UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE PSICOLOGÍA

TESIS DOCTORAL

Deterioro cognitivo subjetivo como marcador preclínico fiable en enfermedad de Alzheimer

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Marina Ávila Villanueva

Directores

Miguel Ángel Fernández Blázquez
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Madrid
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A mi madre, por haberme inculcado la importancia de valerse por uno mismo y sentirse orgulloso de ello.

A mi padre, por su apoyo incodicional eterno.

A Miguel, gracias por tanto.

Agradecimientos: a mis directores de tesis, a mis compañeros de la Fundación CIEN, a la Fundación Reina Sofía y, especialmente, a los 1.213 voluntarios del Proyecto Vallecas.

RESUMEN

La demencia es un síndrome clínico caracterizado por un deterioro cognitivo progresivo, lo suficientemente severo como para impedir un funcionamiento autónomo a nivel personal y social del individuo. La enfermedad de Alzheimer (EA) representa la primera causa de demencia en nuestro país. Según datos del Centro Nacional de Epidemiología, el 7,3% de la población mayor de 65 años podría padecer esta enfermedad en la actualidad. En total, la EA constituye alrededor del 75% de la etiología de las demencias, bien de forma aislada bien en combinación con la patología cerebrovascular. Como consecuencia del incremento de la esperanza de vida y del progresivo envejecimiento de la población en los países occidentales, la demencia representa un enorme reto para los sistemas de salud públicos. En nuestro país, se estima que en el año 2050 un tercio de la población tendrá más de 65 años, por lo que aproximadamente un millón de españoles podría padecer demencia.

La EA es una enfermedad silenciosa que comienza con una degeneración cerebral muchos años antes de que se emita el primer diagnóstico clínico. Hay una larga fase asintomática. La transición desde un estado cognitivamente sano a la demencia debida a EA es un proceso continuo en el que pueden reconocerse diferentes estadios preclínicos y prodrómicos. Dichos estadios se caracterizan por la presencia de un deterioro cognitivo incipiente que incrementa la probabilidad de conversión a demencia en el futuro. Una intervención terapéutica eficaz en estas fases previas a la EA podría eventualmente retrasar la evolución del deterioro y disminuir así la prevalencia de la enfermedad. Por este motivo, uno de los desafíos a los que se enfrenta actualmente la investigación es el desarrollo de instrumentos útiles que permitan el diagnóstico precoz de la EA.

El hallazgo de nuevos marcadores para esta fase podría facilitar la prevención de la enfermedad. En este trabajo, hemos analizado el deterioro cognitivo subjetivo (DCS), como marcador temprano adecuado para describir la fase asintomática de la EA. El DCS se define como la experiencia individual de deterioro cognitivo expresado a través de quejas cognitivas, que podría ser importante para predecir la transición desde la normalidad a la aparición del deterioro cognitivo leve (DCL). Sin embargo, la operativización de estas quejas no es homogénea en la literatura ya que pueden verse afectadas por diversos factores. Una forma de obtener datos fiables es observar una cohorte amplia de sujetos en su transición a

DCL. En nuestro estudio hemos analizado alrededor de mil personas en una franja de edad comprendida entre 70 y 85 años en riesgo de dicha transición.

Para evitar la heterogeneidad que existe a la hora de recoger dichas quejas, hemos seguido las indicaciones de la Subjective Cognitive Decline Iniciative (SCD-I). Este grupo, propone una serie de características que podrían ser de interés para evaluar el riesgo de transición a DCL.

Nuestro punto de partida fue analizar la estructura subyacente del cuestionario Everyday Memory Questionnaire (EMQ), a partir de ahí, extrajimos dos importantes conclusiones: i) las quejas involucran distintos dominios cognitivos, no exclusivamente memoria y ii) determinadas quejas cognitivas son capaces de distinguir entre sujetos sanos y con DCL.

El siguiente paso, fue utilizar en nuestra cohorte las características indicadas por la SCD-I para clasificar a nuestra población en función de la presencia o ausencia de quejas cognitivas. Para ello, dividimos a los participantes en tres grupos: a) Sin quejas (NC), b) con quejas cognitivas de diverso tipo (SCD) y c) con quejas cognitivas que además cumplen una serie de características que incrementan la severidad de las mismas (SCD-Plus). Nuestros resultados concluyen que el grupo SCD-Plus tiene un riesgo 4 veces mayor de desarrollar DCL que los sujetos sin quejas en solo un año.

Sin embargo, apreciamos que las medidas para operativizar el DCS tomadas en una sola ocasión pueden no ser lo suficientemente robustas y, por tanto, podrían carecer de adecuada consistencia interna. Dicho de otro modo, sería importante tener la certeza de que las quejas son estables a lo largo del tiempo, ya que dos puntuaciones tomadas en dos momentos distintos pueden ser diferentes. Para ello, llevamos a cabo un estudio longitudinal a tres años para analizar si el DCS es un marcador preclínico fiable en EA. Por último, el estudio de la dinámica temporal de las quejas cognitivas mediante cadenas de Markov da evidencia de que el grupo de SCD define realmente un periodo preclínico perteneciente a la fase asintomática de la EA. Especialmente los individuos que se clasifican dentro del grupo SCD-Plus necesitan de una atención especial y son óptimos candidatos para una intervención terapéutica temprana. Aportamos un modelo teórico que describe de una forma dinámica cómo se producen las distintas transiciones en la fase preclínica en el continuo de la EA.

ABSTRACT

Alzheimer's disease (AD) is the leading cause of dementia in our environment. According to the National Epidemiology Center, 7.3% of the population over 65 years could suffer from this disease nowadays. In total, AD constitutes about 75% of the etiology of dementias, either alone or in combination with cerebrovascular disease. Because of increased life expectancy and the progressive aging of the population in Western countries, dementia represents a huge challenge for public health systems. In our country, it is estimated that by 2050 a third of the population will be over 65 years, so that approximately one million Spaniards could have dementia by then.

AD is a silent disease that begins with brain degeneration many years before the first clinical diagnostic is noticeable. There is a long asymptomatic phase. The transition from a cognitively healthy stage to an AD-type dementia is a continuum in which some intermediate stages, preclinical and prodromal can be recognized. These stages are characterized by the presence of an incipient cognitive impairment that increases the probability of conversion to dementia in the future. An effective therapeutic intervention in these phases prior to AD could eventually slow the progression of deterioration and thus reduce the prevalence of the disease. For this reason, one of the challenges currently faced by research is the development of useful tools that allow early diagnosis of AD.

The finding of new markers for this phase could facilitate the prevention of the disease. In this work, we have analyzed Subjective Cognitive Decline (SCD), as a suitable early marker for the asymptomatic phase of Alzheimer disease. SCD is a self-experienced persistent decline in cognitive capacity that could be important to predict the transition from a normal status to the onset of mild cognitive impairment (MCI). However, the measurements of cognitive complaints could be heterogeneous since could be affected by several factors. One way to get reliable data is to look at a large cohort of subjects in the transition to MCI In our study, we have analyzed around on thousand people in an age (70-85) with risk for that transition.

To avoid the present heterogeneity in the measured features, we have taken into account some of the recommendations of the Subjective Decline Initiative (SCD-I). The SCD-I proposed a set of features that could be of interest to know the risk for MCI transition.

Although, there are different questionnaires to evaluate SCD, at first we used the Every Memory Questionnaire (EMQ). From that first step, we concluded there is an underlying structure that involves different cognitive domains (not only memory, although its name) and also, that there are cognitive complaints that can distinguish between healthy controls and MCI.

By using the set of features that could indicate a higher risk of transition to MCI, as indicated by the SCD-I, we have divided in our study, the participants in 3 different groups: a) without complaints (NC), b) with some complaints that could be different among the subjects of the group (SCD) and c) subjects with the same set of factors expressed in all the members of the group (SCD-Plus). Our results indicated that SCD-Plus have about four times higher risk for developing prodromal AD in just 1 year, than subjects without complaints.

However, we found that SCD measurements performed only once could not be robust enough and could lack internal consistency. Thus, it will be important to have stability of complaints over the time since some scores collected in two different occasions may be different. Thus, we performed a longitudinal study to look for SCD as a robust and stable (for three years period) factor that could be used as a reliable preclinical marker for AD, and we found that this is the case. Thus, we described that SCD subjects with stable complaints could define a new prodromal period, taking phase in the asymptomatic phase for AD and in particular SCD-Plus subjects may need special attention and can be an optimal candidate for a possible therapeutic intervention. We close this work providing a theoretical tracking of the preclinical phase in AD, not in a static manner, instead we draw the dynamic transitions that take place across de AD continuum across time.

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ACRONYMS

Aβ	Amyloid beta
AD	Alzheimer´s Disease
APOE	Apolipoprotein E
CSF	Cerebrospinal Fluid
EMQ	Everyday Memory Questionnaire
MCI	Mild Cognitive Impairment
MRI	Magnetic Resonance Imaging
NIA-AA	National Institute on Aging and the Alzheimer's Association
SAD	Sporadic Alzheimer´s Disease
SCD	Subjective Cognitive Decline
SCD Plus	Subjective Cognitive Decline Plus
SCD-I	Subjective Cognitive Decline Initiative
SMC	Subjective Memory Complaints
SMD	Subjective Memory Decline

1. INTRODUCTION

1.1. Dementia and Alzheimer´s Disease

The increase in the quality of Medicine during the last century has allowed increasing the length of the human life. This increase has favored the presence of a higher proportion of cases for those diseases having aging as main risk factor. One of these disorders is dementia which is a clinical syndrome characterized by a progressive cognitive impairment severe enough to affect personal and social functioning of an individual.

According to the Spanish Confederation of Alzheimer´s Associations (CEAFA), the number of cases that suffer from AD nowdays in Spain ranges 500,000-800,000 (*Estado del Arte EA - Publicaciones - Ceafa*, 2017). Alzheimer's disease (AD) is the most prevalent cause of dementia in our environment. In total, AD constitutes about 75% of the etiology of dementias, either alone or in combination with cerebrovascular disease. Because of increased life expectancy and the progressive aging of the population in Western countries, dementia represents a huge challenge for public health systems. In our country, it is estimated that by 2040 1.5 million of people over 65 will suffer from dementia(*Worldwide Cost of Living 2017 - The Economist Intelligence Unit*, n.d.). The impact of dementia is not only produced directly on the patient, but also has a great influence his/her family and social environment concerning affective, organizational and economic aspects. In this sense, dementia should be understood as a social problem that must be approached in a comprehensive manner.

The transition from a cognitively healthy stage to an AD-type dementia is a continuum in which some intermediate stages, preclinical and prodromal, can be recognized. These stages are characterized by the presence of an incipient cognitive impairment that increases the probability of conversion to dementia in the future. An effective therapeutic intervention in these phases prior to AD could eventually slow the progression of deterioration and thus reduce the prevalence of the disease. For this reason, one of the challenges currently faced by research is the development of useful tools that allow early diagnosis of AD.

The clinical continuum of AD

Current knowledge understands AD as a continuum process starting 10 to 20 years before the onset of clearly noticeable symptoms. A high degree of agreement has been reached around the different stages of the disease, namely preclinical phase (brain changes without symptoms), prodromal mild cognitive impairment (MCI) phase (mild symptoms and signs without dementia), and dementia phase which usually lasts 10-15 years and leads to a total dependence and finally patient's death (Amariglio, Townsend, Grodstein, Sperling, & Rentz, 2011; Dubois et al., 2010; Rami et al., 2011; Sperling et al., 2011b). Figure 1.1. shows the trajectories of these three AD stages. The preclinical phase is defined by the incipient presence of amyloid plaques, but objective cognitive function remains normal. At the end of this phase, the individual might subjectively experience some kind of cognitive decline (Sperling et al., 2011b). The second stage, called prodromal AD or MCI implies objective cognitive deficits, but not severe enough to significantly affect everyday activities (Albert et al., 2011). And finally the last dementia phase in which the extent of the cognitive deterioration leads to a functional impairment that defines a dementia syndrome (Rovio et al., 2005). This stage usually lasts 10-15 years, leading to total dependence and, eventually patient´s death (Dubois et al., 2010).

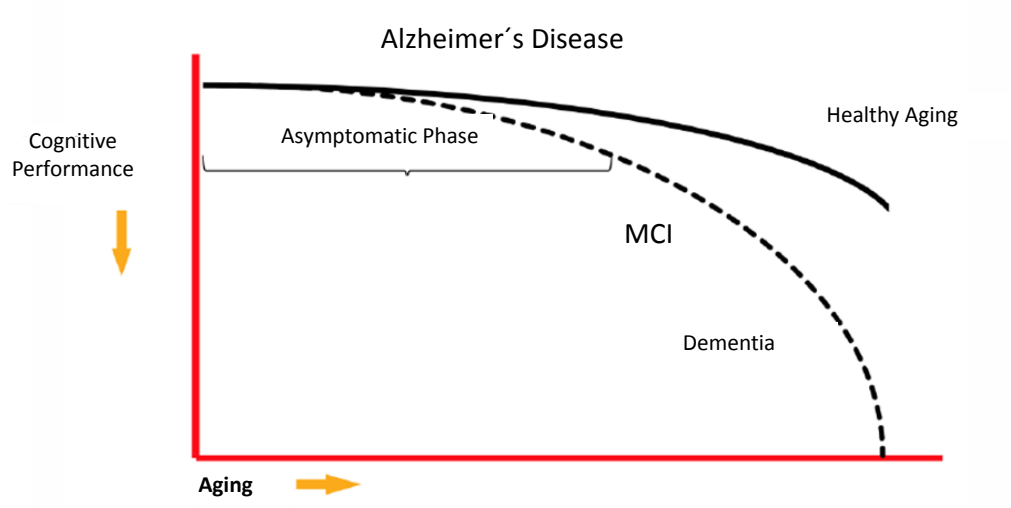

Figure 1.1. *Alzheimer´s Disease stages (adapted from* Sperling et al., 2011b)

MCI: Mild Cognitive Impairment

In practical terms, the recognition of the clinical and biological features of people that convert from a cognitively normal state to MCI and from MCI to dementia is of utmost interest, as well as the factors that may accelerate or prevent those transits. The transition from cognitive normality to early, mild signs of cognitive impairment is specifically difficult to categorize and isolate. It has been proposed that a potential state may arise during the preclinical phase, although this hypothetical state has received different names (for example, "*subjective memory complaints*", "*subjective memory impairment*" or "*subjective cognitive impairment*"). Possibly also due to the lack of a precise definition, studies addressing the conversion from normal cognition to MCI have been scarce, with incidence rates varying between 51 and 77 per 1,000 persons a year. The most frequently reported risk factors for incident MCI are higher age, lower education, and hypertension (Luck et al., 2010). Conversion from MCI to dementia has been more widely studied, with duration of 7-10 years for the MCI stage and annualized conversion rates of 8-17 per 100 persons a year (Ward, Tardiff, Dye, & Arrighi, 2013). Additionally, cognitive performance, cortical amyloid deposition, hippocampal atrophy, hypometabolism in the parietotemporal cortex, and alteration in the cerebrospinal fluid (CSF) levels of 42-aminoacid amyloid beta peptide (Aβ-42) and phosphorylated tau protein (p-tau) have been consistently associated with higher conversion rates from MCI to AD dementia (Brooks & Loewenstein, 2010; Heister et al., 2011). Other markers or co-morbidities (e.g., vascular factors, sleep disturbance) may also be of relevance in the transition from healthy state to AD, particularly in the very old (Frisardi et al., 2010).

Two types of AD, the familial (FAD) and the sporadic (SAD) Alzheimer´s diseases (Colin L Masters et al., 2015)might be distinguished. FAD is a presenil dementia that usually appears below 65 years old, whereas SAD is a senil form of dementia whereby aging is one of the most important risk factors. Since SAD accounts for more than 99% of the cases of AD, we are mainly focusing in this type of disease. In any case, both forms of AD follow similar phases for the development of the neurological disorder (Hampel, Lista, & Khachaturian, 2012).

In AD there are some common anatomic features like the presence of two aberrant brain structures, senile plaques and neurofibrillary tangles and the presence of neuronal death (Stelzmann, Norman Schnitzlein, & Reed Murtagh, 1995). The main component of senile plaques is aggregates of the beta amyloid (Aβ) peptide (C L Masters et al., 1985) whereas

the main component of neurofibrillary tangles is tau protein, a neuronal protein (Grundke-Iqbal et al., 1986). The appearance of senile plaques, at least in FAD, takes place very early in the asymptomatic phase, and that neurofibrillary tangles take place few years before the transition to mild cognitive impairment (Bateman et al., 2012). The case of FAD is well known and it is related to mutations in three different genes: app, psen-1, and psen-2. The consequence of those mutations was to increase the amount of Aβ and based on that, it was suggested the amyloid cascade hypothesis as a possible initial cause for the onset of FAD (Hardy, 2006).

1.2. Biomarkers

Even now with all the advances in the Alzheimer´s research context one of the most common questions from the general population is "What´s the difference between AD and dementia? In 1984 the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer´s Disease and Related Disorders Association (now the Alzheimer´s Association) published a diagnostic criteria, the NIDNCDS-ADRA Criteria to help answer that question (McKhann et al., 1984). Since that, science has made notable discoveries. Using certain biomarkers, we can now distinguish between AD and other causes of dementia (Karlawish, Jack, Rocca, Snyder, & Carrillo, 2017). The National Institute on Aging-Alzheimer´s Association (NIA-AA) working group and the International Work Group (IWG) have proposed guidelines that use biomarkers and also clinical symptoms to define dementia caused by AD and also preclinical AD and MCI due to Alzheimer (Blennow, 2004; Mattsson et al., 2009).

The fact of the evolving biomarker research made possible to identify the disease even at the preclinical stage before the occurrence of the first clinical symptoms, and as a matter of fact, to change the perspective, from a pathology consequence of lesions in the brain has made cognitively impaired individuals to a continuum disease in which we find an asymptomatic phase before the onset of the clinical phenotype, the initial clinical symptoms in mild impairment in specific cognitive functions, and the severe cognitive deficits making impossible independent daily living.

AD is a silent disease having a long asymptomatic phase in which there is brain damage without clinical diagnosis (Bateman et al., 2012). Then, it will be suitable to get early

markers for the disease at that stage to allow a prompt prevention of the neurological disorder. In the case of FAD, it is well-established that the presence of specific mutations for app, psen-1 or psen-2, will result in the appearance of the disease at the 40s and 50s. Thus, from the birth time it is possible to have a precise genetic biomarker. In the case of SAD it is not possible to have very early biomarkers like that and, usually, the combination of clinical neuropsychological studies, cerebrospinal fluid (CSF), structural and functional neuromaging techniques (MRI, PET, MEG, etc.), may facilitate an early prediction for the transition from the asymptomatic to a MCI stage.

Lumbar puncture, using a needle, may facilitate the collection of CSF from a subject. In that CSF sample mainly two biomarkers are usually analyzed: Aβ and tau (total or in phosphorylated form) proteins. Cognitive decline should correlate with a decrease in the amount of amyloid peptide and an increase in the amount of tau protein in CSF (Herukka et al., 2017; Olsson et al., 2016). Also, analysis of CSF could facilitate the diagnostic evaluation of dementia (Simonsen et al., 2017). Less reliable is, at the present, the analysis in other fluids like blood.

About neuroimaging techniques, mainly overall macroscopic changes throughout the brain are measured by MRI (Weiner et al., 2017); Aβ or tau aggregates may be quantified by positron emission tomography (PET), using specific compounds that could bind either amyloid or tau aggregates (Rinne et al., 2010; Shoghi-Jadid et al., 2002)

1.3. Subjective Cognitive Decline

The construct subjective cognitive decline (SCD) refers to a self-experienced persistent decline in cognitive abilities in comparison with a previously normal status and independently of the objective performance on neuropsychological tests. SCD has been a focus of debate within the research literature during the past two decades because of its potential clinical relevance in predicting the onset of future dementia in older adults. In fact, it has been described that subjects might experience some type of cognitive decline up to 15 years before they develop MCI an AD (Reisberg et al., 2008). Furthermore, in the absence of objective cognitive impairment, evidence has been reported about the relationship between SCD and some AD biomarkers such as brain amyloid deposition and cerebral hippocampal hypometabolism (Vannini et al., 2017).

Despite cognitive complaints have been traditionally treated as equivalent to memory failures, SCD is in fact heterogeneous and might affect all cognitive domains from a neuropsychological point of view. For instance, there are symptoms that may be specifically relate to memory ("forgetting recent events"), while others are associated with attention (being unable to follow a lecture) or executive functions ("perseverations in old daily routines). Although episodic memory impairment is usually the first cognitive manifestation of MCI due to AD, perception of memory failures might not be the primary complaint of cases with preclinical AD. Attentional symptoms like "following the thread of a conversation" have been proved to be more associated with risk of dementia than forgetfulness itself such as "problems to recall recent information" (Amariglio et al., 2011).

Since the expression of cognitive complaints is affected by various factors (e.g. aging, personality, mood, drug side effects, etc.) SCD is not necessarily present in all AD patients. Nevertheless, cross–sectional (Rami et al., 2011) and longitudinal studies (Dufouil, Fuhrer, & Alpérovitch, 2005; Reisberg, Shulman, Torossian, Leng, & Zhu, 2010) have provided strong evidence of SCD occurring at preclinical AD.

There are overwhelming epidemiological data in favor of the relationship between SCD an incident cognitive impairment. A meta-analysis focused on the longitudinal value of SCD for detecting later MCI and dementia has shown that, independently of the objective memory performance, 6.6% of older adults with SCD develop MCI per year (Mitchell, Beaumont, Ferguson, Yadegarfar, & Stubbs, 2014). In addition, the rate of progression to dementia among those who report complaints about their own cognitive performance is twofold during a 5 year follow-up period.

Despite its emerging role as a marker of preclinical AD, the concept of SCD is not free from some limitations which are necessary to address. For instance, in Figure 1.2 appears the terminology evolution across time such as subjective memory complaints, subjective cognitive decline, or subjective memory impairment that have been used indistinctly to refer to the same concept.

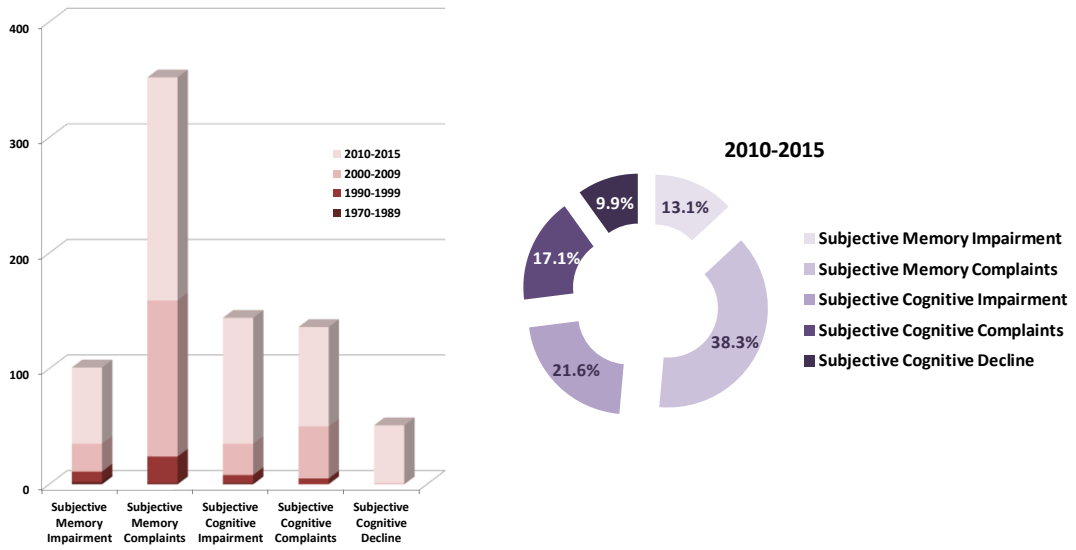

***Figure 1.2**. Number of records by term of self-perception of cognitive decine. Search in PubMed (term inTittle/Abstract) filtering by date of publication.*

This lack of consensus on a single definition of SCD affects to the comparison of findings from different investigations and epidemiological studies. In addition, there is no accepted approach among researchers about the assessment of SCD, including the mode of administration, the cognitive domains to be examined, the number of items to be used, and the optimal way to respond the items. Finally, it becomes difficult to determine which complaints underlie AD because there is a close relationship between SCD and subjective variables such as depression (Crane, Bogner, Brown, & Gallo, 2007), anxiety (Comijs, Deeg, Dik, Twisk, & Jonker, 2002), personality (Pearman & Storandt, 2004) or quality of life (Montejo, Montenegro, Fernández, & Maestú, 2011). In sum, the heterogeneity in definitions and the different approaches for measuring SCD emphasize the necessity of searching for shared terminology and common standards of evaluation. This feature is expected to be progressively solved by using SCD as the most prevalent term.

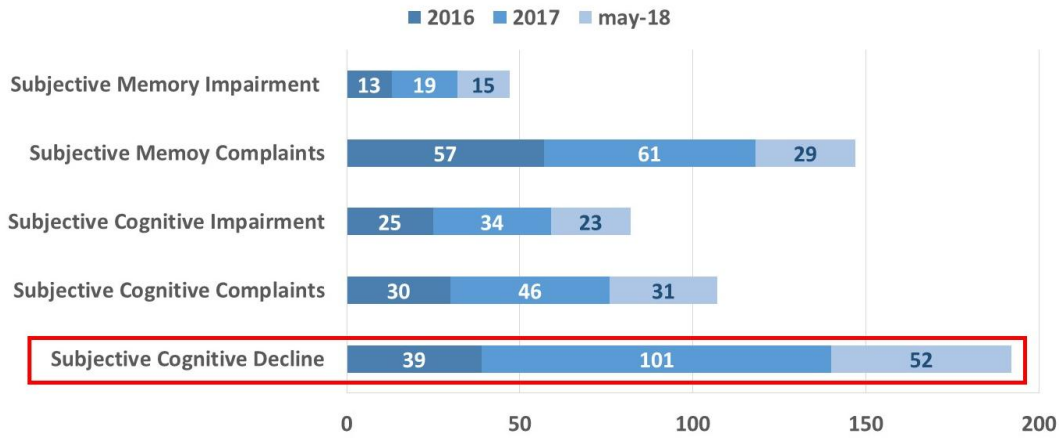

***Figure 1.3**. Terminology used to refer to the same concept. Search in PubMed (term inTittle/Abstract; date of publication 2016-May 2018).*

To assess the potential usefulness of SCD for epidemiological studies and clinical trials, an international working group, the Subjective Cognitive Decline Initiative (SCD-I), agreed to a common terminology and research procedures to identify individuals with SCD at risk of preclinical AD (Jessen et al., 2014). The SCD-I aimed at knowing whether the self-experience of decline in cognition could actually represent the first manifestation of AD. In order to demonstrate that, some common specific features are required to stablish a complete profile of SCD and to characterize two distinct groups of individuals in accordance with such profile.

