UNIVERSITÀ
DI PAVIA

PhD IN BIOMEDICAL SCIENCES

DEPARTMENT OF BRAIN AND BEHAVIORAL SCIENCES

UNIT OF NEUROPHYSIOLOGY

NEUROPSYCHIATRIC SYMPTOMS OF DEMENTIA AND BIOMARKERS OF NEURODEGENERATION: CSF TAU AND MRI

PhD Tutor: Prof. Alfredo Costa

PhD dissertation of

Matteo Cotta Ramusino

N. 474921

a.a. 2020/2021

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Chapter 1: Introduction

1.1 Epidemiology

In the DSM-V the term dementia has been replaced by the term Major Neurocognitive Disorder, which emphasizes the presence of a decline from a previous level of cognitive performance, no longer recognizing as a core symptom the memory deficit, characteristic of Alzheimer's disease, because other cognitive domains (language, cognitive functions, etc.) may be compromised before the memory (1). The main manifestations of dementia are represented by cognitive deficits in multiple domains with a progressive course. However, in addition to cognitive symptoms, often fundamental for the diagnosis, patients with dementia frequently present with neuropsychiatric syndromes, characterized by personality and mood disorders and psychosis. These psycho-behavioral manifestations, called "behavioral and psychological symptoms of dementia" (BPSD), represent the main determinants of patient institutionalization and are also the main target of dementia pharmacological therapy. BPSDs are defined as signs and symptoms related to alterations in perception, thought content, mood, or behavior, and include disorders such as agitation, depression, apathy, psychosis, aggression, sleep disturbance, wandering, and a variety of disorders deemed socially inappropriate (2).

In 2019, the World Health Organization (WHO) estimated a global prevalence of dementia at around 50 million, with an upward trend that will triple this estimate by 2050. Considering that BPSDs are a very common occurrence, affecting 98% of patients with dementia at some point during the course of the disease, the impact of both cognitive and neuropsychiatric disorders on the quality of life of patients and caregivers will be massive (3). The Cache County study found the prevalence of BPSD at the fifth year of illness to be 97%, with greater occurrence of apathy, depression, and anxiety. A lower frequency was found in patients living at home 53% (4). BPSDs are also frequently present in early stages of disease and in forms of mild cognitive impairment (MCI) (5) and are often associated with an increased

neuropathological burden and a more rapid evolution towards full-blown dementia. To confirm this, patients with amnestic MCI and BPSD, particularly apathy, have an almost sevenfold increased risk of progression to Alzheimer's dementia (6). A recent study on a cohort of 181 subjects selected from the Alzheimer's Disease Neuroimaging Study (ADNI) also confirmed an inverse correlation between the presence of BPSD and cognitive and functional outcomes in patients with Alzheimer's Disease, supporting the importance of early recognizing psycho-behavioral disorders to optimize pharmacological and non-pharmacological management of the disease (7). Therefore, BPSDs appear to be reliable predictors of progression, alongside widely known biological predictors such as apolipoprotein E ε4 polymorphism, CSF amyloid β/tau ratio, and hippocampal volumetric measurements. Patients with at least one (clinically significant) BPSD and poor general condition are most likely to progress to severe forms of dementia, greater functional disability, and the exitus (8). Most patients with dementia are cared for at home by non-professional family caregivers, who often complain of stress and depression, as well as presenting difficulties in maintaining their employment, with consequent reduction in quality of life (9). The BPSDs perceived as most stressful by caregivers are aggression, anxiety, disinhibition, and depression, whereas eating and sleep disorders seem to be less stressful (10). Thus, BPSDs represent the most complex and costly aspects of the entire caregiving process, and they correlate with a negative disease outcome, implying increased morbidity and mortality, longer hospital stays, and earlier institutionalization (11).

1.2 Clinical features

The main neuropsychiatric changes that occur in patients with dementia include personality disorders, delusions, hallucinations, mood disorders, anxiety, psychomotor activity disorders,

and various mixed behavioral alterations, including sleep and appetite disorders, sexual behavior alterations, and Kluver-Bucy syndrome.

There are several ways in which BPSDs can be classified; in 1996, a Consensus Statement (2) proposed to distinguish them into:

- symptoms usually and primarily obtained from interviewing the patient or caregivers (including anxiety, depression, hallucinations, delusions);
- symptoms inferred from observation of the patient's actions and behavior (aggression, restlessness, agitation, wandering, disinhibition, eating disorders).

One or more of these manifestations occurs almost invariably in individuals with dementia at some time during the course of their illness. In particular, BPSDs tend to occur in syndromic clusters, which can be distinguished as follows:

- Psychotic cluster (delusions and hallucinations);
- Affective cluster (depression, anxiety);
- Personality cluster (apathy, disinhibition, euphoria);
- Neurotic cluster (irritability, agitation/aggression, psychomotor activity disorders);
- Neurovegetative cluster (sleep and eating disorders).

Delusions represent the most common neuropsychiatric symptom of dementia and, among them, paranoid ideation is the most frequent form. The most frequent delusional ideas involve false beliefs of theft or home invasion, spousal infidelity, abandonment and ideas of persecution. Delusions of misidentification are also common (present in approximately 33-36% of individuals with dementia). Eleven variants can be distinguished, the most frequent concern home misidentification (i.e., the belief that the patient's home is not one's own home), person splitting (e.g., while talking to the spouse, the patient talks to her/him as if she/he were talking to another person), and reduplicative paramnesia (e.g., the patient is convinced that his or her house is located in two different places in the same city), as well as the classic mirror delusions (the patient believes that the reflected image belongs to another person, usually with persecutory

implications) and television delusions (the television protagonists are actually present in the room). Finally, in Capgras' and Fregoli's syndrome the patient is convinced respectively that familiar objects or people are replaced by duplicates or impostors, or conversely that strangers are actually family members in disguise (12).

Visual hallucinations are the most common perceptual disorder, followed by auditory or combined auditory and visual hallucinations. Typical visual hallucinations include people from the past (e.g., dead parents), intruders, animals, complex scenes, or inanimate objects. Auditory hallucinations are often persecutory and usually accompany delusions (13). On the side of mood disorders, depressive symptoms and emotional lability are common manifestations. Although criteria for major depressive episodes are met in few cases, depression is very common in individuals with dementia and achieves clinical significance in approximately 40-50% of individuals over the course of the disease. Crying episodes may be prominent, feelings of being weightless and worthless may be expressed, but suicide is rare. Patients who present with depression during the course of the disease often have family histories of depressive disorders (14). Anxiety has been described in 40% of patients and may occur along with other neuropsychiatric symptoms (e.g., in association with depressive symptoms) or alone. The most common manifestations of anxiety in dementia include repeated questions/seeking for reassurance, concerns about finances, the future, and health, and fear of being left alone (13). Apathy has been described in 72% of subjects and is associated with greater functional impairment, poorer quality of life, more severe cognitive impairment and risk of further functional decline. Agitation is a relatively unspecific term used to describe a range of behaviors common to many forms of dementia. Manifestations are variable and may include vocalizations or inappropriate behaviors that cannot be explained by the subject's needs or by a confusional state (15). Disturbances of psychomotor activity and harassing behaviors are common and become more prominent as the disease progresses. Wandering is a pervasive behavior in the intermediate and late stages of the illness, and providing safe, contained spaces for these

patients is the major difficulty for residential facilities. Restlessness is described in 60% of cases and aggressive behavior is seen in 20% of patients (16). Inappropriate sexual behavior (ISB) is defined as unexpected, inappropriate, and stressful behaviors that interfere with patient care. They can be classified into three forms: language (adoption of vulgar language that is inconsistent with the patient's personal history), explicit sexual acts (touching, grabbing, exhibitionism, masturbation in public or private places), and implicit sexual acts (reading pornographic material or requesting unnecessary genital care) (17). The prevalence of ISB varies widely in studies (1.8-25%) conducted primarily on patients housed in nursing homes, where there is little privacy and ease of favorable situations. Eating behavior also changes during the course of the disease: progressive cognitive-behavioral changes can be seen, which may involve alterations in meal planning, food handling/manipulation, bucco-linguo-facial coordination, processing of sensory afferents such as taste and smell, and food preferences (18). Finally, sleep disturbances manifest as frequent interruptions of nocturnal sleep and occur in 40-70% of cases (19).

Although these symptoms are present in all forms of dementia, often regardless of etiology, some types of dementia are more often associated with particular symptoms. For example, depression is more common in vascular dementia, while hallucinations are more frequently associated with Lewy body dementia than with Alzheimer's disease. In frontotemporal dementia, more specifically in the behavioral variant, the behavioral disorder represents the very "core" of the symptomatology and subjects often develop behaviors typical of the loss of executive functions, such as disinhibition, wandering, socially inappropriate attitudes, and apathy (20). Moreover, not in all cases the severity of dementia is associated with more severe BPSD. Indeed, in vascular dementia, the severity of cognitive impairment does not seem to have any impact on the frequency of BPSDs, except for apathy and aberrant motor activity. On the other hand, in dementia with Levy bodies the frequency of delusions, hallucinations, disinhibition, and sleep disturbances increases with dementia severity; as well as in Alzheimer's

disease, dementia severity is often associated with an increased frequency of clinically relevant BPSDs (21).

