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"COGNITIVE AND CLINICAL PHENOTYPE IN DEMENTIA WITH LEWY BODIES"

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Abstracts

This dissertation describes the research studies I have most occupied during my Ph. D course. Due to the limited number of studies addressing which clinical and cognitive features are most useful in diagnosing early dementia with Lewy bodies (DLB) (Jicha et al. 2010; Boeve et al. 2012) I initially focused on the visuo-constructional impairments in prodromal DLB (study 1); afterwards on visual-spatial and visual-perceptual deficits (study 2) in a different sample of prodromal DLB. Later I investigated which cognitive impairments could be predictive of DLB in regards to the memory domain (Study 3 and 4). Here below the abstract for each study.

Study 1. Assessment of visual-constructional deficits in MCI-DLB.

Introduction: Visual-constructional deficits are a prominent feature of dementia with Lewy bodies (DLB) that may contribute to the differential diagnosis with Alzheimer's Disease (AD). The analysis of the pentagon copy included in the MMSE could be a promising tool for the diagnosis of DLB since its early stages (Ala et al. 2001).

Aim: To assess the pentagon copy performance in prodromal stage of DLB with the Qualitative Scoring MMSE Pentagon Test (QSPT) (Caffarra et al., 2013).

Methods: 30 patients with non amnestic-Mild Cognitive Impairment diagnosed as prodromal DLB (MCI-DLB) and 23 patients with amnestic-MCI diagnosed as prodromal AD (MCI-AD) were enrolled. All patients obtained a MMSE score $\geq$ 26/30. The diagnosis of DLB and AD was confirmed at 3-year follow-up visit according to established criteria. Each MMSE test was examined with the QSPT which is based on the assessment of different parameters of the pentagon drawing. A broad standard neuropsychological assessment was also performed.

Results: The percentage of subjects who were unable to determine the correct number of angles in the pentagon copy test was 45.1% of MCI-DLB and 8.3% of MCI-AD patients (sensitivity 41.1%; specificity 91%). Attentive/executive functions and visual-spatial abilities were worse in the MCI-DLB group, while episodic memory impairment was greater in MCI-AD. Subtle extrapyramidal signs (63%) and RBD symptoms (56%) were the most frequent clinical features supporting the diagnosis of MCI-DLB.

Conclusions: We suggest that a poor performance in determining the number of angles when performing the pentagon copying test, together with the presence of subtle extrapyramidal signs and symptoms of RBD may serve as a predictive tool for early DLB.

Output of the study: This study has been published in parkinsonism and Related Disorders, 21(3), 303–305. (2015). *High specificity of MMSE pentagon scoring for diagnosis of prodromal dementia with Lewy bodies.* Cagnin, A., **Bussè, C.,** Jelcic, N., Gnoato, F., Mitolo, M., & Caffarra, P.

Study 2. Assessment of visual attention, visual-spatial and visual-constructional deficits in MCI-DLB.

Background: Patients with prodromal dementia with Lewy bodies (DLB) may display a different cognitive pattern from Alzheimer's disease (AD) with more severe impairment of attentive and visuo-spatial abilities (Mc Keith, 2005).

Objective: to investigate which cognitive functions could be predictive of the diagnosis of DLB and AD in patients with mild cognitive impairment (MCI).

Methods: Fifty-three patients with MCI were followed over 3-years until a diagnosis of DLB (MCI-DLB: n=25) and AD (MCI-AD: n=28) were made according to standard criteria. At the first assessment patients underwent a thorough cognitive assessment, including MMSE-QSPT (Caffarra, 2013;), attention, memory, executive functions, constructive apraxia, visuo-perceptual abilities (VOSP battery).

Results: The best clinical predictor of DLB was the presence of soft extrapyramidal signs (mean UPDRS score: 4.04 ± 5.9) detected in 72% of patients, followed by REM sleep behavior disorder (60%) and fluctuations (60%). Wrong performances in the pentagon's number of angles were obtained in 44% of DLB and 3.7% of AD patients and correlated with speed of visual attention. Executive functions, visual attention and visuospatial abilities were worse in DLB, while verbal episodic memory impairment was greater in AD. Deficits in the visual-perceptual domain were present in both MCI-DLB and AD.

Conclusions: Poor performance in the pentagon's number of angles is specific of DLB and correlates with speed of visual attention. The dorsal visual stream seems specifically more impaired in MCI-DLB with respect to the ventral visual stream, the latter being involved in both DLB and AD. These cognitive features, associated with subtle extrapyramidal signs, should alert clinicians to a diagnostic hypothesis of DLB.

Output of the study: This study has been published in Dementia and Geriatric Cognitive Disorders Extra 2015. *Clinical and Cognitive Phenotype of Mild Cognitive Impairment Evolving to Dementia with Lewy Bodies.* Cagnin, A., **Bussè, C.,** Gardini, S., Jelcic, N., Guzzo, C., Gnoato, F., et al. 5 (3), 442–9.

Study 3. Analysis of memory domain: specific verbal memory indices of the Rey's Auditory Verbal Learning Test.

Introduction: Several neuropsychological tests exploring episodic memory can't always give a careful contribution to the differential diagnosis of degenerative diseases. The serial position effects, rather than the traditional scores, along with some important learning characteristic measures, could contribute to address towards a specific type of dementia.

Objective: To comprehend if any of the specific measures of verbal memory obtained with the RAVLT, as the verbal learning (VL), verbal forgetting (VF) and the serial position effects, could be of value in distinguishing DLB from AD.

Method: thirty-two AD and twenty-nine DLB patients were enrolled in the study and followed longitudinally for 3 years until the diagnosis was made according standard criteria. Twenty-eight normal elderly subjects served as controls. All subjects underwent baseline neuropsychological assessment including RAVLT. Specific verbal memory measures were evaluated: verbal learning (VL) [immediate recall test: trial 5 *minus* trial 1], verbal forgetting (VF) [trial 6 of delayed recall *minus* trial 5 of immediate recall], percentage of verbal forgetting (VF%) [% verbal forgetting/trial 5] and the serial position effects of immediate recall (trial 1).

Results: The performances of DLB and AD were comparable in the immediate and delayed recall of the RAVLT (IC=95%). However, VL was higher in DLB than AD (DLB=0.25±0.1, AD=0.19±0.1; $p<0.05$) while VF% was greater in AD (AD=65.85%±41.3, DLB=42.97±33.0%; $p=0.001$). Logistic regression analysis showed that VF% may be considered a predictive marker of disease group allocation (T=-0.02, Wald $_{(1)}$=4.85, $p=0.03$). With a VF% cut-off ≥75%, AD and DLB patients were differently distributed (Choerish=5.1), being 58% of AD versus 21% of DLB above this cut-off. Considering the serial position effects, recency effect was significant higher in AD than DLB (AD=2.09±1.1, DLB=1.34±1.2; $p<0.05$). Number of recalled words in the recency domain correlated positively with scores of the digit span backward test ($r=0.54$, $p=0.005$) and digit span forward ($r=0.25$, $p=0.02$) only in DLB (digit span backward: AD: $r=0.88$, $p=0.88$; NC: $r=-0.02$, $p=0.94$; digit span forward: AD: $r=0.12$, $p=0.52$; NC: $r=0.24$; $p=0.22$).

Discussion: DLB patients showed different memory performances from AD at the RAVLT. In details, DLB had better performances of verbal learning and worse verbal forgetting and recency effect. These specific measures of verbal memory could be used as diagnostic marker in the differential diagnosis between DLB and AD.

Output of the study: This study is in submission.

Study 4. Analysis of memory domain in mild cognitive impairment: investigating the efficacy of the Free and Cued Selective Reminding Test (FCSRT).

Objective: The main goal was to comprehend the efficacy of the Free and Cued Selective and Reminding Test (FCSRT) in differentiating patients with mild cognitive impairment converting to dementia with Lewy bodies (MCI-DLB) from patients with MCI due to Alzheimer's disease (MCI-AD).

Materials and methods: Thirty-five participants with MMSE≥26 were included in the study. Fifteen were diagnosed as probable DLB (MCI-DLB: n=15) and twenty as probable AD (MCI-AD: n=20) according to current criteria (Ferman et al. 2013; Albert et al. 2011). Patients underwent a comprehensive cognitive evaluation including the FCSRT for the episodic memory assessment.

Results: At the FCSRT, MCI-DLB were significantly more spared than MCI-AD regarding the total memory recall (ITR) and the index of sensitivity of cueing (ISC) (ITR: DLB=35.13±1.26, AD=29.95±1.08, p=0.01; ISC: DLB=0.94±0.04, AD=0.76±0.04, p=0.00). Moreover, MCI-DLB performed worse than MCI-AD in digit cancellation task (DLB=45.49±1.43; AD=49.83±1.22; p=0.03), number of angles of Mini Mental (MMSE) pentagons copy (DLB=3.11±0.17; AD=3.72±0.15; p=0.01) and Rey figure copy (DLB=23.77±1.47; AD= 27.90±1.26; p= 0.05).

Discussion: At early stage DLB showed to benefit more than AD from the controlled learning through category cues, exhibiting a greater ISC. MCI-DLB showed poorer performances in attentive and visuo-constructional tasks respect to AD.

Conclusions: The FCSRT has shown its utility in the distinction between DLB and AD at early stage increasing the diagnostic accuracy. Moreover, since the main characteristic of the FCSRT is to assess verbal episodic memory isolating the storage capacities of the patients, we exclude a storage memory impairment in MCI-DLB patients.

Output of the study: This study is in preparation.

Introduction

Dementia with Lewy bodies (DLB) is the second most common cause of neurodegenerative dementia after Alzheimer's disease (AD). To improve the differential diagnosis of DLB, consensus criteria have been developed establishing possible and probable levels of clinical diagnostic accuracy (McKeith et al. 2005). However, to diagnose DLB is still difficult, given that the diagnostic criteria has a sensitivity of 32% for pure DLB and it is even lower for mixed DLB and AD pathology (Nelson et al. 2010).

The focus of growing clinical research is the detection and characterization of cognitive deficits associated with DLB, often in the differential diagnosis with AD. Several studies suggested that DLB patients have greater executive, attention and visuospatial dysfunctions than AD patients. (Kondo *et al.*, 2016; Ferman *et al.*, 2013; Guidi *et al.*, 2006). However, relatively little is known about the profile of neuropsychological deficits in DLB regarding different aspects of memory, and limited information is available about prodromal DLB state.

As stated by Ferman *et al.* (2013), when dementia severity is mild or mild to moderate, cognitive comparisons reveal a consistent dissociation between clinically probable AD and DLB. The interest is now driven by the need to accurately detect the onset of cognitive changes and to differentiate among neurodegenerative disorders with overlapping features.

Considering DLB, the distribution of neuropathologic changes may be quite similar to AD, so it is not surprising that the two disorders may result in similar dementia syndromes. Moreover, the two neuropathological processes (amyloid and alpha-synuclein deposition) may be present in association in brains of elderly patients showing comorbid conditions. Memory impairment is often an earliest features of both and, due to these similarities, patients with DLB are often clinically diagnosed as having a probable or possible AD.

Given the difficulty in clinically differentiating DLB form AD, the aim of this thesis is to better comprehend the cognitive profile of patients with DLB, especially in the early stages of the disease, when the diagnosis of dementia is not yet full-blown and cognitive difficulties are still very subtle and not easily detectable.

The main goal was to investigate which early aspects of cognitive deficits in DLB could better contribute to a differentiation from AD. Since standard neuropsychological tests may often not have a discriminative property in the mild stage of DLB, specific neuropsychological tests as well as particular sub-items of these instruments have been carefully analyzed and selected among a wide range of cognitive tools.

To this purpose, this thesis has been organized as follows:

- ✓ In **chapter 1** the definition of dementia has been introduced and the description of both DLB and AD pathologies through a clinical perspective is addressed.
- ✓ In **chapter 2** the concept of mild cognitive impairment (MCI), not yet fully defined in DLB, is explained.
- ✓ **Chapter 3** includes the studies conducted during the ph.D period (two of which have already been published, one is *submitted* and the last is in preparation for final submission), which are here summarized:
    - **In study 1 visual-constructional deficits** were evaluated in MCI-DLB respect to MCI-AD, focusing on the application of a large-scale tool used at the screening phase (Mini Mental State Examination - MMSE). In particular the MMSE crossed pentagons item, which was qualitatively analyzed through the new Qualitative Scoring Pentagons Test (QSPT), was studied as sensitive instrument for detecting DLB during the MCI phase. The aim of this study was to verify the hypothesis that testing visuo-construction abilities with the QSPT in the early stage might be a useful and valid tool for differentiating between DLB and AD.
    - **In study 2 visual-spatial, visual-constructional and visual-perceptual aspects** have been separately investigated using a specific and wide cognitive battery (Visual Object and Space Perception Battery - VOSP, Warrington et al. 1991) whose tasks are related to the involvement of different cortical regions in MCI DLB patients.

      The aim of this study was to assess which aspects of visual spatial and perception impairments could be better diagnostic of DLB at the early stages and which is associated with the performance at the QSPT.

      The results increased our understanding of the relationship between the sub-components of the visuospatial domain, exploring which specific aspects might best distinguish between DLB and AD at the early stage. We were able to define that the visuoconstructional and spatial abilities are most specifically impaired in DLB, while object perception deficits, although very sensitive, are not specific cognitive signatures among DLB and AD in the prodromal stage. None of the sub-components of visual abilities associated with the performance at the QSPT.
    - In **studies 3 and 4** the aims shifted on the investigation of memory disorders in patients with DLB. In fact, episodic memory has been much investigated in AD as one of the core cognitive deficits, while little was known concerning DLB. Through a widely-used "list of words" test (Rey's Auditory Verbal Learning Test - RAVLT) specific measures of verbal memory calculated from the RAVLT aimed to discriminate DLB from AD

(study 3). In another population of patients a new instrument (Free and Cued Recall Test - FCSRT) never tested before in the MCI-DLB patients was studied (study 4). The FCSRT has been already described as a valid tool in distinguishing patients with AD from patients with non-AD dementias (Grober *et al.*, 2010) and to predict the progression to AD in a group of MCI (Lemos *et al.*, 2015; Sarazin *et al.*, 2007). We mainly aimed to comprehend the discriminative value of the FCSRT in a very early pre-dementia stage.

- ✓ **In Chapter 4** are present the discussion and conclusion of the results of the studies.

1. Evaluation of dementia

Dementia is a disorder characterized by a decline in cognition involving one or more cognitive domains (learning and memory, executive function, complex attention, perceptual-motor, social cognition, language). A necessary feature that characterizes dementia regards the presence of deficits which must represent a decline from previous level of function and has to be severe enough to interfere with normal life functioning and independence. This characterization of dementia reflects a 2013 revision of earlier definitions, which is discussed in more detail below (DSM-V, American Psychiatric Association, 2013).

With aging, the burden of dementia is universally increasing. Considering the United States in 2012, an estimated 5.2 million individuals over 65 years had AD, a number that is expected to reach 6.7 million by 2025 (Hebert et al. 2003). Detecting dementia is a common problem in clinical practice. In fact, as we will see, memory loss does not represent a necessary feature from patients. It is often a relative who takes the problem to clinician attention. Nevertheless, family members are often delayed in recognizing the signs of dementia presence, usually inaccurately ascribed to "aging."

Clinicians must diagnose it accurately and manage especially the early cognitive manifestations, with particular regarding new pharmacological treatments. The pretest probability of dementia could be dependent on patient characteristics such as age and race and it is estimated to be at least 60 percent in elderly with reported memory loss.

In this sense a comprehensive neuropsychological assessment could be useful in identifying and evaluate patients with dementia. According to DSM-V released in 2013, the criteria for dementia (or major neurocognitive disorder) include:

● Evidence from the history and clinical assessment indicating significant cognitive impairment in at least one of the following cognitive domains:

- Learning and memory
- Language
- Executive function
- Complex attention
- Perceptual-motor function
- Social cognition

● Impairment(s) must be acquired and represent a significant decline from a previous level of functioning.

● The cognitive deficits must interfere with independence in everyday activities.

● In the case of neurodegenerative dementias, such as Alzheimer disease, the disturbances are of insidious onset and progressive, based on evidence of history or serial mental-status examinations.

● The disturbances are not occurring exclusively during the course of delirium.

● The disturbances are not better accounted for another mental disorder (i.e., major depressive disorder, schizophrenia).

In all previous DSM definitions of dementia, memory is an essential feature for the diagnosis. The definition of DSM-V differs substantively from prior versions, as the cognitive domains have been renamed and expanded to include social cognition and attention. In addition, the centrality of memory dysfunction as sine qua non conditio has been resized, with the same weight of all cognitive domains. The change from DSM-IV to DSM-V probably reflects the fact that some types of non-AD dementia did not meet definitional criteria because of an intact or relatively intact memory.

It is important for a clinician to distinguish dementia from delirium and depression (Jorm et al. 1993):

● Delirium is usually acute or subacute onset and associated with a clouding of the sensorium; delirium and dementia can often overlap.

● Patients with depression tend to complain more memory problems than people with dementia and often come alone. Also they give poor effort on testing ("I just can't do this") and present psychomotor slowing. Depression and dementia can both coexist.

It is also very important to distinguish the different forms of dementia: the major dementia syndromes include (Morris et al.2003) Alzheimer disease (AD), dementia with Lewy bodies (DLB), Frontotemporal dementia (FTD), and Vascular (multi-infarct) dementia (VaD), Parkinson disease with dementia (PDD).

Other less common disorders include progressive supranuclear palsy (PSP) and Huntington disease can be associated with dementia. Comorbidities and "mixed dementias" exacerbating poor cognition are common in elderly patients with dementia.

Diagnostic process

The initial phase in the evaluation of a patient with suspected dementia should focus upon the history given by family members or other who knows the patient well. They could be helpful resources for understanding adequately any type of cognitive and behavioral changes (Knopman et al. 2003).

The assessment of cognitive functions should be arranged with a complete physical examination and neurological examination. The subsequent work-up may include laboratory and imaging studies (Geldmacher et al. 1996¸ Karlawish et al. 2003). Cognitive testing batteries may be useful in identifying patients with dementia, especially if administered to those who may be at risk of cognitive impairment. Screening for B12 deficiency and hypothyroidism and for depression in patients with dementia is recommended. Genetic testing for the apolipoprotein E epsilon 4allele is not currently recommended, nor is genetic testing for other potential causes of dementia.

The use of neuroimaging is still controversial. Some guidelines that have been published do not recommend imaging studies routinely, but may include clinical prediction rules to identify patients that can be diagnosed with imaging studies (i.e., subdural hematoma, normal pressure hydrocephalus, treatable cancer) (Knopman et al. 2001). The prediction rules vary, including factors such as younger age (<60 years), focal signs, short duration of symptoms. However, the sensitivity and specificity of these prediction rules is low (Gifford et al. 2000). The American Academy of Neurology (AAN) recommends structural neuroimaging with either a noncontrast head CT or MRI in the initial evaluation routine (Knopman et al. 2001). The use of positron emission tomography (PET) and other functional neuroimaging techniques in differential diagnosis of neurodegenerative dementia is under investigation. Identification of the underlying etiologic subtype (i.e., Alzheimer dementia, dementia with Lewy bodies, etc.) is then necessary for the treatment approach.

Neuropsychological testing

Many clinicians make use of short standardized mental status scales to document the presence and progression of dementia. The Montreal Cognitive Assessment (MoCA) (Nasreddine et al 2005) is a brief screening assessment tool very sensitive to executive and language dysfunctions compared with other brief tests such as the Mini-mental state examination (MMSE).

Compared to the MMSE, the MoCA is more sensitive for the detection of mild cognitive impairment including a wider range of items for different cognitive domains, i.e. delay memory recall, language, attention, visuospatial and executive functions. The typical cut-off score for normal performance on the MoCA is 26 (i.e., 25 and below is considered abnormal). Cut-offs should be adjusted based on appropriate norms, including education adjustment (Rossetti et al. 2011). The role of neuropsychological assessment may be clear to help in differentiating among the different forms of neurodegenerative diseases, especially in early onset, involving extensive evaluation of multiple cognitive domains.

In 2001 the AAN reviewed a number of studies of neuropsychological testing for dementia and concluded the utility of neuropsychological batteries in identifying patients with dementia, particularly those with higher risk of memory impairment (Petersen et al. 2001).

However, while various causes of dementia can affect preferentially different cognitive functions, it has been demonstrated the limited utility of neuropsychological testing for differentiating among the cause of dementia, as there are substantial overlaps in test performance (Hutchinson et al. 2007). Recent efforts are still aimed in recognizing cases of dementia with Lewy bodies (DLB) having coexisting Alzheimer pathologies (AD) (McKeith et al. 2016). In this regard, the aim of my research was to contribute to better identify DLB patients through cognitive assessment. Given the overlap in the cognitive performance of patients diagnosed with DLB and AD, cognitive tests have been selected cautiously, in conjunction with medical history, clinical information, neurological evaluation, behavioral observations and information from relatives.

1.1 Dementia with Lewy bodies

Dementia with Lewy bodies (DLB) is clinically recognized as the second most common type of degenerative dementia after Alzheimer disease (AD). Since the first descriptions of the 1960s DLB ha been always presented as a mixed clinical pathology with clinical and cognitive features shared with other forms of degenerative dementias.

Because of the complexity in identifying cortical Lewy bodies (LB) nowadays it is still challenging to define DLB as a distinct entity differentiable from other degenerative disorders. Although DLB is still often under recognized, the diagnostic clinical criteria continue to be refined to increase specificity and sensitivity.

Consensus criteria for the diagnosis were developed by the third report of DLB Consortium and have been successively revised to improve sensitivity and specificity (McKeith et al. 2005). Table 1 outlines the clinical diagnostic criteria for probable and possible DLB.

Central features

The presence of *dementia* is necessary to diagnose DLB. Other clinical manifestations are structured into a hierarchy of diagnostic specificity of core, suggestive, and supportive features.

DLB is characterized by early *impairments in attention, executive and visuospatial functions.* Unlike Alzheimer's disease (AD), which typically presents memory loss as its first and most prominent cognitive deficit, DLB *memory impairments* are affected later during the course of the disease (Salmon et al. 1996, Ala et al.2001). Bedside tests of cognitive functions are not enough reliable and specific to distinguish between types of dementia. In particularly, neuropsychological tests, while helpful in quantifying and qualifying neurologic deficits, may not have clear discriminative capacity for DLB and AD syndromes.

