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Improving the understanding of cognitive impairment

Insights from cross-sectional and longitudinal assessment

Luis Ruano

Tese de Doutoramento em Saúde Pública
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I. **Development of a self-administered web-based test for longitudinal cognitive assessment.**
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II. **Prevalence and causes of cognitive impairment and dementia in a population based cohort from Northern Portugal**
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IV. **Age and disability drive cognitive impairment in multiple sclerosis across disease subtypes.**
Ruano L, Portaccio E, Goretti B, Niccolai C, Severo N, Chisari C, Gallo P, Grossi P, Ghezzi A, Roscio A, Mattioli F, Stampatori C, Simone M, Viterbo RG, Amato MP. *Multiple Sclerosis. 2017 Aug;23(9):1258-126.*

V. **Patients with pediatric-onset multiple sclerosis are at higher risk of cognitive impairment in adulthood: an Italian collaborative study.**
Ruano L, Branco M, Portaccio E, Goretti B, Niccolai C, Patti F, Cilia S, Gallo P, Grassi P, Ghezzi A, Roscio M, Mattioli F, Stampatori C, Trojano M, Viterbo RG, Amato MP. *Multiple Sclerosis 2017 1:1352458517717341.*

VI. **Tracking cognitive impairment in multiple sclerosis using the Brain on Track test: a validation study**
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Em cumprimento com o disposto no referido Decreto-Lei, declaro que participei ativamente na definição dos objetivos de todos os trabalhos que constituem esta tese, na aquisição dos dados dos estudos descritos nos artigos I, II, III e VI, e fui pela análise dos dados, interpretação dos resultados e redação da versão inicial de todos os manuscritos.

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List of abbreviations

AOMS - Adult onset multiple sclerosis

AUC - area under the receiving operator curve

BoT - Brain on Track

CNS - Central nervous system

EDSS - Expanded disability scale

MMSE - Mini mental state examination

MCI - Mild cognitive impairment

MoCA - Montreal cognitive assessment

MRI - Magnetic resonance imagining

MS - Multiple sclerosis

POMS - Paediatric onset multiple sclerosis

PP - Primary progressive

RR - Relapsing remitting

SP - Secondary progressive

SDMT - Symbol Digit Modalities Test

Abstract

Background

Cognitive impairment arises from potentially any pathological process that damages the central nervous system (CNS), imposing a heavy burden on affected patients, their families and society. Two particular groups are at a high risk: 1) the older age group, with high incidence of neurodegenerative and vascular disorders, and 2) patients with early onset brain disorders, such as multiple sclerosis. There are many unanswered questions regarding the natural history and determinants of cognitive dysfunction in these different models of CNS disease. Screening and monitoring for cognitive impairment in these groups at risk could be improved by a longitudinal approach, aiming at measuring changes in state, rather than the current state of cognitive performance.

The global aim of this thesis is to contribute for a better understanding of the natural history and determinants of cognitive impairment, by using standard cross-sectional cognitive assessment tools and by developing novel longitudinal approaches to monitor cognitive performance in two models of cognitive impairment; the older population, at risk for mild cognitive impairment (MCI) and early dementia; and patients with MS.

The next paragraphs describe the specific objectives defined for the thesis, along with the corresponding methods and results.

1. *To develop a tool for web-based cognitive monitoring*

We developed a self-administered computerized test intended for longitudinal cognitive screening and monitoring, Brain on Track (BoT). The test can be performed from a home computer and is composed of several subtests directed at different cognitive domains. An initial (A) and a refined version of the test (B) were applied to patients with MCI or early dementia (n=88) and age and education-matched controls. A subsample of a population-based cohort (n=113) performed the test at home every three months to evaluate test-retest reliability.

The test's Cronbach's alpha was 0.90, scores were significantly different between patients and controls (p=0.001), the area under the receiver operating characteristic curve (AUC) was 0.75 and the test was able to identify teste clinically significant differences. In the test-retest reliability analysis 9/10 subtests showed intraclass consistency correlation coefficient >0.70.

2. *To contribute to a better understanding of cognitive impairment and dementia in the general population:*

2.1. *To describe the prevalence and most common causes of MCI and dementia in a Portuguese population-based cohort (EPIPorto)*

Participants aged ≥55 years in the 2013-2015 revaluation of the EPIPorto cohort (n=730) underwent cognitive screening testing with the Mini Mental State Examination and the Montreal Cognitive Assessment. Those scoring bellow cut-points adjusted for the Portuguese population were further evaluated by a trained Neurologist to assess the presence of MCI and dementia and define their most probable aetiology.

The standardized prevalences of dementia and MCI were 1.0% and 4.0% (Portuguese population, direct method). The most common cause of MCI and dementia was vascular (52.8%), followed by Alzheimer's disease (36.1%).

2.2. *To prospectively assess and compare the variation of cognitive performance using BoT in healthy individuals from the general population and patients with MCI.*

We recruited 30 consecutive patients with probable MCI from a memory clinic and 377 healthy controls, a sub-sample of the population-based cohort EPIPorto. All participants performed a neuropsychological assessment and the BoT test at baseline, including two new subtests with difficulty adapted to the participants' expected level of performance. The BoT test was repeated remotely from home every three months for one year. A linear mixed-effects model was built to describe the variation in cognitive performance in each group. The overall accuracy of BoT single use distinguish between MCI patients and matched controls was assessed through the AUC for single and repeated use.

All participants increased their scores in the first tests, but after 120 days those with MCI presented a decline, with a statistically significant higher rate when compared with the general population. The AUC to detect MCI in the single use of BOT was 0.86, while the repeated measurements reached an AUC of 0.96 in the one year monitoring.

3. *To contribute to a better understanding of cognitive impairment in MS*

3.1. *To describe the prevalence, profile and clinical determinants of cognitive impairment in patients with MS and* ***3.2*** *To assess the impact of a paediatric disease onset of MS (POMS) on the long term cognitive outcomes*

Consecutive patients with MS observed in six Italian centres in a period of six months were assessed using the Brief Repeatable Battery and the Stroop Test. A total of 1040 patients where included in the study, 167 with clinically isolated syndrome (CIS), 759 with relapsing remitting (RR), 74 with secondary progressive (SP) and 40 with primary progressive (PP) disease course.

Overall, the prevalence of cognitive impairment was 46.3%. By clinical subtype the prevalence was in CIS 34.5%, in RR 44.5%, in SP 79.4% and in PP 91.3%. In multivariable logistic regression analysis, the presence of cognitive impairment was significantly associated with higher EDSS and older age, with no independent effect by disease duration or clinical subtype.

To analyse the impact of a paediatric onset in cognitive impairment, a subset of 119 patients with POMS and 712 adult onset MS (AOMS) with relapsing or SP forms were compared.

The prevalence of cognitive impairment was 48.0% in AOMS and 44.5% in POMS; with similar neuropsychological profile. However, when adjusting for age, we found a significantly increased risk for cognitive impairment (OR=1.71; p=0.02) and for impairment in information processing speed (OR=1.86; p<0.01) in patients with POMS. A higher EDSS was also identified in POMS (p=0.03) compared with AOMS patients.

3.2. *To assess and compare the cognitive performance in patients with MS and healthy individuals using BoT.*

The BoT test was applied in 30 consecutive patients with MS and 30 age and education matched-controls, first under supervision in a hospital clinic, and then one week later from home. The patients were also assessed using a standard neuropsychological battery, and repeated the test from home every 4 weeks for three months.

The Cronbach's alpha was 0.89, test scores were significantly different between patients and controls (p<0.01; Cohen's d=0.87). Among patients with MS, test scores were significantly lower in patients with CI when compared with their cognitively preserved counterparts; p<0.001, with a large effect size (Cohen's d=2.0). The test scores presented a good correlation with standard neuropsychological tests, particularly with measures of information processing speed. In the test-retest reliability analysis 10/11 subtests presented a intraclass consistency correlation coefficient >0.70.

Conclusion

In this thesis, we were able to provide new insights into the epidemiology and determinants of cognitive impairment in the older population and in patients with MS, as well as to design, develop and validate a novel strategy to monitor cognitive performance in these settings.

The results from the prevalence study on MCI and dementia in the EPIPorto cohort highlight the importance of vascular cognitive impairment in Portugal, with potential for impact in Public Health strategies.

Regarding cognitive impairment in MS, we documented an important presence of cognitive impairment since the earlier stages of disease and contributed to a clarification of the role of patient age, rather than disease duration, in determining the risk for cognitive impairment. Furthermore, we found evidence that MS patients with a history of POMS are at an increased risk for cognitive impairment and physical disability.

The BoT test showed good reproducibility, correlation with existing cognitive tests, ability to identify clinically relevant differences, and high test-retest reliability when performed from home. We were able to improve its diagnostic accuracy by implementing an adaptive part, and demonstrated the feasibility of its use for longitudinal assessment from home, both in MCI and MS patients. The BoT test could be a suitable tool for screening and monitoring cognitive impairment in these settings, providing a low-cost strategy with potential for easy diffusion through the health system.

Resumo

Introdução

O declínio cognitivo pode surgir, potencialmente, de qualquer processo patológico que provoque dano no sistema nervoso central (SNC), com forte impacto nos doentes, nas suas famílias e na sociedade. Dois grupos estão em risco particularmente aumentado para declínio cognitivo: 1) a faixa etária mais idosa, com marcada incidência de doenças neurodegenerativas e doença vascular cerebral, e 2) doentes com patologia crónica do SNC de início precoce na vida adulta, como a esclerose múltipla (EM). Existem muitas questões mal-esclarecidas no que toca à história natural e determinantes da deterioração cognitiva nestes diferentes modelos de doença do SNC. As atuais estratégias de rastreio e monitorização de deterioração cognitiva nestes grupos poderiam ser melhoradas através uma abordagem longitudinal, medindo variação do desemprenho cognitivo, em vez do estado cognitivo em determinado momento.

O objetivo global desta tese é contribuir para uma melhor compreensão da história natural e determinantes do declínio cognitivo, utilizando ferramentas de avaliação cognitiva transversal e desenvolvendo novas abordagens longitudinais para monitorizar o desempenho cognitivo em dois modelos de deterioração cognitiva: a população mais idosa, em risco de declínio cognitivo ligeiro (DCL) e demência; e doentes com EM.

Os parágrafos seguintes descrevem os objetivos específicos da tese, assim como os correspondentes métodos e resultados.

1. *Desenvolver uma ferramenta de monitorização cognitiva à distância.*

Foi desenvolvido um teste computorizado autoaplicado para rastreio e monitorização cognitiva, Brain on Track (BoT). O teste pode ser feito a partir de qualquer computador pessoal, sendo composto por subtestes dirigidos aos diferentes domínios cognitivos. Uma versão inicial (A) e uma versão refinada do teste (B) foram aplicadas a doentes com DCL ou demência em estadio inicial (n=88) e em controlos emparelhados por idade e escolaridade. Uma subamostra de uma coorte de base populacional (n=113) realizou o teste a partir de casa de três em três meses para avaliar a fiabilidade teste-reteste.

O alfa de Cronbach foi de 0,90, as pontuações foram significativamente maiores nos controlos que nos doentes (p=0,001), a área sob a curva característica de operação do recetor (ASC) foi de 0,75 e o teste foi capaz de identificar diferenças clinicamente significativas. Na análise de fiabilidade teste-reteste, 7/8 subtestes apresentaram coeficiente de consistência da correlação intraclasse >0,70.

2. *Contribuir para uma melhor compreensão do DCL e demência na população geral*

2.1. *Descrever a prevalência e as causas mais comuns de DCL e demência numa coorte de base populacional portuguesa (EPIPorto)*

Os participantes com ≥55 anos na reavaliação de 2013-2015 da coorte EPIPorto (n=730) foram avaliados com o Mini Mental State Examination e Montreal Cognitive Assessment. Os que ficaram abaixo dos pontos de corte ajustados para a população portuguesa foram avaliados por neurologista, para determinar a presença de DCL e demência e definir a etiologia mais provável.

As prevalências padronizadas de demência e DCL foram 1,0% e 4,0%, (população portuguesa, método direto). A causa mais comum de MCI e demência foi doença vascular cerebral (52,8%), seguida da doença de Alzheimer (36,1%).

2.2. Avaliar *e comparar prospectivamente a variação do desempenho cognitivo usando Brain on Track em indivíduos saudáveis da população geral e doentes com DCL.*

Foram recrutados 30 doentes com suspeita de DCL consecutivos de uma consulta de memória e 377 controlos saudáveis, uma subamostra da coorte EPIPorto. Todos os participantes realizaram uma avaliação neuropsicológica e o teste *Brain on Track* no início do estudo, que incluía dois novos subtestes com dificuldade adaptada ao desempenho esperado dos participanres. O teste BoT foi repetido a partir de casa a cada três meses por um ano. Um modelo de efeitos lineares mistos, ajustados pela máxima probabilidade restrita, foi construído para descrever e comparar o desempenho cognitivo entre os grupos. A precisão diagnóstica do teste *BoT* para uso único e repetido foi avaliada através da ASC.

Todos os participantes aumentaram as pontuações nos primeiros testes, mas após 120 dias, aqueles com DCL apresentaram declínio a uma taxa estatisticamente significativamente maior do que os da população em geral. O AUC para detetar DCL no uso único do BoT foi de 0,86, enquanto que para uso repetido na monitorização de um ano a AUC foi de 0,96.

3. *Contribuir para uma melhor compreensão do défice cognitivo na EM*

3.1. *Descrever a prevalência, o perfil e os determinantes clínicos do défice cognitivo numa amostra de doentes com EM* e **3.2** *Avaliar o impacto do início pediátrico de EM (EMIP) no desemprenho cognitivo a longo prazo.*

Doentes consecutivos com EM observados em seis centros italianos durante um período de seis meses foram avaliados utilizando a *Brief Repeatable Battery* e o *Stroop Test*. Um total de 1040 doentes foram incluídos no estudo, 167 com síndrome clinicamente isolado (SCI), 759 com forma surto-remissão (SR), 74 com forma secundária progressiva (SP) e 40 com forma primária progressiva (PP).

A prevalência de défice cognitivo foi de 46,3% na amostra. Por fenótipo clínico foi de 34,5% para o SCI, 44,5% para SR, 79,4% para SP e 91,3% para PP. Num modelo de regressão logística múltipla, a presença de défice cognitivo foi associada ao EDSS e idade avançada, sem efeito independente por duração da doença ou fenótipo clínico.

Para avaliar o impacto do início pediátrico de EM no desempenho cognitivo, foi comparado o grupo de 119 doentes com EMIP com o grupo de 712 doentes com início em idade adulta (EMIA), com formas SCI, SR ou SP.

A prevalência de défice cognitivo foi de 48,0% na EMIA e 44,5% na EMIP; com perfil neuropsicológico semelhante. No entanto, ao ajustar para o efeito da idade, foi encontrado um risco significativamente aumentado de défice cognitivo (OR=1,71; p=0,02) e de défice na velocidade de processamento de informação (OR=1,86; p<0,01) em doentes com EMIP. Um EDSS mais elevado também foi identificado em doentes com EMIP (p=0,03) em comparação com EMIA.

3.2. *Avaliar e comparar prospectivamente a variação do desempenho cognitivo usando o Brain on Track em doentes com EM e indivíduos saudáveis.*

O BoT foi autoaplicado por 30 doentes consecutivos com EM e 30 controlos emparelhados para idade e escolaridade, primeiro sob supervisão em ambiente hospitalar, e uma semana depois a partir de casa. Os doentes foram também avaliados usando uma bateria neuropsicológica e repetiram o teste a cada quatro semanas durante três meses, a partir de casa.

O alfa de Cronbach foi de 0,89, com resultados do BoT significativamente diferentes entre doentes e controlos (p<0,01; Cohen's d=0,87 de Cohen). No que respeita aos doentes com EM, o desempenho no BoT foi significativamente inferior em doentes com défice cognitivo, quando comparados com os doentes cognitivamente preservados (p <0,001; Cohen's d=2,0). O BoT apresentou boa correlação com testes neuropsicológicos padrão, particularmente com medidas de velocidade de processamento de informação. Na análise de fiabilidade teste-reteste 10/11 subtestes apresentaram coeficiente de correlação de consistência intraclasse> 0,70.

Conclusão

Na presente tese foram apresentados novos dados sobre a epidemiologia e determinantes do declínio cognitivo na população mais idosa e em doentes com EM, e foi possível desenvolver e validar o uso de uma nova ferramenta para monitorizar o desempenho cognitivo em ambos cenários.

Os resultados do estudo de prevalência de DCL e demência na coorte EPIPorto relevam a importância da deterioração cognitiva de etiologia vascular em Portugal, com impacto ao nível das estratégias de Saúde Pública.

No que toca à EM, foi documentada a presença de défice cognitivo desde os estadios iniciais e foi possível contribuir para o esclarecimento do papel da idade do doente, ao invés da duração da doença, no risco de deterioração cognitiva. Adicionalmente, foi demonstrado que doentes com início pediátrico de EM têm maior risco de défice cognitivo e incapacidade.

O teste BoT mostrou boa reprodutibilidade, correlação com testes cognitivos existentes, capacidade de identificar diferenças clinicamente relevantes e boa fiabilidade teste-reteste realizado a partir de casa. Foi possível melhorar a sua precisão diagnóstica introduzindo uma componente adaptativa, e demonstrada a viabilidade do seu uso para avaliação longitudinal, tanto em doentes com MCI como em doentes com EM. O teste BoT pode constituir-se como uma ferramenta adequada para rastreio e monitorização de défice cognitivo nestes cenários clínicos, fornecendo uma estratégia de baixo custo e com potencial para uma fácil difusão através do sistema de saúde.

1. Introduction

1.1 Cognition and cognitive impairment

Cognition is the essential and defining trait of human condition. The specialization of cells into neurons, the organization of neuronal tissue, the development of functional brain networks and the emergence of the cortical association areas resulted from an evolutionary process that spanned over millions of years, providing the human species with unmatched cognitive skills[1]. These skills allow humans to be self-aware, to judge situations and make decisions, to predict potential events and consequences, to set and accomplish meaningful goals, to understand and modify their surroundings, to communicate with others, using reason and emotion, and to build interpersonal and social relations[2-5]. The loss of any of these abilities has a profound impact in the autonomy of the individual, in the ability to grow and maintain human relations and in his/her potential contribution to the society.

Cognitive impairment potentially originates as the result of any pathological process that damages the central nervous system (CNS) (e.g., neurodegenerative, traumatic, inflammatory, vascular). These processes can be characterized by the disruption of neuronal networks, loss of neuronal

connections and/or cellular death. After the neurodevelopmental stage, functional impairment may develop either through significant cellular death in specialized cortical areas or interruption of neuronal connections and networks. Most frequently, cognitive impairment arises after cumulative brain damage from a combination of pathological processes[6].

There are two population groups at a particularly high risk for cognitive impairment:

a) The elderly, due to the combination of an increased risk for age related neurodegenerative and cerebrovascular disease with cumulative life-time exposure to multiple brain insults;

b) Young patients affected by early onset progressive chronic brain disorders, such as multiple sclerosis (MS), neurosarcoidosis, neurolupus and schizophrenia.

The conditions that contribute to cognitive impairment in both these groups have an undeniable public health impact. Alzheimer's disease and cerebrovascular dementia strongly affect the quality of life of millions of patients and their families worldwide, imposing a major social and economic burden in western societies[7,8]. Furthermore, these diseases, closely associated with ageing and vascular risk factors, are set to become main global public health priorities as less affluent countries undergo demographic and epidemiological transition[9]. The effect of early onset neurologic disorders, such as MS, in cognitive functions has been historically underestimated, but in the recent decades the presence of cognitive impairment among patients

with these conditions has been extensively documented[10]. Cognitive impairment is present in the early and later stages of MS and across its different subtypes[11], and can be the presenting feature of the disease[12]. Its prevalence ranges from 40% to 65%[10], and it is an important predictor of quality of life, physical independence, competence in daily activities and symptom management in patients with MS[13]. All these issues highlight the importance of cognitive impairment in MS and the need for increased efforts in this research field.

Both for elderly patients with neurodegenerative and cerebrovascular disorders and young patients with CNS diseases at risk for cognitive impairment, the timely identification of cognitive deficits can be crucial to guide current therapeutic interventions[14,15], cognitive training[16-18], functional rehabilitation[19] and symptom control[20]. Identifying cognitive impairment at an early stage could also be the key to the successful trial and implementation of curative treatments[21-23]. However, there are still many unanswered questions regarding the natural history and determinants of cognitive impairment in these different models of progressive brain disease.

In the next sections, we will discuss challenges and relevant research questions that pertain to cognitive research in dementia and multiple sclerosis, and how they can be addressed, using standard and innovative solutions for cognitive testing.

1.2 Challenges and unanswered questions in neurocognitive research

Like other biological functions, cognitive abilities change with aging[24]. A slow decline in performance is observed, particularly in cognitive domains such as memory and information processing speed[25]. However, unlike many other biological functions, cognitive abilities are highly variable in the general population[26]. Furthermore, cognitive performance in healthy adults is better determined by their performance at a very young age than by their lifelong occupation, educational attainment or measures of brain damage[27,28]. This high variability in cognitive abilities, the difficulty to predict a level of expected performance based on the occupation and educational attainment and its changes over the life course, constitute a challenge for an accurate assessment and classification of the cognitive impairment in a given subject[26]. Different healthy individuals can have vastly different cognitive performance and decline at different rates, as depicted in Figure 1. Although the reference values of cognitive tests are usually stratified by age and educational attainment, these, as discussed above, are not the only determinants of cognitive performance, and fail to explain a large proportion of its variability[27,28]. Other determinants, such as the cognitive enrichment through the life course and the structural and functional cognitive reserve can be challenging or impractical to measure[29,30].

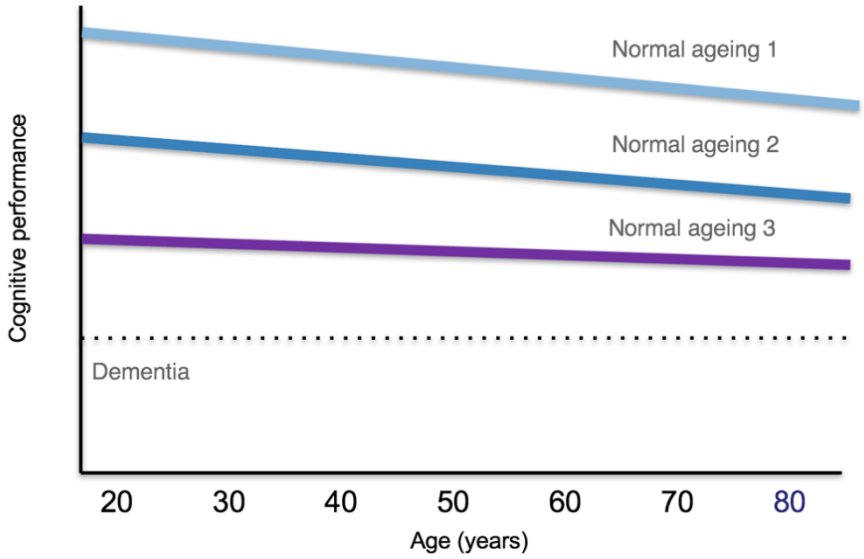

Figure 1 – Model of cognitive aging, illustrating the heterogeneity of cognitive performance in the general population

Another important challenge in cognitive research is that cognitive impairment usually results from a long and slow process of lesion accumulation and disruption of cognitive networks, with partial compensation by neuroplasticity[31]. This process can last for many years before leading to a disruption of function. Therefore, its beginning and initial stages are frequently unnoticed by the patients, relatives and physicians[32-34]. Cross-sectional studies can provide multiple snapshots into the natural history of cognitive impairment; however, for most nosological entities, the pre-symptomatic phase, the pattern and rate of progression are not well-known.

1.2.1 Unanswered questions in dementia research

Dementia is the end stage of several neurological disorders, most of them slowly progressive and associated with aging, with Alzheimer's disease, vascular dementia, Lewy body disease and Parkinson's disease dementia being the most common in Europe[35].

Dementia is characterized by a progressive loss of cognitive functions, among which memory is the most frequently affected domain, though usually it is not the only one involved[36]. The onset of dementia is often preceded by a long pre-symptomatic phase in which cognitive reserve, behavioural and social adaptations are able to compensate for the progressive loss of neurons and their connections[34]. In progressive disorders, this stage further evolves to mild cognitive impairment (MCI), where cognitive complaints and an objective decrease in cognitive performance exist in the absence of any loss of independent function, and then to dementia, after irrevocable loss of autonomy[36]. A model for this progression is depicted in Figure 2.

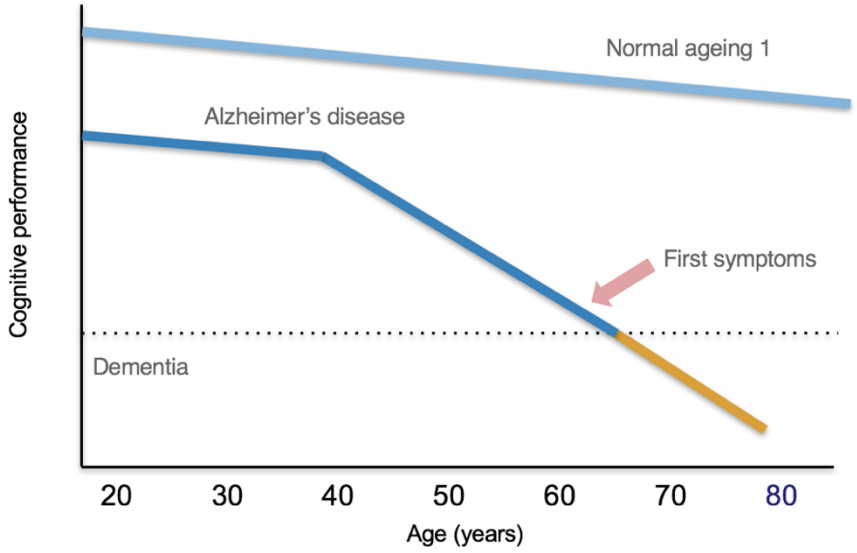

Figure 2 – Model of the progression of cognitive performance in a patient with Alzheimer's disease.

Despite being a major public health problem, there are still scarce data on the prevalence of MCI and dementia in Portugal. The only published epidemiological study was performed in 2003, identifying a prevalence of 2.7% for dementia and 12.3% for all causes of cognitive impairment, including psychiatric and congenital disorders, in the population between 55 to 79 years[37]. Interestingly, vascular dementia presented a relatively high frequency (38.7%), being equal with the prevalence of Alzheimer's disease (38.7%) as the most frequent causes of dementia, in contrast with what is generally described in western countries, where Alzheimer's disease is the leading cause of dementia[35]. Additional studies are needed to replicate these findings in different populations, and to monitor their variation over time.

There are some important challenges in dementia research, mostly related to the slow pace of the pathological changes of most diseases that lead to dementia and the long latency period of these disorders, both

combined with technical difficulties in studying the pathology of the brain *in vivo*, as well as to the difficulties in accurately assessing cognitive performance. As a result, the management of most disorders leading to dementia involves complex and expensive diagnostic workups that are less accurate than desirable, and therapeutic approaches that have some impact in individual patients[38], but fall short of producing relevant public health outcomes[21-23]. Additionally, in clinical practice, it is often challenging to distinguish the age-associated cognitive decline from the early phases of a progressive neurodegenerative disorder. Memory complaints are very frequent in the older population, and can be easily disregarded as a natural consequence of aging. Although they can be just that, patients with memory complaints are frequently found to have minor cognitive deficits in the formal neuropsychological assessment: while some could be in the pre-clinical stages of neurodegenerative dementia, in others, the minor cognitive deficits result from discrete insults to the brain during the life course, and will not progress.

A longitudinal, rather than a discrete assessment of cognitive performance, may be a promising strategy to better understand and characterize the early stages of cognitive deterioration, and to further increase our ability to distinguish static from progressive cognitive impairment. This strategy would allow to identify and describe the different patterns of cognitive decline in patients with progressive cognitive disorders, patients with static cognitive impairment and the cognitive changes associated with ageing. Indeed, recent work has showed that longitudinal neuropsychological

assessment can identify gene-specific pre-symptomatic patterns of decline in familial fronto-temporal dementia, confirming the potential prognostic value of neuropsychological assessment as a clinical biomarker[39]. However, these findings have yet to be demonstrated in other nosological contexts.