1.3 Pathophysiology

The relationships linking BPSD and cognitive symptoms are still largely obscure today. Different mechanisms have been proposed, among them not mutually exclusive:

1) etiological mechanism, whereby BPSDs cause pathophysiological changes in the brain associated with the development of dementia;

2) common neuropathological mechanism, whereby BPSD as well as cognitive symptoms are a direct manifestation of the neurodegenerative process that affects key brain areas for the processing of behavior, emotions and sensory afferences;

3) mechanisms based on a reactive psychological condition: a subject experiencing cognitive decline may develop depression, anxiety or other neuropsychiatric symptoms due to the awareness of the gradual loss of his cognitive and functional abilities;

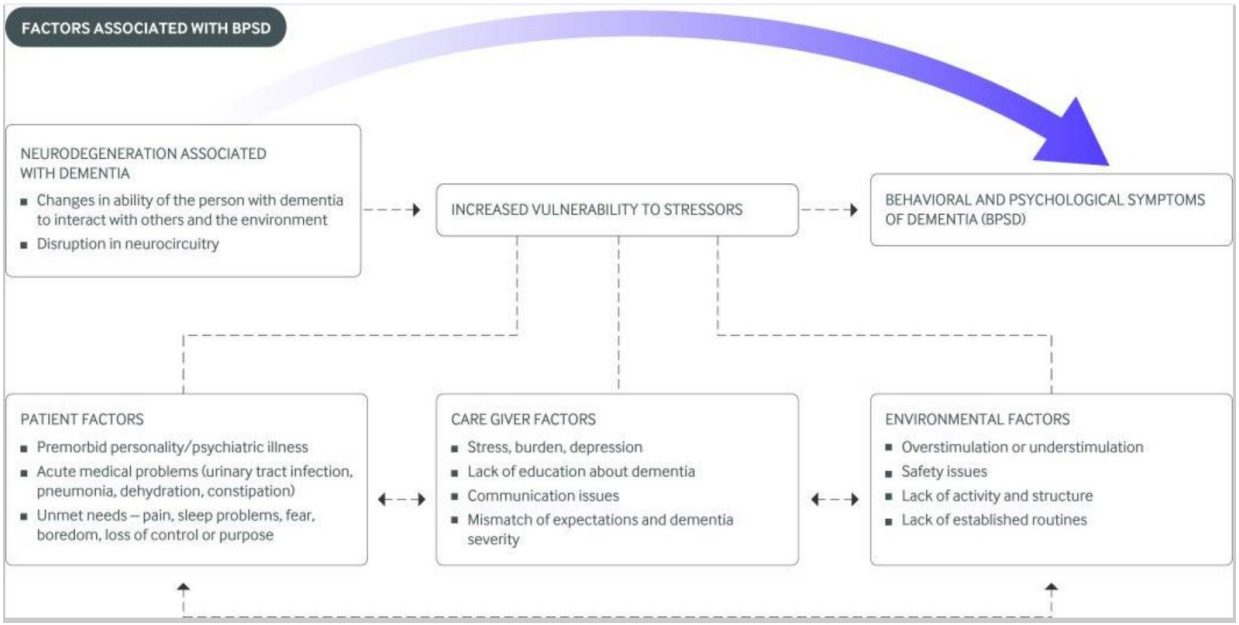
4) mechanisms of synergistic interaction between BPSD and biological factors, which may lower the threshold and promote the onset of mild cognitive decline or dementia.

These possible mechanisms are not mutually exclusive, but on the contrary probably act in combination in a multifactorial scenario (22). Frontal subcortical circuits, including the prefrontal, orbital, and limbic cortex, basal ganglia, and thalamus, control the behavior by influencing planning and executive functions (dorso-lateral circuit), the initiative (prefrontal circuit), the inhibitory control and the compliance with social rules (orbitofrontal circuit). BPSDs could result from synaptic disconnection of part of these circuits (23). Ascending monoaminergic systems (serotonin, noradrenaline, and dopamine), which through the medial forebrain bundle carry terminations to several of the above cortical and subcortical areas, could

certainly contribute to this pathophysiological framework by regulating emotions, feeling of reward, and behavior in general. There is a protein-phenotype correlation in the BPSDs of some neurodegenerative diseases, due to accumulation of the pathogenic protein and subsequent dysfunction of certain brain regions. In alpha-synucleinopathies, in which the progression of damage first involves the brainstem and then the limbic cortex and neocortex, hallucinations, delusions, REM sleep disorders are particularly frequent. In tauopathies, in which instead dysfunction of the frontal cortex and basal ganglia is early, disinhibition, apathy and compulsive behaviors are mainly present (24). Clinical experience also suggests that personality traits may influence the development of BPSD, as the loss of inhibitory control may accentuate preexisting pathological personality traits. Preexisting mental disorders, such as depression, anxiety disorders, bipolar disorder, and psychotic disorders, and their treatment with antidepressants, anxiolytics, mood stabilizers, and antipsychotics, may similarly influence their development. BPSDs are also affected by the relational complexity between patient and caregiver: psychological stress and depression complained of by the caregiver, combined with poor communication styles and caregiving strategies may worsen symptoms (25). Because patients with dementia are characterized by a progressive difficulty in processing and coping with environmental stimuli, they have an increasingly low threshold for stressors, with disturbances appearing even for simple changes occurring in living habits or in the environmental, physical, or social variables.

Figure 1 shows a conceptual model whereby neurodegeneration associated with dementia changes patients' ability to interact with the others (primarily their caregivers) and the environment (20). Dementia may also directly cause BPSDs by disrupting brain circuits involved in the behavior control. Finally, caregivers and environmental factors may determine the onset of BPSDs independently or by interacting with neurodegenerative mechanisms.

Figure 1. Theoretical framework of factors involved in the BPSD development (from Kales HC et al, 2015 (20))



Neuropathological evidence related to BPSDs:

Psychotic Symptoms

Recent studies on the neuropathological correlates of psychosis in autopsy-confirmed AD cases have shown a significant increase in dendritic plaques and neurofibrillary tangles in the presubiculum and middle frontal gyrus, respectively, with a trend toward increased density of these lesions in other cortical areas (entorhinal and superior temporal). In addition, an increased load of neurofibrillary tangles at the hippocampal level, in the absence of other morphological changes, has been associated with increased severity of aggressive behaviors (26). In dementia with Lewy bodies, visual hallucinations are associated with alpha-synuclein deposition in the limbic system, particularly in the anterior cingulate gyrus, entorhinal cortex, amygdala, and basal nucleus of Meynert. In addition, a higher density of Lewy bodies in parahippocampal and inferior temporal regions is strongly associated with well-structured, early-onset visual hallucinations. In contrast to what is observed for AD, patients with DLB and hallucinations tend to have a lower representation of neurofibrillary pathology at neocortical temporal

structures, with an inverse relationship. In fact, if present, the tangles make hallucinations less persistent and the clinical profile of patients more similar to that of AD (27). In frontotemporal dementia, only 2.3% of patients present with delirium or paranoid ideation, and hallucinations are even rarer. The rare occurrence of psychotic symptoms could be associated with the limited involvement of limbic and temporomesial areas by the disease. Cases presenting with schizophrenia-like features before the onset of dementia often recognize TDP-43 or ubiquitin-related pathology.

Depression

Recent clinicopathologic studies of non-demented elderly subjects with late-onset major depression have found no association between depression and cerebrovascular or Alzheimer's disease. In contrast, an association has been reported between depression and neuronal loss in the hippocampus and some subcortical structures. Patients with Alzheimer's disease and depression have greater deposition of neurofibrillary tangles and senile plaques, whereas vascular lesions and Lewy bodies were equally distributed between depressed and non-depressed subjects (28).

Apathy

Neuropathology studies of subjects with apathy and autopsy-defined Alzheimer's disease found increased deposition of neurofibrillary tangles in the anterior cingulate gyrus, particularly on the left side, with no significant differences in terms of senile plaques or Lewy bodies (29).

1.4 Biomarkers

Biomarkers, or biological markers, are indicators that reflect metabolic, cytologic, or tissue morpho-functional changes that occur during the pathophysiologic process of a given disease.

Their utility lies in the fact that they are detectable and measurable in vivo, usually noninvasively or minimally invasively, and are repeatable.

1.4.1 Cerebrospinal fluid

The levels of $A\beta_{42}$, total tau (t-tau) and phosphorylated tau (p-tau) in the cerebrospinal fluid reflect the neuropathophysiological changes that occur during Alzheimer's disease in brain tissue, and are particularly useful for the characterization of preclinical stages of the disease, as well as for the formulation of an early etiological diagnosis. CSF levels of $A\beta_{42}$ in AD patients are decreased, presenting an inverse correlation with the amyloid load in the brain: the increased deposition of amyloid and its sequestration in senile plaques would lead to lower levels of soluble protein. According to the most recent ATN classification system (30), the finding of cerebral amyloidosis is a necessary condition (signature) to define the presence of AD pathology, but not sufficient to speak of Alzheimer's disease. To define the latter in vivo, it is necessary a concomitant increase in the levels of p-tau, a key component of the paired helical filaments that are anatomopathological markers of AD together with senile plaques. Levels of t-tau are commonly increased, correlate with the amount of NFTs, but are not disease-specific, being more of an indicator of the extent of neurodegeneration.