It has been widely known that in DLB early signs on neuropsychological testing may include deficits in visual-spatial and visual-perception abilities (Ala et al. 2001; Mosimann et al. 2004; Bradshaw at al. 2006). However, measures of attention and executive functions (i.e., Trail-Making Test, Wisconsin Card-Sorting Test, letter and category verbal fluency tests) may also be impaired early in AD (Mormont et al. 2003). When memory becomes impaired in DLB, memory retrieval may be more affected than acquisition or encoding (McKeith et al. 1996). DLB patients with prominent AD pathology (i.e., neurofibrillary tangles) may have a cognitive profile that is more characteristic of AD. Differences in cognitive patterns of the two pathologies are described further in a specific section.

TABLE 1 DIAGNOSTIC CRITERIA FOR DLB (MCKEITH ET AL. 2005).

1. **Central features** (essential for a diagnosis of possible or probable DLB).
- Dementia defined as progressive cognitive decline of sufficient magnitude to interfere with normal social or occupational function
- Prominent or persistent memory impairment may not necessarily occur in the early stages but is usually evident with progression
- Deficits on tests of attention, executive function, and visuospatial ability may be especially prominent

2. **Core features** (two core features are sufficient for a diagnosis of probable DLB, one for possible DLB).
- Fluctuating cognition with pronounced variations in attention and alertness
- Recurrent visual hallucinations that are typically well formed and detailed
- Spontaneous features of parkinsonism

3. **Suggestive features** (If one or more of these is present in the presence of one or more core features, a diagnosis of probable DLB can be made. In the absence of any core features, one or more suggestive features are sufficient for possible DLB. Probable DLB should not be diagnosed on the basis of suggestive features alone).
- REM sleep behavior disorder
- Severe neuroleptic sensitivity
- Low dopamine transporter uptake in basal ganglia demonstrated by SPECT or PET imaging

4. **Supportive features** (commonly present but not proven to have diagnostic specificity).
- Repeated falls and syncope
- Transient, unexplained loss of consciousness
- Severe autonomic dysfunction, e.g., orthostatic hypotension, urinary incontinence Hallucinations in other modalities
- Systematized delusions
- Depression
- Relative preservation of medial temporal lobe structures on CT/MRI scan
- Generalized low uptake on SPECT/PET perfusion scan with reduced occipital activity Abnormal (low uptake) MIBG myocardial scintigraphy
- Prominent slow wave activity on EEG with temporal lobe transient sharp waves

5. A diagnosis of DLB is **less likely.**
- In the presence of cerebrovascular disease evident as focal neurologic signs or on brain imaging
- In the presence of any other physical illness or brain disorder sufficient to account in part or in total for the clinical picture
- If parkinsonism only appears for the first time at a stage of severe dementia

6. **Temporal sequence of symptoms.**

DLB should be diagnosed when dementia occurs before or concurrently with parkinsonism (if it is present). The term Parkinson disease dementia (PDD) should be used to describe dementia that occurs in the context of well-established Parkinson disease. [..]

Core clinical features

In addition to dementia, a patient with probable DLB must have at least two of three of "core clinical features" of DLB: cognitive fluctuations, visual hallucinations (VHs), and parkinsonism. (Table1).

Fluctuations

Fluctuations in cognition and levels of vigilance are estimated to be in 60-80% of cases (McKeith et al. 1996). Fluctuations are quite varied in patients and different for severity, duration and type of symptoms. In fact, episodes of fluctuations can be sometimes subtle, as in a transitory failure to perform an activity of daily living, or may be intense enough to bring into question the hypothesis of stroke. Usually fluctuations are described by the familiars as episodes in which patients become confused or behave in a bizarre manner, appear to "blank out" or lose consciousness, have speech or motor arrest, or become extremely sleepy or lethargic. These episodes can last seconds to days and can be alternated with usual functioning.

The identification of fluctuations is very hard to assess. Caregivers may not volunteer these features and often it is probable to elicit false positive responses (Bradshaw at al. 2004). Some structured questionnaires (i.e., Clinical Assessment of Fluctuation, One Day Fluctuation Assessment Scale) could help in the detection. Given that, one or more example of a fluctuations episode with specific descriptive details may be recommended (Walker et al. 2000).

Fluctuating cognition is qualitatively distinct in DLB and AD. DLB fluctuations are more often spontaneous and episodic, apparently related to an interruption of awareness or attention and impact everyday functional ability (Walker et al. 2000). Fluctuations in AD are usually described unclearly as "good days and bad days" and are often explained by external stressors. Episodes that include at least three of the four following features (daytime sleepiness, daytime naps lasting more than two hours, prolonged staring spells and episodes of disorganized speech) are more likely to occur in patients with DLB than with AD (Bradshaw et al. 2000).

Visual hallucinations

Visual hallucinations (VHs) occur in about two-thirds of DLB patients and are relatively rare in AD (Galvin et al. 2006). VHs usually appear early in DLB and may precede parkinsonism. In Tiraboschi et al. (2006) VHs at presentation were the best positive predictor of DLB at autopsy in respect to AD (positive predictive value: 83% versus 32% or less for all other variables) while lack of visuospatial impairment was the best negative predictor (negative predictive value: 90%). Among DLB patients, those with VHs appear to have more severe deficits in visual attention and

executive function compared with those without VHs, but similar degrees of visuospatial and visual-perceptual impairment. Visual attention is the main cognitive determinant for the genesis of VHs (Cagnin et al. 2013).

Descriptions of VHs often concern both well-formed images of people or animals and more abstract visions, such as shapes or colors. Patients could range from simple to extremely complex descriptions, such as seeing something briefly out of the corner of their eye, or an ongoing dialog with a dead loved one. Patients may also describe visual misperceptions, such as moving objects zooming toward or away or shape changing.

Even for fluctuations, if not specifically solicited, VHs are often under reported. Patients may or may not have insight into the nature of the hallucinations and reactions may vary from fear to indifference.

Parkinsonism

In DLB parkinsonian symptoms are brady and akinesia, limb rigidity and/or gait disorder. Frequency is estimated in approximately 70 to 90% of patients. Furthermore, parkinsonian features can be as severe as in idiopathic Parkinson disease (PD) (Galvin et al. 2006). Tremor may occur in DLB but less frequently and less severe than it occurs in PD (Burn et al. 2006). Despite these features are observed in clinical practice, there is no individual characteristic symptom that reliably distinguishes the motor parkinsonism of PD versus DLB.

Suggestive features

These features include REM sleep disorder, severe neuroleptic sensitivity and low dopamine transporter uptake in the basal ganglia on single photon emission computed tomography (SPECT) or positron-emission tomography (PET). The presence of one of these in combination with one core clinical feature supports the diagnosis of probable DLB. The presence of one or more suggestive features in the absence of a core clinical feature suggests possible DLB (table 1).

REM sleep behavior disorder

Rapid eye movement (REM) sleep behavior disorder (RBD) is a parasomnia characterized by the intermittent loss of electromyography atonia normally present during REM sleep and the emergence of purposeful complex motor activity associated with vivid dreams. During REM sleep subjects may produce recurrent sleep-related vocalization and/or complex motor behaviors correlating with dream mentation. RBD movements do not last long in time (less than 60 seconds) and appear very focused such throwing a ball or being not able to protect oneself. Sometimes they could be benign

hand gestures but also violent punching and kicking. Sleep-related injuries could appear as jumping out of bed or striking a bed partner. RBD is commonly related with DLB in 85 % of persons and may be a prodromal symptom of the neurological disease (Ferman et al. 2011). RBD can precede the clinical diagnosis by up to 20 years. Some autopsy-confirmed data proposes the inclusion of RBD as a core clinical rather than a suggestive feature to improve the diagnostic accuracy (Boeve et al. 2002). The management of sleep disturbances in patients with dementia is a complicated and enormously important clinical and community problem. All patients with RBD and bed partners should be counseled to modify the sleeping environment in order to prevent injury.

Other sleep disorders may include insomnia, daytime hyper somnolence, sleep apnea (obstructive or central), periodic limb movements of sleep and restless legs syndrome/Willis-Ekbom disease (Ferman et al. 2014). In one recent study daytime sleepiness is more likely to occur in persons with DLB than in those with AD (81 % DLB versus 39 % AD) (McKeith et al. 1992).

Severe neuroleptic sensitivity

Approximately 30 to 50% of patients with DLB have severe sensitivity to neuroleptics and this feature has been included in clinical diagnostic criteria for this type of dementia (Walker et al. 1999). Acute reactions include severe and sometimes irreversible parkinsonism and impaired consciousness, even in individuals without parkinsonism at baseline. Severe sensitivity to neuroleptics is not dose-related. Neuroleptic medications may also precipitate or worsen confusion or autonomic dysfunction, and their use has been associated with a two to three-fold increase in mortality. Severe reactions to neuroleptics are less common in patients with Parkinson disease and have not been described in AD. Despite the high specificity of this finding, deliberate pharmacologic challenge as a diagnostic strategy is obviously very imprudent.

Supportive features

The following clinical features are common in DLB but do not have clear diagnostic specificity (Table 1).

Repeated falls

Recurrent falls occur in more than one third of patients with DLB and may be among the earliest symptoms (Horimoto et al. 2003). Falls may seem to occur with or without provocation and could be related to parkinsonism, cognitive fluctuations or orthostatic hypotension.

Syncope or transient loss of consciousness

Episodes of altered or loss of consciousness are very commonly labeled. Patients may lose consciousness transiently, or they may be awake but mute and staring blankly. Episodes may even resemble cataplexy, in which patients develop sudden atonia and fall to the floor.

These could be a result of orthostatic hypotension, which has been reported in 28 to 50% of patients, and which can be severe enough to mimic multiple systems atrophy (Kenny et al. 2004). Episodes may also represent an extreme cognitive fluctuation or may be analogous to the motor "freezing" seen in idiopathic PD. Other etiologies such as seizures, stroke, transient ischemic attack, or cardiac arrhythmia should also be ruled out.

Autonomic dysfunction

Autonomic dysfunction is frequent in DLB and may include urinary incontinence or retention, constipation, other gastrointestinal symptoms and impotence (Kenny et al. 2004). Urinary incontinence is often an early sign in DLB (Akaogi et al. 2009) and occurs at late stages of AD when dementia is severe.

Hallucinations in other modalities

DLB patients may experience also hallucinations in other modalities, as auditory or olfactive. Auditory hallucinations may be well formed, such as hearing identifiable speech or music, or they may be less distinct, such as having the impression of hearing a television, voice, or telephone ringing in another room. Olfactory hallucinations can be pleasant (i.e., flowers or food) or unpleasant. Patients have described also tactile hallucinations such as the feeling of insects on their skin or a cat brushing against their leg. The presence of these hallucinations may also prompt a workup for partial seizures, psychotic disorders or substance intoxication.

Systematized delusions

Delusions (false, fixed beliefs) occur commonly in DLB (in as many as 75% of cases) and may be elaborate, specific and systematic (Onofrj et al 2010). Delusions are often embedded in hallucinations or visual misperceptions that the patient experiences. Common themes may include: the partner or caregiver as an impostor, the house as not their home, people in the television or mirror speaking to them. Somatoform disorder is a related but distinct psychiatric syndrome that may be observed in patients with DLB.

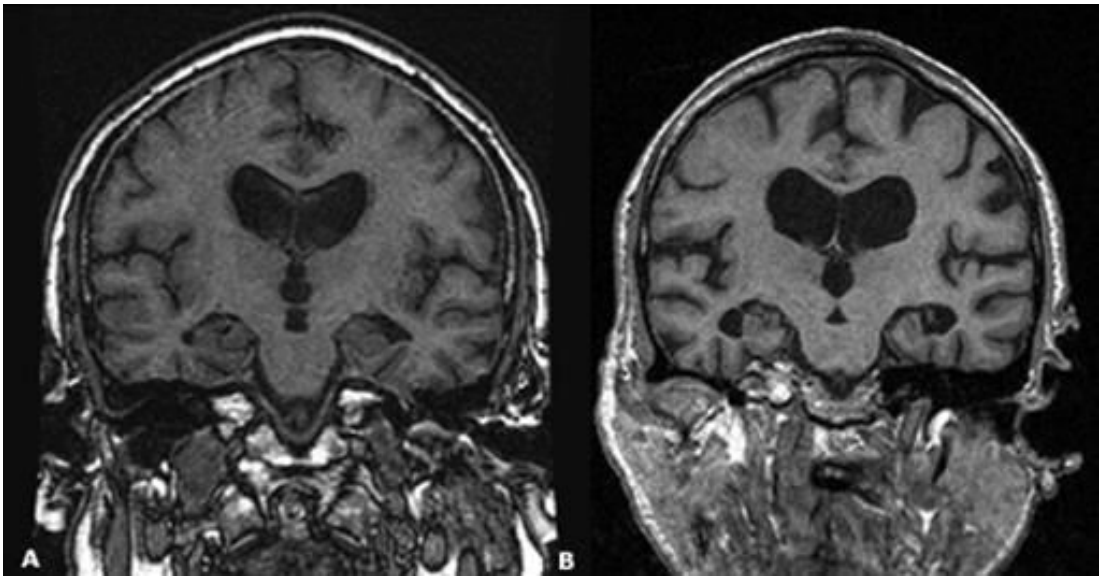
Depression

Most patients with DLB experience depressive symptoms. Up to 40% have a major depressive episode. This is higher in one study than the rate seen in AD (Burton et al. 2002). Depression is less likely than other features of DLB to persist over time (Onofrj et al 2010).

Radiologic features

Radiologic features are not essential but may provide supporting evidence to the diagnosis of DLB. Magnetic resonance imaging **(MRI)** may identify patterns of regional atrophy specific for DLB, even if generalized atrophy and white matter lesions are nonspecific findings in dementia. In particular, MRI coronal sections through the hippocampi usually show a greater degree of hippocampal atrophy in Alzheimer disease (AD) compared with DLB (Figure 1) (Beyer et al. 2007). Volumetric analyses of MRI scans have also suggested atrophy of the putamen and dorsal mesopontine gray matter in DLB compared with AD (Beyer et al. 2007). Despite the changes in the occipital lobe on functional imaging discussed below, regional occipital atrophy is generally not observed on MRI in DLB. However, despite a similar level of dementia severity, patterns of DTI changes in DLB and AD differed significantly. The selective involvement of the visual association areas and subcortical structures and the significant clinical correlations highlight the potential importance of white matter tract change in the pathogenesis of DLB (Mirzaei et al. 2003).

FIGURE 1 MRI APPEARANCE OF DLB COMPARED TO AD. THIN SECTION CORONAL T1 WEIGHTED IMAGES FORM 61 YEAR OLD MALE WITH PATHOLOGICALLY DLB (A) AND A 69 YEAR OLD MALE WITH AD (B). THERE IS RELATIVE PRESERVATION OF THE MEDIAL TEMPORAL LOBES AND HIPPOCAMPAL STRUCTURES IN DLB PATIENT AS COMPARED WITH THE AD PATIENT. MRI: MAGNETIC RESONANCE IMAGING.



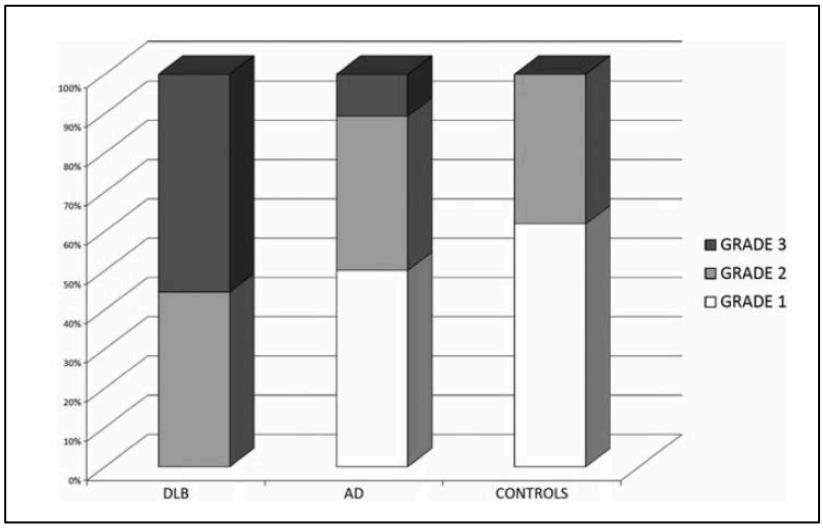
Single-photon emission computed tomography **(SPECT)** and positron emission tomography **(PET)** scans show generalized decreased perfusion and metabolism, respectively, which is most noticeable in the occipital areas. The changes in the occipital lobe appear, at least in small series, to have potential diagnostic utility in DLB, with a sensitivity and specificity for SPECT of 65 and 87% and for PET of 90 and 80% (Pasquier et al. 20002; Firbank et al. 2016).

In another series relationship between hallucinations and FDG-PET in DLB was found: reduced metabolism, frequently seen in the occipital lobes, correlated with the frequency and severity of visual hallucinations (Hu et al. 2000). Using specific ligands for dopamine transporter, SPECT and PET studies have demonstrated low dopaminergic activity in the striatum in DLB (Walker et al. 2002; McKeith et al 2007).

A study in 326 patients with dementia reported a sensitivity of 78% and a specificity of 90% of ioflupane I-123 dopamine transporter SPECT imaging (DaTscan) in DLB (Donaghy et al 2015). This test result is considered a suggestive feature in the diagnosis of probable or possible DLB (Table 1). However, this technique is not widely available for clinical use. Amyloid PET shows increased binding in a subset of patients with DLB and may correlate with cognitive impairment, although further studies are needed (Yoshita et al 2001).

An emerging technique widely used to visualize alterations in the substantia nigra (SN) in Parkinson's disease is the Transcranial Sonography **(TCS)** of the brain parenchyma. Only few studies have been carried out on SN echogenicity in DLB and PD patients showing bilateral and symmetrical marked SN enlargement in 80% of DLB and in one-third of PD patients, whereas in the latter group an asymmetric SN hyperechogenicity was found more frequently (Walter et al. 2006).

FIGURE 2 THE HISTOGRAMS REPRESENT THE DIFFERENT DISTRIBUTION OF SUBSTANTIA NIGRA ECHOGENIC SIZE (NORMAL, MODERATE AND MARKED HYPERECHOGENICITY) AMONGST AD, DLB AND CONTROLS.



In a recent study of our group (**Figure 2**) TCS revealed specific SN echogenic alterations (SN hyperechogenicity) in 100% of DLB compared to 50% AD and 30% controls. This study validates TCS as a effective supportive tool to distinguish DLB from AD at early stages (Favaretto el at. 2016).

1.2 Alzheimer disease

Alzheimer disease (AD) is the most common neurodegenerative disorder (Ballard et al. 2011). AD is typically diagnosed in the eighth or ninth decade of life but early onset forms of the disease may appear even in the fifth decade. Average survival is about 10 years but varies widely depending on the age of onset, the severity of cognitive impairment, the presence of comorbidities and on other factors (DSM-V, 2013).

The diagnosis of AD depends on the clinical criteria outlined below. The role of laboratory and imaging investigations is mainly to exclude other diagnoses. Neuropsychological testing may provide confirmatory information and aid in patient management. Biomarker data can be supportive of the diagnosis of AD and is most useful in patients with atypical clinical presentations or early-onset disease. Definitive diagnosis of AD requires histopathologic examination.

Criteria for the diagnosis of probable AD dementia have been established by the National Institute on Aging and the Alzheimer's Association (NIA-AA) and most recently updated in 2011 (McKhann, 2011).

Probable AD dementia: Core clinical criteria (McKhann et al .2011)

Probable AD dementia is diagnosed when the patient:

1. Meets criteria for dementia **(a)** and in addition, has the following characteristics **(b):**

(a) The diagnosis of dementia. Dementia is diagnosed when there are cognitive or behavioral (neuropsychiatric) symptoms that:

1. Interfere with the ability to function at work or at usual activities; and
2. Represent a decline from previous levels of functioning and performing; and
3. Are not explained by delirium or major psychiatric disorder;
4. Cognitive impairment is detected and diagnosed through a combination of (1) history-taking from the patient and a knowledgeable informant and (2) an objective cognitive assessment, either a “bedside” mental status examination or neuropsychological testing. Neuropsychological testing should be performed when the routine history and bedside mental status examination cannot provide a confident diagnosis.
5. The cognitive or behavioral impairment involves a minimum of two of the following domains: a. Impaired ability to acquire and remember new information—symptoms include: repetitive questions or conversations, misplacing personal belongings, forgetting events or appointments, getting lost on a familiar route. Impaired reasoning and handling of complex tasks, poor judgment–symptoms include: poor understanding of safety risks, inability to

manage finances, poor decision-making ability, and inability to plan complex or sequential activities. c. Impaired visuospatial abilities—symptoms include: inability to recognize faces or common objects or to find objects in direct view despite good acuity, inability to operate simple implements, or orient clothing to the body. d. Impaired language functions (speaking, reading, writing)—symptoms include: difficulty thinking of common words while speaking, hesitations; speech, spelling, and writing errors. e. Changes in personality, behavior, or comportment— symptoms include: uncharacteristic mood fluctuations such as agitation, impaired motivation, initiative, apathy, loss of drive, social withdrawal, decreased interest in previous activities, loss of empathy, compulsive or obsessive behaviors, and socially unacceptable behaviors.

b. Following characteristics:

A. Insidious onset. Symptoms have a gradual onset over months to years, not sudden over hours or days;

B. Clear-cut history of worsening of cognition by report or observation;

C. The initial and most prominent cognitive deficits are evident on history and examination in one of the following categories.

   a. Amnestic presentation: It is the most common syndromic presentation of AD dementia. The deficits should include impairment in learning and recall of recently learned information. There should also be evidence of cognitive dysfunction in at least one other cognitive domain.

   b. Non-amnestic presentations:

      - Language presentation: The most prominent deficits are in word-finding, but deficits in other cognitive domains should be present.
      - Visuospatial presentation: The most prominent deficits are in spatial cognition, including object agnosia, impaired face recognition, simultanagnosia, and alexia. Deficits in other cognitive domains should be present.
      - Executive dysfunction: The most prominent deficits are impaired reasoning, judgment, and problem solving. Deficits in other cognitive domains should be present.

D. The diagnosis of probable AD dementia should not be applied when there is evidence of (a) substantial concomitant cerebrovascular disease, defined by a history of a stroke temporally related to the onset or worsening of cognitive impairment; or the presence of multiple or

extensive infarcts or severe white matter hyperintensity burden; or (b) core features of Dementia with Lewy bodies other than dementia itself; or (c) prominent features of behavioral variant frontotemporal dementia; or (d) prominent features of semantic variant primary progressive aphasia or non-fluent/agrammatic variant primary progressive aphasia; or (e) evidence for another concurrent, active neurological disease, or a non-neurological medical comorbidity or use of medication that could have a substantial effect on cognition.

The Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria for AD are also commonly used. Criteria for AD were revised *(American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V), 2013)* (Table 2).