***Table 1.1**. Features suggested for coding in studies on SCD (adapted from Jessen et al. 2011).*

Features to be collected	How to collect those features
Setting in which SCD is expressed	Clinical setting, population-based study, etc.
Association of SCD with medical help seeking	Yes/No
How SCD is reported	Spontaneously/On request
How long SCD is present	Number of months or years
Age at onset of SCD	Age in which SCD appeared
There is self-perception of memory decline	Yes/No
There is self-perception of other non-memory decline	Yes (specify)/No
There are concerns associated with SCD	Yes/No
There is a feeling of worse performance than peers	Yes/No
SCD is associated with experience of impairment	Yes/No

Confirmation of cognitive decline by an informant	Yes/No
Symptoms of depression or anxiety	Score on scale
APOE genotyping	No alleles ε4/Heterozigous ε4/ Homozigous ε4

The SCD-I recommendeds to collect information regarding features regarding cognitive complaints, which are summarized in Table 1.1. In addition, the SCD-I proposed a set of particular features which be helpful to identify individuals at risk of clinical progression, namely SCD Plus group (Table 1.2.).

***Table 1.2**. Features that increase the likelihood of preclinical AD in individuals with SCD: SCD Plus (adapted from Jessen et al. 2011).*

- Subjective decline in memory rather than other cognitive domain
- Onset of subjective cognitive decline within the last 5 years
- Age at onset of subjective cognitive decline ≥ 60
- Concerns associated with subjective cognitive decline
- Feeling of worse performance than others of the same age group
- If available: Confirmation of cognitive decline by an informant Presence of APOEε4 genotype Biomarker evidence for AD

1.4. The Vallecas Project for early detection of Alzheimer's disease

The transition from normality to early mild signs of cognitive impairment is difficult to characterize and isolate. A potential feature appearing during this transition is the SCD state. Perhaps due to the heterogeneity in the investigation of SCD that was commented previously in section 1.3., the prevalence rates of cognitive complaints in older adults vary from 11.5% to 95% (Garcia-Ptacek et al., 2016). Table 1.3. shows some differences among classical studies that have focused on complaints.

Clearly the targets for early AD identification should be the stages of SCD and MCI, along with the characterization of those subjects with high risk of conversion to dementia. Under a multicausal model of aging-associated, late onset SAD, multiple markers are expected to be relevant for the detection of the groups of interest, and therefore, the most cost-effective and safest procedures should be prioritized.

The main objective of the Vallecas Project is therefore to elucidate, through tracking of progression, the best combination of clinical parameters and complementary tests (imaging and laboratory) that allow deciphering at medium- and long-term features that distinguish

those who will develop cognitive impairment (MCI and dementia) from those who will not. Thus, it intends to identify various markers to determine eventually the potential risk that each individual could have to develop the disease in the future. The study is focused on cognitive testing, comorbidities, multy-modal MRI and systematic blood collection. The final objective of this project is to facilitate testing of disease modifying therapies and next the treatment of the population at risk, before they become cognitively impaired or demented.

***Table 1.3**. Epidemiological characteristic of subjective cognitive decline.*

Study	Context	Age	Prevalence	Evaluation
Schofield et al., 1997	Community dwelling	-	31.0%	Do you have problems with your memory?
Geerlings et al., 1999	Community dwelling	65-84	11.5%	Do you have complaints abot your memory?
John & Montgomery, 2002	Community dwelling	>65	21.0%	Did you have memory loss in the past years?
Glodzik-Sobanska et al., 2007	Volunteers	>65	81.3%	GDS2
Visser et al., 2009	Clinical	>55	26.9%	NR
Nunes et al., 2010	Clinical	55-82	34.9%	SMC scale
Reisberg et al., 2010	Community dwelling	>40	76.9%	GDS
Sachdev et al., 2010	Community dwelling	70-90	95.5%	MCA-Q and IQCODE
Jessen et al., 2011	Community dwelling	>75	57.7%	Do you fell your memory is becoming worse?
Montejo et al., 2011	Community dwelling	>65	24.0%	How would you describe your memory?
Fernández Blázquez et al., 2016	Community dwelling	70-85	69.6%	Clinical interview and questionnaire

With those premises in 2011 CIEN Foundation Alzheimer´s Research Center launched the Vallecas Project, a single-center, community-based longitudinal cohort study with yearly evaluations to identify subjects at the initial stages of AD and to clinically and biologically characterize the transitions between healthy cognition, SCD, MCI and AD dementia (Olazarán et al., 2015). The participant recruitment phase for the Vallecas project lasted from October 2011 to December 2013. By then, 1,213 individuals of both genders, aged 70-85 years were initially recruited and evaluated at baseline. Before entering the study, volunteers interested in participating in it were subjected to an initial assessment to determine whether they meet the criteria for inclusion and/or whether an exclusion criterion exists. Overall, all volunteers were required to meet four inclusion criteria in order to be considered for entering the study: i) signing an informed consent; ii) be aged between

70 and 85 years old; iii) availability and ability to reach the Alzheimer Centre for visits; and iv) visual and hearing abilities that allow conducting the study tests. In addition, a number of exclusion criteria were established, including the following: i) suspected or diagnosed dementia; ii) inability to perform neuroimaging studies; iii) alcohol abuse; iv) mental retardation; or v) history of certain psychiatric or neurological diseases (eg schizophrenia, stroke, severe head trauma, central nervous system infections, uncorrected vitamin deficiencies, etc.). The general procedure of the Vallecas Project is shown in Figure 1.2.

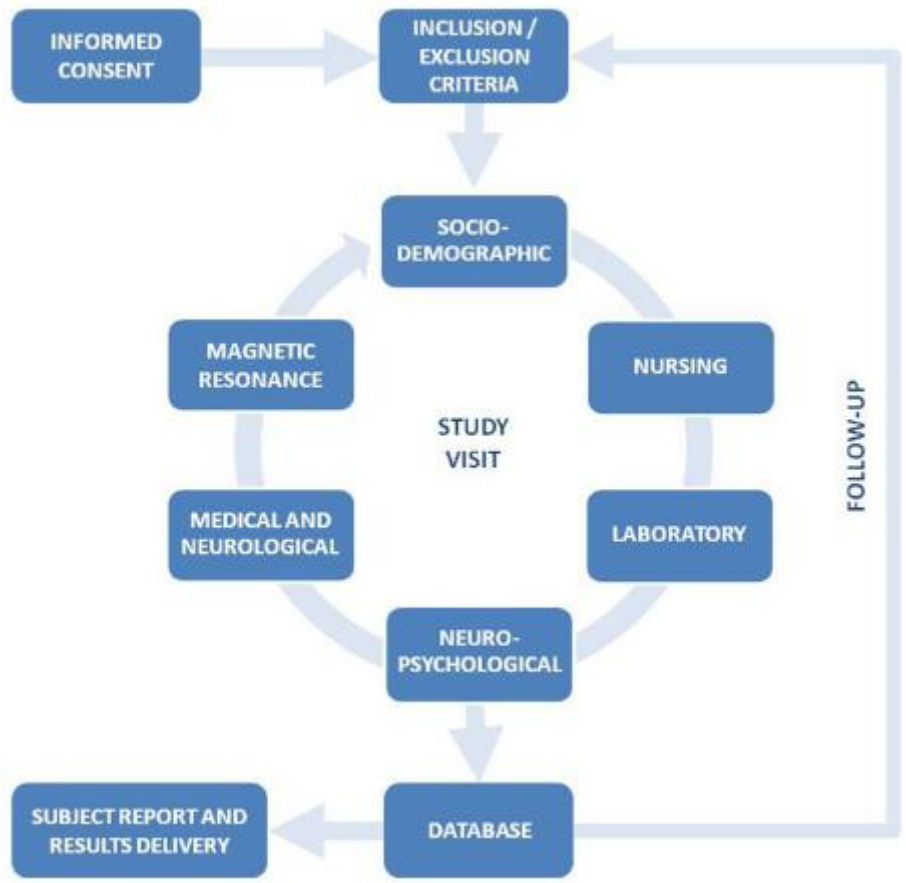

***Figure 1.4**. General procedure and different parts of the study visit of the Vallecas Project. (Olazarán et al., 2015).*

Only 1,172 individuals fulfilled all inclusion and exclusion criteria and therefore were finally admitted to participate in the study. Once included in the study, all participants undergo an annual follow-up in order to assess their evolution trajectories, specifically identifying those that develop cognitive impairment and/or dementia. This is an ongoing study as the Vallecas Project has currently finished the fifth visit for the entire cohort and is about the

middle of the sixth and the beginning of the seventh visits. Table1.4. shows the figures of clinical evaluations conducted to date.

Table 1 4*. Longitudinal clinical diagnoses of the Vallecas Project cohort during the first five visits.*

	Baseline	1-year follow-up	2-year follow-up	3-year follow-up	4-year follow-up
Sample Size	1,172	963	865	767	546
Drop-outs	-	209 (18%)	100 (10%)	98 (11%)	-
Controls	1091	869	768	680	486
MCI	81 (7%)	93 (10%)	88 (10%)	62 (8%)	36 (7%)
Dementia	-	1	9 (1%)	25 (3%)	24 (4%)
New MCI		52	23	17	6
New Dementia		1	9	17	12

In every visit participants undergo a detailed protocol of evalution comprising sociodemographic data, lifestyle scales, vital signs, blood samples, neuropsychological assessment, neurological exam, and MRI study. The complete study visit is carried out in a single day with a total duration of 4 hours including convenient breaks.

a) Sociodemographic profile and lifestyle: a wide sort of information is collected by means of a semi-strutured interview regarding gender, date of birth, marital status, number of children, type and amount of income, occupation, educational attainment, hobbies, leisure activities, diet, physical exercise, quality of life and subjective well being (mobility, personal care, daily activities, pain/discomfort, anxiety/depression, perceived health status).

b) Vital signs and morphometry: blood pressure (seated and standing), height and weight, and head and waist circumferences are measured by a nurse during all the study visits.

c) Medical and neurological assessment: a semi-structured medical interview is conducted by a neurologist focusing on vascular risk factors, neurological disorders, psychiatric disorders, current medications, family history of dementia, and sleep. Also all subjects undergo a general and neurological standard examination: cranial nerves, muscle balance, coordination, extrapyramidal system, gait, osteotendinous reflexes, and midline release reflexes. Gait disturbances and some brief motor tasks

are specially analyzed(Ashendorf, Vanderslice-Barr, & McCaffrey, 2009; Podsiadlo & Richardson, 1991).

d) Neuropsychological assessment: although the neuropsychological battery focuses especially on the evaluation of memory processes, attention and executive functions as potential early markers of AD, the neuropsychological profile is completed by getting information related to other cognitive domains such as language, visuospatial ability and visuoconstruction. All these data allow identifying the strengths and weaknesses in the cognitive profile and characterizing, if necessary, the type of cognitive impairment that an individual presents. Table 1.5 shows the cognitive tests which comprise the neuropsychological battery of the Vallecas Project.

***Table 1.5**. Neuropsychological assesment in the Vallecas Project.*

TEST	DESCRIPTION
Mini Mental State Examination (MMSE)	This is a test of global cognitive assessment. It consists of 20 items that gather a rough information on the level of orientation, attachment, attention, calculation, recall, language and viso-constructive praxis of the subject. The score for this test is made over a maximum of 30 points to the extent that all items are answered correctly. Cognitive impairment diagnosis is performed based on a score of 24 points as the cutoff.
Rey-Osterreith Complex Figure	It is a classic neuropsychological evaluation task consisting in performing a copy of a complex pattern (the time it takes for copying is recorded) and subsequent immediate recall (within 3 minutes), after performing a distraction task, delayed (after 30 minutes) and a recognition task. This test allows to evaluate a large number of cognitive processes related to planning, visoconstruction, impulsiviness, episodic memory, incidental learning, etc. It has also been adapted and rated in the Spanish population over 60 years of age.
Functional Activities Questionnaire (FAQ)	It is a classic questionnaire to assess autonomously performing of instrumental activities of daily living. The questionnaire should be answered by a reliable informant. It consists of 11 items with 4 response options to assess the degree of dependence or independence of the subject in different daily tasks (managing finances, shopping, doing housework, preparing meals, pay attention and discuss news, remembering dates, managing medication or going out alone on the street). The diagnosis of Alzheimer disease occurs from a score of 6 as the cutoff point.
Free and Cued	It is based on the assessment of learning ability and verbal episodic

Selective Reminding Test	memory. The test consists of the consecutive presentation of 4 sheets with 4 words written each (a total of 16 words) that the subject must learn. To facilitate this task, the examiner provides a key for each of the words that will be helpful later to recall more items. After a simple 20 seconds task interference people are asked to remember as many words as possible spontaneously. After 90 seconds, clues to help the memory of those words that did not recalled by himself/herself will be provided. Then the words he/she could not recall with the help of the clue are reminded of and another interference task is proposed. This procedure is performed three times, so that there are three free recall tests and three facilitated recall through the clues. After 30 minutes the delayed free and with clues recall condition is carried out. The indexes that are considered in this test are the total free recall, the total learning, free delayed recall and the overall delayed recall. The test has Spanish ratings
Semantic lexical evocation	The task consist in providing the highest number of words beginning with a certain letter (P, M, and R) or belonging to a specific category (animals, fruits/vegetables, and cookware) for one minute. Furthermore, in the case of phonological evocation the contribution of people names or words that share the same lexical root is not allowed. The number of responses that the subject provides in periods of 15 seconds is recorded, as well as the total number of correct responses, intrusions and perseverations in the minute-long test. This task allows the systematic assessment of both the language proficiency as the semantic system of the subject. Moreover, it must be highlighted that this task has been validated and rated on Spanish population over 60 years.
Clock drawing test	It is an easily applicable screening test to evaluate both the visoconstructive ability as the semantic component associated with the knowledge of the hour. The subject is asked to draw the face of a clock, with all numbers in the correct place and with the hands pointing to 11 and 10. The score of the drawing is based on criteria related to the quality of the clock face, the presence and sequence of numbers, as well as the presence and location of the hands. The maximum score corresponds to 10, considering 6 as a cutoff for the diagnosis of cognitive impairment.
Reading test of intelligence	This test provides a measure of the level of pre-morbid intelligence of the patient through a reading task contained 60 words in the dictionary of the Royal Spanish Academy. An important feature of this test is that the items have a low frequency of use in our country, those who should carry written accent do not carry it and foreign words are also included between them. The subject's task is to read the words in the right way, for what is allowed to rectify if deemed appropriate. The test raw score is the number of words read correctly.
Global Depression Scale	Is a self-reported scale to evaluate depressive symptoms. It consists of 15 questions related to the state of mind to which the subject must respond dichotomously (yes/no). The cutoff point beyond which the likelihood of major depressive disorder increases is 5

Digit-Symbol	This is part of the Wechsler Adult Intelligence Scale (WAIS) for assessing intelligence. Natural numbers from 1 to 9, each of them associated with a different symbol, are presented on a test sheet. Below appear random numbers from 1 to 9 without any associated symbol. The task of the subject is to write the symbols for each number as quickly as possible for one minute. To avoid interference of possible memory alterations on test performance, the model with numbers and symbols for each of them remain in the top of the sheet. This test provides a measure of information processing speed and procedural learning ability to the extent of it will become less necessary for the subject to look at the model because unconscious learning
State-Trait Anxiety Inventory (STAI)	This self-reported test evaluates anxiogenic symptoms related to both a specific time and intensity variable period (anxiety state) as well as a more stable personality pattern tending to perceive situations as threatening (anxiety trait). Thus, there are two scales of this test, each consisting of 20 items with 4 response options (scored by a Likert type scale of 0-3). The total score is the sum of the individual scores for each item. Spain has recently adapted this test in nonclinical populations. After the second visit the neuropsychological examination protocol suffered a slight transformation in order to optimize collection of cognitive information. For this purpose, a series of assessment tests that allow to obtain more information on attention, language, praxis and executive functions from all selected study subjects.
Boston Naming Test	It is a reduced version of the classic subtest included in the Boston test for the diagnosis of aphasia. The Boston Naming Test is used in clinical consultations to assess the ability of naming visual stimuli by visual confrontation. The subject's task is to name each of the 15 drawings that are presented, for which he/she is given a maximum of 20 seconds per image. If the subject does not give the correct answer spontaneously, the examiner provides a semantic or phonological clue if the above is not enough. Total score is the sum of correct spontaneous responses and the number of drawings called using the semantic hint. The correct answers after the phonological key are considered as an indicator of the kind of difficulty to name drawings.
Symbolic Gesture	This test is part of the Revised Barcelona Test (RBT). It explores the performance of a series of symbolic gestures of communication. They are simple, intransitive gestures made with a single upper limb. The primary endpoint of the test is the body position in relation to space and the body.
Imitation of Bilateral Postures	Also as a part of part of the RBT, this test consists in the imitation by the subject of a number of postures that the examiner performed with both hands. This test evaluates the integrity of ideomotor praxis.
Forward and reverse digits	This test allows to evaluate the hearing attentional amplitude and the individual's central executive of the working memory. The subject's task consists in repeating the growing sequences of numbers that the evaluator

	presents at one digit per second. The test is divided into two separate subtests, so that repetition of the first digit is applied in the same order of presentation (Direct digits) and then in reverse order (Inverse digits). The task ends when the subject is not able to repeat two sequences of the same length of digits. In both subtests, the number of correct repetitions and the maximum amplitude of digits that the subject is able to repeat are counted.
Rule Card Shift	This test is part of the Behavioral Assessment of the Dysexecutive Syndrome (BADS). It involves the presentation of a sequence of 21 cards from the French card deck. The subject must respond "yes" or "no" as fast as he/she can and as accurately as possible according to a rule that is in plain view. In the first part of the test rule is to respond "yes" when the card is red and "no" when is black. The second part introduces a variation of the first rule that the subject must respond "yes" when the card is the same color as above and "no" when it is a different color. The number of errors made by the subject in the second part of the test is registered and the score based on such errors is recorded. This test assesses the ability to fulfill one simple rule and the subject flexibility to adapt to a new different rule
Five Points Test	This is a test that measures the subject's cognitive flexibility regarding the ability to design novel visual shapes in a DIN A4 sheet of paper with 40 identical matrices of 5 dots arranged in eight rows and five columns isprovided. The subject's task is to produce for 3 minutes as many figures as possible by connecting the dots within each matrix and the following rules: i) the figures may not be repeated; ii) only straight lines in any direction (horizontal, vertical or diagonal) can be used to connect the dots; and iii) it is not necessary to join the 5 points of the matrix.

e) Laboratory: three types of evacuated blood collection tubes for serum, plasma and blood cells are obtained at each study visit. All individuals are genotyped for Apolipoprotein E from genomic DNA extracted from whole blood. The blood samples are labeled and kept refrigerated at -80 Celsius degrees, for future determinations.

f) Neuroimaging: all studies are carried out in a 3 Tesla MRI (Signa HDxt GEHC, Waukesha, USA) equipped with a gradient system of 50mT/m. A phased array 8 channels brain coil is used for all the subjects. The VP protocol includes a structural study with T1 sequences for volumetry and FLAIR and T2* sequences to assess white matter (WM) lesions and microhemorrages. Perfusion study with arterial spin labeling (ASL) technique was conducted to check for functional alterations and diffusion tensor imaging (DTI) study was performed for analysis of the anisotropy of WM. In addition, the DTI sequence permits to measure structural connectivity. A

resting state functional study is also conducted with blood oxygen level dependent (BOLD) sequences (rs-fMRI) to analize functional connectivity.

Formal feedback is provided in the form of written clinical reports of the study that participants receive at home. These reports include general results of neuropsychological tests and MRI study, and, if clinical relevant, results from the nursing, medical and neurological assessments. Furthermore, if any procedure of the Vallecas Project reveals findings requiring medical or psychiatric attention, the subject is referred to the appropriate assistance resource or is contacted by telephone to provide information and the steps that must follow.

1.4.1. SCD assesment in the Vallecas Project

According to the evidence and the recommendations discussed in section 1.3., the Vallecas Project has combined different approaches to measure SCD accurately. Thus, there are four specific points that have been borne in mind when this longitudinal study was methodologically designed:

1. To use both open-ended questions and structured questionnaires to measure different aspects of SCD. Specific questions inquire about clinical details of the self-experienced cognitive decline (e.g. age at onset, seeking medical help, memory performance compared to other people, etc.) as well as concerns and frequency of particular cognitive complaints (e.g. forgetting recent events, being unable to follow the thread of a story, difficulties to retrieve the adequate word, etc.). In addition a multiple choice approach varies from dichotomic to ordinal Likert-type scales to grasp the dimensionality of SCD in the best way possible.

2. To collect information in different ways to ensure a greater internal consistency. Specifically, it has been combined the adquisition of self-perceived data by means of face-to-face interviews with a healthcare professional along with self-administered questionnaires. Since it is relatively frequent that an individual reports qualitatively different features of SCD after a short period of time, this procedure ensures the evaluation of the stability of those complaints.

3. To inquire about SCD with regard to different timeframes (e.g. last months, last years, youth, etc.) in order to examine the change over time of the self-experienced cognitive decline.

4. To include items to cover all cognitive and non-cognitive domains, not only episodic memory. Some cases of atypical AD or even non-AD dementias may begin with symptoms distinct from memory loss (e.g. problems to inhibit behavior may be indicative of frontotemporal dementias rather than AD). For this reason, it is important to request subjective information about the whole spectrum of cognition and other neuropsychiatric variables.

1.4.1.1. Assessment of SCD during neurological and neuropsychological interviews

Responses to every question of SCD are directly provided by the participants since family members are not available in all cases. To ensure the greatest reliability and internal consistency, SCD is assessed twice and independently within the same visit at the Vallecas Project. First, during the neurological examination participants are asked the following nine questions regarding specific cognitive domains:

1) Attention: “Are you easily distracted?”

2) Spatial orientation: “Do you get lost in familiar surroundings or have trouble finding your way when driving?”

3) Episodic memory: “Do you often forget recent information or events?”

4) Autobiographical memory: “Do you often forget autobiographical information?”

5) Visual recognition: “Do you have trouble recognizing objects or faces?”

6) Speech: “Do you have word-finding difficulties for people’s names or common words?”

7) Language comprehension: “Do you understand simple verbal and written instructions?”

8) Executive functions: “Do you have difficulty driving, managing finances or planning daily activities?”

9) Praxis: “Do you have difficulty sequencing movements (e.g. taking the necessary steps to prepare a bath)?”

It is important to note that all those previous questions are merely tentative; thus, they are opened and spontaneously reported in such a way that alternative questions within the same cognitive domain could arise during the interview. Ultimately, subjective experience of complaints for every cognitive domain is coded in a dichotomic way (yes/no) based on the global impression of the neurologist. Additionally, there are other questions regarding psychiatric and behavioral symptoms which are a complement to the nine questions on cognitive complaints (see section 1.4.1.3.).

Second, during the neuropsychological assessment individuals also complete an ordinal scale of cognitive complaints composed of four items with four points each (ranged 0-3). This scale addresses the following questions to be responded:

1) “How do you perceive your memory in comparison with that of others of your age?”

(“3-bad”; “2-somewhat worse”; “1-somewhat better”; “0-excellent”);

2) “How do you perceive your memory today compared with your young adulthood?”

(“0-better”; “1-equal”; “2-somewhat worse”; “3-much worse”);

3) “Do you perceive your memory today is worse than compared with ten years ago?”

(“0-no”; “1-a little worse”; “2-somewhat worse”; “3-much worse”);

4) “Do you perceive your memory today is worse than compared with one year ago?”

(“0-no”; “1-a little worse”; “2-somewhat worse”; “3-much worse”).

The sum of these items resulted in a total score of cognitive concerns ranging from 0 (no complaints at all) to 12 (maximum complaints). Furthermore, five more open-ended questions are also collected during the neuropsychological interview:

5) Age at onset of cognitive complaints: “How old were you when your cognitive performance began to decline?"

6) Years of SCD´s progression: “How long do you believe you are experiencing cognitive complaints?”

7) Worries associated with self-perceived complaints: “Are you worried about your cognitive decline?”

8) Type of onset of cognitive complaints: “How did you perceive the beginning of the cognitive decline?” (e.g. suddenly, progressive, etc.)

9) Self-experienced functional impairment associated with SCD: “Do you believe your cognitive failures are impeding your daily life activities?”

1.4.1.2. Assessment of SCD by means of self-administered questionnaire

In addition to the whole information collected in both clinical interviews, individuals must accomplish a SCD scale, namely the Everyday Memory Questionnaire (EMQ) (Sunderland, Harris, & Gleave, 1984). This questionnaire is selected because it has been previously validated in our country and showed adequate psychometrical properties for older adults (Montejo et al., 2011). In our study, EMQ is self-administered by following the instructions provided in the validation study and always in the presence of a member of the research team; individuals are required to ask any doubt may arise.

The EMQ comprised 28 items about cognitive failures that occur in everyday life. The items must be responded according to the frequency in which they are experienced by a subject. All items are scored pursuant to a 3-point Likert-type scale, with 0 indicating “never, rarely”, 1 “occasionally, sometimes”, and 2 “frequently, almost always”. The total score range from 0 to 56, with lower scores indicating fewer SCD.

1.4.1.3. Assessment of non-cognitive complaints during medical interview

As discussed earlier, non-cognitive symptoms may represent an initial manifestation of cognitive decline due to different etiologies. Therefore, open-ended questions should be included in the context of the medical interview in order to assess behavioral, functional, and psychiatric self-reported complaints. Specifically, the aspects that should be covered may be the following:

a) Depression: “Do you feel sad, lonely and depressed most of the time?”

b) Anxiety: “Do you feel worried and anxious most of the time?”

c) Apathy: “Do you feel a lack of emotion, motivation or interest in hobbies and previously activities enjoyed?”

d) Disinhibition: “Do you feel difficulties to control your own behavior in some situations?”

e) Irritability: “Do you feel in an irritable mood most of the time?”

f) Hallucinations: “Do you feel you see or hear strange things which maybe are unreal?”

g) Sleep: “Do you feel your sleep routine has changed?”

h) Falls: “Have you had falls recently?”

i) Gait: “Do you feel you have problems to walk?”

1.4.1.4. Assessment of demographic, clinical and cognitive variables

The assessment of demographic, clinical and cognitive variables should be inseparable from SCD whether one´s goal is to determine the properties of cognitive concerns for detecting preclinical AD and individuals at high risk of later MCI. To this end, the Vallecas Project cohort undergoes a detailed survey and assessment protocol to gather information on demographics (age, gender, level of education, marital status, living situation, socioeconomic status, occupation, etc.), lifestyle (physical activity, social support, eating and sleep habits, etc.), quality of life (well-being, perceived health, etc.), medical history (vital signs, physical symptoms, clinical anamnesis, medication, neurological examination, etc.), family history of dementia, and neuropsychological assessment.

Cognitive diagnosis is always agreed between healthcare professionals at clinical consensus meetings. Every individual must be independently diagnosed according to his/her age, gender, cognitive reserve, functional information, and cognitive scores. Nevertheless, rather than psychometrically invariable cut-offs, diagnosis must be based on clinical impression. NIA-AA´s criteria (Albert et al., 2011) can be applied since they are very useful to diagnose core MCI and mild dementia. Cognitively healthy subjects are given a score of 0

in the global Clinical Dementia Rating (CDR) (Hughes, Berg, & Danziger, 1982) while MCI and mild dementia must score 0.5 and 1 respectively.

The comprehensive neuropsychological battery is administered by trained neuropsychologists. This battery includes complete information about all cognitive domains such as visual perception, attention, memory, language, praxis, and executive functions.