1.2.2 Unanswered questions in multiple sclerosis cognitive research

Multiple sclerosis is a progressive multifocal inflammatory disease of the CNS. It evolves mainly through acute inflammatory demyelinating relapses, often associated with the onset of motor, visual, sensitive or dysautonomic symptoms. The prevalence of cognitive impairment in MS available from published reports varies widely, from 40% to 65%[10]. These estimates are even more variable when considering the different MS clinical subtypes, and it is still not clear how the frequency of impairment differs between them[10,11,40,41]. Additionally, while the overall neuropsychological profile of impairment in patients with MS is now relatively well described, few studies investigated the differences in the profile of cognitive impairment across disease subtypes and presented heterogeneous results[11,40,42,43].

A better understating of the independent effects of age, disability, disease duration and disease subtype could prove central to provide a valuable insight on the potential role and interaction of cognitive reserve, brain aging and disease severity for determining cognitive deterioration in MS. However, the association of cognitive impairment with these different demographic and clinical variables is not well established, as inconsistent results have been reported[44-47]. Particular populations of patients with MS, like the patients with paediatric onset MS (POMS), could be at an increased risk for cognitive impairment, given the potential harmful effects of disease activity

in neurodevelopment. However, there is scarce information on their long-term cognitive outcomes. The few longitudinal studies published to date have relatively short follow up periods (1-5 years) and present heterogeneous results concerning the presence and rate of cognitive worsening over time [48-50]. A single previous study compared the cognitive performance in patients with POMS and their adult counterparts, using only the Symbol Digit Modalities Test (SDMT), with the results showing a worse test performance in patients with POMS[51].

The natural history of cognitive impairment in MS is still not well-known. Cognitive decline in MS patients could be explained by the combination of lesions over trans-cortical white matter connexions and progressive cortical neurodegeneration, but the relative contribution of each neuropathological process is uncertain. Grey matter lesions and cortical atrophy are increasingly being reported in neuropathology series[52] and imaging studies [53]. In addition, clinically silent white matter lesions have long been described in cerebral magnetic resonance imaging (MRI) of patients with MS [54], with a frequency that exceeds that of clinical relapses by a factor of two to 10[55]. These silent demyelinating lesions could be the reason behind the onset of new cognitive deficits and their early recognition could prove decisive to prevent further cognitive deterioration. Evidence of probable isolated cognitive relapses has been found[56], and these could be related to the silent demyelinating lesions. However, some of the few cognitive longitudinal studies performed in patients with MS have described a more progressive pattern of decline[57-59], while others were not able to identify cognitive decline at all[60-62]. These conflicting results

could be attributed, at least in part, to the short time span, small sample sizes and/or learning effects of the cognitive assessment tools used.

If we assume that cognitive impairment in MS is mainly driven by cognitive relapses, it would follow a relapsing remitting course, with acute worsening and, at least initially, close to complete recovery between relapses, with progression in cognitive impairment being the result of the accumulation of lesions and incomplete recovery (Figure 3).

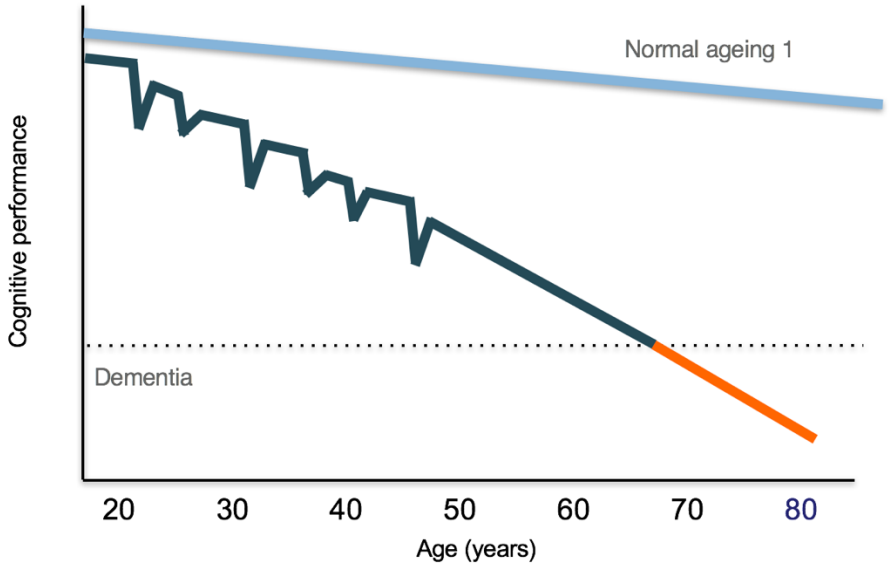

Figure 3 – Model of a relapsing remitting course of cognitive performance in a patient with multiple sclerosis

Another possibility is that cognitive impairment in MS may follow a continuous slow progression, mainly driven by degenerative processes, namely Wallerian degeneration and cortical atrophy (Figure 4).

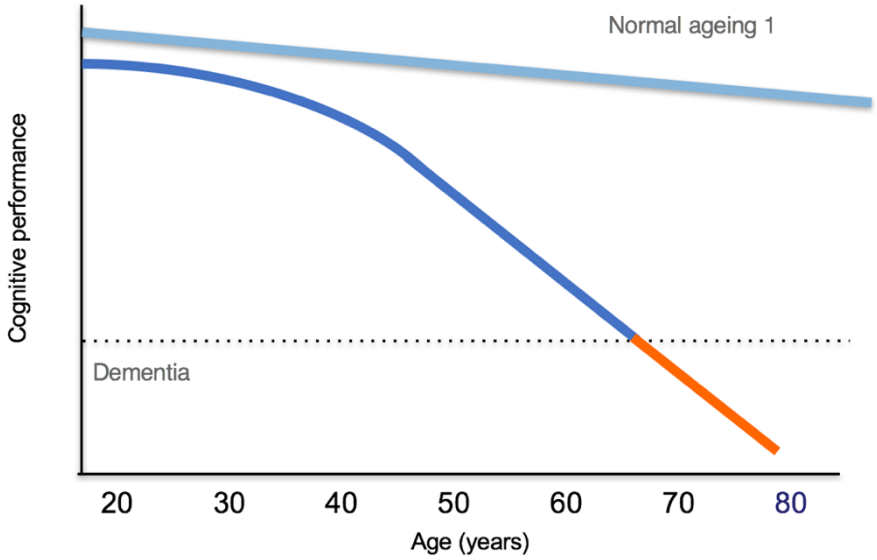

Figure 4 – Model of a progressive course of progression of cognitive performance in a patient with multiple sclerosis

The elicitation of the natural history of cognitive decline in MS would have a meaningful impact in the design of effective measures to prevent, treat and rehabilitate cognitive impairment in MS. However, to clarify these questions, it is decisive to correctly measure the progression of cognitive performance in patients with MS, by increasing the periodicity of assessment, while trying to minimize learning effects. A possible strategy to reach this aim will be laid out in the next sections.

1.2 Improving the assessment of cognitive impairment

The assessment of cognitive functions through formal testing is the only suitable approach to identify and monitor the progression of cognitive impairment, as there are no good imaging or biochemical surrogates of cognitive performance. The gold standard for cognitive assessment is an extensive evaluation of cognitive performance by a trained professional[63], using multiple neuropsychological tests. These tests rely on specific tasks, designed to target different cognitive domains, and are applied through standardized procedures. There are several of these test batteries validated for clinical use, which present a relatively high sensitivity (80-98%) and specificity (44-98%) for the detection of cognitive impairment[64]. Neuropsychological test batteries are also an important tool for neurocognitive research. They can provide a valuable insight into the profile of cognitive impairment in different diseases and disease subtypes and allow for a comprehensive characterization of the differences between stages of disease. Furthermore, they can contribute to the differential diagnosis of cognitive disorders in the clinical setting. However, given the need for the intervention of specialized human resources and their lengthy time of application, they are not suitable to be used as the basis of a screening strategy in the general population, or to monitor patients at risk for cognitive impairment.

Several brief cognitive tools have been developed with the aim of providing a more practical alternative to neuropsychological test batteries, being shorter in duration and offering the possibility to be applied by any health professional with minimal training. The most widely used are probably the mini mental state examination (MMSE)[65] and the Montreal cognitive assessment (MoCA)[66]. The MMSE was developed in 1975, primarily as a tool for a standardized and simplified examination of cognitive mental status in patients with delirium, psychiatric disorders or dementia. It evaluates five cognitive domains: orientation, attention, memory, language and visuo-construction abilities, with no assessment of executive functions or working memory[65]. The MoCA test was developed in 2004, with the goal of an improving the sensitivity to detect MCI and early dementia, and including all the domains assessed in the MMSE, plus executive functions or working memory)[66]. In all, cognitive screening instruments have reached widespread acceptance in many settings, and can certainly contribute to improve the referral of patients to specialized care. However most of the available tools lack the discriminative ability to accurately identify mild cognitive impairment and predict its conversion to dementia[67,68].

The challenges of diagnosing early cognitive impairment based on cross-sectional assessment are illustrated in Figure 5. This model depicts the results of assessing different individuals with a different cognitive reserve, two with normal aging and one with progressive cognitive impairment. As exemplified, a patient with pre-symptomatic progressive decline of several years can present a higher cognitive performance than an individual with lower cognitive

reserve in a discrete cross-sectional assessment. As previously mentioned, the cognitive reserve of the individual can be predicted, to a certain degree, by the educational level, and the accuracy of cognitive tests can be improved using cut-points adjusted for this variable. However, this measure alone is unable to explain most of the variability in cognitive performance[25-27].

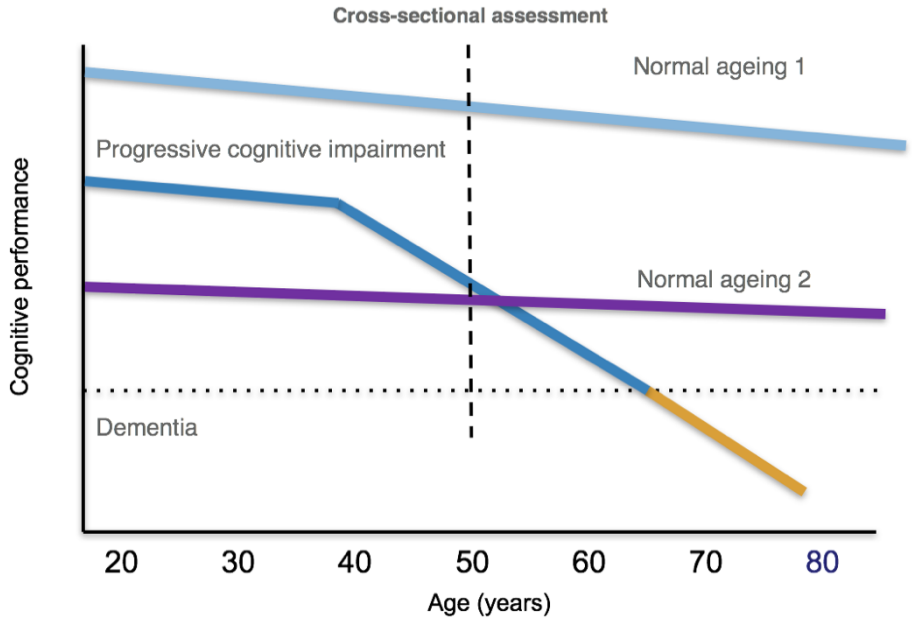

Figure 5 – Model of cross-sectional assessment of cognitive impairment in individuals with different cognitive reserve

The clinical definition of cognitive deterioration and dementia implies a decline in performance from a pre-morbid cognitive function, leading some authors to suggest that cognitive measurements should record changes in state, rather than the current state[69]. Accordingly, follow-up cognitive testing has been recommended to improve the diagnostic reliability for MCI[63] and to monitor cognitive deterioration in patients with MS[70]. Using the model described in Figure 5 to illustrate this concept, assessing cognitive

performance by repeated testing could identify decline from a previous state, providing for the earlier identification of progression (Figure 6). This longitudinal approach could complement the current strategies of cognitive testing, and contribute to overcome some of their limitations.

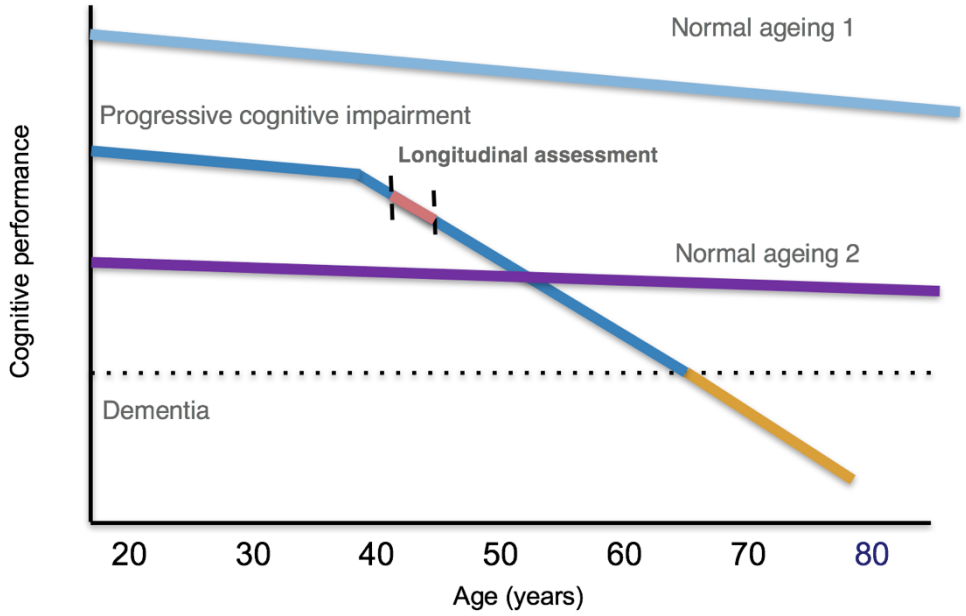

Figure 6 – Model of longitudinal assessment of cognitive impairment in individuals with different cognitive reserve

A longitudinal approach to cognitive testing could provide solid ground for the implementation of novel strategies with improved discriminative ability for screening and monitoring cognitive impairment. It would potentially impact the research field and clinical fields, by allowing the early detection of pre-symptomatic cognitive impairment, by providing better case finding and classification of cognitive impairment in large epidemiological studies, and by improving the measurement of cognitive outcomes in therapeutic trials.

Neuropsychological test batteries, given their lengthy time of application, can hardly be used repeatedly as a long-term monitoring strategy. Very few of these tools have been designed for longitudinal cognitive monitoring[67,68], and they still depend on a trained external evaluator and periodic clinical visits[67,71]. The large majority of brief cognitive tests, namely some of the most widely used such as MoCA and MMSE, have also not been clinically validated for this purpose[67,68]. Furthermore, both neuropsychological test batteries and brief cognitive tests are prone to learning effects[72]. In repeated testing, individuals can learn the correct answer, and show improved results over time, which limits the sensitivity of tests for cognitive impairment in serial use[73]. Indeed, widely used tests such as the MMSE and MoCA present significant learning effects[74], even in large time spans such as 12 months[75].

In the next sections, we will discuss the potential benefits and limitations of computerized tests, and how they could provide some advantages over pen and paper tools to design clinical and epidemiological approaches based on longitudinal cognitive testing.

1.2.1 Computerized cognitive assessment

Computerized cognitive tests have been available for several decades, with well-known advantages for use in clinical and research settings, including lower costs, the ability to accurately measure and store test responses and latency times, the minimization of examiner subjectivity and the potential for multiple test versions, that can minimize learning effects and allow for adaptive testing[76,77]. Some of the computer-based batteries have already shown an overall reliability and discriminative ability comparable to traditional neuropsychological testing[76,77]. However, there are also potential limitations for computerized cognitive testing, namely the lack of familiarity with computer interfaces, that could have a negative effect on test response[78] and a perceived lack of adequately established psychometric standards[79].

Most of the existing computerized cognitive tools have been designed to closely mirror the pen and paper tests[79]. On the one hand, this has the advantage of using established neuropsychological paradigms for cognitive testing, but, on the other hand, these tests do not take advantage of the flexibility and additional potential for interaction of computer-based platforms. Additionally, using these paradigms demands the presence of a trained professional to provide the cues and evaluate the answers, also not taking advantage of the potential for dissemination and diminished costs of computer based testing. Furthermore, most of the available computerized cognitive tests are intended as comprehensive neuropsychological assessment batteries[79],

not being designed for screening or monitoring cognitive impairment[80]. Several computerized cognitive tools aimed at screening for cognitive impairment have also been developed. Some of these tests have shown good diagnostic accuracy and have entered clinical use, such as the National Institutes of Health Toolbox Cognition Battery[81], the CogState[82] and the Cognitive Stability Index[83]. However, while these tests can replace the existing pen and paper screening tests like MoCA and MMSE with some potential advantages, they still require a trained evaluator and a patient visit to a clinic.

In the last years, a few cognitive tests have been developed and validated that allow for self-administration and remote testing, such as the Computer Assessment of Mild Cognitive Impairment[84], MicroCog[85] and COGselftest[86]. While these tests showed good neuropsychological parameters when the tests were conducted in a clinical setting, they were primarily designed for single use and were not validated for follow-up testing or cognitive monitoring[77,87]. To the best of our knowledge, none short cognitive computerized test has been specifically designed for longitudinal use[77,87].

1.2.2 The potential of longitudinal web-based cognitive assessment

In the last sections, we have discussed the potential contribution of a longitudinal testing strategy to identify abnormal patterns of cognitive decline, and the possible advantages of computerized testing to achieve such a strategy.

Particularly amongst cognitive computerized tests, a repeatable web-based test could be the basis for a successful implementation of such a longitudinal strategy of cognitive assessment. The flexibility of the web platform would provide for continuous test development, adaptive testing, and easy monitoring and rapid adjustment of diagnostic standards. The use of web-based software is also an advantage for easier multiplatform implementation. The possibility to perform and repeat the test from home and in different personal computers would allow to improve patient adhesion to cognitive monitoring, demanding less human and financial resources than the current alternatives. A web-based computerized test could also contribute to minimize the learning effects, by providing multiple alternative versions and by using random elements and sequences in the tests. Such a strategy for cognitive assessment would have also a great potential for pervasive diffusion through web based technologies, could improve patient access to cognitive diagnosis and treatments, facilitate the development of patient based outcomes and ultimately reduce the costs for health systems and allow for the implementation of payment models based on outcomes assessment.

In all, this strategy could be an important complement to the current standard of cognitive assessment based on comprehensive testing by an experienced neuropsychologist, that would still retain its important role for establishing the definitive diagnosis, as a part of the etiologic study and for the characterization of the neuropsychological profiles in the research setting.

2. Aims

The global aim of this thesis is to contribute for a better understanding of the natural history and determinants of cognitive impairment, by using standard cross-sectional cognitive assessment tools and by developing novel longitudinal approaches to monitor cognitive performance. Two groups that are at an increased risk for cognitive impairment were selected: the older population, with incident MCI and early dementia and patients with MS. The specific objectives are the following:

1. To develop a tool for web-based cognitive monitoring - Brain on Track (BoT) – **Paper I**.

2. Among the general population at risk for incident MCI and dementia:

2.1. To describe the prevalence and most common causes of cognitive impairment in a population-based cohort (EPIPorto) – **Paper II.**

2.2. To prospectively assess and compare the variation of cognitive performance using BoT in individuals from the general population (EpiPorto cohort) and patients with MCI – **Paper III.**

3. Among patients with MS:

3.1. To describe the prevalence, profile and clinical determinants of cognitive impairment – **Paper IV**.

3.2. To assess the impact of a paediatric disease onset on the long term cognitive outcomes– **Paper V**.

3.3. To prospectively assess and compare the variation of cognitive performance using BoT– **Paper VI.**

3. Methods

This thesis was developed through a sequential and incremental research process, involving several studies in different settings, with the support of a multidisciplinary team, and involving different research and clinical institutions. To accomplish the objectives several research methodologies and study designs were used, in population and clinical settings. The detailed methods for each of the studies will be detailed in depth the following sections, but follows a brief overview.

In Paper I we describe the development of the BoT test and its validation, using two sequential samples of patients with MCI, recruited in the Memory Clinic at *Centro Hospitalar Entre Douro e Vouga* and matched controls (n=88), and then the longitudinal application of the test, to assess test-retest reliability in a sub-sample of the population based cohort EPIPorto (n=113). This cohort is based on a representative sample of the adult population of Porto selected by random digit dialling of landline telephones in 1999-2003, and it is based in the *Instituto de Saúde Pública da Universidade do Porto*. Software development for the test based was in a start-up company dedicated to the development of new tools for cognition assessment and rehabilitation (Neuroinova).

For Paper II we assess the prevalence, determinants and main causes of cognitive impairment and dementia in the normal population, using data from the 2014-2016 revaluation of the population based cohort EPIPorto. All the

participants older than 55 years that attended this evaluation (n=730) were assessed using standard cognitive screening tools, and those with possible cognitive impairment were referred for clinical assessment by a Neurologist; the participants' electronic health records were also searched to identify established clinical diagnosis.

In Paper III we describe the application of the BoT test in two settings, patients with probable MCI (n=30) recruited from the Memory Clinic at *Centro Hospitalar Entre Douro e Vouga* and a sub-set of healthy individuals from the population based cohort EPIPorto (n=312). All participants performed a neuropsychological assessment and the BoT test at baseline, including two new subtests with difficulty adapted to the patients' expected level of performance, and were asked to perform the test from home every three months. The trajectories of cognitive performance measures using Brain on Track over one year were compared between the two groups and the overall accuracy of BoT to distinguish between MCI patients and matched controls was assessed, both for single and repeated use.

Papers IV and V are based in a multicentre Italian cross-sectional study in which consecutive patients with MS were recruited in six different centres (n=1040). Cognitive performance was assessed through the Brief Repeatable Battery and the Stroop test, clinical and demographic patient data was collected using a common database shared among the participating centres. This study results from a collaboration with the NEUROFARBA research unit, of the *Università degli Studi di Firenze*, where I performed a three months clinical and research internship in 2015.

In Paper VI we describe the application of the BoT test in patients with MS, recruited in the MS Clinic of *Centro Hospitalar de Entre Douro e Vouga* and *Hospital de Braga*, compared with age and education-matched community controls (n=60). We further compare the performance of patients with MS in BoT with the results from cognitive screening tests and a neuropsychological test battery.

4. Development and validation of the Brain on Track test

4.1 Development of a self-administered web-based test for longitudinal cognitive assessment (Paper I)

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Development of a self-administered web-based test for longitudinal cognitive assessment

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Sequential testing with brief cognitive tools has been recommended to improve cognitive screening and monitoring, however the few available tools still depend on an external evaluator and periodic visits. We developed a self-administered computerized test intended for longitudinal cognitive testing (Brain on Track). The test can be performed from a home computer and is composed of several subtests, expected to evaluate different cognitive domains, all including random elements to minimize learning effects. An initial (A) and a refined version of the test (B) were applied to patients with mild cognitive impairment or early dementia (n = 88) and age and education-matched controls. A subsample of a population-based cohort (n = 113) performed the test at home every three months to evaluate test-retest reliability. The test's final version Cronbach's alpha was 0.90, test scores were significantly different between patients and controls ($p = 0.001$), the area under the receiver operating characteristic curve was 0.75 and the smallest real difference (43.04) was lower than the clinical relevant difference (56.82). In the test-retest reliability analysis 9/10 subtests showed two-way mixed single intraclass consistency correlation coefficient >0.70. These results imply good internal consistency, discriminative ability and reliability when performed at home, encouraging further longitudinal clinical and population-based studies.

The timely identification of cognitive deficits can be crucial to guide therapeutic intervention[1], cognitive training[2,3] and functional rehabilitation[4] in patients with neurodegenerative disorders, cerebrovascular dementia and young patients with central nervous system (CNS) diseases such as multiple sclerosis (MS) and traumatic brain injury. The standard for cognitive assessment relies on an extensive evaluation of multiple cognitive domains by a trained professional[5]. These neuropsychological test batteries have a high sensitivity and specificity for the detection of dementia[6]; however, their application is time and resource consuming and therefore not a practical strategy for cognitive screening in the general population or for monitoring cognitive function in patients with CNS diseases. Brief low-cost tools have been developed for these aims, but mostly lack the desired discriminative ability to predict the progression to dementia[7]. As the clinical definition of dementia implies a significant decline in performance from a pre-morbid cognitive function, some authors have suggested that cognitive measurements should record alterations in state, rather than the current state[8]. Accordingly, follow-up cognitive testing has been recommended to improve the diagnostic reliability for mild cognitive impairment (MCI)[5] and to monitor cognitive deterioration in patients with MS[9]. However, few screening tools have been clinically validated for this purpose, and still depend on a trained external evaluator and periodic clinical visits[10,11]. A self-administered web-based cognitive test that could be repeated periodically would present some advantages to address these issues. Namely, it could be performed at home, therefore being more cost-effective and convenient for the patient, allow the use of random elements and alternate sequences to minimize learning effects, and offer the possibility of adapting the testing difficulty to the baseline cognitive performance of the patient. A strategy based on such a tool could be useful for the screening of patients with subjective memory complaints in primary care and to monitor

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patients with CNS diseases at risk for cognitive deterioration. It could also prove useful to identify patients in prodromal phases of progressive neurodegenerative diseases to enroll in clinical trials.

Computerized cognitive tests have existed for several decades, they have several known advantages for use in clinical and research settings: the reduced costs, the ability to accurately measure and store test responses and latency times, the minimization of examiner subjectivity and the potential for multiple test versions, allowing for adaptive testing[12,13]. Some of the existing computerized batteries have already shown an overall reliability and discriminative ability comparable to traditional neuropsychological testing[12,13]. There are also potential limitations for computerized cognitive testing, namely the effect of previous experience with computer interfaces on test response[14] and a perceived lack of adequately established psychometric standards[15].

Most of the existing computerized cognitive tests have been designed to mirror the comprehensive neuropsychological assessment batteries[15], applied by a trained professional in a clinical setting and not designed for screening or monitoring cognitive impairment[16]. In recent years, several shorter cognitive tools aimed at screening for cognitive impairment have been developed; nevertheless most of them still require a health professional to be started. To the best our of knowledge, none has been specifically designed for longitudinal use[13,17]. Therefore, we aimed to develop a web-based self-administered test intended for longitudinal cognitive screening and monitoring.

Methods

Rationale and principles for test development. The Brain on Track test was designed to take full advantage of the features and flexibility of a web-based interface, rather than to replicate the existing pen and paper cognitive tests. As an initial base for subtest development, we used simple computerized cognitive training exercises from an existing online platform (Cogweb), being developed by elements from the same research team since 2005[18]. This web-based platform includes more than 60 cognitive training exercises that target different cognitive domains, allowing for remote cognitive training programs in the patient's living environment. These exercises already passed through extensive usability testing in a wide spectrum of ages and disease models, and it was demonstrated that patients could use them independently and repeatedly from their home computers[18]. We expected that exercises based on goal-oriented tasks would have some advantages as a model for computerized self-administered cognitive subtests. This task-based structure could allow for a better understanding of the objective of each subtest and it would motivate the patient to perform at his/her best level. The stimuli in the subtests were optimized through an iterative process; most of them include simultaneous visual and audio cues, all were designed with high contrast graphics and large font sizes. A pool of 50 potential subtests, most of them adapted from the existing Cogweb exercises, was initially developed. As the Brain on Track was intended to be used repeatedly, random elements and sequences were used to minimize learning effects. All of the subtests include at least a random element in each task or compose of multiple predefined similar tasks that are randomly selected and ordered for each trial. For example, in the Opposite subtest, the participant must press the keyboard arrow in the opposite direction to that shown by a large arrow on screen; the direction of the arrow is randomly generated. Another example, in the Puzzles subtest, there are 40 alternate puzzles of similar design and difficulty; the puzzle selection and order is randomly generated at the start of each trial. Furthermore, the subtests were designed with several versions with different levels of difficulty, to offer the possibility of adaptive testing. Each subtest begins with a set of written instructions that are shown on the screen and read by a pre-recorded voice and has a limited duration of two minutes, including the tests instructions. During that time, the participant must perform the tasks described in Appendix 1. The number of tasks presented to the participant within each subtest is limited only by the time limit. The subtest score is the number of tasks performed correctly in each subtest and varies from 0 to the maximum number of tasks the participant can perform successfully within the time limit.

The subtests were designed and programmed to be light on data usage transmitted over the web and of local computer resources. Before the start of each subtest, the data needed for its completion is loaded into the local browser; only then is the participant able to signal using a dialog box if he/she is ready to start. The system was tested and optimized to work in the different versions of the four most used browsers (Google Chrome, Internet Explorer, Firefox and Safari).