The DSM-V definition of probable AD (now called major neurocognitive disorder due to AD) differs from prior versions in that the cognitive domains have been renamed and expanded to include learning and memory, language, executive function, complex attention, perceptual-motor and social cognition. Previously, the criteria recognized five domains (memory, aphasia, apraxia, agnosia, and executive function). Like prior versions, the criteria continue to require both memory impairment and evidence of decline in at least one other cognitive domain. New to the criteria is the recognition of genetic testing results, if known, as supportive of a diagnosis of probable AD.

TABLE 2 CRITERIA FOR MAJOR NEUROCOGNITIVE DISORDER DUE TO ALZHEIMER DISEASE (DSM-V).

A. Evidence of significant cognitive decline from a previous level of performance in one or more cognitive domains*:
Learning and memory.
Language.
Executive function.
Complex attention.
Perceptual-motor.
Social cognition.
B. The cognitive deficits interfere with independence in everyday activities. At a minimum, assistance should be required with complex instrumental activities of daily living, such as paying bills or managing medications.
C. The cognitive deficits do not occur exclusively in the context of a delirium.
D. The cognitive deficits are not better explained by another mental disorder (i.e., major depressive disorder, schizophrenia).
E. There is insidious onset and gradual progression of impairment in at least two cognitive domains.
F. Either of the following:
Evidence of a causative Alzheimer disease genetic mutation from family history or genetic testing.
All three of the following are present:
1) Clear evidence of decline in memory and learning and at least one other cognitive domain.
2) Steadily progressive, gradual decline in cognition, without extended plateaus.
3) No evidence of mixed etiology (i.e., absence of other neurodegenerative disorders or cerebrovascular disease, or another neurological, mental, or systemic disease or condition likely contributing to cognitive decline).

Atypical presentations of Alzheimer disease

A minority of patients with AD do not present in the classic fashion with progressive amnestic dementia (Galton et al. 2000). Distinguishing these cases from other causes of dementia can be challenging.

- ***Primary progressive aphasia***

Primary progressive aphasia (PPA) is a heterogeneous group of neurodegenerative disorders with progressive language difficulty, relative sparing of memory and other cognitive functions, at occurring early in the disease course. PPA is further sub classified into three variants depending on the type of language impairment: nonfluent, semantic or logopenic. The clinical variant of PPA most likely to be due to AD is the logopenic variant that is characterized by frequent word-finding pauses and no major grammar or comprehension deficits (Mesulam et al. 2008; Gorno-Tempini et al. 2004).

Structural imaging of patients with logopenic variant PPA often shows predominant left posterior perisylvian or parietal atrophy (Gorno-Tempini et al. 2011). Fluorodeoxyglucose positron emission tomography (FDG-PET) and single photon emission computed tomography (SPECT) may highlight hypometabolism or hypoperfusion in the same areas and amyloid imaging may reveal AD as the cause rather than FTLD.

- ***Posterior cortical atrophy***

This syndrome manifests with progressive cortical visual impairment (Galton et al. 2000). Patients are often first evaluated by optometrists for visual complaints, such as difficulty reading or driving (Levine et al. 1993; Alladi et al. 2007). Neurologic evaluation may elicit features of the Balint syndrome: simultanagnosia (inability to integrate a visual picture despite adequate acuity); optic ataxia (inability to reach accurately under visual guidance) and ocular apraxia (incapacity to direct accurately to a new target, frequently leading to reading difficulty).

Other features include visual agnosia, ideomotor, constructional, and dressing apraxia; prosopagnosia and visual field neglect. This constellation of findings implicates the dorsal visual stream in the lateral occipital and parieto-occipital regions, although ventral stream deficits also occur. In most patients, neuropathological examination reveals Alzheimer pathology with an exceptional distribution involving visual association areas and even primary visual cortex (Renner et al. 2004; Alladi et al. 2007). A minority of patients with this syndrome have alternative pathologies, such as cortical Lewy bodies, corticobasal degeneration, frontotemporal lobar degeneration, or prion disease (Renner et al. 2004).

- ***Dysexecutive or "frontal" variant***

A subsection of patients with AD present with prominent deficits in executive function related to amnesia.

Cognitive impairment in Alzheimer disease

Selective memory impairment is the most essential clinical manifestation of AD and often one of the earliest manifestations. Memory deficits can be elicited in most patients with AD at the time of presentation. Declarative episodic memory – memory of events occurring at a particular time and place – is usually affected. This type of memory is deeply related to the hippocampus and other medial temporal lobe structures. Declarative memory, as defined by Tulving in 1972, regards both semantic and episodic memory. Within episodic memory, there is a distinction between immediate recall (i.e., mental rehearsal of a phone number), memory for recent events (which is related when the material is conscious and must be recalled) and remote memory (memory of distant events). Memory for recent events, served by the hippocampus, entorhinal cortex and related structures in the medial temporal lobe is prominently impaired in early AD (Peters et al. 2009) while remote memory (related to the sensory association and prefrontal cortices) is spared early because of the consolidation for long periods of time (years), which can be recalled without hippocampal function. In contrast to episodic memory, procedural memory and motor learning, dependent on the subcortical system, are relatively spared in the early phase. Semantic memory could be impaired somewhat later and is supported by neocortical temporal regions, particularly in the anterior temporal lobe.

Executive impairments are common in early AD and are not just a feature characteristic of a subgroup of patients. Complex attentional skills are more frequently affected than other executive functions. There is, however, considerable heterogeneity among AD patients in the pattern of executive dysfunction (Stokholm et al. 2006). Cognitive anosognosia explained as unawareness of cognitive deficit is a variable common feature at early stage (Barrett et al. 2005) and could increase over time (McDaniel et al. 1995). Patients tend to underestimate the deficit and could offer alibis in its description. Those with relatively preserved insight are more likely to be depressed; those with more impaired insight are likely to be agitated, disinhibited, and exhibit psychotic features (Harwood et al. 2000; Mizrahi et al. 2006).

Other symptoms

Neuropsychiatric symptoms are common in AD mainly in the middle and late course of disease. These can begin as subtle symptoms including apathy, social disengagement, and irritability. Apathy can be difficult to distinguish from depression. Apraxia usually occurs later in the disease after deficits in memory and language are clear (Parakh et al. 2004). Changes in olfactory function are common and have been explored as a diagnostic tool. However, the predictive value of simple odor detection testing is limited (Rahayel et al. 2012; Sun et al. 2012). Sleep disturbances occur commonly (Ju et al. 2014). Patients with AD have more fragmented sleep compared to older adults without AD. Such changes may occur very early in the disease process, including in patients who are cognitively normal but have biomarker evidence of beta-amyloid (Aβ) deposition (Brown et al 2016). Pyramidal and extrapyramidal motor signs, myoclonus and seizures do occur in patients with AD, these are typically late-stage findings (Portet et al. 2009). If these are clinically apparent in early to middle stages, an alternative diagnosis should be considered (McKhann et al. 1984).

Neuroimaging and role of biomarkers

The most characteristic focal finding in AD is a reduction of hippocampal volume or medial temporal lobe atrophy. However, because hippocampal volumes decline in normal aging, age-specific criteria are needed (Barkhof et al. 2007). The finding of hippocampal atrophy provides incremental support for AD diagnosis, but it is not sufficiently specific to contribute significantly to the accuracy of the diagnosis over the clinical assessment alone (Wahlund et al. 2005).

Functional brain imaging with F18- FDG-PET, functional MRI (fMRI), perfusion MRI, or SPECT reveals distinct regions of low metabolism (PET) and hypoperfusion (SPECT, fMRI). These areas include the hippocampus, the precuneus (mesial parietal lobes) and the lateral parietal and posterior temporal cortex (Peters et al. 2009; O'Brien et al 2010). In practice, FDG-PET may be most useful in distinguishing AD from frontotemporal dementia in patients with atypical presentations (Rabinovici et al 2011). Amyloid PET tracers (F18-florbetapir, F18-flutemetamol, F18-florbetaben) measuring amyloid lesion in the brain have been developed as aiding tools in the diagnosis of AD in vivo and differentiate AD from other causes of dementia (Rabinovici et al 2011; Clark et al. 2012). Amyloid imaging is not appropriate in patients who meet the core clinical criteria for probable AD and have a typical age of onset, and such a scan should not be used to determine dementia severity (Johnson et al. 2013).

There are several widely investigated biomarkers for the molecular and degenerative process of AD that can be supportive of a diagnosis of AD but are not yet recommended for routine diagnostic purposes. Such testing can add incremental confidence to a clinical diagnosis of AD and can be

useful in certain circumstances, including early-onset dementia and atypical presentations of AD in which the differential diagnosis includes other non-amyloid neurodegenerative diseases such as frontotemporal dementia. Molecular biomarkers of Aβ protein deposition include:

- Low cerebrospinal fluid (CSF) Aβ42 (or Aβ42:Aβ40 ratio)
- Positive amyloid PET imaging using one of the amyloid PET tracers
- Biomarkers of tau deposition (a key component of neurofibrillary tangles) include:
- Increased CSF total tau and phospho-tau
- Evidence of cerebral tau using a tau-specific PET tracer (in development) (Johnson et al. 2016).

In addition to molecular biomarkers there are several topographic biomarkers used to assess the downstream brain changes that correlate with the regional distribution of neuronal dysfunction (Dubois et al. 2010). These include medial temporal lobe atrophy on MRI and reduced glucose metabolism in the parieto-temporal regions on FDG-PET. In general, the topographic biomarkers are less specific than the molecular biomarkers but correlate better with the emergence of clinical symptoms. Research criteria have incorporated both molecular and topographic biomarker data into the research definitions of both symptomatic and presymptomatic forms of AD, anticipating that once biomarkers become more standardized they will be incorporated into clinical diagnostic algorithms for AD (Morris et al. 2014). At present the use of biomarkers is limited primarily to investigational studies and clinical trials, and testing is not universally available. Decreased apolipoprotein E (APOE) and APOE ε4 plasma levels as well as a variety of other serum and CSF proteins have been assessed for predictive value for AD in non-demented subjects and in patients with MCI (Bateman et al. 2012). Such markers might also distinguish AD from other forms of dementia and may recognize patients at risk for a rapidly progressive course of AD. However, a role for these measurements in clinical practice has not been established (Galasko et al. 2003).

1.3 Overlap between dementia with Lewy bodies and Alzheimer disease

The primary entity considered in the differential diagnosis for DLB is AD. In fact it is not rare that diffuse Lewy bodies (LB) pathology coexist with other processes, including Alzheimer pathology (McKeith et al. 2007, Yoshita et al. 2001). The two dementia syndromes are often clinically confused, especially in the advanced stages, as the underlying pathophysiological processes compromise neural structures to a greater extent.

The diagnosis of DLB is still difficult to make given that the current diagnostic criteria (McKeith et al. 2005) has a sensitivity of 32% for pure DLB and it is even lower for mixed DLB and AD

pathology (Nelson et al. 2010). It is particularly true because the clinical syndrome of DLB is modified by the severity of Alzheimer neuritic plaque pathology, although total b-amyloid load has no effect (Tiraboschi et al. 2015). This is consistent with the possibility of a patient having the typical DLB clinical syndrome is ‘‘directly related to the severity of Lewy-related pathology and inversely related to the severity of concurrent AD-type pathology” (McKeith et al. 2005).

Given that AD pathology is frequently present in DLB, there always are a significant number of patients who will be very hard to identify. This group is best described pathologically as **AD with LB** or as **LB variant of AD (LBVAD)** (Hansen et al. 1990). The identification of the LBVAD/AD with LB group would is still problematic, since the anticipated characteristics of the clinical presentation remain uncertain (McKeith et al. 2016).

The combination of two pathologies can influence the clinical presentation and is still presenting diagnostic challenges (McCleery et al. 2015). The engagement with the AD diagnostic community over this important overlap cases should be a priority.

Cognitive phenotypes in DLB the overlap with AD

The difficulty in distinguishing neurocognitive profiles in DLB from AD is particularly true at an early stage. Few studies have dealt with these aspects so far. As stated by Ferman et al. (2013), when dementia severity is mild or mild to moderate, cognitive comparisons reveal a consistent dissociation between clinically probable AD and DLB.

We have already reported that clinical diagnostic criteria for DLB still lack sensitivity. In affected patients with AD, prominent memory loss can coexist with one or more features of DLB, including visual hallucinations, fluctuations, parkinsonism, and falls. When memory becomes impaired in DLB, memory retrieval may be more affected than acquisition (Mormont et al. 2003).

DLB patients with prominent AD pathology may have a cognitive profile that is more characteristic of AD. Patients with DLB may display a different pattern of cognitive decline in comparison to AD. It has been widely known that DLB early neuropsychological signs may include visuospatial, visuo-perceptual deficits (Ballard et al. 1999) and visuospatial/constructional impairments (Salmon et al. 1996; Ala e t. al. 2001). DLB usually have worse performances on attentional and executive tasks (Ballard et al. 2001); however, attention and executive functions (i.e., Trail-Making Test, Wisconsin Card-Sorting Test, letter and category verbal fluency tests) may also be impaired early in AD (Bradshaw et al. 2006). Specifically, these topics are treated more deeply in the following chapter.

Episodic memory involvement in DLB in overlap with AD

Several studies have compared memory impairment in AD and DLB showing less severe memory impairment in DLB than in AD (Economou et al., 2016; Guidi et al., 2006; Hamilton et al., 2004). Differences in memory dysfunction is strongly supported also in MCI studies, which usually refer to memory complaints as a core feature of pre-dementia stage of AD, that is therefore called amnestic MCI (Ferman et al., 2013; Petersen et al., 2004). At the opposite, memory impairment is often observed later in the course of DLB, however few studies reported that MCI with impairment in multiple domains including memory may precede DLB too (Kondo et al., 2016).

In general, in early stage of dementia, DLB subjects perform better on tests of episodic memory than AD patients. This is thought to be dependent on the qualitative different memory processes involved. DLB's poor performances seem to be related to a prominent deficit of retrieval strategies, while AD is characterized by a defective consolidation (Economou et al., 2016; Hamilton et al., 2004). These findings are supported by studies in which free and cued paradigm has been proposed to assess episodic memory functioning (Economou et al., 2016; Hamilton et al., 2004). Memory consolidation of DLB is preserved in front of a systematic retrieval impairment of successfully stored information.

Short story and word list recall are widely used to assess memory abilities in dementia patients. Although both tests provide indexes of immediate and delayed recall, permitting quantification of both initial learning and subsequent memory decay, they strongly differ as to the type of material to be remembered. Word list tests call for the faithful reproduction of a list of typically unrelated words, which requires an active effort to organize memoranda at both encoding and retrieval. Differently, in story recall the information is already logically and semantically organized. This allows more passive learning and the implementation of less demanding retrieval strategies (Desgrandges et al. 1998). In Perri et al. 2013 all demented groups, including DLB and AD, performed similarly on both immediate and delayed recall of the word-list. Conversely, AD patients performed worse than all the other dementia groups on the immediate prose recall. On delayed prose recall, AD patients performed similarly to DLB patients. However, results are not still clear.

Memory functioning in DLB and AD is quite consistent with the different brain structures that are mainly affected. As reported earlier, since the early stage of the disease, AD patients have a greater involvement of temporal structures (e.g., hippocampus, entorhinal cortex; McKhann et al, 2011; Albert et al., 2011), underlying the neuroanatomical network episodic memory. According to the hippocampal/subcortico-frontal dissociation reported by Sarazin et al. (2010), the hippocampus is more involved in encoding and storage processes. In DLB usually these areas are relatively preserved (McKeith et al., 2005). Differently, DLB show a more severe frontostriatal pathology

(Hamilton et al., 2004), which is known to be responsible of executive dysfunction and retrieval deficit (Sarazin et al., 2010).

Given these premises, a part of my studies focused on a more specific assessment of memory impairment. The working hypothesis is that qualitative analysis of different memory processes as the encoding, consolidation, and retrieval strategies could be helpful for the differential diagnosis of AD and DLB. The three macro-areas previously briefly described relate the most important cognitive aspects between DLB and AD which I have most evaluated during my Ph. D course.

2. Evaluation of mild cognitive impairment

Mild cognitive impairment (MCI) may occur as a prodromal to several neurodegenerative dementias and could be considered as an intermediate state between normal cognition and dementia. Even if specific cognition changes could occurred even in normal aging, there is increasing evidence that patients who meet the criteria for MCI can be differentiated from healthy and those with very mild AD. This chapter resumes the different definitions and presents the clinical subtypes of MCI.

Definitions of MCI

MCI refers to cognitive impairment that does not meet the criteria for dementia. In the past MCI was thought as a prodromal stage typical of AD with memory complaint as core feature for the diagnosis of MCI (Petersen et al., 1999). Later it has been recognized a non-obviously progression from MCI to AD and several possible dementia outcomes have been studied. In this regards MCI was recognized as a heterogeneous construct in terms of definitions, clinical presentation, aetiology, prognosis and prevalence (Petersen et al. 2003, Voisin et al. 2003). In this heterogeneity, however, the construct of MCI share some overlapping features and a measurable deficit in cognition in at least one domain, in the absence of dementia or impairment in activities of daily living.

In 2013 the construct of MCI was introduced in the Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (DSM-V) as mild neurocognitive disorder. **Mild neurocognitive disorder** is very similar to MCI and for the clinician the two entities are equivalent. Mild neurocognitive disorder refers to a condition involving cognitive impairment in one or more domains, often memory, with relative preservation of activities of daily living and the absence of dementia.

Age-associated memory impairment and **age-associated cognitive decline (AACD)** are also commonly used and well-known terms. However, these terms differ from MCI in that they were originally devised to define normal age associated memory and cognitive changes in older adults as referenced to young normal adult individuals (Petersen et al. 1999). AACD was developed to better defining cognitive changes in elderly patients compared to age-adjusted norms.

Studies of "**preclinical AD**" should be distinguished from studies of MCI (Small et al. 2003). The construct preclinical AD refers to individuals with normal cognition who have positive biomarkers for AD, such as a positive amyloid PET scan or evidence of AD biomarkers in the cerebrospinal fluid (Sperling et al. 2011), while in MCI refers to patients with cognitive criteria for diagnosis followed prospectively to assess for conversion to AD.

Suspected non-AD pathology (SNAP) is a term that is increasingly used in research to represent individuals who meet criteria for MCI, show no evidence of amyloid pathology based on CSF biomarker and/or amyloid PET imaging, but have evidence of neuronal injury as measured by either medial temporal lobe atrophy or hypometabolism on FDG-PET (Caroli et al. 2015; Petersen et al. 2013). The SNAP designation may be relevant prognostically as well (Caroli et al. 2015).

Clinical subtypes of MCI have therefore been proposed to extend the scope of MCI to other cognitive domains, thereby extending the early detection of other dementias in their prodromal stages (Petersen et al. 2001; Petersen et al. 2004; Salmon et al. 2005): amnestic MCI and non-amnestic MCI.

- ***Amnestic MCI***

  Firstly it has been made a distinction between amnestic MCI (aMCI) and non-amnestic MCI (naMCI), depending on the presence or absence of memory disturbance. Secondly, aMCI and naMCI are further divided into single-domain or multiple-domain types depending on how many cognitive domains are affected (Petersen et al., 2004). Each subtype is characterized by deficits typically related to the specific cognitive domains that are most commonly affected in the associated dementia outcome. Amnestic MCI is often thought of as a precursor to AD (Morris et al. 2001). A-MCI single-domain refers to those individuals with significantly impaired memory who do not meet criteria for dementia. A-MCI multiple-domain refers to those with aMCI complain plus additional subtle impairments in other cognitive domains that are revealed with careful neuropsychological testing (Salmon et al. 2005). These individuals often progress to meet criteria for AD dementia; in a minority of cases, the cognitive profile may simply reflect normal aging.

- ***Non-amnestic MCI***

  NaMCI single domain is characterized by a relatively isolated impairment in a single non-memory domain, such as executive functioning, language, or visual spatial skills (Petersen et al. 2003). Depending upon the domain, individuals with this subtype of MCI may progress to other syndromes, such as DLB, frontotemporal dementia (FTD), primary progressive aphasia progressive supranuclear palsy, or corticobasal degeneration. NaMCI multiple domain refers to patients affected in multiple domains with a relative sparing of memory problems. The substrate of multi-domain naMCI is felt to be that of degenerative disorders associated with tau and alpha-synuclein such as FTD and DLB (Molano et al. 2010).

Cognitive complaints

Patients with MCI, not in particularly only the amnestic subtype, complain primarily impaired memory. Difficulties include certain aspects of memory such as recalling names, frequently reported also by normal elderly patients. It is important to determine that impairments represent a changement over baseline. In contrast to the impaired awareness of deficits commonly present in patients in AD, patients with MCI are often particularly troubled by their symptoms (Steward et al. 2008). However, over time, MCI converting to AD shift to a relatively greater preponderance of informant-reported symptoms over self-reported symptoms (Tabert et al. 2002). This phenomenon may be helpful in following the individual progression to dementia. The relationship between depression and cognitive impairment is complicated. Cognitive impairment may be a presenting symptom of depression, so-called pseudodementia. Depression may also be an early manifestation of cognitive impairment.

Neuropsychological testing

A brief screening evaluation of the patient's cognitive profile is recommended to evaluate patients with suspected cognitive impairment. Screening cognitive evaluations could include the Folstein Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MoCA).

Even if there are no uniformly accepted criteria for the diagnosis of MCI using neuropsychological testing, the Quality Standards Subcommittee of the American Academy of Neurology has provided practice parameters, including evaluation guidelines for MCI (Petersen et al. 2001). Some suggest a 1.5 standard deviation (SD) threshold value for tests of memory impairment; others have used 1 SD (Petersen et al. 1999; Petersen et al. 2001). These appear to be the appropriate range for most individuals diagnosed with MCI, but these data must be used individually. In amnestic MCI, other cognitive domains may be impaired, but test abnormalities are generally milder and are usually within 0.5 SD of appropriate comparison groups. In multiple-domain MCI, several cognitive domains may be impaired in the 0.5 to 1.0 SD range. These ranges may be not used as cut-off values but just to provide a sense of the impairments seen in MCI.

Neuroimaging

The role of neuroimaging in the evaluation of MCI is evolving at present. MRI is more reliable than CT in assessing focal patterns atrophy, such as temporal lobe volume loss; this latter finding may indicate an individual at particular high risk of converting from MCI to dementia. In addition, MRI may be more sensitive than CT in identifying cerebrovascular disease, another finding that may

identify patients a higher risk of progressing to dementia. The role of other neuroimaging tests, including positron emission tomography (PET), functional MRI, PET amyloid imaging and single photon emission CT (SPECT) in assessing the risk for neurodegenerative dementia is investigational. From the Alzheimer Disease Neuroimaging Initiative document is emerging the utility of imaging biomarkers such as amyloid PET in predicting progression (Petersen et al. 2014).