In addition, scales for measuring functional activities and neuropsychiatric variables are very important. For instance, to this end the Functional Activities Questionnaire (FAQ; Pfeffer, Kurosaki, Harrah, Chance, & Filos, 1982) can be administered to collect data with regard to instrumental activities of daily living; the Geriatric Depression Scale (GDS; Yesavage et al., 1982) and the State-Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, & Leshene, 1970) might be also administered as part of the neuropsychological battery to quickly estimate mood and anxiety symptoms.

1.4.2. Classification of individuals in SCD groups in the Vallecas Project.

Finally, information about subjective complaints is examined according to the guidelines proposed by the SCD-I (Jessen et al., 2014). Following these guidelines, individuals are grouped in three different categories pursuant to the extent of SCD reported:

1. No complaints (NC) when individuals do not report complaints with sufficient intensity;

2. Subjective Cognitive Decline (SCD), when subjects report some kind of cognitive complaint; and

3. Subjective Cognitive Decline Plus (SCD-P), when individuals show complaints in memory plus another cognitive domain and additionally they fulfill the rest of the criteria for SCD Plus.

The classification in any of the three groups of SCD may be carried out in two steps (a full description of this procedure is shown on Figure 1.5.). Initially, based on the overall information gathered both in clinical interviews and in self-administered EMQ, SCD may be operationally defined as the self-rated presence of cognitive deterioration using two criteria: i) at least a positive response to any yes/no-type question regarding complaints in

any cognitive domain from the neurological interview; and ii) scores above 1 on the SCD scale administered in the neuropsychological assessment AND above 8 on the self-administered EMQ. To be classified as SCD individuals have to mandatorily accomplish both conditions. Thus, subjects who only fulfill one criterion or neither of them will be considered as NC.

The second step of classification is only applied for those cases categorized as SCD. For these individuals some specific features must be considered such as: age at onset of SCD beyond 60 years, turning up of complaints within the last 5 years, worry associated with SCD, and feeling of worse performance than others of the same age group. When all these conditions accompany the self-experience of decline then an individual is classified as SCD-P.

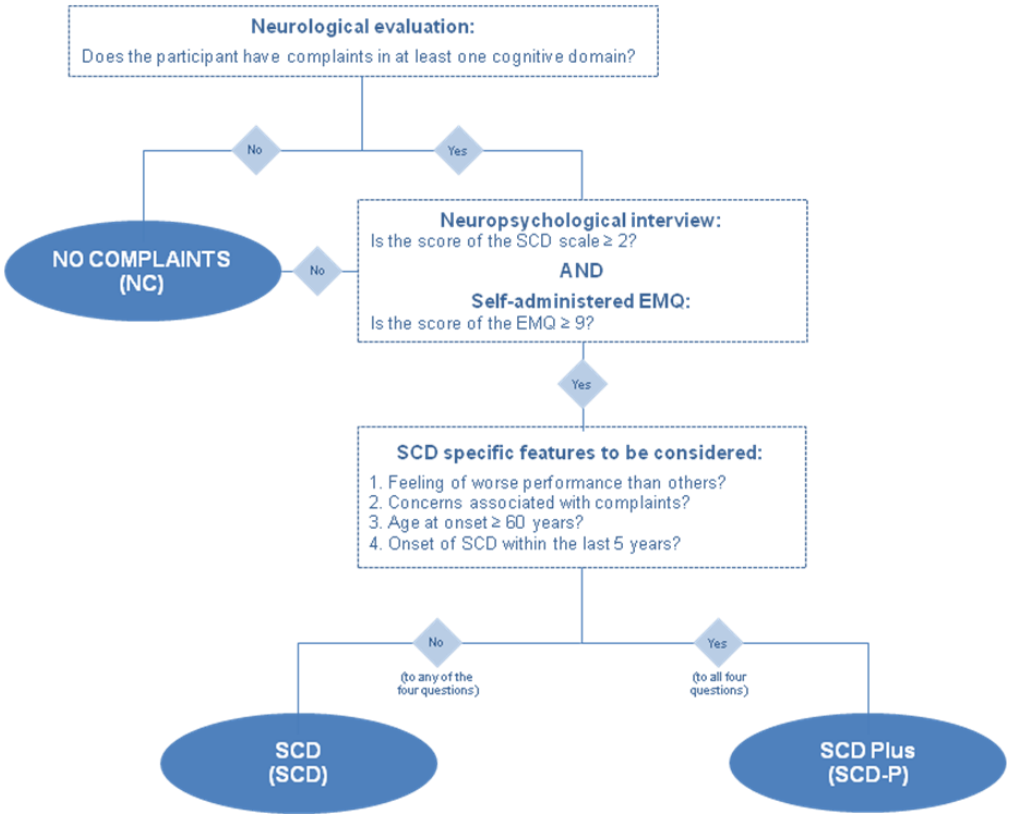

***Figure 1.5**. Flow diagram for SCD classification.*

EMQ: Everyday Memory Questionnaire; SCD: Subjective Cognitive Decline

2. OBJECTIVES AND HYPOTHESIS

2.1. General Objective

TO DEFINE THE ROLE OF THE SUBJECTIVE COGNITIVE DECLINE IN THE ALZHEIMER´S CONTINUUM.

2.2. Specific Objectives and Hypothesis

Five specific and consecutively goals as well as their respective hypothesis can be considered in this investigation:

1. *TO STUDY THE UNDERLYING STRUCTURE OF THE SCD IN A SAMPLE OF OLDER ADULTS.*

The purpose of this aim was to analyze whether SCD has a unidimensional structure or on the contrary there is a factorial underlying structure. Historically researchers have implicitly assumed that the self-reported complaints are a monolithic concept which is referred almost exclusively to memory. Proof of that is there is barely research about the nature and implication of different types of complaints on objective cognitive impairment. The clinical evidence however seems to indicate that there may be distinct types of cognitive complaints beyond memory that could have different clinical values in practice. We propose to analyze the underlying structure of a widely used scale focused on assessing memory complaints, the EMQ, in a sample of older adults to confirm whether subjective complaints are uni or multidimensional.

HYPOTHESIS 1: *If we analyze a listed items of complaints as used in the EMQ, then we would find a multidimensional underlying structure of SCD.*

2. *TO EXAMINE THE CROSS-SECTIONAL CLINICAL VALUE OF THE SCD IN DISTINGHISING BETWEEN HEALTHY ELDERLY CONTROLS AND INDIVIDUALS WITH MCI.*

As a second step we will test whether some specific cognitive complaints are more outstanding than others to distinguish between healthy ederly controls and individuals with MCI. To probe that, we will apply the underlying structure obtained from the previous EMQ´s factor analysis and study if those dimensions are able to differentiate between MCI

and healthy controls. The hypothesis which will be tested is patients with MCI have a different profile of cognitive complaints compared to cognitively healthy individuals.

HYPOTHESIS 2: *If we examine different types of complaints as those suposely obtained in objective 1, we will find some of them are able to distinguish between healthy ederly controls and individuals with MCI.*

3. *TO ANALYZE THE LONGITUDINAL PREDICTIVE VALUE OF THE SCD AS A MARKER OF FAST CONVERSION TO MCI IN A SAMPLE OF OLDER ADULTS.*

   As the second objective is confirmed, this goal will search for specific features of SCD that could predict conversion to MCI over time. The Vallecas Project cohort will be classified according to the guidelines provided by the SCD-I in three groups based on SCD´s features: No complaints, SCD, and SCD-Plus. Then, we will analyze whether the different subtypes of SCD are related to cognitive trajectory and are able to predict earlier the onset of MCI.

   HYPOTHESIS 3: *If we use SCD-I recommendations and classify subjects in No SCD- SCD and SCD-Plus, then specific features that define SCD-Plus will predict fast conversion to MCI.*

4. *TO INVESTIGATE THE RELIABILITY AND TEMPORAL STABILITY OF THE MEASUREMENTS OF THE SCD OVER TIME IN A SAMPLE OF OLDER ADULTS.*

   Measures of subjective variables like SCD are actually assessing two different types of information: i) the construct of interest (i.e. SCD in our case); and ii) errors of measurement which comprised both the error variance and short-term fluctuations due to shifts in self-perception itself. In psychometry it is a well-established fact that when repeated subjective measurements are collected from an individual the scores on two different occasions may be different. If this were the case in the majority of individuals, the subjective variable would lack internal consistency. In other words, if two longitudinal measures are quite different and they do not converge, which one is the real to characterize the individual? This lack of temporal stability in measures, which can affect preferably to subjective variables rather than objective performance, represents an important bias to investigation. Specifically, if a construct do not probe to be stable enough over time it should not be considered as a target for research. In this

way, making use that SCD is systematically and repeatadely collected in the Vallecas Project, we will analyze the temporal stability of self-reported complaints over time for the whole cohort. We hypothesize that SCD may have enough internal consistency if the construct is operationally defined and consistently assessed.

HYPOTHESIS 4*: If we operationally define and consistenly assess SCD over time, then it will be a stable, reliable and robust concept.*

5. *TO DETERMINE THE EXPRESSION AND THE TEMPORAL DYNAMICS OF THE SCD THROUGH THE AD CONTINUUM.*

This last objective depends on having previously demonstrated the temporal stability of SCD. If the latter were probed we will analyze the validity of the SCD as a very early marker of AD examining the dynamic transition from the preclinical to prodromal AD stages. Also we will examine whether diferent SCD subtypes could correlate with the different transition rates to MCI.

HYPOTHESIS 5*: If we focus at the preclinical substages of AD proposed by the NIA- AA, then we will find there is a temporal dynamic transition happening in the preclinical phase of AD that goes from NO SCD to SCD to SCD-Plus.*

3. EXPERIMENT 1

Publication title:

CLINICAL RELEVANCE OF SPECIFIC COGNITIVE COMPLAINTS IN DETERMINING MILD COGNITIVE IMPAIRMENT FROM COGNITIVELY NORMAL STATES IN A STUDY OF HEALTHY ELDERLY CONTROLS.

Journal:

FRONTIERS IN AGING NEUROSCIENCE

Year:

2016

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3.1. Summary of objectives

1. To examine the dimensional structure of the EMQ.

2. To report the existence of latent factors on the EMQ structure, not only the overall score.

3 To determine whether specific cognitive complaints are more useful than others to discriminate MCI.

3.2. Abstract

Introduction: Subjective memory complaints in the elderly have been suggested as an early sign of dementia. This study aims at investigating whether specific cognitive complaints are more useful than others to discriminate Mild Cognitive Impairment (MCI) by examining the dimensional structure of the Everyday Memory Questionnaire (EMQ).

Material and Methods: A sample of community-dwelling elderly individuals was recruited (766 controls and 78 MCI). The Everyday Memory Questionnaire (EMQ) was administered to measure self-perception of cognitive complaints. All participants also underwent a comprehensive clinical and neuropsychological battery. Combined exploratory factor analysis and item response theory were performed to identify the underlying structure of the EMQ. Furthermore, logistic regression analyses were conducted to study whether single cognitive complaints were able to predict MCI.

Results: A suitable five-factor solution was found. Each factor focused on a different cognitive domain. Interestingly, just three of them, namely forgetfulness of immediate information, executive functions and prospective memory proved to be effective in distinguishing between cognitively healthy individuals and MCI. Based on these results we propose a shortened EMQ version comprising 10 items (EMQ-10).

Discussion: Not all cognitive complaints have the same clinical relevance. Only subjective complaints on specific cognitive domains are able to discriminate MCI. We encourage clinicians to the EMQ-10 as a useful tool to quantify and monitor the progression of individuals who report cognitive complaints.

3.3. Introduction

Subjective memory complaints (SMC) can be defined as a self-experienced persistent decline in memory or any other cognitive ability in comparison with a previously normal status. Regarding the elderly, the topic of SMC has been a focus of intense debate within the research literature during the past two decades. Perhaps, the reason for that is the clinical importance of SMC in predicting the onset of memory impairment and future dementia. A recent meta-analysis has shown that, independently of the objective memory performance, 6.6% and 2.3% of older people with SMC develop mild cognitive impairment (MCI) and dementia per year (Mitchell et al., 2014), respectively. Since there is increasing evidence that SMC may represent a very early manifestation of Alzheimer´s Disease (AD) (Jessen et al., 2014), little is known about the clinical role of specific complaints on the transition between normal aging to cognitive impairment.

Although SMC increase with age, complaints tend to show only mild or non-significant correlations with objective memory performance. Instead, many cross-sectional studies have reported a close relationship between SMC and other subjective variables such as depression (Crane et al., 2007), anxiety (Comijs et al., 2002), perceived health (Montejo et al., 2014), personality (Pearman and Storandt, 2004) and quality of life (Montejo et al., 2011).

Structured questionnaires are considered the best approach of gaining insight into older adults' SMC (Montejo et al., 2014). Basically, these questionnaires consist of a list of common memory failures that must be rated according to the frequency in which they are experienced by subjects. Although there are many questionnaires that have been proposed to evaluate SMC, the Everyday Memory Questionnaire (EMQ) (Sunderland et al., 1984) is perhaps one of the most extended scales. The EMQ has been used to assess SMC in a variety of populations, including older adults (Garrett et al., 2010; Ossher et al., 2013). It consists of 28 items about memory failures that occur in everyday life. All items must be answered according to a Likert-type scale.

Despite Sunderland et al. (1984) emphasized the unidimensionality of the EMQ, the analysis of its individual items evidences that a high percentage of them do not exactly correspond to memory complaints. Rather, some items would involve various cognitive domains like,

for instance, visual perception ("failed to recognize, by sight, close friends or relatives"), attentional processing ("been unable to follow the thread of a story"), language production ("found that a word is on the tip of your tongue"), or executive functions ("forgotten a change in your daily routine"). This may be the reason for what several studies have reported the existence of various latent factors on the EMQ structure (Calabria et al., 2011; Cornish, 2000; Royle and Lincoln, 2008). In any event, investigations using the EMQ with older adults have exclusively focused on the overall score (Alegret et al., 2015), and have not addressed the role of the specific underlying factors upon differentiation between healthy controls and people with MCI.

This study aims at investigating whether specific cognitive complaints are more useful than others to discriminate MCI by investigating the underlying structure of EMQ´s items in a large community-dwelling older adult sample. We expect to find different cognitive complaints dimensions in the EMQ. Our secondary goals are, to propose a shortened version of EMQ based on discrimination and difficulty parameters of items within each factor, and to examine the ability of these specific dimensions to differentiate between MCI and healthy controls.

3.4. Material and Methods

3.4.1. Participants

The participants of this study comprised 844 community-dwelling individuals aged 70 years and over. All of them were part of the Vallecas Project cohort, a community-based longitudinal investigation for early detection of AD. The Vallecas Project was launched by CIEN Foundation-Queen Sofia Foundation on October 2011 (Olazarán et al., 2015). The study was approved by the Research Ethics Committee of the Carlos III Institute of Health. Written informed consent was obtained by all the participants.

All participants underwent a detailed assessment protocol including past medical history, neurological and neuropsychological examination, as well as biochemical and genetic blood test. The complete visit was usually carried out within four hours, with convenient breaks if necessary.

Every participant was independently diagnosed taking into account age, gender, cognitive reserve, functional information, and neuropsychological scores. Cognitive diagnoses were agreed between neurologists and neuropsychologists at consensus meetings. In all cases, cognitively healthy subjects had to obtain a score of 0 in the global Clinical Dementia Rating (CDR) (Hughes et al., 1982). Criteria from the National Institute on Aging-Alzheimer´s Association (NIA-AA) were used to diagnose MCI (Albert et al., 2011). A total of 766 individuals were classified as controls and 78 met the criteria for MCI.

3.4.2. Subjective complaints assessment

We used the EMQ to measure cognitive complaints. This questionnaire was administered following the instructions provided in a previous Spanish validation study (Montejo Carrasco et al., 2012). Participants were asked to rate the 28 items according to the frequency with which they experienced each complaint. Items were scored on a 3-point scale, with 0 indicating “never, rarely”, 1 “occasionally, sometimes”, and 2 “frequently, almost always”. Thus, the total score ranged from 0 to 56. In all cases, individuals completed the EMQ in the presence of a member of the research team.

3.4.3. Neuropsychological assessment

A comprehensive neuropsychological battery was applied by trained neuropsychologists in order to obtain information about visual perception, attention, memory, language, praxis, and executive functions. A total of eleven cognitive tests were considered: Mini Mental State Examination (MMSE) (Folstein et al., 1975); Clock Drawing Test; Free and Cued Selective Reminding Test (FCSRT) (Buschke, 1984); Lexical and Semantic Verbal Fluency (Peña-Casanova et al., 2009); Forward and Backward Digit Span (Wechsler, 1997); Five Point Test (Lee et al., 1994); Rule Card Shifting Test (Wilson et al., 1996); Boston Naming Test (15-items version) (Fernández-Blázquez et al., 2012); Imitation of Bilateral Postures and Symbolic Gesture (Peña-Casanova, 1990); and Digit Symbol Coding (Wechsler, 1997). In addition, the following three scales were also administered to collect further data with regard to functional performance and mood: Functional Activities Questionnaire (FAQ) (Pfeffer et al., 1982), Geriatric Depression Scale (GDS) (Yesavage et al., 1982), and State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970).

3.4.4. Data analysis

Analyses were conducted using R version 2.15. (R Development Core Team, 2012). Differences between healthy controls and MCIs on baseline characteristics were evaluated with Mann-Whitney tests and Pearson´s χ2 as appropriate. To identify latent constructs in the structure of correlations among the 28 items of the EMQ, an exploratory factor analysis (EFA) was performed using exclusively control subjects. Since response categories were ordinal scores, a polychoric correlation matrix resulted in a preferable approach for EFA (Brown, 2006).

First, a descriptive analysis of items was developed in order to find out their individual distribution. Then, it was determined whether the assumptions of normality and sphericity were met. Due to no prior theory exists regarding the structure of data, Weighted Least Squares (WLS) with oblique Promax rotation was selected as the factor extraction method. The procedure for determining the number of factors was Parallel Analysis. In addition to χ2, the most common indexes of goodness-of-fit, Root Mean Square Error of Approximation (RMSEA) and Standardized Root Mean Square Residual (SRMR) were used. Values no greater than 0.06 for RMSEA and lower than 0.08 for SRMR indicate acceptable fit (Hu and Bentler, 1999).

By means of an Item Response Theory (IRT) approach, we calibrated all retained items using single Graded Response Models (GRM), one for each factor. This kind of models are the most appropriate to examine ordinal items as well as they assume normality of the latent trait. GRM estimate a slope parameter and two location parameters for each 3-category item. After obtaining item´s parameters from the IRT calibration, we used this information to identify a shortened version of the EMQ that maintained adequate content coverage within each factor with maximum precision. To guide selection of items, we examined the item information functions of every single factor. Additionally, two quantitative criteria were established in order to produce the maximum amount of information (discrimination index >1) with optimal difficulty distribution (sum of location parameters ranged from 2 to 4). Therefore, those items that did not fulfill both criteria were excluded from their corresponding factor. Finally, internal consistency was estimated by means of Cronbach's alpha coefficient for ordinal categories.

Since distribution of most variables and components of the EMQ did not fulfill all the assumptions for using parametric statistics, a Spearman correlation analysis was carried out between the resulting factors and demographic and cognitive variables. In addition, Mann-Whitney tests were to study differences between control and MCI groups. As proposed by Cohen (1988), non-parametric adjusted effect sizes were estimated through the approximation of the z distribution associated with the Mann-Whitney test. According to the value of r, a large effect is 0.5, a medium effect is 0.3, and a small effect is 0.1. Additionally, to facilitate the interpretation of results, measures of probability of superiority (PS) were also provided.

Finally, we also performed logistic regressions to examine whether age, education, and gender along with the underlying EMQ´s factors were able to predict cognitive impairment. In order to measure the impact of the model upon data, a special consideration was given to tests of signification for the model estimators. Analysis of residuals and goodness-of-fit statistics were also performed to measure the degree of adjustment of the model to available data.

3.5. Results

3.5.1. Descriptive analysis of the sample

The sample consisted of 766 controls (90.8%) and 78 MCIs (9.2%). Demographic and cognitive data, as well as differences between both groups, are shown in Table 1. Significant differences were found for age and years of education in such a way that MCIs were older and less educated than controls. A larger percentage of males were also classified as MCI. Moreover, as expected, the majority of cognitive variables showed large differences in favor of controls (p-value < 0.001), except for trait anxiety, where no statistically differences were found.

3.5.2. Exploratory factor analysis

First, descriptive statistics of individual items of EMQ were calculated (Table 2). Items 11, 19 and 27 were excluded from further analysis due to their values of skewness and/or kurtosis were over |2.5|. Thus, a symmetrically distribution with the rest of 25 retained items was ensured to be applied the EFA.

***Table 3.1**. Descriptive analysis and mean differences between control and MCI groups.*

	Control (n=766)		MCI (n=78)		p-value
	Mean	SD	Mean	SD	
Age (years)	74.07	3.80	76.08	4.06	< 0.001
Education (years)	11.15	6.69	8.04	6.00	< 0.001
Sex	63% Female		50% Female		0.032
Cognitive performance					
MMSE	28.75	1.46	26.09	2.28	< 0.001
Clock Drawing Test	9.42	1.26	8.20	1.88	< 0.001
FCSRT free immediate	24.90	5.73	13.89	5.26	< 0.001
FCSRT total immediate	42.84	4.45	30.78	8.29	< 0.001
FCSRT free delayed	9.90	2.49	4.50	2.83	< 0.001
FCSRT total delayed	14.79	1.51	10.13	3.35	< 0.001
Lexical Verbal Fluency	39.76	12.95	27.31	10.33	< 0.001
Semantic Verbal Fluency	49.54	10.11	34.57	8.49	< 0.001
Forward Digits	7.40	1.87	6.37	1.55	< 0.001
Backward Digits	4.64	1.85	3.77	1.51	< 0.001
Five Point Test	21.88	8.19	14.77	5.81	< 0.001
Rule Card Shifting	3.02	3.10	7.11	3.10	< 0.001
Boston Naming Test-15 items	12.83	1.85	9.69	3.07	< 0.001
Posture Imitation	7.27	1.20	6.31	1.26	< 0.001
Symbolic Gesture	9.70	1.00	9.47	0.96	0.001
Digit Symbol Coding	39.72	15.10	25.20	10.95	< 0.001
FAQ	0.38	0.68	2.68	2.33	< 0.001
GDS	1.47	2.17	2.73	2.78	< 0.001
STAI state	14.51	8.80	17.76	10.97	0.037
STAI trait	16.77	9.68	17.00	9.57	0.887

Note. MMSE = Mini-Mental State Examination; FCSRT = Free and Cued Selective Reminding Test; FAQ = Functional Activities Questionnaire; GDS = Geriatric Depression Scale; MCI = Mild Cognitive Impairment; SD = Standard Deviation; STAI = State-Trait Anxiety Inventory

The EMQ total score was not normally distributed (Shapiro-Wilk normality test, W=0.93; p<0.001). The mean of the EMQ total score was 13.18±7.84 (range 0-47). We did not obtain significant association between EMQ and gender (W=59,803; p=0.55) nor age (rho=0.04; p=0.28). Nevertheless, the correlation between EMQ total score and years of education was statistically significant (rho=-0.16; p<0.001), which meant that individuals with more years of education tended to report less cognitive complaints. The Cronbach's alpha coefficient for the polychoric correlation matrix comprising the 25 items was 0.93.

***Table 3.2**. Descriptive statistics of individual items of EMQ.*

Items	Mean	SD	Median	Min	Max	Skew	Kurtosis
1	0.89	0.49	1	0	2	-0.23	0.79
2	0.39	0.54	0	0	2	0.97	-0.14
3	0.25	0.49	0	0	2	1.76	2.26
4	0.26	0.50	0	0	2	1.70	2.02
5	0.71	0.55	1	0	2	-0.01	-0.55
6	0.74	0.53	1	0	2	-0.16	-0.38
7	0.51	0.53	0	0	2	0.33	-1.16
8	0.61	0.56	1	0	2	0.23	-0.82
9	0.35	0.54	0	0	2	1.17	0.34
10	0.31	0.51	0	0	2	1.32	0.71
11	0.16	0.43	0	0	2	2.71	6.86
12	0.58	0.61	1	0	2	0.54	-0.63
13	0.89	0.50	1	0	2	-0.22	0.76
14	0.44	0.58	0	0	2	0.91	-0.17
15	0.48	0.55	0	0	2	0.59	-0.75
16	0.29	0.49	0	0	2	1.35	0.76
17	0.46	0.59	0	0	2	0.85	-0.27
18	0.21	0.42	0	0	2	1.66	1.41
19	0.09	0.33	0	0	2	4.02	16.63
20	0.37	0.53	0	0	2	0.99	-0.14
21	1	0.61	1	0	2	0	-0.32
22	0.34	0.53	0	0	2	1.22	0.47
23	0.52	0.62	0	0	2	0.79	-0.39
24	0.73	0.57	1	0	2	0.08	-0.51
25	0.26	0.50	0	0	2	1.71	2.06
26	0.54	0.61	0	0	2	0.66	-0.52
27	0.16	0.40	0	0	2	2.44	5.43
28	0.35	0.53	0	0	2	1.18	0.38

Note. SD = Standard Deviation

Table 3 shows the factor loadings and the communalities and percentage of variance explained for the factors obtained. The measure of sample adequacy was appropriate for developing an EFA (KMO=0.92; Barlett χ21035=4,350.15; $p<0.001$). Although the Parallel Analysis determined six dimensions as the optimal solution from a statistical point of view, we finally adopted an explanation with five components because of it proved more reasonable in biological terms. The first factor corresponded to the items 2, 6, 8, 13, 15, 16, 17, 20, 21, 23, and 28 and explained 17% of the total variance. This component was called Forgetfulness of Immediate Information (FII). The second component comprised the items 3, 4, 5, 9, 10, and 12 and explained 11% of the total variance; it was termed as Executive

Functions (EF). A third component, named as Prospective Memory (PM), retained the items 7, 14, 18 and 22. Finally, the fourth and fifth factors comprised, respectively, items 1 and 24 and items 25 and 26. They were called Forgetfulness of Common Objects (FCO) and Spatial Orientation (SO). The analysis of the polychoric correlation matrix by using Mardia´s tests revealed data to be reached a suitable multivariate normality (skew statistic = 7146.36 with $p < 0.001$; kurtosis statistic = 36.02 with $p < 0.001$). Likewise, reliability of all factors was considered appropriate.