Measurement of these markers can be used to discriminate patients with AD from non-demented individuals of the same age and from patients with other morbid conditions. However, a large overlap demonstrated on autopsy examination between AD, dementia with Lewy bodies, and vascular dementia, precludes the possibility of achieving 100% specificity and sensitivity. In a 2017 Cochrane pooling prospective studies of subjects with mild cognitive impairment, both t-tau and p-tau showed wide variability in sensitivity (51-90%, 40-100%) and specificity (48-88%, 22-86%), which was only partially improved by the use of $A\beta_{42}$ ratios (sensitivity 80-96%, specificity 33-95%) (31). Furthermore, in non-Alzheimer dementias, elevated t-tau values were found in patients with DLB, FTLD, or VaD, compared with controls,

whereas p-tau was found to be increased only in DLB. This affects the diagnostic accuracy of these biomarkers in distinguishing AD from other forms of dementia: lower levels of tau differentiated DLB with a sensitivity of 73% and specificity of 90%, FTLD with a sensitivity and specificity of 74%, and VaD with a sensitivity of 73% and specificity of 86%; whereas lower levels of p-tau differentiated FTLD with a sensitivity of 79% and specificity of 83%, and VaD with a sensitivity of 88% and specificity of 78% (32). Heterogeneity in the performance of CSF biomarkers leads to a state of uncertainty regarding the diagnosis of Alzheimer's disease, which therefore still needs special attention to the risk of misdiagnosis or overdiagnosis in clinical practice.

Recent studies have investigated a possible link between the occurrence of particular neuropsychiatric symptoms, single or in clusters, and the levels of CSF biomarkers frequently used in clinical practice. In cognitively unimpaired elderly subjects, high values of the t-tau/$A\beta_{42}$ ratio have been identified as predictors of the development of negative emotions, such as anxiety and depression (33). In subjects with MCI, Ramakers and colleagues observed a correlation between high levels of t-tau and anxiety, and between low levels of $A\beta_{42}$ and agitation, irritability, and anxiety (34). Similarly, low levels of $A\beta_{42}$ correlated with the presence of depression in another MCI population (35). In patients with mild-to-moderate Alzheimer's dementia, high levels of t-tau and p-tau were variably associated with different BPSD, particularly agitation and apathy (36), whereas an inverse correlation was observed between $A\beta_{42}$ and aggression (37). This evidence, although not always reproducible and concordant, reflects at the CSF level the neuropathological alterations observed at the cortical level. In particular, t-tau and p-tau levels are an expression of neurodegeneration and deposition of neurofibrillary tangles at the limbic and temporal lobes.

1.4.2 Neuroimaging

Visual rating scales are a widespread and easy-to-use tool to assess atrophy, for both clinical and research purposes (38-39). In this regard, very little is known on the correlation among visual rating scales and BPSD: in AD, high scores of medial temporal atrophy (MTA) were significantly associated with apathy and disinhibition (40), while posterior atrophy (PA) on the right hemisphere was associated to agitation and aggression (41). Previous neuroimaging studies, assessing morphological, perfusion and metabolic changes in the brain of AD patients, found that BPSD, such as delusions, apathy and depression, were particularly associated with a frontal region involvement, predominantly of the anterior cingulate cortex (ACC) and orbitofrontal cortex (OFC) (42). Studies of volumetric magnetic resonance showed an association between visual hallucinations and decreased occipital-to-whole brain ratio. Voxel-based morphometry (VBM) from T1-weighted MRI revealed association of delusions with decreased gray matter (GM) density in the left frontal lobe, right frontoparietal cortex, and left claustrum. Apathy was associated with atrophy in the anterior cingulate regions, frontal lobe bilaterally, the head of left caudate nucleus and bilateral putamen. Finally, agitation was associated with decreased GM values in the left insula and anterior cingulate bilaterally (43). Similarly, studies in subjects with MCI reported a link between apathy and hypoperfusion of the frontal, temporal, occipital lobes and atrophy in the inferior temporal gyrus and anterior cingulate (44). Similar investigations were conducted also in non-AD dementias: in the behavioral variant of frontotemporal dementia (bvFTD), a close correlation was observed between disinhibition and atrophy of specific frontotemporal areas (ventromedial orbito-frontal, medial frontal and anterior temporal lobe) (45), while in dementia with Lewy bodies (LBD) a dysfunction of both associative visual areas and limbic areas was found to be associated with the occurrence of hallucinations (46).

Chapter 2: Experimental study (Part 1)

Given the variability concerning the evidence on BPSD so far available in the literature, this study aimed to expand the knowledge of the biological correlates of the neuropsychiatric symptoms, evaluating the impact of BPSD in different forms of cognitive impairment (MCI, AD, FTD, LBD and Vascular Dementia), and searching for potential correlations between BPSD and CSF biomarkers and cortical visual rating scales.

2.1 Materials and Methods

2.1.1 Patients

Participants were cognitively impaired patients undergoing diagnostic workup at the Behavioral Neurology Unit of the IRCCS Mondino Foundation. Inclusion criteria were: a diagnosis of MCI (amnestic or non-amnestic / single or multiple domain) (47) or dementia; age between 50 and 90 years and an available informant with at least 10 hours per week of contact with the patient. No limit of severity of dementia was set, as long as the patient was able to perform a formal cognitive assessment. The vision and hearing acuity of patients were sufficient for compliance with testing procedures. Patients were excluded if they had a history of psychiatric disease or epilepsy, or any uncontrolled medical condition that could contribute to the subject's cognitive impairment (e.g., nephropathy, liver disease, brain tumor, alcohol or drug abuse, normal pressure hydrocephalus). None of the patients were receiving medications for dementia, such as cholinesterase inhibitors or antipsychotic drugs, at the time of the diagnostic workup. Previously, 10 patients had taken antipsychotic drugs, and 11 had been on cholinesterase inhibitors.

2.1.2 Study design

The study was designed as a single-site cross-sectional study. Enrolled patients underwent complete clinical, neurological and neuropsychological assessment, brain imaging (magnetic resonance imaging, MRI, or computed tomography CT), CSF collection (for the assay of $A\beta_{42}$, total tau and phospho-tau levels) and Neuropsychiatric Inventory (NPI) assessment. The neuropsychological examination included tests for global cognitive efficiency (Mini Mental State Examination, MMSE), and for memory (Verbal Span, Digit Span, Corsi Test, 15 Item Memory Test, Story Recall Test, Rey Complex Figure delayed recall), logical and executive functioning (Raven's Colored Matrices, Frontal Assessment Battery), attention (Trail Making Test A/B, Attentive Matrices, Stroop Test), language (Semantic and Phonemic fluency tests) and visual-spatial perception (Rey Complex Figure copy). NPI assessment was performed asking the caregiver to indicate via the screening questions whether the patient had experienced any domain-related neuropsychiatric symptom over the previous month. If the screening questions were validated, the caregiver was then asked to provide a domain rating for frequency, severity, and level of distress, and the total domain score was the product of the ratings for frequency and severity (48). Four main NPI clusters (hyperactive behaviors, psychosis, affective behaviors and apathy) were defined according to Aalten and coll. (49). "Hyperactive behaviors" cluster included agitation, euphoria, disinhibition, irritability, aberrant motor behaviour and night-time behaviour disturbances; "psychosis" cluster included delusions and hallucinations; "affective behaviors" cluster included anxiety and depression; finally, "apathy" cluster included apathy and appetite/eating abnormalities.

Once diagnostic workup was completed, patients were classified into two syndromic categories, MCI and dementia, and the latter patients received an appropriate etiological diagnosis according to the most recent diagnostic criteria. MCI subjects were diagnosed according to NIA-AA criteria (47), and had clinical dementia rating (CDR)=0.5. Subjects with

dementia received an etiological diagnosis of typical AD (50), behavioral variant of frontotemporal dementia (bvFTD) (51), Lewy body dementia (LBD) (52) or vascular dementia (VD) (53), and had CDR>=1. All patients with non-vascular dementia had a score<4 on the Modified Hachinski Ischemic Scale. Despite the advanced diagnostic workup including morphological and CSF biomarkers, 6 demented patients could not receive an etiological diagnosis with high confidence, and therefore were classified into a separate group, as not otherwise specified dementias (Dem NOS).

2.1.3 Neuroimaging

MRI and CT scans were acquired at the Neuroradiology Unit of IRCCS Mondino Foundation. For this study we analyzed 76 3D T1-weighted sequences acquired with Magnetom Skyra 3T (Siemens Healthcare), and 15 tomograms acquired with Somatome Perspective CT (Siemens Healthcare). MTA, PA and the global cortical atrophy–frontal (GCA-F) scales were rated on 3D T1-weighted MR images, according to the original descriptions (38-39, 54), on the both hemispheres. The MTA scale assesses the width of the choroid fissure and of the temporal horn, as well as the height of the hippocampus; the PA scale assesses the width of the posterior cingulate- and parieto-occipital sulci, and the atrophy of the parietal lobe and precuneus; the GCA- F evaluates the severity of the atrophy of the frontal lobes. On CT images, only MTA scale was rated. Visual ratings were collegially performed by two raters with more than 2 years of experience in the visual rating and over 900 neuroimages assessed from LANE dataset (55). The raters were blind to diagnosis and CSF profile. Nine scans did not undergo visual rating due to the presence of artifacts or excess motion.

2.1.4 Cerebrospinal fluid

In 87 participants, lumbar puncture was performed at the level of the L3/L4 or L4/L5 intervertebral space, according to the standard procedure used for patients with cognitive

disorders in our clinic. CSF samples were centrifuged for 10 minutes at 1800 rpm at 4°C within 3 hours of collection. The samples were then divided into aliquots of 0.5 mL in polypropylene tubes and stored at −80°C. Measurement of CSF $A\beta_{42}$, t-tau, and p-tau was performed using chemiluminescence enzyme immunoassay (Lumipulse G600II, Fujirebio); only for 14 participants $A\beta_{40}$ was available due to the relatively recent introduction of this assay in our laboratory. Biomarker profile was considered suggestive of AD pathology if $A\beta_{42}$<599 pg/mL, t-tau>404 pg/mL and p-tau>56.5 pg/mL, or $A\beta_{42}$/t-tau<1.27, $A\beta_{42}$/p-tau<8.10 and $A\beta_{42}/A\beta_{40}$<0.069 (56-57).