Diagnosis

A workgroup on diagnostic guidelines from the National Institute on Aging-Alzheimer's Association identified the following core clinical features that indicate **MCI due to AD** (NIA-AA, Albert et al. 2011). It should be noted that these criteria only refer to the subset of all MCI that is thought be due to AD, and not to MCI due to other etiologies, i.e. vascular disease, other neurodegenerative conditions, or psychiatric conditions (Petersen et al. 2004). These include:

- Cognitive concern reflecting a change in cognition reported by patient or informant or observed by clinician
- Objective evidence of impairment in one or more cognitive domains, typically including memory
- Preservation of independence in functional abilities
- Not demented

In addition, the etiology of MCI should be evaluated to identify the cause as most likely to be Alzheimer disease:

- Rule out vascular, traumatic, medical causes of cognitive decline, where possible
- Provide evidence of longitudinal decline in cognition, when feasible
- Report history consistent with AD genetic factors, where relevant.

The specific designation of MCI due to AD is used when a biomarker associated with AD (i.e., CSF testing of Aβ, tau, phospho-tau), amyloid imaging, *APOE* genotype, or functional scan consistent with AD is present in the affected subject (Table 3). Using the NIA-AA criteria on a cohort of research subjects with MCI, individuals with cognitive impairment and both amyloid and neuronal injury biomarkers had an approximately 60 percent conversion rate to AD dementia over three years (Vos et al. 2015). As part of the MCI due to AD criteria, biomarkers are used to augment clinical suspicions that the clinical syndrome of MCI is due to AD (Albert et al. 2011). At present, however, the use of biomarkers is limited to investigational studies and clinical trials.

TABLE 3 SPECTRUM OF WIDELY INVESTIGATED BIOMARKERS FOR ALZHEIMER DISEASE. AB: BETA-AMYLOID; MRI: MAGNETIC RESONANCE IMAGING.

	Pathophysiologic marker	Topographic marker	Marker of neuronal injury/degeneration
Cerebrospinal fluid			
Aβ42 or Aβ42:Aβ40 ratio	Yes	No	No
Total tau, phospho-tau	Yes	No	Yes
PET			
Amyloid tracer uptake	Yes	No	No
Fluorodeoxyglucose	No	Yes	Yes
Structural MRI			
Medial temporal lobe atrophy	No	Yes	Yes

2.1 Mild cognitive impairment converting into dementia with Lewy bodies

Recently there have been many efforts to determine the characteristics of prodromic AD. On the opposite, very few studies addressed the same topic in the progression of MCI to DLB. The rate of progression of MCI to DLB has been demonstrated to be 24.5% among non-amnesic MCI (naMCI) (Ferman et al., 2013). Evidences of prodromal DLB are not yet clearly defined (Donaghy et al. 2014); specific criteria that characterize MCI due to DLB have not been established yet as, by definition, the consensus clinical diagnostic criteria for DLB exclude individuals in a phase of pre-dementia. This failure could depend on, whether retrospective studies may be useful for evaluating the development of symptoms, a relatively small number of patients convert to to DLB making some limits to the generalization of the findings.

However, the diagnosis of prodromal DLB is in growing interest, providing an important opportunity to investigate the earliest stages of DLB. We already know that DLB clinical criteria have high specificity but low sensitivity (Nelson et al. 2010) and the presence of biomarkers of LB disease it's becoming necessary to optimize the diagnostic accuracy moving towards earlier diagnosis. As shown in Table 4, presenting features of DLB can be generally synthetized in three main categories: *cognitive impairment, behavioral/psychiatric phenomena, and physical symptoms:*

- *Cognitive impairment:* DLB can be preceded by amnestic or non-amnestic cognitive impairment, although cases involving non-memory domains (that is, attention/executive, visuospatial or language) are more likely to progress to DLB than single-domain amnestic MCI (Ferman et al. 2013; Jicha et al. 2006). Fluctuations are at least a common core

symptom in the prodromal phase, present in 2/7 cases (Molano et al. 2010) and 3/9 cases (Jicha et al. 2010) in two longitudinal studies. Delirium, however, is reported as prodromal features of DLB and may represent the earliest manifestations of cognitive fluctuations (Vardy et al. 2014).

- *Behavioral/psychiatric phenomena*: DLB may initially present with noncognitive symptoms such as visual hallucinations (VHs), depression and RBD (Auning et al. 2011).
- *Physical symptoms:* parkinsonism, a core feature of DLB, may be a presenting symptom in around one-quarter of patients. α Synuclein deposition in the olfactory bulb, peripheral nervous system and brainstem is associated with a decreased sense of smell (hyposmia), constipation, orthostatic dizziness and increased salivation (Auning et al. 2011).

Mild cognitive impairment and neurological deficits are often present in early stages; however, the combination with neuroimaging, disease progression or LB disease-specific symptoms, i.e. RBD, is required.

In a very recent revision (McKeith et al. 2016) further efforts have been made in considering the MCI-DLB as varied and distinct entity, taking into account not just the prodromal DLB but also its diagnostic overlap with AD.

Table 5 shows suggested early, intermediate and later clinical features as indicative of early LB disease. As shown, REM sleep behavior disorders and the autonomic involvement have been recognized as part of MCI-DLB spectrum (McKeith et al. 2016). However, cognitive fluctuations of attention, recently recognized as predictable of DLB in one of our studies (Cagnin et al., 2015) were not be considered in this review among the early symptoms.

This lack could be due to the fact that the identification of fluctuating confusion is verisimilarly very hard to evaluate. Consistent with other studies (Cagnin et al., 2015, Molano et al. 2010 and Jicha et al. 2010), REM sleep behavior disorder (RBD) and subtle parkinsonism have indeed been confirmed as early feature of DLB. It is important to note that the identification of these suggested symptoms, however, occurred by asking patients their personal experience with symptoms in the 10-15 years before and this method could be considered limited in its validity.

A starting point for the consideration of the DLB pre-dementia and its progression in an eventual dementia state (Figure 3). It's evident that prodromal DLB may progress either to typical DLB or to atypical DLB, and data suggest these events as almost equally. The term " typical DLB" refers to the identified clinically cases meeting the consensus criteria for probable or possible DLB. In many cases, a confident diagnosis of DLB will be made without a biomarkers confirmation. In other cases, when the clinician is less confident, additional biomarker evidences may be required. It is apparent that there will be a need of biomarker confirmation of LB pathology also for atypical cases

of prodromal DLB and also for dementia stage. It's not clear, however, how to denominate the atypical MCI-DLB as regards the most of the cases where mixed pathologies are concomitant. It is good to know that another important question regards, moreover, the usefulness of types of biomarker(s) to detect DLB early.

Proposal of a broader classification: Prodromal DLB Subtypes (McKeith et al.2016)

Turning on a consideration of early/prodromal diagnosis of both typical and atypical DLB cases and revisiting DLB diagnosis, McKeith et al. (2016) suggested at least three prototypical forms of early DLB. Prodromal DLB should be supposed when early clinical symptoms are suggestive of a higher involvement of cognitive or psychiatric features. Other early symptoms can be interpreted as indicative of prodromal LB disease in general, or in the case of early motor symptoms, of prodromal Parkinson disease.

Differently to AD where MCI is generally considered as the main prodrome, it has been suggested that DLB may have one of several general clinical profiles. Three prodromal DLB subtypes are considered, as shown in Figure 4:

- **MCI variant (DLB-MCI)** presents usually a non-amnestic profile, multidomain and probably associated with early visual-perceptual and attentional deficits (Ferman et al. 2013). Conversion to DLB of these subtype respect to conversion to AD in amnesic MCI may occur more frequently. The DLB-MCI generally corresponds to the DSM-V category of mild NCDLB (DSM-V).
- **Delirium-onset DLB** characterized by provoked or spontaneous delirium that occurs months or years prior to dementia onset. It is present in up to 25% of cases with DLB compared to only 7% of cases with AD (Vardy et al. 2014).
- **Psychiatric DLB disorder** not widely recognized, with principal presentation as psychosis or a late-onset affective disorder (McKeith et al.1992) typically treatment refractory and with adverse sensitivity to neuroleptics.

Prodromal DLB criteria will meet clinical criteria for one of these prototypic syndromes (MCI, delirium onset, or psychiatric onset) as a minimum require, accompanied by at least 1 biomarker supportive of LB disease (McKeith et al. 2016).

TABLE 4. EXAMPLES OF PRESENTING SYMPTOMS IN DLB (DONAGHY AND MCKEITH, 2014).

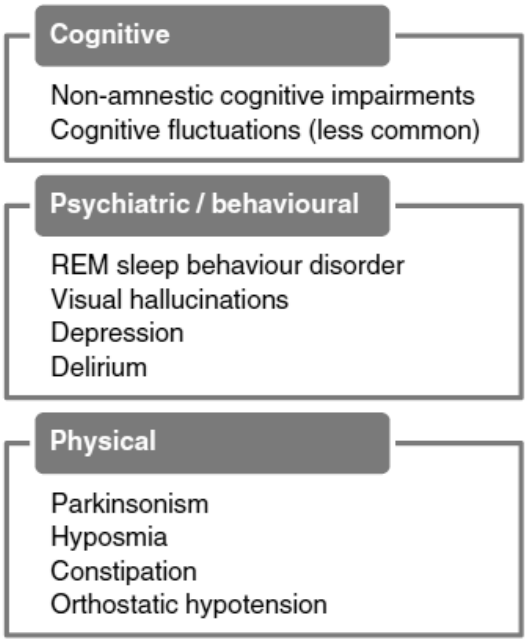


TABLE 5 CLINICAL FEATURES THAT HAVE BEEN SUGGESTED AS INDICATIVE OF EARLY LB DISEASE (MCKEITH ET AL. 2016).

Early symptoms (typically 5-15 years pre-dementia)
Decreased sense of smell
REM sleep behavior disorder
– Nightmares
– Crying or shouting during sleep
– Limb movements during sleep
Constipation
Dizziness on standing
Urinary incontinence
Increased saliva
Increased sweating
Intermediate symptoms
Delirium: provoked or unexplained
Late onset psychiatric disorder
– Psychosis
– Depression
Later symptoms
Cognitive impairment (nonamnestic mild cognitive impairment)
Visual hallucinations, illusions, and misconceptions
Parkinsonism

Abbreviations: LB, Lewy body: REM, rapid eye movement.

FIGURE 3. GRAPHIC OF THE RELATIONSHIPS BETWEEN PRODROMAL AND DEMENTIA STAGES OF DLB (MCKEITH AL. 2016).

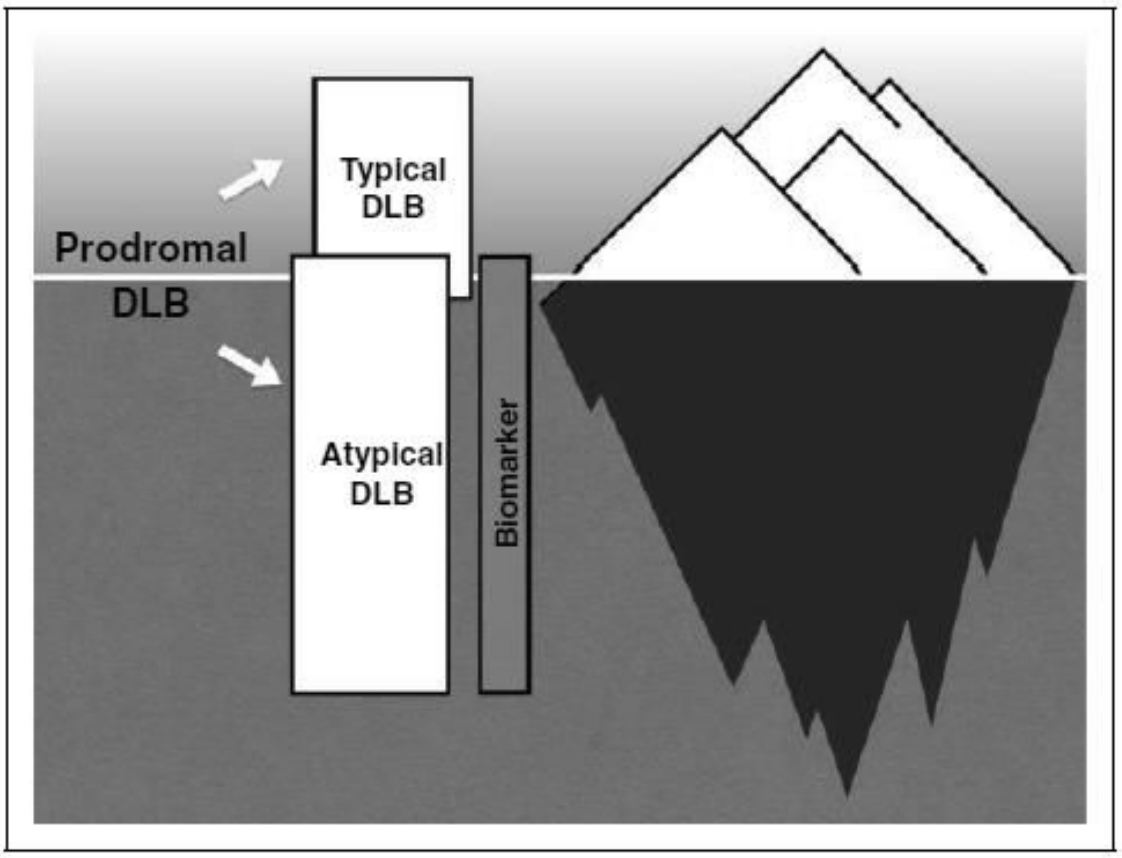
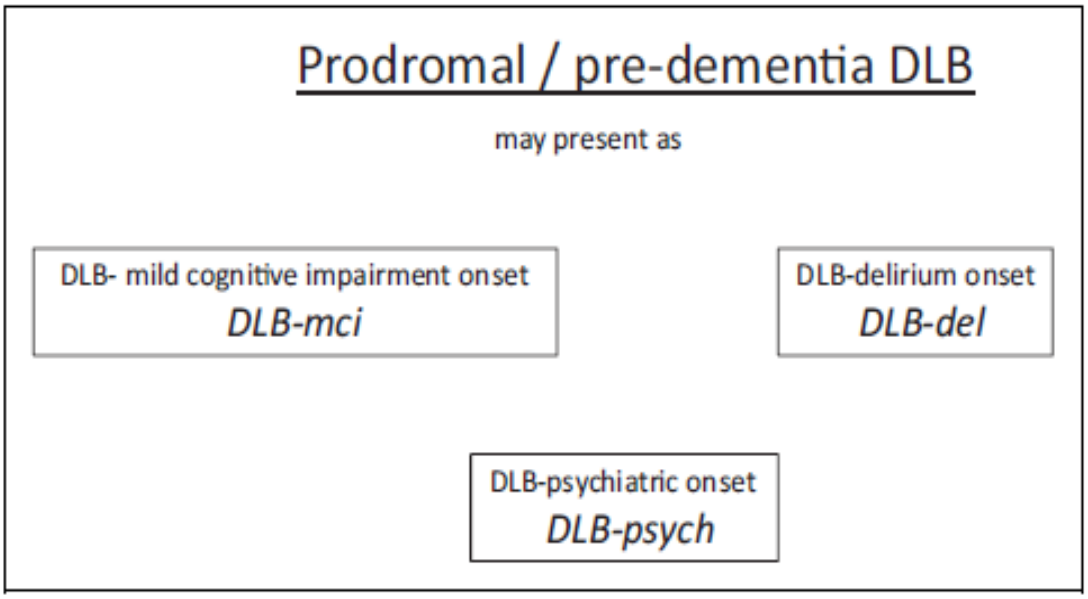


FIGURE 4. PROPOSED CLASSIFICATION OF PRODROMAL DLB SUBTYPES



Cognitive profile in MCI-DLB / Prodromal DLB

The cognitive pattern of impairment preceding DLB dementia has been recently identified in very few studies. Early impairments in visual abilities and attentional domain have been reported (Kondo et al., 2016; Cagnin et al., 2015; Ferman et al., 2013) and, between MCI progressing to clinically probable DLB, attention and/or visuospatial impairment are concomitant with memory difficulty (Ferman et al. 2013). A visual-attentional impairment associated with executive dysfunction (Ferman et al. 2013; Cagnin et al., 2015) appears to be the only best clinical marker of conversion to DLB dementia until now.

Little is known about memory domain in prodromal DLB and actually there is still some disagreement about the severity and extent of memory impairment. General memory complaints were observed in MCI-DLB (Ferman et al. 2013; Kondo et al.2016) and the 66.7% of aMCI-m converting into DLB presented a visual memory impairment, supporting Ferman et al.'s findings (2013) (Kondo et al. 2016). However, visual memory impairment has been interpreted by Kondo (2016) as closely related to a visual cognition and attention deficit.

An impairment of visual abilities and attentional functions associated with executive dysfunction (Ferman et al. 2013; Cagnin et al., 2015) appears to be the only best clinical marker of conversion to DLB dementia until now. Summarily, regarding the cognitive phenotype of MCI converting into DLB, results are scarce, needing further studies.

3. EXPERIMENTS

Study1.

Assessment of visual-constructional deficits in MCI-DLB.

As already reported, there are a limited number of studies addressing which clinical and cognitive characteristics are most useful in diagnosing early DLB (Jicha et al. 2010; Boeve et al. 2012). We already know that in the initial stage the presence of executive and visual-spatial and perceptual deficits appears to provide a better predictive value for the diagnosis of DLB.

In a recent paper by Mitolo et al. (2014) the Qualitative Scoring method for the Pentagon copy test (QSPT) (Caffarra et al. 2016) of the Mini-Mental State Examination (MMSE) was applied to a cohort of DLB patients whose diagnosis was subsequently confirmed neuropathologically, demonstrating that an alteration of the number of angles in the copy intersecting-pentagon item was present in 33.3% of patients with DLB at the time of first evaluation, compared to 6.3% of AD patients, with a progressive deterioration in the DLB group.

We expected that QSPT parameters could reveal a lower performances in early DLB patients, supporting the hypothesis that early neuropsychological signs in DLB may include visuospatial and visuospatial/constructional impairments (Salmon et al. 1996; Ala e t. al. 2001) more specifically than in AD. In our first study we aimed to investigate whether the QSPT could be a valid screening tool in discriminating between DLB and AD in subjects with mild cognitive impairment (MCI). QSPT is shown in the appendix.

Patients and Methods

Patients were screened at the Memory Clinic of the University of Padova (Italy). Thirty patients with MCI without dementia whose clinical diagnosis of probable DLB was confirmed at follow-up visits (MCI-DLB) were recruited. Twenty-three patients with MCI ultimately diagnosed as probable AD (MCI-AD) served as a control group. At baseline the diagnosis of MCI was made according to established criteria (Winblad et al. 2004) considering the results of an extensive cognitive test battery and clinical history. Inclusion criteria were: absence of dementia at the baseline visit with a cutoff MMSE score $\geq$ 26/30 and absence of functional impairment in everyday life. The clinical diagnosis of probable DLB or AD was made according to current diagnostic criteria after at least a 3-year follow-up (McKeith et al. 2005, McKhann et al., 1984). Exclusion criteria were: inability to establish the final diagnosis at follow-up visits and cognitive decline due to comorbidities. At study entry subjects underwent full clinical history and neurological evaluation including the screening for extrapyramidal signs with the Unified Parkinson's Disease Rating Scale (UPDRS) motor score

and the assessment of behavioral disorders with the Neuropsychiatric Inventory (NPI) questionnaire. The presence of fluctuations in cognition and attention was determined using the Mayo Fluctuation Questionnaire (Ferman et al. 2004) and the presence of symptoms suggestive of Rem Sleep Behavior Disorder (RBD) was determined with the Mayo Sleep Questionnaire (Boeve et al. 2011). At baseline all patients underwent a neuropsychological evaluation by an experienced neuropsychologist. Global cognitive performance was assessed using the MMSE test. Each pentagon drawing of the MMSE test was retrospectively examined with the QSPT by a neuropsychologist (MM) not involved in the formal cognitive assessment and blind to the diagnosis (Caffarra et al. 2013). The QSPT is based on the assessment of five parameters of the pentagon copy test, i.e. number of angles, distance/intersection, closure/opening, rotation and the presence of closing-in (see appendix). Specific cognitive domains were investigated using the following neuropsychological tests: Digit Cancellation (visual search) and Trail Making Test e part A for the evaluation of attention; Digit span forward and backward for short term memory and prose memory (immediate and delayed recall) test for long term memory; Letter fluency test for the study of word generation and executive functioning; Clock Drawing test for the evaluation of visual-constructional abilities and executive functions; Rey-Osterrieth Complex Figure (ROCF) test for the assessment of visual-constructional abilities (ROCF copy) and long term visual-spatial memory (ROCF delayed recall) (Caffarra et al. 2002).

The Student t test for independent sample was used for the analysis of normal variables, the Mann-Whitney U test for ordinal variables and the Chi Square test for categorical variables. Linear correlations between the cognitive test scores were tested using the Spearman's rho correlation coefficient. The significance level was set at $p < 0.05$. The study received approval by the institution review board and all patients' signed informed consent.

Bonferroni correction was applied on 4 multiple comparisons based on the main cognitive domains investigated: attention and executive function, working memory, episodic memory, visual-spatial and visual-perceptual functions. The significance level was set at $p = 0.0125$ according to Bonferroni correction.

Results

The MCI-DLB and MCI-AD groups were matched for age (DLB: 75.4 ± 6.2; AD: 72.6 ± 6 years; p= 0.09), gender (DLB: M/F = 14/16; AD: 10/13, p= 0.8) and education (DLB: 9.4 ± 4.8; AD: 10.5 ± 4.7 years; p= 0.4). In the MCI-DLB group, the presence of visual hallucinations (VHs) was reported in 9/30 patients (30%), fluctuations of cognition and alertness in 53% of patients (16/30) and RBD in 56.6% of patients (17/30). Parkinsonism was the most frequent supportive feature

present in 19/30 MCI-DLB patients (63%), although often as subtle extrapyramidal signs (mean UPDRS score: 3.2 ± 5.3), with 15/19 patients with UPDRS score ≤ 5. Sixteen MCI-DLB patients had only one or no clinical core features diagnostic for DLB. Of the remaining 14 MCI-DLB patients, 12 had 2 core features (7 patients: subtle extrapyramidal signs and fluctuations in attention and cognition; 3 patients: VH and fluctuations; 2 patients: VH and extrapyramidal signs) and 2 patients presented with all three clinical core features. None of the MCI-AD patients had VHs, fluctuations in attention/cognition, RBD-like symptoms or extrapyramidal signs ($p < 0.001$). Neuropsychological test results of the MCI-DLB and the MCI-AD patients are reported in Table 1. The mean MMSE score was 28/30 for both groups ($p= 0.46$). For the QSPT, the total score did not differ between the two groups, while the number of angles was significantly worse in the MCI-DLB patients ($p= 0.009$). For the MCI-DLB patients 45.1% (12/30) showed an impaired number of angles item of the QPST (scoring <4) compared to 8.3% (2/23) of MCI-AD ($p =0.005$). The sensitivity of the impaired performance on this parameter was 41.4% (95% CI: 23%- 59%) and the specificity of 91% (95% CI: 79% - 100%) in the differential diagnosis between prodromal DLB and AD. The positive predictive value was 85.7% (95% CI: 67%-100%) and negative predictive value was 54% (95% CI: 38%-70%).