Table 3.3*. Exploratory Factor Analysis of the EMQ with component loadings of each item.*

	I	II	III	IV	V	Communalities
Item 21	**0.710**	-0.254				0.37
Item 8	**0.618**	0.193	0.211		-0.288	0.57
Item 20	**0.605**			-0.156		0.40
Item 13	**0.576**	-0.148	0.153			0.40
Item 15	**0.528**		0.347			0.54
Item 17	**0.502**	0.425	-0.236			0.56
Item 6	**0.502**			0.144		0.37
Item 16	**0.431**	0.267				0.52
Item 28	**0.339**		0.208	-0.107	0.109	0.31
Item 2	**0.328**	0.313	-0.108			0.40
Item 23	**0.312**		0.237	-0.176		0.26
Item 3	-0.106	**0.786**				0.53
Item 4	-0.249	**0.718**	0.221			0.51
Item 9	0.400	**0.437**	-0.155		-0.127	0.41
Item 10	0.125	**0.407**			0.179	0.37
Item 12	0.109	**0.383**			0.146	0.34
Item 5	0.211	**0.318**		0.282	-0.118	0.44
Item 18	0.180		**0.692**	-0.149		0.52
Item 22	0.252	0.182	**0.501**			0.61
Item 14	0.400		**0.430**		-0.127	0.62
Item 7	-0.123		**0.349**	0.328	0.188	0.42
Item 1			-0.128	**1.002**		0.85
Item 24	0.265	-0.220	0.120	**0.568**		0.60
Item 25	-0.150				**0.955**	0.91
Item 26	0.109		-0.105		**0.688**	0.51
Eigenvalue	4.23	2.76	2.00	1.67	1.65	
Proportion variance	0.17	0.11	0.08	0.07	0.07	
Cronbach coeficient	0.80	0.66	0.63	0.65	0.65	

3.5.3. IRT calibration

Preliminary non-parametric Mann-Whitney tests were carried out in order to ascertain whether the five components of the EMQ were able to distinguish between healthy controls and MCIs. Thereby, FII (U=16510; p<0.001), EF (U=17176; p<0.001) and PM (U=19932.5; p=0.016) were found to differentiate between both groups. However, FCO (U=24588; p=0.381) and SO (U=23920; p=0.258) did not show relevant differences. Thus, according to the aims of this work, only FII, EF and PM were finally analyzed. FCO and SO were excluded for further analyses.

Table 3.4*. Parameters estimated from the three Graded Response Models (GRM).*

	a	b1	b2
FII			
Item 2	1.320	0.547	3.361
Item 6	1.403	-0.820	2.765
Item 8	1.887	-0.245	2.470
Item 13	1.345	-1.451	2.373
Item 15	1.714	0.168	2.867
Item 16	1.816	0.787	3.107
Item 17	1.278	0.332	2.911
Item 20	1.353	0.601	3.444
Item 21	1.174	-1.567	1.542
Item 23	0.942	0.231	3.164
Item 28	1.115	0.302	3.681
EF			
Item 3	1.698	1.053	2.889
Item 4	1.473	1.076	3.100
Item 5	1.300	-0.805	2.792
Item 9	1.068	0.831	3.781
Item 10	1.238	0.315	3.612
Item 12	1.224	-0.075	2.703
PM			
Item 7	1.094	0.046	4.181
Item 14	2.273	0.327	2.203
Item 18	1.581	1.214	4.092
Item 22	2.408	0.594	2.456

Note. a = discrimination parameter; b1 = difficulty parameter response option 1; b2 = difficulty parameter response option 2; AIC = Akaike Information Criterion; FII = Forgetfulness of Immediate Information; EF = Executive Functions; PM = Prospective Memory.
AIC FFI: 11,292.92; AIC EF: 5,938.43; AIC PM: 3,742.51

The parameter estimates from the three GRM calibrations are shown in Table 4. The slope values for all items ranged from 0.942 to 2.408, indicating a considerable variation in discrimination among them. However, items 14 and 22 showed the best discrimination for

PM, while values for items of FII and EF were more homogeneous. On the other hand, despite the range of location parameters reflected a sizeable range of underlying cognitive complaints (-1.567 to 4.181), the majority of item response categories were selected by participants who had more complaints than average. These results pointed out that items allowed to differentiating among individuals at the end of the complaints continuum.

Then, we selected the best combination of items within each factor to maximize the amount of information with optimal difficulty distribution. We used both discrimination and difficulty parameters of items to carry out the selection. To that end, items should have discrimination indexes greater than 1 and location parameters ranged from 2 to 4. These criteria were adopted because of the objectives of this work (the easiest or the most difficult items were considered not advisable to discriminate between controls and MCI). Overall, 10 items were finally selected as follows: i) FII: items 2, 8, 15, 16, 17 and 28; ii) EF: items 3, 10 and 12; and iii) PM: items 14 and 22. A final score of this shortened EMQ, called EQM-10, was also calculated by adding up these 10 items.

3.5.4. Multivariate study

As shown in Figure 1, FII, EF and PM correlated among them in a range from 0.33 to 0.53. Regarding to neuropsychological tests, the three factors showed low-moderate correlation coefficients with psychiatric symptoms, while the correlation with cognitive performance was mainly low. For depression and anxiety, the coefficients were positive, indicating that complaints increased as depression and anxiety scores were higher. On the contrary, the relationship with objective cognition showed negative coefficients, meaning larger complaints as cognitive performance decreased. Interestingly, FII was more associated with episodic memory (FCSRT), while EF was stronger related to language production (Fluency, BNT) and executive components (number of errors in RCS). Correlation coefficients between MMSE and every factor were very similar to those obtained in neuropsychological tests. Age and education showed low correlation coefficients with all factors.

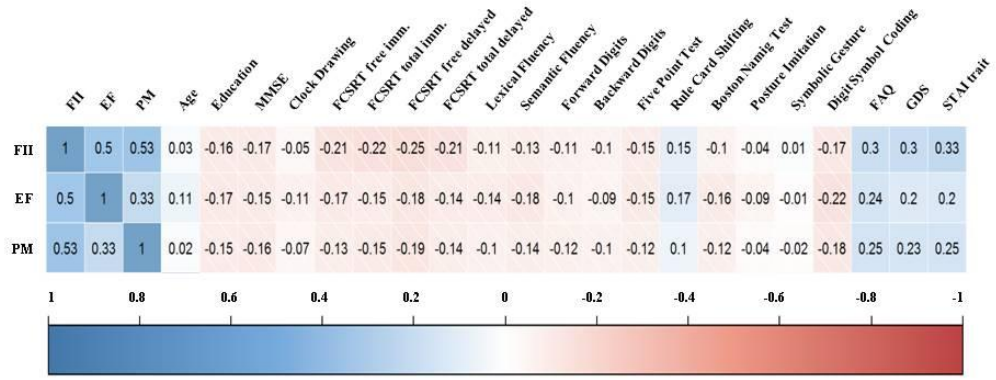

Figure 3.1*. Correlogram between the factors of the EMQ and demographic and cognitive variables.*

Note. FII = Forgetfulness of Immediate Information; EF = Executive Functions; PM = Prospective Memory; MMSE = Mini-Mental State Examination; FCSRT = Free and Cued Selective Reminding Test; FAQ = Functional Activities Questionnaire; GDS = Geriatric Depression Scale; STAI = State-Trait Anxiety Inventory.

Non-parametric Mann-Whitney tests were performed to determine whether the three factors were able to distinguish between healthy controls and MCIs. Figure 2 shows the scores of both groups for each component. FII (U=17,175; p<0.001), EF (U=17,651; p<0.001) and PM (U=19,015; p<0.001) were found to differentiate between both groups. According to the non-parametric effect size, FII, EF, and PM showed respectively the following sizes: 0.16, 0.14, and 0.12. Total score of the EMQ-10 was also significant (U=14,098; p<0.001) and showed a mild increase in the effect size (r=0.18).

Finally, four logistic regression models were carried out to study the impact of cognitive complaints upon the diagnostic of MCI. All these five models were adjusted for age, education and gender as covariates (Table 5). The three cognitive factors proved to be significant in their respective models after controlling for demographic variables. In addition, estimates of all factors were positive, what indicated that expressing complaints was associated with MCI. FII showed the best determination coefficient (model 1; R2=0.14) followed by EF (model 2; R2=0.10) and PM (model 3; R2=0.10). Indeed, total score of EMQ-10 (model 4; R2=0.14) did not improve the association with diagnostic showed by FII. Hence, although the values of these determination coefficients were not too high, demographic variables and cognitive complaints were effective in distinguishing between cognitively healthy individuals and MCI.

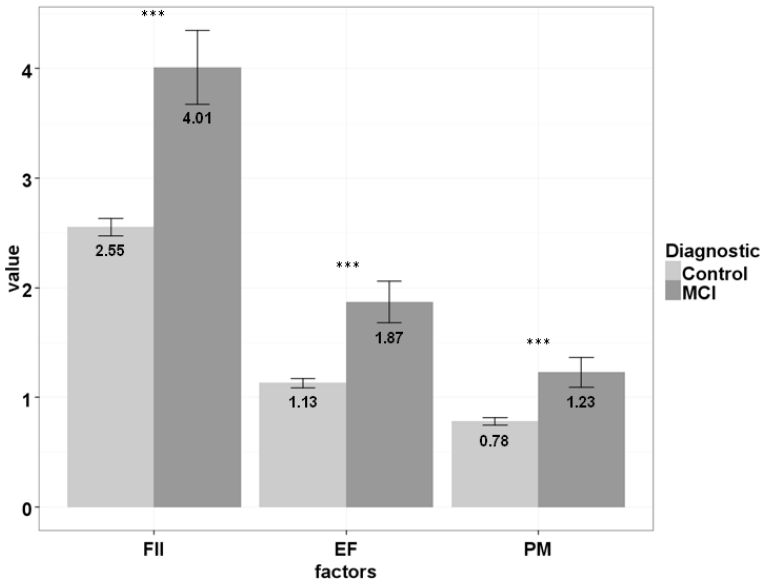

***Figure 3.2**. EQM-10 factors scores differences between controls and MCIs.*

Note. MCI = Mild Cognitive Impairment; FII = Forgetfulness of Immediate Information; EF = Executive Functions; PM = Prospective Memory.
*** p-value < 0.001

3.6. Discussion

In the current study, we have examined the latent structure of the EMQ and the ability of specific cognitive complaints to differentiate between MCI and healthy controls. To that end, it was analyzed a sample of 844 community-dwelling individuals above 70 years old who voluntarily participated in a longitudinal investigation for early detection of AD. Of them, only the 766 control individuals were used to study the factor structure of the EMQ. To our knowledge, this is the first study that investigates the dimensional structure of the EMQ and compares how well specific cognitive complaints are able to discriminate MCI.

Our results highlight an adequate internal consistency of the EMQ, as well as a factorial structure. This outcome does not fit well with the Sunderland´s assumption on the unidimensionality of the questionnaire (Sunderland et al., 1984). Indeed, as already reported by other authors, the EMQ has proved to have an underlying structure composed of three (Calabria et al., 2011), four (Royle and Lincoln, 2008) or even five factors (Cornish, 2000). Rather than a specific questionnaire focused on memory complaints, the EMQ seems to be a more complex scale that is able to measure various domains of subjective cognitive impairment.

***Table 3.5**. EQM-10 factors scores differences between controls and MCIs.*

Model 1: Age, Education, Sex and Forgetfulness of Immediate Information				
Variables	B	SE	z value	Sig.
(Intercept)	-10.88	2.55	-4.28	< 0.001
Age	0.12	0.03	3.71	< 0.001
Education	-0.10	0.03	-3.68	< 0.001
Female	-0.94	0.27	-3.50	< 0.001
FII	0.24	0.05	4.78	< 0.001
Null Deviance=474.50 on 773 dg; Residual Deviance=410.23 on 769 dg; AIC: 420.23				
Model 2: Age, Education, Sex and Executive Functions				
Variables	B	SE	z value	Sig.
(Intercept)	-9.25	2.47	-3.75	< 0.001
Age	0.10	0.03	3.13	0.002
Education	-0.09	0.03	-3.37	< 0.001
Female	-0.70	0.27	-2.60	0.009
EF	0.34	0.10	3.49	< 0.001
Null Deviance= 466.38 on 779 dg; Residual Deviance= 417.76 on 775 dg; AIC: 427.76				
Model 3: Age, Education, Sex and Prospective Memory				
Variables	B	SE	z value	Sig.
(Intercept)	-10.88	2.49	-4.37	< 0.001
Age	0.12	0.03	3.82	< 0.001
Education	-0.07	0.02	-3.05	0.002
Female	-0.75	0.27	-2.83	0.005
PM	0.38	0.12	3.09	0.002
Null Deviance=467.31 on 784 dg; Residual Deviance=421.76 on 780 dg; AIC: 431.76				
Model 4: Age, Education, Sex and EQM-10				
Variables	B	SE	z value	Sig.
(Intercept)	-11.43	2.62	-4.37	< 0.001
Age	0.12	0.03	3.72	< 0.001
Education	-0.08	0.03	-3.11	0.002
Female	-0.79	0.28	-2.82	0.005
EQM-10	0.15	0.03	4.78	< 0.001
Null Deviance=444.97 on 739 dg; Residual Deviance=384.68.76 on 735 dg; AIC: 394.68				

Note. Dg = degrees of freedom; AIC = Akaike Informative Criterion; Objects; SE = Standard Error; FII = Forgetfulness of Immediate Information; EF = Executive Functions; PM = Prospective Memory.

In our study, items 11 (failed to recognize, by sight, close friends or relatives), 19 (forgotten important details about yourself) and 27 (repeat to someone what you have just told them) were excluded from the EFA since their skewed distribution. The reason for that exclusion may have to do with the fact that these three items seem to reflect severe symptoms which appear in mild dementia rather than in earlier stages (preclinical or prodromal phases). The

final solution with the remaining 25 items comprised of a five-factor structure which explained up to50% of EMQ´s total variance: i) FII was associated with fails in immediate retrieval, as well as naming impairment; ii) EF was related to distractibility, inhibition errors and monitoring; iii) PM referred to things that someone has to recall in the next future; iv) FCO had to do with forgetting personal details; and v) SO was associated with difficulties for spatial orientating.

One crucial aim of the present study was to examine the association of SMC with neuropsychological performance and clinical diagnosis. EMQ´s factors exhibited higher correlation coefficients with psychiatric symptoms than with cognitive performance as other studies have already demonstrated (Balash et al., 2013). Global cognitive status assessed by means of MMSE was negatively correlated with all factors. In addition, as shown in Figure 1, FII and EF proved to be the factors that correlated higher with cognitive performance, especially episodic memory in the case of FII, and executive functions for EF. This outcome provides concurrent validity to the latent structure of the EMQ because the internal content of the factors is directly related to the cognitive domain supposedly assessed. Furthermore, the use of an IRT approach allowed us to find out the best 10-items that maximize the collection of information on cognitive complaints.

Regarding the clinical implications of this work, it has been suggested that cognitive complaints are able to distinguish between cognitively healthy elders and MCI (Buckley et al., 2013). In our study, three types of cognitive concerns are able to discriminate between controls and MCI. Higher scores in specific complaints on retrieval of immediate events, executive functioning, and prospective memory are related to prodromal stages of dementia. Indeed, our results indicate that their effect sizes give a PS of nearly 60%. That is, if two individuals, one control and one MCI, were selected at random, the score in any of these three factors would be higher for the MCI patient on 60% of times. The fact that both forgetfulness of objects and spatial orientation do not show differences in control subjects and MCI could be explained because of the first of them refers to a high prevalent oversight in the elderly population (“Forgetting where you have put something”, “Forgetting where things are normally kept or looking for them in the wrong place”) and the other one is an idiosyncratic sign of mild dementia (“Getting lost or turning in the wrong direction on a journey, on a walk, or in a building where you have been before”). All these findings

emphasize that not all cognitive complaints have the same clinical significance for prediction of cognitive impairment.

Concerning the limitations of the present study, the cross-sectional nature of our research is perhaps the most important one. Although our results suggest that specific cognitive complaints discriminate between controls and MCI, it remains unclear whether those specific complaints may be used to detect individuals at high risk of conversion to MCI in the future. Given that the Vallecas Project is still in progress, this is an important issue that shall be addressed in next visits. Another limitation is that family members of the participants were not available in all cases in order to confirm the severity of the cognitive complaints reported by subjects with MCI. This information could be very useful in future studies to minimize the effect of anosognosia, a common symptom in MCI that might somewhat bias the results. Finally, it should be desirable to study the link between cognitive complaints and other variables such as cognitive reserve that may influence on cognitive performance (Freret et al., 2015; Mondini et al., 2016). Since cognitive reserve has been positively related to both episodic and working memory (Lojo-Seoane et al., 2014), it could be hypothesized that self-perception of subjective deterioration could be increased in those individuals with low cognitive reserve.

In summary, not all cognitive complaints are effective in distinguishing healthy elderly individuals from those with MCI. Specific complaints related to episodic memory, executive functions and prospective memory discriminate between controls and cognitive impaired subjects. Individuals who present these particular complaints and do not yet have a diagnosis of MCI may need special attention in terms of close clinical follow-up or an early cognitive intervention. The use of the EMQ-10 is highly recommended to quantify subjective decline and to monitor the longitudinal progression of individuals who report those cognitive complaints.

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Authors contributions

MAV, ARV, MV, MFB collected the data. All authors drafted the manuscript. MFB, JRS, MAV conducted the statistical analysis. All authors interpreted the data and critically edited the manuscript. All authors approve the submitted version of the manuscript and are accountable for the accuracy and integrity of the work.

3.7. Summary of conclusions

1. EMQ has an adequate internal consistency, as well as a well-stablished factorial structure.

2. EMQ is able to measure various domains of cognitive complaints.

3. The SCD construct has a multidimensional structure.

4. Three types of cognitive concerns are able to distinguish between contols and MCI subjects (retrieval immediate events/executive functioning/ prospective memory).

5. Complaints associated with these three types of concerns give us a profile of a subject at a higher risk of developing MCI.

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4. EXPERIMENT 2

Publication title:

SPECIFIC FEATURES OF SUBJECTIVE COGNITIVE DECLINE PREDICT FASTER CONVERSION TO MILD COGNITIVE IMPAIRMENT

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4.1. Summary of objectives

1. To evaluate the clinical significance of SCD on the conversion from healthy stage to MCI.

2. To assess the potential usefulness of SCD on its different subtypes.

3. To determine SCD association with cognitive performance.

4. To examine the risk of SCD for objective cognitive decline.

4.2. Abstract

Background: Alzheimer´s disease (AD) is a silent disorder that needs the earliest possible intervention in order to reduce its high economic and social impact. It has been recently suggested that Subjective Cognitive Decline (SCD) appears at preclinical stages many years before the onset of AD. Therefore, SCD could become an ideal target for early therapeutic intervention.

Objective: The goal of this study was to evaluate the clinical significance of SCD on the conversion from a cognitively healthy stage to a Mild Cognitive Impairment (MCI) in one-year follow-up.

Methods: A total of 608 cognitively intact individuals from the Vallecas Project´s cohort, a community-based prospective study to identify early markers of AD, were enrolled in this study. Participants were classified in three groups: i) No Complaints (NCg), ii) Subjects with complaints in one or more cognitive domains (SCDg), and iii) Subjects who, besides complaints, fulfilled the features of SCD Plus proposed by the International Working Group of SCD (SCD-Pg).

Results: Individuals were followed up for a mean of 13.1 months (range 10.7-22.4). During this time, 41 volunteers developed MCI (6.7% of total sample). The conversion rate for SCD-Pg (18.9%) was significantly higher than SCDg (5.6%) and NCg (4.9%).

Conclusion: Specific features associated with SCD may help to identify individuals at high risk of fast conversion to MCI. These results highlight the importance of a close follow-up of subjects with SCD-P and include them in early intervention programs because of their increased risk for the development of MCI.

4.3. Introduction

Sporadic Alzheimer´s Disease (AD) is a multifactorial neurodegenerative disorder that begins affecting the brain many years before cognitive impairment is noticeable. According to the National Institute on Aging-Alzheimer Association (NIA-AA) workgroups, there are three different stages of AD´s progression over time. First, there is a preclinical phase in which some of the disease hallmarks in the brain have taken place, such as the presence of amyloid plaques, but no objective cognitive impairment is present [1]. A second stage, called prodromal AD or Mild Cognitive Impairment (MCI) due to AD, involves minor cognitive changes which are noticeable to the patient and/or to others, but are not severe enough to significantly affect everyday activities [2]. Finally, there is a third stage in which the intensity of the cognitive disorder leads to a functional impairment that ends up with a dementia syndrome [3]. The difficulty in pharmachologically altering the progression of AD stages has fostered the growing consensus that therapeutic interventions are more likely to be effective at the earliest possible phase [4]. Currently, treatment efforts between stages 2 and 3 have led to negative results. Thus, the search for early markers of preclinical AD is of paramount importance since disease-modifying therapeutic approaches are being developed for future use in at risk populations [5].

In dominantly inherited AD patients, the studies on pathophysiological changes occurring several years before symptoms onset support the existence of preclinical stages [6]. Biomarkers, based on the analysis of cerebrospinal fluid (CSF) samples or brain imaging [7], have shown evidence of neuropathological features in those preclinical (silent) stages of AD. However, these abnormalities do not seem to be accompanied by a clear cognitive marker. In fact, there is still no full consensus on the clinical significance of possible entities such as the Subjective Cognitive Decline (SCD) [8].

SCD refers to a self perception of progressive deterioration of cognitive abilities independently of the objective performance on neuropsychological tests. Since cognitive complaints are heterogeneous and their expression could be affected by various factors (e.g. normal aging, personality traits, depression, drug side effects, neurological disorders, etc.), SCD is not necessarily present in all prodromal cases. Nevertheless, both cross-sectional [9–11] and longitudinal studies [12–14] have provided strong evidence of SCD occurring at preclinical stage of AD. Indeed, the combination of SCD and CSF biomarkers

has also proved to be the best predictors of clinical progression from preclinical to prodromal and dementia stages [15]. For all these reasons, during the last decades there has been an increasing interest in the study of SCD as a potential early sign of cognitive decline and future progression to AD [16,17].

To assess the potential usefulness of SCD for clinical trials, the Subjective Cognitive Decline Initiative (SCD-I) has recently agreed to a common terminology and research procedures to identify individuals with SCD at risk of preclinical AD [18]. The SCD-I aims at knowing whether the self-experience of decline in cognition may represent the first symptomatic manifestation of AD. In order to demonstrate that, some common specific features are needed to better establish the profile of SCD. Thus, the SCD-I recommended to collect information regarding features such as settings in which cognitive complaints are expressed, association of SCD with medical help seeking, number of years and age at onset of SCD, subjective decline in memory and non-memory domains, and association of SCD with experience of impairment. In addition, the SCD-I proposed a set of particular features which could be helpful to identify individuals at risk of clinical progression. Those features include a more acute subjective memory decline than any other cognitive domain, onset of complaints within the last 5 years, age at onset over 60 years, worries about SCD, and feeling of worse performance than other people from the same age group. This set of features makes up a more severe form of SCD referred to as SCD Plus. This new category could allow us to explain the transition from a non-symptomatic stage to the first symptomatic manifestation of AD.

In the present study we have assessed the validity of different subtypes of SCD and to determine its association with neuropsychiatric and cognitive performance. Furthermore, we have examined the risk of SCD for objective cognitive decline rather than subjective one. SCD-I´s guidelines [18] were used in order to analyze whether specific features of SCD were more related to cognitive impairment.

4.4. Materials and methods

4.4.1. Participants

Subjects participating in this study were part of the Vallecas Project cohort, a community-based prospective study on early detection of AD, recently launched by CIEN Foundation and Queen Sophia Foundation (Madrid, Spain) [19].

Inclusion criteria for the Vallecas Project included community-dwelling individuals from 70 to 85 years of age without dementia or any other mental disorder impeding daily functioning at the beginning of the study. After participants signed proper consent forms, trained neurologists and neuropsychologists conducted structured clinical interviews in order to collect demographic, clinical and cognitive data. The complete visit was usually carried out within four hours, with convenient breaks.

Baseline and follow-up diagnosis were agreed between neurologists and neuropsychologists at consensus meetings. Every participant was independently diagnosed according to clinical criteria and taking into account age, gender, cognitive reserve, functional information, and cognitive scores. In all cases, cognitively healthy subjects were given a score of 0 in the global Clinical Dementia Rating (CDR). Criteria from NIA-AA [2] were used to diagnose core MCI.

For the purposes of this study, 608 individuals were enrolled. To be considered eligible for participating in this investigation, subjects had to have been diagnosed as cognitively intact at baseline as well as they had to have completed all questions about subjective complaints. After follow-up, 41 participants progressed to MCI.

4.4.2. Subjective Cognitive Decline assessment

Questions on cognitive concerns were coded according to the guidelines suggested by SCD-I [18]. Responses to every question were directly provided by the participants since family members were not available in all cases. Cognitive complaints were assessed twice and independently in the Vallecas Project. First, during the neurological interview participants were asked the following nine yes/no-type questions regarding specific cognitive complaints: 1) Are you easily distracted? 2) Do you get lost in familiar surroundings or have trouble finding your way when driving? 3) Do you often forget recent information or

events? 4) Do you often forget autobiographically information? 5) Do you have trouble recognizing objects or faces? 6) Do you have word-finding difficulties for people's names or common words? 7) Do you understand simple verbal and written instructions? 8) Do you have difficulty driving, managing finances or planning daily activities? 9) Do you have difficulty sequencing movements (e.g. taking the necessary steps to prepare a bath)?

Second, during the neuropsychological interview participants also completed an ordinal scale of cognitive complaints (SCD scale) composed of four items with four points each (ranged 0-3). This SCD scale addressed the following questions: a) How do you perceive your memory in comparison with that of others of your age? (with four response alternatives scoring "3-bad"; "2-somewhat worse"; "1-somewhat better"; "0-excellent"); b) How do you perceive your memory today compared with your young adulthood? ("0-better"; "1-equal"; "2-somewhat worse"; "3-much worse"); c) Do you perceive your memory today is worse than compared with ten years ago? ("0-no"; "1-a little worse"; "2-somewhat worse"; "3-much worse"); d) Do you perceive your memory today is worse than compared with one year ago? ("0-no"; "1-a little worse"; "2-somewhat worse"; "3-much worse"). The sum of these items resulted in a total score of cognitive concerns ranging from 0 to 12 (lower scores meaning fewer complaints). Information concerning age at and years of SCD onset, as well as worries associated with SCD, were also collected in the same neuropsychological interview.

Based on information obtained in both clinical interviews, SCD was operationally defined as the self-rated presence of cognitive deterioration using two criteria: i) a positive response to any yes/no-type complaint question, and ii) scores above 1 on the SCD scale. Therefore, according to both criteria, the sample was classified in three different SCD groups: i) No Complaints group (NCg); ii) Subjective Cognitive Decline group (SCDg), involving some self-reported cognitive complaint; and iii) Subjective Cognitive Decline Plus group (SCD-Pg), when memory and any other cognitive complaint was expressed and all the rest of the main criteria proposed by the SCD-I were fulfilled [18].

4.4.3. Objective Cognitive assessment

A comprehensive neuropsychological battery was administered to assess all relevant cognitive domains. Overall, six tests were used at baseline: Mini Mental State Examination

(MMSE) [20]; Rey-Osterrieth Complex Figure (ROCF) [21,22]; Free and Cued Selective Reminding Test (FCSRT) [23–25]; Lexical and Semantic Verbal Fluency [26]; Clock Drawing Test [27]; and Digit Symbol Coding [28]. Moreover, Functional Activities Questionnaire (FAQ) [29] and Clinical Dementia Rating (CDR) [30] complemented the neuropsychological battery.

For statistical purposes, individual indexes of ROCF (i.e. time and score of copy, and immediate and delayed recall) and FCSRT (i.e. total performance within each trial; free and total immediate recall; and free and total delayed recall) were separately assessed.