The studies reported in this paper have been approved by the appropriate ethics committees. The web-based system for data collection has been approved by the Portuguese Data Protection Authority. All of the data transmission was encrypted, there was no personal data transmission over the Internet and the participants' identity information was stored in a separate off-line database. All of the participants in the studies gave their informed consent prior to their inclusion; in participants with cognitive impairment the caregiver consent was also requested. The statistical package used was SPSS Statistics 22.0.

Development of the Brain on Track test (Study I). *Development of the first test version – Test A.* From the initial pool of 50 potential subtests, a group of experienced neuropsychologists and neurologists defined and developed a group of 9 subtests, expected to evaluate attention, memory, executive functions, language, constructive ability and spatial processing (Test A), with a total duration of 18 minutes (Word categories task, Attention task II, Sequences, Visual memory task I, Puzzles, Written comprehension, Shopping task, Verbal memory task, Inhibitory control). In the subtest development, we identified the use of the keyboard for word input as a major difficulty in the elderly, being more dependent on the level of previous computer experience than the use of the mouse, and a common source of error through input mistakes when the participant performed the test autonomously. For these reasons, the subtest interface was designed to depend solely on the mouse or on pressing unique keyboard keys, therefore the episodic verbal memory subtest is based on cued recall and a verbal fluency subtest was not included. Subtest description can be found in Appendix 1.

For a cognitive test in the adult population, the minimal important clinical difference (MID) that should be detected corresponds to the change from healthy status to early stage cognitive disorders with clinical complaints.

All participants	
• ≥18 years of age • No physical impairment precluding using a computer and mouse interface	
Study I	
Patients	**Controls**
Mild cognitive impairment • Subjective cognitive complaints over a period of at least 6 months • One cognitive domain 1.5 standard deviations (SD) below norm in the neuropsychological evaluation • No clinical depression • No impairment in daily activities **Or** Mild dementia • Complying DSM-V criteria for major neurocognitive disorder[20] (significant cognitive impairment in at least one cognitive domain representing a significant decline from a previous level of functioning that interferes with independence in everyday activities) • Score of 1.0 in the clinical dementia rating scale[21]	• Absence of any neurological, psychiatric or systemic disease that could impair cognition (except for stable depressive symptoms) • Absence of drugs that could impair cognition in the past 3 months • Absence of alcohol or substance abuse in the previous 2 years • No subjective memory complaints
Study II	
• Montreal Cognitive Assessment scores above the cut point stratified by age and educational attainment for the Portuguese population (1.5 SD below mean)[29] • Access to a computer at home • Being able to use a computer and mouse interface without external help	

Table 1. Inclusion criteria for participants.

Therefore, we applied Test A in two groups: 1) consecutive patients referred to a memory clinic with mild cognitive impairment (MCI) or early stage (mild) dementia; 2) a convenience sample of community controls, matched for age group (±10 years) and educational attainment level (groups: 1–4; 5–9; 10–12; and >12 years) recruited from adult learning centers in the hospital region, healthy hospital volunteers, patient relatives and health workers.

The overall inclusion criteria for patients were: ≥18 years of age and no physical impairment precluding using a computer and mouse interface (Table 1). Mild cognitive impairment was defined as the presence of subjective cognitive complaints over a period of at least 6 months reported by the patient or family members, one cognitive domain 1.5 standard deviations (SD) or more below age-corrected norms in the neuropsychological evaluation, without clinical depression and without impairment in daily activities[19]. For mild dementia, the inclusion criteria were fulfilling the DSM-V definition for major neurocognitive disorder[20] (significant cognitive impairment in at least one cognitive domain representing a significant decline from a previous level of functioning that interferes with independence in everyday activities) and a mild dementia, defined as a score of 1.0 in the clinical dementia rating scale[21] (Table 1). The initial clinical classification was confirmed after at least 6 months of clinical follow-up by a neurologist. Every patient had a complete diagnostic workup, including blood analysis for treatable causes of dementia, imaging studies and a complete neuropsychological evaluation.

The inclusion of community controls was determined based on an interview with a neurologist and a review of previous medical history. The inclusion criteria were: ≥18 years of age, absence of any neurological, psychiatric or systemic disease that could impair cognition (except for stable depressive symptoms), absence of drug use that could impair cognition in the past 3 months, absence of alcohol or substance abuse in the previous 2 years, no physical impairment precluding the use of a computer and mouse interface and no subjective memory complaints.

The tests were self-administered in a hospital clinic, under the observation of a member from the research team. Difficulties in understanding or performing the tests and failure to complete the test battery were systematically registered.

Refinement to the second test version – Test B. A second version of the test (Test B) was defined after analysis of the results from Test A, retaining 7 subtests from Test A and introducing 5 new subtests (Word categories task, Attention task I, Auditory memory task, Opposite task, Visual memory task II, Attention task II, Sequences, Calculus task, Visual memory task I, Puzzles, Written Comprehension, Shopping Task; subtest description can be found in Appendix 1). Test B was self-administered by a group of patients and matched controls, using the same study protocol, setting and inclusion criteria already described for test A.

Statistical analysis. Principal component analysis was performed to evaluate dimensionality of the subtests[22]. The acceleration factor that corresponds to the numerical solution to the elbow of the scree plot was used to define the number of components retained. Subtests with high factor loading (factor loading >0.50) were retained. The internal consistency was assessed using Cronbach's alpha to discard subtests that lowered the overall internal consistency and/or with lower item-total correlation (<0.50)[23].

The subtest scores were standardized to a t-score using the mean and standard deviation of the healthy controls as the reference. The final test scores are the total sum of the subtests' scores. To compare the differences in age, education and test scores between the two groups Student's T test for independent samples was used, since all variables presented a normal distribution ($p > 0.05$ in Kolmogorov–Smirnov test). Linear regression analysis was used to assess the correlation of the Brain on Track test scores to the scores of the Montreal Cognitive Assessment (MoCA) and the Mini Mental State Examination (MMSE). A multivariable linear regression model was used to identify a possible effect of age, gender and education on test scores, independent of test groups (patient vs. control, and their potential interactions with the test group). To estimate the predictive accuracy of test scores to distinguish between patients and controls and calculate the areas under the corresponding receiver operating characteristic curves (AUC)[24], logistic regression models were fitted using test group (patient vs. control) as the dependent variable, test scores as the independent variable and adjusting for factors associated to test scores (age, education, and interaction between education and test scores).

Considering the use of Brain on Track to detect cognitive impairment in the adult population, the minimal relevant status change to be detected can be defined as the difference between healthy individuals and patients when first presenting with memory complaints caused by early stage cognitive disorders. By selecting the two test groups that fit these criteria (healthy controls vs. patients with early stage cognitive impairment), we estimated the MID as the difference in the average test score between these two groups. To assess the test ability to detect clinically important changes over time, the difference between the MID and the smallest real difference (SRD) was calculated[25,26]. The SRD was estimated using the following formula: SRD = Standard Error of Measurement (SEM)*1.96. The SEM was calculated as SEM = SD*$\sqrt{}$(1–Cronbach's alpha)[27].

To validate the test, we hypothesized that the older, less educated and the patients affected with MCI/early dementia would have lower scores. For the comparison between patients and controls, we hypothesized that the test would have at least an acceptable predictive ability to detect MCI/Mild dementia in a single use (AUC$\geq$0.70) and, more importantly for a repeatable test, that it would be sensitive to status change (SRD $<$ MID).

Test-retest from home (Study II). *Design.* The refined version of Test B with the 8 subtests retained after Study I (Word categories task; Opposite task; Visual memory task II; Attention task II; Sequences; Calculus task; Puzzles and Written comprehension) was used to assess test-retest reliability, with the test being self-applied at home in a sub-sample of participants from the EpiPorto cohort. The EpiPorto cohort was assembled between 1999 and 2003, as a representative sample of adult ($\geq$18 years) community dwellers of Porto, an urban center in the northwest of Portugal, with approximately 300,000 inhabitants at that time[28]. Households were selected by random digit dialing of landline telephones. Within each household, a permanent resident aged 18 years or more was selected by simple random sampling. The initial number of participants in the cohort was 2485 (70% participation). In the 2013–2014 revaluation of the EpiPorto cohort, the first 300 consecutive participants were invited to participate in the Brain on Track test-retest study. The inclusion criteria were MoCA scores above the cut-point stratified by age and educational attainment for the Portuguese population (1.5 SD below the mean)[29] to exclude participants with cognitive impairment, access to a computer at home, and being able to use a computer and mouse interface without external help (Table 1).

After accepting to participate, each participant performed the test under the supervision of an element from the research team in a clinical lab. This session had two main goals: a) teaching the participant how to login to the Brain on Track web page and accustoming the participant with the user interface and b) guaranteeing that the participant understood the instructions and mechanics of each game, so that subsequent testing would not be as dependent on learning effects.

One week after the training session, the participants were asked to perform the test at home by e-mail and SMS. The participants accessed the web site from their home computer and performed the testing autonomously. They were asked to repeat the test a 2nd time, 3 months later, and a 3rd time 6 months after the first trial.

Statistical analysis. Test-retest reliability for each subtest was assessed using consistency two way mixed single intraclass correlation coefficient (ICC)[26,30]. We hypothesized that most of the subtests would have good test-retest reliability (minimum ICC of 0.70). Additionally, learning effects between trials were also tested using Student's T test for related samples, since all variables presented a normal distribution ($p > 0.05$ in Kolmogorov–Smirnov test). Ethical approval of research: Study I was approved by the ethics committee of Hospital São Sebastião, Centro Hospital de Entre o Douro e Vouga, Santa Maria da Feira, and Study II by the ethics committee of Hospital de São João, Porto and the methods were conducted in accordance with the approved guidelines. All patients and caregivers were provided with information about the purpose and procedures of the study and provided written informed consent.

Results

Study I. A total of 176 individuals were recruited for Study I, 98 performed Test A (49 patients and 49 controls), 78 performed Test B (39 patients and 39 controls). There were no significant differences between patients and controls regarding age and education (Table 2). Participants that performed Test B were older, with a significant difference (mean age difference = 4.52 years; $t(174) = -2.97$; $p = 0.003$), and slightly less educated, but with a non-significant difference (average education difference = 0.51 years; $t(174) = -1.29$; $p = 0.200$).

	Age	Education	MMSE	MoCA
	Mean (Standard Deviation), *years*		Mean (Standard Deviation), *test scores*	
Test A				
Controls (n = 49)	67.9 (11.9)	4.9 (3.0)	27.8 (1.7)	21.9 (3.2)
MCI/Mild Dementia (n = 26/n = 23)	68.2 (11.8)	4.6 (2.6)	26.4 (3.3)	17.3 (5.6)
p-value (Student's T test)	0.90	0.60	0.02	0.01
Test B				
Controls (n = 39)	72.2 (7.2)	4.1 (2.5)	25.8 (2.5)	19.4 (3.7)
MCI/Mild Dementia (n = 18/n = 21)	73.0 (7.5)	4.2 (2.4)	24.0 (3.8)	15.0 (4.5)
p-value (Student's T test)	0.64	0.89	0.03	0.001

Table 2. Participant demographics and cognitive screening test scores. MCI – mild cognitive impairment; MoCA – Montreal Cognitive Assessment; MMSE – Cognitive Assessment and Mini Mental State Examination.

	Correct answers Mean (Standard Deviation) % *Correct response*			Principal component analysis	
	Patients	Controls	Reliability analysis	Corrected Item-Total Correlation	Cronbach's Alpha if Item Deleted
Word categories task	8.24 (4.49) *81.4%*	11.59 (4.03) *92.1%*	0.818	0.744	0.888
Attention task II	19.94 (10.79) *93.7%*	22.16 (7.79) *98.0%*	0.698	0.614	0.900
Sequences	5.24 (3.53) *88.0%*	8.80(4.16) *97.0%*	0.777	0.704	0.892
Visual memory task I	5.59 (2.68) *84.8%*	6.22 (1.82) *93.6%*	0.495[A]	–	–
Puzzles	2.00 (1.37) *100%*	2.71 (1.79) *100%*	0.693	0.614	0.900
Written comprehension	14.18 (3.22) *88.0%*	15.35 (3.49) *97.9%*	0.789	0.717	0.891
Shopping task	3.90 (2.88) *88.0%*	6.29 (3.22) *96.0%*	0.803	0.728	0.890
Verbal memory task	4.02 (2.14) *89.1%*	4.96 (2.36) *96.8%*	0.774	0.704	0.892
Inhibitory control	23.94 (8.46) *86.1%*	26.18 (8.08) *97.7%*	0.819	0.750	0.888

Table 3. Principal components analysis and reliability analysis for Test A. Overall Cronbach's Alpha = 0.91; Variance explained by the first component was 55.8%. [A]Subtest discarded after observing the principal component analysis results (factor loading < 0.50).

In the principal component analysis the solution defined by the scree plot criteria was of one principal component in both tests. For Test A the eigenvalue for the first factor was 5.49, corresponding to 55.8% of the explained variance, the second factor had an eigenvalue value of 1.39, corresponding to 11.6% of the explained variance. Therefore, one principal component was defined, including the subtests Word categories task, Attention task II, Sequences, Puzzles, Written comprehension, Shopping task, Verbal memory task, Inhibitory control; one subtest (Visual memory task I) did not reach the predefined factor loading of 0.50 and was discarded (Table 3). For Test B the eigenvalue for the first factor was 5.87, corresponding to 55.8% of the explained variance, the second component had an eigenvalue value of 8.8, corresponding to 9.8% of the explained variance. Therefore, one principal component was defined, including the subtests Word categories task, Attention task I, Auditory memory task, Opposite task, Visual memory task II, Attention task II, Sequences, Calculus task, Visual memory task I, Puzzles, Written Comprehension, Shopping Task; two subtests (Attention task I and Auditory memory task; Table 4) did not reach the predefined factor loading of 0.50 and was discarded (Table 3).

Concerning internal consistency, the subtests retained after principal component analysis from Test A had good internal consistency (Table 3), but one subtest from Test B (Visual memory task I) did not meet the predetermined standard (Table 4). The final versions of the two tests showed high internal consistency, with Cronbach's alpha of 0.91 for Test A and 0.90 for Test B.

The average score for Test A was 9.03 (95% Confidence Interval (CI): −7.20; 25.26) in patients with MCI/Mild Dementia and 50.00 (95%CI: 31.49; 68.50) in controls, showing a significant difference ($t(96) = 3.35$; $p = 0.001$; Table 5). For Test B, the average scores were −6.56 (95%CI: −34.96; 21.84) in MCI/Mild Dementia and 50.00 (95%CI: 27.50; 75.52) in controls, also with a significant difference ($t(76) = 3.16$; $p = 0.02$). There was a moderate to strong positive statistically significant correlation between Brain on Track test scores and the test scores from MoCA (Test A: $p < 0.001$; R = 0.52 $\beta = 0.04$ (95% CI: 0.03; 0.06); Test B: $p < 0.001$; R = 0.62 $\beta = 0.03$ (95% CI: 0.02; 0.04): and MMSE (Test A: $p < 0.001$; R = 0.39 $\beta = 0.02$ (95% CI: 0.01; 0.02); Test B: $p < 0.001$; R = 0.52 $\beta = 0.02$ (95% CI: 0.01; 0.03).

In the linear regression analysis, there was a significant association between the test scores and age, Test A: $p < 0.001$; $\beta = -0.20$ (95% CI: −0.29; −0.11); Test B: $p = 0.041$; $\beta = -0.20$ (95%CI: −0.38; −0.01) and also between the test scores and educational attainment, Test A: $p = 0.001$; $\beta = 0.67$ (95% CI: 0.30; 1.04); Test B: $p = 0.007$; $\beta = 0.80$ (95% CI: 0.29; 1.37), while no significant effect was identified for gender (Test A: $\beta = -2.86$ $p = 0.78$; Test B: $\beta = 9.20$ $p = 0.591$). In Test A, a significant interaction was found between test group (patient vs. control) and educational attainment: in the more educated individuals the differences in test scores between

	Correct answers Mean (Standard Deviation) % *Correct response*		Reliability analysis	Principal component analysis	
	Patients	Controls		Corrected Item-Total Correlation	Cronbach's Alpha if Item Deleted
Word categories task	8.15 (5.01) *79.8%*	11.64 (4.09) *96.0%*	0.726	0.635	0.885
Attention task I	2.97 (1.42) *82.1%*	4.67 (2.26) *84.8%*	0.251[A]	–	–
Auditory memory task	2.77 (1.81) *68.8%*	3.95 (2.38) *80.3%*	0.489[A]	–	–
Opposite task	23.77 (19.43) *64.6%*	26.18 (13.07) *92.3%*	0.639	0.528	0.892
Visual memory task II	13.23 (5.51) *81.8%*	18.05 (3.46) *98.6%*	0.686	0.611	0.887
Attention task II	15.72 (10.25) *91.1%*	21.03 (9.62) *98.8%*	0.817	0.779	0.875
Sequences	13.72 (8.63) *90.4%*	14.38 (3.57) *98.6%*	0.770	0.713	0.880
Calculus task	15.62 (9.26) *90.0%*	17.18 (6.53) *96.1%*	0.754	0.670	0.883
Visual memory task I	15.13 (3.83) *94.6%*	16.74 (3.41) *99.7%*	0.512	0.441	0.898[B]
Puzzles	1.08 (0.90) *100%*	2.49 (1.59) *100%*	0.712	0.628	0.885
Written Comprehension	13.77 (5.74) *88.7%*	15.69 (5.15) *98.9%*	0.742	0.677	0.882
Shopping Task	6.28 (4.14) *88.8%*	10.51 (4.28) *98.1%*	0.781	0.713	0.880

Table 4. Principal components analysis and reliability analysis for Test B. Overall Cronbach's Alpha = 0.90; Variance explained by the principal component was 45.5%. [A]Subtest discarded after principal component analysis (factor loading <0.50). [B]Subtest discarded after internal consistency analysis (item-total correlation <0.50).

	Test A	Test B
Cronbach's Alpha	**0.91**	**0.90**
T-scores*		
Mean [95% Confidence Interval]		
Controls	50.00 [31.49; 68.05]	50.00 [27.50; 72.52]
MCI/Mild Dementia	9.03 [−7.20; −25.26]	−6.56 [−34.69; 21.84]
Correlation with MMSE score	0.39	0.52
Spearman's R *(linear regression p-value)*	($p < 0.001$)	($p < 0.001$)
Correlation with MoCA score	0.52	0.62
Spearman's R *(linear regression p-value)*	($p < 0.001$)	($p < 0.001$)
Minimal important difference (MID)	40.97	56.82
Smallest real difference (SRD)	37.89	43.04
Difference of MID and SRD (%)	7.5%	23.9%
Area under the ROC curve	0.74	0.75

Table 5. Standardized total test scores after item selection. MCI – mild cognitive impairment; ROC – Receiving operator characteristic; MoCA – Montreal Cognitive Assessment; MMSE – Cognitive Assessment and Mini Mental State Examination. *Sum of the standardized subtest scores transformed to a T-distribution.

test groups are higher than in those with lower education levels ($p = 0.011$; $\beta = -0.89$ (95%CI: −1.57; −0.21)) (Fig. 1). Although a similar trend can also be observed in Test B (Fig. 1), the interaction was not statistically significant ($p = 0.172$; $\beta = -0.76$ (95%CI: −1.87; 0.34)).

To estimate the predictive accuracy of the test to distinguish between patients and controls, and using a logistic regression model adjusted for the parameters associated with test scores (age, education and the interaction between age and education), the AUC was 0.741 for Test A and 0.753 for Test B.

The smallest real difference (SRD) between test groups was 37.89 for Test A and 43.04 for Test B (Table 5), lower than the predefined clinically relevant differences (MID) for both tests (4.00 and 4.82 respectively). The difference between the SRD and MID was higher in Test B (22.9%) than in Test A (7.5%).

The Verbal Memory and Inhibitory Control subtests presented a floor effect in the control participants with lower to average education and were perceived to be the most difficult subtests from Test A by the participants and neuropsychologists. For this reason, and given the worst discriminative ability of Test A in patients, they were replaced by simpler alternatives in Test B: the Verbal memory task was replaced by the Auditory memory task and the Visual memory task II as alternative tests for episodic memory; the Inhibitory Control task was replaced by the Opposite task and the Calculus tasks as alternative tests for inhibitory control/executive function. Difficulties in understating the goal were reported by some patients and controls in two of the subtests from Test B (Shopping task and Visual Memory task I), so these tests were excluded from the refined version of Test B in which the test-retest analysis was completed. The remaining test instructions and mechanics were well understood by the patients and controls.

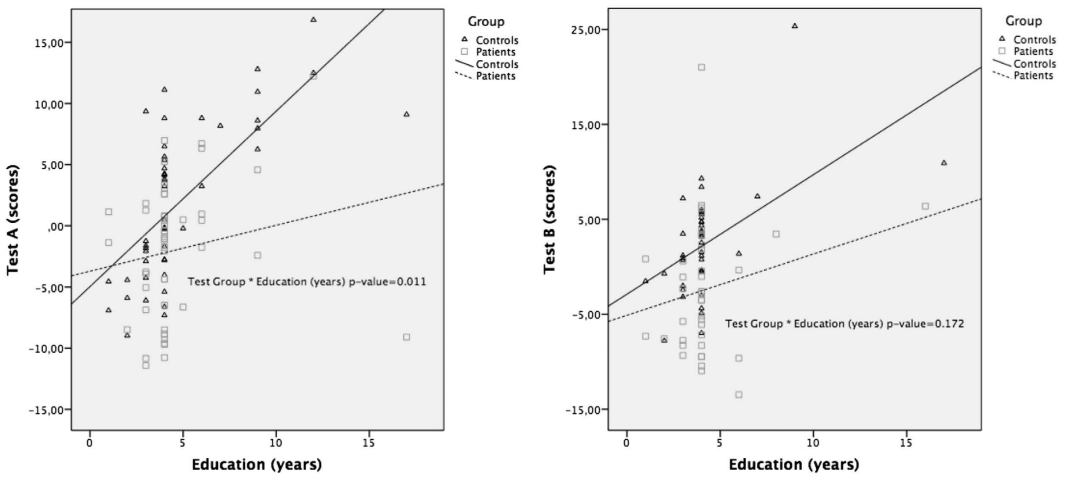

Figure 1. Association between test scores and education by test group (Controls vs. MCI/Mild Dementia) in Test A and Test B.

	Subtest scores Mean (standard deviation)			ICC	
	Trial 1	Trial 2	Trial 3	2 trials	3 trials
Word categories task	14.81 (5.69)	15.06 (5.11)	15.80 (4.96)	0.797	0.836
Opposite task	30.80 (22.94)	32.06 (21.87)	34.18 (20.30)*	0.754	0.814
Visual memory task II	13.40 (3.01)	13.27 (2.91)	14.31 (3.29)	0.700	0.790
Attention task II	21.97 (8.78)	23.68 (8.50)	24.54 (7.40)	0.406	0.547
Sequences	11.28 (5.61)	10.96 (5.53)	11.31 (5.72)	0.795	0.855
Calculus task	19.64 (9.67)	20.35 (9.08)	20.52 (9.54)	0.847	0.880
Puzzles	4.97 (3.82)	5.04 (2.23)	5.02 (2.42)	0.610	0.768
Written comprehension	14.01 (5.18)	14.05 (3.78)	14.92 (3.61)*	0.660	0.811

Table 6. Results from the test-retest study (consistency two way mixed single intraclass correlation coefficient). *Statistically significant difference between trials 2 and 3 in Student's T test for related samples ($p < 0.05$).

Study II. From the 300 potential participants, 63 (21%) were not eligible because of MoCA test scores below the defined cut-point. From the remaining participants, 73 (24%) were excluded because they did not have continuous access to a computer at home, 18 (6%) could not use a computer and mouse interface without external help and 17 (6%) refused to participate in the test-retest study from home. The study was initiated by 129 participants, from whom 113 completed the 3 trials at home (87.6%). The mean (SD) age and years of schooling of the study participants were 64.8 (6.0) and 11.8 (4.6). There was a slight upward trend in subtests scores (Table 6), which was statistically significant between trials 2 and 3 of the Opposite ($t(112) = 2.89$; $p = 0.005$; mean difference = 1.08; sd = 2.30) and the Written Comprehension ($t(112) = 3.03$; $p = 0.003$; average difference = 0.87; sd = 2.3) tasks. In the analysis of the test-retest reliability for 3 consecutive trials, only 1 subtest showed a low intraclass correlation (Attention task II), all of the other subtests showed high ICC, with 6 of 10 tests with ICC higher than 0.80 (Table 6).

Discussion

In this paper, we describe the assembling of Brain on Track, a web-based self-administered test intended for longitudinal cognitive testing, and present the results of its early validation process. The second version (Test B) was able to improve the initial version (Test A) and showed good internal consistency, reproducibility, positive correlation with existing cognitive screening tests and ability to identify clinically relevant differences. The subtests showed high test-retest reliability when performed at home, notwithstanding a small learning effect between trials was identified in some subtests. Future longitudinal studies with longer follow-up will allow us to address the potential impact of additional trials in learning effects and test-retest reliability. The education level of patients in this study is lower than what is usually found in the literature. This is not surprising, given that the Portuguese population is one of the least educated in Europe, especially in the elderly groups[31]. The fact that the Brain on Track test could be successfully applied in this setting underlines its potential as an inclusive tool for cognitive testing. On the other hand, this could also represent a potential limitation for the generalization of the test to more educated populations. However, the differences in test scores between patients and controls increased among the more educated when compared with the least educated. Consequentially, the predictive accuracy is higher in the more educated group.

The Brain on Track tool shares potential advantages with the other computerized cognitive tests: the cost-effectiveness, the ability to accurately record and store the responses, the minimization of examiner bias and the potential for adaptive testing[12,13]. The main criticisms of these tools relate to the lack of adequately established psychometric standards and the potential difficulties in the response for older adults unfamiliar with computer interfaces[12,13]. In a population-based cohort of adult individuals in Portugal, 70% of could be included in this strategy and 87.6% of these participants were able to complete 3 test sessions without external help, suggesting a good usability in this setting. We expect the resistance and lack of familiarity with computers to decrease in the near future, as the number of adults with access and experience in computer use increases. Performing the tests at home without supervision also creates the potential issue of non-compliance, which if not controlled could represent a potential limitation. The usability and the impact of non-compliance will be explored in future studies with larger groups of patients and healthy controls using qualitative interviews and focus groups with patients and relatives and by alternating observed and non-observed testing sessions in the long-term monitoring plan.

There are some major technological hurdles in the development process of computerized tests performed at home that can become potential limitations if not properly addressed, namely the different hardware, software and Internet speed of the patients' computers. We are confident we were able to minimize their impact on test results by 1) using web-based instead of locally installed software, allowing to control the subtest duration and the latency times in real time and thus guarantying homogeneity in the different platforms and 2) preloading all of the data needed for each subtest before its initiation, resulting in Internet speed affecting the waiting time between the subtests, but not the duration of each subtest, nor the latency between the tasks within each subtest.

Notwithstanding all of the challenges their implementation entails in the real world, computerized cognitive tests can present a diagnostic accuracy comparable to traditional neuropsychological testing[12,13]. In the last decade, the field has increasingly expanded in the direction of shorter screening tests[13], able to address the unmet need for a cost-effective diagnostic approach for the increasing number of individuals at risk for dementia in the general population. Several such tests have shown good diagnostic accuracy and have entered clinical use, such as the National Institutes of Health Toolbox Cognition Battery[32], the CogState[33] and the Cognitive Stability Index[34]. However, while these tests can replace the existing pen and paper screening tests like MoCA and MMSE with some potential advantages, they still require a trained evaluator and a patient visit to a clinic. Other approaches for expanding the accessibility to cognitive screening have been proposed, namely the Audio Recorded Cognitive Screen[35], that relies on an audio recording to provide testing instructions and can be applied without an external evaluator, though its use was not yet validated for repeatable testing or for remote self-administration. In the last years, a few cognitive tests have been developed and validated that allow for self-administration and remote testing, such as the Computer Assessment of Mild Cognitive Impairment[36], MicroCog[37] and COGselftest[38]. These tests, like the Brain on Track test, showed good neuropsychological parameters when the tests were conducted in a clinical setting, but they were primarily designed for single use and are not validated for longitudinal follow-up[13,17]. For a first use of the Brain on Track test in a longitudinal screening strategy (i.e.: patients with memory complaints in primary care), a cut-point optimized for positive likelihood ratio could be defined (specificity = 0.90; sensitivity = 0.54; positive likelihood ratio = 4.73; negative likelihood = 0.46) and patients falling below would be classified as probably affected and referred to a neurologist. It is important to emphasize that the AUC and other discriminative statistics can serve as proof of concept for the test's discriminative ability but they do not accurately assess the test performance for repeated use; the positive difference between the SRD and MID and the test-retest reliability of the Brain on Track test are good indicators that the tool will be able to identify this cognitive decline over time. To the best of our knowledge, this is the first development process for a computerized repeatable cognitive test where test selection was performed based on the test-retest reliability from home. Moving forward to longitudinal validation, we plan to test different strategies to identify possible cognitive impairment: 1) test scores falling bellow an expected performance threshold for each age/education group and 2) a pattern of decline in individual performance.