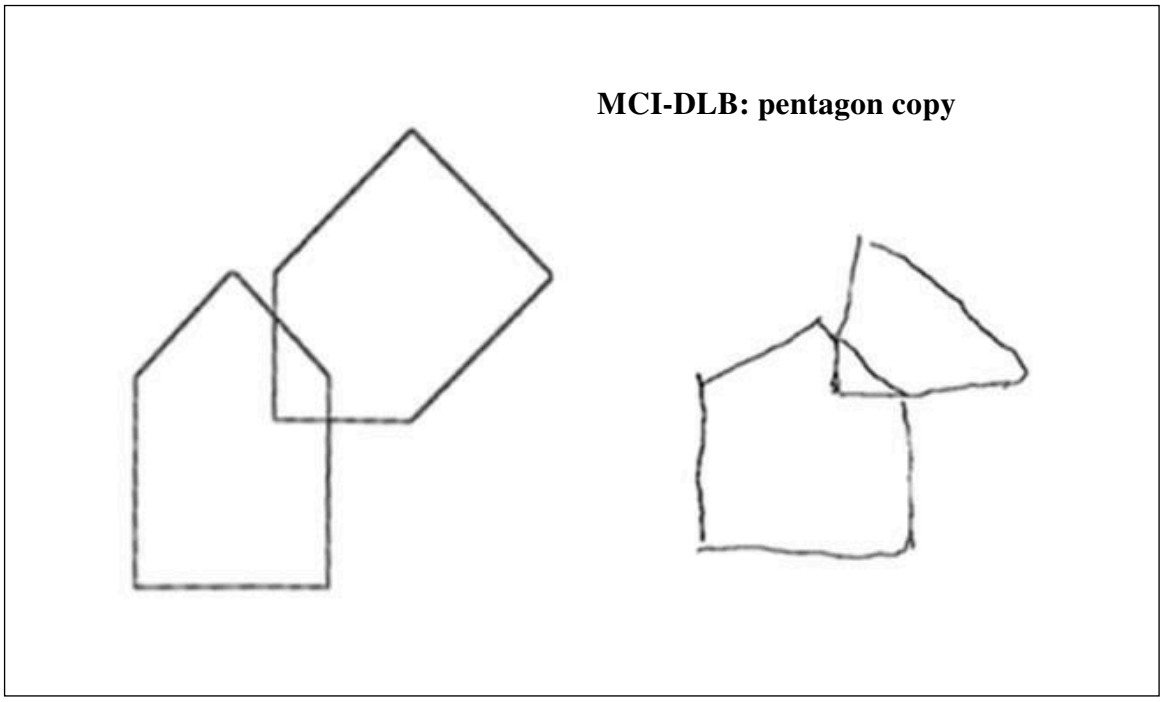
An example of impaired performance by a DBL patient in the pentagon's number of angles is shown in Fig. 5. Patients in the MCI-DLB group showed greater impairment in speed of attention, working memory and visual-constructional abilities, and performed worse than MCI-AD in the Trail Making Test e part A ($p =0.026$), in the Digit span backward ($p =0.003$) and Clock drawing tests ($p =0.029$) (Table 6). MCI-AD patients, instead, showed greater impairment in tests exploring memory, with lower scores in memory delayed recall test ($p =0.009$). The QSPT number of angles score correlated only with the TMT-A test score in both groups (DLB: $r = -0.45$, $p =0.01$; AD: $r = 0.57$, $p =0.003$). Inability to determine the number of angles in pentagon copy test was not associated with the presence of any of the typical clinical characteristics of DLB: namely parkinsonism ($p =0.49$), RBD-like symptoms ($p =0.46$), fluctuation in attention ($p =0.46$) and VHs ($p =0.56$). The combination of impaired number of angles with subtle signs of parkinsonism was present in 10 MCI-DLB patients, with RBD in 8, with fluctuations in attention in 8 and with VHs in 3 patients. In more than 50% of the MCI-DLB patients only one core clinical feature ($n =12$) or no core clinical features ($n =4$) were detectable at baseline. Of these 16 patients, 5 had an impaired performance in the pentagon copy test and in 2 cases was the only characteristic suggestive of DLB. With Bonferroni correction, the QSPT number of angles, Digit Span backwards and Prose memory delayed recall were significant ($p= 0.0125$).

TABLE 6 NEUROPSYCHOLOGICAL TEST SCORES IN MCI-DLB AND MCI-AD PATIENTS.

Test scores mean (SD)	MCI-DLB (n=30)	MCI-AD (n=23)	*p*
MMSE	28.2 (1.6)	27.9 (1.1)	0.43
Digit Cancellation	42.9 (9.2)	42.2 (11.2)	0.82
Trail making Test – A (sec)	91.2 (61.7)	61.2 (24.1)	**0.03**
Digit span			
Forward	4.8 (0.9)	5.2 (0.9)	0.14
Backward	3.0 (0.7)	3.7 (0.8)	**0.003***
Prose memory			
Immediate recall	11.2 (5.8)	8.4 (4.4)	0.05
Delayed recall	12.3 (5.7)	8.1 (5.6)	**0.009***
Letter fluency	25.2 (9.2)	30.0 (10.0)	0.07
Clock drawing	6.3 (3.2)	8.1 (2.6)	**0.03**
Rey-Osterrieth Complex Figure			
Copy	27.4 (7.8)	30.9 (5.3)	0.06
Delayed recall	10.1 (5.6)	9.3 (5.2)	0.61
QSPT			
Numbers of angles	3.2 (1.1)	3.9 (0.3)	**0.009***
Distance/Intersection	3.6 (0.5)	3.6 (0.5)	0.65
Closure/Opening	1.2 (0.7)	1.7 (0.8)	0.85
Rotation	1.2 (0.8)	1.7 (0.6)	0.08
Closing-in	0.9 (0.3)	0.9 (0.2)	0.27
Total Qualitative Score	10.7 (0.3)	11.4 (1.4)	0.15

MMSE: Mini- Mental State Examination. QSPT: Qualitative Scoring Pentagon test. *p = 0.0125 with Bonferrroni correction.

FIG. 5 EXAMPLE OF A PENTAGON COPY TEST BY A PATIENT WITH MCI-DLB (MMSE= 28/30)



Discussion

This study aimed at evaluating some features not yet investigated in patients with prodromal DLB: (I) whether an impaired performance on the pentagon copy item of the MMSE is detectable in the pre-dementia stage and which errors are most frequent; (ii) whether differences performing this cognitive task could be useful in differentiating patients developing DLB from AD, considered as single variable or in association with other clinical features; and (iii) which cognitive tests correlate with the pentagon copy test. In an autopsy-verified sample of DLB and AD we were able to demonstrate the ability of the QSPT to differentiate DLB from AD patients with a mean MMSE score of 24/30 (Mitolo et al. 2014), while the present results extend this possibility backward to the early stage of prodromal DLB with a good specificity. In a clinical setting, the high specificity versus a low sensitivity of the pentagon copy score means that having an impaired pentagon copy performance adds valuable information to the clinical assessment pointing towards a suspicion of DLB, while a normal performance leaves the possibility of both DLB and AD diagnoses. However, given that the performance of the pentagon copy test is a variable independent of other clinical features, the combination of cognitive and clinical information may decrease the false negative diagnoses. These data confirm that a different cognitive profile, with a relatively worse ability performing the executive/attentive and visual-construction testing for DLB and worse performance of memory testing for AD, is detectable in the MCI stage (Jicha et al. 2010, Boeve et al. 2004). In addition to previous studies (Mitolo et al. 2014), these results extend the power of the QSPT in differentiating DLB from AD at the MCI stage (MMSE 28/30). Through the QSPT method, we explored five different aspects of drawing production, but the only parameter that showed a significant difference between DLB and AD patients was the number of angles. These results confirm previous evidence (Mitolo et al. 2014; Caffarra et al. 2013) that this specific parameter may be considered consistent and useful in the differential diagnosis between DLB and AD and that this deficit may occur at a very early stage of the disease. The only correlation of the QSPT number of angles was found with the TMT-A score, possibly due to a common mechanism involving visual attention including the speed of ocular scanning and mental tracking (Salthouse, 2011). Overall, the present results support the view that testing visuo-construction abilities might be a useful and valid tool for differentiating between DLB and AD. We therefore propose that poor performance in the copy test, together with presence of subtle extrapyramidal signs and symptoms of RBD, may be of additional value to the clinical evaluation as a predictive screening tool for early DLB. However, future research should focus on extending the assessment to other visuospatial functions to increase our understanding of the relationship between the sub-components of the visuospatial domain, and to explore which specific aspects might best distinguish between DLB and AD at the early stage.

We therefore propose that poor performance in the copy test, together with presence of subtle extrapyramidal signs and symptoms of RBD, may be of additional value to the clinical evaluation as a predictive screening tool for early DLB.

Output of the study

This study has been published in parkinsonism and Related Disorders, 21(3), 303–305. (2015). *High specificity of MMSE pentagon scoring for diagnosis of prodromal dementia with Lewy bodies.* Cagnin, A., **Bussè, C.,** Jelcic, N., Gnoato, F., Mitolo, M., & Caffarra, P.

Study 2.

Assessment of visual attention, visual-spatial and visual-constructional deficits in MCI-DLB.

The previous study from our group demonstrated that a reduced number of angles of the MMSE pentagon copy could be a marker of MCI-DLB with a specificity of 91% in the discrimination with MCI-AD, suggesting that visuoconstructional impairment may serve as an early cognitive marker of DLB (Mitolo et al. 2014). In a neuropathologically confirmed study, it has been shown that in the prodromal stage of DLB, the performance in visual attention was worse than in AD (Jicha et al. 2010).

To our knowledge there are no studies assessing the subfield of visuospatial and perceptual abilities in the MCI stage due to DLB. Thus, the aim of this study was to determine which clinical and cognitive characteristics could help better identify MCI patients evolving to DLB from those evolving to AD, with particular emphasis on visual space and object perception deficits.

By applying a specific cognitive battery as The Visual Object and Space Perception (VOSP, Warrington et al. 1991), we expected that the assessment of visuospatial and perceptual abilities may reveal a typical early DLB phenotype, supporting the hypothesis of early 'dorsal visual stream' involvement in DLB. In particular, we expected a) lower scores in visuospatial VOSP subtests for MCI-DLB. A specific impairment in VOSP "number location" and "cube analysis", which rely on the 'dorsal visual stream', was found in clinically manifested DLB (Calderon et al. 2001); b) a relationship between QSPT and VOSP subtests; c) a relationship between the sub-components of the visuospatial domain and attention; in particular, a correlation between visuoconstructional items and attention tasks could tests this hypothesis.

Patients and Methods

Patients were enrolled and studied at the neurological departments of the Universities of Padova and Parma (Italy). Twenty-five patients with mild cognitive deficits in the predementia stage that were eventually diagnosed with probable DLB at the follow-up visits (MCI-DLB) and twenty-eight patients with mild cognitive deficits ultimately diagnosed as probable AD (MCI-AD) were included in the study. The charts of the patients were retrospectively evaluated, and data from clinical and neuropsychological assessments at the first visit (time of predementia stage) were evaluated by dementia experts not involved in the patients' assessments. In this study about 20% of DLB group is different respect to the previous study, and the 90% of the AD group. Inclusion criteria were: absence of dementia (cutoff MMSE score >26/30) and of functional impairment in everyday life at

the first visit, and clinical information available from the first visit to the follow-up visits until a definite clinical diagnosis of probable DLB or AD was reached according to current diagnostic criteria [8, 9] after at least 3 years of follow-up. Exclusion criteria were: inability to establish a definite diagnosis at the follow-up visits and cognitive decline due to comorbidities.

At the first assessment, the patients underwent full clinical history and neurological evaluation including screening for extrapyramidal signs with the Unified Parkinson's Disease Rating Scale (UPDRS) motor score (McKhann et al. 2011), assessment of behavioral disorders with the Neuro-psychiatric Inventory Questionnaire (Fahn et l. 1987), presence of fluctuations in cognition and attention using the Mayo Fluctuation Questionnaire (Cummings et al. 1997) and presence of symptoms suggestive of REM sleep behavior disorder (RBD) by the Mayo Sleep Questionnaire (Ferman et al. 2004).

Baseline neuropsychological evaluation was obtained for each patient. Global cognitive performance was assessed using the MMSE test. Each pentagon drawing of the MMSE test was retrospectively examined with the Qualitative Scoring MMSE Pentagon Test (QSPT) (Caffarra et al. 2013) by a neuropsychologist not involved in the formal cognitive assessment and blind to the diagnosis.

Specific cognitive domains were investigated using the following neuropsychological tests: digit cancellation and Trail Making Test A for the evaluation of visual attention; digit span forward and backward for short-term memory; prose memory (immediate and delayed recall) test for long-term memory; letter fluency test for the study of word generation and executive functioning; clock drawing test for visuoconstructional abilities and executive functions; Rey-Osterrieth Complex Figure (ROCF) test for the assessment of visuoconstructional abilities (ROCF copy) and long-term visual-spatial memory (ROCF delayed recall) (Boeve et al. 2011).

The Visual Object and Space Perception (VOSP) battery was administered to all patients in order to assess visual-perceptual and visual-spatial impairments (Warrington et al. 1991). The VOSP is a neuropsychological battery composed of a screening test, 4 subtests for object perception and 4 subtests for space perception (see appendix). The screening test, evaluating figure/ground perception, includes 20 squares with random patterns, on half of which a degraded 'X' is inserted. The subject is asked to judge whether the 'X' is present or not (maximum score 20). For object perception, in the fragmented letters task (subtest 1), the subject has to recognize 20 degraded capital letters (maximum score = 20); in the silhouettes task (subtest 2), the subject is asked to identify 30 black silhouettes presented in an unusual view (maximum score = 30); in the object decision task (subtest 3), the subject has to judge which of the 4 drawings represents a real object, while the 3 other objects are nonsense drawings (maximum score = 20); in the progressive

silhouettes task (subtest 4), 2 series of 10 drawings progressively easier to be identified were presented (maximum score = 20). For space perception, the dot counting subtest (subtest 5) is composed by 10 arrays of black dots, and the subject has to count the number of dots in each item (maximum score = 10); in the position discrimination task (subtest 6), 2 squares are presented, one with a black dot printed in the center and the other one with a black dot printed out of the center: the subject has to point to the dot perceived in the center of the square (maximum sore = 20); in the number location task (subtest 7), each item consists of 2 squares, one containing a random pattern of numbers and the other one containing a single black dot, corresponding to the position of one of the numbers: the subject has to point to the number that corresponds to the position of the dot (maximum score = 10); in the cube analysis (subtest 8), the subject has to count how many solid bricks there are in a series of drawings, each representing a tridimensional structure of bricks (maximum score = 10).

The Student t test for independent groups was used for the analysis of normally distributed variables, the Mann-Whitney U test for ordinal variables and the χ 2 test for categorical variables. Linear correlations between the cognitive test scores were tested using Spearman's ρ correlation coefficient. Logistic regression model analysis was used to find the independent cognitive variables related to the diagnosis. The significance level was set at $p < 0.05$. The study received approval by the institutional review board, and all patients signed informed consent.

Bonferroni correction was applied on 4 multiple comparisons based on the main cognitive domains investigated: attention and executive function, working memory, episodic memory, visual-spatial and visual-perceptual functions. The significance level was set at $p = 0.0125$ according to Bonferroni correction.

Results

At the MCI stage, the DLB and AD groups were matched for age (DLB: 76.5 ± 4.9 years, age range: 67–86; AD: 73.4 ± 6.7 years, age range: 57–83; p = 0.06), gender (male/female = DLB: 14/11; AD: 16/12; p = 0.9) and education (DLB: 9.3 ± 4.5 years; AD: 11.4 ± 4.7 years; p = 0.11). In the DLB group, the presence of visual hallucinations was reported in 9/25 patients (36%), fluctuations of cognition and alertness in 60% (15/25) and RBD in 60% (15/25). In the AD group, 1 patient had visual hallucinations, and none manifested fluctuations or RBD. Presence of extrapyramidal signs was the most frequent supportive feature detected in 18/25 DLB patients (72%), although often subtle (mean UPDRS score: 4.04 ± 5.9), with 13/18 patients with UPDRS score ≤ 5. Only 4/24 (17%) patients with prodromal AD showed subtle extrapyramidal signs (mean

UPDRS score: 0.25 ± 0.64). Neuropsychological test results of the MCI-DLB and MCI-AD groups are reported in table 7. The mean MMSE score was 27.8 ± 1.3 for DLB and 27.2 ± 1.3 for AD ($p = 0.07$). In the QSPT, the score for number of angles was significantly lower in the DLB patients ($p = 0.01$). An impaired number of angles (angle item score < 4) were present in 44% (11/25) of DLB patients compared to 3.7% (1/27) of AD ($p < 0.001$). No significant difference was found for the total QSPT score and its remaining subscores.

Patients of the DLB group showed greater impairment than those with AD in speed of visual attention (Trail Making Test A, $p = 0.03$), working memory (digit span backward, $p = 0.001$) and executive functions (verbal fluency, $p = 0.002$). AD patients, instead, showed greater impairment in verbal episodic memory, with lower scores in prose memory delayed recall ($p = 0.02$).

As for visuospatial abilities investigated with the VOSP battery, patients with MCI-DLB presented with significantly more severe visuoconstructional deficits (cube analysis subtest) and visual-attentional deficits (number location subtest) than AD patients (table 7). Deficits in the visual-perceptual domain were detected in MCI-DLB as well as in MCI-AD, particularly in the fragmented letters, silhouettes and object decision items, and therefore did not appear to be disease specific, although more DLB patients than AD patients scored below the cutoff value of normality (i.e. silhouette item <cutoff value = DLB: 72%, AD: 43%) (figure 6).

The QSPT score for number of angles correlated positively with the Trail Making Test A test score ($r = –.34$, $p = 0.01$) and cube analysis of VOSP ($r = 0.28$, $p = 0.04$), and negatively with the prose memory immediate recall test score ($r = –0.36$, $p = 0.008$) in the whole sample.

The QSPT scores for number of angles restricted to the DLB group correlated negatively only with the prose memory immediate recall ($p = 0.01$).

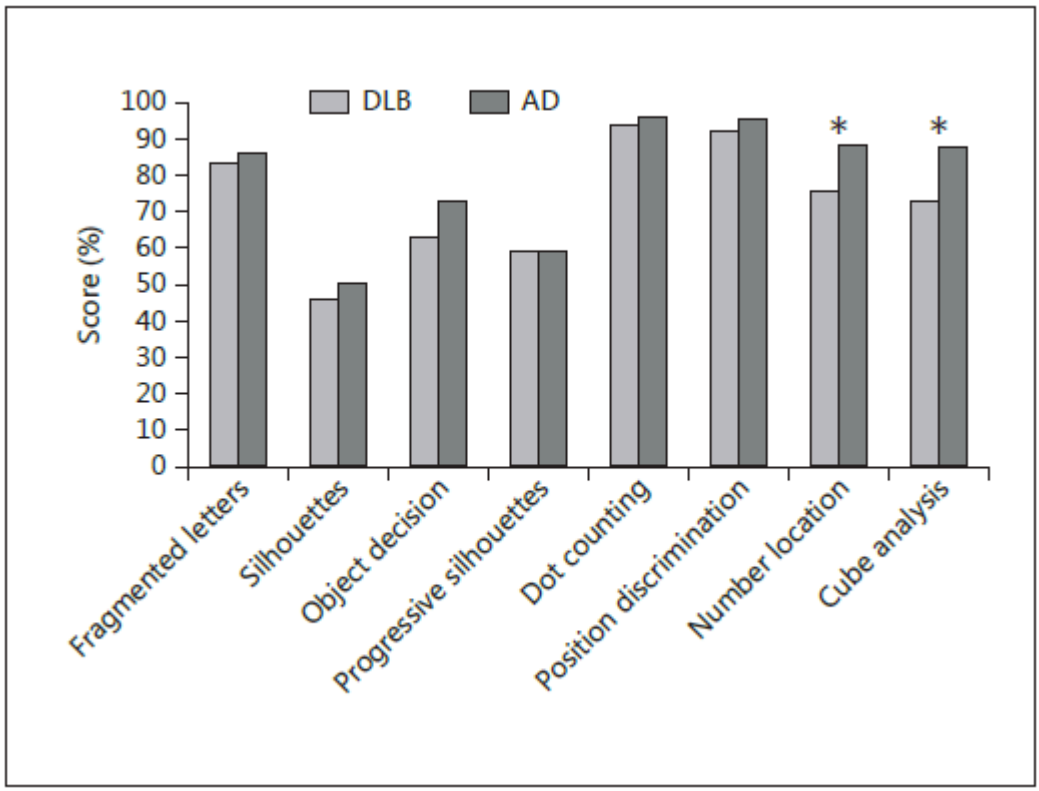
In the multivariate analysis using a logistic regression model, we considered only the test scores that were significantly different between DLB and AD ($p \leq 0.01$): number of pentagon angles, digit backward, verbal fluency and cube analysis of the VOSP battery. The results of this analysis showed that only the score for number of pentagon angles and the digit backward test score remained significantly correlated with the diagnosis. Using both the score for number of angles and the digit backward tests score, the specificity (88%) and sensitivity (54%) of the differential diagnosis did not change significantly with respect to using only the number of angles (specificity 96%, sensitivity 42%). The positive predictive value was 81% using both variables and 91% using only the score for number of angles, while the negative predictive value was 67% and 63%, respectively.

TABLE 7 COGNITIVE TESTS SCORE DIFFERENCES BETWEEN PRODROMAL DLB AND AD PATIENTS.