4.4.4. Neuropsychiatric assessment

The neurological protocol included three questions about self-reported depression (Do you feel sad, lonely and depressed most of the time?), anxiety (Do you feel worried and anxious most of the time?), and apathy (Do you feel a lack of emotion, motivation or interest in hobbies and previously activities enjoyed?). These three symptoms were collected as dichotomous questions (coded yes/no). In addition, Geriatric Depression Scale (GDS) [31] and State-Trait Anxiety Inventory (STAI) [32] were also administered as part of the neuropsychological battery.

4.4.5. Apolipoprotein E ε4 genotyping

APOE gene polymorphism status was studied with total DNA isolated from peripheral blood following standard procedures. Genotyping of APOE polymorphisms (rs429358 and rs7412) was performed by Real-Time PCR [33]. APOE was coded 1 for the APOE ε4 carriers, and 0 for non-carriers.

4.4.6. Statistical analyses

We performed a preliminary analysis of demographic, cognitive and clinical variables at baseline to find out their distribution and possible associations with SCD. Diagnoses at baseline and follow-up, as well as conversion rate to MCI, were relevant variables to study the relationship between SCD groups and cognitive status.

Associations between categorical variables were analyzed with the Pearson´s $\chi 2$ and Fisher´s test when appropriate. In addition, due to differences of sample size groups and

that most variables were not adjusted to the parametric assumptions, analysis of variance was based on non-parametric Kruskal-Wallis tests. SCD groups were treated as an independent variable with three levels (NCg, SCDg, and SCD-Pg) whereas demographic, cognitive and neuropsychiatric data at baseline were used as dependent variables. Although all neuropsychological variables were analyzed, special emphasis in memory tests was made. A multivariate-adjusted Cox proportional hazard regression models was used to study the relationship between SCD groups at baseline and conversion rate to MCI. Time to event was calculated as date of entry into the study to date of MCI diagnosis. Overall, according to the previous analyses, several covariates were adjusted for studying the impact of SCD upon progression to MCI in three consecutive models: a) Model 1: age, gender and years of education; b) Model 2: Model 1 covariates plus depression, anxiety and apathy; c) Model 3: Model 2 covariates plus FCSRT free immediate recall, FCSRT free delayed recall and APOE. A final model was calculated by retaining the covariates that met the following two conditions: first, they were found significant in any of the three previous models, and second, the minimum number of them had to explain the most part of conversion rate´s variance. These covariates proved to be gender, FCSRT free immediate recall and APOE. All the results were presented as hazard ratio (HR) with a 95% confidence interval (CI).

We used 2-sided significance tests for all analyses, with statistical significance set at p-value < 0.05. The proportional hazards assumption was assessed for all variables using the Schoenfeld residuals graphs. Neither violation of assumptions in individual variables nor in the global model was found. All statistical analyses were performed using R version 2.14.2 [34].

4.5. Results

Participants were followed up for a mean of 13.1 months (SD 1.3, median 12.9, range 10.7-22.4). During this time, 41 volunteers developed MCI (6.7% of the total sample). Regarding cognitive concerns, 69.6% of participants reported some type of complaint in any of the nine yes/no type questions. As expected, SCD scale showed significant differences between groups, especially when compared NCg and SCD-Pg. In addition, the correlation between the baseline score of the SCD scale and the follow-up SCD score showed a positive

correlation (ρ=0.66; p<0.001). This outcome was taken as indicative of an appropriate stability of cognitive concerns.

***Table 4.1**. Baseline characteristics of the study sample by Subjective Cognitive Decline groups.*

	Total Sample (n=608)		NCg (n=185)		SCDg (n=370)		SCD-Pg (n=53)		p-value	Post-hoc
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Age (years)	74.14	3.83	74.23	3.96	74.21	3.83	73.32	3.36	0.344	
Education (years)	11.03	6.66	11.00	6.72	11.18	6.73	10.10	5.94	0.530	
Gender	62% Female		64% Female		59% Female		75% Female		0.079	
APOE ε4 (%)	17.7		16.5		16.7		29.4		0.074	
FAQ	0.42	0.80	0.22	0.54	0.48	0.86	0.68	0.94	< 0.001	b
SCD										
SCD score	5.07	2.00	3.92	1.78	5.46	1.82	6.46	2.00	< 0.001	a,b,c
Years of onset	7.30	6.13	6.58	6.86	8.10	6.06	3.28	1.35	< 0.001	a,b,c
Age at onset	66.88	7.13	67.90	7.71	66.03	7.15	70.04	3.73	< 0.001	c
Neuropsychiatric symptoms										
GDS	1.63	2.22	1.05	1.69	1.75	2.29	2.89	2.75	< 0.001	a,b,c
STAI state	14.33	8.73	12.39	8.51	14.65	8.40	18.75	9.89	< 0.001	a,b
STAI trait	17.20	9.77	14.16	8.20	17.70	9.97	24.17	9.30	< 0.001	a,b,c
Depression (%)	21.2		7.0		26.2		35.8		< 0.001	a,b
Anxiety (%)	15.5		4.3		19.2		28.3		< 0.001	a,b
Apathy (%)	10.0		1.6		11.9		26.4		< 0.001	a,b,c
Cognitive performance										
MMSE	28.61	1.49	28.60	1.63	28.61	1.43	28.57	1.45	0.734	
Clock Drawing Test	9.33	1.12	9.43	0.99	9.31	1.16	9.12	1.27	0.326	
ROCF time of copy	249.09	117.81	247.46	114.83	250.31	120.83	246.19	108.38	0.986	
ROCF copy	30.05	6.67	29.60	7.55	30.24	6.32	30.32	5.78	0.929	
ROCF immediate recall	12.68	6.17	12.82	6.33	12.81	6.14	11.25	5.80	0.186	
ROCF delayed recall	12.51	6.26	12.66	6.35	12.62	6.28	11.25	5.79	0.305	
FCSRT trial 1	12.57	2.65	12.97	2.61	12.52	2.59	11.55	2.98	0.001	b
FCSRT trial 2	13.81	2.16	14.09	1.98	13.81	2.08	12.85	2.90	0.003	b
FCSRT trial 3	14.64	1.70	14.75	1.55	14.69	1.58	13.87	2.57	0.008	
FCSRT free immediate	23.62	6.30	24.69	6.68	23.43	6.05	21.12	5.92	0.002	b
FCSRT total immediate	41.09	5.55	41.81	5.41	41.03	5.52	39.00	5.74	0.002	b
FCSRT free delayed	9.48	2.59	9.93	2.54	9.45	2.54	8.10	2.64	< 0.001	b,c
FCSRT total delayed	14.33	1.82	14.46	1.78	14.35	1.81	13.75	1.90	0.018	b
Phonemic Verbal Fluency	36.32	11.60	36.66	12.31	36.11	11.39	36.53	10.73	0.806	
Semantic Verbal Fluency	18.59	4.75	50.85	10.30	48.59	9.17	48.08	9.15	0.079	
Digit Symbol Coding	19.20	7.53	19.93	8.42	18.82	7.17	19.23	6.60	0.413	
CDR sum of boxes	0.11	0.21	0.08	0.19	0.10	0.20	0.23	0.27	< 0.001	b
Annual conversion rate to MCI (%)	6.7		4.9		5.6		18.9		0.001	b,c

APOE: Apolipoprotein E; CDR: Clinical Dementia Rating; FAQ: Functional Activities Questionnaire; FCSRT: Free and Cued Selective Reminding Test; GDS: Geriatric Depression Scale; MCI: Mild Cognitive Impairment; MMSE: Mini Mental State Examination; NCg: No Complaints group; ROCF: Rey-Osterrieth Complex Figure; SCD: Subjective Cognitive Decline; SCDg: Subjective Cognitive Decline group; SCD-Pg: Subjective Cognitive Decline Plus group; SD: Standard Deviation; STAI: State-Trait Anxiety Inventory.

Post-hoc analyses: a = NCg vs SCDg; b = NCg vs SCD-Pg; c = SCDg vs SCD-Pg.

Table 4.1. summarizes the descriptive statistics of the total sample and the three SCD groups at baseline. No differences in age or years of education among groups were found. Nevertheless, there was a trend for significance concerning gender and APOE status, being female and ε4 carrier more frequent for SCD-Pg than for other groups. SCDg and SCD-Pg reported more depression, anxiety, and apathy compared to NCg. Furthermore, GDS and STAI scores were also significantly lower in NCg. Differences in apathy, GDS, and STAI trait were also found between SCDg and SCD-Pg. Overall, these results highlighted that neuropsychiatric symptoms were more frequent in both SCD groups compared to NCg; and these symptoms were especially marked for SCD-Pg. Regarding cognitive assessment, statistical differences between groups were only found for instrumental activities of everyday (FAQ), verbal episodic memory (FCSRT), and clinical rating (CDR sum of boxes). Interestingly, the SCD-Pg showed the worst cognitive performance, while neuropsychological tests did not significantly differ between NCg and SCDg. Finally, conversion rate to MCI was significant especially high for SCD-Pg (18.9%) compared to NCg (4.9%) and SCDg (5.6%).

A specific analysis between SCD-Pg converters (n=10; 19%) and SDC-Pg non-converters (n=43; 81%) was developed with the aim of finding out what variables could directly influence upon the diagnosis of MCI. In this case, due to the small sample size, a robust bootstraping procedure was carried out along with the standard non-parametric analysis (Table 4.2.). Significant differences were found in SCD scale, where converters reported more concerns than non-converters. Surprisingly, no differences were found in neuropsychiatric symptoms, neither depression nor anxiety questionnaires. Among cognitive variables, several indexes of FCSRT (trial 2, and free immediate and delayed recall) differed between groups. Figure 4.1. shows the magnitude of the differences between non-converters and converters through three types of variables: subjective cognitive complaints, objective cognitive performance and psychiatric variables. All these results suggested that subjective and objective cognitive variables, but not psychiatric ones, were actually involved in conversion from SCD-Pg to MCI.

***Table 4.2**. Baseline comparisons between SCD-Pg non-converters and SCD-Pg converters.*

	Non Converters (n=43)		Converters (n=10)		p-value
	Mean	SD	Mean	SD	
Age (years)	73.05	3.27	74.50	3.66	0.230
Education (years)	9.89	5.76	11.00	6.96	0.608
Gender	77% Female		70% Female		0.692
APOE ε4 (%)	24.39		50.00		0.173
FAQ	0.60	0.95	1.00	0.82	0.088
SCD					
SCD score	6.19	1.68	8.00	3.06	0.033
Years of onset	3.23	1.34	3.50	1.43	0.568
Age at onset	69.81	3.65	71.00	4.11	0.356
Neuropsychiatric symptoms					
GDS	2.65	2.68	3.90	2.96	0.146
STAI state	18.09	9.56	21.60	11.29	0.466
STAI trait	23.53	9.52	26.90	8.20	0.339
Depression (%)	32.56		50.00		0.465
Anxiety (%)	23.26		50.00		0.123
Apathy (%)	25.58		30.00		0.999
Cognitive performance					
MMSE	28.65	1.41	28.20	1.62	0.443
Clock Drawing Test	9.28	1.11	8.45	1.71	0.152
ROCF time of copy	257.63	108.30	197.00	99.11	0.053
ROCF copy	30.35	5.63	30.20	6.70	0.732
ROCF immediate recall	11.43	5.70	10.50	6.44	0.439
ROCF delayed recall	11.69	5.80	9.40	5.66	0.165
FCSRT trial 1	11.84	2.57	10.30	4.27	0.448
FCSRT trial 2	13.42	2.00	10.40	4.65	0.038
FCSRT trial 3	14.28	1.65	12.10	4.61	0.068
FCSRT free immediate	21.95	5.70	17.11	5.56	0.040
FCSRT total immediate	39.53	5.42	36.44	6.84	0.303
FCSRT free delayed	8.42	2.63	6.56	2.19	0.045
FCSRT total delayed	13.91	1.97	13.00	1.32	0.080
Phonemic Verbal Fluency	36.05	10.81	38.60	10.68	0.517
Semantic Verbal Fluency	49.16	9.02	43.40	8.59	0.106
Digit Symbol Coding	19.21	6.35	19.30	7.97	0.847
CDR sum of boxes	0.198	0.27	0.35	0.24	0.080

APOE: Apolipoprotein E; CDR: Clinical Dementia Rating; FAQ: Functional Activities Questionnaire; FCSRT: Free and Cued Selective Reminding Test; GDS: Geriatric Depression Scale; MMSE: Mini Mental State Examination; ROCF: Rey-Osterrieth Complex Figure; SCD: Subjective Cognitive Decline; SD: Standard Deviation; STAI: State-Trait Anxiety Inventory.

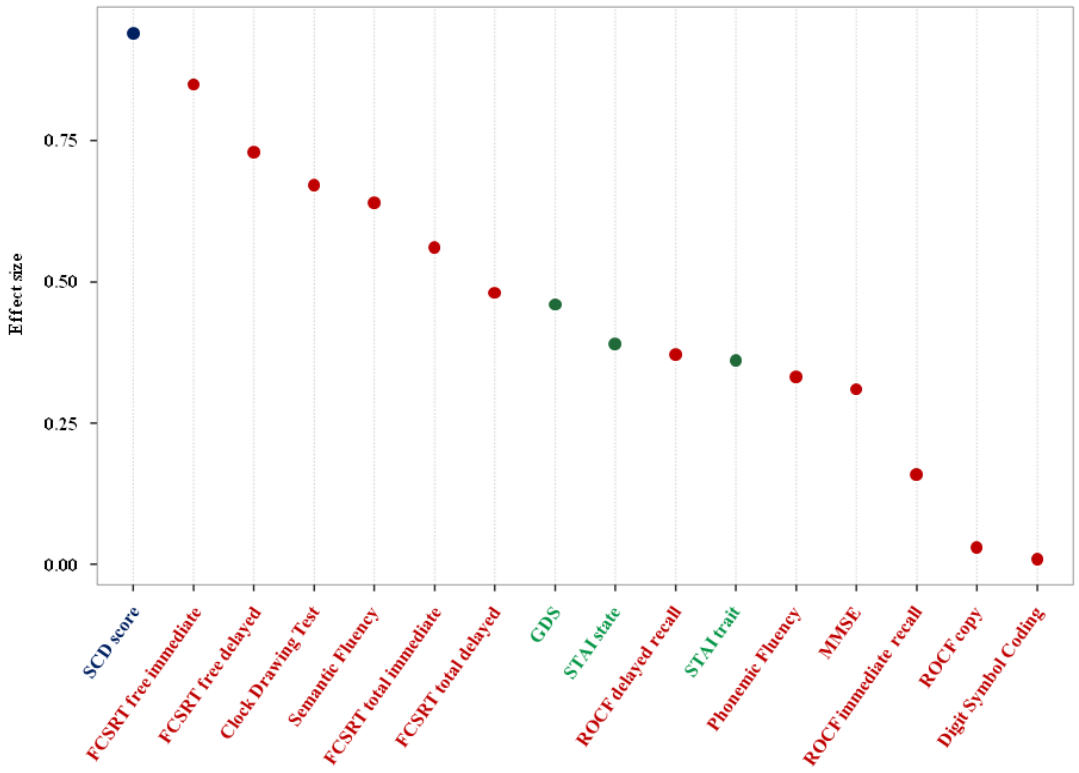

***Figure 4.1**. Effect size of individual variables at baseline on conversion from SCD Plus to MCI.*

This graphic represents the effect size of the individual variables at baseline on the profiles of SCD-Pg non-converters and SCD-Pg converters. Variables are clustered in three categories: i) subjective cognitive complaints (blue), ii) objective cognitive performance (red), and iii) psychiatric symptoms (green). As shown, SCD score had the largest effect size (Cohen´s d=0.94) among groups followed by cognitive performance variables, especially free recall of verbal information. Magnitude of depression and anxiety scores resulted less important to discriminate SCD-P subjects at risk of conversion to MCI.
FCSRT: Free and Cued Selective Reminding Test; GDS: Geriatric Depression Scale; MMSE: Mini Mental State Examination; ROCF: Rey-Osterrieth Complex Figure; SCD: Subjective Cognitive Decline; STAI: State-Trait Anxiety Inventory.

In unadjusted Cox proportional hazard regression models, SCDg had about a 32% increased risk of progression to MCI compared to NCg, whereas the increased risk for SCD-Pg was about 360%. Kaplan-Meier plots showed clear differences in risk by SCD-Pg after 14 months of follow-up (Figure 4.2.). On the other hand, adjusted Cox regression models were also conducted to control the influence of sociodemographic, neuropsychiatric, neuropsychological and genetic variables upon SCD and its association with conversion to MCI. As shown in Table 4.3., after adjustment for all fitted models SCDg did not display differences compared to NCg. However, SCD-Pg showed a significant high risk of MCI, especially in Model 1 when control was only made for age, gender and education (HR 5.44, 95% CI = 2.16-13.75). Additional adjustment for depression, anxiety and apathy (Model 2) yielded similar results for SCD-Pg. Finally, additional adjustment for both free immediate

and delayed memory, as well as APOE genotyping (Model 3), only marginally decreased the HR values for SCD-Pg. Hence, as expected, memory performance (HR 0.83; 95% CI 0.76–0.91; p-value < 0.001) was the most significant predictor of progression to MCI, in such a manner that the lower the memory score, the higher the risk to convert to MCI.

In order to confirm these outcomes, we conducted a more parsimonious final model controlling for gender, FCSRT free immediate recall and APOE as covariates. The reason for considering these three covariates and no others was because only those ones proved to be significant in their respective models. As a result, in our final model, the multivariate adjusted HRs in participants who reported SCD-P were 4.17 (95% CI: 1.52–11.43) compared to NCg, while SCDg did not differ from NCg. The value of the determination coefficient was 0.13, almost the same as Model 3 with 9 covariates. This small value could indicate that other variables apart from SCD account for the rate of conversion to MCI.

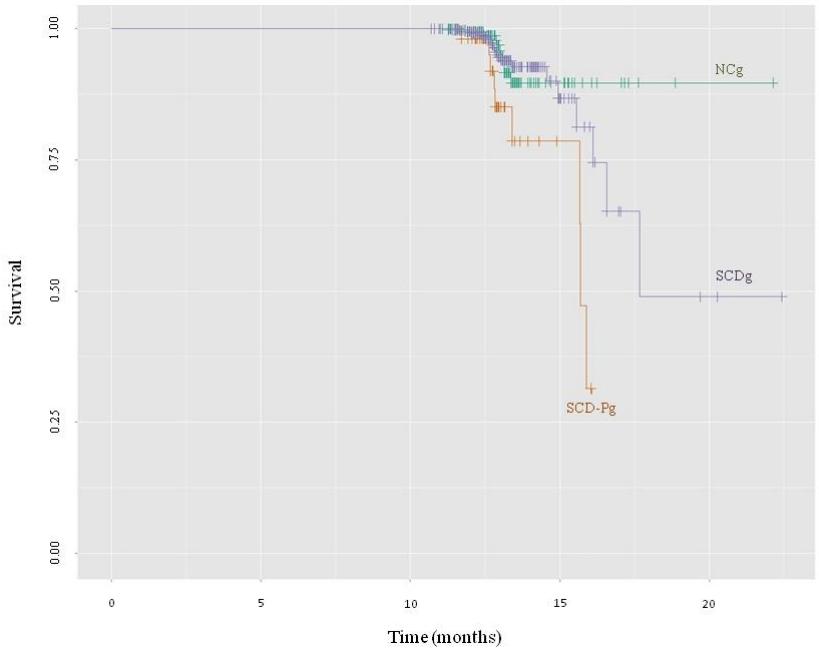

***Figure 4.2**. Kaplan-Meier survival curves for unadjusted rates of MCI by SCD groups. SCD-Pg shows the highest risk of conversion to MCI.*

***Table 4.3**. Cox proportional hazard regression models of conversion rate to MCI with additional adjustment for potential mediators.*

	HR (95% CI)		
Adjustment Model	NCg	SCDg	SCD-Pg
Model 1	1 [Reference]	1.30 (0.60-2.82)	5.44 (2.16-13.75)***
Model 2	1 [Reference]	0.10 (0.49-2.48)	4.40 (1.65-11.72)**
Model 3	1 [Reference]	0.89 (0.35-2.22)	2.92 (0.96-8.86)
Final Model	1 [Reference]	1.17 (0.52-2.65)	4.17 (1.52-11.43)**

Model 1: adjusted for age, gender and education (years). R^2=0.04
Model 2: adjusted for Model 1 covariates and depression, anxiety and apathy. R^2=0.05
Model 3: adjusted for Model 2 covariates and FCSRT free immediate, FCSRT free delayed and *APOE*. R^2=0.14
Final Model: adjusted for sex, FCSRT free immediate and *APOE*. R^2=0.13

CI: Confidence Interval; FCSRT: Free and Cued Selective Reminding Test; HR: Hazard Ratio; NCg: Non Complaint group; SCDg: Subjective Cognitive Decline group; SCD-Pg: Subjective Cognitive Decline Plus group.
** p<0.01; *** p<0.001

4.6. Discussion

The role of the clinical significance of SCD in the transition from a cognitively intact stage to MCI has been analyzed in a cohort that was followed-up approximately for 13 months. According to the guidelines proposed by the SCD-I [18], subjects were classified depending on their cognitive concerns. Thus, 69.6% of the sample reported some type of complaint, what is slightly higher than the common prevalence of SCD in the general population [35], but consistent with this age group [36,37]. Finally, based on self-reported complaints, three different groups of SCD were established (NCg, SCDg, and SCD-Pg).

Although no statistical differences were found with regard to gender and APOE, a trend for significance was obtained between groups. Indeed, female gender and ε4 carrier were more associated with SCD-Pg than the two other groups. Since both features have been proved to increase the risk of AD [38], cognitive complaints could be somehow related to them. This relationship might occur at preclinical stages, before anosognosia and objective impairment appear. Nevertheless, to isolate the role of SCD as early sign of progression to MCI, gender and APOE were controlled in further analysis.

Potential differences between groups were analyzed using neuropsychiatric and cognitive variables. Symptoms of depression, anxiety and apathy were strongly associated with SCD, and they were able to identify the NCg from the rest of SCD groups. This finding has been highlighted in a large number of studies [39–41], suggesting that complaints correlate more

closely to depression and anxiety than cognitive performance; thus, they could play a mediating role between mood and cognitive status [42]. However, our results did not support the fact that the combination of SCD and specific psychiatric symptoms increased the risk of conversion to MCI. Indeed, according to the Cox regression Model 2, psychiatric covariates neither resulted significant nor provided additional information to SCD. Perhaps, the explanation of these outcomes has to do with the fact that SCD would begin to decline the cognitive status some years before that of psychiatric symptoms. This explanation is somehow consistent with the literature since while depression and anxiety are mainly suggested to be risk factors for dementia in prodromal AD [43–45], SCD is proposed to be an early sign of MCI in preclinical stages [46,47]. Otherwise, although cognitive measures were related to SCD in a lesser degree than neuropsychiatric variables as expected [36,48,49], low memory performance at baseline allowed to better differentiate SCD-Pg. In addition, and most importantly, controlling for depression, anxiety and apathy, episodic memory performance was associated with a faster rate of MCI conversion in individuals with SCD Plus.

In line with previous studies that have found an increased risk of AD in healthy subjects who reported SCD seven years earlier [12–14], our annual conversion rate to MCI for subjects with SCD Plus at baseline was almost 20%. This outcome was significant and differed from SCDg, which displayed a conversion rate of 5.6%. Indeed, while the HR obtained for SCDg were similar to other studies [50,51], and it did not significantly differ from NCg, individuals who reported SCD Plus had a higher risk of developing MCI (HR=4.17) compared to those subjects without complaints. In addition, the inclusion of gender, memory performance and APOE genotyping in the survival analyses did not decrease the predictive power of the SCD-Pg.

Taking into account that the follow-up period covered less than two years, these results indicate that some particular features associated with cognitive complaints (i.e. onset of complaints within the last 5 years, age at onset over 60 years, worries associated with complaints, and feeling of worse performance than other people from the same age group) may identify those individuals at high risk of fast conversion to MCI. These findings have paramount implications for clinical settings. For instance, cognitive training programs should be implemented in subjects meeting all features of SCD Plus proposed by SCD-I [18].

Regarding the weaknesses of the present study, it is recognized that the short follow-up could be seen as an important limitation. Nevertheless, we consider that the elapsed time is enough to examine the main objective of this paper, that is, to seek for the minimum time required for conversion to MCI in healthy subjects who report SCD. Our outcomes suggest the existence of different rates of conversion to MCI that depend on the features of SCD. Thus, SCD-Pg seems to exhibit a larger and faster risk of MCI. Given that the Vallecas Project is still in progress, the association between SCD and MCI shall be analyzed in next visits to explore deeply our present results. In addition, since the SCD data were collected in a volunteer sample of elderly people, generalization of results should be treated with caution.

Although biomarkers have a clear value for diagnosis of early AD, we should not look down on the complementarity that behavioral markers hold for the disorder. Age-dependent neuropsychological and cognitive assessment has provided evidence that a decline in speed of information processing, executive function (working memory, task switching, inhibitory function) and reasoning goes along with normal aging. Longitudinal studies assessing cognitive function prior to dementia have also steadily shown a gradual cognitive decline in episodic memory as well as non-memory domains up to a decade before dementia onset. Interestingly, the preclinical path suggests a long and slow rate of presymptomatic changes as well as a period of acceleration of performance decline that may begin several years before MCI onset. However, current neuropsychological tests do not seem to be sensitive enough to discriminate SCD converters and non-converters which lead to the necessity of developing more sensitive tools for these preclinical stages of the disease.

Self-report of subtle cognitive decline, even in the absence of significant objective impairment on testing, may predict future decline in older individuals. Subjects who report SCD Plus might need special attention in terms of an early cognitive or pharmacological intervention. Thus, combining biomarkers with measures sensitive to detect very subtle cognitive decline in longitudinal studies of older individuals could be extremely useful in coming years. For instance, it will be needed to prove whether individuals at preclinical stage with subjective complaints and positive biomarkers have a major risk of AD progression. If so, since at this preclinical phase the brain is supposed to be recoverable, the effectiveness of new clinical trials with AD modifying therapies should be examined.

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4.7. Summary of conclusions

1. Self-report of cognitive decline may predict future cognitive decline in older individuals.

2. Mainly five specific features associated with SCD (SCDplus features) may help to identify individuals with a high risk of conversion to MCI.

3. These individuals need frequent clinical monitoring.

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5. OPINION PAPER

Publication title:

SUBJECTIVE COGNITIVE DECLINE AS A PRECLINICAL MARKER FOR ALZHEIMER´S DISEASE: THE CHALLENGE OF STABILITY OVER TIME

Journal:

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5.1. Comment

Subjective Cognitive Decline (SCD) during preclinical phase of Alzheimer´s Disease (AD) refers to a self-experienced persistent decline in cognitive abilities in comparison with a previously normal status and independently of the objective performance on neuropsychological tests (Jessen et al., 2014). This is an important construct because is supposed to be a sign of the preclinical AD stage (Figure 5.1.). During the past decades, researchers have analyzed the clinical value of SCD as a predictor of the onset of future AD in older adults (Rabin et al., 2017). Thus, a recent meta-analysis has shown that, independently of the objective memory performance, almost 25% of older adults who report SCD will progress to prodromal AD four years later. In addition, the rate of progression to dementia among those individuals who report SCD is two-fold during a 5-year follow-up period (Mitchell et al., 2014).