2.1.5 Statistical analysis

Shapiro-Wilk test was used to investigate the distribution normality of the different variables. Demographic and clinical characteristics among diagnostic groups were compared using ANOVA or Kruskall-Wallis test for continuous variables, and Chi-square test ($\chi 2$) or Fisher's exact test for categorical variables. Differences in BPSD and syndromic clusters' distribution among diagnostic groups were assessed with Kruskall-Wallis test. Bonferroni correction was used to control for multiple comparisons in post hoc analyses performed through Dunn tests. Correlations between NPI scores and CSF biomarkers or visual atrophy brain scales were calculated using Pearson coefficient. For non-parametric variables, a confirmation was obtained using Spearman coefficient. Differences in atrophy scores' distribution between patients with or without any of BPSD (e.g. agitation vs non-agitation) were assessed with Fisher's exact tests. A beta regression model with backward elimination was used to evaluate the relations of the dependent variable, NPI total score, with the following baseline predictors: age, gender, education, MMSE, diagnosis, t-tau, Aβ42, left and right MTA, left and right PA, left and right GCA-F. The variable p-tau was removed after a first correlation analysis between the predictors. After the normalization of the dependent variable, the beta distribution was tested through a Kolmogorov-Smirnov test. Statistical computations were performed using R v. 3.5.3

(The R Foundation for Statistical Computing). Two-sided p-values <0.05 were considered to indicate significance. Due to the heterogeneous nature of the sample, p-values between 0.05 and 0.1 were also reported suggesting possible significance in further studies.

2.2 Results

2.2.1 Patients' characteristics

Demographic and clinical characteristics, and biomarker measurements of the study population are shown in Table 1. Seventy-four patients were diagnosed with dementia (mean age, 74.5±7.0 years; 60% female) and 26 with MCI (72.7±6.5 years; 46%). Mean MMSE score was 17.3±5.2 (range: 4.9-26.1) in patients with dementia and 26.8±2.0 (range: 23-30) in subjects with MCI. In the dementia group, 48 patients were diagnosed as AD, 7 as FTD, 4 as LBD, 9 as VD and 6 as Dem NOS. No significant difference was found among AD, non-AD and MCI groups with respect to age, gender and education. As expected, AD group showed lower $A\beta_{42}$ levels, and higher p-tau and t-tau levels, compared to non-AD and MCI groups. No significant difference was found among the three groups with regard to atrophy scales scores.

Table 1. Sociodemographic features, cognitive status and biomarker measures in the total sample and in 3 main diagnostic groups

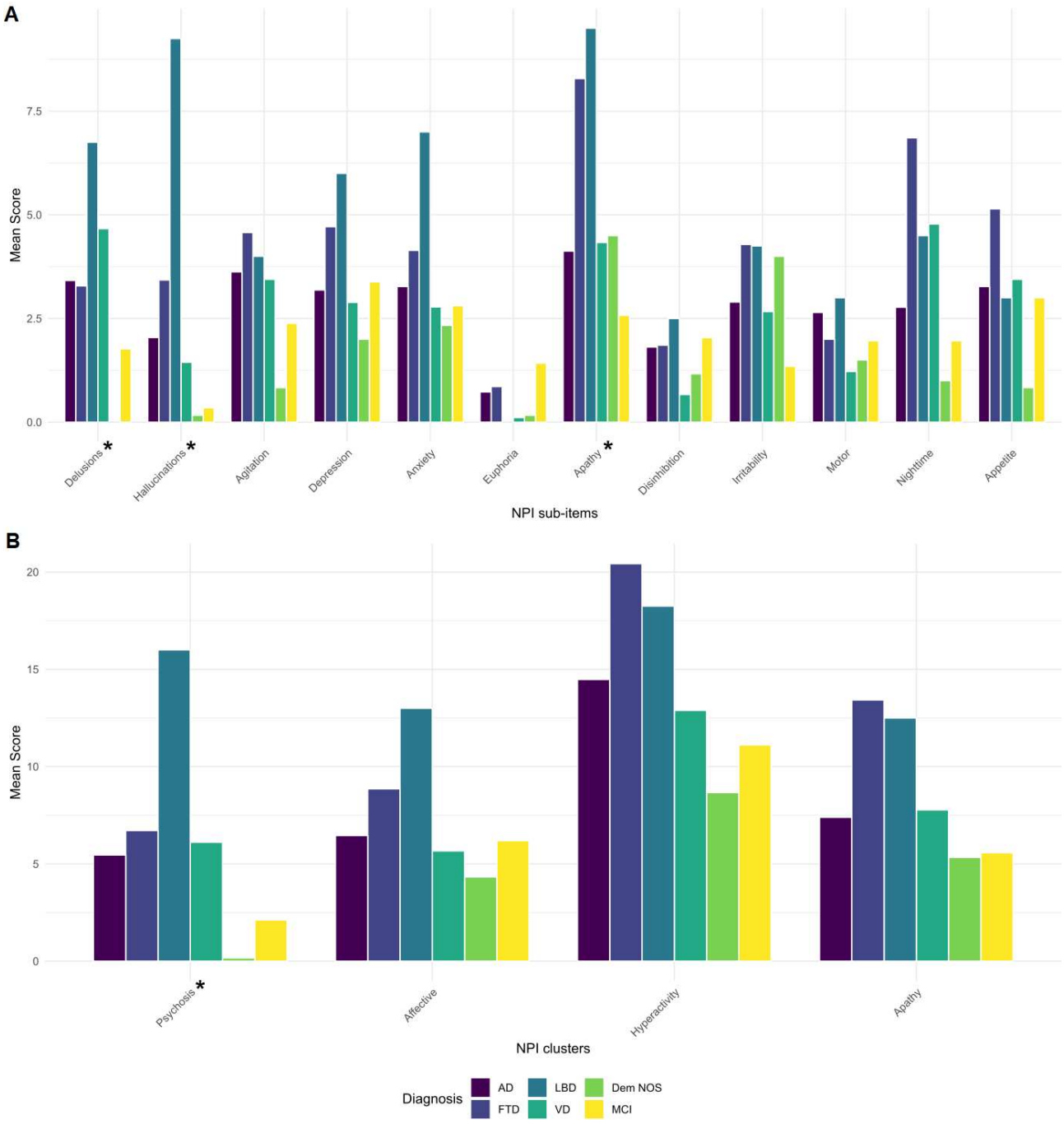
	Total Sample (N=100)	Dementia (N=74)		MCI (N=26)	p-value[a]
		AD (N=48)	non-AD (N=26)		
Age, mean (sd),y	74.1 (6.9)	74.4 (6.5)	74.8 (8.0)	72.7 (6.5)	0.31
Female, N. (%)	44 (44)	30 (62.5)	14 (53.8)	12 (46.2)	0.45
Education, mean (sd), y	7.8 (3.6)	7.5 (3.8)	7.8 (4.1)	8.2 (2.6)	0.43
MMSE, mean (sd)[b]	19.9 (6.2)	16.7 (5.4)	18.6 (4.7)	26.8 (2.0)	<0.001
NPI tot, mean (sd)	32.6 (30.0)	33.8 (31.8)	38.0 (29.3)	25.0 (26.7)	0.33
CSF biomarkers, mean (sd), pg/ml					
$A\beta_{42}$[b]	707.9 (360.2)	528.9 (230.9)	904.8 (394.0)	838.7 (376.5)	<0.001
p-tau[b]	72.5 (39.4)	95.7 (39.9)	44.6 (15.7)	57.4 (29.5)	<0.001
t-tau[b]	541.3 (351.8)	761.3 (360.3)	306.5 (117.2)	375.0 (235.3)	<0.001
MTA, N.	91	44	23	24	
0 L/R	13/16	6/6	2/3	5/7	0.07/0.30
1 “	16/24	7/11	3/4	6/9	
2 “	36/28	16/15	8/7	12/6	
3 “	22/19	14/10	7/7	1/2	
4 “	4/4	1/2	3/2	0/0	
GCA, N.	75	35	17	23	
0 L/R	18/18	7/7	2/2	9/9	0.24/0.30
1 “	27/28	13/14	6/6	8/8	
2 “	24/26	12/11	6/9	6/6	
3 “	6/3	3/3	3/0	0/0	
PA, N	76	36	17	23	
0 L/R	20/25	5/7	5/6	10/12	0.05/0.09
1 “	31/36	13/18	9/8	9/10	
2 “	21/11	14/7	3/3	4/1	
3 “	4/4	4/4	0/0	0/0	

AD= Alzheimer’s disease; CSF= cerebrospinal fluid; GCA= global cortical atrophy; L= Left; MCI= mild cognitive impairment; MMSE= Mini-Mental State Examination; MTA= medial temporal atrophy; NPI= Neuropsychiatric Inventory; PA= posterior atrophy; R= right. a) Significance tests used were Fisher test for categorical variables and Kruskal-Wallis test for continuous variables. b) Post-hoc pair-wise comparisons with Bonferroni correction of diagnostic groups: MMSE: MCI > AD and non-AD ($p < 0.001$); Aβ1-42: AD < non-AD and MCI ($p < 0.001$); p-tau: AD > non-AD and MCI ($p < 0.001$); t-tau: AD > non-AD and MCI ($p < 0.001$)

2.2.2 Neuropsychiatric symptoms among diagnostic groups

NPI total score was not significantly different among diagnostic groups. With regard to the sub-items, delusions, hallucinations and apathy were differently distributed among the diagnostic groups ($p<0.05$, <0.001 and <0.01 respectively), as well as the psychosis cluster when NPI clusters were computed ($p<0.05$) (Figure 2). After post-hoc analyses, hallucinations showed higher scores in LBD compared to MCI and AD patients ($p<0.001$ and $p<0.05$, respectively), and psychosis cluster also displayed higher scores in LBD with respect to MCI subjects ($p<0.05$).