These initial results are encouraging and validate the Brain on Track test as a valid cognitive test. The undergoing clinical based and population longitudinal studies will allow for further development, refinement and validation for longitudinal clinical use.

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Author Contributions

Study concept and design: L.R., N.L., J.P. and V.T.C. Acquisition of data: L.R., A.S., I.A., R.B., C.M., S.M. and J.P. Analysis and interpretation of data: L.R., M.S., N.L. and V.T.C. Critical revision of the manuscript for important intellectual content: All authors. Administrative, technical and material support: L.R., A.S., N.L., E.C., M.C., V.B., J.P. and V.T.C. Study supervision: L.R., N.L., J.P. and V.T.C.

Additional Information

Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: V.T.C. and J.P. have a shareholder position at Neuroinova, Lda a *start-up* company that conceived Brain on Track, holds registered trademark and commercialization rights and also provided funding for part of the study. E.C., V.B. and M.C. received fees for parts of the technological development; A.S. and C.M. received fees for patients' cognitive assessments. The author(s) declare no other competing financial interests.

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5. The natural history of cognitive decline and dementia

5.1 Prevalence and causes of cognitive impairment and dementia in a population based cohort from Northern Portugal (Paper II)

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Keywords: Dementia; Epidemiology; Cognitive impairment; Prevalence.

List of tables and figures:

Abstract

Background: Vascular dementia might play a particularly important role in the epidemiology of cognitive impairment in countries with a high stroke burden, such as Portugal. The aim of this study is to assess the prevalence and main aetiologies of cognitive impairment in a population based cohort from Northern Portugal.

Methods: 730 individuals aged ≥55 years from the 2013-2015 follow-up wave of the EPIPorto cohort underwent cognitive screening using Mini Mental State Examination and Montreal Cognitive Assessment. Those scoring below age and education adjusted cut-points for the Portuguese population were evaluated by a neurologist to assess the presence of dementia or mild cognitive impairment(MCI), and define their most probable aetiology.

Results: 36 cases of MCI and dementia were identified, corresponding to a crude prevalence of dementia of 1.0% and of MCI 4.0% and adjusted estimates of 4.1% and 1.3%. The most common cause of MCI and dementia was vascular (52.8%), followed by Alzheimer's disease (36.1%).

Conclusion: This study highlights the importance of vascular cognitive impairment in the epidemiology of dementia in Portugal. These findings carry an important public health message regarding the prevention and management of cognitive impairment in Portugal, and possibly other countries with a high burden of stroke.

Background

Cognitive impairment and dementia are worldwide increasingly frequent, impacting the quality of life of millions of patients and their families[1]. Dementia is estimated to affect 2-3% of the individuals aged 70-75 years, and 20-25% of those aged 85 years or more, globally[2]. In western societies, the age-standardized prevalence among those older than 60 years has been estimated at 6-7%[3], and is expected to remain at this level in the next decades[4], contributing to an increasing number of cases in the population, due to the demographic aging. Alzheimer's disease (AD) is the most frequent type of dementia in western countries, while vascular cognitive impairment and dementia (VaD) is generally described as the second cause[5].

Epidemiological data is needed to assess the potential for preventive interventions and for resource distribution, towards the most adequate health responses. Based on extrapolations from international data, the number of people living with AD in Portugal is estimated at around 160 thousand, with associated costs of 37 M€/year, only considering drug treatments[6]. However, the only published epidemiological study on the frequency of cognitive impairment and dementia in Portugal was performed in 2003, showing a prevalence of 2.7% for dementia and 12.3% for all causes of cognitive impairment, including psychiatric and congenital disorders, in the population between 55 to 79 years[7]. Additional studies are needed to replicate these findings in different populations, and to monitor their variation over time.

The present study aims to assess the prevalence of cognitive impairment and dementia in the EPIPorto population based cohort, and to identify their most frequent causes.

Methods

Study design and protocol

The EPIPorto is a population based closed cohort assembled between 1999 and 2003 in the city of Porto, representative of dwellers ≥18 years (n=2485)[8]. Porto is the second largest urban centre in Portugal, with a heterogeneous socio-demographic population consisting of approximately 300 thousand inhabitants at the time[8]. Random digit dialling of landline telephones was used to select households. Then, within each household, a permanent resident aged at least 18 years was selected by simple random sampling.

The present study was based on the 2013-2015 re-evaluation of the cohort. Among 1,126 cohort members aged ≥55 years, a total of 730 were evaluated (63.3% participation) in two steps, namely a screening phase and a clinical evaluation; those not evaluated were older (mean difference in age, 7.8 years, 95% confidence interval [95%CI]: 6.8-8.8) and less educated (mean difference in schooling, 1.6 years, 95% CI: 1.0-2.1), with higher prevalence of hypertension (43.1% in non-participants vs. 29.8% in participants, $p<0.001$) and diabetes (9.6 vs 4.3%, $p<0.001$) and no significant differences in the prevalence of dyslipidaemia (44.2 vs 39.6%, $p<0.001$) and sex (38.3 vs. 38.5% of males, $p=0.94$).

Screening was performed using the Portuguese validated versions of the Mini Mental State Examination (MMSE)[9] and the Montreal Cognitive Assessment (MoCA)[10] tests; the Beck Depression Inventory (BDI)[11] and other instruments and questionnaires aimed at assessing the current health status and socio-demographic determinants were also used at this evaluation. Participants that scored below cut-points validated for the Portuguese population in any of the cognitive screening tests (MMSE: 22 for 0-2 years; 24 for 3-6 years and 27 for ≥7 years of schooling[9]; MoCA:

age- and education-adjusted defined as 1.5 SD below mean of the normative sample[12]) were selected for the clinical evaluation. This comprised a clinical interview and examination, performed by a trained Neurologist using a standard clinical protocol, including the clinical assessment of higher cognitive functions, a complete anamnesis and the standardized search for memory complaints using the Portuguese version of the subjective memory complaints scale (SMC)[13]. The participants were asked to bring a close relative or other surrogate to assess the presence and impact of cognitive impairment in daily activities. The clinical records of all the participants selected for the clinical examination were reviewed to identify any previously established diagnoses of neurological or psychiatric disorders, as well as results from brain imaging and relevant lab results. This search was performed in the three public hospitals of Porto (*Hospital de São João*, *Hospital de Santo António* and *Hospital Magalhães Lemos*). Based on the results from the clinical evaluation, the cognitive screening tests results and the clinical records the participants were classified by a Neurologist as having 1) no psychiatric or neurologic affection; 2) depression or anxiety; 3) static/reversible cognitive impairment; 4) progressive cognitive impairment, further classified as mild cognitive impairment (MCI) or dementia. MCI was defined as the presence of subjective cognitive complaints over a period of at least six months, reported by the patient or family members, in the presence of impairment according to the MoCA test (1.5 standard deviations (SD) or more below age-corrected norms), without clinical depression and without impairment in daily activities[14]. Dementia was considered present when the participants fulfilled the DSM-V definition for major neurocognitive disorder[15] (significant cognitive impairment in at least one cognitive domain representing a significant decline from a previous level of functioning that interferes with independence in everyday activities). The probable etiology was defined by the Neurologist that performed the neurological assessment, using also all the

clinical, imaging and lab data retrieved from health records, based on the DSM-V criteria for each nosological entitity[15]. When a new diagnosis was established in this clinical assessment, the Neurologist wrote a letter to the participant general practitioner, providing the clinical information and recommending an investigation and management plan, including complementary studies (that were later retrieved for etiological diagnosis). In the individuals that did not participate in the clinical evaluation, any relevant diagnosis identified in the clinical records search that was established by a neurologists or psychiatrists and complied with the previously defined criteria was also included in the estimates.

Figure 1 depicts the flow of the participants through the steps of the study. From the 730 participants screened, 133 (18.2%) presented a score suggestive of a possible cognitive impairment. Among the latter, 94 were evaluated by a neurologist to confirm and classify the cognitive impairment and clinical evaluation could not be performed in 39 participants, who were classified regarding the presence of cognitive impairment using only data from clinical records.

Ethical issues

All the participants provided written consent, and specifically allowed access to their electronic clinical records and referral of the diagnosis and investigation plan to the general practitioner, with the possibility to opt out of any of these procedures. In cases of cognitive impairment, written consent was also sought from a valid surrogate. The study was approved by the institutional ethics committee and by the national data protection authority.

Statistical analysis

Comparisons of continuous variables between sample groups were performed using the Student's t- test or the Mann-Whitney U test, whether the distribution of the values was a bell-shaped curve or not, respectively. For categorical variables, the Pearson's chi-squared test or the Fisher's exact test were used.

The age-standardized prevalence of MCI and of dementia were computed using the direct method. Data from the last census, in 2011, of the Portuguese population was used as the standard populations for the city of Porto and for the population of Portugal[16,17]. For the European population, the European Standard Population 2013[18] was used.

The statistical analysis was performed using Stata version 11.2 (StataCorp. 2009. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP).

Results

From the 94 participants assessed in the clinical evaluation, cognitive impairment was confirmed in 58. In the 39 participants that missed the clinical evaluation, the review of electronical records resulted in a diagnosis in 10 cases, while the others had an unremarkable medical history. In all, a total of 68 participants (47 women and 21 men) were classified has having cognitive impairment (Figure 1).

Regarding the distribution of the scores of the two screening tests used (Figure 2), the MoCA scores present a nearly normal distribution for all the participants and, as expected, a shift to the left is observed in the case of those with cognitive impairment. For the MMSE, the distribution of the scores is asymmetric and suggests a ceiling effect, with most of the results equal to the maximum value of the test. Although a shift to the left is seen among participants with a psychiatric or a neurologic affection in relation to participants without these conditions, a considerable proportion of the MMSE scores are at the maximum value and there is substantial overlap in the scores between cognitively affected subjects and the remaining participants.

The prevalence of all causes of cognitive impairment, including static and reversible aetiologies was 9.3% (7.5% in men and 10.5% in women), 10.3% when standardized for the Porto population, 9.6% for the Portuguese population and 9.8% for the European standard population.

In 32 participants (47%), the cognitive impairment was attributed to a static or reversible affection, the most common being anxiety/depression (n=27) followed by hypothyroidism (n=3), with one case of learning disability and one of obstructive sleep apnoea.

A total of 36 cases of cognitive impairment due to MCI or dementia were identified, corresponding to prevalence of 4.0% (5.3% in men and 3.1% in women) for MCI, and 1.0% (0.4% in men and 1.3% in women) for dementia. The age-standardized prevalences were 4.1% for MCI and 1.3% for dementia, when using both the standard populations of Porto or Portugal. When standardizing these results for the European population, the estimates were 4.0% and 1.0%. A probable diagnosis of AD was established in 13 cases (36.1%), whereas 19 were diagnosis with probable VaD (52.8%). One patient presented dementia in the context of Parkinson's disease. There were two cases with a clear history of progressive MCI after radiotherapy and chemotherapy treatments for cancer, and one patient with MCI due to chronic alcoholism. Using the education-adjusted MMSE cut-off points, only 17.7% of the participants later classified with MCI and dementia were correctly identified as positive in the screening strategy. For the MoCA, the age and education-adjusted 2.0 SD cut-off points correctly identified as positive 61.8% and the 1.5 SD cut-off points 97.1% of the participants with MCI and dementia. Among the participants selected in the screening step scoring below the 1.5 SD cut-off points, the frequency in which MCI and dementia were not confirmed was (77.1%).

The socio-demographic characteristics of participants with MCI and dementia in comparison with those having no cognitive impairment are presented in table 1. The former were significantly older and less educated, and presented lower scores for the screening tests MoCA and MMSE.

Discussion

In this study, we identified an age and sex standardized prevalence of 1.3% for dementia and 4.1% for MCI, with VaD contributing to an important part of these cases.

In the previous survey of MCI and dementia in a Portuguese population, the prevalence of dementia was higher, at 2.7%[7]. This could be explained by the higher socioeconomic and educational level, and the younger average age of the population of the city of Porto[16,17]. Indeed, the study conducted in 2003 found a prevalence of 1.6% for dementia when including participants from the urban setting alone. When considering only the cases of cognitive impairment with no dementia due to neurological causes, it yielded a prevalence of MCI of 3.9% for the urban and 4.3% in the rural populations, also in line with our current observations. Both studies report a lower overall prevalence of dementia and MCI in Portugal than usually described for western Europe, where the average prevalence, standardized for the European population, ranges from 1.6% in the 60-64 years age group to 24.7% in 85-89, with 6.9% for those ≥60 years[3]. When looking more closely at the regional context of Mediterranean countries, the prevalence of dementia in Italy ranges from a minimum of 5.9%[19] (for a sample with range of 65–97 years) to a maximum of 28.4%% (for a sample with age ≥75 years)[20], while in Spain the dementia prevalence ranges from a minimum of 5.9%[21] to a maximum of 14.9%[22], in populations aged ≥65 years. Several factors could explain the observed differences in prevalence. In what concerns environmental factors, there is evidence that the consumption of omega-3 and omega-6 acids[23], particularly in fatty fish is associated with reduced risk of dementia and AD. Portugal is the country with the highest seafood consumption in Europe, higher than in Italy or Spain, particularly concerning fatty fish.[24,25] A similar scenario is observed in Japan, where the consumption of fish also is very high[26] and AD

prevalence is low.[27] Another additional factor that may contribute to the decreased prevalence of dementia and AD is the seemingly lower prevalence of carriers and homozygous for the ε4 allele of the APOE gene in Portugal when compared with other European countries, with the only prevalence estimate being 9.8%[28], compared with the 12.7% European average[29].

We found two cases of progressive cognitive impairment related to post-radio and chemotherapy. It is known that patients undergoing certain forms of cancer chemotherapy may develop cognitive impairment ("chemo-brain").[30] Post-radio cognitive impairment has been reported even in cases where such therapy was not directed to brain areas.[31] In a study performed in the same setting of Northern Portugal, the incidence of cognitive impairment at one year after diagnosis was estimated in 8.1% in women with breast cancer[32]. Since the incidence of most types of cancer increases with age[33], similar to cognitive impairment and dementia, and taking into account the increase of life expectancy in high-income countries[34], cancer related cognitive decline may truly become a public health issue. More investigation in this field is needed, in order to determine the types of cancer and therapeutic agents more likely to cause this effect, as well as means of prevention and treatment.

The main cause of MCI and dementia identified in this study was vascular cognitive impairment (52.8% for VaD vs. 36.1% for AD). The only previous study performed in Portugal also showed a high prevalence of VaD, equal with that of AD (38.7%)[7] as a cause of dementia, and adding up the reported prevalence of all vascular causes amounted to 48% of cognitive impairment.[7] Taken as a whole, the results emphasize the role of vascular disease in the epidemiology of MCI and dementia in the Portuguese population. It is interesting to note that these findings are different from the results of studies performed in another Southern European populations[35]. A study aimed to assess the incidence and subtypes of dementia in

three elderly (age 65 years and older) populations of central Spain revealed that most participants had AD (71.4%), while only 11.2% had VaD.[36] An Italian study of prevalence of clinically diagnosed dementing disorders over age 59 found a prevalence of 2.6% for AD and 2.2% for multi-infarct dementia.[37] Another Italian study, performed with individuals aged 65-84, showed that AD was the most common type of dementia (53%), while VaD accounted for 27% of the overall number of cases.[38] While the younger age of participants enrolled in the present study could contribute to a lower prevalence of AD in relation to VaD, these findings are not so surprising if we take into account that Portugal presents a considerably higher incidence of stroke than other similar Western European regions[39], as stroke is both a marker of uncontrolled vascular disease and a contributing factor for vascular cognitive impairment[40], and cerebrovascular disease is the main cause of death[41], unlike Spain or Italy, where the main cause of death is ischemic heart disease.[42]

An explanation for such a high risk of cerebrovascular disease and vascular dementia in Portugal is lacking. The prevalence of hypertension, a major risk factor for stroke and VaD[43] is high (42.2%)[44], but within the figures reported in other European countries. However, it is estimated that the percentage of younger patients are not medicated, and the percentage of patients under monotherapy are far above the European average.[45] This may help to explain the high frequency of VaD in Portugal. Another possible explanation is the high prevalence of atrial fibrillation (AF) in the Portuguese population, with a reduced frequency of anticoagulant therapy utilization[46], as AF is a very important risk factor for stroke and potentially for VaD.[41]

Only in a few regions in the world present a higher prevalence of VaD than AD, namely Japan and the Middle East[27]. Japan seems to have the lowest prevalence of dementia in general and AD in particular among developed countries[27]. Most VaD

cases in Japan are due to multiple lacunar infarcts or small vessel disease, while VaD secondary to large cortical infarcts represents a minor percentage. This is probably due to a higher incidence of lacunar stroke in Japan comparing to European countries, where thromboembolism plays a major role in stroke etiology.[47] A previous epidemiological study showed that lacunar infarcts represented 39.1% of the total of ischemic infarcts in a Portuguese population.[48] This is a high percentage comparing to the results of other European studies, where the prevalence is heterogeneous, but does not reach 30% in any study.[49] Since cerebral small vessel disease is the most prevalent vascular lesion associated with vascular cognitive impairment[50], the high prevalence of lacunar stroke in Portugal and Japan may, at least in part, explain the burden of vascular dementia in both countries.

We cannot discard that the erosion in the participation in the EpiPorto cohort contributed to an underestimation of the prevalence of dementia and MCI, as participants with cognitive impairment could be less prone to participate in the cohort re-assessment. This is supported by the older age and less education of participants not assessed in the cohort re-evaluation. Additionally, and although we performed a complete revision of their electronical medical records for relevant diagnosis, there could be some missed cases in the participants that did not attend the clinical evaluation, particularly of MCI.

The study design overestimates the sensitivity of MMSE and MoCA for MCI and dementia, as the test scores were also used to classify participants, resulting in verification bias. Nevertheless, the frequency of those with MCI and dementia correctly identified by the education-adjusted cut-off points of the MMSE was very low in this sample. This frequency was still less than desirable for the most widely used 2.0 SD cut-offs of the MoCA test, but high for the 1.5 SD cut-off points. However, and based on estimates from this sample, a screening strategy based in the 1.5 SD MoCA cut-

points would result in a considerably high number of individuals with a positive screening not having MCI or dementia (77.1%). These results indicate that there is need for better tools to screen for these conditions in the Portuguese population.

In conclusion, the results of this study highlight the importance of VaD in the epidemiology of cognitive impairment in Portugal, and carry an important public health message regarding the potential for its prevention and management. Indeed, measures of primary prevention, like the promotion of healthy diet, regular practice of exercise have the potential to avert a great part of the dementia epidemic in Portugal and other countries with a higher burden of cerebrovascular disease[51]. Of particular potential, and a suitable target for public health programs, are the lack in awareness, control and compliance of treatment of hypertension[52]. Furthermore, directed multidomain interventions, involving changes in of diet, exercise, cognitive training, and control of vascular risk factors, could prevent further cognitive deterioration in patients with early and pre-symptomatic vascular cognitive impairment[53]. It is important that coordinated efforts are directed to implement such measures to lessen the burden on patients, families and society.

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Tables

Table 1. Socio-demographic characteristics of participants.

	General population, no CI		MCI and dementia		p value
	No.	% or (p25-p75)	No.	% or (p25-p75)	
Sex					
Women	402	60.7	20	55.6	0.537
Men	260	39.3	16	44.4	
Age, years	66.0	(62.0 - 73.0)	71.5	(65.5 - 78.0)	0.007
Age group					
55-64	262	39.6	8	22.2	0.037
65-74	270	40.8	14	38.9	0.822
75-84	108	16.3	12	33.3	0.020
≥85	22	3.3	2	5.6	0.354
Education, years	9.0	(4.0 - 13.0)	6.0	(4.0 - 10.0)	0.037
Education					
<12	453	68.4	31	86.1	0.025
≥12	209	31.6	5	13.9	
MoCA score	24.0	(21.0 - 26.0)	17.0	(15.0 - 19.0)	<0.001
MMSE score	29.0	(27.0 - 29.0)	27.0	(26.0 - 28.0)	<0.001

MCI – mild cognitive impairment; MoCA – Montreal cognitive assessment; MMSE – mini mental state examination

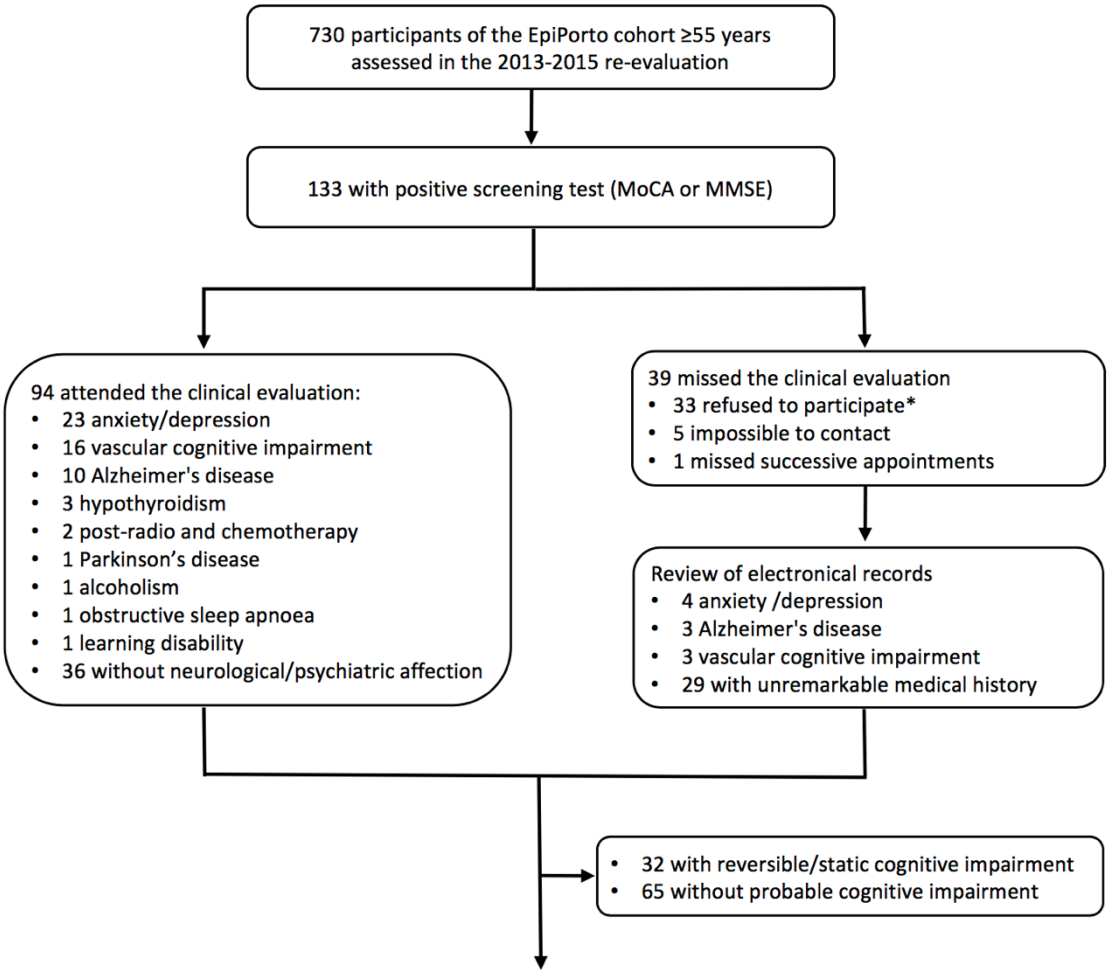
Figure legends

Figure 1. Flow-chart of participants through the steps of the study and final results on the frequency of MCI and dementia.

Figure 2. Distribution of the MoCA and MMSE scores for participants with or without cognitive impairment.

Figures

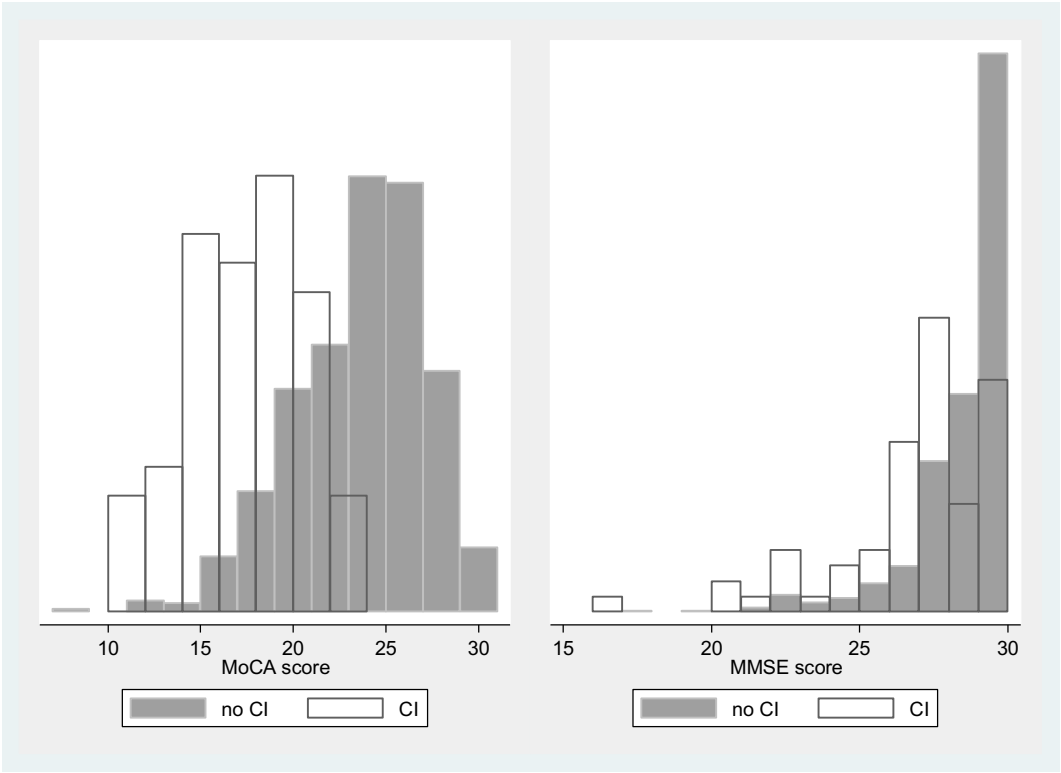
Figure 1



	MCI (n)	Dementia (n)	All n (%)
Vascular cognitive impairment	16	3	19 (27.9)
Alzheimer's disease	10	3	13 (19.1)
Post-radio and chemotherapy	2	0	2 (2.9)
Parkinson's disease	0	1	1 (1.5)
Alcoholism	1	0	1 (1.5)
Total	29	7	36 (100.0)

* Reasons for refusal: not interested in participating because they did not feel any cognitive problem n=15; transportation/mobility difficulties n=14; patients already followed by neurologist n=4. MoCA – Montreal Cognitive Assessment; MMSE - Mini Mental State Examination; MCI – Mild cognitive impairment.

Figure 2



Supplementary Material

Supplementary table 1. Observed prevalence of MCI and dementia cases by sex and age group

	MCI						Dementia					
	all		women		men		all		women		men	
	n	%	n	%	n	%	n	%	n	%	n	%
Age (y)												
55-59	3	2,7	1	1,5	2	4,3	0	0,0	0	0,0	0	0,0
60-64	4	2,4	1	0,9	3	4,9	1	0,6	0	0,0	1	1,6
65-69	5	3,0	3	2,8	2	3,3	1	0,6	1	0,9	0	0,0
70-74	7	5,5	5	6,0	2	4,5	1	0,8	1	1,2	0	0,0
75-79	6	7,0	2	4,2	4	10,5	3	3,5	3	6,3	0	0,0
>=80	4	5,8	2	5,3	2	6,5	1	1,4	1	2,6	0	0,0
All	29	4,0	14	3,1	15	5,3	7	1,0	6	1,3	1	0,4

5.2 Tracking cognitive performance in the general population and in patients with mild cognitive impairment with a self-applied computerized test (Brain on Track) (Paper III)

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Keywords: Cognition Disorders, Dementia, Neuropsychology, Cognitive Assessment, Computer-Assisted Decision Making

List of tables and figures:

Abstract

Background: There is dearth of research on the use of repeated measurements to identify longitudinal trends of cognitive performance. Brain on Track (BoT) is a computerized test developed for self-administered cognitive screening and monitoring.

