.09 (1.20)	0.488	0.12
Clock Draw	0.05 (1.49)	−0.37 (1.53)	0.216	0.28
Trail Making Test A	−1.31 (1.70)#	−1.36 (1.85)#	0.896	0.02
Trail Making Test B	−1.97 (1.84)#	−1.63 (1.79)#	0.413	−0.18
• **initiative**				
Semantic Fluency	−0.07 (1.33)	−0.86 (1.48)	**0.004***	0.54
Motor Initiative	−0.27 (1.80)	−0.70 (1.90)	0.238	0.23
Graphomotor Initiative	0.05 (0.76)	−0.13 (1.00)	0.319	0.21
• **reasoning**				
Raven Coloured Progressive Matrices	0.05 (1.06)	−0.60 (1.43)	**0.009***	0.48
Interpretation of Proverbs	0.73 (1.23)	0.34 (1.82)	0.211	0.21
• **orientation**				
Personal, Spatial and Temporal Orientation	−2.32 (2.45)#	−2.23 (2.35)#	0.846	−0.04
• **calculation**				
Basic Written Calculation	−0.47 (1.00)	−0.59 (1.17)	0.582	0.08
• **visuo-constructional abilities**				
Cube Copy	1.54 (1.95)	1.33 (2.37)	0.656	0.11
• **language**				
Token Test	−0.59 (1.11)	−1.17 (1.83)#	0.113	0.36
• **memory and learning**				
Visual Reproduction	1.45 (1.30)	0.58 (0.99)	0.150	0.77
Digit Span Forward	0.55 (1.30)	0.42 (1.34)	0.622	0.08
Word Recall	−1.25 (1.44)#	−1.77 (1.57)#	0.093	0.35
Logical Memory (immediate recall)	−1.17 (1.13)#	−1.92 (1.53)#	**0.005***	0.53
Logical Memory (delayed recall)	−1.99 (1.40)#	−2.64 (0.93)#	0.056	0.53
Forgetting Index [(1)]	−1.23 (2.38)#	−1.79 (2.78)#	0.266	0.26
Verbal Paired-Associate Learning	−1.18 (1.20)#	−1.58 (1.54)#	0.139	0.25
CVLT 5 Trials Total Recall	−3.14 (1.36)#	−3.69 (0.95)#	0.077	0.42

Means of z scores calculated according to the equation [$z=(x-\text{mean})/\text{SD}$]; Group comparisons were performed with independent samples Student's t test. *Statistically significant $p<0.05$. #Presence of impairment (z score<−1). [(1)]Forgetting Index = [(LM delayed recall −LM immediate)/LM immediate)]*100. CVLT, California Verbal Learning Test.

0.834–1.155, $p=0.821$; Raven Coloured Progressive Matrices: HR = 1.217, CI: 1.005–1.475, $p=0.045$). Only the clinical and neuropsychological measures that differentiate the groups were included as predictors. In the first model, only the clinical predictor (age) by the method enter was included. Age at baseline was not associated with time to event (conversion to dementia). Neuropsychological predictors were subsequently subjected to multivariate Cox proportional hazards regression analysis (Table 3). The Semantic Fluency was added to the model and was a significant predictor (HR = 0.762, CI: 0.634–0.916, $p=0.004$), whereas the Logical Memory (immediate recall) in the presence of Semantic Fluency did not reach significance as predictor (HR = 0.852, CI: 0.704–1.031, $p=0.099$) (Table 3). However, the Logical Memory (immediate recall) was a significant predictor if entered first in the model (data not shown in Table 3; HR = 0.797, CI: 0.663–0.957, $p=0.015$). When the Raven Coloured Progressive Matrices was added to the model, the other predictors lost their significance (Semantic Fluency: HR = 0.835, CI: 0.691–1.009, $p=0.062$; Logical Memory (immediate recall): HR = 0.898, CI: 0.738–1.092, $p=0.281$). In the final model, only the Raven Coloured Progressive Matrices, a test of non-verbal reasoning, remained significant as a predictor of time to conversion to dementia (HR = 0.712, CI: 0.566–0.894, $p=0.004$). A decrease of one unit (z score) in Raven Coloured Progressive Matrices was associated with a 29% increase in the risk of conversion to dementia (Table 3).