Figure 2. Barplot of mean scores of NPI sub-items (A) and NPI clusters (B) by diagnostic groups



AD= Alzheimer's disease, FTD= frontotemporal dementia; Dem NOS= demented not otherwise specified; LBD= Lewy body dementia, MCI= mild cognitive impairment, NPI= Neuropsychiatric Inventory, VD= Vascular dementia.* Significance tests used were Kruskal-Wallis: Delusions ($p<0.05$), Hallucinations ($p<0.001$), Apathy ($p<0.01$), Psychosis cluster ($p<0.05$) Post-hoc pair-wise comparisons with Bonferroni correction: Hallucinations: LBD > MCI and AD ($p<0.001$ and 0.05, respectively) Psychosis cluster: LBD > MCI ($p<0.05$)

2.2.3 Neuropsychiatric symptoms and t-tau

When searching for correlations between NPI and t-tau levels in patients with primary dementia (AD, LBD and FTD), we found a trend toward a negative correlation ($R = -0.25$, $p = 0.08$) (Figure 3). Moreover, after backward elimination, the beta regression model indicated a significant association among NPI total score and t-tau ($p<0.01$) (Table 2). As far as each sub-item of NPI was concerned, a significantly negative correlation was found between nighttime disturbances and t-tau both in patients with primary dementia ($R= -0.29$, $p<0.05$) and in total sample ($R = -0.22$, $p<0.05$).

Figure 4 illustrates the relationship between t-tau and NPI total score according to the stage of disease (scored with MMSE). A positive non-significant relationship was observed in MCI subjects, while a negative relationship was found in patients with dementia. Although the relationship is stronger in lower MMSE score, it shows a greater slope for higher MMSE score (MMSE=12-23: $R=-0.07$ $p=0.657$; MMSE=0-11: $R=-0.55$ $p=0.083$). The heterogeneity of the diagnostic groups included in the sample could partly explain the lack of significance observed in the correlations between t-tau and NPI total score (both in the whole sample and in the subgroups by MMSE range).

Figure 3. Scatterplot of Pearson's correlation between CSF t-tau and NPI total score in patients with primary dementia (AD, FTD and LBD).

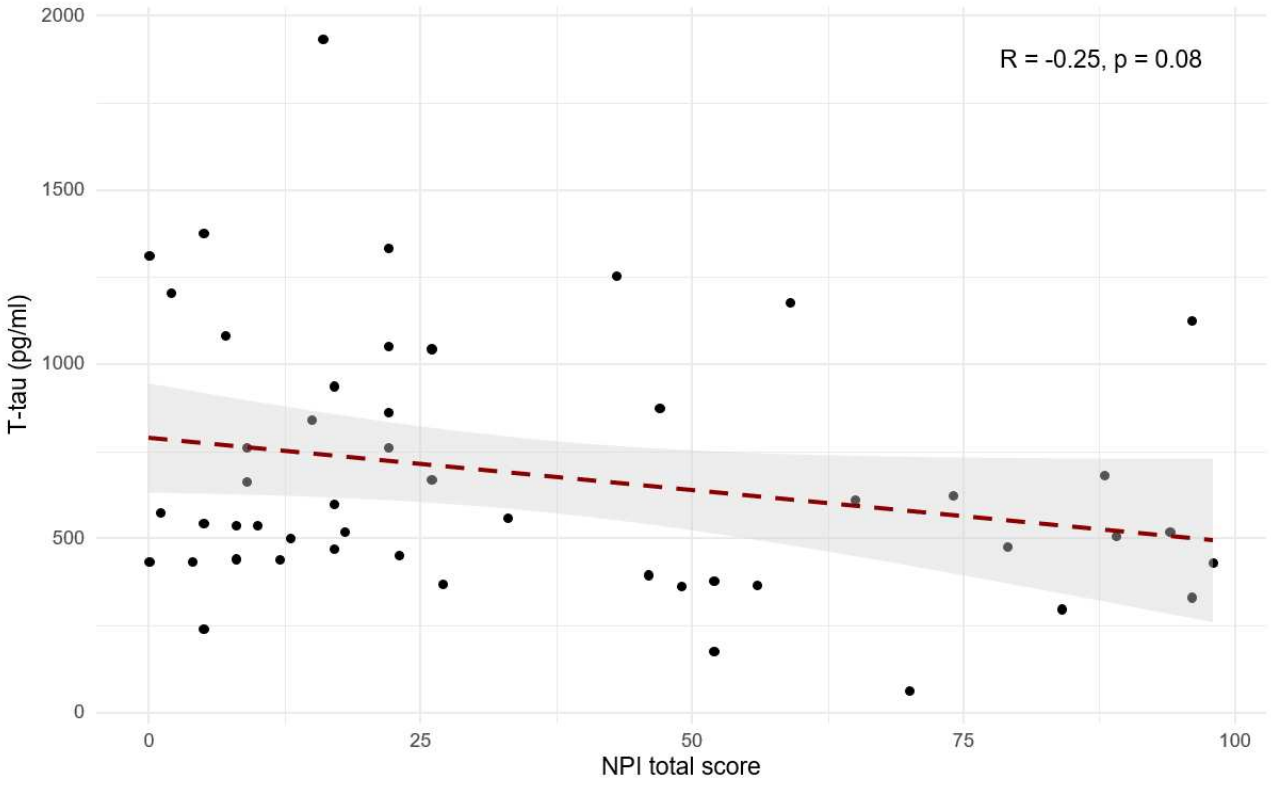
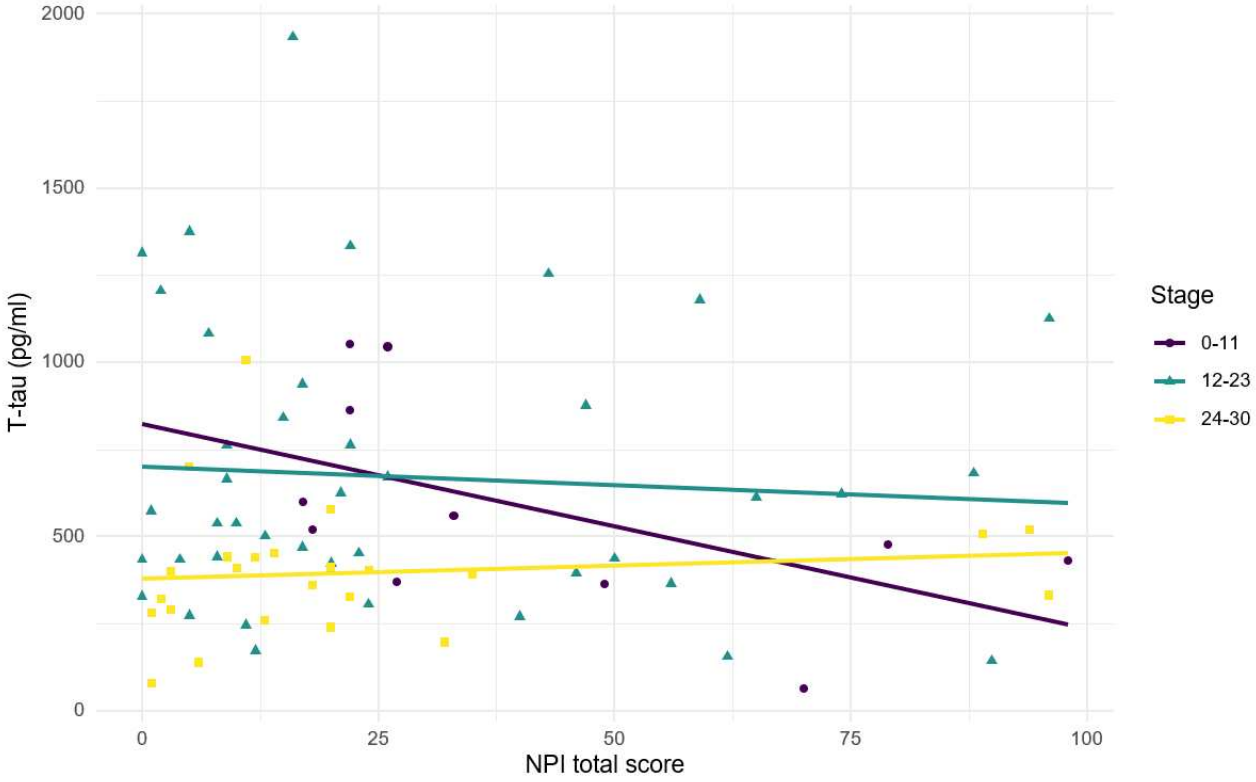


Table 2. Beta regression model of association of NPI total score and sociodemographic and biomarkers variables, displaying predictors retained in the final model after backward elimination.