Cognitive tests	**Prodromal DLB (n=25)**	**Prodromal AD (n=28)**	***p***
MMSE	27.8 (1.3)	27.2 (1.3)	0.07
QSPT			
Total score	10.8 (2.1)	11.6 (1.2)	**0.1**
Number of angles	3.28 (1.1)	3.96 (0.2)	**0.01**
Number of angles < 4 (%)	44	3.70	**<0.001***
Digit Cancellation	41.8 (8.2)	43.7 (9.1)	0.56
Trial Making Test - A (s)	99.0 (70.8)	66.4 (28.0)	**0.03**
Digit Span			
Forward	4.7 (1.0)	5.1 (0.8)	0.10
Backward	3.0 (1.2)	3.9 (0.6)	**0.001***
Prose Memory			
Immediate recall (IR)	10.7 (4.6)	8.2 (5.9)	0.08
Delayed Recall (DR)	11.4 (5.8)	7.7 (5.4)	**0.02**
Verbal Fluency	22.3 (10.1)	30.5 (9.0)	**0.002**
Rey Complex Figure			
Copy	27.1 (4.9)	31.1 (7.2)	0.05
Delayed Recall	8.9 (4.5)	8.6 (5.4)	1
VOSP			
Incomplete Letters (1)	16.7 (4.2)	17.3 (3.7)	0.51
Silhouettes (2)	13.9 (7.2)	15.2 (5.1)	0.31
Object Decision (3)	12.6 (3.1)	14.6 (3.8)	0.05
Progressive Silhouettes (4)	11.9 (2.3)	11.8 (2.6)	0.96
Dot-Counting (5)	9.4 (0.7)	9.6 (1.3)	0.81
Position Discrimination (6)	18.5 (1.8)	19.1 (1.8)	0.25
Number Location (7)	7.6 (1.8)	8.9 (2.5)	**0.03**
Cube-Analysis (8)	7.3 (2.0)	8.8 (2.2)	**<0.001***

Data are presented as mean (SD) except where indicated otherwise. Bold *p* values indicate statistical significance. *p = 0.0125 with Bonferrroni correction.

FIG. 6 MEAN PERCENTAGE OF CORRECT SCORES FOR THE VOSP SUBTESTS IN MCI-DLB (LIGHT GREY COLUMNS) AND AD (DARK GREY COLUMNS).



Discussion

Cognitive features

Prodromal AD showed a disproportionate involvement in long-term episodic memory, while prodromal DLB manifested a poorer verbal working memory (digit span backward) and a prominent difficulty in the speed and ease of verbal production (verbal fluency), as a consequence of more frontal/dysexecutive dysfunction.

Our study supports the hypothesis that the visuoconstructional and object/space perception deficits typical of overt DLB are already present in the prodromal stage of the disease. However, visuoconstructional and visual motor tracking deficits were more severe in early DLB patients than in AD, while visual-perceptual abilities investigated by the fragmented letters, silhouettes, object decision and progressive silhouettes items of the VOSP battery were impaired in both prodromal DLB and AD. We were also able to demonstrate in prodromal DLB a specific impairment in number location and cube analysis, which rely on the 'dorsal visual stream', similarly to what Calderon et al. (2001) found in clinically manifested DLB. In other words, the ventral visual pathway ('what') seems to be involved in the early stages in both DLB and AD, while functional impairment of the dorsal pathway ('where') is more specific of DLB.

Presence of visual-spatial/visuoconstructional deficits has been considered very common in autopsy-confirmed DLB patients at their first assessment (Tiraboschi et al. 2006). The authors of this study proposed that the best model to predict DLB is the presence of visual hallucinations (positive predictive value: 83%), while lack of visual-spatial deficit represented a highly negative predictor of DLB (negative predictive value: 83%). However, in this study the patients were demented (mean MMSE score 24 ± 4.2) and not in their prodromal stage. Our data referred to a disease stage antecedent to that described by Tiraboschi et al. (2006) and strengthened the previous finding by Ferman et al. (2013) which longitudinally followed a cohort of MCI-DLB patients describing a pattern of attentional and visuospatial involvement since the prodromal stage of the disease.

Although the retrospective nature of our study may be a limitation, it has the advantage to investigate the full spectrum of visual-spatial perception and attention by applying a specific cognitive battery. As such, we were able to define that the visuoconstructional and spatial abilities are most specifically impaired in DLB, while object perception deficits, although very sensitive, are not specific cognitive signatures among DLB and AD in the prodromal stage.

The pentagon copying of the MMSE was first investigated in neuropathologically proven DLB and AD patients (Jicha et al. 2010). In the study by Ala et al. (2001), 2 of 17 DLB patients and 17 of 26 AD patients drew the pentagons regularly (10 angles, of which 2 intersecting), reaching an 88% sensitivity and a 58% specificity in discriminating these two conditions. However, the data referred to a group of patients in the full spectrum of disease severity, while in the earlier stages specificity could increase at the expense of sensitivity.

In an autopsy-proven study of pure AD (n = 66) and DLB (n = 9) patients, as opposed to the combination of AD and DLB (n = 57), it was demonstrated that pure AD patients performed worse in the verbal memory domain, while pure DLB patients presented spared verbal memory and a more altered visuospatial domain (Johnson et al. 2005). The AD/DLB comorbidity influences the cognitive pattern by further impairing only the visuospatial domain. Therefore, co-occurrence of DLB and AD pathology did not influence the speed of disease progression but only the type of cognitive impairment. We acknowledge that the main limitation of our study is the lack of neuropathological confirmation and the impossibility to confirm the above-mentioned data.

We found that prodromal DLB patients with reduced number of angles of the pentagon copy task, an index of altered visuoconstructional ability, have better verbal memory immediate recall scores than DLB patients with preserved number of angles.

We hypothesized that this subgroup of DLB patients may represent the pure form of DLB. This suggestion is supported by the finding of Yoshizawa et al. (2013) that pure DLB patients have

worse visuoconstructional deficits with respect to patients with AD and also mixed AD/DLB since the early stage of the disease. The confirmation of clinical diagnosis by brain neuropathology will validate this hypothesis.

Clinical features

In the MCI stage, the best clinical predictor of the developing disease (DLB vs. AD) was presence of soft extrapyramidal signs, detected in 72% of DLB patients, followed by presence of RBD (60%) and fluctuations (60%). These findings confirmed that some core features of DLB are already present before an overt dementia syndrome is manifested (Cagnin et al. 2015). Moreover, 52% of MCI-DLB patients presented at the first assessment with 2 core features (fluctuation and extrapyramidal involvement). However, these features may be underestimated since they could be very mild (very subtle motor involvement) or not sufficiently enquired (RBD and fluctuations). On the other side, only 4 patients with AD had 1 core feature of DLB.

These data are in line with those reported in a neuropathology case series by Jicha et al. (2010), in which 8 of 9 patients with MCI developing brain pathological changes supportive of DLB had at least 1 symptom among the core features of DLB versus none of the MCI subjects developing later AD.

The study by Molano et al. (2010) underscores the importance to investigate symptoms and signs beyond cognition in the prodromal DLB stages and suggests that RBD may be the most sensitive predictor of developing DLB. We confirm this statement, although in our study, the most frequent predictor of DLB was the presence of mild parkinsonism. Postuma et al. (2012) studied the development of parkinsonism in a cohort of patients with idiopathic RBD and demonstrated that motor changes, assessed with the UPDRS scale, were present for 6 years before the diagnosis of DLB, although with slow progression (UPDRS increase: 2 points per year). They concluded that extrapyramidal signs in prodromal DLB have a more indolent course than in Parkinson's disease and proposed that this may be, at least in part, due to the involvement of extranigral structures. In our study, extrapyramidal signs were subtle; mostly with a UPDRS motor score ≤ 5, suggesting the need to have a trained specialist to be intercepted.

It would be interesting to assess whether mild extrapyramidal signs detectable in AD patients, such as in the 4 subjects of this study, are due to the comorbid presence of α-synuclein pathology in the substantia nigra or whether they are caused by alterations of extranigral structures. In a recent retrospective cohort study of 531 patients with neuropathological verification, AD with Lewy bodies had poorer motor performance and more behavioral problems than AD without Lewy bodies (Chung et al. 2015).

Although the main limitation of this study is the lack of autopsy confirmation, we are confident that the clinical diagnosis of DLB would have mirrored the neuropathological changes, since the likelihood of occurrence of the clinical syndrome of DLB has been found to be positively related to the extension of cortical Lewy bodies and negatively to neuritic pathology in AD (Tiraboschi et al. 2015).

Output of the study

This study has been published in Dementia and Geriatric Cognitive Disorders Extra 2015. *Clinical and Cognitive Phenotype of Mild Cognitive Impairment Evolving to Dementia with Lewy Bodies.* Cagnin, A., **Bussè, C.,** Gardini, S., Jelcic, N., Guzzo, C., Gnoato, F., et al. 5 (3), 442–9.

Study 3.

Analysis of memory domain: specific verbal memory indices of the Rey's Auditory Verbal Learning Test.

Although declarative memory loss is not considered a core feature of cognitive impairment in dementia with Lewy bodies (DLB) (McKeith et al., 2005), some aspects of episodic memory, such as immediate or delayed free recall, may be compromised as severely as in Alzheimer's disease (AD) (Salmon et al., 1996; Hamilton et al. 2004, Perri et al., 2013).

Some authors demonstrated that both DLB (Perri et al. 2013; Filoteo et al. 2009) and AD patients (Estévez-Gonzalez et al. 2003; Perri et al. 2013) performed worse than healthy controls in word list tests. Considering the standard scores of the Rey Auditory Verbal Learning test (RAVLT), DLB patients did not perform differently from AD (Perri et al. 2013; Perri et al. 2014).

However, different anatomical brain structures are involved in the neuropathological changes typical of DLB and AD. The amnesic syndrome in AD, in fact, is associated with damage to the mesial-temporal cortical regions, including the hippocampus (Squire et al. 2004). In DLB, instead, the hippocampus is affected to a lesser degree than AD, while the neuropathological changes are widespread in neocortical regions (Burton et al. 2012, Perri et al. 2013).

In AD, memory impairment affects the storage processes, while in DLB consolidation and elaborative encoding/strategic retrieval mechanisms are both affected (Perri et al. 2013). Memory systems are differentially affected in the two diseases; however, to what extent these differences could be taken into account for clinical diagnosis remains an open question.

In the present study we investigated the possibility of differentiating DLB from AD by using the RAVLT, a widely used tool to assess episodic memory (Perri et al. 2014; Perri et al. 2013, Tierney et al. 2001). In particular, we aimed at assessing if any of the specific measures of verbal memory obtained with the RAVLT, as verbal learning (VL), verbal forgetting (VF) and the serial position effects, could be of value in distinguishing DLB from AD.

We expected that specific measures of memory may reveal a greater learning capacity for DLB and a greater forgetting measure for AD, supporting the hypothesis of 'AD amnesic syndrome', characterized by deficient learning and rapid memory decay, as reported by Squire et al. (2004). Considering the serial position effects, a reduction of primacy and a normal (Bayley et al., 2000; Martín et al., 2013; Orru et al., 2009) or increased recency (Howieson et al., 2011; Jones, Greer & Cox, 2011) could be expected for AD. No evidence in regional performances between AD and DLB were previously investigated.

Patients and Methods

Eighty-nine participants were enrolled in the study: 29 diagnosed as probable DLB, 32 with probable AD and 28 healthy elderly subjects used as normal control (NC) group. Patients were evaluated at the Neurological Department of the University of Padua (Italy) and followed longitudinally for at least three years till the diagnosis were made according to standard criteria (McKhann et al. 2011, McKeith et al., 2005). A different sample of patients respect to the previous studies has been recruited. Initial and follow-up examinations included medical history, neurological examination, global cognitive function evaluation and extensive neuropsychological assessment (memory, language, visuospatial and constructive abilities, attention, and executive functions). NC participants were healthy elderly volunteers recruited from programs of community based social engaging activities. Inclusion criteria for NC were Mini Mental State Examination (MMSE) score $\geq$ 27 and absence of the following features: active or previous neurological/psychiatric disorders, medical disorder that could be associated with cognitive impairment, subjective memory disturbance, behavioral changes, and history of alcohol or drug abuse. The three groups were comparable for age (years: DLB=74.7±7.5, AD=72.2±8.3, NC=72.8±3.9; $F_{(2.86)}=1.04$; p=0.36) and gender distribution ($F_{(2.2)}=6.04$; p=0.05). Educational levels were different among groups ($F_{(2.86)}=25.91$, $p<0.001$), with the NC group more educated than both DLB and AD (DLB= 8.4±4.2, AD= 10.2±3.8; NC= 15.3±3.1; $p<0.001$ in all comparisons). As expected, mean MMSE scores also differed among groups ($F_{(2,86)}=30.92$, $p< 0.001$), whereby NC had a higher mean MMSE score than both patient groups, as shown in Table 8. To adjust these differences, educational level and MMSE score were used as covariates in the other comparisons.

Participants underwent baseline neuropsychological examination, which included the MMSE test. Specific cognitive domains were investigated using the following neuropsychological tests: digit cancellation and Trail Making Test A for the evaluation of visual attention; digit span forward and backward for short-term memory; prose memory (immediate and delayed recall) test for long-term memory; letter fluency test for the study of word generation and executive functioning; clock drawing test for visuoconstructional abilities and executive functions; Rey-Osterrieth Complex Figure (ROCF) test for the assessment of visuoconstructional abilities (ROCF copy) and long-term visual-spatial memory (ROCF delayed recall) (Boeve et al. 2011).

Each pentagon drawing of MMSE was retrospectively examined with the Qualitative Scoring of the pentagon copy test (QSPT) (Caffarra et al., 2013).

For the evaluation of specific measures of episodic verbal memory, RAVLT was administrated (Carlesimo et al., 1996). RAVLT consists of 15 nouns read aloud by the examiner (with a 1-sec.

interval between each word) for five consecutive trials (trials 1 to 5) [see appendix]. Each trial is followed by a free-recall test. Patients are instructed that they would hear a list of 15 words and those they should listen to it carefully because they would be asked to repeat as many words as possible. The order of presentation of words remains fixed across trials. After a 20-min delay period, each subject is again required to recall the words (trial 6). Other psychometric activities were carried out during the 20-min delay period. RAVLT standard scores include the proportion of items correctly recalled for each trial and the sum of them for the immediate recall performance (i.e. Trial 1= number of words correctly recalled in trial 1 *1/15; total immediate recall: =number of words correctly recalled in trial 1+ number of words correctly recalled in trial 2+ [t3..+t4+t5] *1)/75. 75 is the result of 15 item* 5 lists.

The following verbal memory indices were calculated according to previous literature (Gainotti and Marra, 1994; Antonelli-Incalzi et al., 1995; Estévez-Gonzalez et al. 2003): *a) Verbal Learning* (VL) corresponding to the result of the difference between the total words recalled after trial 5 *minus* the total words recalled after trial 1; *b) Verbal Forgetting* (VF) calculated as the difference between the total words recalled in trial 5 *minus* the total words recalled in the delayed performance trial (trial 6); *c) Percentage of Verbal Forgetting* (%VF) calculated as the ratio VF/total words recalled in trial 5 in percentage. This last index represents the level of VF loaded for the number of words recalled after the last trial of immediate verbal memory performance. d) To study the *serial position effects*, the 15-word list of trial 1 was divided as follows: the first 4 words represented the primacy region, the next 7 words represented the middle region, and the last 4 words represented the recency region of the list. Since the primacy, middle and recency regions contained a different numerosity, a proportion has been calculated as number of words recalled in each region divided by the total number of items presented in each region, (i.e. primacy = number of words recalled in primacy region *1)/4).

Statistical analysis was performed using IBM SPSS Statistics. A comparison of the neuropsychological performances of the three groups (DLB AD and NC) has been done using a one-way ANCOVA for each test and MMSE and education as covariates. Logistic regressions were made separately with the VL, VF and %VF as independent variables and patient group as dependent variables, in order to estimate the probability of the group membership outcome (AD or DLB).

A Chi-squared test served to investigate the relationship between the group and %VF $\geq$ a cut-off of 75 %. Regional scores of the first trial of the RAVLT were assessed using a one-way applying the Bonferroni correction. Linear correlations between the recency effect and attentive/executive test scores were tested in the three groups using the Spearman's ρ correlation co efficient.

Results

Neuropsychological tests differences among the three groups are summarized in Table 8. DLB group obtained worse scores than AD only in the Digit cancellation task (DLB=34.78±11.1, AD=42.26±10.3; p< 0.001) and in the rotation of pentagons copy (DLB= 1.16±0.7, AD=1.68±0.5 <0.001), while in all the other tests they were similar. Particularly in the tests exploring declarative memory, standard scores were nor significantly different between DLB and AD.

The mean values of recalled words in each trial of the RAVLT were comparable (IC=95%) and impaired respect to controls in DLB and AD, as illustrated in Figure 7. Regarding VL, VF and %VF, we found a significant effect of the group on all the three measures (VL: $F_{(4,1)}$=17.84, p<0.001; VF: $F_{(4,1)}$=9.15, p<0.001; %VF: ($F_{(4,1)}$=22.27, p<0.001). Considering the VL, post-hoc showed better performances of DLB vs AD (VL: DLB= 0.25±0.1, AD=0.19±0.1; p<0.05). Considering the VF, AD and DLB were similar (VF: AD=0.19±0.1, DLB=0.25±0.1; p>0.05). In the %VF, AD obtained a greater %VF than DLB (%VF: AD=65.84±41.3, DLB=42.96±33.0; p=0.001). The %VF it has been revealed the only marker in predicting the type of disease (T=-0.02, Wald $_{(1)}$ =4.85, p=0.028). DLB performed equally in all the memory measures (all p>0.05) respect to NC. AD performed similarly to NC only in VL (p>0.05) but differently in VF and %VF (VF: NC=0.11±0.1, p<0.01; %VF: NC=13.67±14.3, p=0.001).

The relationship between percentage of patients falling above/below a VF-75%-cut-off and the patient group membership was significant (χ^2=8.72, p<0.01) with a significant distribution (Choerish = 5.1); in particular, the number of AD patients with ≥75 % VF (AD=18) is three times greater than the number of DLB (n=6) lying above the cut-off (Table 9).

In the serial position effects, results revealed an effect of group on the proportion of words recalled both in the primacy ($F_{(2, 86)}$ =15.83, p<0.001) and in recency regions ($F_{(2, 86)}$ =3.76, p<0.05). Considering the primacy effect, post-hoc revealed a stronger primacy for the NC (p<0.001) while AD and DLB were similar (Primacy: NC=2.07±1.1, DLB=0.89±0.8, AD=0.75±0.9). Considering the recency effect, AD and DLB was different (p<0.05) with an increasing slope at the end of the curve for the AD and reduction for the DLB (Recency: AD=2.09±1.1, NC=1.78±0.9, DLB=1.34±1.2; Figure 9). Number of recalled words in the recency domain correlated positively with scores of the digit span backward test (r=0.54, p=0.005) and digit span forward (r=0.25, p=0.02) only in DLB (digit span backward: AD: r=0.88, p=0.88; NC: r=-0.02, p=0.94; digit span forward: AD: r=0.12, p=0.52; NC: r=0.24; p=0.22).

TABLE 8 DIFFERENCES OF NEUROPSYCHOLOGICAL TESTS SCORES (MEAN VALUES ± SD) AMONG PATIENTS WITH ALZHEIMER'S DISEASE (AD), DEMENTIA WITH LEWY BODIES (DLB) AND NC PARTICIPANTS.

Test scores (Mean± SD)	DLB (n=29)	AD (n=32)	NC (n=28)	p
MMSE	24.3±3.2&	24.6±3.3*	29.4±0.9	< 0.001
Digit cancellation task	34.78±11.1$	42.26±10.3	51.78±4.4	<0.001
Trial making-A (time")	118.35±77	96.47±57.4	51.14±28	<0.001
Trial making-A(errors)	0.87±1.7	0.83±1.2	0.07±0.3	<0.01
Letter fluency	20.14±10&	22.63±9.7*	41.96±7.1	<0.001
Semantic fluency	23.61±6.3&	24.68±11.6*	46.71±8.1	<0.001
Digit span forwards	4.92±0.8	5.23±0.8	5.5±0.8	<0.05
Digit span backwards	3.15±0.8	3.39±0.8	4.29±0.8	<0.001
Prose immediate recall	8.21±4.5	6.76±4.5	11.4±3.7	<0.001
Prose delayed recall	8.57±5.5	6.86±5.2	13.2±3.2	<0.001
RAVLT immediate recall	28.27±1.5	27.82±1.4	45.72±1.8	<0.001
RAVLT Delayed Recall	4.60±0.5	3.19±0.4	9.30±0.6	<0.001
ROCF Delayed Recall	8.49±9&	7.05±6*	19.8±4.8	<0.001
Clock drawing	6±3.5	5.98±3.2	9.65±0.4	<0.001
ROCF copy	20.9±10.3	25.12±9.5	32.3±2.1	<0.001
QSPT:				
Distance/Intersection	3.00±0.8&	3.43±0.8	3.93±0.3	<0.001
Rotation	1.16±0.7$&	1.68±0.5	1.96±0.2	<0.001
Total score	10.00±2.7	11.14±2.6	12.70±0.7	<0.01

$: DLB FROM AD; &: DLB FROM NC; *: AD FROM NC.

FIG. 7 REY AUDITORY VERBAL LEARNING TEST (RAVLT) CURVES DERIVED FROM NUMBER OF RECALLED WORDS (%) ACROSS THE SIX TRIALS IN NC, AD, AND DLB GROUPS.

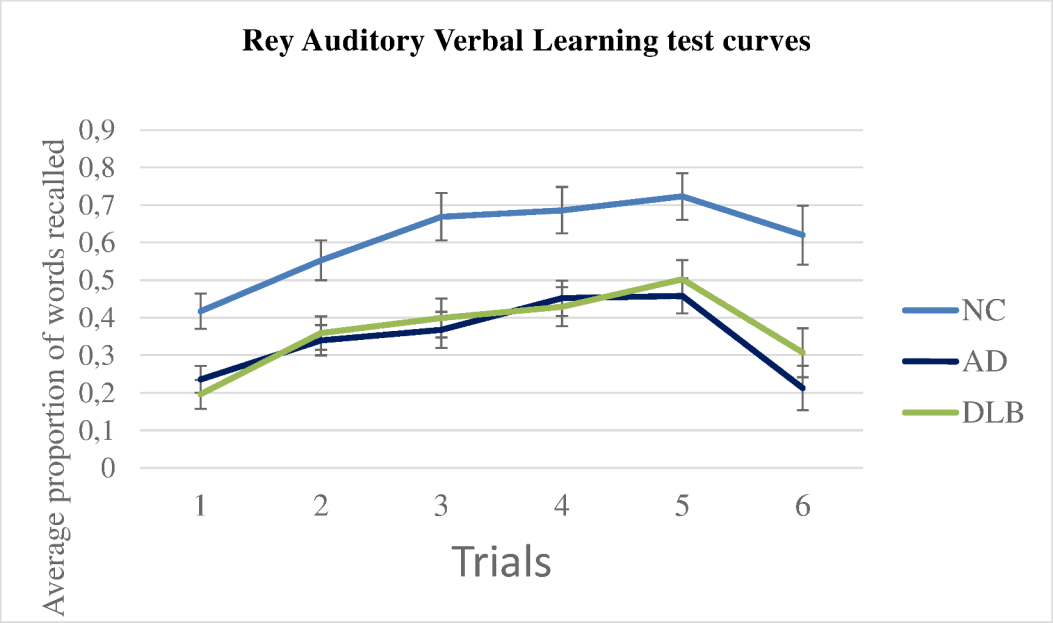


TABLE 9 NUMBER AND PERCENTAGE OF AD AND DLB PATIENTS ABOVE/BELOW THE CUT-OFF VALUE (75%) IN PERCENTAGE OF VERBAL FORGETTING.