Objective: To describe the variation of cognitive performance using BoT in patients with mild cognitive impairment (MCI) and individuals from the general population, and to assess the ability of BoT to discriminate between MCI and healthy controls for single and repeated use.

Methods: We recruited 30 consecutive patients with probable MCI from a memory clinic and 377 controls, a sub-sample of the population-based cohort EPIPorto. At baseline, all participants performed a neuropsychological assessment and BoT. The BoT test was repeated from home every three months, for one year. A linear mixed-effects model was built to describe the variation in cognitive performance in each group. The overall accuracy of BoT single use distinguish between MCI patients and matched controls was assessed through the area under the curve (AUC), both for single and repeated use.

Results: All participants increased their scores in the first tests, but after 120 days those with MCI presented a decline, with a statistically significant higher

rate when compared with the general population. The AUC to detect MCI in the single use of BOT was 0.86, while the repeated BoT measurements reached an AUC of 0.96 in the one year monitoring.

Conclusion: In this this study, we were able to identify a distinct longitudinal pattern of performance in patient with MCI using the BoT computerized self-applied test. The test presented high discriminative ability for single use, that was improved with the 12-month monitoring strategy.

Background

Cognitive performance is expected to decline with aging, as many other biological functions[1]. Results from cross sectional studies suggest a gradual age-associated decline in most cognitive functions of normal aging elders[2]. However, few studies have reported the results of repeated cognitive testing in individuals from the general population and there is still limited research on the use and interpretation of repeated measurements to identify longitudinal trends of cognitive deterioration.[1,2] Such data would allow for a better understanding of the pattern and rate of age-associated cognitive changes, and is also a promising strategy to identify pre-symptomatic or early symptomatic cognitive decline.

A comprehensive neuropsychological battery performed by a trained professional is the gold standard for detecting cognitive impairment[3], however it is not a cost-effective strategy for periodic cognitive testing in large groups of individuals. The brief cognitive screening tools currently available lack the desired discriminative ability to identify mild cognitive impairment (MCI) and still require a trained external evaluator[4,5]. Furthermore, most tasks included in both comprehensive batteries and brief screening tests were not specifically designed to minimize the practice effects of repeated testing[6]. Computerized cognitive tests have the potential to overcome these limitations, by allowing the use of multiple test versions and self-administered testing, and have shown neuropsychological parameters similar to traditional tests[7,8]. Additionally, they offer the potential for easier implementation of adaptive testing, in which test

difficulty can be tailored to the individual performance[9]. Nevertheless, most of the existing computerized cognitive tests were designed to mirror the pen and paper testing[10]; they require a trained professional, do not take advantage of the potential for adaptive testing, and are not intended for monitoring longitudinal cognitive performance[11].

The Brain on Track test (BoT) is a computerized cognitive test developed for self-administered web-based longitudinal cognitive screening and monitoring[12]. It was previously shown to have good reproducibility, significant correlation with existing cognitive tests, ability to identify clinically relevant differences for MCI and early dementia and high test-retest reliability when performed from home[12].

The objective of this study is to describe the variation of cognitive performance over one year using BoT in patients with mild cognitive impairment (MCI) and individuals from a population based cohort. We also assessed the ability of BoT to discriminate between MCI and healthy controls in single and repeated uses.

Methods

The Brain on Track test

The development and validation of the BoT test resulted in a version with seven subtests[12]. After critical review of these results by the neurologists and neuropsychologists of the development team, it was decided to expand the assessment of memory, executive functions and information processing speed, due to the lack of subtests assessing those domains, and four additional subtests were added: Colour Interference Task (executive function, inhibitory control), Delayed Verbal Memory Task (delayed verbal memory), Verbal Memory Task II (immediate verbal memory) and Attention Task III (Attention, information processing speed). Furthermore, in previous work, the test showed a better discriminatory ability in individuals with middle and higher education, when compared with individuals with less than four years of schooling. Therefore, the Delayed Verbal Memory Task and Attention Task III were designed with three different levels of difficulty, adapted to the expected baseline cognitive performance of the participants, based on the educational attainment[12]. We expected that these changes would improve the discriminatory ability of the test. The total duration of the BoT test is 24 minutes, and the description of the subtests is detailed in the Appendix.

Study design and protocol

This is a longitudinal study in which a group of patients with probable MCI and a group of individuals from the general population were monitored with Brain on Track for one year. Overall inclusion criteria were: a) ≥18 years of age; b) access to a computer at home and c) being able to use a computer and mouse interface without external help.

Patients with probable MCI and participants from the EPIPorto cohort performed the same baseline neuropsychological evaluation, using a battery of cognitive tests validated for the Portuguese population, including the Montreal Cognitive Assessment (MoCA) test[13], the Mini Mental State Examination (MMSE)[14] the Wechsler Memory Scale III[15], Trail Making Test A and B[16], Stroop Test[17], Clock Drawing Test[18] and Token test[19].

Patients with probable MCI were recruited in the Memory Outpatient Clinic of *Centro Hospitalar de Entre Douro e Vouga*. Eligibility criteria included the presence of progressive cognitive complaints over a period of at least six months, as reported by the patient or family members, impairment in at least one cognitive domain in a neuropsychological evaluation and no limitation in daily activities[20]. Eligible patients who attended the Neurological outpatient clinic in the second semester of 2015 and complied with inclusion criteria were consecutively invited to participate in the study. We recruited 30 patients with a clinical diagnosis of probable MCI from a memory clinic, from which 24 completed the one-year follow-up (80.0%). From these, 16 were confirmed as having a progressive clinical deterioration compatible with MCI in the one-year

clinical re-assessment (nine due to probable Alzheimer's disease, six due to probable vascular cognitive impairment and one due to probable Lewy body disease), while in seven the final clinical diagnosis was anxiety/depression and in one obstructive sleep apnoea syndrome. Those without MCI were included in the general population group for the analyses.

The individuals from the general population represent a subset from the EPIPorto population-based cohort[21]. This cohort was assembled between 1999 and 2003 as a representative sample of adult (≥18 years) dwellers from the city of Porto. Participants were selected by random digit dialing of landline telephones[21]. In the 2013-2015 revaluation of the cohort, the first 300 consecutive participants were invited to participate in the test-retest study of the first version of BoT[12], while the remaining who attend the re-evaluation (n=676) were invited to participate in the present study. From the latter, 75 refused to participate and 289 were excluded, because they did not have continuous access to a computer connected to the internet at home (n=182) or because they could not use a computer and mouse interface without external help (n=107). Therefore, a total of 312 participants were enrolled, from whom 259 completed the one-year follow-up (83.0%). Participants from the EPIPorto cohort who presented impairment in any domain in the neuropsychological assessment were also evaluated by a neurologist to verify if they complied with the criteria for MCI; one participant from the cohort was considered to have MCI, due to probable Alzheimer's disease, and included in the MCI group for data analysis.

All the patients with probable MCI and the one from the EPIPorto study who was classified as having MCI performed lab studies for and brain imaging, and were re-evaluated at the end of the one year follow up, by repeating the neuropsychological evaluation and the clinical observation by neurologist, both blinded for the results of BoT. The final analysis comprises a total of 17 patients with confirmed MCI and a total of 267 healthy individuals.

Assessment with BoT

All participants underwent the first testing with BoT in the hospital clinic or research lab; the test was self-administered, though under the observation of a member from the research team. This session had two main goals: a) teaching the participant how to login to the BoT web page and accustoming the participant with the user interface and b) guaranteeing that the participant understood the instructions and mechanics of each subtest, to minimize learning effects in subsequent testing. One week after the training session, and then every three months for one year, the participants were asked, by e-mail and mobile text messaging, to access the web site from their home computer and to perform the test autonomously.

Statistical analysis

Final test scores of the Brain on Track test were calculated by summing the subtests' z-scores (standardized using the mean and standard deviation (SD) of the general population sample as the reference), and then standardizing

this sum to a t-score (using the mean and standard deviation of the general population sample as the reference, and then multiplying by 10 and adding 50). To compare the differences in age, education and test scores between MCI patients and controls Student's T test for independent samples was used, since all variables presented a normal distribution ($p>0.05$ in Kolmogorov–Smirnov test).

Linear mixed-effects models (LMEM) fit by restricted maximum likelihood were built to describe and compare BoT scores between patients with MCI and individuals from the general population over one year. To build the model, we included, *a priori*, the variables age, education and MCI vs. non-MCI in the model, and separately tested linear and quadratic factors of time, retaining them in the model if they reached statistical significance ($p<0.05$). Then, we separately tested interaction factors between all the variables in the model (MCI, age, education, the linear and quadratic terms for time) retaining the interaction factors in the final model if they reached statistical significance ($p<0.05$).

To estimate the discriminative ability of a screening strategy for early cognitive impairment in individuals with memory complains based on the BoT test we performed a direct comparison between patients with MCI and age and education-matched controls and estimated the area under the curve (AUC) of the BoT test scores to identify MCI. Best matched controls for education and age with each patient with MCI using the nearest neighbor matching propensity score method[22]. The proposed screening strategy comprises two cut points for the first BoT test: all the subjects scoring above

the high cut point would be considered probably not affected and dismissed from further testing; subjects scoring below the low cut point would be classified as probably affected and immediately referred to a Memory Clinic; subjects scoring between these two points would be monitored through regular repetitions of the test. The high cut point was defined to reach the highest possible sensitivity, so that none affected subject was dismissed, and the low cut point for a specificity of 85%, so that those immediately referred to the Memory Clinic have a high probability of being affected.

To estimate the AUC to distinguish between MCI and controls based on the 12-month follow-up with BoT, we first built a LMEM in the matched sample, to estimate the trend in time of BoT scores in MCI vs. matched controls using natural cubic splines with one knot (fixed for all the sample) and random effects by for each spline and intercept individual.[23] To estimate the fixed knot that allowed for the best fit of the data one-dimension optimized function defined using Bayesian-information criteria was used.[23] Then, the random effects of the LMEM were used to predict the probability of MCI. These probability measures were used to define the AUC and a cut point for the 12-month monitoring strategy with BoT, with the higher possible sensitivity, to guarantee that no affected subject was ruled out.

Statistical analysis was performed in R statistical package.

Ethics

The research protocol was approved by the institutional ethics committees of the hospitals where the study was performed. The web-based system for data collection of the Brain on Track test is encrypted and anonymized, and its use has been approved by the Portuguese Data Protection Authority. All subjects provided written informed consent for participation.

Results

At baseline, patients with MCI were older, less educated and had worse performance in cognitive screening tests than healthy controls. The performance in BoT was also significantly worse in patients (Table 1).

When analysing the performance in BoT using the LEEM model, patients with MCI presented, on average, an overall significantly worse performance than healthy individuals. There was also a significant association of older age and lower education with lower average scores on BoT (Table 2). When looking at the longitudinal evolution over time, there was a significant trend to a linear increase in performance in both patients and controls, with a slope that did not differ significantly between the groups ($p=0.34$ for interaction). The quadratic term for time reached statistical significance, even after including the linear effect of time in the model ($p<0.001$). The quadratic term presented a negative concavity, denoting a decrease in performance after the initial increase. Moreover, there was a significant interaction between the quadratic term for time and having MCI, implying that in patients with MCI the decrease is significantly more pronounced than in healthy controls. There was no significant interaction between time (linear or quadratic) with education and age.

In Figure 1, the predicted model scores are depicted, comparing the performance over one year in patients with MCI and healthy controls. The peak in performance in patients with MCI is at around 120 days (coinciding

approximately with the 3rd test from home), with a decline after that, while in controls the performance tends to stabilize at around 180 days (coinciding approximately with the 4th test from home).

Concerning the diagnostic accuracy of BoT for single use, the AUC to identify MCI was 0.862. Based on this, we propose a rule-in cut point, for immediate referral to a Memory Clinic, with a specificity of 88.3% and sensitivity of 76.5%, while the rule-out cut point, for dismissing subjects from further testing, with a sensitivity of 100% and a specificity of 47.0%. Using the data collected over one year in the monitoring strategy, the AUC increased to 0.944, while the single cut point for rule-out would have a sensitivity of 100% and a specificity of 73%.

Discussion

In this study, we were able to implement a cognitive monitoring strategy based on the BoT computerized self-applied test in healthy individuals from a subset of a population-based cohort and in patients with probable MCI from a memory clinic. After an initial increase in test scores in all participants, patients with MCI presented a significant cognitive decline, when compared with controls, after a peak at 120 days. The repeated BoT measurements reached an AUC of 0.94 in the one year monitoring, compared with 0.86 in for single use.

One of the biggest challenges faced in clinical practice and dementia research is to distinguish the age-associated cognitive decline from the early onset of dementia, particularly in patients with memory complaints, but without the interference in the daily performance or social activities that defines dementia. These results highlight the potential of a screening monitoring strategy to identify patients with MCI from the pool of elderly individuals with early memory complaints. Nevertheless, there are still some issues concerning its use. One important potential limitation of all monitoring strategies is practice effects. These are a major concern on longitudinal cognitive monitoring, because of the capacity of the individual to learn and adjust, and therefore individuals perform better at cognitive function tests with repeated testing, interfering on the results interpretation.[24,25] This can be illustrated in the few studies in which the MoCA test was applied repeatedly, at different intervals of time, in patients with MCI. While in a follow-up of 3.5 years 42% of

MCI patients declined in the MoCA, with an average of 1.7 points[26], in shorter time spans, such as 12 months, the MoCA test result increases, demonstrating important practice effects[27]. Taking this limitation into account, it is important to know the factors that can minimize or enhance this effect. One of this factors is the task familiarity[28]. We tried to optimize this by starting the monitoring strategy with a self-administered BoT test in the hospital clinic or research lab, under the observation of a member from the research team, who repeated the instructions in case of any difficulty. Another strategy to minimize this problem is the use of alternate forms[28]. The BoT subtests are designed with a wide variety of elements and different combinations of these elements, so that each trial is different from test to test. The frequency of the evaluations is also an important factor. A previous study in healthy individuals compared two groups with high (baseline, weeks 2-3, week 6, week 9 and month 3) and low (month 6 and month 12) cognitive test frequency over one year, with the high frequency group presenting with prominent practice effects.[25] In our study, we opted for an intermediate frequency (every 3 months for 1 year), which we considered to be low enough to minimize practice effects, but high enough to make an efficient monitoring and to detect changes in the cognitive status of the participants over time.[25] Despite of the implemented measures, our results show that practice effects probably played a role in the performance of both groups in the initial evaluations. The initial slope of the linear increase was similar in patients and controls, but posteriorly, the MCI group started to decline, following a parabola like trajectory that was significantly different from the healthy controls, that maintained a more stable performance. We cannot

discard that, at least in part, the apparent practice effect in patients with MCI could be due to a cognitive improvement secondary to the effects of anti-dementia medication, as most patients in the sample have started cholinesterase inhibitors and/or memantine close to the start of the cognitive monitoring. Ultimately, some degree of learning effects are unavoidable, for that reason the existence of control groups that undergo the same protocol is essential[28], as it allows a direct comparison between the two groups for each successive trials.

Another key point to the efficiency of cognitive testing is addressing the individual pre-morbid differences in cognitive performance, known as cognitive reserve. A possible solution for this problem is the application of adaptive testing, in which the difficulty grade of a question is determined by the performance in the previous question, therefore adapting the test to the patient's abilities. Several authors have argued in favor of that strategy and proposed theoretical models of adaptive testing in the cognitive assessment of the elders.[9,29] However, although adaptive tests have already been developed to monitor the development of young children[30], such tools have never been used in the monitoring of cognitive changes over time in an elderly population. In this study, we performed a first step towards adaptive testing, by adjusting the difficulty of some subtests to the expected performance of the participants, based on academic achievement, making the evaluation process more adapted to each individual. This could be a crucial feature for successful long term cognitive monitoring by limiting ceiling and ground effects, allowing shorter testing sessions without sacrificing precision, and the

possibility to monitor patients with some degree of previous impairment. The inclusion of additional subtests to BoT resulted in an increase in the diagnostic accuracy in single use, with the AUC improving from 0.75 in the previous version[12] to 0.86 in the present version. We aim to further explore this strategy in future studies.

There are some limitations to this study. The number of individuals enrolled with probable MCI that had anxiety/depression and not a neurologic disorder was higher than expected, resulting in a relatively small sample of patients with definitive MCI. Furthermore, it would be interesting to better characterize their pattern of cognitive performance over time and compare them with the MCI and healthy controls, but the small number of patients in this group prevented any meaningful analysis.

The adherence to the monitoring strategy was quite high in the study, similar in both settings, and represents and interesting proof of concept for the feasibility of monitoring patients with cognitive impairment. Nevertheless, 42% of the general population sample did not participate in the study because they did not have access to a computer with internet connection or lacked familiarity with this interface. This is still a considerable number, but it is expected to decrease as the penetration of technology increases and as the younger, more educated strata of the population reaches older age.

In all, the results from this paper suggest that the BoT test could be a suitable tool for an early identification and monitoring of cognitive impairment in elderly individuals, and hopefully improve the current approaches to manage individuals with early memory complaints in the primary care setting and their

referral for specialized care. Additionally, this tool could prove useful to identify candidates for future pre-symptomatic or early symptomatic treatments for Alzheimer's disease. Pre-symptomatic cognitive decline has been demonstrated in unimpaired presenilin-1 carriers using a composite score of neuropsychological tests over five years of follow-up[31]. If, as hoped, pharmaceutical treatments for Alzheimer's disease, currently under phase 2 and 3 clinical trials[32], prove successful in the pre-symptomatic phase, monitoring the population at risk with BoT could effectively identify individuals with probable early cognitive impairment, who would then perform more expensive confirmatory imaging or molecular biomarker tests to demonstrate beta-amyloid pathology, and start treatments with potential to delay or avoid the evolution to dementia.

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Disclosures

V.T.C. and J.P. have a shareholder position at Neuroinova, Lda a start-up company that conceived Brain on Track, holds registered trademark and commercialization rights and also provided funding for part of the study. A.S. received fees for patients' cognitive assessments. The authors declare no other competing financial interests.

Tables

Table 1 - Participant demographics and test scores at baseline

	Patients with confirmed MCI (n=17)	Healthy controls	
		Matched sample (n=17)	Whole sample (n=267)
Age, mean (SD), *years*	70.2 (8.0)	66.5 (7.3)	57.4 (11.4)
Sex (female), *n (%)*	64.7%	58.8%	49.9%
Education, mean (SD), *years*	5.3 (1.9)	5.7 (2.6)	13.6 (4.5)
BoT score	27.8 (5.4)	45.4 (8.8)*	50.0 (10.0)
MMSE score	26.0 (2.4)	28.9 (1.0)*	29.3 (1.0)
MoCA score	16.5 (4.9)	24.7 (3.3)*	26.3 (2.8)

*$p<0.01$ when compared to patients with MCI

Table 2 - Linear mixed-effects model for the test scores of the Brain on Track test over one year

Variables in the model	Linear coefficient	Standard error	p-value
Age *(years)*	-0.37	0.04	<0.001
Education			
4-9 years	6.09	1.66	>0.001
≥10 years	11.96	1.52	>0.001
MCI	-29.75	9.28	>0.01
Time *(years)*	5.11	1.13	>0.001
Time^2	-0.11	0.004	>0.001
Time^2*MCI	0.11	0.004	>0.01

MCI – Mild cognitive impairment

Table 3 – Sensitivity and specificity for single use and for repeated use of the Brain on Track test

	Single BoT test at baseline	BoT 12-month follow–up*
Area under the ROC curve	0.862	0.944
Higher cut-point for referral to specialized care		
Sensitivity	76.5%	
Specificity	88.3%	
Lower cut point for dismissal from further testing		
Sensitivity	100.0%	100.0%
Specificity	47.0%	73.0%

BoT – Brain on Track; ROC – receiver operating characteristic

* Probability of MCI defined using a linear mixed-effects model to estimate the trend in time of BoT scores using natural cubic splines with one fixed knot and random effects for intercept and splines by individual.

Figures

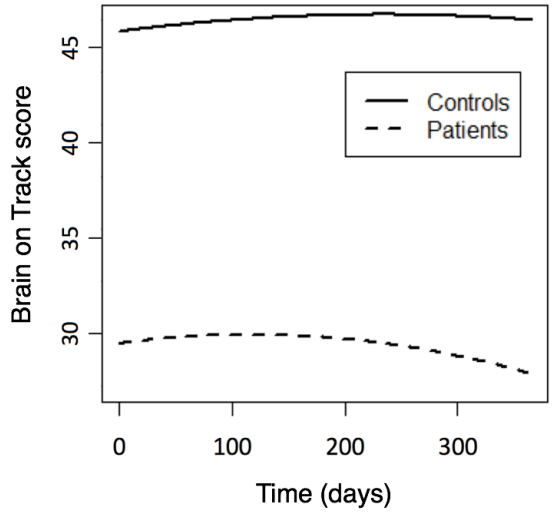

Figure 1 – Trajectories of cognitive performance over one year in the Brain on Track test in patients with mild cognitive impairment and healthy individuals

Appendix

Appendix - Description of Brain on Track subtests

Name	Target Domains	Subtest Description
Puzzles	Constructive ability	The screen is divided into two parts: on the right the target image is shown, on the left several of the image composing pieces are scattered. The purpose of the task is to complete the image using the scattered pieces.
Opposite Task	Inhibitory control Executive functioning	In a central position of the screen, a large arrow is shown. The participant must press the keyboard arrow in the opposite direction to that shown by the large arrow.
Visual Memory Task II	Attention Short term memory	On the screen, three cubes of different colors light up in a random sequence. The participant must memorize this sequence and reproduce it using the mouse to click on the cubes in the correct order.
Calculus Task	Calculus	The participant should perform the numerical calculation shown on screen and input the number via keyboard or by using the mouse to click on a keypad with numbers on screen. The operation should be completed before a balloon reaches the top of the screen.
Sequences	Executive function Abstract thought	The upper part of the screen displays a set of figures that follows a certain logic sequence. The participant should select the figure that completes the sequence from four possible figures.
Verbal Memory Task II	Immediate verbal memory	The participant is asked to memorize a list of four words. After a short delay, 10 words are shown on screen (four correct and six distracters) and the participant must click on the correct words.
Written Comprehension	Language comprehension	On the screen, there are several sets of geometric objects of different shapes and colors. The participant must select the set that matches the description of the written command.
Word categories	Language	The participant must select the correct category for the word that is shown on the screen by dragging the word to the corresponding box. If the word does not belong to any of the categories, the participant must drag it to the garbage can.
Colour Interference task	Executive function, inhibitory control	A name of a colour is shown inside a coloured frame. The colour name, the colour of the word font and the colour of the frame are random. In the first set, the participant must select YES when the word and the colour of the frame match, and NO when they are different. In the middle of the subtest, a new instruction appears on screen, and now the participant must select YES when the colour of the frame matches the colour of the word font.
Delayed Verbal Memory Task	Short term memory	The participant is asked to memorize a list of two, three and five words on the first, second and third levels, respectively. After this, the participant is asked to recall the words after 90 seconds, after 180 seconds and after 360 seconds, performing an interference task between recalls. In the recall, four, six or 10 words, respectively for each level, are shown on screen. Half the words on screen are correct and half are distracters, the participant must click on the correct words. The words are randomly selected from a list of 50 words for each level, with increasing complexity.
Attention task III	Attention, information processing speed	Two pictures are shown on screen, each picture is composed of with several geometrical shapes of different colours, three shapes in each picture on level one; eight shapes on level two; and 15 words on level three. The participant must decide if the two pictures are equal or different. The shapes and colours are randomized for each trial.

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6. Understanding cognitive impairment in multiple sclerosis

6.1 Age and disability drive cognitive impairment in multiple sclerosis across disease subtypes (Paper IV)

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Keywords: Multiple Sclerosis, cognitive impairment, disease course, epidemiology

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Original Research Paper

Age and disability drive cognitive impairment in multiple sclerosis across disease subtypes

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Abstract

Background: There is limited and inconsistent information on the clinical determinants of cognitive impairment (CI) in multiple sclerosis (MS).

Objective: The aim of this study was to compare the prevalence and profile of CI across MS disease subtypes and assess its clinical determinants.

Methods: Cognitive performance was assessed through the Brief Repeatable Battery and the Stroop test in consecutive patients with MS referred to six Italian centers. CI was defined as impairment in ⩾2 cognitive domains.

Results: A total of 1040 patients were included, 167 with clinically isolated syndrome (CIS), 759 with relapsing remitting (RR), 74 with secondary progressive (SP), and 40 with primary progressive (PP) disease course. The overall prevalence of CI was 46.3%; 34.5% in CIS, 44.5% in RR, 79.4% in SP, and 91.3% in PP. The severity of impairment and the number of involved domains were significantly higher in SP and primary progressive multiple sclerosis (PPMS) than in CIS and RR. In multivariable logistic regression analysis, the presence of CI was significantly associated with higher Expanded Disability Status Scale (EDSS) and older age.

Conclusion: CI is present in all MS subtypes since the clinical onset and its frequency is increased in the progressive forms, but these differences seem to be more associated with patient age and physical disability than to disease subtype per se.

Keywords: Multiple sclerosis, cognitive impairment, disease course, epidemiology

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Background

Cognitive impairment (CI) is known to be present in all stages of multiple sclerosis (MS); however, the prevalence estimates vary considerably between studies, ranging from 40% to 65%.[1] The profile of CI in the overall MS population is now relatively well known, involving mainly complex attention, information processing speed, episodic memory, and executive functions.[1,2] Therefore, brief neuropsychological batteries for MS[3] and newly developed assessment tools[4] mainly focus on the assessment of these functions. However, few studies investigated the differences in the prevalence and profile of CI between the different MS disease subtypes, providing heterogeneous results.[5–9] Many of these studies included small clinical samples and focused mainly on relapsing remitting (RR) or progressive forms. Moreover, the association of CI with several clinical features, such as physical disability, sex, and disease duration, is not well established, since inconsistent results have been reported in the literature.[10–13] The heterogeneity of the published literature could be, at least in part, attributable to small sample size and dissimilarities in the clinical characteristics of the studies' samples. Exploring the independent effects of age, physical disability, disease duration, and disease subtype could prove central to provide a better understanding of the potential role and interaction of cognitive reserve, brain aging, and disease severity for determining CI in MS.

The aims of this collaborative, nationwide, cross-sectional study were to describe the prevalence and

profile of CI in a large sample of patients with MS, with a specific focus on prevalence and neuropsychological profiles across different disease subtypes, and to assess the association between CI and the main demographic and clinical features.

Methods

Study design and setting

We invited all consecutive MS patients attending their regular clinical follow-up visits in six Italian MS Centres during the study period (January–October 2010) to participate in the study. The inclusion criteria were (a) diagnosis of MS based on the 2001 McDonald criteria[14] and (b) being between 18 and 70 years old. The exclusion criteria were as follows: (a) presence of current or past neurological disorder other than MS; (b) active major psychiatric illness (such as schizophrenia, bipolar disorder, and major depressive disorder); (c) history of learning disability; serious head trauma, alcohol or drug abuse; and (d) relapse and/or corticosteroid use within 4 weeks preceding the neuropsychological assessment. The classification of disease subtypes was based on the 1996 Lublin's definition.[15] All the participants provided informed consent and the study was approved by the ethics committees of the different institutions.

Clinical and neuropsychological assessment

Patient data were collected using a common database shared among the participating centers and included disease course, age at onset, disease duration, relapses in the previous year, current treatment with disease modifying drugs, and education (complete years of formal schooling). Physical disability was assessed using the Expanded Disability Status Scale (EDSS),[16] a scale validated to monitor disease progression in MS.[17] Fatigue was assessed using the fatigue severity scale (FSS), a scale developed and validated for MS[18] that is composed of nine items with a score range of 9–63. Depression was assessed using the Montgomery and Asberg Depression Scale (MADRS), a standardized measure of mood disorder, with scores ranging from 0 to 60.[19] The FSS and MADRS scales were not part of the initial study protocol; nevertheless, they were routinely used in several of the study centers, resulting in FSS being applied in 728/1040 and MADRS in 356/1040 patients at the time of the study assessment.