For the Kaplan-Meier curves, the comparison was between the highest and the lowest terciles of the Raven Coloured Progressive Matrices scores to assess the differences in time to conversion to dementia. Because at baseline both groups showed normative results, the presentation of Kaplan-Meier curves comprised the lowest and the highest terciles, instead of impaired and unimpaired z scores,

Table 3
Multivariate Cox proportional-hazard regression models for predictors of conversion to dementia

Predictors (n = 130; event/conversion to dementia = 61; censored = 69)	HR	95%CI	p
Multivariate analysis			
Model 1 – demographic variable (enter method)			
Age (mean, y)	0.984	0.950–1.019	0.376
Model 2 – cognitive predictors (enter method)			
Semantic Fluency (mean, z score)	0.762	0.630–0.916	**0.004***
Model 3 – cognitive predictors (enter method)			
Semantic Fluency (mean, z score)	0.804	0.664–0.974	**0.026***
Logical Memory (immediate recall) (mean, z score)	0.852	0.706–1.031	0.099
Model 4 – cognitive predictors (enter method)			
Semantic Fluency (mean, z score)	0.835	0.681–1.009	0.062
Logical Memory (immediate recall) (mean, z score)	0.898	0.738–1.092	0.281
Raven's Coloured Progressive Matrices (mean, z score)	0.712	0.566–0.894	**0.004***

CI, Confidence Interval; HR, Hazard Ratio; y, years; *Statistically significant ($p < 0.05$).

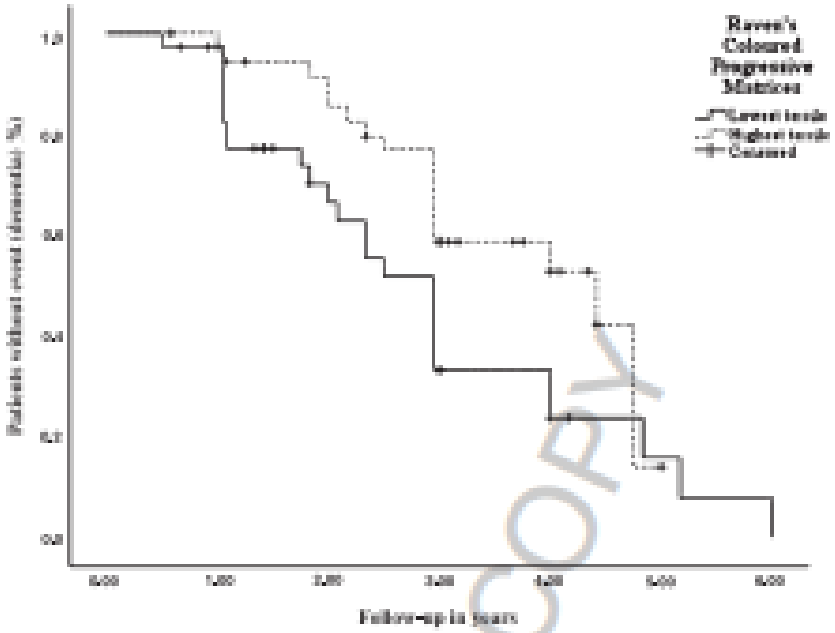
to offer a more balanced sample size curves (Fig. 2). According to the Kaplan-Meier curves, for z scores in the lowest tercile (z score range: –2.88 to –0.96) after 3 years of follow-up approximately 50% of patients had converted to dementia, whereas for the highest tercile (z score range: 0.59 to 1.82) the conversion of approximately 50% of patients occurred later, after 4 years of follow-up. Accordingly, a significant difference between Kaplan-Meier curves was found ($\chi^2(1) = 6.131$; $p = 0.013$).

DISCUSSION

Patients with MCI due to AD that converted to dementia during the follow-up period were more impaired at the baseline in neuropsychological tests assessing verbal fluency, non-verbal reasoning, and episodic memory, as compared to non-converters. An interesting result is that only non-verbal reasoning, assessed through Raven Coloured Progressive Matrices, remained significant as a predictor of time to conversion to dementia in a multivariate model.

Several studies have previously evidenced the predictive value of neuropsychological measures to assess time to conversion to dementia in MCI patients with unknown biomarker status [22, 52–54]. Noteworthy, some studies highlighted that not only episodic memory performance but also other cognitive areas, namely executive functions and language tests, are associated with a higher likelihood of progression from MCI to dementia during follow-up [14, 21, 55–57]. Thus, it would be plausible to expect a similar contribution of neuropsychological testing for patients with MCI due to AD.