	Estimate	Std. Error	Z value	p value= pr(>|z|)
(Intercept)	4.204	2.144	1.961	0.050
Age	-0.053	0.026	-2.018	**0.044**
Male gender	-1.661	0.444	-3.741	**0.0002**
Education	-0.099	0.051	-1.932	0.053
MMSE	0.036	0.030	1.198	0.231
t-tau	-0.002	0.001	-2.855	**0.004**
$A\beta_{42}$	-0.001	0.001	-1.820	0.069
GCA L	-1.178	0.831	-1.418	0.156
GCA R	1.844	0.912	2.022	**0.043**

GCA= global cortical atrophy; L= Left; MMSE= Mini-Mental State Examination; R= right.

Figure 4. Relationship between CSF t-tau and NPI total score according to disease stages.



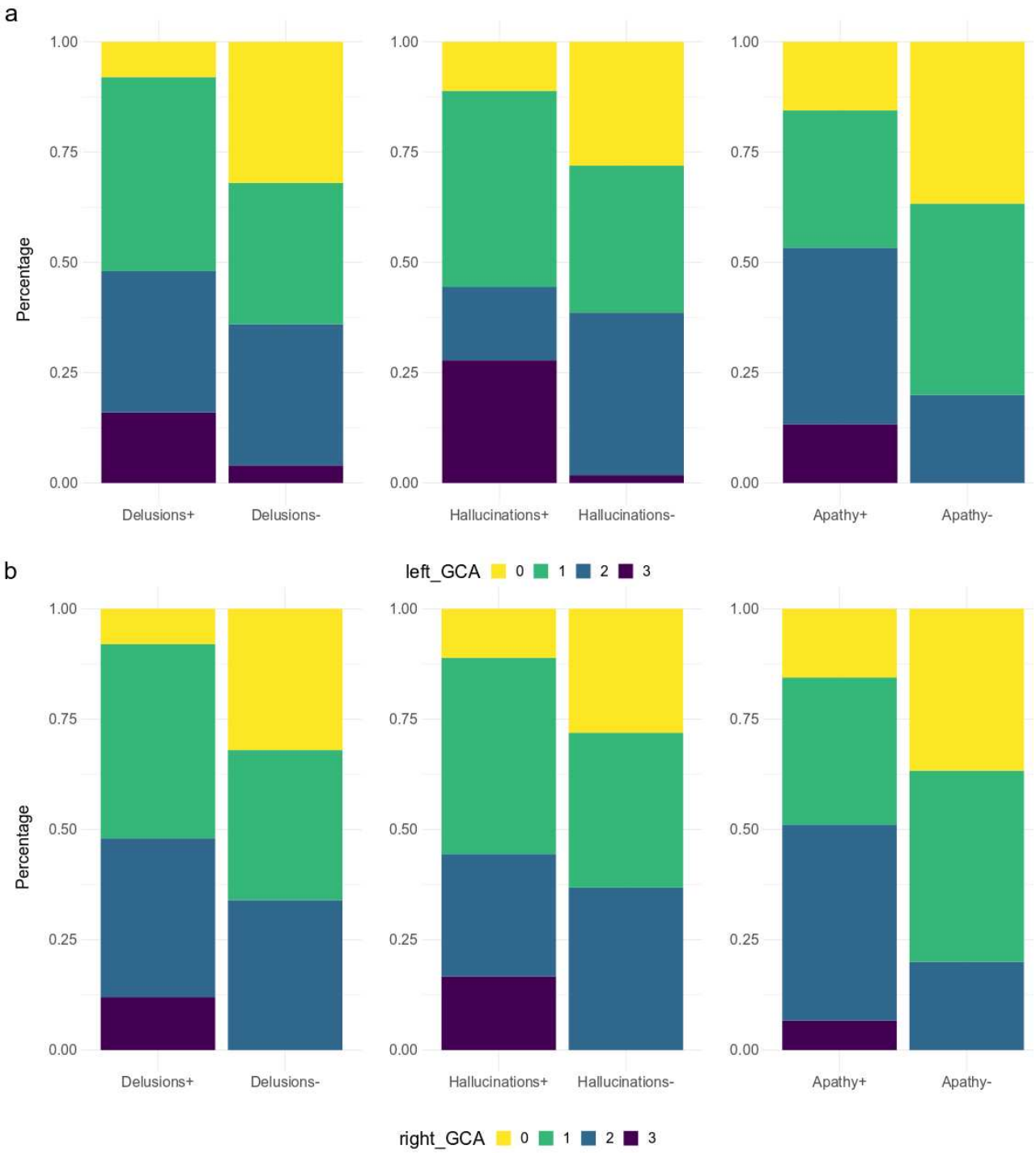
Stage was defined according to MMSE score. 24-30, mild cognitive impairment; 12-23, mild-to-moderate dementia; 0- 11, moderate-to-severe dementia. Correlations: stage 24-30: R=0.11 p=0.597; stage 12-23: R=-0.07 p=0.657; stage 0-11: R=-0.55 p=0.083

2.2.4 Neuropsychiatric symptoms and brain atrophy

When patients were dichotomized in two groups based on the presence or absence of a specific NPI domain (e.g. agitation positive vs agitation negative), significantly higher GCA-F scores were found in patients with the following symptoms (Figure 5): delusions ($p<0.05$, on both sides), hallucinations ($p<0.01$ and $p<0.05$, on left and right side, respectively) and apathy ($p<0.05$, on both sides). No significant differences were found regarding MTA and PA scores.

From correlation analyses, a positive correlation emerged between GCA-F scores and delusions (right: $p<0.05$), agitation/aggression (left: $p<0.05$), and psychosis cluster (right: $p<0.05$). Conversely, nighttime disturbances were positively correlated with both GCA-F and MTA scores on both sides (left: $p<0.01$; right: $p<0.05$). Finally, in accordance with the correlation analyses, the beta regression model indicated a significant association between NPI total score and right GCA-F score ($p<0.05$) (Table 2).

Figure 5. GCA-F score distribution by NPI sub-items.



GCA-F= global cortical atrophy; NPI= Neuropsychiatric Inventory. GCA-F scores were reported only for NPI sub-items with significant comparative changes (panel A for the left hemisphere, and panel B for the right hemisphere). Significance tests used was Fisher test: Delusions ($p<0.05$), Hallucinations ($p<0.01$), Apathy ($p<0.05$)

Chapter 3: Experimental study (Part 2)

In order to confirm and extend the evidence emerging in part 1 of the experimental study, we investigated potential correlations between neuropsychiatric symptoms and quantitative measures of atrophy, in particular cortical thickness (CT) and volume.

3.1 Materials and Methods

3.1.1 Patients

Participants were cognitively impaired patients undergoing diagnostic workup at the Behavioral Neurology Unit of the IRCCS Mondino Foundation between June 2018 and February 2021. Inclusion criteria were: a diagnosis of MCI (amnestic or non-amnestic / single or multiple domain) (47) or dementia; age between 50 and 90 years and an available informant with at least 10 hours per week of contact with the patient. No limit of severity of dementia was set, as long as the patient was able to perform a formal cognitive assessment. The vision and hearing acuity of patients were sufficient for compliance with testing procedures. Patients were excluded if they had a history of psychiatric disease or epilepsy, or any uncontrolled medical condition that could contribute to the subject's cognitive impairment (e.g., nephropathy, liver disease, brain tumor, alcohol or drug abuse, normal pressure hydrocephalus). None of the patients were receiving medications for dementia, such as cholinesterase inhibitors or antipsychotic drugs, at the time of the diagnostic workup.

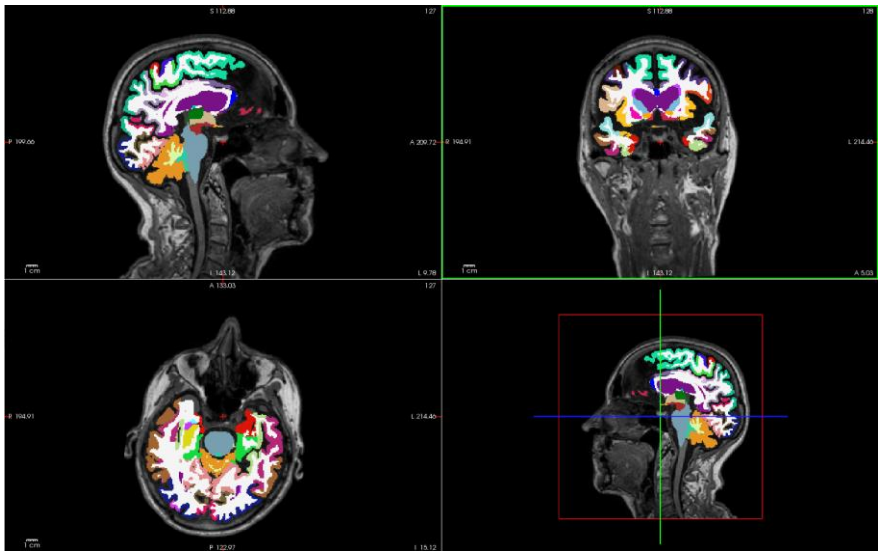
3.1.2 Neuroimaging

Images were acquired on a 3T (n=85) Siemens Skyra 3T, both equipped with a 32-channel coil at the Department of Neuroradiology, IRCCS Mondino Foundation, Pavia, Italy. Imaging parameters for the 3D T1-weigthed sequence were as follows: magnetization-prepared rapid acquisition with gradient echo (MPRAGE) with time of repetition = 2300 ms, echo time = 2.98

ms; inversion time = 900 ms; flip angle = 9°; voxel size = 1.0 x 1.0 x 1.0 mm (n= 78) or 1.2 x 1.2 x 1.2 mm, (n= 24) with no interslice gap; matrix size = 256 x 256.MRI scans were acquired at the Neuroradiology Unit of IRCCS Mondino Foundation.