A neuropsychological evaluation was performed using the Brief Repeatable Battery (BRB)[3] and the Stroop test.[20] The BRB incorporates tests of verbal memory acquisition and delayed recall (Selective Reminding Test (SRT)); visual memory acquisition and delayed recall (10/36 Spatial Recall Test (SPART)); attention, concentration, and speed of information processing (Paced Auditory Serial Addition Test (PASAT); Symbol Digit Modalities Test (SDMT)); and verbal fluency on semantic stimulus (Word List Generation (WLG)). The neuropsychologists involved in the study had participated in a common training session in which test administration and scoring procedures had been clarified and agreed upon. Test failure was defined as a score below the 5th or above the 95th percentile, when appropriate, according to age, sex, and education-adjusted Italian norms.[21] Impairment in a given cognitive domain was defined as failure in at least one test assessing that domain, namely, SRT for Verbal Learning, SPART for Visuospatial Learning, SDMT and PASAT for Information Processing Speed, and WLG and the Stroop tests for Executive Function. CI was defined as impairment in at least two cognitive domains.

Statistical analysis

Group comparisons were performed using Student's *t*-test for independent samples, the non-parametric Kruskal–Wallis test or χ^2 test with *z*-test adjusted for multiple comparisons (Bonferroni method), where appropriate. The tests were two-sided, with a significance level of 0.05. To confirm the theoretical cognitive domains assessed by the cognitive tests, we performed principal component analysis.

To measure the association between the presence of CI and the different clinical and demographic variables, we calculated crude and adjusted odds ratio (OR), using simple and multivariate logistic regression. We built an a priori model (Model 1), including the demographic variables and education, and estimated the adjusted OR of the other variables. In Model 2, we adjusted to all variables that in Model 1 had a *p*-value lower that 0.1. Finally, we fitted in Model 3 the two variables that remained significant in Model 2 (age and EDSS), and estimated the adjusted OR of the other main clinical variables (disease duration and clinical course). We also assessed the presence of interactions between the variables in Model 2 and Model 3, and tested the inclusion of quadratic factors for each continuous variable, to check the presence of a non-linear relation between the independent variables and the log odds. The presence of multi-collinearity was assessed by calculating the correlation matrix between the main variables, and the variance-inflation (VIF) and generalized variance-inflation factors (GVIF) for logistic regression. The

Table 1. Clinical and demographic characteristics of the study patients.

	Total sample ($n=1040$)	CIS ($n=167$)	RR ($n=759$)	SP ($n=74$)	PP ($n=40$)	p-value
Age, mean (SD) (years)	40.1 (11.0)	33.9 (9.8)	39.9 (10.2)	51.6 (9.5)	49.3 (10.9)	<0.001[a,b]
Sex (female), *n* (%)	704 (67.7)	111 (66.5)	529 (69.7)	43 (58.1)	21 (52.3)	0.062
Education, mean (SD) (years)	12.2 (3.7)	12.7 (3.3)	12.3 (3.7)	11.0 (4.1)	10.2 (3.4)	<0.001[a,c]
Age at onset, mean (SD) (years)	29.7 (9.8)	32.5 (9.4)	28.6 (9.4)	32.2 (11.1)	36.4 (10.7)	<0.001[d]
Disease duration, mean (SD) (years)	10.3 (9.1)	1.4 (2.2)	11.2 (8.4)	19.4 (10.0)	12.8 (6.7)	<0.001[a,e,f]
Relapses in the previous year, mean (SD)	0.9 (1.0)	1.0 (0.5)	0.9 (1.1)	0.3 (0.6)	0.0 (0.0)	<0.001[b,g]
EDSS, median (IQR)	0.2 (2.5; 3.5)	1.5 (1.0; 2.0)	2.0 (1.5; 3.5)	6.0 (4.5; 6.5)	5.25 (5.0; 6.0)	<0.001[a,b]
Treatment with DMDs, *n* (%)	658 (62.7)	28 (16.8)	571 (75.2)	41 (55.4)	9 (22.5)	<0.001[d,h]

CIS: clinically isolated syndrome; RR: relapsing remitting; SP: secondary progressive; PP: primary progressive; SD: standard deviation; EDSS: Expanded Disability Status Scale; IQR: interquartile range; DMDs: disease modifying drugs.
Superscript letters denote significant differences between groups, adjusted for multiple comparisons with the Bonferroni method (adjusted p-value = 0.008):
[a]CIS versus RR, SP, and PP.
[b]RR versus SP and PP.
[c]RR versus PP.
[d]RR versus CIS, SP, and PP.
[e]RR versus SP.
[f]SP versus PP.
[g]PP versus CIS and SP.
[h]SP versus RR, CIS, and PP.

goodness of fit of the models was assessed using the Hosmer–Lemeshow test and the discrimination power was using the C-statistic. The same steps were replicated to fit logistic regression models for impairment in each cognitive domain (final models shown). Statistical analysis was performed using IBM SPSS Statistics Version 23.0.

Results

The study sample consisted of 1040 patients, 167 clinically isolated syndrome (CIS), 759 RR, 74 secondary progressive (SP) and 40 primary progressive (PP) MS patients. The main demographic and clinical characteristics of the sample are depicted in Table 1. The refusal rate in the largest study center (Florence) was 14.5%. Although exact records of refusals are not available for the other centers, the feedback was that the vast majority of the patients agreed to participate.

In the principal component analysis of the items from the neuropsychological evaluation, the variance explained by the four retained components was 69% (Supplementary Table 1). For component 1 (23% variance), the items with a high factor loading corresponded to SRT test; for component 2 (17% variance), to the PASAT test; for component 3 (15% variance), to the WLG and Stroop tests; and for component 4 (14% variance), to the SPART test (Supplementary Table 1), while that of the SDMT presented a moderate loading factor for both components 2 (0.44) and 3 (0.59). These components corresponded approximately with the theoretical cognitive domains: component 1 to verbal learning, component 2 to information processing speed, component 3 to executive function, and component 4 to visuospatial learning; based on these results and on the previous literature, we retained the theoretical construct for the cognitive domains, including the SDMT in the information processing speed domain.

In the whole study sample, the prevalence of CI was 46.3%; 34.5% in CIS, 44.5% in RR MS, 79.4% in SP, and 91.3% in patients with PP. The differences in prevalence were statistically significant in the comparisons of CIS versus SP, CIS versus PP, RR versus SP, and RR versus PP ($p<0.001$) (Table 2). Overall, information processing speed was the most commonly affected cognitive domain (47.9%). There were no significant differences between patients with CIS and RR regarding the frequency of impairment in the different domains (Table 2). On the whole, in patients with SP and PP courses, the presence of CI, as well as impairment on different cognitive domains, was approximately twofold increased when compared to CIS and RR (Table 2). There were no significant differences between the prevalence of impairment by domain between SP and PP patients.

Considering the whole sample, patients with CI were older, had a longer disease duration, higher disability levels on the EDSS, and an older age at MS onset.

Table 2. Prevalence and profile of cognitive impairment in the study sample.

	Total sample ($n=1040$)	CIS ($n=167$)	RR ($n=759$)	SP ($n=74$)	PP ($n=40$)	p-value
Cognitive impairment (⩾2 domains)	46.3%	34.5%	44.5%	79.4%	91.3%	<0.001[a]
Verbal learning	31.1%	27.1%	28.7%	57.7%	46.2%	<0.001[a]
Visuospatial learning	20.5%	14.5%	19.9%	35.3%	31.6%	<0.001[a]
Information processing speed	47.9%	41.2%	45.7%	79.4%	66.7%	<0.001[a]
Executive function	40.8%	41.8%	36.2%	76.4%	92.3%	<0.001[a]
Number of impaired domains (impaired patients), *mean (SD)*	2.6 (0.7)	2.7 (0.7)	2.5 (0.7)	2.8 (0.8)	2.5 (0.6)	0.056
Number of impaired domains (all patients), *mean (SD)*	1.4 (1.2)	1.2 (1.2)	1.4 (1.2)	2.4 (1.1)	2.3 (0.7)	<0.001[a]

CIS: clinically isolated syndrome; RR: relapsing remitting; SP: secondary progressive; PP: chronic progressive; SD: standard deviation.
Superscript letters denote significant differences between groups, adjusted for multiple comparisons with the Bonferroni method (adjusted p-value=0.008):
[a]CIS versus SP; CIS versus PP; RR versus SP; and RR versus PP.

Table 3. Comparison of clinical and demographic characteristics between impaired and non-impaired patients.

	Without cognitive impairment ($n=486$)	With cognitive impairment ($n=422$)	p-value
Age, mean (SD) (years)	36.9 (9.8)	43.2 (11.2)	<0.001
Sex (female), *n* (%)	334 (68.7%)	283 (67.1%)	0.320
Education, mean (SD) (years)	12.52 (3.3)	12.12 (4.0)	0.109
Age at onset, mean (SD) (years)	28.5 (8.9)	30.7 (10.5)	0.001
Disease duration, mean (SD) (years)	8.4 (7.8)	12.5 (10.0)	<0.001
Relapses in the previous year, mean (SD)	0.93 (0.99)	0.82 (0.99)	0.128
EDSS, mean (SD)	2.1 (1.4)	3.0 (1.8)	<0.001
Treatment with DMDs, *n* (%)	289 (59.5%)	266 (63.0%)	0.276

EDSS: Expanded Disability Status Scale; DMDs: disease-modifying drugs; SD: standard deviation.

There were no significant differences in sex, education, and relapses in the previous year between cognitively preserved and impaired patients (Table 3).

In the univariate logistic regression, there was a significant association between the presence of CI and older age (OR (10 years)=1.75; $p<0.001$), longer disease duration (OR (10 years)=1.68; $p<0.001$), and higher disability levels on the EDSS (OR (2 points)=1.99; $p<0.001$). There were no significant differences regarding sex (OR=1.08; $p=0.59$), education (OR=0.97; $p=0.12$), and clinical disease activity (OR=0.76; $p=0.05$). In the subset of patients with fatigue data ($n=728$), there was a significant association between higher FSS score and CI (OR (5 points)=1.05; $p=0.03$), while in the subset with depression data ($n=356$), no association was found between the MADRS score and CI (OR (5 points)=1.02; $p=0.07$). When adjusting for the effect of the demographic variables in the a priori model, disease duration, EDSS, clinical course, and relapses in the previous year presented an association of $p<0.1$ and were fitted in Model 2. In this model, the presence of CI was significantly associated only with older patient age, while the association with other variables was non-significant (Table 4). When adjusting the OR of disease duration and clinical course to age and EDSS (Model 3), the association with CI is non-significant ($p=0.47$ and $p=0.30$, respectively). It is important to note the decrease in the OR of disease duration and disease course when they are fitted in the model with EDSS and age, while the OR for these two latter variables stays approximately the same (Table 4). The VIF and GVIF for the variables (Table 4) are well below the conservative cut point of 5.0,[22] indicating a relatively low multi-collinearity. There was no significant effect of the quadratic terms of the continuous variables or of interaction factors between the variables.

Table 4. Logistic regression models of the prevalence of cognitive impairment in patients with MS.

	Univariate regression		Model 1[a]		Model 2[b]		Model 3[c]	
	OR (95% IC)	*p*-value	OR (95% IC)	*p*-value	OR (95% IC)	*p*-value	OR (95% IC)	*p*-value
Age (10 years)	1.75 (1.75; 2.00)	<0.001	1.76 (1.53; 2.01)	<0.001	1.49 (1.25; 1.77)	<0.001	1.62 (1.42; 1.86)	<0.001
Education (years)	0.97 (0.94; 1.01)	0.12	1.06 (0.79; 1.42)	0.92	1.02 (0.98; 1.06)	0.42		
Sex (female)	1.08 (0.82; 1.43)	0.59	1.06 (0.79; 1.42)	0.69	0.94 (0.68; 1.30)	0.72		
Disease duration (10 years)	1.68 (1.44; 1.97)	<0.001	1.28 (1.07; 1.53)	0.08	1.17 (0.95; 1.45)	0.14	1.08 (0.89; 1.30)	0.47
EDSS (2 points)	1.99 (1.68; 2.36)	<0.001	1.84 (1.53; 2.21)	<0.001	1.75 (1.39; 2.20)	<0.001	1.80 (1.51; 2.15)	<0.001
Clinical course		<0.001		<0.001		0.34		0.30
CIS vs RR	1.52 (1.06; 2.17)	0.02	1.18 (0.81; 1.71)	0.38	0.98 (0.61; 1.58)	0.93	0.91 (0.62; 1.34)	0.63
CIS vs SP	7.29 (3.66; 14.52)	<0.001	3.59 (1.73; 7.46)	0.001	1.34 (0.39; 3.27)	0.53	1.29 (0.56; 2.97)	0.56
CIS vs PP	19.89 (4.50; 87.88)	<0.001	10.66 (2.35; 48.40)	0.002	3.77 (0.76; 18.79)	0.11	3.30 (0.69; 15.89)	0.14
Relapses in the previous year	0.76 (0.58; 1.00)	0.05	1.17 (0.87; 1.59)	0.09	1.07 (0.76; 1.49)	0.71		
FSS (5 points)	1.05 (1.00; 1.10)	0.03	1.00 (0.95; 1.05)	0.87				
MADRS (5 points)	1.12 (0.99; 1.26)	0.07	1.07 (0.03; 1.22)	0.34				
Current treatment with DMDs	1.16 (0.89; 1.52)	0.27	1.24 (0.94; 1.64)	0.14				

MS: multiple sclerosis; OR: odds ratio; IC: Interval of Confidence; EDSS: Expanded Disability Status Scale; FSS: Fatigue Severity Scale; MADRS: Montgomery and Asberg Depression Scale; DMDs: disease modifying drugs; CIS: clinically isolated syndrome; RR: relapsing remitting; SP: secondary progressive; PP: primary progressive.
p-value for Hosmer–Lemeshow goodness of fit test: Model 1 = 0.47; Model 2 = 0.10; Model 3 = 0.08.
C-statistic: Model 1 = 0.66; Model 2 = 0.71; Model 3 = 0.70.
Variation inflation factors: age = 1.39; disease duration = 1.68; EDSS = 1.34; disease subtype = 1.70.
Generalized variation inflation factors: age = 1.18; disease duration = 1.29; EDSS = 1.16; disease subtype = 1.09.
[a]Variables in the model adjusted for sex, education, and age.
[b]Variables in the model adjusted for sex, education, age, disease duration, EDSS, clinical course, and relapses in the previous year.
[c]Variables in the model adjusted for age and EDSS.

Moreover, in an analysis focusing on single cognitive domains, both higher physical disability on the EDSS and older age were associated with increased prevalence of impairment, even after adjusting for the other variables of interest (Table 5). Executive function was the only cognitive domain in which impairment remained associated with disease subtype (Table 5) after adjusting for the other variables in the model (PP and SP > CIS > RR).

Discussion

In this large, collaborative study, we assessed the cognitive performance of MS patients using a neuropsychological battery specifically developed and validated for the disease. Although the study was clinic-based rather than population-based, it involved the main national MS centers, thus providing a reasonably good representation of the population of MS patients in the country.

The prevalence of CI in our study was found to be 46.3%, a figure in line with what has been reported in the recent literature.[1,2,8] The overall profile of CI was also consistent with what has been described,[2] particularly concerning the frequent impairment in information processing speed and episodic memory. However, the prevalence of impairment in executive function was higher than what has been reported in some of the previous literature.[1,2] The two tests used for assessing aspects of executive functions in this study were the Stroop test and the WLG test: notably, a component of speed in information processing cannot be ruled out in these tests. To address this issue, we performed principal component analysis to confirm the theoretical cognitive domains. We found four main components, with the WLG and the Stroop tests having a high factor loading for the same component (0.78 and 0.66, respectively). Additionally, using healthy controls from a previously published normative sample,[21] we performed an exploratory logistic regression analysis to determine if the differences in the Stroop and WLG scores between patients and controls remained significant after adjusting for the SDMT. We found that adjusting for SDMT did not change the OR of the associations between these test scores and patient status (Stroop: crude OR = 1.32 ($p < 0.001$); adjusted OR = 1.23 ($p < 0.001$); WLG: crude OR = 0.31 ($p < 0.001$); adjusted OR = 0.37 ($p < 0.001$)). The results from this analysis indicate that the ST and the WLG tests have an ability to distinguish between patients and controls that is not greatly reduced after controlling for the processing speed component assessed by the SDMT, suggesting they have a potential value in assessing executive function in MS. Overall, these findings suggest the importance of assessing executive function in patients with MS and advocate for an inclusion and further evaluation of tools such as the WLG test in future studies of CI in MS.

CI was more frequent in patients with RR than CIS (44.5% vs 34.5%); however, the difference was not statistically significant. Patients with RR and CIS presented a similar cognitive profile, with a more frequent involvement of information processing speed and executive function compared with other cognitive domains. In comparison with CIS and RR, the prevalence of CI was significantly higher in the progressive forms, as was the number of affected cognitive domains. Indeed, when compared with patients with CIS and RR, our patients with PP and SP had an approximately twofold higher prevalence of impairment in the distinct cognitive domains, with no particular domain disproportionately represented. There is some controversy in the literature regarding the prevalence of CI in the secondary compared with the PP forms, with different authors reporting patients with SP as more, equally, or less affected than patients with PP.[1,5,7] As for the neuropsychological profile, efforts to define distinct cognitive profiles between SP and PP patients have revealed only subtle, often inconsistent, differences.[1,5,7] In this study, patients with SP and PP presented similar prevalence and profile of CI: several cognitive domains were affected in a sizeable proportion of patients, with higher prevalence of impairment in information processing speed and executive function followed by verbal learning. It should be acknowledged, however, that a potential under-representation of the PPMS subtype in our study population can suggest some selection of study participants, since patients with PPMS—for whom no disease modifying drugs are available—may be less likely referred to specialized MS centers.

In the multivariable analysis, we found that the main determinants of overall CI were increased physical disability on the EDSS and older patient age, rather than disease duration or subtype per se.

Additionally, the multivariable analysis by cognitive domain confirmed increased physical disability and older age as the two main determinants of impairment, the effect of disease subtype only remaining significant in the executive function domain. These findings support a prominent effect on cognitive functioning of aging and disease severity, rather than of different pathogenetic mechanisms related to each disease subtype. It is interesting to note that agreeing results have been found in a large single center study,

Table 5. Logistic regression models of the prevalence of impairment by cognitive domain in patients with MS.

	Verbal learning		Visuospatial learning		Information processing speed		Executive function	
	Crude OR (95% CI)	Adjusted OR[a] (95% CI)	Crude OR (95% CI)	Adjusted OR[b] (95% CI)	Crude OR (95% CI)	Adjusted OR[b] (95% CI)	Crude OR (95% CI)	Adjusted OR[a] (95% CI)
Age (10 years)	1.66 (1.46; 1.49)	**1.56 (1.33; 1.82)**	1.42 (1.24; 1.64)	**1.29 (1.24; 1.64)**	1.59 (1.41; 1.80)	**1.40 (1.21; 1.63)**	1.34 (1.18; 1.52)	**1.19 (1.02; 1.39)**
Education (years)	1.00 (0.97; 1.05)	–	1.00 (0.96; 1.04)	–	0.99 (0.96; 1.03)	–	0.89 (0.86; 0.83)	–
Sex (female)	0.63 (0.47; 0.85)	**1.59 (1.18; 2.14)**	0.80 (0.57; 1.11)	–	0.97 (0.75; 1.27)	–	1.53 (1.13; 2.06)	**2.00 (1.44; 2.78)**
Disease duration (10 years)	1.25 (1.08; 1.46)	1.11 (0.91; 1.35)	1.64 (1.41; 1.90)	1.10 (0.89; 1.36)	1.43 (1.22; 1.68)	1.2 (0.89; 1.36)	1.43 (1.22; 1.68)	1.07 (0.87; 1.33)
EDSS (2 points)	1.70 (1.45; 1.98)	**1.54 (1.25; 1.98)**	1.61 (1.35; 1.92)	**1.49 (1.19; 1.86)**	1.70 (1.45; 2.00)	**1.49 (1.22; 1.82)**	1.69 (1.42; 2.00)	**1.57 (1.28; 1.56)**
Clinical course								
RR vs CIS	0.92 (0.63; 1.34)	1.58 (1.00; 2.45)	0.68 (0.43; 1.09)	1.09 (0.65; 1.85)	0.83 (0.59; 1.17)	1.48 (0.99; 2.20)	1.27 (0.89; 1.80)	**1.95 (1.28; 2.96)**
RR vs SP	3.39 (2.06; 5.57)	0.94 (0.51; 1.74)	2.20 (1.30; 3.73)	0.88 (0.46; 1.68)	4.57 (2.44; 8.55)	1.53 (0.75; 3.12)	5.69 (2.99; 10.84)	**2.61 (1.25; 5.44)**
RR vs PP	2.13 (1.11; 4.07)	0.61 (0.28; 1.32)	1.86 (0.92; 3.78)	0.92 (0.40; 2.08)	2.38 (1.17; 4.82)	0.81 (0.36; 1.81)	21.15 (2.73; 163.72)	**15.02 (1.85; 122.12)**
Relapses in the previous year	0.75 (0.57; 0.99)	–	0.77 (0.56; 1.06)	–	0.76 (0.59; 0.99)	–	0.85 (0.64; 1.13)	–
FSS (5 points)	1.02 (0.95; 1.05)	–	1.04 (0.99; 1.09)	–	1.05 (1.00; 1.09)	–	1.00 (0.95; 1.05)	–
MADRS (5 points)	1.01 (0.89; 1.14)	–	1.08 (0.94; 1.23)	–	1.09 (0.98; 1.22)	–	1.10 (0.97; 1.25)	–
Current treatment with DMDs	0.97 (0.94; 1.00)	–	1.02 (0.99; 1.05)	–	0.96 (0.94; 0.99)	–	1.01 (0.98; 1.05)	–

OR: odds ratio; IC: Interval of Confidence; EDSS: Expanded Disability Status Scale; DMDs: disease modifying drugs; CIS: clinically isolated syndrome; RR: relapsing remitting; SP: secondary progressive; PP: primary progressive; FSS: Fatigue Severity Scale; MADRS: Montgomery and Asberg Depression Scale.
[a]Variables in the model adjusted for age, sex, disease duration, EDSS, and clinical course.
[b]Variables in the model adjusted for age, disease duration, EDSS, and clinical course.
Bold values denote a significant association ($p<0.05$).

where clustering by disease subtype did not show any differences in the cognitive profile of CI.[23]

Regarding the relation between physical disability and CI, there is some heterogeneity in the published literature.[10–12] The results from this study clearly imply an association between increasing degrees of physical and cognitive disability, that is also supported by the few available longitudinal series, with smaller sample size.[24,25] The observed relationship may be an effect of disease severity, progression, and biological changes associated with aging, with increasing burden of lesions in the brain, atrophy, and diffuse changes in the white and gray matter, as depicted by imaging and pathological studies.[26] A recent study has also suggested the existence of isolated cognitive relapses that can be detected only through periodic cognitive assessment and may contribute to the burden of CI in the long run.[27]

The absence of an independent effect of disease duration in overall CI is another noteworthy finding from this study. On one hand, age and disease duration are correlated and it may be difficult to disentangle the effect of these two variables. However, the correlation between patient age and disease duration in this patient sample is not particularly strong ($r=0.54$; Supplementary Table 2), resulting in low multi-collinearity between the variables (Table 4). On the other hand, it is interesting to note the parallel between our cross-sectional cognitive findings and what has been reported in large natural history studies on disease prognosis, where physical disability and disease progression are more related to patient age than to the duration of the disease or the clinical phenotype at onset,[28,29] suggesting, as in this study, that disease duration is not an accurate predictor of disease progression. Overall, these results support the hypothesis that in MS the shift from a predominantly inflammatory phase, dominated by clinical relapses, to a predominantly neurodegenerative phase, dominated by irreversible progression of neurological disability, may be mainly driven by biological factors related to aging. Furthermore, the results concur with the hypothesis of cognitive reserve, as aging has previously been associated with decreased plasticity and capability of functional reorganization in MS that probably results from the interaction between cerebral aging and the accumulation of structural brain damage.[30]

As for the role of sex, the published research usually points to an overall worse functional prognosis in males with MS when compared to females.[31] Some previous studies have suggested this also applies to cognitive outcomes,[32] but the issue is controversial in the literature, as most recently published large series have found no significant differences in the prevalence of overall CI.[8,12,13,23] In our sample, in spite of a higher physical disability level in males, we were not able to confirm any significant effect of sex in the prevalence of overall CI, neither as a first order association nor when adjusting for other predictors. Nevertheless, sex-related differences were found in the verbal learning and executive function domains. The better performance of women in verbal learning tests had already been reported, and could perhaps contribute to explain the higher prevalence of CI in males in some of the published literature, as tests designed to evaluate executive functions, in which females performed worst in this study, are not always used to assess patients with MS. Nevertheless, the presence of sex-related differences in some cognitive domains could hint at an interaction between sexual hormones, disease activity, and neurodegeneration, as hypothesized by some authors.[32]

There was also no association of CI with the use of disease modifying drugs. This may be accounted for by the discontinuation or absence of treatment in the older and more disabled patients with the progressive phenotypes. It is also possible that patients with RR with more active and severe disease are more likely to be treated, which renders it difficult to determine the impact of disease modifying drugs on cognition. Longitudinal, controlled studies are needed to shed some light on this score.

As for the association of progressive course and higher impairment in executive function, this is mainly driven be the Stroop test results. We can speculate that this relationship is due to increased frontal dysfunction[33] and frontotemporal lobe atrophy[34] in patients with progressive forms compared with patients with RR. However, the higher impairment in executive function found in CIS patients was mainly driven by a worse performance on the WLG test, which is consistent with findings obtained in a small clinical series.[8]

One limitation of our study is the partial data on depression and fatigue that are well-known potential confounders for cognitive performance in MS.[1] However, performing a sensitivity analysis in the subsets of patients with available data we found that fatigue and depression scores were not retained in the multivariable analysis. These results suggest that fatigue and depression were not major contributors to MS-related CI in these patients.

The model using age and physical disability alone (Model 3) presented an accuracy of 70% to classify patients as having CI, implying that there are other factors that could explain the remaining variability in the subject cognitive outcome, such as genetic determinants, environmental factors, comorbidities, as well as different individual resilience to brain damage due to intellectual enrichment and cognitive reserve.[12,35] Indeed, previous studies have found an association between CI and measures of cognitive reserve, such as the cognitive reserve index,[35] which is composed of education and an assessment of premorbid IQ and premorbid leisure activities. The use of these measures should probably be expanded in future studies, as education alone is probably not a good enough surrogate of cognitive reserve in many populations, as suggested by the results from the present and several of the previous studies, which have reported no direct association of CI and education.[8,13]

In conclusion, the findings obtained from this large clinical series strongly imply that the presence of CI is more related to patient age and disease severity than to disease duration or subtype per se. Furthermore, this study clearly documents a significant presence of CI since the earlier stages of MS, which increases in frequency and severity in the progressive stages. It also adds evidence to previous clinical studies[5–9] and therapeutic trials in CIS,[36] pointing to the need for systematic neuropsychological assessment since the beginning of MS and monitoring throughout the disease course, suggesting that prompt diagnosis and management strategies should ideally be pursued at a younger patient age, when compensatory abilities, brain plasticity, and cognitive reserve may better mitigate the effects of pathological damage in the brain.

Author Contributions

The first two authors contributed equally to the manuscript.

Declaration of Conflicting Interests

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6.2 Patients with paediatric-onset multiple sclerosis are at higher risk of cognitive impairment in adulthood: an Italian collaborative study (Paper V)

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Keywords: Multiple Sclerosis, cognitive impairment, paediatric onset, epidemiology

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Original Research Paper

Patients with paediatric-onset multiple sclerosis are at higher risk of cognitive impairment in adulthood: An Italian collaborative study

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Abstract

Background: Patients with paediatric-onset multiple sclerosis (POMS) could be at an increased risk for cognitive impairment (CI), given the potential harmful effects of disease activity in neurodevelopment. However, there is scarce information on their long-term cognitive outcomes.

Objective: To compare the prevalence and profile of CI between adults with a history of POMS and those with classic, adult-onset multiple sclerosis (AOMS).

Methods: Cognitive performance was assessed through the Brief Repeatable Battery (BRB) and the Stroop Test in consecutive patients referred to six Italian MS centres. CI was defined as impairment in ⩾2 cognitive domains.

Results: In all, 119 patients with POMS and 712 with AOMS were included in this analysis. The prevalence of CI was 48.0% in AOMS, 44.5% in POMS; with similar neuropsychological profile between the two groups. However, when adjusting for current age, we found a significantly increased risk for CI (odds ratio (OR) = 1.71; $p = 0.02$) and for impairment in information processing speed (OR = 1.86; $p < 0.01$) in patients with POMS. A higher Expanded Disability Status Scale (EDSS) was also identified in POMS ($p = 0.03$) compared with AOMS patients.