According to our results, cognitive areas associated with reasoning and fluid intelligence, that reveal little decline until more advanced phases of AD, as can be seen in the normative results of our MCI patients, can contribute significantly to predict time to conversion. As previously mentioned, only non-verbal reasoning, assessed through Raven Coloured Progressive Matrices, remained significant as a predictor of time to conversion to dementia in a multivariate model. For each standard deviation reduction in the z score of Raven Coloured Progressive Matrices score the risk of conversion to dementia increased approximately 29%. This test is a measure of fluid intelligence that demands several abilities as visual-perceptual, process integration, logical reasoning, and cognitive flexibility [58]. The contribution of the Raven Coloured Progressive Matrices to predict time to conversion to dementia has been, to the best of our knowledge, largely neglected in the literature. Fluid intelligence has been addressed as a proxy of cognitive reserve [59]. In AD patients, a higher cognitive reserve was associated with slower clinical progression in predementia stages, but after the onset of dementia it appears to have the opposite effect and accelerate the cognitive decline [60]. Interestingly, in a different cohort study from the same memory clinic in Lisbon, in amnestic MCI patients without amyloid status information, an association of performance in Raven Coloured Progressive Matrices with long-term (10 years) diagnostic stability was also found [61]. Likewise, a large community-based study with non-demented subjects, the Framingham cohort prospective study, showed that a test of abstract reasoning was a strong predictor of long-term (22 years) conversion to dementia [62]. In the present study, the Raven Coloured Progressive Matrices test was found to be the stronger predictor of conversion to dementia at a shorter (3 years) term in patients with MCI due to AD.



Number at risk					
	2 year	3 years	4 years	5 years	6 years
Lowest tercile (n=37)	27	20	12	7	3
Highest tercile (n=37)	35	29	22	6	0

Fig. 2. Kaplan-Meier analysis of the incidence of dementia among patients in the lowest and in the highest tercile of the $ scores.

As foreseeable, most of the MCI due to AD patients converted during the follow-up period. Remarkably patients that converted to dementia during follow-up were younger at baseline than patients that did not convert, with no differences being found in duration of symptoms, presence of depressive symptoms, and years of formal education. This result seems to be in contradiction to longitudinal studies of conversion from MCI to AD that commonly report higher risk of conversion to dementia for the older patients [63, 64]. However, the influence of age in cognitive decline for AD patients is not straightforward and some studies have revealed that AD patients starting the symptoms earlier had a less benign course with higher rate of cognitive decline [65]. Notwithstanding the difference at baseline, age was not a significant predictor of time to conversion.

The present study has some limitations that might be addressed in future studies. Obtaining a longer follow-up would be important. Replication of the present findings in other studies recruiting patients at a similar clinical stage would be needed. The genotyping of Apolipoprotein E (*APOE*) ε4 is not recommended in a clinical context [66] and for that reason was not available, and this is a limitation of the present study. Patients did not undergo all neuronal injury biomarkers, so it was not possible to assess their predictive value on time to future conversion to dementia. Not all patients with MCI undergo the diagnostic procedures with biomarkers, which are costly and invasive, thus the patients diagnosed with MCI due to AD are not representative of the AD population in a memory clinic.

The major strengths of the present study are the sample high likelihood of having AD neurodegen-

eration according to the diagnostic criteria and the minor loss to follow-up of the cohort. As future perspectives, predicting conversion of MCI due to AD to dementia might be improved by machine learning techniques, namely by a feature selection ensemble approach to automatically choose the best neuropsychological predictors of future conversion, as was already done for MCI patients without amyloid status information [67]. Anticipating a precision medicine approach, it would important to refine risk models that can provide reliable prognostic information to the individual patient with MCI due to AD [68].

It has been an extraordinary recent advance being able to diagnose AD at an early clinical stage. Still, after being diagnosed with MCI due to AD, patients and families need to make important life decisions and future planning, and expectedly wish to get a reliable estimation of the disease progression. To the best of our knowledge, the present study is the first to explore the differential contribution of routine neuropsychological tests to predict time to conversion to dementia among patients diagnosed with MCI due to AD. Neuropsychological tests, namely assessing verbal fluency, episodic memory, and particularly non-verbal reasoning assessed with the Raven Coloured Progressive Matrices, may contribute to predict stability or conversion to dementia at a clinically meaningful time window.

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