Percentage of Verbal Forgetting		<75 %	≥75 %	total
DLB	n	23	6	29
	%	79	20.7	100
AD	n	13	18	31
	%	41.9	58.1	100

FIGURE 8. PERCENTAGE OF AD AND DLB PATIENTS ABOCE THE CUT-OFF VAUE (75%) IN PERCENTAGE OF VERBAL FORGETTING

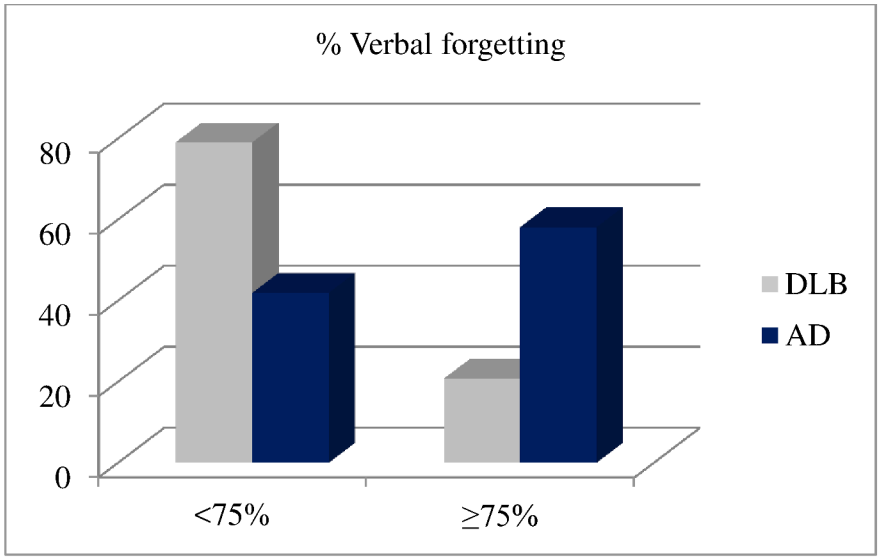
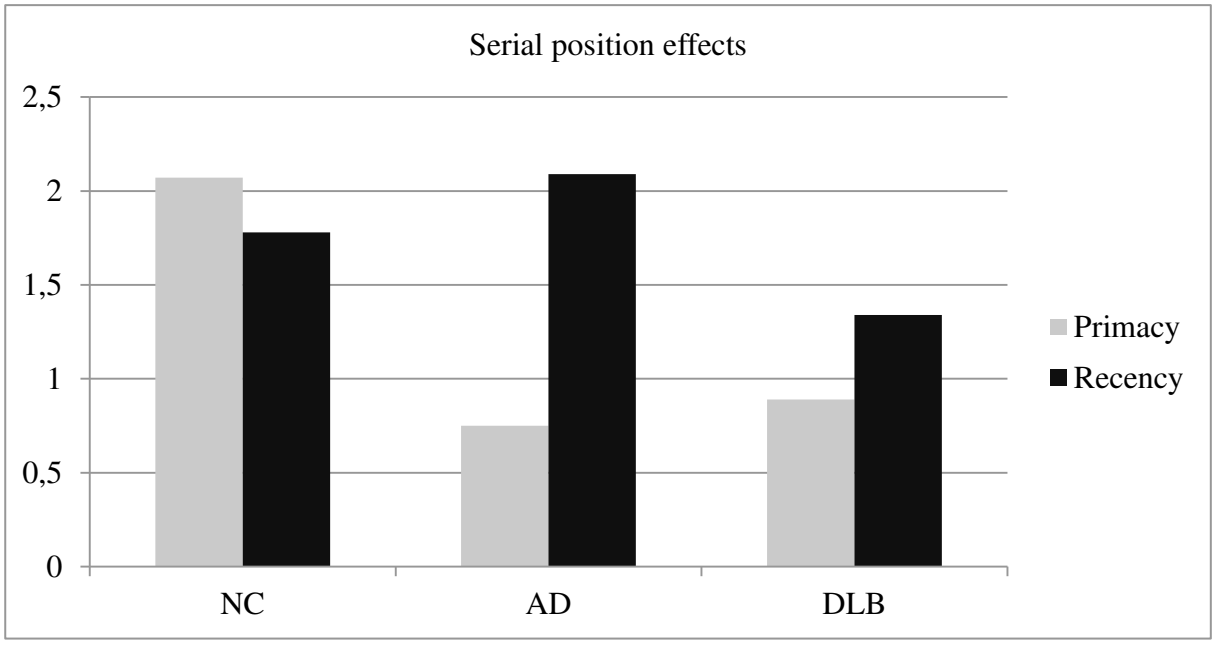


FIG. 9 MEAN VALUES OF THE NUMBER OF WORDS RECALL (PROPORTION) IN THE PRIMACY AND RECENCY REGIONS OF THE TRIAL 1 OF THE RAVLT.



Discussion

A comparison of cognitive performance in mild DLB and AD reported differences only in visual attention. This could indicate that the profile of cognitive impairment becomes similar in a mild-moderate dementia stage of both neurodegenerative diseases, being visuo-spatial, executive and memory functions similarly impaired. This is in line to the fact that neuropsychological tests may not have a discriminative property in differentiating DLB from AD in the mild stage.

This is particularly true for the prose memory and for the RAVLT test. While a standard memory test as the RAVLT is not enough specific in distinguishing DLB from AD, specific measures of verbal memory computed in the RAVLT could help in discriminating between these two conditions. Patients with DLB demonstrated a greater learning curve than AD while, on the contrary, AD had a greater forgetting curve on the %VF measure, the last holding the better discriminating ability.

The %VF measure represents the loss of recall items over time respect to the amount of items previously learned. In fact, isolating the number of items recalled previously, where AD and DLB were similar, AD showed a rate of forgetting higher than DLB. This value probably better highlights the amnesic syndrome of AD, characterized by deficient learning and rapid memory decay, consistently with Squire et al. (2004). In fact, poor VF and %VF respect to healthy controls would possibly be suggestive of probable AD (Estévez-Gonzalez et al. 2003). DLB patients instead could possibly benefit more than AD from the repeated exposure to items enhancing storage capacity and retrieval.

The power of this study was to identify the %VF as a predictable marker of the type of disease (DLB or AD). In this regard, patient with higher scores of %VF have more probability to be AD where lower scores could be suggestive for DLB. Regarding this value, we found that the number of AD patients over the threshold were three times greater the number of DLB, with a 20.7 % DLB lying above the cut-off. This case probably represents the "mixed DLB/AD conditions", with DLB characterized by a concomitant AD pathology. So, the 75% threshold value could help in facilitating discrimination between pure DLB to DLB-AD mixed.

The study of the serial position effects (Murdock et al, 1962) offered the possibility to better understand the different processes involved in the episodic memory deficits. To our knowledge a reduction of primacy effect and a normal (Bayley et al., 2000; Martin et al., 2013; Orru et al., 2009) or increased recency effect (Howieson et al., 2011; Jones, Greer, & Cox, 2011) could be prognostic of AD. No evidences in regional performances between AD and DLB were previously investigated.

In this study, while both patients groups are similarly impaired respect to healthy subjects in the primacy effect, AD and DLB groups performed differently for the recency. DLB showed a recency reduction respect to AD. We can conclude that the recency effect could be another good marker of DLB to be considerate while administrating the RAVLT. Finally, since the recency effect correlates with attentive span properties, a reduction of recency effect in DLB could be driven by encoding deficits due to attentional impairment.

In this study, while both patients groups are similarly impaired respect to healthy subjects in the primacy effect, AD and DLB groups performed differently for the recency, where DLB showed a recency reduction respect to AD. The traditional dual-store model (Talmi et al.2005) and the unitary-store model (Campos et al. 2015) are two opposite perspective to explain the serial position effects. In a single-store perspective, the reduction of the recency effect is due to an impairment of activation of attentional/perceptive processes of working memory (WM). This interpretation may explain the recency reduction in DLB. Primacy effect, instead, is generally related to the higher activation level of working memory (named FA - Focus of attention) allowing the implementation of strategies of rehearsal during the input. A similarity of primacy effect between AD and DLB could be easier to explain in a dual-store interpretation (Talmi et al.2005). Dual-store models implies that the recognition of early items is activated by left hippocampal areas, associated with long term memory (LTM), and frontal and parietal cortical areas, associated WM. In this model, the primacy effect is similar between AD and DLB for the engagement of both LTM and WM processes that are respectively strongly impaired. On the opposite, being a recency effect not associated with hippocampal activation, but only with WM processes, AD is normal and DLB impaired.

Irrespective of the cognitive models, the performance at the recency effect could be considered another good marker of DLB. Finally, since the recency effect correlates with attentive span properties, a reduction of recency effect in DLB could be driven by encoding deficits due to attentional impairment.

Output of the study

This study is in submission.

Study 4.

Analysis of memory domain in mild cognitive impairment: investigating the efficacy of the Free and Cued Selective Reminding Test (FCSRT).

Only few studies considered the cognitive phenotype of DLB and AD at the MCI stage (Guidi et al. 2006, Kondo et al. 2016) and no studies specifically address type of memory deficits in MCI due to DLB. It is widely known the involvement of three different processes in memory functioning, such as recording/encoding, storage and retrieval of information. The impairment of at least one of these processes could lead to low memory performance. Whether low performance depends on a primary effect of memory deficit (in encoding, storage or retrieval) or, instead, is secondary to an inefficient ability to recover strategies, i.e. in the case of an executive dysfunction, the result will be the same: low memory performance. In order to investigate separate components of episodic memory, the Free and Cued Selective Reminding test (FCSRT) (Grober e Buschke, 1987; Grober et al. 2010; Frasson et al. 2011) has been demonstrated to be a useful psychometric instrument able to separate the assessment of the underlying mechanisms: 1) a controlled encoding of items to be registered 2) the following immediate and delayed spontaneous recall of the information and 3) the checking of the recall by providing semantic cues facilitating the access to information previously stored (see appendix).

It has been demonstrated that the amnesic dysfunction in AD is related to poor information storage, not improved by controlled learning and cued recall (Economou et al., 2016). Therefore, patients with AD usually obtain low performances in total recall scores of FCSRT. In contrast, low FCSRT scores in non-AD patients are due to a secondary effect of the executive dysfunction that adversely affects strategies for encoding and retrieval (Grober et al. 2010).Furthermore, considering that the FCSRT has already been shown to be efficient at distinguishing between AD dementia and non-AD dementia even at MCI stage (not focusing on DLB in particular) (Grober et al., 2010), we expected FCSRT scores to be significantly different in MCI converting to DLB respect to AD, supporting the hypothesis of lower performances in MCI-AD.

Thus, the aim of the present study was to compare cognitive performances at the FCSRT in MCI patients converting to DLB (MCI-DLB) or AD (MCI-AD).

Materials and methods

Thirty-five patients with MCI without dementia were recruited at the Memory Clinic of the University of Padova (Italy). At follow-up visits, fifteen patients had a clinical diagnosis of

probable DLB (MCI-DLB), while twenty patients had a clinical diagnosis of probable AD (MCI-AD). The diagnosis of MCI was made according to established criteria (Petersen *et al.*, 2004; Albert et al. 2011; McKeith et al. 2005) considering the results of an extensive cognitive test battery and clinical history. The clinical diagnosis of probable DLB or AD was made according to current diagnostic criteria (McKeith *et al.*, 2005; McKhann *et al.*, 2011).

Inclusion criteria were: absence of dementia at the baseline visit with a cut-off MMSE score≥ 25/30; no evidence of (a) substantial concomitant cerebrovascular disease, defined by a history of stroke temporally related to the onset or worsening of cognitive impairment; or the presence of multiple or extensive infarcts or severe white matter hyper intensity burden; or (b) core features of other dementing disorders apart from dementia itself; or (c) prominent features of semantic variant primary progressive aphasia or nonfluent/agrammatic variant progressive aphasia; or (d) evidence for another concurrent, active neurological disease, or a non-neurological medical comorbidity or use of medication that could have a substantial effect on recognition; finally, the cognitive and behavioral dysfunctions are not explained by delirium or a major psychiatric disorder.

At baseline all patients underwent a neuropsychological evaluation by an experienced neuropsychologist. Global cognitive performance was assessed using the Mini Mental State Examination (MMSE, Magni *et al.*, 1996). Specific cognitive domains were investigated using the following neuropsychological tests:

Attention and executive functions:

- Digit span Cancellation (Della Sala *et al.*, 1992): the patient is asked to search some target numbers in a sheet. We considered the number of right targets found in 45 sec in the three matrices.
- Trail Making Test - part A (TMT-A; Giovagnoli *et al.,* 1996): the subject is instructed to connect a set of 25 dots as quickly as possible while still maintaining accuracy.
- Verbal fluency (Mondini *et al.*, 2011): the patient is asked to generate as many words as possible from a category in a given time. Both letter (i.e., words that begin with letters “c”, “p”, “s”; Mondini *et al.*, 2011) and semantic (words belonging to animals, fruits and brands of cars categories; Novelli *et al.*, 1986) fluency tasks were assessed. We considered the numbers of correct words for each category in 60 seconds.

Memory:

- Digit span forward and backward (Monaco *et al.*, 2013): subjects hear a sequence of numerical digits and are tasked to recall the sequence correctly (in normal or reverse order), with increasingly longer sequences being tested in each trial.

We considered the longest number of sequential digits that can accurately be remembered. The test evaluates short term and working memory.

• Prose Memory Test (Mondini *et al*., 2011)*:* it consists of listening to a short passage followed by an immediate recall; successively, the patient is presented the same passage and a recall is required after 10 minutes. We considered the different scores of these two trials. The test evaluates long term memory.

• Free and Cued Selective Reminding Test (FCSRT) (Frasson *et al.*, 2011): Five scores were derived from the test: immediate free recall (0 to 36 points); immediate total recall, which is the sum of the words recalled with cues and those recalled without cues (0 to 36 points); delayed free recall (0 to 12 points); delayed total recall, which is the sum of the words recalled with cues and those recalled without cues (0 to 12 points); and Index of sensitivity of cueing (cut-off score ≥ 0.90). The test evaluates episodic memory and the type of process involved.

• Rey-Osterrieth Complex Figure (ROCF) delayed recall test (Caffarra *et al.*, 2002): patients are asked to reproduce a complicated line drawing that they copied earlier in the neuropsychological assessment. The test evaluates long term visual-spatial memory.

Visual-spatial and visuoconstructional abilities:

• Pentagon drawing of MMSE: each pentagon was examined with the Qualitative Scoring MMSE Pentagon Test (QSPT; Caffarra *et al.*, 2013). In particular, only the number of angles reported was taken into consideration (Table 10).

• Clock Drawing test (Mondini *et al.,* 2011): patients have to draw numbers and hands of the clock in a large pre-drawn circle printed on a sheet of paper.

• ROCF copy test (Caffarra *et al.*, 2002): patients are asked to reproduce a complicated line drawing, first by copying it freehand (recognition).

TABLE 10. QUALITATIVE SCORING METHOD FOR THE PENTAGON COPYING TEST (QPST). SUB-SECTION FOR THE SCORING OF NUMBER OF ANGLES

Parameters	Performance scores	Assigned scores
Number of angles	10	4
	10±1	3
	10±2	2
	7-5	1
	<5 or >13	0

Statistical analysis was performed using IBM SPSS Statistics. Pearson's chi-squared test was used to compare the distribution of gender, visual hallucinations, symptoms of parkinsonism, and cognitive fluctuation between the groups. Independent samples t-test were used to compare MCI-DLB and MCI-AD on MMSE score, education and age at time of the assessment. Performances of the neuropsychological tests were considered as dependent variables (DV). Since age and education were found to have a significant relation with the DVs, an analysis of covariance (ANCOVA) was performed applying the Bonferroni correction. This allowed to statistically controlling for the effects of continuous variables that were not of primary interest, i.e., covariates (CV). MMSE, education and age were considered as CVs.

Results

Demographics data are shown in table 11. The two groups were matched for age (MCI-DLB: 74.60±4.26 years; MCI-AD: 69.55±10.08 years; p= 0.05), gender (MCI-DLB: M/F= 7/8; MCI-AD: M/F= 9/11; χ^2 (1, 35) = 0.01; p= 0.92), education (MCI-DLB: 10.20±4.13 years; MCI-AD: 12.45±4.02 years; p= 0.12). Visual hallucinations (χ^2 (1, 30) = 12.96; p< 0.001), symptoms of parkinsonism (χ^2 (1, 30) = 15.77; p <0.001), cognitive fluctuations (χ^2 (1, 30) = 15.77; p<0.001) were found to be significantly different in the two disorders, i.e., they were typical features of DLB patients. Neuropsychological test results of the MCI-DLB and MCI-AD groups are reported in Table 12. The mean MMSE score was 26.80±1.27 for MCI-DLB and 27.15±1.39 for MCI-AD (p= 0.44).

Memory

Performances of prose memory test did not differ significantly between MCI-DLB and MCI-AD, either in the immediate recall (F (1, 27) = 0.92; p= 0.35) nor in the delayed recall (F (1, 27) = 0.39; p= 0.54).

Similar performances were observed also in the digit span forward (F (1, 30) = 0.29; p= 0.59) and backward (F (1, 30) = 0.27; p= 0.61) tasks. ROCF delayed recall was also not significantly different between the two disease groups (F (1, 30) = 0.61; p= 0.44).

As for the FCSRT instead, significant differences were found in two variables: MCI-AD patients had worse performances in the immediate total recall (ITR; F (1, 30) = 9.07; p= 0.01) and also lower sensitivity to cueing (ISC; F (1, 30) = 10.75; p < 0.001) compared to MCI-DLB (Figures 10 and 11).

On the contrary, no significant differences were observed in the immediate free recall scores between the two groups (IFR; F (1, 30) = 2.45; p= 0.13). The disease group did not influence the

delayed recall, neither delayed free recall (DFR; F (1, 30) = 1.89; *p*= 0.18) or delayed total recall (DTR; F (1, 30) = 2.95; *p*= 0.10).

Visuospatial and visuoconstructional abilities

The number of angles reported in pentagon-copying was significantly worse in MCI-DLB patients (F (1, 30) = 6.87 *p*=0.01). MCI-DLB group showed a trend of greater impairment than those with MCI-AD even in ROCF copy task (F (1, 30) = 4.26 *p*=0.05), supporting the dysfunction of visuoconstructional abilities. On contrary, similar performances were observed in Clock drawing (F(1, 28) = 0.65, *p*=0.43).

TABLE 11. DEMOGRAPHICS OF PATIENTS WITH MCI-DLB AND MCI-AD (BOLD * P VALUES INDICATE STATISTICAL SIGNIFICANCE)

Demographics	**MCI-DLB** (n=15)	**MCI-AD** (n=15)	***p***
Gender (m/f)	7/8	9/11	0.92
Age	74.60±4.26	69.55±10.08	0.05
Education	10.20±4.13	12.45±4.02	0.12
VH %	54.5	0	**0.00** *
Parkinsonism %	63.6	0	**0.00** *
Cognitive fluctuations %	63.6	0	**0.00** *

TABLE 12. NEUROPSYCHOLOGICAL TEST SCORES IN MCI-DLB AND MCI-AD PATIENTS (BOLD * P VALUES INDICATE STATISTICAL SIGNIFICANCE).

Cognitive tests	**MCI-DLB** (n=15) M ± SD	**MCI-AD** (n=20) M ± SD	***p***
MMSE (raw score)	26.80 ± 1.27	27.15 ± 1.39	0.44
QSPT (Number of angles)	3.07 ± 0.80	3.75 ± 0.44	**0.01** *
Digit Cancellation	44.53 ± 6.01	50.55 ± 5.49	**0.03** *
Trail making test A, sec	96.07 ± 41.57	63.45 ± 37.64	0.10
Fluency			
Phonemic	27.20 ± 6.19	32.95 ± 12.19	0.49
Semantic	29.47 ± 8.70	34.47 ± 14.29	0.74
Digit Span			
Forward	5.45 ± 1.19	5.33 ± 0.98	0.59
Backward	3.65 ± 1.09	3.47 ± 0.99	0.61
Prose memory			
Immediate recall	8.53 ± 3.46	8.00 ± 5.48	0.35
Delayed recall	9.40 ± 4.62	7.76 ± 5.89	0.54
ROCF			
Copy	22.33 ± 6.93	28.97 ± 5.81	0.05
Delayed recall	9.53 ± 5.73	8.65 ± 6.49	0.44
Clock drawing	5.97 ± 0.84	7.83 ± 0.76	0.43
FCSRT			
IFR	22.60 ± 5.89	18.05 ± 9.89	0.13
ITR	34.93 ± 1.75	30.10 ± 6.15	**0.01** *
DFR	7.73 ± 3.85	5.35 ± 4.02	0.18
DTR	11.33 ± 1.35	9.95 ± 2.82	0.10
ISC	0.94 ± 0.08	0.75 ± 0.19	**0.00** *

Fig 10. Immediate Total Recall (ITR) scores of MCI-DLB and MCI-AD groups.