Conclusion: Patients with a history of POMS appear to be at higher risk of physical and cognitive disability than AOMS patients, after correcting for age effects, with particular involvement of information processing speed.

Keywords: Multiple sclerosis, cognitive impairment, paediatric onset, epidemiology

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Background

Paediatric-onset multiple sclerosis (POMS) occurs in 3%–10% of the whole multiple sclerosis (MS) population[1] and is usually characterized by a relapsing remitting (RR) course. An increased inflammatory activity seems to be present in paediatric patients, when compared with adult-onset MS (AOMS) patients.[2]

Childhood and adolescence represent a critically important period for both brain development and formal academic training. Cognitive impairment (CI) is known to be present in patients with POMS, being consistently reported in approximately one-third of patients,[3] while in the AOMS population 40%–65% of patients present CI.[4] The most affected cognitive domains in the paediatric population with MS are similar to those observed in adults, with a predominant involvement of memory, complex attention, information processing speed, executive functions and visual-spatial abilities.[3–6] Additionally, in POMS subjects, there is accumulating evidence of involvement of linguistic faculties[1,6] and lower intellectual efficiency in terms of intelligence quotient (IQ), particularly in those with younger age at MS onset.[7] An early MS disease onset can have a negative influence in school achievements and overall quality of life,[8–11] but it is unclear if it could lead to an increased risk for CI in later life.[3] Some studies suggest a negative

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impact of relapses, disease duration and physical disability in the cognitive performance of patients with POMS,[8,9,12] but these issues remain controversial.[10,11]

There is little information about the long-term cognitive outcome in patients with POMS. Longitudinal studies published to date have relatively short follow-up periods (1–5 years), and suggest cognitive worsening, with variable rates of decline, although there are patients who may exhibit stable or improved cognitive function over time.[3,10,11] Some of the heterogeneous results found in the literature are probably related to differences in the demographic and clinical composition of the study samples, as well as to the assessment tools used. The observed improvement in cognitive performance in a subgroup of POMS subjects may be due, in principle, to more efficient brain plasticity and compensatory abilities playing a protective role against CI.[3,7] Furthermore, there is little data comparing the cognitive performance and profile of CI of adult patients with POMS and AOMS. A single previous study compared the cognitive performance of these two groups, using the Symbol Digit Modalities Test (SDMT), with the results showing a worse test performance in patients with POMS.[13] A worse performance in language abilities could also be expected, given the more prominent language deficits in the paediatric population.[1,6]

In this cross-sectional, collaborative study, we aimed at comparing the differences in the prevalence and profile of CI in adulthood between adult patients with a history of POMS and patients with classic AOMS. We also explored potential differences between these two groups in terms of other clinical variables, such as disability, fatigue and depression.

Methods

Study design and setting

The setting of the study was a nationwide Italian collaborative initiative. During a 6-month period, consecutive patients with MS from six Italian Centres were recruited and underwent a standardized clinical and neuropsychological assessment. The detailed methodology has been previously described.[14] For this study, a subsample was defined, including the group of adult patients with a history of POMS (diagnosis ⩽18 years old) and AOMS, confirmed using the 2001 McDonald criteria,[15] with an RR or secondary progressive (SP) course, defined based on the 1996 Lublin's definition.[16] The exclusion criteria were current or past neurological disorder other than MS, relapse and/or corticosteroid use within 4 weeks preceding the neuropsychological assessment, major psychiatric illness, history of learning disability before MS onset, alcohol or drug abuse and serious head trauma. Demographical and clinical data including Expanded Disability Status Scale (EDSS) levels and therapeutic information were collected using a common database shared among the participating Centres. In each Centre, a standardized neuropsychological evaluation was performed by a well-experienced neuropsychologist using the Brief Repeatable Battery (BRB)[17] and the Stroop Test.[18] The BRB is a widely used and extensively validated for patients with MS. The BRB includes the Selective Reminding Test (SRT), a test that assesses verbal learning by six learning trials and delayed recall of 12 words; the 7/24 Spatial Recall Test (SPART), a test that assesses visuospatial learning by three consecutive trials and a delayed recall of the position of 10 checkers in a checkerboard; the Paced Auditory Serial Addition Test (PASAT), a measure of sustained attention and speed of information processing in which the subject hears single digit numbers and is asked to add each digit to the one preceding it; the SDMT, another measure of sustained attention and speed of information processing in which the subject must associate a pseudorandomized sequence of the symbols with a single digit as quickly as possible, using a key of symbols and digits; and the word list generation (WLG), that explores verbal fluency by asking the subject to produce as many words as possible belonging to a semantic category.[17] The Stroop Test was selected to complement the BRB in order to provide an additional measure of complex attention and executive function. The neuropsychologists involved in the study participated in a common training session in which test administration and scoring procedures were clarified and agreed upon. Test failure was defined as a score below the 5th or above the 95th percentile, when appropriate, using age, sex and education-adjusted Italian norms.[19] Impairment in each cognitive domain was defined as failure in a test assessing that domain, namely SRT for Verbal Learning; SPART for Visuospatial Learning; SDMT and PASAT for Information Processing Speed; and WLG and the Stroop test for Executive Function. These theoretical cognitive domains were previously confirmed by principal component analysis in the whole sample.[14] CI was defined as impairment in at least two cognitive domains. The fatigue severity scale (FSS)[20] and the Montgomery and Asberg Depression Scale (MADRS),[21] while not part of the initial study protocol, were routinely used in several of the study centres, and were also collected during the evaluation to assess fatigue and depression. Data on FSS were available in 96/119 patients with POMS

and in 502/712 patients with AOMS, MADRS was available in 33/119 and 280/712, respectively. A cut-off of ⩾4 was used to classify patients as fatigued on the FSS[22] and of ⩾20 to classify patients as moderately or severely depressed on the MADRS.[23] All the participants provided their written informed consent and the study was approved by the ethics committees of the different institutions.

Statistical analysis

Group comparisons were performed using Student's t-test for independent samples and the nonparametric Mann–Whitney test or χ^2 test, where appropriate. The tests were two-sided, with a significance level of 0.05. To compare the occurrence of CI and impairment in each cognitive domain between patients with POMS and AOMS, we calculated crude and adjusted odds ratio (OR) using logistic regression. This analysis was performed adjusting for the effect of age and EDSS that have found to be the independent predictors of CI in a previous analysis of the complete sample.[14] Furthermore, we tested the inclusion of disease duration in the logistic regression models adjusted for age, EDSS and POMS, given that a different disease duration is also expected in patients with POMS and AOMS of the same age.

Additionally, and to further clarify the impact on CI of the different disease duration between patients with POMS and AOMS, we performed a complementary analysis of the data by selecting a patient with POMS for each patient with AOMS, best matched for (1) disease duration, (2) age, (3) EDSS and (4) sex, and compared the differences in the prevalence of CI and impairment in each cognitive domain between these matched groups. Statistical analysis was performed using IBM SPSS Statistics for OS X, Version 23.0.

Results

From the total sample of 1040, 831 adult patients with RR and SP MS were included in this study, 712 with AOMS and 119 with a history of POMS, the excluded participants being clinically isolated syndromes or having a primary progressive course. The refusal rate in the largest study centre (Florence) amounted to 14.5%, other study centres did not keep exact records of refusals; nevertheless, the feedback was that the vast majority of the patients agreed to participate. The main demographic and clinical characteristics of these two groups are shown in Table 1. Median age at onset in patients with POMS was 16.4 years, while it was 29.7 years in patients with AOMS ($p < 0.001$). The education, sex distribution and frequency of current treatment with disease-modifying drugs (DMDs) were also similar between the groups (Table 1).

The prevalence of CI was 48.0% in AOMS and 44.5% in POMS ($p = 0.49$). The prevalence of impairment in the different cognitive domains was also similar between the two groups (Table 2), the only significant difference being a higher frequency of verbal learning impairment in patients with AOMS (32.9% vs 21.8%; $p = 0.02$).

Patients with POMS had longer disease duration, higher EDSS levels and higher number of relapses in the previous year than patients with AOMS (Table 1). When stratifying by age group, the EDSS level remained consistently higher in patients with POMS (Figure 1). The prevalence of patients with FSS ⩾ 4 was higher in patients with AOMS ($p = <0.01$), while the MADRS scores showed no significant differences between groups.

Patients with POMS who exhibited CI in their adulthood ($n = 53$) were older ($p = 0.03$) at the time of examination, had higher EDSS ($p < 0.01$) and longer disease duration ($p = 0.02$) as compared with cognitively preserved POMS patients ($n = 66$). There were no significant differences in sex, education, age at onset and relapses in the previous year (Table 3).

In the univariate logistic regression, there were no significant differences in the occurrence of CI between patients with POMS and AOMS (OR = 0.87; $p = 0.49$; Table 4). However, adjusting for the effect of age, we found a significantly increased risk for CI in patients with POMS (OR = 1.71; $p = 0.02$). As depicted in Figure 2, the frequency of CI within each age group was higher in POMS than in AOMS. Performing the same age adjustment for impairment in information processing speed, the OR of POMS increased from 0.96 to 1.86 and the association also became significant ($p < 0.01$). The OR of POMS for impairment in visuospatial learning and executive functions also increased after adjustment although the association remained non-significant ($p = 0.09$ and $p = 0.24$, respectively) (Table 4). Contrarily, the increased frequency of impairment in verbal learning in patients with AOMS was no longer significant after adjusting for the effect of age, with a crude OR of 0.57 ($p = 0.02$) and an adjusted OR of 1.02 ($p = 0.95$). Adjusting the OR for both age and EDSS, the association between POMS and CI was no longer significant (Table 4). Further adjusting the models for disease duration does not result in a relevant decrease in the OR of POMS for CI (OR adjusted for age and EDSS = 1.38; OR

Table 1. Clinical and demographic characteristics of the study sample.

	Adult-onset MS ($n=712$)	Paediatric-onset MS ($n=119$)	p value
Age, median (IQR), years	41.9 (35.0; 49.2)	29.7 (24.4; 37.9)	<0.001
Sex (female), *n* (%)	67.7%	73.0%	ns
Education, mean (SD), years	12.1 (3.8)	12.8 (3.4)	ns
Age at onset, median (IQR), years	29.7 (24.4; 36.4)	16.4 (14.6; 17.9)	<0.001
Clinical course (relapsing remitting), *n* (%)	90.3%	95.8%	ns
Disease duration, median (IQR), years	9.2 (4.7; 16.6)	13.2 (8.1; 21.5)	<0.01
Relapses in the previous year, mean (SD)	0.83 (1.0)	1.29 (1.1)	<0.001
EDSS, median (IQR)	2.0 (1.5; 4.0)	2.5 (1.5; 4.0)	0.03
FSS (score ⩾4), *n* (%)	66.7%	79.5%	<0.01
MADRS (score ⩾20), *n* (%)	11.1%	3.0%	ns
Treatment with DMDs, *n* (%)			
No treatment	31.0%	24.8%	
Glatiramer acetate	9.8%	5.5%	
Interferons	40.0%	46.8%	
Natalizumab	11.2%	16.5%	
Fingolimod	0.6%	0.9%	
Immunosuppressant[a]	7.4%	5.5%	ns

MS: multiple sclerosis; SD: standard deviation; EDSS: Expanded Disability Status Scale; IQR: interquartile range; DMDs: disease-modifying drugs; ns: not significant ($p>0.07$).
[a]Azathioprine, mitoxantrone, cyclophosphamide and methotrexate.

Table 2. Prevalence and profile of cognitive impairment.

	Adult-onset MS ($n=712$)	Paediatric-onset MS ($n=119$)	p value
Cognitive impairment (⩾2 domains)	48.0%	44.5%	ns
Impairment in verbal learning	32.9%	21.8%	0.02
Impairment in visuospatial learning	20.9%	22.2%	ns
Impairment in information processing speed	48.5%	47.4%	ns
Impairment in executive function	39.8%	37.4%	ns
Number of impaired domains (impaired patients), *mean, SD*	2.6 (0.7)	2.5 (0.7)	ns
Number of impaired domains (all patients), *mean, SD*	1.4 (1.2)	1.3 (1.2)	ns

MS: multiple sclerosis; SD: standard deviation; ns: not significant ($p>0.2$).

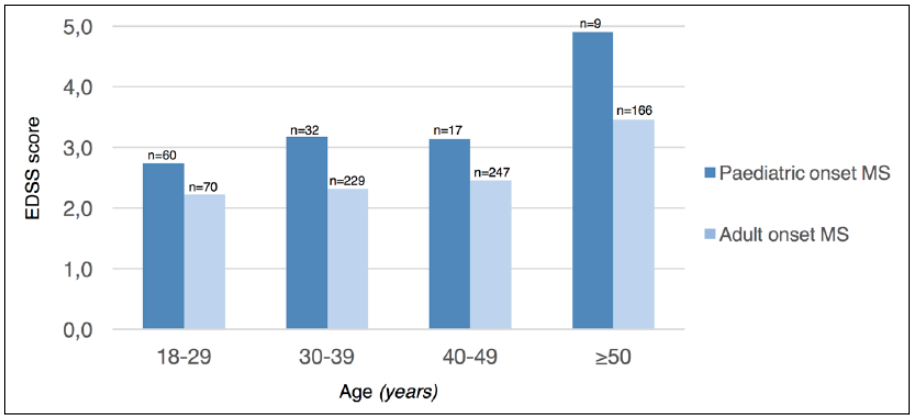

Figure 1. Comparison of the average EDSS score by age group in patients with paediatric- and adult-onset multiple sclerosis.

Table 3. Comparison of clinical and demographic characteristics between impaired and non-impaired patients with paediatric-onset multiple sclerosis.

	POMS without cognitive impairment ($n=66$)	POMS with cognitive impairment ($n=53$)	p value
Age, median (IQR), years	27.5 (22.7;35.7)	32.3 (27.0;39.8)	0.02
Sex (female), *n* (%)	75.0%	72.9%	ns
Education, mean (SD), years	13.2 (3.1)	12.5 (3.8)	ns
Age at onset, median (IQR), years	16.4 (14.7;17.9)	16.9 (14.2;17.8)	ns
Disease duration, median (IQR), years	10.7 (6.2;19,3)	15.4 (11.0;22.8)	0.03
Relapses in the previous year, mean (SD)	1.3 (1.1)	1.4 (1.0)	ns
EDSS, median (IQR)	2.25 (1.5;3.5)	3.5 (2.0;5.0)	<0.01
Treatment with DMDs, *n* (%)			
No treatment	28.3%	18.2%	
Glatiramer acetate	6.7%	4.5%	
Interferons	40.0%	52.3%	
Natalizumab	20.0%	13.6%	
Fingolimod	0%	2.7%	
Immunosuppressant[a]	5.0%	9.1%	ns

POMS: paediatric-onset multiple sclerosis; EDSS: Expanded Disability Status Scale; DMDs: disease-modifying drugs; IQR: interquartile range; ns: not significant ($p>0.1$).
[a]Azathioprine, mitoxantrone. cyclophosphamide and methotrexate.

Table 4. Odds ratio of paediatric-onset multiple sclerosis for cognitive impairment and impairment in the different cognitive domains.

Outcome for POMS	Unadjusted OR (95% CI)	p value	OR adj. for age (95% CI)	p value	OR adj. for age and EDSS (95% CI)	p value	OR adj. for age, EDSS and duration (95% CI)	p value
Cognitive impairment (⩾2 domains)	0.87 (0.57; 1.03)	ns	1.71 (1.07; 2.74)	0.02	1.38 (0.85; 2.23)	ns	1.32 (0.77; 2.23)	ns
Impairment in verbal learning	0.57 (0.36; 0.91)	0.02	1.02 (0.62; 1.68)	ns	0.82 (0.49; 1.38)	ns	0.71 (0.40; 1.26)	ns
Impairment in visuospatial learning	1.01 (0.63; 1.63)	ns	1.56 (0.93; 2.63)	ns	1.32 (0.77; 2.27)	ns	1.29 (0.71; 2.35)	ns
Impairment in information processing speed	0.96 (0.65; 1.42)	ns	1.86 (1.19; 2.90)	<0.01	1.53 (0.97; 2.41)	ns	1.31 (0.79; 2.17)	ns
Impairment in executive function	0.90 (0.59; 1.38)	ns	1.32 (0.83; 2.11)	ns	1.07 (0.66; 1.74)	ns	1.06 (0.61; 1.82)	ns

POMS: paediatric-onset multiple sclerosis; EDSS: Expanded Disability Status Scale; ns: not significant ($p>0.07$); adj.: adjusted.

adjusted for age, EDSS and disease duration = 1.32; Table 4). Moreover, when testing this addition of disease duration to the models with age, EDSS and POMS for CI and impairment in the different domains, the association of disease duration with CI and impairment in different domains is non-significant ($p>0.08$).

In the analysis of patients with AOMS and POMS matched for disease duration, age, EDSS and sex the prevalence of CI was 28.7% in POMS and 44.5% in AOMS ($p=0.02$). The prevalence of impairment in each cognitive domain was also higher for patients with POMS although the only difference to reach statistical significance was visuospatial learning (Table 5). Comparing the cognitive test scores and Cohen's *d* values of the two matched groups, we found significant differences in tests of information processing speed, with moderate effect size for the PASAT test (Cohen's *d* value = 0.47); Supplementary Table 1).

Table 5. Comparison of adult and paediatric-onset MS patients, matched for disease duration, age, gender and EDSS.

	Adult-onset MS ($n=119$)	Paediatric-onset MS ($n=119$)	p value
Disease duration, median (IQR), years	12.3 (7.3; 16.7)	13.2 (8.1; 21.5)	ns
Age, median (IQR), years	34.9 (19.1; 38.6)	29.7 (24.4; 37.9)	0.01
Sex (female), *n* (%)	63.6%	73.0%	ns
EDSS, median (IQR)	2.5 (1.5; 4.0)	2.5 (1.5; 4.0)	ns
Cognitive impairment (⩾2 domains)	28.7%	44.5%	0.02
Impairment in verbal learning	24.2%	21.8%	ns
Impairment in visuospatial learning	11.7%	21.2%	0.04
Impairment in information processing speed	36.8%	47.4%	ns
Impairment in executive function	36.2%	37.4%	ns

MS: multiple sclerosis; EDSS: Expanded Disability Status Scale; IQR: interquartile range; ns: not significant ($p>0.09$).

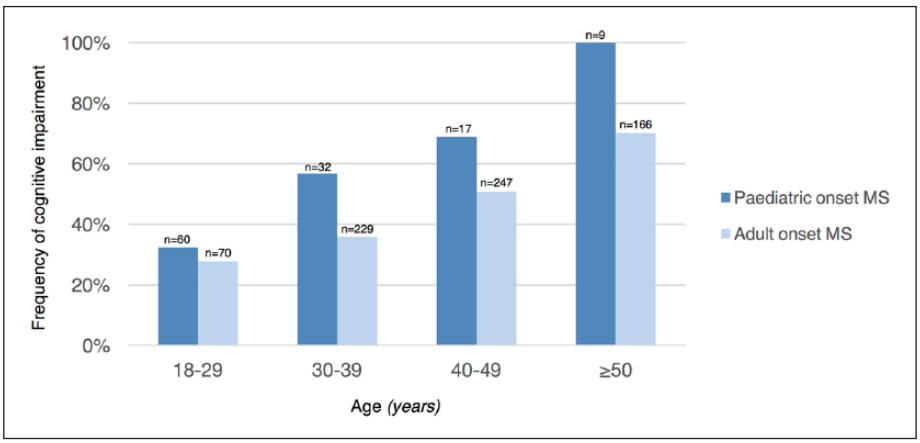

Figure 2. Comparison of the prevalence of cognitive impairment by age group in patients with paediatric-and adult-onset multiple sclerosis.

Discussion

In this study, we compared the prevalence and profile of CI in a large sample of adult patients with POMS and AOMS, with RR and SP forms, using a standardized neuropsychological battery specifically validated for MS patients.

Our study showed a prevalence of CI of 48.0% in AOMS and 44.5% in POMS. These results are in line with what is generally reported in the literature for adult patients with MS (40%–65%).[4] The prevalence of CI obtained for the adult patients with a history of POMS was higher than that generally found by previous studies in children and adolescents with MS.[3] This finding reinforces the notion, coming from a few longitudinal studies performed up to date, that, in subjects with a paediatric onset, CI may tend to increase over the years, and those not affected during childhood seem to be at an increased risk to develop cognitive dysfunction later in adulthood,[3] at an earlier age than their AOMS counterparts. It is important to note that this earlier onset of CI in patients with POMS could have a negative influence in several aspects of the patient life, namely educational achievements, career making and family planning. Patients with POMS and AOMS presented a similar cognitive profile, with information processing speed being the most commonly affected domain in both groups.

While the crude prevalence of CI was similar between the two groups, after adjusting for the effect of age, we found that patients with POMS had a significantly higher risk for CI than patients with AOMS. This effect was particularly prominent for impairment in information processing speed, with POMS patients having two times increased risk for impairment in this domain than AOMS patients of the same age, and a worse performance in both SDMT and PASAT when compared to matched AOMS patients (Supplementary Table 1). A worse performance of adult patients with POMS in SDMT has been previously described,[13] suggesting that information processing speed could

be disproportionally affected in the long term after an early disease onset. Overall, these findings suggest that adult patients with a history of POMS have worse cognitive outcomes than patients with AOMS of the same age. On the one hand, the onset of MS in a young age, with the cerebral structures and cognitive faculties still in development, could be the main responsible for the increase risk for CI. On the other hand, these results could be explained by the longer disease duration in POMS patients. Since there was a significant association between a paediatric onset of MS and longer disease duration, it was not possible to completely disentangle the independent effect of these two variables in a logistic regression model, also due to the sample size. However, it is interesting to note that further adjusting the model for disease duration does not result in a relevant decrease in the OR for CI of POMS. Furthermore, in the comparison of patients with AOMS and POMS best matched for disease duration, age, EDSS and sex the prevalence of CI was significantly higher POMS than in AOMS (44.5% vs 28.7%; $p=0.02$). Although the large available sample of AOMS patients, it was not possible to groups were not perfectly match simultaneously for age and disease duration, given that the relative smaller number of younger patients with longer disease duration in the AOMS population when compared with the POMS population, resulting in and older median age for patients with AOMS in this analysis (Table 5). However, given the increased risk of CI in older patients with MS, any effect of this unbalance in the comparison would be to overestimate the prevalence of CI in AOMS. Consequently, the results from this matched comparison support the notion that the higher risk for CI in patients with POMS is probably more related to the paediatric onset than to longer disease duration. Additionally, in our previous analysis of the larger sample from which the subsample was selected, we could not find an independent effect of disease duration in the prevalence of CI in the overall MS population.[14] Taken as a whole, these findings support the hypothesis that the paediatric onset *per se* has an important role in determining the cognitive outcomes in adulthood, and that disease duration is probably not the main reason behind the differences in CI performance between adult patients with POMS and AOMS. Additional studies comparing these two groups can be useful to clarify this issue.

Our finding of a similar cognitive profile between the two groups is in accordance to the literature. Previous studies have described language deficits in children with MS[1,3] although this topic is controversial.[24] To explain our results, several considerations should be taken into account. The BRB, used in our study, does not include a specific evaluation of linguistic faculties, which limits our capability to drive any firm conclusion at this regard. In our sample, there were no differences in the two groups in terms of verbal fluency. These results suggest that the verbal fluency deficits described in the paediatric population with MS could be attenuated in adulthood. This may be due to adaptive processes, which are thought to play a role in the improvement of cognition in such cases,[10,11] and we can hypothesize that formal training through academic education could be related to improved linguistic skills in adulthood. Finally, it is important to note that linguistic deficits are described especially in POMS patients with early disease onset (before 10 years of age),[3] who represented a minority of subjects in our sample (4.2%). Long-term follow-up studies of patients with POMS from the diagnosis to adulthood, considering different classes of age at onset, are needed to shed some light on the evolution of different cognitive faculties over the disease course in this special population of patients.

A higher physical disability level was significantly associated with CI in the previously published analysis of the whole study sample.[2] In this context, it is important to note that the subgroup of POMS patients with CI also presented higher disability levels although not by a large magnitude, whereas previous studies reached heterogeneous conclusions about the association of physical disability with cognitive dysfunction in patients with POMS.[8,10,12] It must be noted, however, that the AOMS patients in this sample are significantly older than POMS patients, and when stratifying by age group, the EDSS level remained consistently higher in patients with POMS (Figure 1). In previous prognostic studies, POMS patients appeared to reach irreversible EDSS milestones with a delay of nearly 10 years of disease duration when compared with AOMS patients although these irreversible levels of disability were achieved at a younger age in POMS.[25]

Patients with POMS presented a higher physical disability level than patients with AOMS although they did not exhibit worse results in depression and fatigue scores. Although the fatigue and depression data were incomplete in this sample, the results agree with those of a previous study that compared depression and fatigue between adults of the two groups.[13] Other studies comparing children with POMS and adult patients with AOMS also showed lower prevalence rates of depression and fatigue in the first group.[1] This is an interesting finding because higher depression and fatigues scores in adult POMS

compared to AOMS patients might be expected, due to the reported association with higher physical disability[26,27] and recent report of an association between psychiatric morbidity and cognitive dysfunction in POMS.[28] We can hypothesize that these patients could develop better coping strategies, due to living with the diagnosis since early age. Alternatively, the results could also be explained, at least in part, by reduced insight, possibly related with young age at onset and/or higher levels of CI, and a consequent decreased perception of the severity of the disease. Indeed, although a deficit in disability self-awareness has not been extensively researched in patients with MS, some reports have described its presence.[29,30]

In interpreting the study findings, we should consider a few limitations, namely, the cross-sectional design and absence of neuropsychological assessment of the patients at the time of the diagnosis, as well as possible selection of more severe POMS cases referred to specialized MS Centres for adults. Furthermore, it would be interesting to have magnetic resonance imaging (MRI) data to analyse potential differences between the groups and to assess possible correlations of imaging and CI measures.

Despite the above considerations, the results from the study add to previous evidence in the field suggesting that patients with a history of POMS, as compared with their adult-onset counterpart, may be especially vulnerable to the negative consequences of the disease and present worse physical and cognitive outcomes in the long run, particularly regarding impairment in information processing speed. These findings highlight the need for early screening and systematic monitoring of cognitive functioning in the paediatric MS population, aimed at providing prompt counselling and intervention strategies in everyday practice. The development of effective approaches for rehabilitation and prevention of cognitive deterioration in this population remains a priority for future research in this area.

Declaration of Conflicting Interests

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6.3 Tracking cognitive impairment in multiple sclerosis using the Brain on Track test: a validation study (Paper VI)

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Keywords: Multiple Sclerosis, cognitive impairment, cognitive assessment, Computer-Assisted Decision Making

Abstract

Background: The accurate and regular monitoring cognitive performance in multiple sclerosis (MS) patients is critical to develop prevention and management strategies for cognitive impairment (CI). The Brain on Track test (BoT) is a self-administered web-based tool developed for cognitive screening and monitoring.

Objective: The objective of this study was to validate the use of the BOT in MS, by assessing its ability to distinguish between MS patients and matched controls, as well as detect CI among MS patients, its correlation with standard cognitive tests and its reliability and learning effects in repeatable use.

Methods: The BoT was applied in 30 patients with MS consecutively selected and 30 age- and education-matched controls, first in a hospital clinic, under supervision, and then one week later from home. After these first two trials, MS patients repeated the test from home every four weeks for three months. A standard neuropsychological battery was also applied to MS patients at baseline.

Results: The Cronbach's alpha was 0.89. Test scores were significantly different between MS patients and controls (Cohen's $d=0.87$; $p<0.01$). Among MS patients, scores were significantly lower in those with CI documented in the standard neuropsychological battery than in their cognitively preserved

counterparts (Cohen's d=2.0; p<0.001). The BOT scores presented a good correlation with standard neuropsychological tests, particularly for information processing speed. Regarding test-retest reliability, 10/11 subtests presented two-way mixed single intraclass consistency correlation coefficients >0.70.

Conclusion: The BOT showed good neuropsychological parameters in MS patients, endorsing the use of self-administered computerized tests in this setting.

Background

Cognitive impairment (CI) is known to be present in all multiple sclerosis (MS) subtypes since the clinical onset, with the prevalence ranging from 40% to 65%[1]. The frequency of CI seems to increase with patient age and physical disability[2], but those with early onset forms are at a higher risk for CI at older age[3].