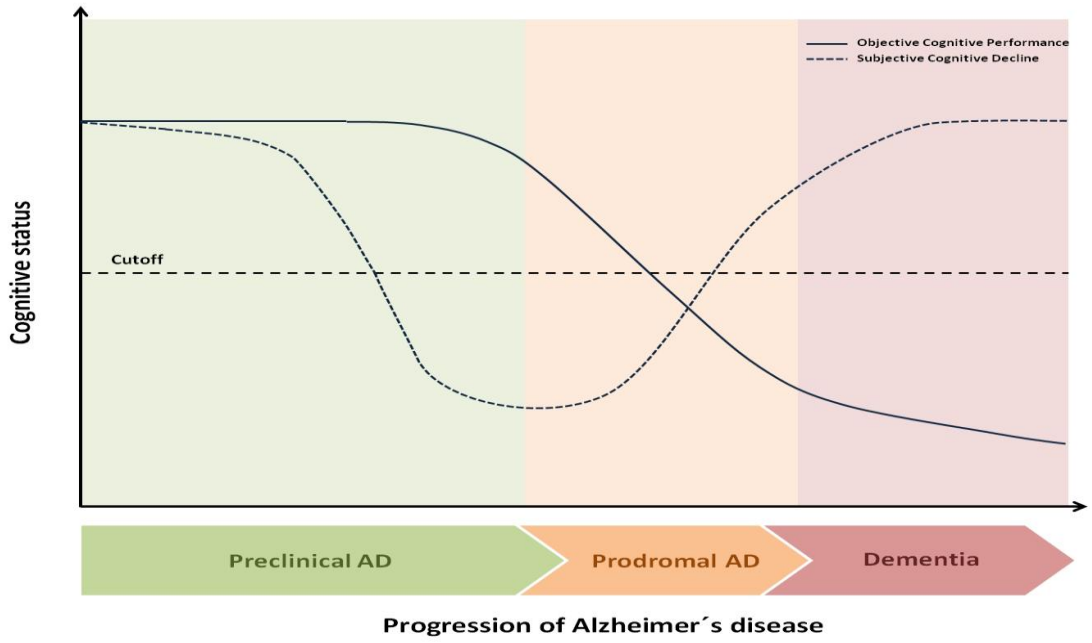

Figure 5.1*. Theoretical temporal dynamic of objective and subjective cognitive decline in AD continuum.*

This graphic represents the hypothetical differences in AD progression over time between Subjective Cognitive Decline (SCD) and Objective Cognitive Performance (OCP). At final stage of preclinical AD, SCD seems to be a better marker than OCP. As disease progresses, cognitive performance tend to decrease and at prodromal stage both SCD and OCP are below cutoff. However, at last phase of prodromal AD SCD usually disappear leading to a deficit of self-awareness about the own disabilities namely anosognosia.

To increase the potential usefulness of SCD the international working group called Subjective Cognitive Decline Initiative (SCD-I) agreed to a common framework and research procedures to study the role of SCD as a preclinical marker of preclinical AD (Jessen et al., 2014). As a result of these indications, cognitively healthy individuals who accomplish certain conditions of SCD have been proved to have a four times higher risk for progressing to prodromal AD in just one-year compared to those subjects without complaints (Fernández-Blázquez et al., 2016). Despite its outstanding clinical value, recently the SCD-I also pointed out some limitations of SCD when it comes to investigate this concept (Molinuevo et al., 2017; Rabin et al., 2015). These limitations could be summarized in three different blocks:

1. Terminology has not been homogeneous across studies and terms such as "subjective memory complaints", "subjective cognitive complaints", "subjective cognitive decline" or "subjective memory impairment" have been used interchangeably to refer to the same underlying concept. This lack of consensus on a single definition of SCD might affect to the comparison of findings from different investigations.

2. Methodology and tools to assess SCD are also heterogeneous what involves the context in which the sample is recruited (clinical versus community-based), the mode of administration of measures (structured interview conducted by a examiner versus self-reported questionnaires), the cognitive domains that must be examined (memory versus non-memory domains), the number of items to be used (one or two questions versus scales with a large number of items), the way to respond the questions (opened questions versus multiple choice), and the timeframe to collect data (shorter versus longer periods of time). This heterogeneity may lead to contradictory results of the studies.

3. Operational criteria and cutoffs to consider who is truly reporting SCD have been totally different across studies.

This heterogeneity in definitions, in approaches for measuring SCD, and in operational criteria emphasizes the necessity of searching for shared terminology and common frameworks of evaluation. To settle these limitations the SCD-I also proposed some

recommendations (Molinuevo et al., 2017). As a first step, it is important to select the most appropriate measures that should be related to the characteristics of the target population. Cognitive complaints may have different implications depending on the research context where they are gathered. For instance, the concerns on SCD in clinical samples may be higher than in community-dwelling individuals. Moreover, it would be suitable to rely on tools with adequate psychometric properties for the reference population. As a second step, the SCD´s measures must have appropriate content coverage regarding to the target population. In this way, all items should be well-written and understandable, avoiding double meanings and inquiring for difficulties often found in daily life. In a third step, measures should explore different cognitive and non-cognitive domains because the earliest symptoms of AD may affect beyond memory. In the fourth step, the response options for all measures should be selected depending on the aim of our study. When the purpose is to distinguish between groups, dichotomous items may be enough. However, if we are interested in monitoring the change of SCD over time, ordinal response options should be preferred. Finally, another critical point is to determine the reference period of time in which we want to examine the SCD. Generally, inquiring over short periods of time (no longer than one year) allow us to reduce problems with retrospectively recall or estimation of SCD. Nevertheless, we can of course ask for longer periods if we want to study the progression of SCD through the lifetime.

However, as far as we know, there is another crucial limitation with regard SCD that has not been properly considered yet. This issue is related to the concept of the temporal stability of complaints over time. In psychometrical terms, when we are measuring subjective variables like SCD we are actually obtaining two different types of information: i) the construct of interest (i.e. SCD in our case); and ii) errors of measurement which comprised the error variance and include information regarding other irrelevant constructs (e.g. depressive symptoms associated with a particular complaint, personality traits, etc.) as well as short-term fluctuations due to shifts in self-perception itself. Thus, when repeated subjective measurements are collected from an individual the scores on two different occasions may be quite different (Nesselroade and Salthouse, 2004). If this occurs along the majority of subjects, then it means that the subjective variable does not have enough internal consistency. In other words, if two longitudinal measures are quite different and they do not converge, which one is the real to characterize the individual? This lack of

temporal stability, which can affect preferably to subjective measures rather than objective performance, represents an important bias to investigation. Thus, if a construct do not probe to be stable enough over time it should not be considered as a target for research. Only demonstrating that SCD is a robust and stable concept it could become a reliable preclinical marker for AD.

To prove the stability of SCD, it is necessary to harmonize a protocol to gather all relevant information about cognitive complaints and to compare longitudinally the responses of the individuals. Specifically, we suggest collecting information by means of different approaches to ensure the greatest possible internal consistency. An interesting position would be to gather self-perceived data using two procedures: a face-to-face interview with a healthcare professional and a self-administered questionnaire of cognitive complaints. Likewise, it should be desirable combining both open-ended questions and structured scales in order to measure different features of SCD. The use of questionnaires is highly recommended to quantify SCD somehow and to monitor the progression of cognitive complaints over time. Additionally, a multiple choice approach should vary from dichotomic to ordinal Likert-type scales to grasp the dimensionality of SCD in the best way possible.

Regarding the content of SCD to be collected, it should be measured clinical details of the self-experienced cognitive decline. Variables such as age at onset, time of progression, memory performance compared to other people, concerns associated with SCD, and frequency of particular cognitive complaints are relevant data that must be carefully obtained. Moreover, SCD should not be examined in isolation to examine the effect of complaints upon AD. Demographic variables such as age, gender, and education, as well as medical and lifestyle variables can be gathered very easily by means of a survey. These variables have the greatest interest due to their possible implication in the expression of SCD. Additionally, objective cognitive performance and diagnosis are critical to establish the current stage of an individual in the continuum of AD and the relationship between SCD and risk of developing MCI and AD. Finally, neuropsychiatric variables should be collected as well because of their mediator role between SCD and cognitive decline.

In conclusion, self-report of subtle cognitive decline has been proposed to appear at the end of the preclinical phase of AD even in the absence of significant objective impairment detectable on standardized neuropsychological assessment (Rabin et al., 2017). This fact

explains why SCD is gaining an increasing prominence in neurodegenerative research as a potential marker for future MCI due to AD. In any event, this construct must deal with some limitations that have been already pointed out by the SCD-I (Molinuevo et al., 2017). However, in our opinion SCD has to face up to another challenge not brought to the table so far that is related to the temporal stability of complaints over time. If SCD does not probe to have enough internal consistency, then this construct cannot be considered as a reliable preclinical marker for AD.

Authors contributions

MAV and MAFB are responsible for the conceptualization, reviewing the literature, and critically editing the manuscript. Both authors approve the submitted version of the manuscript and are accountable for the accuracy and integrity of the work.

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5.2. References

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Rabin, L. A., Smart, C. M., Crane, P. K., Amariglio, R. E., Berman, L. M., Boada, M., et al. (2015). Subjective Cognitive Decline in Older Adults: An Overview of Self-Report Measures used Across 19 International Research Studies. J. Alzheimers. Dis. 48 Suppl 1, S63-86. doi:10.3233/JAD-150154.

6. EXPERIMENT 3

Publication title:

INTERNAL CONSISTENCY OVER TIME OF SUBJECTIVE COGNITIVE DECLINE: DRAWING PRECLINICAL ALZHEIMER´S TRAJECTORIES

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6.1. Summary of objectives

1. To examine the role of SCD as a possible precursor for MCI development.

2. Since, single point measurements of SCD could result in high variability, our second goal is to look for a SCD stable data.

3. To obtain robust results for this measure we have carried out a longitudinal study.

4. To evaluate the clinical significance of SCD during the development of cognitive impairment through AD continuum.

6.2. Abstract

Background: Early intervention to prevent, or delay, the transition from healthy cognition to cognitive impairment in older adults is an important goal. In this way, it is critical to find sensitive, reproducible and early markers to use low cost methods for the detection of that transition. One of those early markers for symptomatic manifestation of AD is Subjective Cognitive Decline (SCD).

Objective: To examine the internal consistency of the concept of SCD and to evaluate its clinical significance on the progression through the Alzheimer´s disease continuum.

Methods: 1,091 cognitively healthy individuals from the Vallecas Project´s cohort were followed for three years. Cognitive complaints were systematically collected and analyzed along with clinical data. All participants were classified in three groups at every visit based on specific features of their complaints.

Results: Concordance analyses showed a good agreement in longitudinal classification of Subjective Cognitive Decline. The Multi-state Markov Model highlighted a unidirectional transition from the status of no cognitive complaints to Subjective Cognitive Decline. Interestingly, a more severe condition of Subjective Cognitive Decline, namely Subjective Cognitive Decline Plus, showed the highest risk of progression to mild cognitive impairment.

Conclusions: The concept of Subjective Cognitive Decline is stable over time when it is operationally defined and consistently assessed. It provides not only a fast identification of individuals at higher risk of future mild cognitive impairment, but also it allows us to track longitudinal trajectories.

6.3. Introduction

The investigation using biomarkers has pointed out that the first physiopathological changes associated with amyloid-β deposition might be observed several decades before the clinical diagnosis of Alzheimer´s Disease (AD), even in absence of symptoms [1]. As a result, the progression of AD is currently conceived as a continuum starting 20 years before the onset of clearly noticeable symptoms [2]. The National Institute on Aging-Alzheimer Association (NIA-AA) established three different stages of AD as progression occurs over time. First, there is a preclinical phase which is defined by the incipient presence of amyloid plaques, but objective cognitive function remains normal. At the end of this preclinical phase the individual might experience some kind of Subjective Cognitive Decline (SCD) that increases the risk of future dementia [3]. A second stage called prodromal Mild Cognitive Impairment (MCI) due to AD refers to a cognitive impairment that is not severe enough to significantly affect everyday activities. Finally, there is a third phase in which cognitive impairment worsens progressively leading to a functional impairment that defines an AD dementia syndrome. Since the publication of these guidelines in 2011, data have continued confirming that changes of biomarker measures represent a continuous process. Following this model, positive biomarkers can antecede the clinical symptoms by 15-20 years, and that fact changed the assumption of three clinical defined entities to a conception of disease continuum. In practical terms, the detection of the clinical and biological AD hallmarks of people who will convert from a cognitively healthy state to a latter MCI phase is a challenge of great interest, conceptualized as a research framework to investigate AD continuum [4].

Early intervention to prevent, or delay, the transition from healthy cognition to cognitive impairment in older adults is an important goal. In this way, it is critical to find sensitive, reproducible and early markers to use low cost methods for the detection of that transition. One of those early markers for symptomatic manifestation of AD is SCD [5]. This condition has been proved to precede MCI due to AD and it may serve for an early intervention in the onset of AD [6]. Indeed, a recent meta-analysis has shown that almost 25% of older adults who report SCD will develop prodromal AD four years later [7]. In addition, the rate of progression to dementia among those individuals who report SCD is twofold during a 5 year following period.

SCD during preclinical phase of AD refers to a self-experienced persistent decline in cognitive abilities in comparison with a previously normal status and independently of the objective performance on neuropsychological tests. To increase the potential usefulness of SCD the international working group called Subjective Cognitive Decline Initiative (SCD-I) agreed to a common framework and research procedures to study the role of SCD as a preclinical marker of AD [8]. As a result of these indications, cognitively healthy individuals who accomplish certain conditions of SCD, namely SCD-Plus, have been proved to have a four times higher risk for progressing to prodromal AD in just one-year compared to those subjects without complaints [9]. Despite its outstanding clinical value, the identification and measurement of SCD is not an easy task because of some limitations pointed out by the SCD-I [5,6]. These limitations could be summarized in three different blocks: i) terminology has not been homogeneous across studies; ii) methodology and tools to assess SCD are also heterogeneous; and iii) operational criteria and cutoffs to consider who is truly reporting SCD have been totally different across studies. Thus, for instances, the previous limitations are probably the reason whereby the prevalence of SCD in older adults may range from 10 to 80% depending on the studies [10–13]. Additionally, another issue which is pending to be solved is the stability over time of SCD [14].

In this work, we will test the internal consistency of SCD across time. As the stability is demonstrated, SCD could become a reliable, low-cost preclinical marker for AD. We therefore hypothesize that in a series of longitudinal evaluations the self-report of cognitive impairment would be stable across time. Additionally, we will analyze the validity of the SCD as a very early marker of AD examining the dynamic transition from the preclinical to prodromal AD stages.

6.4. Materials and methods

6.4.1. Participants

The participants of this study comprised 1,091 cognitively healthy community-dwelling individuals aged 70 years and over at baseline. All of them were part of the Vallecas Project cohort, a community-based longitudinal investigation for early detection of AD [15]. The participants were volunteers that were recruited through radio and TV campaigns, leaflet distribution, and visits of the research team to social centers for the elderly. The study was

approved by the Research Ethics Committee of the Carlos III Institute of Health, Madrid, Spain. Informed written consent was obtained from all participants enrolled in this study.

The participants underwent a detailed assessment protocol annually for three years. The protocol included past medical history, neurological and neuropsychological examination, as well as biochemical and genetic blood test. The complete visit was usually carried out within four hours with convenient breaks. The neuropsychological battery included complete information about all cognitive domains that covered the whole spectrum of cognition. For the purposes of this work we only analyzed the performance in the following tests: Free and Cued Selective Reminding Test (FCSRT) [16], Digit-Symbol Coding [17] and Clinical Dementia Rating (CDR) [18]. In addition, the Geriatric Depression Scale (GDS) [19] and the State- Trait Anxiety Inventory (STAI) [20] were also administered as part of the neuropsychological battery to quickly estimate mood and anxiety symptoms.

Clinical diagnoses were always agreed between neurologists and neuropsychologists at consensus meetings; all diagnoses were always blind to previous diagnosis. MRIs were done to rule out the presence of macroscopic lesions or significant vascular damage which could interfere with cognitive performance. Then, every individual was independently diagnosed after each visit according to his/her age, gender, cognitive reserve, functional information, and cognitive scores. Nevertheless, rather than psychometrically invariable cut-offs, diagnosis were based on clinical impression. NIA-AA´s criteria [21] were applied to diagnose core MCI and mild dementia. Cognitively healthy subjects were given a score of 0 in the global CDR while MCI and mild dementia must score 0.5 and 1 respectively. All participants were diagnosed as cognitively healthy at baseline.

6.4.2. SCD assessment

SCD was assessed twice and independently within the same visit at the Vallecas Project. First, during the neurological examination participants were asked the following nine questions regarding specific cognitive domains: 1) Attention (“Are you easily distracted?”); 2) Spatial orientation (“Do you get lost in familiar surroundings?”); 3) Episodic memory (“Do you often forget recent information or events?”); 4) Autobiographical memory (“Do you often forget autobiographical information?”); 5) Visual recognition (“Do you have trouble recognizing objects or faces?”); 6) Speech (“Do you have word-finding difficulties for

people's names or common words?"); 7) Language comprehension ("Do you understand verbal and written instructions?"); 8) Executive functions ("Do you have difficulty driving, managing finances or planning daily activities?"); and 9) Praxis ("Do you have difficulty sequencing movements (e.g. taking the necessary steps to prepare a bath)?"). The previous questions were coded in a dichotomic way (yes/no).

Second, during the neuropsychological assessment individuals also completed an ordinal scale of memory complaints composed of four items with four points each (ranged 0-3): 1) "How do you perceive your memory in comparison with that of others of your age?" ("3-bad"; "2-somewhat worse"; "1-somewhat better"; "0-excellent"); 2) "How do you perceive your memory today compared with your young adulthood?" ("0-better"; "1-equal"; "2-somewhat worse"; "3-much worse"); 3) "Do you perceive your memory today is worse than compared with ten years ago?" ("0-no"; "1-a little worse"; "2-somewhat worse"; "3-much worse"); and 4) "Do you perceive your memory today is worse than compared with one year ago?" ("0-no"; "1-a little worse"; "2-somewhat worse"; "3-much worse"). The sum of these items resulted in a total score of cognitive concerns (SCD scale) which ranged from 0 (no complaints at all) to 12 (maximum complaints). Furthermore, five more open-ended questions were also collected: 5) Age at onset of cognitive complaints ("How old were you when your cognitive performance began to decline?"); 6) Years of SCD´s progression ("How long do you believe you are experiencing cognitive complaints?"); 7) Worries associated with self-perceived complaints ("Are you worried about your cognitive decline?"); 8) Type of onset of cognitive complaints ("How did you perceive the beginning of the cognitive decline? (e.g. suddenly, progressive, etc.)"); and 9) Self-experienced functional impairment associated with SCD ("Do you believe your cognitive failures are impeding your daily life activities?").

6.4.3. Classification of individuals in SCD groups

We followed the guidelines proposed by the SCD-I [8] to examine the implication of specific SCD features as early signs of AD. According to these guidelines, individuals were grouped in three different categories pursuant to the extent of SCD reported in both clinical interviews: i) No complaints group (NCg); ii) Subjective Cognitive Decline group (SCDg); and iii) Subjective Cognitive Decline Plus (SCD-Pg). We decided to adopt a conservative criterion in order to ensure the most stable categorization possible of SCD groups. First, our primary

measure for SCD was the SCD scale because it was composed of items that delve into the self-perception of complaints over time compared to others and oneself. Therefore, scores 0-1 on the SCD scale was considered as non-indicative of SCD while scores ranging 4-12 were conceived as a strong signal of SCD. Intermediate 2-3 scores were thought to be at borderline and in those cases the information from the 9 yes/no-type questions was taken into account as a secondary measure to classify the participants. Only if the response was affirmative in at least one of those questions the individual was considered as SCDg. Otherwise, the individual was included in NCg. Specifically, the three SCD groups were operationally defined as follows: i) NCg: scores 0-1 on the SCD scale administered in the neuropsychological assessment OR scores 2-3 on the SCD scale, but none positive response to any of the 9 yes/no-type question from the neurological interview; ii) SCDg: scores 4-12 on the SCD scale administered in the neuropsychological assessment OR scores 2-3 on the same scale plus at least a positive response to any cognitive domain from the neurological interview; and iii) SCD-Pg: only for cases categorized as SCDg specific features were considered such as: age at onset of SCD beyond 60 years, turning up of complaints within the last 5 years, worry associated with SCD, and feeling of worse performance than others of the same age group. When all these conditions accompany the self-experience of decline then an individual was classified as SCD-Pg.

6.4.4. APOE genotyping

APOE gene polymorphism status was studied with total DNA isolated from peripheral blood following standard procedures. Genotyping of APOE polymorphisms (rs429358 and rs7412) was performed by Real-Time PCR [22]. APOE was coded 1 for the APOE ε4 carriers, and 0 for non-carriers.

6.4.5. Statistical analyses

Analyses were conducted using R version 3.1.1. [23], specifically packages mice [24] for multiple imputation and msm [25] for multi-state modeling. We used 2-sided significance tests for all analyses, with statistical significance set at p-value < 0.05.

We performed a preliminary analysis of demographic, cognitive and clinical variables at baseline to find out their distribution and explore the nature and distribution of missing values. Nearly 10% of data were missed, but no profiles of missingness were identified (i.e.

the missingness spread over many individuals, variables and study visits). We therefore conducted a multiple imputation procedure under a fully conditional specification method in order to impute values as closer as possible to ideal predicted observations. Those imputed values were generated on the basis of existing variables through four different databases, one for each study visit; a total of four imputation procedures were thereby conducted. In this sense it should be noted that those individuals who did not attend to any visit were excluded from the corresponding databases. The imputation procedure replaced each missing observation with a set of plausible values representing uncertainty about the appropriate value to impute. The procedure was repeated five times and generated the corresponding five data sets whose coefficients varied from one set to another. The imputed data sets were analyzed using the usual procedure for complete data. Finally, the results of these analyses were combined to produce valid statistical inferences of data.

We then applied the SCD´s operational criteria to classify the whole cohort in every visit. SCD groups were treated as independent variables with three levels (NCg, SCDg, and SCD-Pg) whereas demographic, cognitive and neuropsychiatric data were used as dependent variables. Associations between categorical variables were analyzed with the Pearson´s $\chi 2$ test when appropriate. In addition, due to differences of sample size among SCD groups, analyses of variance and post-hoc analyses was based on non-parametric Kruskal-Wallis tests with Bonferroni corrections when necessary. As estimates of the effect size partial eta squared ($\eta p2$) for quantitative and Cramer´s V for categorical variables were calculated.

In order to test the hypothesis related to the temporal stability of the self-experienced cognitive decline, we first calculated the Intraclass Correlation Coefficient (ICC) among the scores of the SCD scale over time insofar as this is a quantitative variable. Then the percentage of incongruent classifications over time was obtained. Since SCD groups were categorical, we used the Pearson´s $\chi 2$ for analyzing the association among them and also obtained squared weighted Cohen´s kappa and Fleiss´ kappa concordance coefficients for two or multiple observations respectively. For a variety of reasons weighted Cohen´s kappa has been considered a good statistic for that because it provides an estimate of the percentage agreement between ratings corrected for chance (i.e. target values are random). Indeed, the weighted kappa with squared function is identical to the Concordance Correlation Coefficient (CCC) for continuous data [26]. According to the values of kappa, it was considered: < 0.20 as poor agreement; 0.21-0.40 as fair agreement; 0.41-0.60 as

moderate agreement; 0.61-0.80; as good agreement; and 0.81-1.00 very good agreement [27].

Then, we conducted a preliminary analysis of the conversion rate among groups by SCD only with consistently cases identified through the follow-up. Irrespective of MCI diagnosis, those consistent cases were defined as follows: i) individuals who remained stable in the same group during follow-up ("Stable NCg"; "Stable SCDg"; "Stable SCD-Pg"); and ii) subjects who showed a forward transition in the preclinical spectrum from left to right during follow-up ("Transition from NCg to SCDg"; "Transition from SCDg to SCD-Pg"). Otherwise, when backward transitions were detected (i.e. transitions from SCD-Pg to SCDg or from SCDg to NCg) individuals were removed from the analyses. Then, for each group we examined the percentage of individuals who developed or not MCI during follow-up.

Multi-State Markov Model

As this study focused on the temporal dynamics of the preclinical stage of AD, we performed a Multi-state Markov Model in continuous time to better characterize transitions among the following four states: No SCD, SCD, SCD-P, and MCI. Here it is important to note that all MCI statuses considered were confirmed during follow-up. Analyses based on this approach are appropriate for modeling the course of health processes in continuous time because they are able to accurately capture the transition of individuals in forward and backward directions across discrete stages [28]. Then, considering the assumption of the AD continuum, Multi-state Markov Model enables us to describe the process in which individuals move through the preclinical AD stages in continuous time.

In our study, we examined the stage-sequential dynamic of preclinical AD using Multi-state Markov Model in continuous time. The Markov assumption claims that the rate of transition from one state to another depends only on the current state. Although this assumption seems to be restrictive, it is necessary to compute the likelihood for intermittently observed data like ours. An alternative to deal with this assumption is to include time in the current state as an additional predictor in the model. However, since with intermittently observed states it is not possible to know how long a participant has been in a given state, the apparent restrictiveness of the Markov assumption was

ameliorated somehow by introducing age in the model. Thus, the transition matrix was calculated between any two unrounded ages and therefore accommodates variation in the time between participant visits [29].

We modeled therefore a Multi-state Markov Model with forward-backward algorithm to maximize likelihood estimation. Since we only observe states at a finite series of time and time interval between two consecutive visits in our longitudinal study varied across participants, a time-homogeneous model was preferred instead of a discrete one [30]. We then specified a multi-state model with four different states (No SCD, SCD, SCD-P, MCI) as well as the initial values for the transition intensity matrix that corresponded to [(0,0.5,0,0.01), (0.1,0,0.15,0.05), (0.05,0.5,0,0.2), (0,0,0,0)]. This matrix, which is really important because governs the whole model, represents the theoretical probabilities of transition from one state to another independently of the real data. Our model therefore assumed that individuals could move or recover from consecutive states, as well as convert from any state to MCI, which was conceived as the absorbing state. All transitions were interval-censored because we could not know the exact time in which individuals had transitioned. Multi-state Markov Model provided the estimated transition probability matrix and its 95% confidence intervals to evaluate the probability of a change of SCD status membership over time conditional on previous status. The analysis of this matrix allowed us to better understand the temporal dynamics of preclinical AD over time and to test the hypothesis of SCD and SCD-Plus as two different statuses that increase the risk of future MCI.

6.5. Results

The participants were followed-up for a mean of 2.9 years (SD 0.8; median 3.2; range 1.0-4.2). During this time, 84 (7.7%) individuals developed MCI and 16 (1.5%) progressed to dementia. Based on the cognitive trajectories of these 16 participants (marked memory impairment as a primary symptom during follow-up and MRIs excluding significant vascular damage) we could assume that there is a high probability of AD dementia type, but there is no available biomarker confirmation.