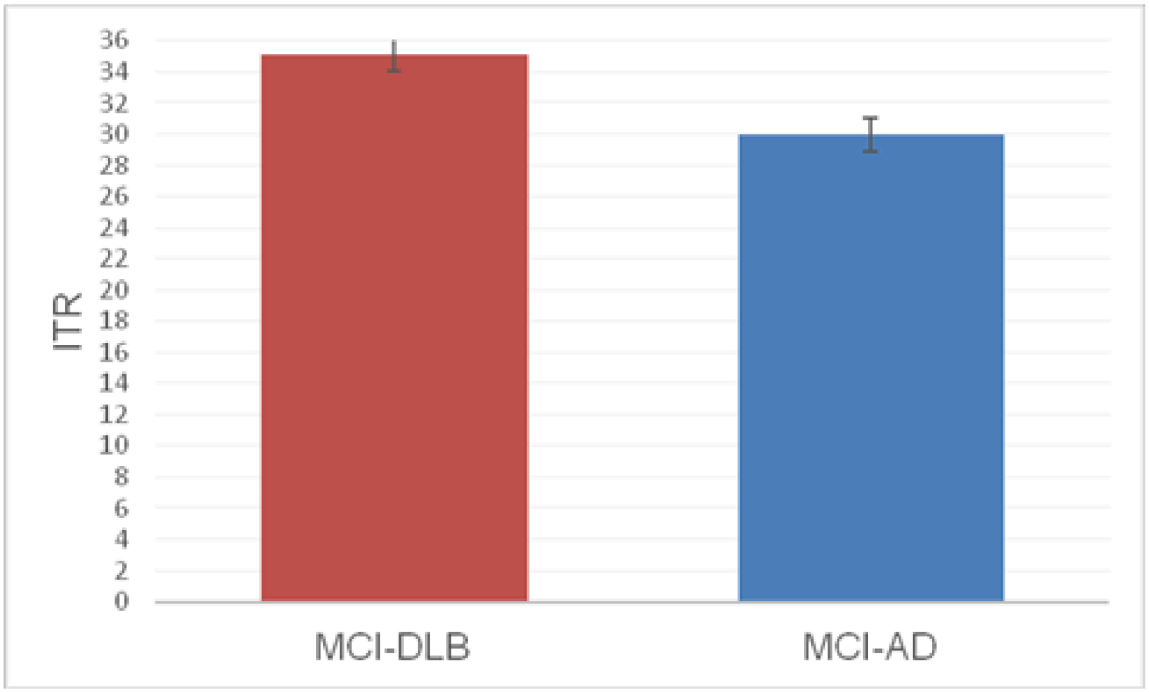
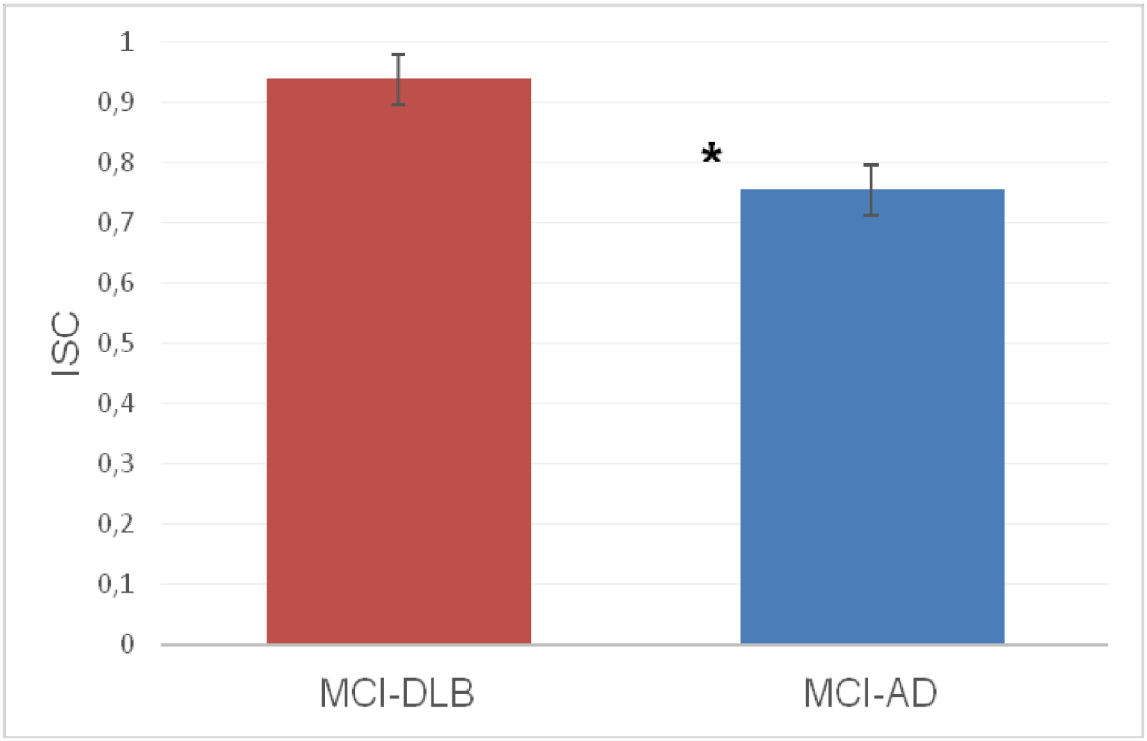


Fig 11. Index of Sensitivity of Cueing (ISC) values in MCI-DLB and MCI-AD groups.



Discussion

The FCSRT has been already described as a valid tool in distinguishing patients with AD from patients with non-AD dementias (Grober *et al.*, 2010) and to predict the progression to AD in a group of MCI (Lemos *et al.*, 2015; Sarazin *et al.*, 2007). With the results of this study, we could demonstrate that the immediate total recall (ITR) and the index of sensitivity of cueing (ISC) are also useful instruments to differentiate AD from DLB in a very early pre-dementia stage. In fact, MCI-DLB patients had significantly greater ITR and ISC values (being in the normal range) than MCI-AD (below normal threshold) (Frasson *et al.*, 2011). Such findings should be clear since sensitivity of cueing is evaluated by taking into consideration the performance of the subjects in the immediate recall task of the FCSRT. Moreover, ISC is a very sensible index that may be impaired when subjects do not recall just one or two items.

Given that encoding condition is equally controlled in all patients thanks to the encoding specificity of the FCSRT, results showed that MCI-DLB had an effective improvement of the performance, aided by provision of cues, which may not occur in MCI-AD.

This suggests that, in controlled encoding conditions, memory impairment, mainly affects retrieval more than consolidation in MCI-DLB. In fact, forgotten items at free recall can be recalled by providing the associated semantic category, after a previous effective consolidation of the items. On the opposite, memory impairment in MCI-AD is due to a defective storage process, which makes information completely unavailable and compensatory strategies cannot help in normalizing the performance. Nevertheless, it should be observed that also AD showed a slight but not sufficient improvement from free to total recall in the immediate condition. We can therefore suggest that consolidation deficit in a very mild pre-dementia stage is not as impaired as it will be as the disease progresses. Overall, these findings not only give further evidence of the nature of memory impairment in MCI-DLB and MCI-AD but also indicates that index of sensitivity of cueing may be a reliable marker for differentiation between DLB and AD at disease onset.

We already mentioned Sarazin *et al.*'s study (2010), in which strong correlations were found between the total score of the FCSRT and measures of the medial temporal lobe atrophy. Considering their findings, we could suppose that in our study MCI-DLB patients may likely have a relative preservation of temporal lobe structures and less severe hippocampal involvement than MCI-AD.

Differently from immediate total recall and index of sensitivity of cueing, no differences were found in the free recall condition for both immediate and delayed recall. Similarly, we observed the same patterns considering the immediate to delayed tasks of another memory test, the Prose memory, where MCI-DLB patients performed equally to MCI-AD. This may be explained by the

fact that general a memory recall task may be not enough sensible to detect the nature of memory impairments which are differently involved in these two types of dementia (Grober *et al.*, 2010). The FSCRT, instead, could be considered more sensible to detect the changing of episodic memory typical of Alzheimer's disease that reflects the amnestic syndrome of the hippocampal type, based on significantly impaired performance in the cued recall with control of encoding test (Dubois et al. 2014).

As for other cognitive variables, significant differences between MCI-DLB and MCI-AD groups were found in some aspects of the visuospatial and visuo-constructional domain. MCI-DLB patients failed in the pentagon copy (QSPT; Caffarra et al., 2013), particularly in the number of angles. These results are in line with other populations of MCI-DLB and MCI-AD in our recent studies (Cagnin et al., 2015).

Concerning attentive functions, in the MCI stage, DLB patients showed a greater impairment compared to AD. In fact, MCI-DLB was able to detect a lower number of digits showing a major impairment of visual selective attention. We could state that attention is one of the first domains affected by DLB, while executive dysfunction may occur later or earlier at the same level of impairment in both DLB and AD. It is worth considering that AD may be characterized by executive deficits to various extents (Stokholm *et al.*, 2006), therefore it can't be ruled out that in this research MCI-AD included a greater number of patients with an executive dysfunction than previous studies.

These findings support previous studies which showed that visuospatial dysfunction together with attentional impairments are one of the main distinguishing features of MCI-DLB from MCI-AD since the earliest stage of the disease (Kondo *et al.*, 2016; Cagnin *et al.*, 2015; Ferman *et al.*, 2013; Guidi *et al.*, 2006).

Output of the study

This study is in preparation.

4. CONCLUSIONS

The four studies reported mainly provide suggestive evidence regarding the feasibility to detect DLB cognitive deficits, especially during the prodromal phase.

As a screening neuropsychological investigation, the Qualitative Scoring Pentagon Test (QSPT) showed a significant difference between prodromal DLB and AD patients. In fact, through the QSPT tool, we explored five different aspects of drawing production, but only the number of angles parameter showed a significant difference between DLB and AD. The impaired number of angles in the cohort of MCI-DLB could be considered a strong variable that remains constant considering all the three studies involving the early-stage dementia.

In an autopsy-verified sample of DLB and AD we were able to demonstrate the ability of the QSPT to differentiate DLB from AD patients with a mean MMSE score of 24/30 (Mitolo et al. 2014), while the present results extend this possibility backward to the early stage of prodromal DLB with a good specificity. The high specificity and a low sensitivity of the pentagon copy score adds valuable information to the clinical assessment pointing towards a suspicion of DLB, while a normal performance leaves the possibility of both DLB and AD diagnoses.

These data confirm that a different cognitive profile, with a relatively worse ability performing the executive/attentive and visual-construction testing for DLB and worse performance of memory testing for AD, is detectable in the MCI stage (Jicha et al. 2010, Boeve et al. 2004). Overall, the present results support the view that testing visuo-constructional abilities might be a useful and valid tool for differentiating between DLB and AD. Moreover, a poor performance in the pentagon copy test, together with the presence of subtle extrapyramidal signs and symptoms of RBD, may give additional value to the clinical evaluation as a predictive screening tool for early DLB.

A brief cognitive assessment to detect early DLB may include also two subtests of the Visual Object and Space Perception Battery (VOSP) for the measure of visuospatial and visuo-constructional abilities. This second research comprehend about 20% of different DLB population that performed even in the VOSP as well as in the pentangons copy and a 90 % novel AD patients. This study extended the assessment to other visuospatial functions, aimed to understand the relationship between the sub-components of the visuospatial domain. We concluded that a specific impairment in number location and cube analysis might best distinguish DLB from AD at the early stage. Specific nature of visual-spatial impairment in DLB, involves the dorsal pathway (‘where’), similarly to what Calderon et al. (2001) found in clinically manifested DLB, while the ventral visual pathway (‘what’) seems to be involved in the early stages in both DLB and AD.

Although the retrospective nature of our study may be a limitation, it has the advantage to investigate the full spectrum of visual-spatial perception and attention by applying a specific cognitive battery. As such, we were able to define that the visuoconstructional and spatial abilities are most specifically impaired in DLB, while object perception deficits, although very sensitive, are not specific cognitive signatures among DLB and AD in the prodromal stage.

The pentagon copying of the MMSE was first investigated in neuropathologically proven DLB and AD patients (Jicha et al. 2010). The study by Ala et al. (2001) reached an 88% sensitivity and a 58% specificity in discriminating these two conditions. However, the data referred to a group of patients in the full spectrum of disease severity, while in the earlier stages specificity could increase at the expense of sensitivity.

The AD/DLB comorbidity influences the cognitive pattern by further impairing only the visuospatial domain. Therefore, co-occurrence of DLB and AD pathology did not influence the speed of disease progression but only the type of cognitive impairment. We acknowledge that the main limitation of our study is the lack of neuropathological confirmation and the impossibility to confirm the above-mentioned data.

We hypothesized that this subgroup of DLB patients may represent the pure form of DLB. This suggestion is supported by the finding of Yoshizawa et al. (2013) that pure DLB patients have worse visuoconstructional deficits with respect to patients with AD and also mixed AD/DLB since the early stage of the disease.

As for episodic memory, two different studies have been done: study 3 included patients in mild stages of dementia, while study 4 involved the prodromal phase. As shown in study 3, as the disease progresses, visuo-spatial, executive and memory functions become similarly impaired in both neurodegenerative diseases. In fact, a comparison of cognitive performances in mild DLB and AD reported differences only in visual attention, indicating a similar cognitive impairment in a mild-moderate dementia stage of both neurodegenerative diseases, where visuo-spatial, executive and memory functions are similarly impaired. This is in line to the fact that neuropsychological tests may not have a discriminative property in differentiating DLB from AD in the mild stage. While a standard memory test as the RAVLT is not sensitive enough in distinguishing DLB from AD, specific measures of verbal memory obtained with the RAVLT helped in discriminating between these two conditions.

Patients with DLB demonstrated a greater learning than AD on the VL while AD had a greater forgetting on the VF% measure, the last holding the better discriminating ability. This measure seems to better describe the amnesic syndrome typical of AD, characterized by deficient learning and rapid memory decay, as previously reported by Squire et al. (2004). DLB patients, instead,

could benefit more than AD from the repeated exposure to items enhancing storage capacity and retrieval. The measure of the serial position effects (Murdock et al, 1962) offered the possibility to better understand the different processes involved in the episodic memory deficits. According to literature, a reduction of primacy effect and a normal (Bayley et al., 2000; Martín et al., 2013; Orru et al., 2009) or increased recency effect (Howieson et al., 2011; Jones, Greer & Cox, 2011) could be prognostic of AD condition. DLB showed a reduction of the recency effect compared to AD. We can conclude that the recency effect could be another good cognitive marker of DLB to be considered using the RAVLT. Finally, we found a correlation between the recency effect and digit span attentive abilities, indicating that a reduction of the recency effect in DLB could be related by encoding deficits due to attentional impairment.

Further clarifications about underlying memory mechanisms emerged also in study 4 in a different population of MCI. The specific ability of the Free and Cued Selective Reminding Test (FCSRT) to detect AD at an early stage (Lemos et al., 2015; Sarazin et al., 2007) and the lack of knowledge about DLB performances regarding the FCSRT, together demonstrated the utility of the FCSRT in increasing the diagnostic accuracy. The FCSRT has been already described as a valid tool in distinguishing patients with AD from patients with non-AD dementias (Grober *et al*., 2010) and to predict the progression to AD in a group of MCI (Lemos *et al*., 2015; Sarazin *et al*., 2007). With the results of this study, we could demonstrate that the immediate total recall (ITR) and the index of sensitivity of cueing (ISC) are also useful instruments to differentiate AD from DLB in a very early pre-dementia stage.

As for other cognitive variables, significant differences between MCI-DLB and MCI-AD groups were found in some aspects of the visuospatial and visuo-constructional domain. MCI-DLB patients failed in the pentagon copy (QSPT; Caffarra et al., 2013), particularly in the number of angles. These results are in line with other populations of MCI-DLB and MCI-AD in our recent studies run with another population (Cagnin et al., 2015). Concerning attentive functions, in the MCI stage, DLB patients showed a greater impairment compared to AD. In fact, MCI-DLB was able to detect a lower number of digits showing a major impairment of visual selective attention.

We could conclude that attention is one of the first domains affected by DLB, while executive dysfunction may occur later or earlier at the same level of impairment in both DLB and AD. It is worth considering that AD may be characterized by executive deficits to various extents (Stokholm *et al.*, 2006), therefore it can't be ruled out that in this research MCI-AD included a greater number of patients with an executive dysfunction than previous studies.

These findings support previous studies which showed that visuospatial dysfunction together with attentional impairments are one of the main distinguishing features of MCI-DLB from MCI-AD

since the earliest stage of the disease (Kondo *et al.*, 2016; Cagnin *et al.*, 2015; Ferman *et al.*, 2013; Guidi *et al.*, 2006).

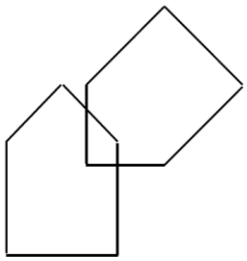
Moreover, we tended to exclude storage memory profile impairment in MCI-DLB patients, better understanding the memory processes involved in DLB. Strength of this set of studies refers to the consistency of results regardless to the different patients samples recruited for each study. Subtle differences in test results among MCI-studies were found, i.e. in Trial Making Test-A or in the digit span backwards test. However, such divergences may be principally due to the intrinsic heterogeneity of the samples.

Another strong point is represented by the novelty of these data in a population, i.s MCI-DLB, not deeply investigated so far. A limitation of these studies relies on the lack of neuropathological confirmation of the diagnosis, however, this point has been partially overcome by a fixed clinical follow-up period needed to confirm the diagnosis.

The prodromal phase of DLB provides a critical opportunity for potential intervention treatments, but only if we are able to detect the disorder at the early phase of the clinical course. To this line, a better understanding of cognitive impairments associated with DLB may provide valuable information not only to the clinicians but even to patients and caregivers.

Appendix

a) **Qualitative scoring method for the pentagon copying test (QSPT) of the Mini Mental State Examination (MMSE) (Caffarra et al. 2013)**



The new scoring method included five criteria of judgment as follows: 1) numbers of angles; 2) distance/intersection between the two figures; 3) closing/opening of the contour; 4) rotation of one or both pentagons; 5) closing-in and a total score corresponding to the sum of individual scores of each parameter, ranging from 0 to 13.

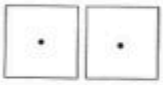
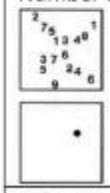
Parameters	Performance scores	Assigned scores
1. Numbers of angles	10	4
	10 ± 1	3
	10 ± 2	2
	7–5	1
	< 5 or > 13	0
2. Distance/Intersection	Correct Intersection	4
	Wrong Intersection	3
	Contact without Intersection	2
	No contact, distance < 1 cm	1
	No contact, distance > 1 cm	0
3. Closure/opening*	Closing both figures	2
	Closing only one figure	1
	Opening both figures	0
4. Rotation**	Correct orientation of both figures	2
	Rotation of one figure (either one figure is absent or it is not a pentagon then it is not assessable)	1
	Rotation of both figures (or both not assessable like pentagons)	0
5. Closing-in	Absent	1
	Present	0
Total	Sum of 1+2+3+4+5	0–13

*Figure is considered close even though two sides do not touch each others but the distance is $\leqslant 1$ mm.
**When there is not a figure or figure is not a pentagon (then rotation is not assessable) score is 0. When rotation is less than 45°, figure is not considered rotated. Tremor is ignored.

b) Visual Object and Space Perception Battery (VOSP) (Warrington et al. 1991)

VOSP is used for the assessing of object and space perception. Consists of eight tests each designed to assess a particular aspect of object or space perception, while minimizing the involvement of other cognitive skills. Tasks are explained in the figure above.

Object Perception (Ventral Stream)	
Incomplete Letters 	• Screening test: subjects name individual letters (1 per card) degraded by 30% • Test items: subjects name individual letters (1 per card) degraded by 70% • Total trials: 20 • **Cut-Off Value for Failure: 16/20***
Silhouettes 	• Subjects identify 15 silhouettes of animals and 15 silhouettes of inanimate objects, presented at unusual angles • Subjects may name, gesture, mimic use, or describe the object (language is minimized) • Test is discontinued after 5 errors for animals and after 5 errors for inanimate objects • Total Trials: 30 • **Cut-Off Value for Failure: 15/30**
Object Decision 	• Subject must select a picture of a real object juxtaposed among 3 nonsense objects Subjects are not required to name the object, and need only identify which one is real • Total Trials: 20 • **Cut-Off Value for Failure: 14/20**
Progressive Silhouettes *not depicted.*	• Cards with shadows of one of two objects, presented at sequentially more recognizable viewpoints • Subjects are shown 10 cards of a gun and 10 cards of a trumpet, with the goal of identifying the object with the fewest number of presented cards • Total Trials: 20 • **Cut-Off Value for Failure: 15/20**

Space Perception (Dorsal Stream)	
Dot Counting 	• Counting random assortments of five to nine scattered dots on a card • If subjects fail the first item, they do not move on to the remainder of the test • Total Trials: 10 • **Cut-Off Value for Failure: 8/10**
Position Discrimination 	• Subjects are shown two cards, each containing a single black dot that is either exactly centered or slightly off-center; subject must specify which card contains the centered dot • Total Trials: 20 • **Cut-Off Value for Failure: 18/20**
Number Location 	• Subjects are presented with two cards, one with scattered numbers (from one to nine) and the other with a single dot, corresponding to the position of an individual number. Subjects must indicate which number corresponds to the single dot. • Total Trials: 10 • **Cut-Off Value for Failure: 7/10**
Cube Analysis 	• Subjects must count stacked blocks (including hidden ones) rendered as drawings on cards. There is one practice item. • Total Trials: 10 • **Cut-Off Value for Failure: 6/10**

c) Rey Auditory Verbal Learning Test (RAVLT) (Carlesimo et al. 1996)

The RAVLT is a neuropsychological assessment designed to evaluate episodic verbal memory. The test is designed as a list-learning paradigm in which the patient hears a list of 15 nouns and is asked to recall as many words from the list as possible. After five repetitions of free-recall, some interferences tasks will follow. After a 15 min delay, the participant is asked to again recall the words from List.

Sheet of paper for collecting word-list recall task data (list A). Italian version.

	IMMEDIATE RECALL					**DELAYED RECALL**
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Delayed 15'**
Tenda						
Tamburo						
Caffè						
Cintura						
Sole						
Giardino						
Baffi						
Finestra						
Fiume						
Paesano						
Colore						
Tacchino						
Scuola						
Casa						
Cappello						
TOTAL						

d) Free and Cued Selective reminding Test (FCSRT) (Italian version: Frasson et al. 2011)

The Free and Cued Selective Reminding Test (FCSRT) is a memory test beginning with a study phase in which subjects are asked to search a card containing four pictures for an item that goes with a unique category cue (e.g., fruit). After all four items are identified; immediate cued recall of just those four items is tested, providing retrieval practice while the items are still in working memory. The search is performed again for items not retrieved by cued recall. The search procedure is continued for the next group of four items until all 16 items have been identified and retrieved in immediate recall. The study procedure is followed by three trials of recall, each consisting of free recall followed by cued recall for items not retrieved by free recall for a maximum score of 48. Items not retrieved by cued recall are re-presented. Each separate trial is followed by 20 seconds of interference. The three measures being evaluated here include free recall (the cumulative sum of free recall from the three trials; range 0-48), total recall (the cumulative sum of free recall + cued recall from the three trials, range 0-48), and cue efficiency (total recall-free recall)/ (48-free recall, range 0.0-1.0).

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