The commercial software FreeSurfer v.6 (https://surfer.nmr.mgh.harvard.edu) was used to perform image segmentation of the whole brain volume. FreeSurfer provides a full processing stream for structural MRI data, such as skull stripping, B1 bias field correction, and an accurate reconstruction of the patient's cortical surface by identifying the gray-white matter boundary. Subsequently, the pial surface is defined and cortical surface is labelled accordingly to anatomical atlases (see Figure 6), more information about FreeSurfer procedure could be found elsewhere (58-59). We chose to use the parcelling results based on the Desikan-Killiany atlas (60), as it gave the necessary details for our analysis. We performed a quality check of the results and the segmentation was visually inspected by a neuroradiology trainee with five years of experience in neuroimaging for any errors. The mean cortical thickness of 20 cortical areas and the volume of four subcortical areas were extracted. An in-house Matlab v.2020b code was used to extract and store patient-specific anatomical measures of interest for subsequent statistical analysis. The chosen 24 regions of interest were: Entorhinal cortex, Parahippocampal gyrus, Temporal pole, Fusiform gyrus, Superior frontal gyrus, Middle frontal gyrus_Caudal middle frontal gyrus, Middle frontal gyrus_Rostral middle frontal gyrus, Inferior frontal gyrus_Pars opercularis, Inferior frontal gyrus_Pars triangularis, Inferior frontal gyrus_Pars orbitalis, Orbitofrontal cortex_Lateral orbital frontal cortex, Orbitofrontal cortex_Medial orbital frontal cortex, Frontal pole, Precentral gyrus, Paracentral lobule, Cingulate cortex_Rostral anterior division, Cingulate cortex_Caudal anterior division, Cingulate cortex_Posterior division, Cingulate cortex_Isthmus division, Corpus callosum, Accumbens, Amygdala, Caudate, Hippocampus, Pallidum, Putamen, Thalamus, Insula.

Figure 6. FreeSurfer segmentation according to Desikan-Killiany atlas (60).



3.1.3 Statistical analysis

Shapiro-Wilk test was used to investigate the distribution normality of the different variables. Demographic and clinical characteristics among diagnostic groups were compared using ANOVA or Kruskall-Wallis test for continuous variables, and Chi-square test ($\chi 2$) or Fisher's exact test for categorical variables. Correlations between NPI scores and cortical thickness (CT) and volume (V) were assessed using Spearman coefficient. Statistical computations were performed using R v. 3.5.3 (The R Foundation for Statistical Computing). Two-sided p-values <0.05 were considered to indicate significance.

3.2 Results

3.2.1 Patients' characteristics

Demographic and clinical characteristics of the study population are shown in Table 3. Fifty-eight patients were diagnosed with dementia (mean age, 73.6±7.1 years; 55% female) and 27 with MCI (73.2±5.7 years; 44%). Mean MMSE score was 18.8±5.0 in patients with dementia and 26.8±2.1 in subjects with MCI. In the dementia group, 41 patients were diagnosed as AD,

5 as FTD, 2 as LBD, 4 as VD and 6 as Dem NOS. No significant difference was found among AD, non-AD and MCI groups with respect to age, gender and education. As expected, AD group showed lower $A\beta_{42}$ levels, and higher p-tau and t-tau levels, compared to non-AD and MCI groups.

Table 3. Sociodemographic features, cognitive status and biomarker measures in the total sample and in the three main diagnostic groups.

	Total Sample (N=85)	Dementia (N=58)		MCI (N=27)	p-value[a]
		AD (N=41)	non-AD (N=17)		
Age, mean (sd), y	73.4 (6.7)	74.1 (7.1)	72.0 (7.3)	73.2 (5.7)	0.50
Female, N. (%)	45 (53)	27 (66)	6 (35.3)	12 (44.4)	0.06
Education, mean (sd), y	8.1 (3.5)	7.6 (3.7)	8.2 (3.5)	8.8 (3.4)	0.30
MMSE, mean (sd)[b]	21.5 (5.5)	18.4 (5.0)	20.3 (4.6)	26.8 (2.1)	<0.001
NPI tot, mean (sd)	25.2 (25.7)	31.4 (29.0)	24.4 (25.9)	16.2 (17.0)	0.14
CSF biomarkers, mean (sd), pg/ml					
$A\beta_{42}$[b]	624.0 (285.4)	526.9 (229.6)	801.3 (352.7)	658.3 (267.6)	<0.001
t-tau[b]	613.5 (431.3)	859.4 (466.8)	325.9 (278.3)	436.4 (220.0)	<0.001
p-tau[b]	87.3 (62.9)	120.7 (71.2)	48.6 (40.7)	63.0 (30.0)	<0.001

AD= Alzheimer's disease; CSF, cerebrospinal fluid; MCI= mild cognitive impairment; MMSE= Mini-Mental State Examination; NPI= Neuropsychiatric Inventory. a) Significance tests used were Chi-square test for categorical variables and Kruskal–Wallis test for continuous variables. b) Post hoc pair-wise comparisons with Bonferroni correction of diagnostic groups: MMSE: MCI > AD and non-AD ($p < 0.001$); Aβ1–42: AD < non-AD ($p < 0.01$); p-tau: AD > non-AD and MCI ($p < 0.001$); t-tau: AD > non-AD and MCI ($p < 0.001$).

3.2.2. Neuropsychiatric symptoms and atrophy measures (cortical thickness and volume)

Among correlation analyses, significant correlations were observed for 4 main neuropsychiatric symptoms: delusions, hallucinations, agitation, and apathy (Table 4). Delusions showed negative correlations with CT and V of frontal areas (dorsolateral and orbital, with a prevalent involvement on the right side) and of areas of the limbic system (anterior and posterior cingulate, isthmus and entorhinal cortex). As well, hallucinations showed an involvement of

the frontal lobe (dorsolateral) and the limbic system (anterior and posterior cingulate, isthmus, fusiform gyrus and hippocampus). A decrease in CT and V of the opercular region (insula and temporal pole) and the limbic system (entorhinal, parahippocampal and fusiform cortex and amygdala) was instead correlated with agitation/aggression. Finally, apathy showed a negative correlation with regions of the frontal lobe (dorsolateral, orbital, opercular, precentral and paracentral) insula and the limbic system (anterior cingulate and isthmus).

Table 4. Correlations between neuropsychiatric symptoms and atrophy measures (cortical thickness and volume).

Delusion			Hallucination			Agitation			Apathy		
Regions of Interest	r_s	p-value	Regions of Interest	r_s	p-value	Regions of Interest	r_s	p-value	Regions of Interest	r_s	p-value
Cortical thickness			*Cortical thickness*			*Cortical thickness*			*Cortical thickness*		
Superiorfrontal-R	-0.26	0.02	Superiorfrontal-R	-0.26	0.02	Insula-R	-0.29	0.01	Superiorfrontal-R	-0.36	0.00
Superiorfrontal-L	-0.23	0.04	Superiorfrontal-L	-0.22	0.05	Entorhinal-L	-0.27	0.01	Superiorfrontal-L	-0.36	0.00
Caudalmiddlefrontal-R	-0.22	0.04	Caudalmiddlefrontal-L	-0.22	0.05	Parahippocampal-L	-0.23	0.04	Caudalmiddlefrontal-R	-0.29	0.01
Parsopercularis-R	-0.29	0.01	Rostralmiddlefrontal-L	-0.24	0.03	Temporalpole-R	-0.25	0.02	Caudalmiddlefrontal-L	-0.31	0.00
Lateralorbitofrontal-L	-0.28	0.01	Parsopercularis-R	-0.27	0.01	Fusiform-R	-0.24	0.03	Rostralmiddlefrontal-R	-0.25	0.02
			Parstriangularis-R	-0.22	0.05				Rostralmiddlefrontal-L	-0.24	0.03
									Parsopercularis-R	-0.35	0.00
									Parsorbitalis-L	-0.32	0.00
									Parstriangularis-R	-0.25	0.02
									Parstriangularis-L	-0.25	0.02
									Lateralorbitofrontal-L	-0.30	0.01
									Medialorbitofrontal-L	-0.37	0.00
									Precentral-R	-0.29	0.01
									Precentral-L	-0.24	0.03
									Paracentral-R	-0.22	0.04
									Paracentral-L	-0.22	0.05
									Caudalanteriorcingulate-L	-0.30	0.01
									Isthmuscingulate-L	-0.24	0.03
									Insula-R	-0.22	0.05
									Entorhinal-L	-0.22	0.05

Volume			*Volume*			*Volume*			*Volume*		
Superiorfrontal-R	-0.24	0.03	Caudalanteriorcingulate-L	-0.29	0.01	Parahippocampal-L	-0.23	0.04	Parsopercularis-R	-0.32	0.0
Parsorbitalis-R	-0.23	0.04	Posteriorcingulate-L	-0.23	0.04	Amygdala-R	-0.25	0.02	Parsopercularis-L	-0.22	0.04
Parsorbitalis-L	-0.24	0.03	Isthmuscingulate-L	-0.23	0.04				Rostralanteriorcingulate-L	-0.23	0.03
Medialorbitofrontal-R	-0.22	0.04	Fusiform-R	-0.22	0.05				Caudalanteriorcingulate-L	-0.29	0.01
Caudalanteriorcingulate-L	-0.22	0.04	Hippocampus-R	-0.25	0.02						
Posteriorcingulate-L	-0.25	0.02									
Isthmuscingulate-R	-0.23	0.03									
Isthmuscingulate-L	-0.24	0.03									
Entorhinal-R	-0.23	0.04									
Fusiform-R	-0.25	0.02									
Hippocampus-R	-0.26	0.02									

R_s= Spearman correlation coefficient

Discussion

This study investigated the distribution of neuropsychiatric symptoms and their CSF and neuroimaging correlates in a large sample of patients suffering from different dementing disorders, diagnosed with the most recent criteria. A significant prevalence of psychotic symptoms (hallucinations and delusions) was found in patients with LBD when compared to MCI or AD. The CSF biomarkers analysis showed a negative trend of t-tau when plotted with total NPI scores in patients with primary dementias, and a positive relationship in patients with MCI, drawing a parabolic trajectory across disease stages of increasing severity. However, the pathological heterogeneity of the sample and the small number of some etiological subgroups may have prevented the statistical significance of some analyses of correlation between t-tau and NPI score. Cortical atrophy (assessed with both visual rating and quantitative measures) in frontal lobe and limbic system was associated with occurrence of delusions, hallucinations, agitation/aggression and apathy.