Regarding cognitive concerns, 78% of participants reported some type of SCD at baseline; that is, they were classified as SCDg or SCD-Pg. The single measure ICC value for the scores

of the SCD scale during the whole follow-up showed a positive correlation of 0.522 (p-value <0.001). On the other hand, the average measure ICC was 0.814. Table 6.1. summarizes the descriptive statistics of the total sample and the three SCD groups at baseline. No differences in age, gender and ApoE among groups were found. Nevertheless, there was a trend for significance concerning education, having less years of education SCD-Pg than NCg. Differences in depression (GDS) and anxiety (STAI) were also found among all three groups. These results highlighted that neuropsychiatric symptoms were more frequent in both SCD groups compared to NCg; and these symptoms were especially marked for SCD-Pg. Regarding cognitive assessment, statistical differences between groups were found for instrumental activities of everyday (FAQ), verbal episodic memory (FCSRT), speed processing (Digit Symbol Coding) and clinical rating (CDR sum of boxes). Interestingly, the SCD-Pg showed the worst cognitive performance, while only two indices of FCSRT differed significantly between NCg and SCDg.

Table 6.1*. Baseline demographic and clinical characteristics of the sample by Subjective Cognitive Decline groups.*

	NCg	SCDg	SCD-Pg	p-value	Post-hoc
n	240	685	166	-	
Age, y, mean (SD)	74.48 (3.84)	74.87 (3.90)	74.39 (3.77)	0.208	
Education, y, mean (SD)	11.56 (5.81)	10.65 (5.84)	9.37 (5.21)	0.002	b
Female, n (%)	155 (64.6)	429 (62.6)	115 (69.3)	0.272	
ApoE ε4, n (%)	41 (17.2)	119 (17.5)	30 (18.1)	0.409	
FAQ, mean (SD)	0.13 (0.38)	0.45 (0.81)	0.67 (0.92)	<0.001	a,b,c
SCD					
SCD score, mean (SD)	1.76 (0.93)	4.68 (1.85)	6.07 (2.07)	<0.001	a,b,c
Years of onset, y, mean (SD)	7.08 (5.15)	8.31 (6.16)	3.12 (1.42)	<0.001	a,b,c
Age at onset, y, mean (SD)	67.40 (6.34)	66.57 (7.32)	71.27 (4.10)	<0.001	b,c
Neuropsychiatric symptoms					
GDS, mean (SD)	0.95 (1.63)	1.62 (2.30)	2.36 (2.58)	<0.001	a,b,c
STAI trait, mean (SD)	13.19 (7.96)	17.00 (9.78)	21.84 (9.53)	<0.001	a,b,c
Cognitive performance					
FCSRT free immediate, mean (SD)	25.00 (6.32)	23.31 (6.25)	22.50 (5.78)	<0.001	a,b
FCSRT total immediate, mean (SD)	42.15 (5.42)	41.02 (5.56)	39.94 (5.49)	<0.001	a,b,c
FCSRT free delayed, mean (SD)	10.02 (2.47)	9.38 (2.69)	8.78 (2.44)	<0.001	a,b,c
FCSRT total delayed, mean (SD)	14.71 (1.69)	14.30 (1.84)	14.02 (1.83)	<0.001	a,b
Digit Symbol Coding, mean (SD)	21.02 (8.19)	19.07 (7.13)	17.86 (6.73)	<0.001	a,b
CDR sum of boxes, mean (SD)	0.00 (0.00)	0.00 (0.03)	0.00 (0.00)	0.553	a,b

ApoE: Apolipoprotein E; CDR: Clinical Dementia Rating; FAQ: Functional Activities Questionnaire; FCSRT: Free and Cued Selective Reminding Test; GDS: Geriatric Depression Scale; NCg: No Complaints group; SCD: Subjective Cognitive Decline; SCDg: Subjective Cognitive Decline group; SCD-Pg: Subjective Cognitive Decline Plus group; SD: Standard Deviation; STAI: State-Trait Anxiety Inventory.

p-values indicate the values assessed with non-parametric Kruskal-Wallis tests for each variable except sex and ApoE ε4, where contingency χ2 tests were performed. Non-parametric post-hoc: a=NCg vs SCDg; b=NCg vs SCD-Pg; c=SCDg vs SCD-Pg

Table 6.2. shows the percentage of concordance and discrepancies related to the classification of individuals in SCD groups. Since SCDg and SCD-Pg are both referred to a self-perception of complaints and that the boundaries between them are permeable, we collapsed them in a single level. Based on the assumption about the linear transition upon the AD spectrum, the agreements were defined according to two parameters: i) individuals who converted from NCg to SCDg; and ii) subjects who remained stable as SCDg over time. Discrepancies were then defined as those cases in which a reversal from SCDg to NCg occurs. As expected, the percentages of discrepancy increased with the duration of follow-up from around 5% at one-year follow-up, to 17% at three-year follow-up. We also conducted some concordance analyses involving Cohen´s and Fleiss´ kappa indices. All values of kappa resulted significant ($p<0.0001$) and over 0.40, ranging from 0.46 for the three-year follow-up to 0.76 for the one-year period. These outcomes indicated that there was a good agreement in the longitudinal classification of SCD groups.

***Table 6.2**. Concordance among Subjective Cognitive Decline groups over time.*

	One-year follow-up			Two-years follow-up		Three-years follow-up
	v0-v1	v1-v2	v2-v3	v0-v1-v2	v1-v2-v3	v0-v1-v2-v3
Concordance	94%	95%	95%	88%	90%	83%
Discrepancy*	6%	5%	5%	12%	10%	17%
Cohen´s kappa (95% CI)†	0.76 (0.71-0.82)	0.71 (0.62-0.79)	0.62 (0.51-0.72)			
Fleiss´ Kappa†				0.53	0.53	0.46

* Discrepancy is only referred to those cases in which transition from SCDg or SCD-Pg to NCg is observed.
† All p-values < 0.0001
Annual mean discrepancy related to SCDg to NCg = 7.7%
Annual mean discrepancy related to SCD-Pg to NCg = 4.7%
v0: baseline visit; v1: one-year follow-up visit; v2: two-year follow-up visit; v2: three-year follow-up visit.

Regarding the association between SCD and conversion rate to MCI, in a preliminary analysis we classified all individuals according to the evolution of their complaints through the follow-up. Considering only consistent cases (i.e. those who remained stable or those who moved forward in the preclinical spectrum from left to right) and irrespective of MCI diagnosis, five new groups were thereby built as shown in Table 6.3. It should be noted that since backward transitions were removed from this analysis, only 591 participants were finally considered. The column “n over time” in Table 6.3. reflects the number of individuals who were retained in each group (for example, 117 participants transitioned from NCg to SCDg at any point of the follow-up). The column “% conversion to MCI” means the

percentage of the participants of each category who developed MCI (as in our previous example, 4 out 117 of the participants in the "Transition from NCg to SCDg" group developed MCI what represents 3.6% versus the 96.4% who remained cognitively intact). The outcomes showed that the percentages of conversion to MCI were increasing as it was observed a theoretical transition from left to right in the continuum: from 0% in "Stable NCg" to 42.9% in "Stable SCD-Pg". Therefore, the rate of conversion to MCI showed a strong relationship to SCD-P.

***Table 6.3**. Cumulative conversion rate to Mild Cognitive Impairment by transition among Subjective Cognitive Decline groups.*

	n over time	% conversion to MCI
Stable NCg	41	0.0%
Transition from NCg to SCDg	117	3.4%
Stable SCDg	331	6.3%
Transition from SCDg to SCD-Pg	88	17.0%
Stable SCD-Pg	14	42.9%

MCI: Mild Cognitive Impairment; NCg: No Complaints group; SCDg: Subjective Cognitive Decline group; SCD-Pg: Subjective Cognitive Decline Plus group

***Table 6.4**. Status prevalence of Subjective Cognitive Decline by visit and transition probability matrix for preclinical AD stages.*

Status prevalence by visit…	No SCD	SCD	SCD-Plus	MCI
Baseline	0.225	0.630	0.145	0.000
One-year follow-up	0.196	0.634	0.120	0.050
Two-year follow-up	0.119	0.681	0.140	0.060
Three-year follow-up	0.101	0.713	0.113	0.073

Probability of transitioning to... conditional on...	No SCD	SCD	SCD-Plus	MCI
No SCD	0.501	0.449	0.044	0.006
SCD	0.072	0.784	0.120	0.025
SCD-Plus	0.032	0.517	0.334	0.117
MCI	0.000	0.000	0.000	1.000

However, transition among states in this preliminary test seemed to be partially biased because of two reasons: the loss of nearly half of the sample after removing backward cases as well as the extensive contingency tables necessary to explore individual trends on the SCD transition. To solve those issues a more sophisticated statistical approach was applied for studying the temporal dynamics of SCD with all cases. We performed a Multi-state Markov Model to better understand the temporal dynamic of preclinical AD stages (-2

log-likelihood=3,972.06; AIC=3,986.06). Table 6.4. shows the transition probability matrix across SCD statuses. These transition probabilities express the incidence of transitioning during the follow-up conditional on earlier membership in any specific the SCD status. Participants who did not report SCD at baseline had 50% of probabilities of remaining as no SCD during the three-year follow-up. In case of transition, they were most likely to transition to the SCD status, but not to SCD-Plus or MCI. Those in SCD status at baseline had 78% of probabilities of remaining there at follow-up and their most likely transition was to SCD-Plus status. Indeed, probability of progression to SCD-Plus was approximately twice than reversion to No SCD status. Individuals in the SCD-Plus status at baseline were the least stable group remaining only 33% of individuals in the same status during the follow-up. Their more likely transition was backward towards SCD status (52% of them) and the probability of reversion to No SCD was very unlikely. Most importantly, these outcomes pointed out that the SCD-Plus was the status in which the likelihood of progression to the following prodromal AD stage, MCI, was higher (12%) followed by SCD (3%) and No SCD (less than 1%). Figure 6.1. shows graphically the fitted survival probability of conversion to MCI over time for No SCD, SCD and SCD-Plus statuses.

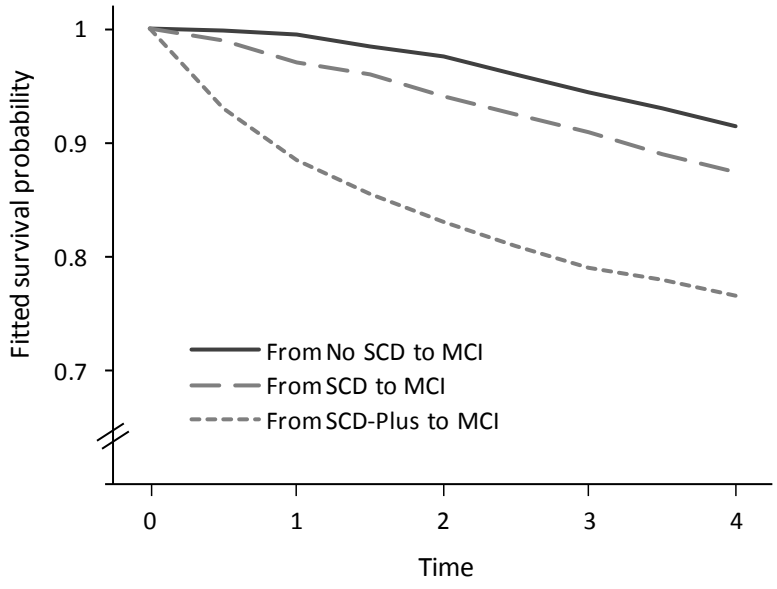

***Figure 6.1**. Kaplan-Meier survival curves for rates of conversion to MCI by SCD statuses.*

SCD-Plus shows the highest risk of conversion to MCI over time.
MCI: Mild Cognitive Impairment; SCD: Subjective Cognitive Decline

6.6. Discussion

The purpose of this study was to determine whether SCD may be a suitable very early marker for AD in a large cohort composed of 1,091 participants aged 70 years and over with uniform ascertainment of risk factor and disease. All participants were cognitively healthy at baseline and during three years of follow-up we examined the appearance of SCD, its longitudinal internal consistency and the transition from normal cognition to MCI stage over time.

Since the study and definition of SCD have been heterogeneously treated in the literature [6], and this is probably the reason whereby the prevalence of SCD in older adults may range from 10 to 80% depending on the studies [10–13], we decided to base our study on the guidelines provided by the SCD-I [8]. We operationally defined a robust procedure to measure and characterize SCD. Cognitive complaints were assessed twice and independently within the same visit; first during the neurological examination and then during the neuropsychological evaluation. The combination of all these data at baseline showed that 78% of individuals reported SCD. Although this prevalence is slightly higher than that of other similar studies [31], this fact could be explained due our cohort is a little older. Interestingly, during the follow-up we found that about half of individuals who did not report complaints at baseline did manifest cognitive concerns in the following years. We interpret this finding as an expected consequence of aging. Although the transition from non complaints status to SCD status have been previously described [32], to our knowledge our study is the first in which the assessment of SCD has been collected annually with a very strict and well-defined operational criteria as well as including two subgroups of complaints, namely SCDg and SCD-Pg, according to the guidelines given by the SCD-I [8].

The preliminary cross-sectional analyses among SCD groups at baseline did not find differences in age, gender and ApoE. Nevertheless, as expected the results did highlight that depressive and anxiety symptoms were more frequent in both SCD groups, especially for SCD-Pg, compared to NCg. Regarding neuropsychological assessment, SCD-Pg showed the worst cognitive performance compared to the other two groups though the scores in tests were in the range of normal cognition.

To test our first aim about proving the stability of the SCD construct over time, we focused on analyzing longitudinally the percentage of concordance and discrepancies related to the classification of individuals in SCD groups. As expected, the number of discrepancies increased with the duration of follow-up what may be explained by the greater number of options to group subjects. The concordance analyses with Cohen´s and Fleiss´ kappa indices showed a good agreement in the classification of SCD groups what confirmed that the concept of SCD has a considerable internal consistency over time when it is operationally defined and consistently assessed. Our hypothesis was therefore confirmed and SCD fulfils one of the requirements to be considered as a potential marker for early detection of AD [14].

For our second goal, to examine the association between SCD and conversion rate to MCI, in a preliminary analysis we obtained that the rate of conversion to MCI was directly related to the presence of SCD-P and dramatically increased up to almost 43% when SCD-P remains stable over time. This result is according to previous findings [33]. However, since the boundaries between the SCD groups may be permeable due to the intrinsic criteria used for classifying individuals, we performed a Multi-state Markov Model to test the AD continuum hypothesis in its preclinical stage and to better identify the underlying trajectory of SCD over time. The results showed a clear sequential trend transitioning from cognitively healthy individuals with No SCD to SCD/SCD-Plus and, finally, MCI. In the three-year follow-up of this study, 50% of participants with No SCD at baseline progressed to a SCD stage characterized by the presence of a self-perception of cognitive failures. From this stage, almost 80% remained as SCD through the whole follow-up and 12% did progress to a SCD-Plus phase which might be conceived as the previous stage of MCI. Interestingly, the possibility of reversion from SCD to No SCD during the follow-up was very unlikely (7%). Finally, we found that the greatest probability to convert to MCI was up to 12 times higher for individuals at SCD-Plus status. Overall, these results about change show a parsimonious and detailed scenario of how individuals move into and out of the different SCD categories. Most importantly, beyond biomarkers this is the empirical demonstration of the existence of a continuum in the preclinical phase of AD with three well-defined statuses that have different probabilities to develop future MCI.

There are some limitations in this work that we want to address. First, the follow-up time may be not large enough to appreciate the expected changes that must appear in a disease

so insidious like AD in which progression may happen in a wider period of time. Since Vallecas Project is still in progress we will minimize this limitation through the longitudinal follow-up of our cohort by further analyzing whether transition probabilities of developing MCI increase for SCD-Plus against SCD and No SCD. Second, in our study we do not have available information on CSF/PET biomarkers to confirm that SCD and MCI are due to underlying AD pathology and thus we cannot rule out other ulterior neurological and psychiatric conditions which are well-known to lead to SCD and MCI. Nevertheless in the context of this project we do rely on complete clinical and cognitive profiles of every participant so we can obtain confirmation of the diagnosis in the follow-up, as well as the clinical trajectories that mostly match with AD.

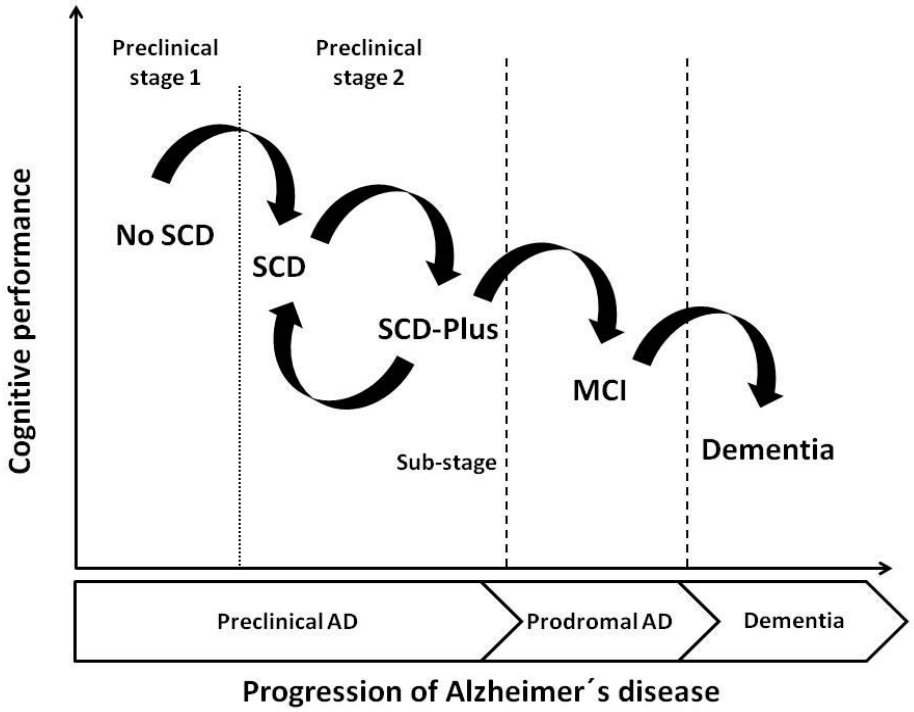

***Figure 6.2**. Hypothetical dynamic transition through AD continuum.*

This graphic represents the hypothetical dynamic of preclinical AD phases according to a major risk of developing future MCI. Overall, two main stages can be identified, namely No SCD and SCD, as well as a sub-stage called SCD-Plus within SCD. Based on our results, progression may occur from No SCD to SCD, but oposite transitions from SCD to No SCD are very unlikely. Once an individual is at the SCD phase there may be a progression to a severe form of cognitive concerns, the sub-stage SCD-Plus, in which the hazard of MCI is the highest compared to No SCD and SCD. Nevertheless, since the criteria for classifying SCD-Plus are very restrictive, permeability is possible between SCD and SCD-Plus and thus individuals might be gone through or back over time.
AD: Alzheimer´s disease; MCI: Mild Cognitive Impairment; SCD: Subjective Cognitive Decline

Our study has a powerful strength regarding the analytic approach to examine the role of SCD on cognitive decline. Unlike the majority of studies that follow a static, traditional, and

cross-sectional methodology -that is, grouping individuals based on SCD features just once at baseline and analyzing their risks ratios of progression through the AD spectrum-, our research implements a novel, comprehensive, and longitudinal approach to further investigate the temporal dynamics of SCD as well as transitions through preclinical stages during the whole follow-up. Thus, to our knowledge this is the first time that it has been observed the existence of a real transition within the preclinical AD stage until prodromal MCI (Figure 6.2.). Additionally, we would like to point out another remarkable strength of our study: the proposal of a methodological, systematic and reproducible way of recording SCD. This provides a consistent, robust and well operationally defined construct that allows monitoring preclinical AD stages.

To summarize, self-report of subtle cognitive decline appears at the end of the preclinical phase of AD even in the absence of significant objective impairment detectable on standardized neuropsychological assessment. As a novel marker, SCD must address some challenges before being considered as a real predictor of future MCI and dementia. The most crucial challenge, internal consistency over time, has been now proved by our pioneer study, which is the first one as far as we know that analyzes the temporal dynamics of SCD. It has been also highlighted that there is a progressive transition from No SCD to SCD and SCD-Plus statuses. Thus every successive preclinical status indicates higher risk of future MCI, focusing on SCD-Plus as the one that requires special attention in terms of early preventive intervention. The strength of SCD measurements is that is a non-invasive, easy, and low-cost method for screening both patients attending to memory units and general population. This marker could facilitate not only a fast and easy identification of individuals at higher risk for a premature cognitive impairment, but it allows us to better track the longitudinal trajectory of individuals. SCD therefore could become extremely useful in coming years to measure the effectiveness of new clinical trials with AD modifying therapies at preclinical phases which are supposed to be still recoverable.

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6.7. Summary of conclusions

1. Measurements of SCD along time allow to determinate the stability of SCD and the strength of SCD data.

2. It is suggested the use of SCD as a possible non-invasive, easy and low cost marker for the transition to MCI.

3. We provided the temporal dynamics of the AD continuum in which SCD plays an important role as an early step.

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7. DISCUSSION

The initial signs of AD regarding to cognitive status can be divided into the objective performance level measurable by tests and the self-perception of cognitive decline. The first has been a hot topic of research for many years, and thus the early manifestation of AD in the objective performance spectrum is well known. The latter subjective component of self-experienced cognitive decline in the context of AD has only recently become a topic of interest. Novel research has suggested that both objective and subjective decline occur through the AD continuum and that their contribution to dementia prediction varies depending on the stage of impairment.

In the present investigation we have tried to stablish a complete background on the state of the art of SCD and studied the nature of the complaints. The following are the key findings found according to the objectives set out for this research in section 2.

Objective 1. TO STUDY THE UNDERLYING STRUCTURE OF THE SCD IN A SAMPLE OF OLDER ADULTS.

Our departure point was to analyze whether the expression of SCD in elderly people has a unidimensional structure or on the contrary there is a factorial underlying structure. Historically it has been implicitly assumed that the self-reported complaints are a monolithic concept referred almost exclusively to memory. The clinical and qualitative evidence however seems to indicate that there may be distinct types of cognitive complaints beyond memory that could have different clinical values in practice.

Then, in our first experiment we examined the underlying structure of a widely used scale which is presumed to assess memory complaints, the EMQ (Sunderland et al., 1984), in a sample of older adults from Vallecas Project cohort. This questionnaire is perhaps one of the most extended scales that have been used to assess subjective complaints in a variety of populations, including older adults (Garrett, Grady, & Hasher, 2010; Ossher, Flegal, & Lustig, 2013). It consists of 28 items about memory failures that occur in everyday life and that must be scored according to a Likert-type scale. Despite Sunderland et al. (1984) emphasized the unidimensionality of the EMQ, the analysis of its individual items evidences that a high percentage of them do not exactly correspond to memory complaints. Rather,

some items would involve various cognitive domains such as visual perception (“failed to recognize, by sight, close friends or relatives”), attentional processing (“been unable to follow the thread of a story”), language production (“found that a word is on the tip of your tongue”), or executive functions (“forgotten a change in your daily routine”). In any event, investigations using the EMQ with older adults have exclusively focused on the overall score (Alegret et al., 2015), and have not addressed the role of the specific underlying factors upon differentiation between healthy controls and MCI.

We used a combination of statistical approaches, namely EFA and IRT, to better capture the dimensionality of the questionnaire. Items 11 (failed to recognize, by sight, close friends or relatives), 19 (forgotten important details about yourself) and 27 (repeat to someone what you have just told them) were excluded from the EFA since their skewed distribution. The reason for that exclusion may have to do with the fact that these three items seem to reflect severe symptoms which appear in mild dementia rather than in earlier stages of AD (preclinical or prodromal phases). The final solution with the remaining 25 items comprised of a five-factor structure which explained up to 50% of EMQ´s total variance. Those factors were called as follows: i) FII was associated with fails in immediate retrieval, as well as naming impairment; ii) EF was related to distractibility, inhibition errors and monitoring; iii) PM refered to things that someone has to recall in the next future; iv) FCO had to do with forgetting personal details; and v) SO was associated with difficulties for spatial orientating. Furthermore the use of an IRT approach allowed us to find out the best 10-items that maximize the collection of information on cognitive complaints.

Our results therefore highlighted an adequate internal consistency of the EMQ, as well as a well-established factorial structure. This outcome did not fit well with the Sunderland´s assumption on the unidimensionality of the questionnaire (Sunderland et al., 1984). Rather than the EMQ seemed to reflect a more complex scale than supposed which is able to measure various domains of cognitive complaints. Thus, these outcomes confirmed our hypothesis on the multidimensional structure of the SCD construct.

Objective 2. TO EXAMINE THE CROSS-SECTIONAL CLINICAL VALUE OF THE SCD IN DISTINGHISING BETWEEN HEALTHY ELDERLY CONTROLS AND INDIVIDUALS WITH MCI.

Once it was demonstrated that SCD is a heterogeneous construct with a solid underlying structure, our second aim was to investigate whether specific cognitive complains were more useful than others to discriminate MCI. In the same experiment we went through to examine the association between the five factors previously obtained with the neuropsychological performance and the clinical diagnosis. As expected, the EMQ factors exhibited higher correlation coefficients with depressive and anxiety symptoms than cognitive performance. This outcome has been already found in different studies (Balash et al., 2013; Jessen, 2014) and it could be explained following the cognitive model of depression(Peckham, McHugh, & Otto, 2010). According to this model, individuals with depressive symptoms might exhibit some sort of information-processing biases toward negative information. Thus many older adults with depression may have a hypersensitivity to perceive their own cognitive failures and this could result in an overreporting of complaints. Moreover, another explanation is that by definition individuals with SCD score within normal ranges on neuropsychological testing. Neuropsychological tests assess performance at a single point in time whereas subtle subjective decline captures longitudinal change (Rabin, Smart, & Amariglio, 2017). In our work, FII and EF were interestingly the factors that correlated higher with cognitive performance. Specifically FII was strongly associated with episodic memory and EF with excutive functions. This outcome highlighted concurrent validity to the latent structure of the EMQ because the internal content of the factors is directly related to the cognitive domain assessed. By contrast, the fact that both FO and SO did not show differences in control subjects and MCI could be explained because the first one referred to a high prevalent oversight in the elderly population (“Forgetting where you have put something”, “Forgetting where things are normally kept or looking for them in the wrong place”) and the second was an idiosyncratic sign of mild dementia (“Getting lost or turning in the wrong direction on a journey, on a walk, or in a building where you have been before”).

Regarding clinical diagnosis, our results proved that three types of cognitive concerns were able to distinguish between controls and MCI. Higher scores in specific complaints on

retrieval of immediate events, executive functioning and prospective memory were related to prodromal stages of dementia. Indeed, our results indicated that if two individuals, one control and one MCI, were selected at random, the score in any of these three factors would be higher for the MCI patient on 60% of times. All these findings emphasize that not all cognitive complaints have the same clinical significance for prediction of cognitive impairment. Individuals who present these particular complaints and do not yet have a diagnosis of MCI may need special attention in terms of close clinical follow-up or an early cognitive intervention.

Then experiment 1 gave an answer to objectives 1 and 2. We demonstrated therefore that SCD is far away to be a monolithic entity. Rather it has an underlying structure that involves different cognitive domains and also that complaints have specific weights in distinguishing between healthy elderly controls and MCI. Complaints associated with episodic memory, executive functions and prospective memory give us a profile of subject at higher risk of developing MCI.