Most papers that aim to describe the profile, determinants and evolution of CI in MS are cross-sectional and the few existing longitudinal studies yielded heterogeneous findings[1]. While some investigations document a progressive pattern of cognitive deterioration from the early stages to the most advanced, progressive stages of the disease[4-8], others show preservation of cognitive skills over the time of follow-up[9-16]. There is also some recent evidence that "isolated cognitive relapses" could play a role in driving CI in MS[17], and these could be associated with clinically silent demyelinating lesions in the magnetic resonance imaging[18]. These heterogeneous results may be due to differences in the patient samples, assessment tools, test-retest reliability and possible learning effects of the repeated use of the cognitive assessment tools. To answer these questions, it would be interesting to increase both the frequency of cognitive assessment and the length of follow-up. Computerized cognitive testing presents some interesting attributes that render it particularly suitable for these aims, by allowing for: 1) remote self-applied testing, reducing the barriers and costs of cognitive assessment; 2) easier implementation of alternative versions and randomized elements in tasks, to reduce the learning effects of repeated testing; 3) adaptation of the

testing difficulty to the expected performance of the individual; and 4) more accurate measures of scores, through improved standardization of testing procedures[19].

Brain on Track is (BoT) a web-based computerized cognitive test, developed for self-administered cognitive screening and monitoring[20]. It showed good reproducibility, correlation with existing cognitive tests, ability to identify clinically relevant differences for mild cognitive impariment and early dementia and high test-retest reliability when performed from the patients' home[20].

The objective of this study was to validate the use of BoT to screen and monitor cognitive impairment in patients with MS, by assessing its ability to distinguish between MS patients and matched controls, as well as identify cognitive impairment in MS patients, its correlation with standard cognitive tests and its reliability and learning effects in repeatable use.

Methods

The Brain on Track test

The BoT test is a self-administered computerized test, which can be performed from any computer with an internet connection, mouse and keyboard, intended for longitudinal cognitive testing. It is composed of several subtests, which have been previously described in detail[20], expected to evaluate different cognitive domains, all including random elements to minimize learning effects, and takes 24 minutes to be completed. Briefly, the initial development and validation of the BoT test[20] resulted in a version with seven subtests, including Visual Memory Task II (attention, visual memory), Calculus Task (calculus), Opposite Task (inhibitory control, executive functioning), Written Comprehension (language comprehension, information processing speed), Word categories (language), Sequences (executive function, abstract) and Puzzles (visuospatial ability). In subsequent work[21], four additional subtests were added: Inhibitory control task (executive function, inhibitory control), Verbal memory task II (immediate verbal memory), Delayed verbal memory task (delayed verbal memory) and Attention task III (attention, information processing speed). The latter two subtests were designed with three different levels of difficulty, to be used for adapting the test difficulty to the patients' educational attainment, which was shown to improve the discriminatory ability of the Brain Track Test in MCI[21].

Study design and protocol

In this study, the BoT test was applied in 30 patients with MS and 30 matched controls. MS patients who complied with the inclusion criteria were consecutively selected among those attending regular follow-up in the outpatient clinics of two MS centres from Northern Portugal (*Centro Hospitalar de Entre Douro e Vouga* and *Hospital de Braga*).Controls, matched for age (10-year groups) and educational level (1-4, 5-9, 10-12 and >12 years), were selected among hospital volunteers and health workers fulfilling the inclusion criteria.

To be eligible, both patients and controls had to be ≥18 years of age and no physical impairment precluding the use of a computer and mouse interface. Specific inclusion criteria for patients were an established diagnosis of MS based on the 2010 MacDonald criteria[22], with a relapsing reminting form[23], and a score in the Expanded Disability Status Scale (EDSS)[24] ≤4.5. Specific inclusion criteria for controls were absence of any neurological, psychiatric or systemic disease that could impair cognition (except for stable depressive symptoms) and normal cognitive performance, defined as performance above the age and education adjusted norms (2.0 standard deviations) for the Portuguese population in the Montreal cognitive assessment test (MoCA)[25].

In the baseline evaluation of all participants a first session of the BoT test was completed under supervision. The subjects were shown how to log to the BoT on-line platform and performed the exercises independently under the observation of a neuropsychologist from the research team. In case of any difficulties the instructions were repeated. This was to guarantee that the participants understood the instructions and mechanics of each exercise, to

be able to access the web site from their home computer and to perform the test autonomously. All participants were asked to perform the BoT test at home one week after the baseline evaluation. Among MS patients, the test was also repeated every four weeks for three months. Reminders via e-mail and mobile text messaging were send to all the participants in the day of the scheduled test.

At baseline, all participants underwent an evaluation with the MoCA test, and MS patients performed a complete clinical evaluation, including the Expanded Disability Status Scale (EDSS)[24], the Modified Fatigue Impact Scale (MFIS)[26] the Beck Depression Inventory (BDI)[27], and were assessed using a battery of cognitive tests validated for the Portuguese population. The battery assessed complex attention on the Trail Making Test (TMT) A and B[28]; information processing speed using the Symbol Search Test (SST) of the Wechsler adult intelligence Scale IV (WAIS-IV)[29]; working memory on the Letter-Number Sequencing (LNS) subtest of the WAIS-IV[29]; verbal memory on the California Verbal Learning Test (CVLT)[30]; executive function on the Stroop test[31] and the Matrix Reasoning Test (MRT) from WAIS-IV[29] and visuospatial abilities on the Clock Drawing Test[32]. Impairment in a cognitive domain was considered present when participants scored less than 2.0 SD below age and education-adjusted norms in at least one test. Impairment in at least two of the cognitive domains above was classified as CI. We also applied two tests not validated for the Portuguese population, but extensively used in MS, the visual memory in the Brief Visuospatial Memory Test (BVMT)[33] and the Symbol Digit Modalities Test (SDMT)[34].

The research protocol was approved by the institutional ethics committees of the hospitals where the study was performed. The web-based system for data collection of the BoT test is encrypted and anonymized, and its use has been approved by the Portuguese Data Protection Authority. All subjects provided written informed consent for participation.

Statistical analysis

Final test scores of the BoT test were calculated by summing the subtests' z-scores (standardized using the mean and standard deviation (SD) of the whole sample as the reference), and then standardizing this sum to a t-score (using the mean and standard deviation of the whole sample as the reference, and then multiplying by 10 and adding 50). To compare the differences in age, education and test scores between patients with MS and controls, Student's T test for independent samples was used, since all variables presented a normal distribution ($p>0.05$ in the Kolmogorov–Smirnov test).

To estimate the predictive ability of the BoT test to distinguish between 1) MS patients *vs.* controls and 2) MS patients with CI *vs.* those cognitively preserved, the areas under the receiver operating characteristic curves (AUC)[35] were estimated using logistic regression models fitted with group as the dependent variable and test scores as the independent variable.

Principal component analysis was performed to evaluate dimensionality of the subtests and the relationship between the BoT scores and the results

from the other neuropsychological assessment tests[36]. The acceleration factor that corresponds to the numerical solution to the elbow of the scree plot was used to define the number of components retained. The internal consistency was assessed using Cronbach's alpha[37]. Test-retest reliability for each subtest was assessed using consistency two way mixed single intraclass correlation coefficient (ICC)[38,39] for the four BoT test trials performed by MS patients (one supervised and three remote). Additionally, learning effects between pairs of consecutive trials were assessed in these patients using Student's T test for related samples, since all variables presented a normal distribution ($p>0.05$ in Kolmogorov–Smirnov test). Statistical analysis was performed in SPSS.

Results

The patients and controls did not differ in age and education, but the proportion of females was significantly higher in the control group (Table 1). There was also no significant difference in the MoCA score between the two groups (p=0.56). Among MS patients, the median (percentile 25; percentile 75 [P25; P75]) was 1.0 (0.0; 2.1) for the EDDS score, 3.5 (2.0; 9.3) for the number of years since the clinical onset of MS. The frequency of CI, as assessed by the battery of cognitive tests, was 22.0% in MS patients, while the mean MFIS score was 32.9 (SD=19.9), the mean BDI score 6.9 (SD=6.3) and 16.7% of the patients were under 2nd line disease modifying drugs (the remaining were all treated with 1st line drugs).

The score of the first BoT test was significantly lower in patients as compared with heathy controls ($p<0.01$), with the difference presenting a large effect size (Cohen's d=0.87; Table 1). The AUC to distinguish patients vs. controls based on the BoT test score was 0.75. In the second BoT trial, performed remotely from home in patients and controls, the difference between groups remained significant, with a moderate effect size (Table 1).

The scores of the BoT subtests were also lower in MS patients; differences were not statistically significant only for two subtests (Opposite Task and Written Comprehension). In six of the subtests, a large effect size was present (Cohen's d>0.8), while in three subtests the effect size was moderate (Cohen's d= [0.5;0.8] Table 2). A high internal consistency was

present, with an overall Cronbach's alpha of 0.89, that would not be increased by removing any of the subtests (Table 2). In the principal component analysis, the solution defined by the scree plot criteria was one principal component for the BoT test, corresponding to 49.0% of the explained variance. All the subtests presented a factor loading close or above 0.50 (Table 2).

Among the patients with MS, the mean score of the first BoT test was significantly lower in the ones with CI (classified based on the neuropsychological evaluation) when compared with their cognitively preserved counterparts; the mean (SD) score were 35.1 (7.2) in patients with CI and 49.0 (6.6) in those without CI ($p<0.001$), corresponding to a large effect size (Cohen's $d=2.0$). The AUC to distinguish cognitively preserved patients with MS from patients with CI, based on the BoT test score, was 0.91.

In the principal component analysis of the items from the neuropsychological evaluation, the solution defined by the scree plot criteria was two principal components, with a variance explained by the two retained components of 66.7%. For component 1, that explained most variance (57.3%), several tests presented a high factor loading (Table 3), particularly those assessing information processing speed and complex attention (SDMT, SST, TMT B), but also some of those assessing visual memory (BVMT) and executive function (MRT), with the Brain in Track test also presenting one of the highest factor loading (0.82). The second component explained far less variance (8.4%), with all the items having a lower factor loading than for component 1, the highest being for the Stroop test (0.46) and the MoCA test (0.45), with the Brain in Track test presenting a factor loading of 0.24. A

significant correlation was found with between the BoT test and all the tests from the neuropsychological battery. Generally, the BoT subtests also showed a significant correlation with the tests from the neuropsychological battery that were expected to assess the same domain. The correlations were higher for the SDMT (r=0.67) and the SST (r=0.71). The correlations of the BoT test with the tests of the neuropsychological battery were generally higher than those identified for the MoCA test, particularly in the measures of information processing speed (Appendix 1).

In the analysis of the test-retest reliability of the four consecutive trials performed by the MS patients, including one supervised test and three tests performed from home, nine out of the eleven subtests showed a ICC higher than 0.80 (Table 4). When assessing learning effects between consecutive trials, there was an upward trend between the first trial and the second of most subtest scores, with significant differences in seven out of eleven (Table 4). Opposite task presented a significant and continuous upward trend across the four trials (Table 4). A significant improvement was also identified between the third and fourth trial of the Calculus Task and Sequences (Table 4).

Discussion

In the present study, we describe the validation of a web-based self-administered test intended for longitudinal cognitive testing (Brain on Track) in a sample of patients with relapsing-remitting MS. The test showed good internal consistency and reliability indexes, as well as ability to identify significant differences, with large effect sizes when comparing the differences in performance between patients with MS and age- and education-matched controls. Furthermore, the test also showed the ability to identify significant differences between MS patients with CI and those cognitively preserved, also with large effect size. The BoT test adds a high factor loading to the principal component defined by the dimensionality analysis of the standard neuropsychological tests in these sample of MS patients, close to the factor loading of the most commonly used tests in these patients, such as SDMT and BVMT. Additionally, the BoT test presented significant correlations with all the tests from the neuropsychological battery, which were particularly high with measures of information processing speed, the most frequently affected domain in MS, particularly in the early disease stages[1,2].

A few computerized cross-sectional cognitive tests have been previously developed and validated in MS[40-44], and have been used to provide new insights into the field[45], but data on repeated use is lacking for most of them. We could find only a single computerized instrument intended for repeated use in MS, the Cognitive Drug Research battery[46], that measures attention, vigilance, working memory, episodic memory and information processing

speed. In a study enrolling forty-three mildly disabled, clinically active RRMS patients, the cognitive tests were performed at day -60 (training), day -30 (training), day 0 (baseline), day 30 and months 3, 6, 12, 18 and 24. The results of the cognitive tests were compared to those of Digit Symbol Substitution Test and Paced Auditory Serial Addition Test, showing a significant correlation. Most subtests from the CDR battery also revealed good test-retest reliability, with the exception of the working and episodic memory indices[46]. However, as most instruments in the field, this test requires repeated clinical visits and depends on a trained external evaluator, potentially increasing costs and possibly leading to lack of adhesion in long term monitoring.

The BoT test showed a high reliability in the test-retest analysis of four consecutive trials in the sample of patients with MS. There was a significant learning effect in most subtests between the first trial, performed in the hospital clinic under supervision, and the second trial, performed one week later from home. In the subsequent trials from home, one and two months after the first test, the scores do not show a significant increase for most subtests, the only exception being the Opposite task, that showed a continuous upward trend. In future studies, we will assess if this upward trend is bound to persist and affects the classification of patients as having CI.

This study has some potential limitations. The sample of healthy controls was not matched for gender, as the most recent large series from literature have found no significant differences in the pattern and prevalence of CI between genders [2,45,47-49]. While appropriate for comparing patients and healthy controls, the sample size is relatively small to compare the results

between patients with and without CI. Moreover, it would be interesting to compare the results with other widely used cognitive tests for MS, such as Rao's Brief Repeatable Battery[50], but at the time of the study initiation these were not adapted and validated for the Portuguese population. It is important to note that the patients included in the sample have relapsing remitting course in early and intermediate stages of disease (EDSS <4.5). A self-applied tool, such as BoT is probably not useful in patients with established motor or visual impairment in the late stages of diseases. Nevertheless, it is in these early stages that the timely detection of CI could most probably lead to effective benefits of rehabilitation treatments, functional interventions and, eventually, of new therapeutic escalation strategies. Future work with larger samples and longer time of follow-up will confirm if the large differences in test performance between MS patients with CI and those cognitively preserved is not diminished by learning effects, and if the tool can identify incident cases of CI.

In all, these results are encouraging and endorse the use of self-administered computerized tests in patients with MS. Using the BoT test in large cohorts of patients with MS could allow for a better understanding of the patterns of cognitive decline, and possible detection of cognitive relapses that currently go unrecognized, and hopefully lead to the development of new strategies for the prevention and therapeutic management of CI in the clinical setting.

Tables

Table 1 – Participant demographics and baseline test scores

	Patients with MS	Controls	*p-value*
Age, *years* mean (SD)	37.0 (8.0)	36.2 (9.2)	0.72
Education, *years* mean (SD)	11.5 (3.1)	12.5 (3.6)	0.29
Sex, *female (%)*	44.4%	85.7%	<0.01
MoCA, *score* mean (SD)	25.4 (2.5)	25.9 (2.6)	0.56
BoT, score mean (SD)			
Test 1 Day 0	45.9 (8.9)	54.0 (9.5)	<0.01
Test 2 Day 7	48.2 (11.5)	55.1 (10.0)	0.02

MS – multiple sclerosis; MoCA – Montreal Cognitive Assessment; BoT – Brain on Track; SD – standard deviation.
Cohen's d: Test 1=0.87; Test 2=0.68

Table 2 – Principal components and Internal consistency analysis for the first trial of the Brain on Track test

	Correct answers Mean (SD)				**PCA**	**Internal consistency**	
	MS patients	Controls	*p-value*	Cohen's D		Corrected item-total correlation	Cronbach's Alpha if item deleted
Attention task III	10.2 (2.3)	12.4(2.5)	<0.01	0.82	0.45	0.36	0.89
Visual memory task II	8.8 (2.7)	10.8 (3.4)	0.03	0.65	0.71	0.64	0.88
Delayed verbal memory task	16.9 (2.7)	19.2 (1.9)	0.01	0.98	0.55	0.47	0.89
Calculus Task	12.1 (3.4)	15.5(3.5)	<0.01	0.99	0.76	0.68	0.88
Colour interference task	17.4 (5.1)	21.5 (6.9)	0.02	0.59	0.73	0.66	0.88
Verbal memory II	8.9 (1.9)	10.8 (2.4)	<0.01	0.88	0.86	0.53	0.89
Opposite Task	51.7 (21.9)	59.0 (21.7)	0.22	0.33	0.62	0.53	0.89
Written Comprehension	10.9 (4.2)	11.9 (4.2)	0.37	0.24	0.62	0.54	0.89
Word categories	17.5 (7.6)	23.4 (5.6)	<0.01	0.88	0.78	0.70	0.88
Sequences	12.8 (4.9)	17.2 (6.1)	<0.01	0.80	0.68	0.81	0.88
Puzzles	4.3 (3.4)	6.1 (3.5)	<0.01	0.52	0.83	0.77	0.87

MS – multiple sclerosis; SD – standard deviation; PCA- principal component analysis;

Overall Cronbach's Alpha=0.89; Variance explained by the principal component was 49.0%

Table 3 – Principal component analysis of the Brain on Track and other neuropsychological tests in patients with multiple sclerosis

	Component 1	Component 2
Brain on Track test	0.82	0.24
MoCA	0.71	0.45
Symbol Digit Modalities Test	0.87	-0.24
Trail Making Test A*	0.79	-0.33
Trail Making Test B*	0.85	-0.30
LNS (WAIS-IV)	0.74	-0.21
CVLT	0.64	0.33
BVMT	0.81	-0.02
MRT (WAIS-IV)	0.81	0.23
Clock Draw Test	0.65	-0.14
Stroop Test	0.53	0.46
Symbol Search (WAIS-IV)	0.81	-0.22
Explained variance (%)	57.3%	8.4%

LNS - Letter-Number Sequencing; WAIS-IV - Wechsler Adult Intelligence Scale IV; CVLT – California Verbal Learning Test; MRT - Matrix Reasoning Test; MoCA - Montreal Cognitive Assessment; BVMT - Brief Visuospatial Memory Test.

* The sign of the scores from these tests were reversed.

Table 4 – Results from the test-retest study in patients with multiple sclerosis

	Subtest scores Mean (standard deviation)				ICC
	Trial 1 Day 0	Trial 2 Day 7	Trial 3 Day 35	Trial 4 Day 68	
Attention task III	10.2 (2.3)	11.2 (2.0)*	11.3 (2.8)	11.6 (2.4)	0.71
Visual memory task II	8.8 (2.7)	10.2 (2.6)*	10.7 (3.6)	10.8 (3.9)	0.88
Delayed verbal memory task	16.9 (2.7)	18 (2.3)	18.2 (2.2)	18.0 (3.0)	0.60
Calculus Task	12.1 (3.4)	13.0 (4.0)	12.7 (4.1)	14.2 (4.2)*	0.87
Colour interference task	17.4 (5.1)	21.9 (7.2)*	23.0 (7.6)	24.0 (7.6)	0.86
Verbal memory II	8.9 (1.9)	8.9 (2.5)	9.0 (3.0)	9.5 (1.4)	0.90
Opposite Task	51.7 (21.9)	61.7 (21.7)*	65.7 (21.4)*	73.4 (19.6)*	0.84
Written Comprehension	10.9 (4.2)	11.8 (4.7)*	11.7 (4.9)	12.7 (5.01)	0.88
Word categories	17.5 (7.6)	19.9 (8.3)	21.9 (8.2)	21. 3 (8.6)	0.80
Sequences	12.8 (4.9)	16.0 (6.0)*	16.0 (6.0)	19.3 (6.5)*	0.81
Puzzles	4.3 (3.4)	6.0 (2.6)*	6.5 (2.5)	6.6 (2.8)	0.88

ICC - consistency two way mixed single intraclass correlation coefficient

* $p<0.005$ in the difference between consecutive trials in Student's T test for related samples

Appendix 1 – Correlations of the Brain on Track subtest scores, total score and MoCA with cognitive tests of the neuropsychological battery at baseline

	Brain on Track subtests											BoT	MoCa
	Word categories	Colour interference task	Opposite Task	Written Comprehension	Visual memory task II	Attention task III	Verbal memory task II	Calculus Task	Puzzles	Delayed verbal memory	Sequences		
TMT A[A]	0.32	0.37	0.15	0.38	0.38	0.38	0.15	**0.41***	**0.58****	**0.63****	**0.44***	**0.52****	**0.42***
TMT B[A]	0.37	**0.50****	0.11	**0.44***	**0.47***	**0.47***	0.27	**0.43***	**0.62****	0.23	**0.43***	**0.60****	**0.53****
LNS (WAIS-IV)	**0.54****	0.22	-0.23	**0.60****	0.27	0.20	0.22	**0.40***	**0.41***	0.31	0.28	**0.43***	**0.38***
CVLT	**0.44***	0.28	0.22	**0.54****	**0.61****	0.33	0.29	0.30	0.38	**0.39***	0.21	**0.47***	**0.46***
BVMT	**0.50****	0.24	0.29	**0.52****	**0.55****	0.38	0.19	**0.48***	**0.65****	**0.45***	**0.53****	**0.62****	**0.53****
MRT (WAIS-IV)	0.36	0.21	0.20	**0.60****	**0.49****	**0.48***	0.17	**0.45***	**0.48***	**0.50****	**0.49****	**0.65****	**0.64****
Clock Test	0.26	**0.43***	0.16	**0.45***	**0.60****	**0.45***	0.19	0.34	**0.48***	0.38	0.17	**0.48***	**0.42***
Stroop Test	0.30	0.35	0.10	**0.48***	0.21	0.07	0.32	0.29	0.35	0.36	-0.05	**0.46***	**0.39***
Symbol Search (WAIS-IV)	0.35	**0.58****	0.21	**0.52****	0.37	**0.40***	**0.46***	**0.41***	**0.54****	**0.52****	**0.51****	**0.71****	**0.51****
SDMT	**0.48***	**0.50****	0.01	**0.57****	**0.48***	**0.47***	0.22	**0.56****	**0.58****	**0.47***	**0.45***	**0.67****	**0.41***

* Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level; A - The sign of the scores from these tests were reversed for clarity. TMT - Trail Making Test; LNS - Letter-Number Sequencing; WAIS-IV - Wechsler Adult Intelligence Scale IV; CVLT – California Verbal Learning Test; MRT - Matrix Reasoning Test; BoT – Brain on Track; MoCA - Montreal Cognitive Assessment; BVMT - Brief Visuospatial Memory Test.

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Disclosures

V.T.C. and J.P. have a shareholder position at Neuroinova, Lda a start-up company that conceived Brain on Track, holds registered trademark and commercialization rights and also provided funding for part of the study. A.S. received fees for patients’ cognitive assessments. IA received fees for study coordination and monitoring. The authors declare no other competing financial interests.

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7. Discussion and conclusions

Main achievements

This thesis aimed to contribute to a better understanding of cognitive impairment in two important groups, patients with MCI and early dementia from the general population, and patients with MS, by using standard cross-sectional cognitive assessment tools and by developing novel longitudinal approaches to monitor cognitive performance. The main achievements and findings of this work are the following:

- We developed a web-based cognitive test (BoT), that showed good correlation with established cognitive tests, ability to identify clinically relevant differences in patients with cognitive impairment, and a high test-retest reliability when performed from home (Paper I).
- The diagnostic accuracy of the BoT test was improved by implementing subtests with different versions of variable difficulty, adapted to the patient expected level of cognitive performance (Paper III, Paper VI).
- The prevalence of dementia and MCI were estimated to be 1.3% and 4.1% (adjusted for the Portuguese population), in a population-based cohort from Northern Portugal (EpiPorto), with the most common cause being

vascular cognitive impairment (52.8%), followed by Alzheimer's disease (36.1%) (Paper II).

- The BoT test was successfully used to monitor the cognitive performance from home in a group of healthy individuals from the EPIPorto cohort and in patients with MCI recruited from a Memory Clinic (Paper III).
- After increasing the BoT test scores in the first few test trials, patients with MCI presented a statistically significant higher rate of decline in performance when compared with healthy individuals of the general population over one year (Paper III).
- The presence of cognitive impairment was documented since the earlier stages of MS in a large multicentre study, with the prevalence increasing in the progressive stages (Paper IV).
- In patients with MS, older patient age and increased physical disability, as measured by the EDSS, are the main clinical factors driving the risk for cognitive impairment (Paper IV).
- Patients with MS and a history of paediatric onset of disease are at an increased risk for cognitive impairment and physical disability, when compared to their adult onset counterparts (paper V).
- The use of the BoT test was validated for patients with MS, and its feasibility in strategies of remote testing from home was demonstrated in this setting (Paper VI).

Discussion and future directions

The findings from the study on the prevalence and etiology of MCI and dementia in the EpiPorto cohort (Paper II) carry an important public health message regarding the prevention and management of cognitive impairment in Portugal, as a large part of the dementia epidemic could be prevented by public health measures[21,23], and its impact greatly minimized by early directed interventions, encompassing diet, exercise, cognitive training and vascular risk monitoring[14]. The effect of these multi-domains interventions requires the timely identification of cognitive impairment in the population at risk, before the damage becomes irreversible[14]. The BoT test could be a suitable tool for this aim, providing a low-cost strategy with potential for easy diffusion through the health system. It also could be useful to monitor progressive small vessel disease and silent brain infarcts, that would otherwise be undocumented.

The results from the large multicentre Italian study performed in MS patients highlight the need for early screening and systematic monitoring of cognitive functioning in these patients. The long-term follow-up of patients with MS, using tools such as BoT, would allow for a better understanding of the patterns of cognitive decline, and possibly to detect cognitive relapses that currently go unrecognized. Besides contributing to clarify the natural history of cognitive impairment over the disease course, closely monitoring patients with MS could be helpful to the development of new prevention, management and therapeutic strategies for cognitive impairment in the clinical setting.

Concerning the possibility of cognitive relapses and their timely identification in patients with MS, some questions come to mind. It would be interesting to assess impact of timely short courses of acute anti-inflammatory treatment and cognitive rehabilitation in these circumstances. Furthermore, they could represent uncontrolled disease that could benefit from second line disease modifying treatment. The BoT test could provide a viable tool to monitor and identify a decline in cognitive performance in MS patients.

An important result from the studies presented in this thesis is the acceptance of the BoT test and the feasibility of the monitoring strategy in the real-world settings, with a successful implementation from the remote location of home (Paper I, IV and VI). The adhesion to the strategy gives confidence that the use of the BoT test can provide a contribution to the unmet needs in different fields. If a fully successful low-cost web-based strategy for repeatable cognitive testing could be implemented, it could have an important impact in different settings:

a) In population-based research, monitoring healthy individuals with such a tool would enable to describe age-associated decline and identify the longitudinal cognitive patterns of individuals that progress to dementia.

b) In clinical research, monitoring the cognitive performance of young patients with CNS diseases could contribute to a better understanding of the natural history of cognitive deterioration in these conditions, and lead to the design of smarter rehabilitation strategies and improved therapeutic-escalation strategies.

c) Specifically, in clinical trials of Alzheimer's disease, a sensitive tool to measure pre-symptomatic cognitive decline could expand the recruitment of patients and provide a more accurate selection, while the longitudinal testing also has the potential to improve the outcomes assessment.

d) In daily clinical activities, improving the identification of cognitive impairment at its onset could lead to better disease management through timely interventions, preventing further deterioration, disability and loss of quality of life.

Nevertheless, there are still many challenges ahead, and further work to implement and refine these strategies towards their full potential. We expect that the resistance and lack of familiarity with computers will only decrease in the near future, as the number of adults with access and experience in computer use increases. Furthermore, the pressure on health systems of the increasing population aging, as well as the expensive costs of the diagnosis and management of these disorders will demand for easily scalable and low-cost strategies to be implemented.

Conclusion

On the whole, the results described in this work contributed to a better understanding of the epidemiology of MCI and dementia in Portugal, of cognitive impairment in MS, and verified that the BoT test could be a suitable tool for screening and monitoring cognitive impairment in these settings, providing a strategy which requires fewer resources than the conventional tools, and with the potential for easy diffusion through the health systems.

The development of new and effective approaches for prevention, treatment and rehabilitation of cognitive impairment largely depends on a better comprehension of its natural history and the pre-symptomatic or early symptomatic identification of impaired cognitive performance. A window of opportunity to improve the lives of affected patients and families resides at this early time in the disease course, when protective and curative interventions may take advantage of brain plasticity to mitigate the effects of pathological damage. This is where we are headed.

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