The high prevalence of hallucinations in our cohort of LBD patients is consistent with the most recent diagnostic criteria, which consider the hallucinations as one of the core clinical features, occurring in up to 80% of patients (52) and predicting post-mortem Lewy pathology with 93% accuracy (61). This key symptom usually occurs in the form of well-structured visual hallucinations (VH), associated to different degree of insight, even though tactile and auditory variants have been also reported (52). Delusions are frequently reported in LBD, approximately in 49% of cases (62), the most frequent of them being represented by misidentification delusions, whose prevalence in LBD is higher than in AD (52% versus 34%) (12). The high frequency of psychotic symptoms observed in this study in LBD patients is therefore consistent with previous reports in literature, supporting a higher prevalence of hallucinations and delusions in synucleinopathies, among which LBD, than in tauopathies, such as AD and FTD (46). Different functional and structural abnormalities have been suggested to underlie psychotic manifestations: parietal and occipital hypometabolism and frontal

atrophy were reported in VH (62-63), while hypoperfusion of frontal, limbic and paralimbic cortex were associated with misidentifications and delusions (64).

Interestingly, in our study the negative trend observed between NPI total score and t-tau in patients with dementia was somewhat unexpected in front of the results of previous studies, which mostly report a positive correlation between t-tau and BPSD severity (33-34, 36, 65). Upon closer examination, these studies investigated populations with milder cognitive impairment than ours, including either healthy subjects (33) or patients with MCI (34) or mild AD (MMSE=24.2±2.3) (65). Conversely, the present study includes patients belonging to more advanced stages of disease and with a more severe cognitive impairment, regardless of the etiological diagnosis (AD or non-AD) (MMSE=16.7±5.4 and 18.6±4.7, respectively). We therefore believe that t-tau decreases in advanced stages of dementia as a result of the decline of the neurodegenerative process involving the brain cortex, as also supported by longitudinal studies (66). Unlike the above studies, Bloniecki and colleagues (36) investigated the relationship between CSF biomarkers and BPSD in a cohort of cognitively more impaired patients (affected from mild-to-moderate AD, MMSE=19.1±4.2), and including a wider range of diagnosis (in addition to AD, also vascular dementia and AD mixed, MMSE=20.2±4.6). They found a positive correlation in the AD group alone, but with a decrease in the correlation coefficient (from 0.35 to 0.13) when the whole sample was analyzed. The inclusion of non-AD dementias, usually characterized by lower t-tau values, could plausibly account for this finding. Similarly, the negative trend observed in our population of demented patients could partly originate from the presence of non-AD dementias (in particular, FTD and LBD), which may contribute to mitigate or reverse the correlation between t-tau and BPSD. On the other hand, the finding of a positive trend in our subjects with MCI appears to be consistent with the evidence present in literature related to early disease stages. Summarizing, after an initial increase directly related to the spread of the neurodegenerative process, CSF t-tau levels may decline in advanced disease stages, as a result of the reduced number of neurons spared by atrophy and still likely to degenerate.

Consistently with this interpretation, Mollenhauer et coll reported a decline in t-tau levels in AD patients in advanced stage (67), while Isoe et coll described a biphasic curve with an increase in t-tau levels at the disease onset, followed by a progressive decline in the final stages (68).

In this study, we report for the first time that cortex visual rating is able to detect more severe atrophy involving the frontal lobe in patients with delusions, hallucinations or apathy. The search for possible links between BPSD and cortical abnormalities is currently an active field of investigation. A recent study identified the reduction of volume of the frontal lobe (in particular, the anterior cingulate cortex and the middle frontal gyrus) as a significant predictor of the occurrence of BPSD, such as apathy, delusions and hallucinations, in AD patients (42). Previously, other studies had supported this link, reporting a positive correlation between frontal atrophy and the occurrence of apathy in AD patients, and an association between frontal lobe dysfunction and initiative reduction in AD, FTD and LBD patients, particularly in late disease stages. The involvement of frontal networks subserving motivation and reward mechanisms, including the anterior cingulate cortex, superior and middle frontal gyri and basal ganglia, provides a possible explanation of how atrophy involving these areas may contribute to the loss of interest and to the development of apathy (23, 69). A number of studies also reported a more frequent finding of frontal atrophy in demented patients with hallucinations. In these subjects, Sanchez-Castaneda et al. precisely described a more severe cortical atrophy involving the inferior frontal gyrus and the precuneus (63), while Heitz et al. reported a functional impairment of both anterior and posterior cortical regions, including the anterior cingulate cortex, the orbitofrontal cortex and the cuneus (70). In addition to other reports in literature, this evidence is endorsed by the results of the present study. The above frontal areas are indeed included in neuronal circuits assigned to inhibitory control and the decision-making mechanisms; their deregulation could prevent the patient from inhibiting the production of internal images, thus representing the pathophysiological substrate for the development of hallucinations (71). Conversely, understanding the neurobiological bases of delusions is more challenging, as few studies addressed systematically this issue. As

mentioned above, frontal atrophy is a frequently encountered finding, that suggests an involvement of many areas of the frontal lobe in the generation of delusions, with particular regard to the orbitofrontal, limbic and paralimbic regions (64, 72). In addition, the significant correlation between delusions' severity and both GCA-F scores and quantitative measures of atrophy in numerous frontal regions on the right side in our sample, confirms the pivotal role of the right frontal lobe in controlling and structuring thought. Based on this assumption, frontal cortical atrophy could promote the development of delusions through the loss of control functions aimed to supervise reality and to compare internal experience with the outer world, leading to the consolidation of false beliefs (73). However, the meaning to attribute to the above findings is still largely speculative, mainly due to the heterogeneity of delusions' presentation, which may reflect the impairment of multiple functional networks located in different areas of the brain. Finally, agitation positively correlated with left-sided GCA-F scores and with the atrophy of mesolimbic and temporal regions on both hemispheres. The few studies of neuroimaging investigating structural and functional correlates of agitation/aggression reported an involvement of the left frontotemporal region in AD patients, associated with a concurrent more severe burden of neurofibrillary tangles in the same region (74-75). These findings are consistent with a large body of neuropsychiatry literature describing a complex brain network of prefrontal, subcortical, and mesolimbic circuitry that mediates and regulates social behaviors, and of frontoinsular circuitry that plays a crucial role in the processing of more complex social emotions such as empathy, compassion, and fairness. Agitation and aggression may therefore be due to the default of this frontotemporal network, leading to the loss of capacity to process and regulate behaviors properly (75).

The evidence emerging from this study, and corroborated by data from the literature, suggests that the limbic system is involved in the pathophysiology of the most stressful and disabling BPSDs. Hallucinations, delusions, agitation, and apathy recognize a dysfunction somewhere in the mesolimbic circuitry composed of the fusiform gyrus, hippocampus, amygdala, orbitofrontal and

prefrontal cortices, and cingulate gyrus. The hippocampus and fusiform gyrus process sensory information from the environment and provide an emotional correlate through networks with the amygdala. All of these sensory and emotional inputs are transmitted to the orbitofrontal region, where the prefrontal cortex exerts an inhibitory barrier role, filtering out unnecessary, incorrect, or invalid data. The information thus cleaned up can be relayed back to the hippocampus via the cingulate. Excess function of the hippocampus-amygdala system, defective control of the prefrontal cortex, or disruption of the cingulate gyrus may promote the occurrence of BPSD.

Limitations

The main limitation of this study is the heterogeneous composition of the population and the small size of some subgroups. The larger number of AD patients may have partly affected the correlation analyses between CSF or neuroimaging biomarkers and BPSD severity. Indeed, primary dementias, such as AD, FTD and LBD, usually show different levels of tau and different cortical atrophy distribution, as results of the underlying neuropathological process and its specific tropism.

Conclusions

This study provides a real-world overview of the most clinically relevant BPSD occurring in patients attending a memory clinic due to dementing conditions. The gathered evidence suggests that, in a future perspective, CSF biomarkers and visual rating scales for cortical atrophy could be hopefully included in a multidimensional evaluation of demented patients, aimed to predict prognosis and occurrence of BPSD. Longitudinal studies on wider and diagnosis-balanced cohorts of patients are however necessary to properly ascertain the actual predictive value of these biomarkers.

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Acknoledgement

I am grateful to Elisabetta Zardini for her significant contribution in CSF immunoassay, to Sara Bernini for the neuropsychological assessment, to Lisa Farina for the MRI scans acquisition and to Elena Ballante for her support in the statistical analyses.