Objective 3. TO ANALYZE THE LONGITUDINAL PREDICTIVE VALUE OF THE SCD AS A MARKER OF FAST CONVERSION TO MCI IN A SAMPLE OF OLDER ADULTS.

The findings from the first experiment showed that specific cognitive complaints were able to distinguishing cognitively healthy individuals from MCI in a cross-sectional manner. Our following step was to search for specific features of SCD that enabled us to predict those individuals at high risk of developing MCI during a short period of time.

The earliest research on SCD was based on longitudinal community-based studies that examined whether memory complaints at baseline predicted progression to dementia, with contradictory findings (Geerlings, Jonker, Bouter, Adèr, & Schmand, 1999; Schmand, Jonker, Hooijer, & Lindeboom, 1996; Tobiansky, Blizard, Livingston, & Mann, 1995). Many of these studies were conducted prior to the formulation of the concept of MCI, so it is likely that some SCD participants met criteria for MCI (Rabin et al., 2017). Thus, interpretation of early longitudinal studies should consider this methodological limitation. More recent studies have found that SCD is able to predict dementia, but over longer follow-up periods (Kaup, Nettiksimmons, LeBlanc, & Yaffe, 2015; Koppara et al., 2015; Reisberg et al., 2010; van Oijen, de Jong, Hofman, Koudstaal, & Breteler, 2007), in individuals with significant concern

or worry (Jessen et al., 2010) or in participants recruited from memory clinics rather than community-based samples (Nunes et al., 2010; van Harten et al., 2013; Visser et al., 2009). Overall, there is overwhelming evidence on the role of SCD as a predictor of dementia when considering long-term periods but less data support its implication in the short-term.

In experiment 2 we then tested whether different subtypes of SCD were able to predict fast conversion from cognitively healthy to MCI. In an attempt to cope with the methodological problems already commented on the heterogeneity of definitions and approaches to measure SCD (Molinuevo et al., 2017), we followed the guidelines from the SCD-I with the aim of harmonizing the study of SCD. According to these guidelines, we used the same common terminology and research procedures to identify and classify individuals with SCD at risk of preclinical AD (Jessen et al., 2014).

Some common specific features are needed to stablish the profile of SCD according to the SCD-I recommendations: i) to collect information regarding features such as settings in which cognitive complaints are expressed; ii) association of SCD with medical help seeking; iii) number of years and age at onset of SCD; iv) subjective decline in memory and non-memory domains; and v) association of SCD with experience of impairment. In addition, a set of particular features could be helpful to identify individuals at risk of clinical progression and then to make up a more severe form of SCD referred to as SCD Plus. Those features include a more acute subjective memory decline than any other cognitive domain, onset of complaints within the last 5 years, age at onset over 60 years, worries about SCD, and feeling of worse performance than other people from the same age group. This new category of SCD-Plus could allow us to explain the transition from a non symptomatic stage to the first symptomatic manifestation of AD.

We replicated these guidelines in our Vallecas Project cohort. The goal of this experiment was to evaluate the clinical significance of SCD on the conversion from a cognitive healthy stage to a MCI in one year of follow-up. A total of 608 cognitively intact individuals were enrolled in the analyses. Depending on some SCD´s features participants were classified in three differente groups: i) No Complaints (NCg); ii) subjects with cognitive complaints in one or more cognitive domain (SCDg); and iii) subjects who besides complaints fulfilled the features of SCD Plus (SCD-Pg).

The results from this experiment showed that the conversion rate for the SCD-Pg (18.9%) was significantly higher than SCDg (5.6%) and NCg (4.9%). So, among other facts, what we found was that when we followed the guidelines proposed by the SCD-I for classifying groups according to their SCD´s features, the conversion rate of SCD-Pg was almost 20% in only one year. This outcome differed significantly from NCg and SCDg. Indeed, while the HR obtained for SCDg were similar to other studies (Geerlings et al., 1999; van Oijen et al., 2007), individuals who reported SCD Plus had almost 5 times higher risk of developing MCI compared to those subjects without complaints. The inclusion of gender, memory performance and APOE genotyping in the survival analysis did not decrease the predictive power of the SCD-Pg.

Overall, experiment 2 demonstrated that self-reported subtle cognitive decline in the absence of significant objective impairment on testing did have the ability to predict future decline in older individuals, even considering just a short period of time. SCD therefore showed a predictive value as an early marker of cognitive impairment. In practical terms, the interpretation of these findings suggests that individuals who might be characterized as SCD-Plus would need special attention regarding early cognitive and pharmacological intervention.

Objective 4. TO INVESTIGATE THE RELIABILITY AND TEMPORAL STABILITY OF THE MEASUREMENTS OF THE SCD OVER TIME IN A SAMPLE OF OLDER ADULTS.

Taking together all previous findings seem to point out cognitive concerns, and specially SCD-Plus, as a reliable marker of future AD when the SCD-I´s recommendations for tackling methological issues are considered (Jessen et al., 2014; Molinuevo et al., 2017). Nevertheless, at this moment of our line of investigation an important new concern arose before considering SCD as a solid target for research. To our knowledge this concern did not have ever brought to the table so far and it had to do with the reliability and temporal stability of the SCD´s measurements over time. In practical terms, if two longitudinal measures do not tend to match, which one would be the real to characterize the individual? This lack of temporal stability would represent an important bias to investigation. Only demonstrating that we can measure SCD as a robust, reliable and stable concept it could become a reliable preclinical marker for AD.

In our opinion paper, we provided some guidelines that should be taken into account in order to prove the stability of SCD. First, we posed that it is necessary to harmonize a protocol to collect all relevant information about cognitive complaints and to compare longitudinally the responses of the individuals. Variables such as age at onset, time of progression, memory performance compared to other people, concerns associated with SCD, and frequency of particular cognitive complaints are relevant data that must be carefully obtained (Jessen et al., 2014; Rabin et al., 2015). Second, it was proposed to gather self-perceived data using two procedures: a face-to-face interview with a healthcare professional and a self-administered scale of SCD. The use of scales and questionnaires is highly recommended to quantify SCD somehow and to monitor the progression of cognitive complaints over time since questionnaires tend to have higher internal consistency and validity than specific questions of SCD (Montejo et al., 2014; Rabin et al., 2015). Additionally, a multiple choice approach should vary from dichotomic to ordinal Likert-type scales to grasp the dimensionality of SCD in the best way possible (Burmester, Leathem, & Merrick, 2015).

Apart from SCD information, demographic variables such as age, gender, and education, as well as medical and lifestyle variables must be gathered by means of a survey. These variables have the greatest interest due to their possible implication in the expression of SCD. Additionally, objective cognitive performance and diagnosis are critical to establish the current stage of an individual in the continuum of AD and the relationship between SCD and risk of developing MCI and AD. Finally, neuropsychiatric variables should be collected as well because of their mediator role between SCD and cognitive decline.

With the previous recommendations in mind, we carried out a third experiment to test if the measurement of SCD over time is reliable and stable enough. Cognitive complaints were assessed twice and indepedently within the same visit at the Vallecas Project: i) neurological interview; and ii) neuropsychological assessment. We followed again the guidelines proposed by the SCD-I and applyed the SCD´s operational criteria to classify individuals in three different categories in every visit pursuant to the extent of SCD reported in both clinical interviews: NCg, SCDg and SCD-Pg.

In order to test the hypothesis related to the temporal stability of the self-experienced cognitive decline, we first calculated the percentage of incongruent classifications over

time. We obtained ≈ 95% concordance comparing visit to visit in one year follow-up, ≈ 89% in two years follow-up, and 83% in three years follow-up. In order to analyze the reliability of the SCD classification over time we conducted concordance analyses involving squared weighted Cohen´s and Fleiss kappa indices. For a variety of reasons weighted kappa has been considered a good statistic for concordance because it provides an estimate of the percentage agreement between ratings corrected for chance (i.e. target values are random; Lin, Hedayat, & Wu, 2012). All values of kappa resulted significant ($p<0.0001$) and over 0.40, ranging from 0.46 for the three years follow-up to 0.76 for the one year period. These outcomes indicated a good agreement in the longitudinal classification of SCD groups, above all considering the subjective nature of the concept, what was taken as a proof of temporal stability. So, this is the first time to our knowledge that SCD has been demonstrated to be a reliable concept and to have appropriate internal consistency when it is measured as here. Thus, the proposal of a methodological, systematic and reproducible way of recording SCD is a remarkable strength of our study. This proposal provides a consistent, robust and well operationally defined construct that allows monitoring preclinical AD stages.

Objective 5. TO DETERMINE THE EXPRESSION AND THE TEMPORAL DYNAMICS OF THE SCD THROUGH THE AD CONTINUUM.

Finally, after proving that SCD is stable over time in a series of longitudinal assessments, we analyzed the validity of the SCD as a very early marker of AD. There is already overwhelming evidence about this fact through the literature, and thus it has been described that individuals might experience some type of cognitive decline up to 15 years before they develop MCI and AD (Reisberg et al., 2008). Furthermore, in the absence of objective cognitive impairment, it has been reported evidence about the relationship between SCD and some AD biomarkers such as brain amyloid deposition and cerebral hippocampal hypometabolism (Eckerström et al., 2017; Vannini et al., 2017). We however examined the role of SCD as predictor of cognitive impairment from an original approach which does not use so far: the analysis of the dynamic transition from preclinical stages of AD. Specifically, we tested the hypothesis of SCD and SCD-Plus as two different statuses that increase the risk of future MCI due to AD.

To do that, we performed a Multi-state Markov Model to better characterize transitions over time among the three supposed preclinical stages (No SCD, SCD, and SCD-Plus) up to MCI. Thus our study has a powerful strength regarding the analytic approach to examine the role of SCD on AD. Unlike the majority of studies that follow a static, traditional, and cross-sectional methodology -that is, grouping individuals based on SCD features just once at baseline and analyzing their risks ratios of progression through the AD spectrum-, a Multi-state Markov Model implements a novel, comprehensive, and longitudinal approach to further investigate the temporal dynamics of SCD as well as transitions through preclinical stages during the whole follow-up. Analyses based on this approach are appropriate for modeling the course of health processes in continuous time because they are able to accurately capture the transition of individuals in forward and backward directions across discrete stages (Jackson, Sharples, Thompson, Duffy, & Couto, 2003). Then, considering the assumption of the AD continuum, a Multi-state Markov Model enables us to describe the process in which individuals move through the preclinical AD stages in continuous time and to better identify the underlying trajectory of SCD over time. The main advantage of a Multi-state Markov Model is that we can assume that individuals can move or recover from consecutive SCD states, as well as convert from any SCD state to MCI.

The results showed a clear sequential trend transitioning from cognitively healthy with No SCD to SCD/SCD-Plus and, finally, MCI. In the three-year follow-up of this study, 50% of participants with No SCD at baseline progressed to a SCD stage characterized by the presence of a self-perception of cognitive failures. From this stage, almost 80% remained as SCD through the whole follow-up and 12% did progress to a SCD-Plus phase which might be conceived as the previous stage of MCI. Interestingly, the possibility of reversion from SCD to No SCD during the follow-up was very unlikely (7%). Finally, we found that the greatest probability to convert to MCI was up to 12 times higher for individuals at SCD-Plus status. Overall, as depicted in Figure 6.2., these results about change showed a parsimonious and detailed scenario of how individuals move into and out of the different SCD categories. Every successive preclinical status indicates higher risk of future MCI, focusing on SCD-Plus as the one that requires special attention in terms of early preventive intervention. Most importantly, beyond biomarkers this is the empirical demonstration of the existence of a

continuum in the preclinical phase of AD with three well-defined statuses that have different probabilities to develop future MCI.

The strength of SCD measurements is that is a non-invasive, easy, and low-cost method for screening both patients attending to memory units and general population. This marker could facilitate not only a fast and easy identification of individuals at higher risk for a premature cognitive impairment, but it allows us to better track the longitudinal trajectory of individuals. SCD therefore could become extremely useful in coming years to measure the effectiveness of new clinical trials with AD modifying therapies at preclinical phases which are supposed to be still recoverable.

Inclusive approach

AD is the most prevalent cause of dementia in old population and, at the present, there is not cure for the disease. Identification of the specific features at early stages of the disease is essential for a possible intervention, or prevention, of the main pathological characteristic related to the disorder: the cognitive impairment.

It has been highlighted that subjects showing a MCI convert with a higher rate to AD than those without MCI. The criteria to define MCI and the parameters used for the identification were deeply discussed, but in 2011 the NIA-AA gave some recommendations that resulted in a clearer way to look at the transition from normal aging to dementia, through MCI (Albert, Dekosky, et al., 2011; see also Cheng, Chen, & Chiu, 2017). This approach opened the door to objectively diagnose MCI patients at the earliest stage of cognitive impairment. Randomized controlled trials were carryed out with those patients, but pharmacological treatments failed to find any effect at delaying cognitive decline. That is one of the reasons why the focus of the investigation for stopping the disease process has moved in the last years to stages before MCI in which it is supposed that the disease-modifying therapy may success.

Since the end of the 20th century it is well known that many patients might experience a subjective decline in memory or other cognitive domains even prior to objective cognitive impairment. Self-report of subtle cognitive complaints have been proposed to appear at the end of the preclinical phase of AD even in the absence of significant objective impairment detectable on standardized neuropsychological assessment (Sperling et al., 2011b). This

self-experience of cognitive decline represented an opportunity to extend the spectrum of AD to at earlier stage. That explains why the study of cognitive complaints was gaining an increasing prominence in the research of neurodegenerative disorders.

Despite the overwhelming epidemiological data in favour of the role of cognitive concerns as a risk factor for subsequent MCI and dementia in older adults (Mitchell et al., 2014), this construct had to face up to some limitations such as the absence of an operational definition as well as the lack of harmonized criteria and assessment protocols. The insight and self-experience of cognitive decline is phenomenologically complex and may differ among individuals. Indeed from 2000 heterogeneous terminology emerged to refer to this sort of pre-MCI stage -"subjective cognitive impairment", "subjective memory complaints", "subjective memory impairment", "subjective cognitive complaints"-. Moreover, there is a great variability in the assessment procedures which is reflected on the different questions, response options, nature of items, and mode of administration across studies. This heterogeneity is likely affecting the outcomes obtained in each study and also determines the comparison of results. Because of this scenario, in 2014 the SCD-I published a position paper agreeing to a common terminology and research procedures to investigate the phenomenom (Jessen et al., 2014). Since then, we rely on a homogeneous framework to better study the implication of cognitive complaints in dementia due to AD. The term SCD was suggested and defined as a self-experienced persistent decline in cognitive capacity in comparison with the subject's previously normal status, during when the subject had normal age-, gender-, and education-adjusted performance on standardized cognitive tests. This represents a new step for the transition from normal cognition to MCI (Rabin et al., 2017). Additionally, based on the evidence the SCD-I suggested the term SCD-Plus, a specific form of SCD that could be at higher risk of later MCI. The main characteristics that define SCD-Plus are as follows: a subjective decline in memory rather than other cognitive domain, onset of SCD within the last 5 years, 60 years of age at SCD onset, worries associated with SCD, and feelings of worse performance than others in the same age group.

Our results were the first in confirming the hypothesis of the higher risk associated with the SCD-Plus criteria. We obtained that the SCD-Plus group had a significantly higher risk of developing MCI in just 13 months (18.9%; adjusted HR=4.2), compared with SCD and No SCD individuals (near 5%). We also confirmed the robustness and reliability of the SCD construct. We insisted on the necessity for both systematic evaluation and operationally

definition of cognitive complaints, and we demonstrated that SCD is stable over time. In our opinion, these results give the construct sufficient robustness and pave the way for further investigation on the implication of SCD through the AD continuum.

In addition, SCD is not only a good predictive marker of deterioration without the need for the use of invasive and expensive biomarkers, but also our results change the concept of the SCD in a certain way. Figure 7.1., which is based on the work of Jack et al., 2010, describes the temporal evolution of AD biomarkers in relation to each other and to the onset and progression of clinical symptoms. What we have introduced in this model is SCD-Plus as one of the earliest phases in AD. We hypothesize that SCD-Plus would be located as the third marker and its appearance would occur after tau deposition, but just before brain structure starts changing.

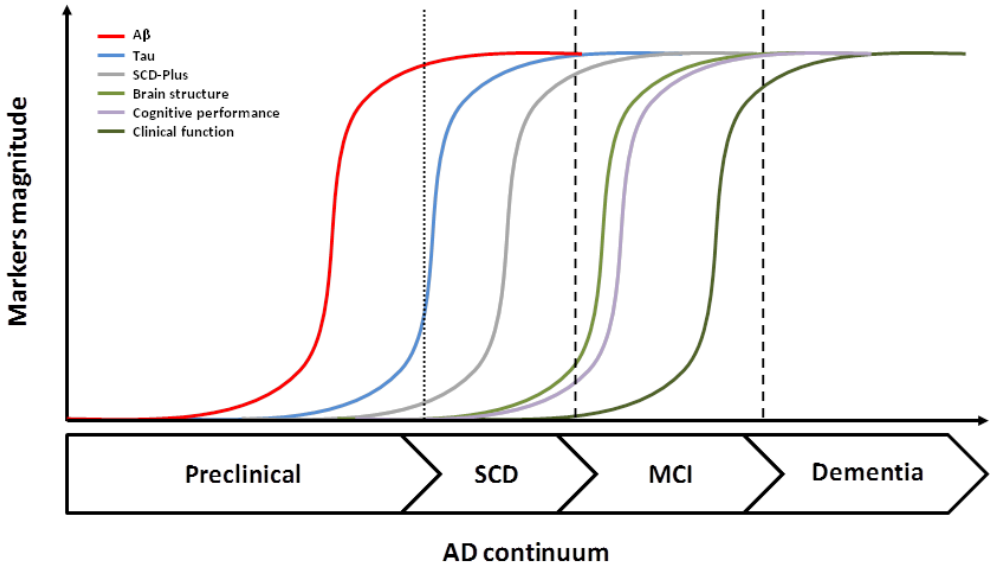

***Figure 7.1**. Revised dynamic biomarkers of the AD pathological cascade model (based on the model proposed by Jack et al., 2010).*

On the basis of the available evidence in 2010, Jack et al. proposed the use of specific AD biomarkers for disease staging in vivo. The disease model embodied the following temporal dynamic: (1) biomarkers become abnormal in a temporally ordered manner as the disease progresses; (2) Aβ identified by CSF Aβ42 or PET amyloid imaging occurs early in the disease, long time before the appearance of clinical symptoms; (3) tau-mediated neuronal injury identified by CSF appears later in the disease spectrum; (4) brain changes captured by structural MRI are the last biomarker to become abnormal; however, those brain changes would retain a closer relationship with cognitive performance than other biomarkers; and (5) cognitive and functional deterioration are the last symptoms that appear in this model. We hypothesize that there would be a third curve namely SCD-Plus that would occur after tau deposition, but just before brain structure starts changing, in the preclinical AD stage.
Aβ: β-amyloid; AD: Alzheimer´s Disease; MCI: Mild Cognitive Impairment; SCD: Subjective Cognitive Decline.

From our perspective SCD goes beyond being considered an unspecific symptom that may or not may appear in the stage prior to MCI, to being considered as a real stage within the continuum of AD in which asymptomatic subjects present a higher risk of conversion. In this way we would reach one of the clear and unanimous objectives in AD research: very early diagnosis when the subject is still asymptomatic to implementation of preventative measures in selected target population, namely SCD-Plus.

Future directions

As confirmed throughout this thesis, SCD must be considered as a stage prior to latter cognitive impairment. Just like us, research has mainly focused on SCD´s features that characterize those individuals who will convert to MCI over time. Although this is a very important point, in our opinion it would be interesting to examine three more groups of individuals and their relationship with SCD and MCI: i) subjects with SCD that do not progress to MCI across time; ii) individuals who do not report any kind of SCD but they do develop MCI; and iii) people who show a backward transition from SCD to No SCD. We firmly believe that the analyses of those three additional groups of individuals will shed light to capture a wider scenario on the SCD and will help to better understand the nature od the SCD.

Also, we would like to underline that nowdays only SCD is an available maker of cognition that has demonstrated to be effective for detecting major risk of future MCI. Thus, although standard cognitive tests have long been considered the gold standard for the diagnosis and prediction of AD progression, there are no objective cognitive tests capable to distinguish susceptible profiles of cognitive impairment. The sensitivity of classical tests is suboptimal at detecting the subtle cognitive changes that characterize the preclinical phase of AD. To account for this fact some reasons can be listed: standard paper-pencil cognitive tests do not allow us to rigorously control for parameters such as stimuli presentation velocity which could be relevant for the assessment; we cannot accurately record some responses nor reaction times neither; and these tests are also frequently affected by ceiling-effects specially for people with presumed high cognitive reserve. Cognitive neuropsychology must be therefore challenged to improve the sensitivity of the current standardized tests through the development of a new generation of cognitive instruments (Rentz et al., 2013). These newly developed measures will need to be simple, cost-effective, and capable of capturing

the subtle cognitive changes that can differentiate healthy aging from preclinical AD. These tests also need to be useful across educational levels as well as proving sensitive to change over the short timeframe of a clinical trial. New cognitive tasks must be supported on the current evidence derived from knowledge gained through translational efforts in neuroscience. Specifically, the instruments must be designed to target the neural pathways involved in the basic components of memory including encoding (learning of new information), retrieval (accessing information) and storage (recognition of information), as well as semantic encoding and top-down high level of attentional processing that may be vulnerable in preclinical AD (Badhwar et al., 2017; Hafkemeijer, van der Grond, & Rombouts, 2012).

We would like to continue this line of investigation by combining it with non-invasive biomarkers and within our reach, which is why the future line of this thesis is directed towards the combination of the complaints groups with the Default Mode Network study. If it were to be replicated, as in our case it is possible to differentiate between groups the characterization of individuals at risk would result robust and detailed.

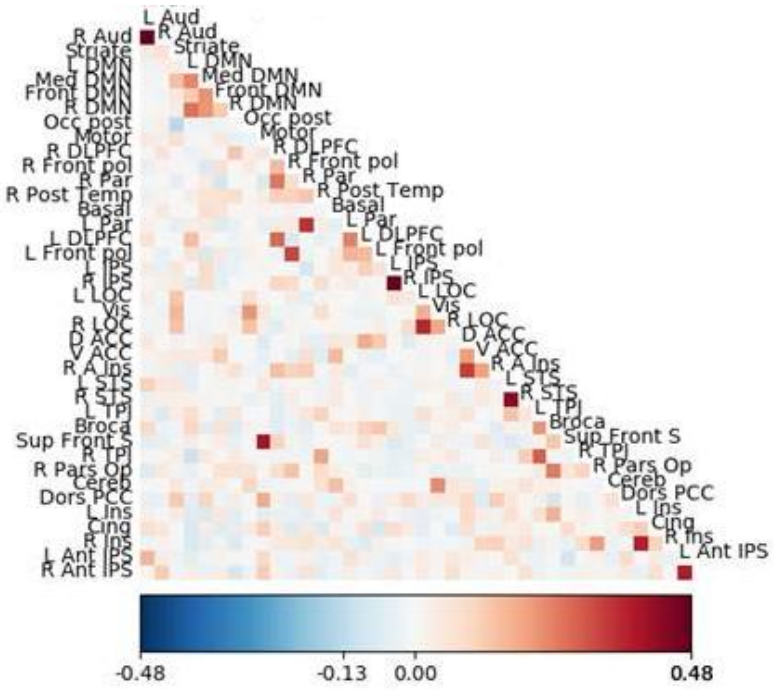

Figure 7.2*. Preliminary data of partial correlations among brain regions according to mean connectome for SCD-Plus. Blue color indicates negative correlation, red color positive correlation (Gómez-Ramírez et al., unpublished observations).*

Overall, the identification of SCD as a dynamic and stable marker of MCI and AD conversion is the major contribution of our investigation. This marker characterizes a stage in the preclinical AD flow and could be used in clinical settings for the early diagnosis of MCI. Currently, we are studying the neurophysiological underpinnings of this marker. The Neuroimaging Department of CIEN Foundation is working on a complementary experiment using resting state fMRI (Figure 7.2.). The hypothesis is that hyperconnectivity is a common network response to any neurological insult. During the earliest asymptomatic stages of AD, for instance the SCD stage, the combined effects of neuronal hyperconnectivity and increase in Aβ production culminates in the accumulation of Aβ deposits which ultimately cause synaptic dysfunction. This hyperconnectivity can be detected using connectivity maps in resting state fMRI and we expect that they correlate with the appearance and progression of SCD.

8. FINAL CONCLUSIONS

This research has focused on the analysis of the association between SCD and AD by means of three different and consecutive experiments. The main final conclusions derived from our research might be summarized in the following points:

1. Self-reported cognitive complaints have a multidimensional structure what means that there exist distinct types of cognitive and non-cognitive complaints beyond memory. We therefore support the notion that SCD is not a monolithic entity when it is measured by using questionnaires that involve a considerable number of different items.

2. Not all cognitive complaints have the same clinical value in distinguishing between cognitively healthy older adults and MCI. Specifically, complaints associated with episodic memory, executive functions and prospective memory seem to be greater in MCI than in controls.

3. In agreement with the SCD-I two profiles of SCD can be distinguished: i) SCD, when individuals report complaints to some extent in one or several cognitive domains; and ii) SCD-Plus, when subjects besides report complaints fulfilled a set of features related to a subjective decline in memory rather than other cognitive domain, onset of SCD within the last 5 years, 60 years of age at SCD onset, worries associated with SCD, and feelings of worse performance than others in the same age group.

4. When implementing the recommendations provided by the SCD-I on the terminology and methology for studying SCD, approximately 70% of older adults aged 70-85 show some type of cognitive complaint as measured in a community-based investigation such as the Vallecas Project. In particular, 61% of individuals can be classified as SCD and 9% as SCD-Plus.

5. The transition from a cognitively intact stage to MCI in just one year of follow-up is mediated by SCD. Thus, the conversion rate was especially high for SCD-Plus (19%) compared to No SCD (5%) and SCD (6%). Adjusted Cox proportional hazard regression models indicate that SCD-Plus has 4 times higher risk of fast conversion to MCI than those older adults who do not report SCD.

6. SCD is a reliable concept and has appropriate internal consistency over time when it is operationally defined and systematically measured.

7. The analysis of the SCD´s temporal dynamics for a three-year follow-up period highlights the existence of two main stages into the preclinical AD phase -No SCD and SCD- as well as a sub-stage called SCD-Plus that falls within SCD.

8. Progression may occur from No SCD to SCD, but oposite transitions from SCD to No SCD are very unlikely. Once an individual is at the SCD phase there may be a progression to a severe form of cognitive concerns, the sub-stage SCD-Plus, in which the risk of MCI is the highest compared to No SCD and SCD. Nevertheless, since the criteria for classifying SCD-Plus are very restrictive, permeability is possible between SCD and SCD-Plus and thus individuals might be gone through or back over time.

9. Our findings therefore provide evidence on the use of SCD as an earlier phase that precedes MCI through the AD continuum. We provide a theoretical tracking of the preclinical AD phase not in a static way instead we draw a dynamic transition of what is occuring across time.

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